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# Hematopathology

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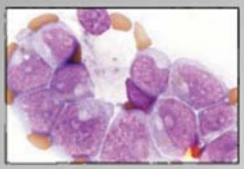

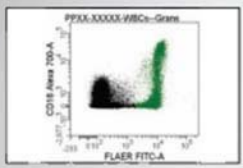


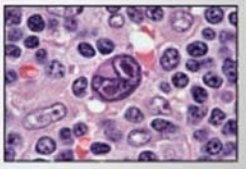

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FOUNDATIONS IN DIAGNOSTIC PATHOLOGY

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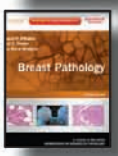


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# HEMATOPATHOLOGY

A Volume in the Series

**FOUNDATIONS IN DIAGNOSTIC PATHOLOGY**

SECOND EDITION

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*To Eileen, Evan, and Ethan, who bring my life*
*into focus (without the need for immersion oil) …*

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# Foreword

Surgical pathology, with all of the subspecialties it encompasses, has become increasingly complex and sophisticated. It is simply not possible for any individual to master the skills and knowledge required to perform all of these tasks at the highest level. Providing a correct diagnosis is certainly challenging enough, but the standard of care has far surpassed merely providing an accurate pathologic diagnosis. Pathologists are now asked to provide large amounts of ancillary information, both diagnostic and prognostic, often on small amounts of tissue, a task that can be daunting even for the most experienced pathologists.

General surgical pathology textbooks are useful resources, but by necessity they cannot possibly cover many of the aspects that pathologists need to know and include in their reports. For this reason, *Foundations in Diagnostic Pathology* was born. This series is designed to cover the major areas of surgical pathology and cytopathology, and each volume is focused on one major topic. The goal of every book in this series, which is now beginning its second edition, is to provide the essential information that any pathologist—whether general or subspecialized, in training or in practice—would find useful in the evaluation of virtually any type of specimen encountered.

My colleague of many years at Cleveland Clinic, Dr. Eric Hsi, a renowned hematopathologist, has yet again edited an outstanding tome on hematopathology that fulfills the goals and philosophy behind *Foundations in Diagnostic Pathology*. This second edition cuts to the essentials of what all pathologists want and need to know about this complex group of disorders. The list of contributors is impressive and includes nationally and internationally renowned hematopathologists who were generous in providing their expertise to this book. By design, there is uniformity in the organization of these chapters, each of which includes practical information and numerous photomicrographs that emphasize the essential diagnostic points. There is a seamless integration of ancillary diagnostic techniques, including immunohistochemistry, cytogenetics, and molecular techniques, which of course are essential to the modern practice of hematopathology.

The book is organized into four major sections: nonneoplastic disorders; lymphomas; leukemias, chronic myeloproliferative disorders, and myelodysplasia; and disorders of histiocytes, mast cells, plasma cells, spleen, and ancillary techniques. This edition features 24 chapters written by leaders in the fields in which they contribute their enormous experience and expertise.

I extend my deepest appreciation to Dr. Eric Hsi and all of the authors who have contributed to this outstanding second edition of the *Foundations in Diagnostic Pathology* series. This second edition improves on what was already an outstanding volume in this series, and I am completely confident you will enjoy this second edition of the *Foundations in Diagnostic Pathology* series.

*John R. Goldblum, MD*

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# Preface

It was with great pleasure that I accepted the kind offer to edit a second edition of this textbook. I was gratified that trainees and practicing pathologists found the first edition informative and useful. However, the field of hematopathology has advanced considerably since the first edition in 2007. With the publication of a second World Health Organization classification of hematologic malignancies, advances in our understanding of the molecular genetics of hematologic diseases (benign and malignant), and continued expansion in the variety of ancillary studies required for the characterization of these diseases in the clinical laboratory, it is time for an update. Although the general format, look, and feel of the first edition is maintained, this second edition contains new information relevant to diagnostic pathologists. Notably, new chapters have been added to cover such topics as flow cytometry and splenic pathology that have been requested by readers. As noted in the preface to the first edition, this book is not meant to be an encyclopedic treatment of hematopathology. I believe we have still kept the book concise while covering the most important information in both neoplastic and non-neoplastic hematopathology and offering useful discussions on differential diagnoses. Of course, this book is only as good as the individual chapters, and I am indebted to the many contributors who really did the "heavy lifting." It is an honor to work with such fine pathologists and educators.

*Eric D. Hsi, MD*

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# Contents


## SECTION 1
## Non-Neoplastic Disorders 1

CHAPTER 1
Red Blood Cell/Hemoglobin Disorders 3
**Steven H. Kroft, MD, and Sara A. Monaghan, MD**

CHAPTER 2
Platelet Disorders 55
**Kandice Kottke-Marchant, MD, PhD**

CHAPTER 3
Non-Neoplastic Morphologic Abnormalities of White Blood Cells and Macrophages 99
**Dennis P. O'Malley, MD, and Eric D. Hsi, MD**

CHAPTER 4
Reactive Lymph Nodes and Castleman Disease 111
**Judith A. Ferry, MD**

CHAPTER 5
Bone Marrow Failure Syndromes 160
**James R. Cook, MD, PhD**

CHAPTER 6
Benign Causes of Bone Marrow Abnormalities Including Infections, Storage Diseases, Systemic Disorders, and Stromal Changes 177
**Dennis P. O'Malley, MD**

## SECTION 2
## Lymphomas 203

CHAPTER 7
Small B-Cell Lymphomas 205
**Paul J. Kurtin, MD**

CHAPTER 8
Diffuse Aggressive B-Cell Lymphomas 261
**Graham W. Slack, MD, Eric D. Hsi, MD, and Randy D. Gascoyne, MD, FRCPC**

CHAPTER 9
Peripheral T-Cell Lymphomas 293
**William R. Macon, MD**

CHAPTER 10
Immunodeficiency-Related Lymphoproliferative Disorders 327
**Jonathan W. Said, MD**

CHAPTER 11
Hodgkin Lymphoma 350
**Bertram Schnitzer, MD**

## SECTION 3

## Leukemias, Chronic Myeloproliferative Neoplasms, and Myelodysplasia 385

CHAPTER 12

B-Cell Leukemias of Mature Lymphocytes 387

**Eric D. Hsi, MD**

CHAPTER 13

Mature T-Cell and Natural Killer Cell Leukemias 403

**William G. Finn, MD, and William R. Macon, MD**

CHAPTER 14

Acute Myeloid Leukemia 419

**Amy E. Heerema-McKenney, MD, and Daniel A. Arber, MD**

CHAPTER 15

Precursor Lymphoid Neoplasms 457

**Mihaela Onciu, MD**

CHAPTER 16

Acute Undifferentiated Leukemia and Mixed-Phenotype Acute Leukemias 471

**Mihaela Onciu, MD**

CHAPTER 17

Myeloproliferative and "Overlap" Myelodysplastic/Myeloproliferative Neoplasms 479

**John Anastasi, MD**

CHAPTER 18

Myelodysplastic Syndromes 523

**Adina M. Cioc, MD, and Phuong L. Nguyen, MD**

## SECTION 4

## Disorders of Histiocytes, Mast Cells, Plasma Cells, Spleen, and Ancillary Techniques 547

CHAPTER 19

Disorders of Histiocytes 549

**Ronald Jaffe, MBBCh, and Kudakwashe Chikwava, MBChB**

CHAPTER 20

Mastocytosis 588

**Dong Chen, MD, PhD, and Chin-Yang Li, MD**

CHAPTER 21

Plasma Cell Neoplasms and Related Disorders 612

**Pei Lin, MD**

CHAPTER 22

Disorders of the Spleen 632

**Robert S. Ohgami, MD, PhD, James M. Ziai, MD, and Daniel A. Arber, MD**

CHAPTER 23

Flow Cytometric Principles in Hematopathology 655

**Steven J. Kussick, MD, PhD**

CHAPTER 24

Molecular Diagnosis in Hematopathology 679

**Shakil H. Merchant, MD, Jennifer L. Oliveira, MD, James D. Hoyer, MD, and David S. Viswanatha, MD**

INDEX 727

SECTION 1

# Non-Neoplastic Disorders

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# 1

# Red Blood Cell/Hemoglobin Disorders

■ **Steven H. Kroft, MD** ■ **Sara A. Monaghan, MD**

## ■ INTRODUCTION

The disorders of red blood cells can be divided into disorders of decreased red blood cell (RBC) mass (anemias) and those of increased RBC mass (erythrocytoses). Most of this chapter is devoted to various types of anemias, with only a short discussion of polycythemia.

From the pathophysiologic standpoint, anemias can be divided into three categories (Table 1-1): anemias of blood loss, anemias of decreased red blood cell production, and anemias caused by increased RBC destruction (hemolysis). Each of these categories can be subdivided further, as illustrated in Table 1-1 and subsequent sections.

This chapter focuses on commonly encountered conditions. Various rare causes of anemia and some disorders lacking characteristic changes in peripheral blood or bone marrow are omitted.

## ■ ANEMIAS OF BLOOD LOSS

Anemia caused by blood loss can be divided into acute and chronic forms. The anemia of acute blood loss, such as associated with major trauma, is not immediately evident based on complete blood cell count parameters, because losses consist of whole blood and the vascular system contracts on the decreased blood volume. However, with intravascular volume repletion, the erythrocytes will be diluted and the degree of RBC and hemoglobin (Hb) loss will be manifest on the complete blood cell count.

Chronic, low-level blood loss is initially compensated by increased marrow RBC production; therefore no anemia will be present, although reticulocytosis may be evident. With prolonged blood loss, body iron stores become depleted, the iron-deficient marrow is no longer able to compensate, and iron deficiency anemia (IDA) develops. Thus, the anemia of chronic blood loss is essentially IDA (see the following section).

## ■ ANEMIAS OF DECREASED RED BLOOD CELL PRODUCTION

Anemias of decreased red blood cell production can be divided into those caused by maturation defects and those caused by proliferation defects. A third category, anemia of chronic disease (ACD), is due to multiple interacting mechanisms and is often considered separately from the first two.

### MATURATION DEFECTS (INEFFECTIVE ERYTHROPOIESIS)

Maturation defects are characterized by defects in the maturation of the developing erythroid precursors in bone marrow. The defect may be primarily one of nuclear maturation (megaloblastic anemia) or cytoplasmic maturation (iron deficiency). Anemias of ineffective erythropoiesis are characterized in general by a failure of the marrow to produce adequate mature erythrocytes, despite erythroid hyperplasia in the marrow, and morphologic abnormalities of erythroid precursors, mature RBCs, or both. Myelodysplastic syndromes, although often characterized by ineffective erythropoiesis, are not discussed in this chapter (see Chapter 18).

### MEGALOBLASTIC ANEMIA

The megaloblastic anemias are macrocytic anemias associated with characteristic morphologic features of hematopoietic cells. Cytopenias are due to impaired DNA synthesis, which leads to abnormal nuclear maturation of hematopoietic precursors and ineffective hematopoiesis. The major causes of megaloblastic anemia are folate deficiency and cobalamin (Cbl; vitamin $B_{12}$) deficiency. Folate in the form of tetrahydrofolate (THF) is a transporter of 1-carbon units, used for synthesis of purines, thymidine, and methionine (Figure 1-1). Cbl is a required intermediate in an intracytoplasmic reaction that converts methyltetrahydrofolate, the

**TABLE 1-1**

**Classification of Red Blood Cell Disorders**

***Anemias***

- Anemias of blood loss
  - Acute
  - Chronic
- Anemias of decreased red blood cell production
  - Maturation defects (ineffective erythropoiesis)
    - Megaloblastic anemia
    - Iron deficiency
    - Myelodysplastic syndromes
  - Proliferation defects
    - Stem cell defects
    - Marrow replacement processes
    - Anemia of chronic kidney disease
    - Anemia of chronic disease
- Hemolytic anemias
  - Intrinsic red cell defects
    - Hemoglobin disorders
      - Structural hemoglobin disorders
        - Sickling diseases
        - Hemoglobin C disease
      - Thalassemias
    - Red cell membrane disorders
      - Hereditary spherocytosis
      - Hereditary elliptocytosis
      - Hereditary pyropoikilocytosis
    - Red cell enzyme disorders
      - Glucose-6-phosphate dehydrogenase deficiency
    - Paroxysmal nocturnal hemoglobinuria
  - Extrinsic red cell defects
    - Autoimmune hemolytic anemia
      - Warm
      - Cold
    - Microangiopathic hemolytic anemia
    - Parasitic hemolysis
      - Malaria
      - Babesia

***Erythrocytosis***

- Relative erythrocytosis (decreased plasma volume)
- Absolute erythrocytosis (polycythemia)
  - Primary
    - Polycythemia vera
    - Primary familial polycythemia
  - Secondary
    - Chronic hypoxia
      - Cardiac disorders
      - Pulmonary disorders
      - High altitude residence
    - Hemoglobin abnormalities
      - High oxygen affinity hemoglobins
      - Increased carboxyhemoglobin (smokers)
    - Aberrant erythropoietin production by tumors

main form of folate transported in plasma and taken up by cells, into THF while transferring the methyl group to homocysteine to generate methionine. Therefore either folate or Cbl deficiency impair thymidine and methionine synthesis. Cbl also serves as a cofactor for intramitochondrial conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A (not illustrated).

Because Cbl is produced in nature only by microorganisms, humans must obtain it from their diet. Good sources include red meat, some dairy products, and some seafood.

Pepsin, which requires acidic pH for action, releases Cbl from food in the stomach. Cbl will then bind haptocorrins (R-proteins) secreted in saliva. Haptocorrins are degraded by pancreatic enzymes in the small intestine, permitting Cbl to bind to intrinsic factor secreted by gastric parietal cells. Intrinsic factor–Cbl binds to receptors specific for this complex on ileal enterocytes and is absorbed. A small proportion of Cbl can also passively diffuse through gastrointestinal mucosa. The fraction of Cbl bound to transcobalamin II (TC II) in the plasma is the biologically active component available for receptor-mediated cellular uptake, but a larger proportion of plasma Cbl is bound to haptocorrins.

The body stores a large amount of Cbl; therefore it may take several years for deficiency to develop, even with the onset of severe dietary insufficiency or malabsorption. Cbl deficiency is most often caused by malabsorption. Autoimmune gastritis resulting in a lack of intrinsic factor (i.e., pernicious anemia) is a common cause, but malabsorption of Cbl may also be due to other gastric disease (e.g., atrophic gastritis, *Helicobacter pylori* infection), ileal disease (e.g., inflammatory bowel disease, tropical sprue), postsurgical malabsorption, pancreatic insufficiency, drugs (e.g., proton pump inhibitors, histamine $H_2$–receptor agonists, biguanides), chronic alcoholism, and competition for dietary Cbl (e.g., intestinal bacterial overgrowth, fish tapeworm). Overt Cbl deficiency is rarely due to isolated dietary insufficiency, but a devastating exception to this occurs among exclusively breast-fed infants of mothers who have subclinical Cbl insufficiency that may be related to a vegan diet or occult malabsorption.

Microorganisms and plants synthesize folates; important dietary sources include vegetables, fruit, and fortified cereals. In contrast to cobalamin, the folate body stores are sufficient for approximately 4 months; therefore folate deficiency is more often related to a poor diet. Other causes of folate deficiency are increased demand (e.g., pregnancy, prematurity, chronic hemolytic anemia, severe exfoliative dermatitis, leukemias), loss due to hemodialysis, drugs, and competition for dietary folates (intestinal bacterial overgrowth). Malabsorption of folate is rare unless there is extensive intestinal disease (e.g., gluten-induced enteropathy, tropical sprue).

## Clinical Features

The hematologic manifestations of Cbl and folate deficiency are the same. The onset of anemia is gradual, and the severity at presentation is extremely variable; some patients exhibit profound macrocytic anemia (Hb levels approaching 2 g/dL), jaundice, markedly increased lactate dehydrogenase, and increased indirect bilirubin. The ineffective hematopoiesis and hemolysis may be

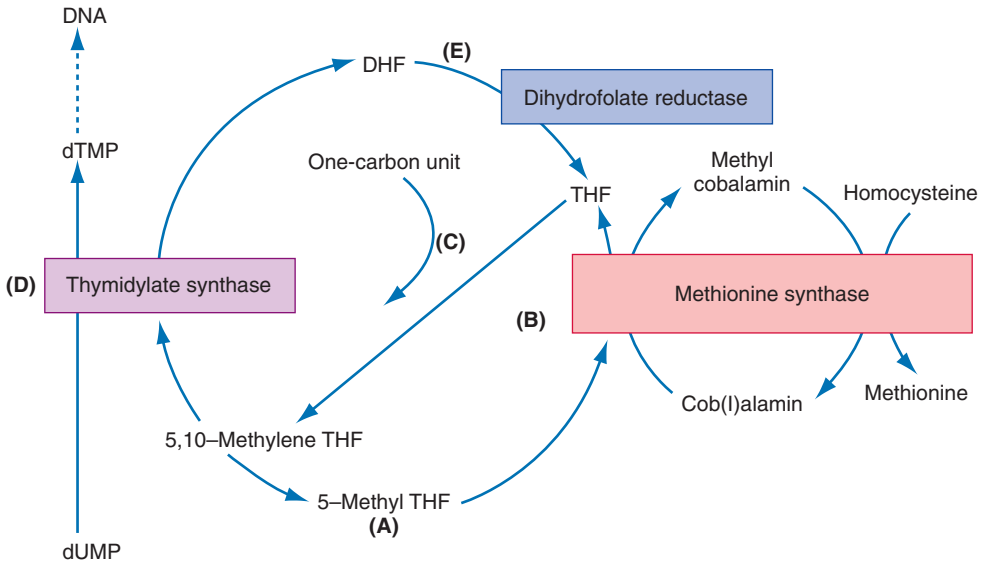


**FIGURE 1-1**

Folate and cobalamin in the synthesis of DNA and methionine. Folates conjugated with multiple glutamic acid residues (folate polyglutamates) are deconjugated during absorption in the duodenum and jejunum. Folate monoglutamates then undergo conversion to N5-methyl tetrahydrofolate (N5-methyl THF), the main form of folate transported in plasma and taken up by cells **(A).** Demethylation of N5-methyl THF **(B)** yields tetrahydrofolate (THF), which undergoes polyglutamation needed for cellular retention of folate. This methyltransferase reaction requires cob(I)alamin as an intermediate with transfer of the methyl group to homocysteine to generate methionine. THF, as an intermediate, accepts a one-carbon unit **(C).** The folate derivative N5,N10-methylene THF donates the one-carbon unit to the reaction catalyzed by thymidylate synthase **(D).** Both thymidylate (dTMP), a required component for DNA synthesis, and dihydrofolate (DHF) are produced. Dihydrofolate reductase reduces DHF back to THF **(E).**

## MEGALOBLASTIC ANEMIA–FACT SHEET

### Definition

- Macrocytic anemia owing to folate deficiency, cobalamin deficiency, and other causes, resulting in impaired DNA synthesis, abnormal nuclear maturation, and ineffective hematopoiesis

### Incidence and Location

- Folate deficiency is rare in countries with mandatory food fortification
- Dietary cobalamin deficiency is rare, except among vegans and populations with low intake of animal-source foods

### Morbidity and Mortality

- Increased risk of neural tube defects
- Peripheral neuropathy, subacute combined degeneration of the spinal cord, and cognitive abnormalities that may be only partially reversible (cobalamin deficiency)
- Increased risk of gastric adenocarcinoma and carcinoid tumor (pernicious anemia)

### Prevalence

- Prevalence estimates of low red blood cell (<140 ng/mL) and serum (<3 ng/mL) folate levels among women of childbearing age in the United States is 4.5% and 0.3%, respectively
- Increased incidence of cobalamin deficiency in breast-fed infants and young children among populations at risk of dietary insufficiency (strict vegans and developing countries)
- Prevalence of cobalamin deficiency owing to malabsorption increases with age

### Clinical Features

- Gradual onset of macrocytic anemia
- Evidence of hemolysis (jaundice, elevated lactate dehydrogenase, elevated total bilirubin)
- Atrophic glossitis
- Neuropsychiatric effects (cobalamin deficiency)
  - Paresthesias
  - Loss of position and vibration sense
  - Ataxia
  - Cognitive impairment (impaired memory, dementia)
  - Psychosis (rare)

### Prognosis and Therapy

- Treatment of the underlying cause of the deficiency
- Intramuscular injection (or large oral doses) of vitamin B12
- Oral folate supplementation (parenteral administration needed in some cases)

so pronounced that haptoglobin is reduced and hemosiderinuria occurs. The mean corpuscular volume (MCV) is characteristically high, with clinically overt megaloblastic anemia, but may also become elevated before the development of overt anemia. However, the MCV may be in the normal range if there is concurrent iron deficiency anemia or thalassemia. The reticulocyte count is low. Leukopenia and thrombocytopenia are often present because impairment of DNA synthesis affects all proliferating cells. The effect on epithelial cells can result in the classic "beefy red smooth tongue" (atrophic glossitis).

In addition to its hematologic effects, Cbl deficiency is often associated with a peripheral neuropathy and demyelinization of the dorsal and lateral columns of the spinal cord (i.e., subacute combined degeneration), leading to paresthesias, loss of position and vibration sense, spastic ataxia, and extensor plantar reflexes. Cognitive impairment may be seen; psychosis is a rare complication. Neurologic complications of Cbl deficiency may be only partially reversible, making early recognition of this disorder essential.

## Pathologic Features

In clinically overt megaloblastic anemia, the peripheral blood film demonstrates marked variability in red cell size (anisocytosis) with large, oval red cells (macroovalocytes), teardrop cells, fragments, microcytes, and other nonspecific abnormally shaped red cells (poikilocytes; Figure 1-2). Polychromasia is characteristically absent. With increasing severity, there is increased red cell fragmentation. Howell-Jolly bodies, nucleated RBCs with megaloblastic morphology, and basophilic stippling are often observed (Figure 1-3). Hypersegmented neutrophils with six or more nuclear lobes are sensitive and specific for megaloblastic anemia (Figure 1-4).

Bone marrow aspirate smears typically demonstrate erythroid hyperplasia and a shift toward immaturity, as well as characteristic cytologic changes in all three myeloid lineages. Maturing cells are larger than normal, with asynchronous nuclear and cytoplasmic maturation. The chromatin of the erythroid precursors appears more granular than during normal maturation, and the most mature erythroid precursors display a prominent fenestrated nuclear appearance that contrasts with the expected complete condensation of chromatin at this stage (Figure 1-5). Erythroid precursors with abnormally shaped, multiple, budding, or fragmented nuclei are often seen (Figure 1-6), resembling those seen in myelodysplastic syndromes. Giant metamyelocytes and bands with abnormally open chromatin are also characteristic (Figures 1-5 and 1-7), while larger than normal megakaryocytes with unattached nuclear lobes or hyperlobated nuclei reflect similar abnormalities in megakaryocytic maturation. Because of the pronounced ineffective hematopoiesis, markedly increased iron stores are seen with a Prussian blue iron stain unless there is concurrent iron deficiency. Bone marrow core biopsy specimens demonstrate a markedly hypercellular marrow with expanded and left-shifted trilineage

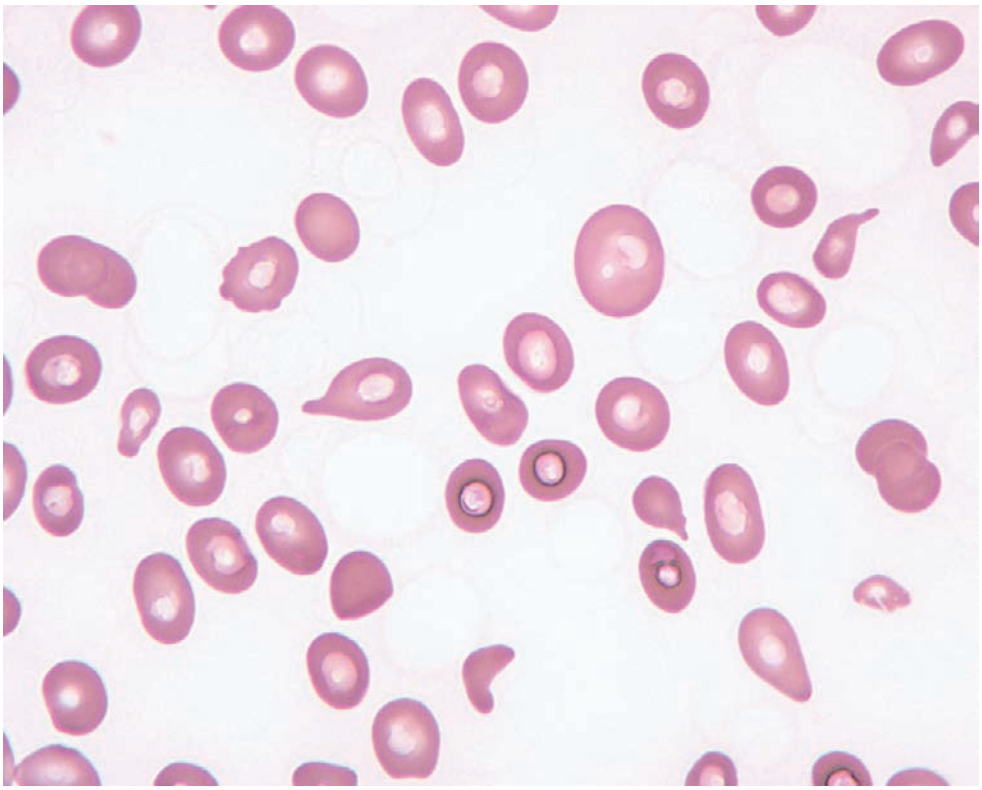

**FIGURE 1-2**

Megaloblastic anemia, peripheral blood findings. This blood smear from a patient with severe megaloblastic anemia demonstrates prominent anisopoikilocytosis, including large oval cells (oval macrocytes), microcytes, and teardrop cells.

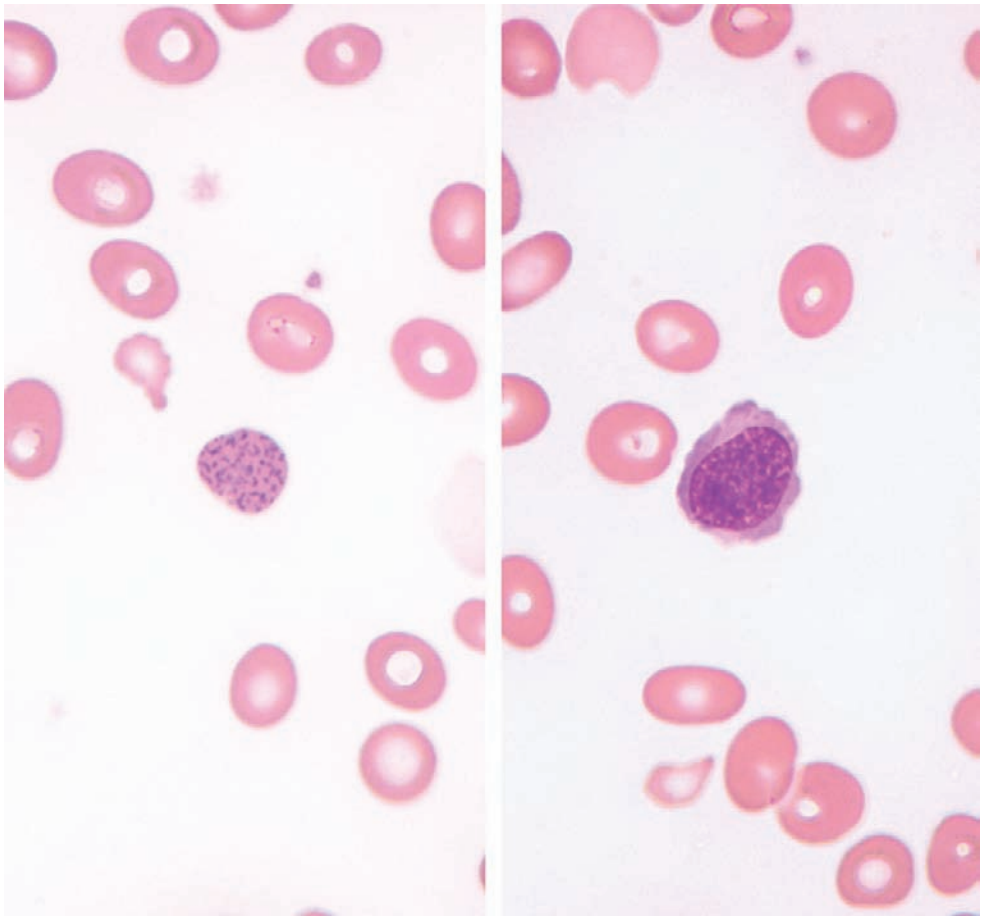

**FIGURE 1-3**

Megaloblastic anemia, peripheral blood findings. Coarse basophilic stippling *(left)* and circulating nucleated red cells *(right)* are common in megaloblastic anemia. The red cell precursor is relatively mature, judging by the pink-gray quality of the cytoplasm. However, the chromatin is quite open, with an abnormal stippled appearance, indicating megaloblastic change.

## MEGALOBLASTIC ANEMIA–PATHOLOGIC FEATURES

### Peripheral Blood

- Anemia, often pancytopenia
- Prominent anisocytosis
- Poikilocytosis
  - Macroovalocytes
  - Teardrop cells
  - Fragments
- Lack of polychromasia
- Hypersegmented neutrophils
- Howell-Jolly bodies, nucleated red cells, basophilic stippling

### Bone Marrow

- Hypercellular marrow with trilineage expansion
- Left-shifted hematopoietic precursors
- Asynchronous nuclear to cytoplasmic maturation
  - Megaloblastic erythroid precursors
  - Giant metamyelocytes
  - Larger than normal megakaryocytes with abnormal nuclei

### Differential Diagnosis

- Other causes of megaloblastic hematopoiesis
  - Drugs
  - Some myelodysplastic syndromes
- Nonmegaloblastic macrocytosis
  - Reticulocytosis
  - Liver disease
  - Alcohol
  - Hypothyroidism
  - Drugs

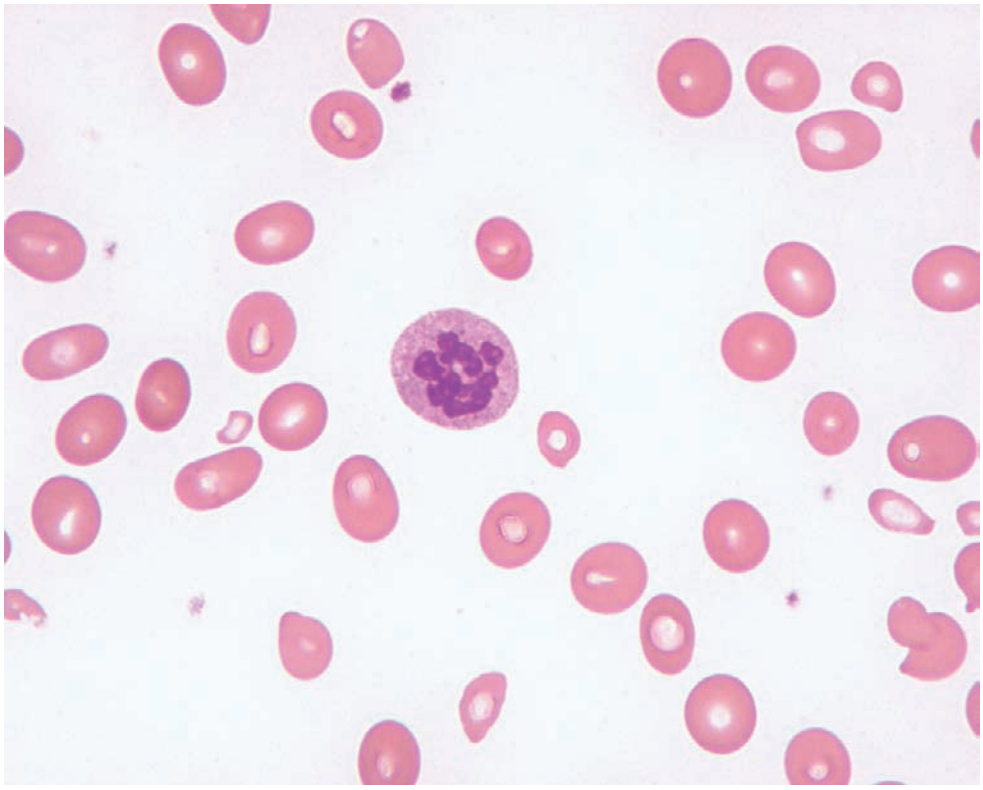

**FIGURE 1-4**

Megaloblastic anemia, peripheral blood findings. A hypersegmented neutrophil in the peripheral blood of a patient with megaloblastic anemia. Neutrophils are generally considered hypersegmented when they contain six or more lobes.

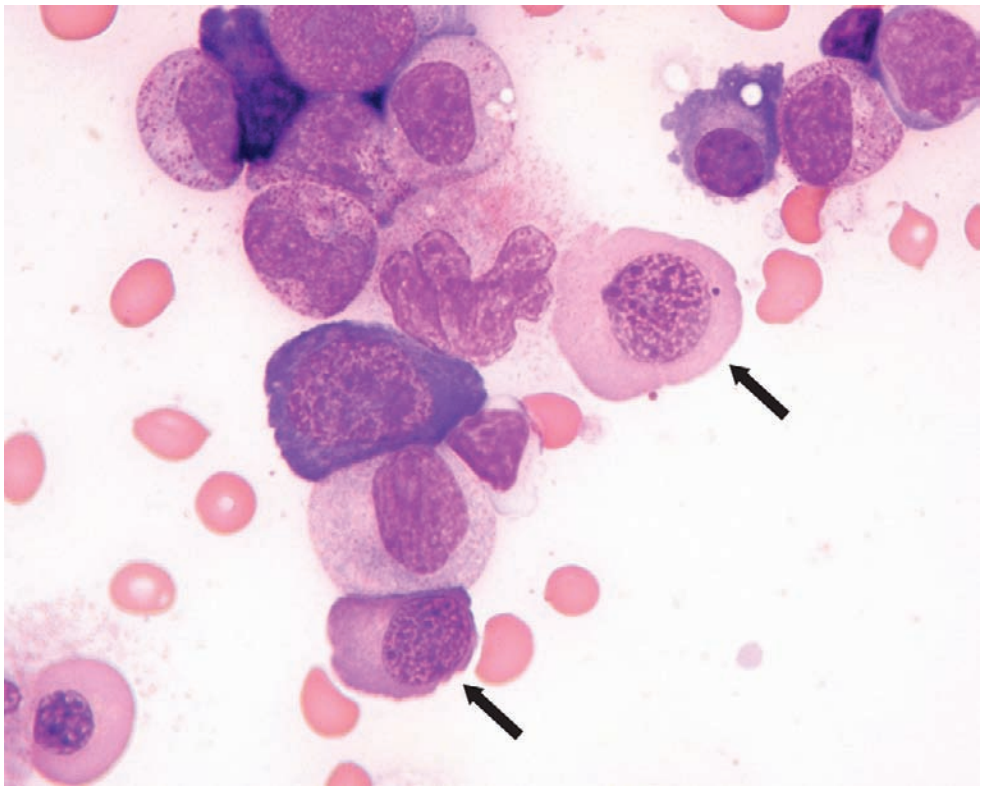

**FIGURE 1-5**

Megaloblastic anemia, bone marrow aspirate smear findings. This marrow aspirate smear image from a patient with severe megaloblastic anemia demonstrates two late-stage erythroid precursors (based on an advanced degree of cytoplasmic hemoglobinization) that are abnormally large and have dispersed, stippled megaloblastic chromatin *(arrows)*. Also present is a giant granulocyte at approximately the metamyelocyte stage with abnormally dispersed chromatin (immediately to the left of upper arrowed megaloblast).

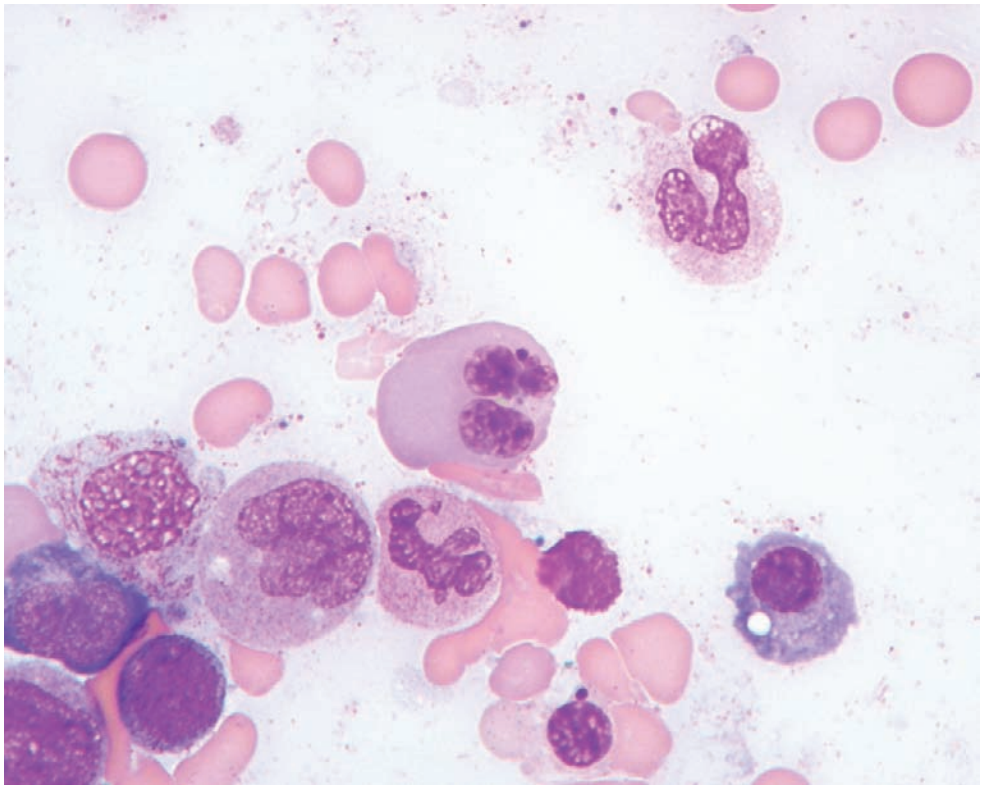

**FIGURE 1-6**

Megaloblastic anemia, bone marrow aspirate smear findings. This image demonstrates a dysplastic-appearing orthochromic erythroid precursor with two nuclei, one irregular, and irregularly distributed chromatin.

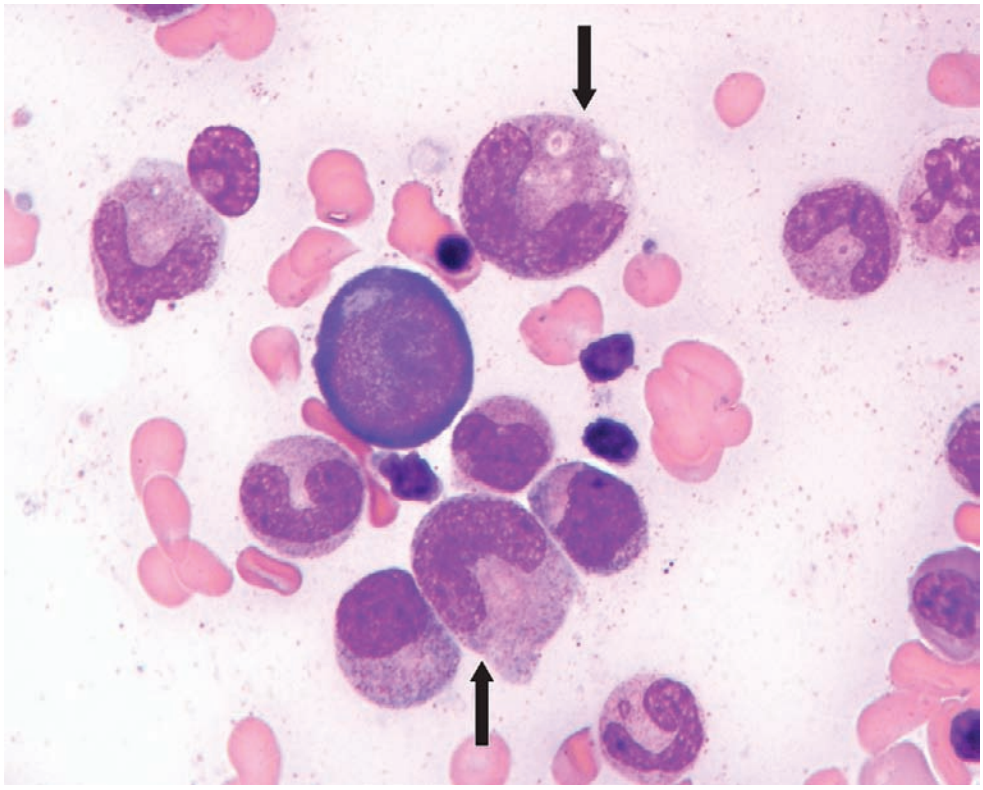

**FIGURE 1-7**

Megaloblastic anemia, bone marrow aspirate smear findings. This marrow aspirate from the same patient as in Figure 1-6 demonstrates two giant bands with abnormally dispersed megaloblastic chromatin. Note that these cells *(arrows)* are larger than the early basophilic normoblast in the field and other granulocytes in the field.

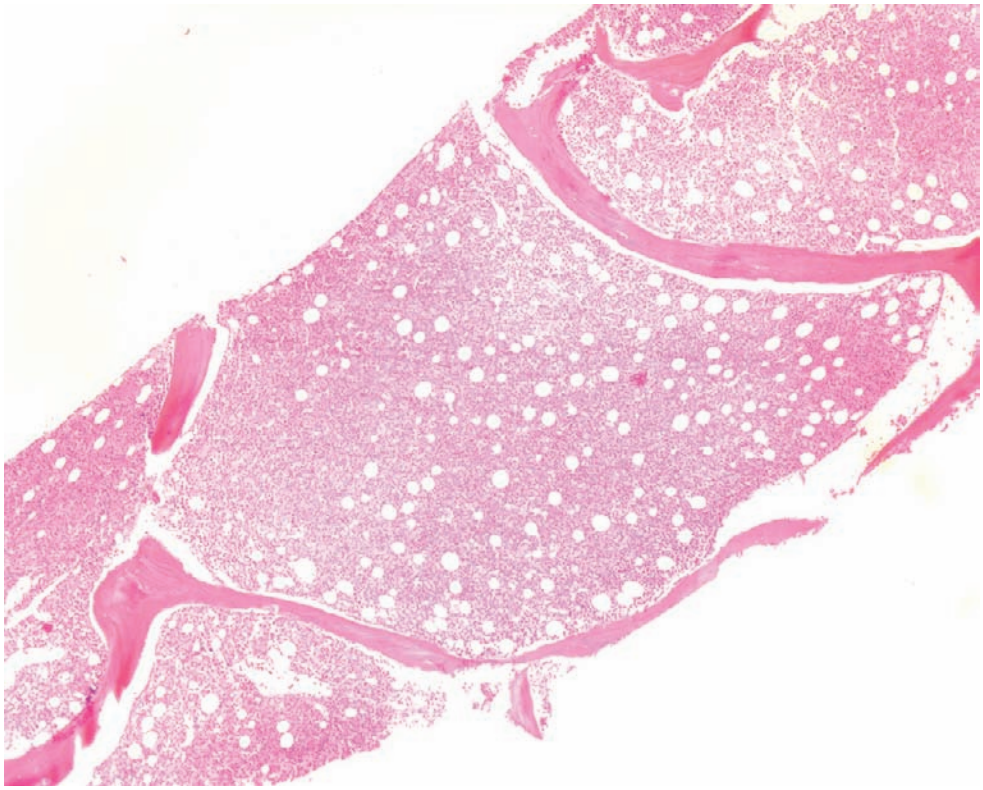

**FIGURE 1-8**

Megaloblastic anemia, bone marrow biopsy findings. The bone marrow biopsy in megaloblastic anemia is hypercellular.

hematopoiesis (Figure 1-8). In particular, islands of immature erythroid precursors with finely stippled chromatin and distinct, irregular, eosinophilic nucleoli are often prominent (Figure 1-9).

## Ancillary Studies

Serum Cbl is fairly sensitive and specific for overt clinical Cbl deficiency. Causes of falsely low serum Cbl include pregnancy, use of oral contraceptives, anticonvulsant administration, human immunodeficiency virus infection, and folate deficiency. The last can complicate the interpretation of the laboratory workup of megaloblastic anemia (Table 1-2). Falsely normal levels of Cbl are rare with clinically overt deficiency, but may be observed in association with myeloproliferative disorders, liver disease, intestinal bacterial overgrowth, and congenital TC II deficiency. Congenital TC II deficiency manifests as severe megaloblastic anemia in infancy.

Folate status may be assessed by either serum or red cell folate assays. Misleading levels of serum folate may occasionally be due to fluctuations related to short-term dietary changes. Hemolysis (in vivo and in vitro) will cause falsely elevated serum folate, whereas alcohol intake can cause transient low levels despite adequate stores. Red cell folate levels better reflect body folate stores and are less prone to short-term fluctuation. Importantly, Cbl deficiency can cause reduced red cell folate levels, but this is usually associated with normal or increased serum levels (see Table 1-2).

Measurement of serum homocysteine and methylmalonic acid can also be useful in the assessment of megaloblastic anemia. Homocysteine accumulates in both Cbl and folate deficiency because of interruption of the biochemical pathway illustrated in Figure 1-1. Serum methylmalonic acid increases in Cbl deficiency, but not folate deficiency, because of interruption of the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A. A normal methylmalonic acid level generally rules out Cbl deficiency; however, both metabolites may be mildly elevated with renal insufficiency. Elevated serum homocysteine may also be seen with vitamin $B_6$ deficiency, riboflavin deficiency, hypothyroidism, some drugs, and genetic mutations affecting metabolism of homocysteine.

## Differential Diagnosis

The major differential diagnoses of megaloblastic anemia owing to folate or Cbl deficiency are megaloblastic changes because of drug therapy and myelodysplastic syndromes. A variety of agents that interfere with DNA synthesis can cause megaloblastic changes in maturing marrow elements, including methotrexate, zidovudine, hydroxyurea, and azathioprine. Megaloblastic

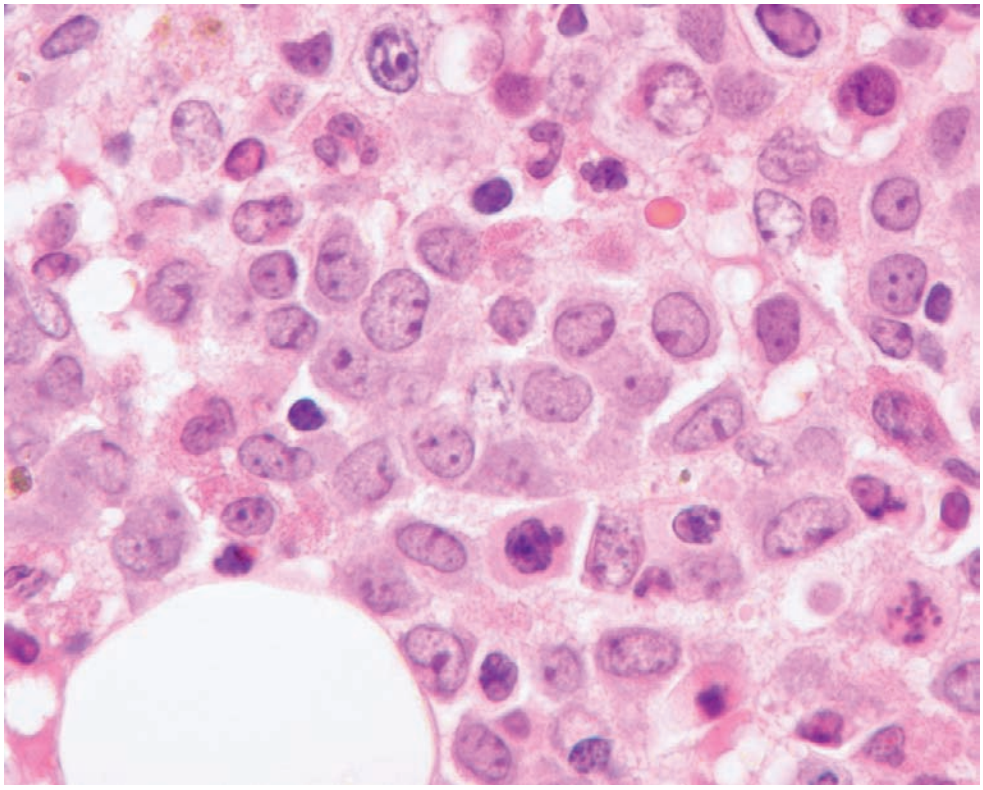

**FIGURE 1-9**

Megaloblastic anemia, bone marrow biopsy findings. Prominent infiltrates of immature erythroid precursors with fine chromatin and distinct nucleoli can be mistaken easily for acute leukemia or large cell lymphoma in biopsy sections.

**TABLE 1-2**

**Ancillary Tests Used to Evaluate Possible Megaloblastic Anemia**

| | $B_{12}$ Deficiency | Folate Deficiency | Mixed $B_{12}$/Folate Deficiency | Myelodysplastic Syndrome |
|---|---|---|---|---|
| Serum folate | Normal/high | Low/normal | Low | Normal |
| Red blood cell folate | Low/normal | Low | Low | Normal |
| Serum cobalamin | Low/normal | Normal/low | Low | Normal/low |
| Methylmalonic acid | High | Normal | High | Normal |
| Homocysteine | High | High | High | Normal |

morphology owing to drugs tends to be confined to the erythroid compartment, although this may not be the case with long-term administration. In general, an accurate drug history and biochemical tests for folate and Cbl deficiency will distinguish this cause of megaloblastosis.

Megaloblastic anemia may be potentially confused with myelodysplastic syndromes because of the potential for dyserythropoiesis in megaloblastic anemia or the potential for megaloblastic changes in myelodysplastic syndromes. However, when megaloblastic changes are seen in the erythroid elements of myelodysplastic syndromes, they will generally be accompanied by a spectrum of other abnormalities that are not a feature of megaloblastic anemia, such as neutrophil hyposegmentation or hypogranularity, hypogranular platelets, hypolobated megakaryocytes, or increased blasts. Furthermore, giant bands and metamyelocytes as seen in megaloblastic anemia are usually not a notable feature of myelodysplastic syndromes. As a general rule, a high MCV (more than 130 fL) is a feature that would favor a megaloblastic anemia owing to Cbl or folate deficiency versus other causes.

In addition to other causes of megaloblastosis, nonmegaloblastic macrocytosis can also enter into the

differential diagnosis of megaloblastic anemia. These diagnoses include reticulocytosis, liver disease, alcohol abuse, drug effects, and hypothyroidism. Reticulocytosis as a cause of macrocytosis is easily discerned either with a reticulocyte count or detection of prominent polychromasia on examination of a blood smear. Other causes of nonmegaloblastic macrocytosis do not produce the degree of anisopoikilocytosis, oval macrocytes, or hypersegmented neutrophils seen in megaloblastic anemia. Liver disease in particular is characterized by a uniform population of round macrocytic cells and target cells.

### Prognosis and Therapy

Standard therapy for Cbl deficiency, when the cause is not dietary deficiency, has traditionally been intramuscular injection of vitamin $B_{12}$; however, oral therapy is effective for some patients. To prevent adverse neurologic consequences because of concurrent or overlooked Cbl deficiency, Cbl status must be established before folate therapy is initiated. This may be difficult to do in practice because folate deficiency can cause low serum Cbl for reasons that are not well understood. If there is any doubt, Cbl supplementation should be administered along with folate therapy. Standard therapy for folate deficiency is oral supplementation. Parenteral administration of folate may be needed in cases caused by intractable malabsorption.

## IRON DEFICIENCY ANEMIA

A sufficient iron supply is important for many biologic processes, but only impairment of Hb production and erythropoiesis owing to iron deficiency are addressed in this chapter. Iron serves as the ligand for binding of molecular oxygen to the porphyrin ring of heme. Anemia results from the inability of erythroid precursors to synthesize adequate Hb because of insufficient iron. Two stages of iron deficiency preceding anemia have been described. The first stage is iron depletion, and it is the earliest period during which other physiologic processes may be affected before an effect on erythropoiesis. Iron-deficient erythropoiesis then begins and, because of the longevity of the iron-replete red cells in circulation, continues for some time before development of overt anemia.

The risk of developing IDA depends heavily on age, diet, and regional or socioeconomic factors. The rapid utilization of iron during infancy, early childhood, and pregnancy as well as blood loss during childbirth and menstruation greatly increases the risk of IDA. Iron deficiency is widely accepted as the most common contributing cause for anemia overall. Recent National Health and Nutrition Examination Surveys (2003 to 2006) from the United States estimated that iron deficiency was present in 14% of children 1 to 2 years old, 3.7% to 4.5% of children 3 to 5 years old and 9% to 16% of nonpregnant females 12 to 49 years old. Inadequate dietary intake of iron is more frequent among impoverished populations. Parasitic infections, especially caused by hookworms and schistosomiasis, resulting in blood loss are major contributing factors for IDA in some developing countries. In affluent societies, chronic blood loss, sometimes occult because of malignancy, is by far the most common cause of IDA. Malabsorption of iron can be due to gastrectomy and bariatric surgery, but may also be a contributing factor for IDA found in association with atrophic gastritis, *Helicobacter pylori* infection, and celiac disease.

### Clinical Features

IDA ranges from mild to profound, with Hb levels as low as 2 g/dL in the most severe cases. IDA is classically a microcytic and hypochromic anemia, reflected as a decreased MCV and mean corpuscular Hb concentration (MCHC), respectively. However, the MCV may be normal in early iron deficiency; the degree of microcytosis roughly correlates with the degree of anemia. Likewise, the MCHC may be normal in early iron deficiency when only a minority of cells are hypochromic.

Most signs and symptoms are those seen in any form of anemia, such as fatigue, dyspnea, and pallor of skin and mucous membranes. Because the anemia develops over a protracted period and compensatory mechanisms have ample time to develop, anemia resulting from iron deficiency is surprisingly well tolerated, even when extremely severe. Pica, koilonychia (i.e., spoon-shaped nails), and esophageal webs are rare but have a strong association with IDA. Angular cheilitis and atrophic glossitis are also seen with IDA but are not specific.

### Pathologic Features

The characteristic findings on the peripheral blood film are small (microcytic), underhemoglobinized (hypochromic) RBCs with variability in size (anisocytosis) and abnormal shapes (poikilocytosis; Figure 1-10). Elliptocytes are prominent poikilocytes in IDA; often they are long and narrow (pencil cells). Prekeratocytes are usually evident and are recognized as red cells with sharp-edged, submembranous vacuoles and preserved central pallor (see Figure 1-10). Target cells, teardrop cells, very small hypochromic microcytes, and various nonspecific poikilocytes are also common. Occasional red cell fragments may be observed, but they are not

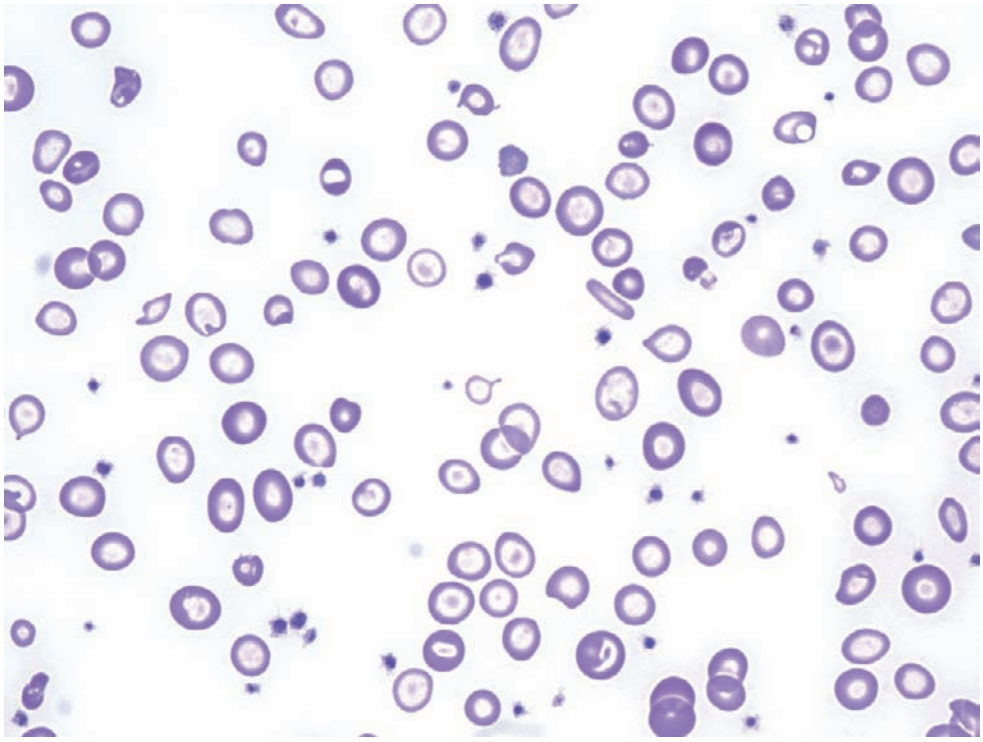

**FIGURE 1-10**

Iron deficiency anemia, peripheral blood findings. In this smear from a patient with severe iron deficiency, there is variation in size and shape of the red cells (anisopoikilocytosis). The red cells are obviously hypochromic, with an enlarged area of central pallor and relatively little remaining hemoglobin. An elongate elliptocyte (pencil cell) is visible slightly to the right and above the center of the field. Also present in the upper right hand corner is a red cell with a sharply defined, submembranous vacuole (prekeratocyte). These poikilocytes are characteristic but nonspecific features of iron deficiency anemia.

**IRON DEFICIENCY ANEMIA–FACT SHEET**

**Definition**
- Anemia owing to inadequate iron supply

**Incidence and Location**
- Most common cause of anemia worldwide

**Morbidity and Mortality**
- Reduced quality of life and reduced productivity
- Probably increased maternal and fetal–neonatal morbidity and mortality
- Probably reduced cognitive development in children

**Gender, Race, and Age Distribution**
- Increased incidence in infants, children, women of childbearing age, and impoverished populations, particularly in underdeveloped countries
- Increased incidence in some minorities compared with white population in the United States

**Clinical Features**
- General signs and symptoms of chronic anemia
- Angular cheilitis, glossitis, pica, koilonychia, and esophageal webs

**Prognosis and Therapy**
- Oral iron supplementation
- Parenteral iron in patients with uncontrolled blood loss, intolerance to oral iron, or intestinal malabsorption

**IRON DEFICIENCY ANEMIA–PATHOLOGIC FEATURES**

**Peripheral Blood**
- Hypochromic, microcytic red blood cells
- Anisocytosis
- Lack of polychromasia
- Poikilocytosis, including elliptocytes, prekeratocytes, and target cells

**Bone Marrow**
- Mild erythroid hyperplasia
- Absent stainable iron on a Prussian blue stain on an aspirate smear

**Differential Diagnosis**
- Thalassemia trait or thalassemia-like hemoglobinopathy
- Anemia of chronic disease

prominent. Thrombocytosis is common, but platelet counts may also be normal or reduced. Variations from the typical findings occur when there has been recent partial repletion of iron or when additional factors, such as Cbl or folate deficiency, are also contributing to anemia.

The bone marrow in IDA may demonstrate erythroid hyperplasia, but this is generally of only a mild degree. Erythroid precursors may have scant cytoplasm with frayed cytoplasmic borders.

## Ancillary Studies

Serum ferritin measurement is the most useful single laboratory test for iron deficiency. Serum ferritin below the lower limit of the reference range (about 12 mg/dL) is essentially diagnostic of iron deficiency. However, ferritin is an acute-phase reactant, and normal levels may be seen when iron deficiency coexists with infection, inflammation, or malignancy. However, when this occurs, the ferritin level will usually be low-normal; therefore low-normal values in an individual with an active inflammatory process support a diagnosis of iron deficiency in the appropriate setting. Ferritin levels may even be higher in patients with acute liver injury, despite iron deficiency. Measurement of serum iron alone is not useful because of diurnal fluctuations of serum iron levels and the rapid changes that may occur with dietary intake, inflammation, and blood loss. Transferrin, measured either directly or indirectly as the total iron binding capacity, is typically increased in IDA, but this response can be blunted in the presence of hypoproteinemia. The transferrin saturation (the ratio of serum iron to total iron binding capacity) is typically less than 15% in iron deficiency, but saturation levels in this range may also be seen in ACD.

Increased serum soluble transferrin receptor (sTfR) is a sensitive marker of iron deficiency, reflecting increased expression of this receptor on RBC precursors as a response to depleted tissue iron. This parameter is elevated during iron-deficient erythropoiesis before the development of overt anemia. Furthermore, inflammation does not raise sTfR levels substantially. However, states with an increased mass of RBC precursors such as hemolytic anemias and ineffective erythropoiesis (e.g., megaloblastic anemia) will manifest elevated sTfR; therefore this finding is not specific for iron deficiency.

When biochemical tests are equivocal, the absence of stainable iron with a Prussian blue stain performed on well-prepared marrow aspirate smear is considered to be the gold standard for the diagnosis of IDA. An iron stain performed on a bone marrow core biopsy may also be helpful, but iron can be leached out during decalcification of the bone marrow biopsy and result in the false interpretation of absent iron stores.

## Differential Diagnosis

Other common causes of hypochromic, microcytic anemia are thalassemia and ACD. Sideroblastic anemia with microcytosis is rarer. The hematologic and biochemical tests that are useful to differentiate the major causes of microcytic anemia are listed in Table 1-3. In addition, mild anemia associated with thalassemia trait or a thalassemia-like hemoglobinopathy (e.g., Hb E) characteristically exhibits a red blood cell count greater than $5 \times 10^{12}$ cells/L, which is unusual for IDA. Hb electrophoresis would help establish the diagnosis of most β-thalassemias and thalassemia-like hemoglobinopathies. Under circumstances that render the interpretation of biochemical studies for iron status particularly difficult, such as inflammation, monitoring the response of Hb or the reticulocyte count to a therapeutic trial of iron supplementation may be sufficient to confirm iron deficiency. Otherwise, any uncertainty about iron status usually can be resolved by an iron stain performed on a bone marrow aspirate smear.

## Prognosis and Therapy

Iron supplementation with oral iron salts, most commonly ferrous sulfate, is standard therapy for

**TABLE 1-3**

**Pertinent Findings and Ancillary Tests for Untreated, Uncomplicated Hypochromic, Microcytic Anemias**

| Laboratory Feature | Iron Deficiency Anemia | Thalassemia | Anemia of Chronic Disease |
|---|---|---|---|
| Increased anisocytosis (elevated RDW) | Present | Absent or present | Typically absent |
| Increased reticulocytes | Typically absent | Absent or present | Absent |
| Bone marrow iron stores | Absent | Normal or increased | Typically increased; sideroblasts reduced |
| Serum ferritin | Typically reduced | Normal or increased | Typically increased |
| Serum iron | Typically reduced | Normal or increased | Typically reduced |
| TIBC | Typically increased | Normal or reduced | Reduced or normal |
| Transferrin saturation | Typically decreased | Normal or increased | Normal or reduced |
| Serum transferrin receptor | High | Normal or high | Normal or increased |

*RDW,* Red blood cell distribution width index; *TIBC,* total iron binding capacity.

uncomplicated IDA. Response to iron supplementation is usually rapid if the iron deficiency is the result of inadequate iron intake. If an appropriate response is not observed or when occult blood loss is more likely, additional evaluation to determine the cause of IDA should be considered. Parenteral iron may be needed for patients with uncontrolled blood loss, intolerance to oral iron, or malabsorption.

Because of the physiologic attempts to compensate for chronic anemia, a rapid blood transfusion can result in hypervolemia and cardiac dilatation in a patient who has gradually developed severe iron deficiency. Therefore red cell transfusions should be reserved for patients with severe anemia causing cardiac compromise.

## PROLIFERATION DEFECTS

This group of anemias results from a decreased pool of erythroid stem cells available for maturation and proliferation or interference or blocking of the proliferation of an otherwise adequate stem cell pool, or both. Disorders belonging to this group include marrow failure syndromes, including aplastic anemia and pure red cell aplasia (discussed in Chapter 5), marrow replacement processes, anemia of chronic kidney disease (ACKD) and ACD. Only the last two are discussed in this chapter.

### ANEMIA OF CHRONIC KIDNEY DISEASE–FACT SHEET

**Definition**

- Anemia of variable degree due primarily to underproduction of erythropoietin

**Prevalence**

- The prevalence and severity of anemia increase as renal function decreases
- The prevalence and severity is increased in patients with diabetes
- Prevalence varies with age, gender, and race or ethnicity
- Higher prevalence of anemia among black versus white persons who have comparable levels of renal insufficiency

**Morbidity and Mortality**

- Associated with reduced quality of life, reduced cognitive function, left ventricular hypertrophy, and increased cardiac morbidity and mortality

**Clinical Features**

- General symptoms of chronic anemia

**Prognosis and Therapy**

- Optimal dialysis and nutrition
- Erythropoiesis-stimulating agent therapy and iron supplementation

## ANEMIA OF CHRONIC KIDNEY DISEASE

ACKD is primarily caused by underproduction of erythropoietin. Toxic metabolites related to uremia also likely contribute to ACKD by suppressing erythropoiesis, reducing red cell survival, and increasing the risk of blood loss due to platelet dysfunction.

There is variation in the level of renal function for which anemia develops, but the prevalence and severity of anemia increase as renal function decreases. Evaluation of recent National Health and Nutrition Examination Surveys (1999 to 2004) estimates that about 13% of the population aged 20 years or older in the United States has chronic kidney disease. The prevalence of anemia is higher among African Americans compared with whites with comparable levels of renal insufficiency. The prevalence of anemia is also higher and more severe among patients with diabetes, manifesting at earlier stages of chronic kidney disease.

### Clinical Features

Progressive uremia leads to plasma expansion and hemodilution; therefore the Hb level immediately before hemodialysis is lower than that immediately afterward. Cardiovascular and renal attempts to compensate for anemia contribute to the development of left ventricular hypertrophy and can lead to congestive heart failure. Treatment with erythropoiesis-stimulating agents (ESAs), such as epoetin alfa or darbepoetin alfa, can improve the general quality of life of these patients. However, the response to these therapies is limited if either absolute or functional iron deficiency develops. Functional iron deficiency is due to depletion of the iron pool immediately available for erythropoiesis. Mobilization of iron stores becomes rate limiting and is inadequate to meet the accelerated demand for iron during aggressive ESA therapy.

### Pathologic Features

In ACKD, the peripheral blood film shows a normochromic, normocytic anemia with minimal polychromasia and minimal anisocytosis (Figure 1-11). Occasional echinocytes and other poikilocytes may be observed, such as acanthocytes and rare fragments. There is no increase in polychromasia.

The bone marrow is usually normocellular or slightly hypocellular, and in the absence of ESA therapy it contains normal to slightly reduced erythroid precursors. The amount of sideroblastic iron and storage iron will depend on overall iron status. Morphologic features of

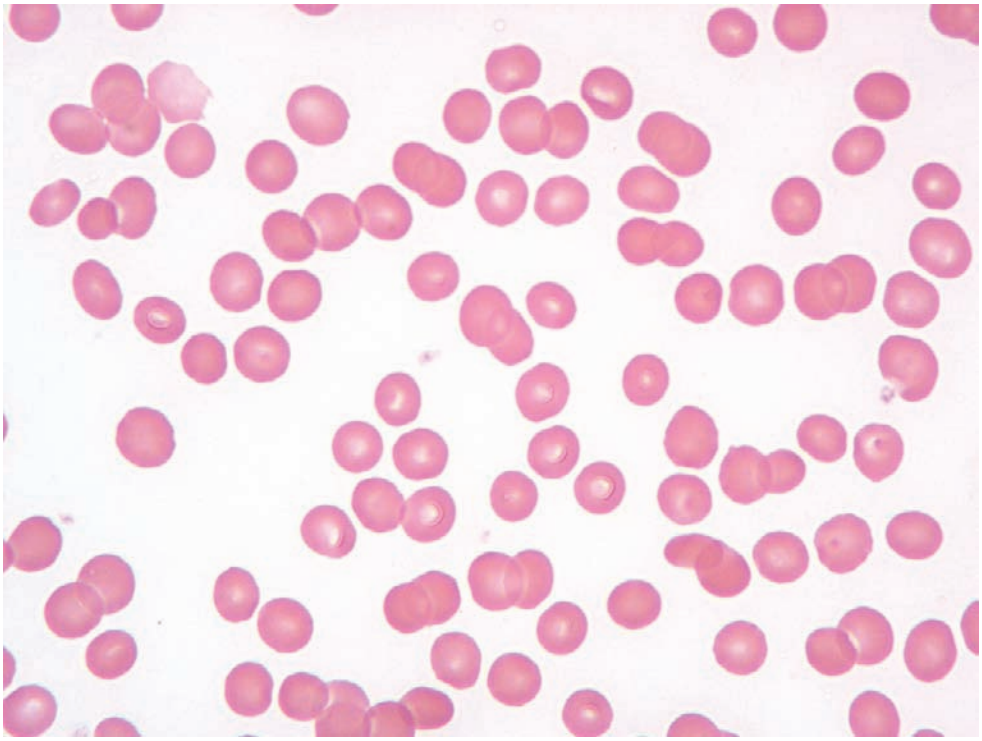

**FIGURE 1-11**

Anemia of renal failure, peripheral blood findings. This blood smear from a patient with renal failure demonstrates a bland, normochromic–normocytic anemia with minimal anisopoikilocytosis and no appreciable polychromasia.

**ANEMIA OF CHRONIC KIDNEY DISEASE–PATHOLOGIC FEATURES**

**Peripheral Blood**

- Normocytic, normochromic anemia
- Minimal polychromasia
- Occasional poikilocytes, including echinocytes, rare acanthocytes, and fragments

**Bone Marrow**

- Normocellular to slightly hypocellular marrow without an increase in erythroid precursors
- Bony changes related to renal osteodystrophy may be observed

**Differential Diagnosis**

- Iron deficiency anemia
- Megaloblastic anemia
- Anemia of chronic disease
- Hypothyroidism
- Secondary hyperparathyroidism
- Aluminum toxicity
- Pure red cell aplasia

renal osteodystrophy may be noted on a bone marrow core biopsy specimen, especially in association with end-stage renal disease and in patients who have been treated with long-term dialysis. Renal osteodystrophy is a spectrum of abnormalities of bone turnover that variably exhibits thinning or thickening of bony trabeculae, excess osteoid without mineralization (osteomalacia), stromal fibrosis, and osteitis fibrosa cystica (Figure 1-12).

## Ancillary Studies

Plasma erythropoietin concentration and the reticulocyte count are inappropriately low for the degree of anemia in ACKD. An increase in reticulocytes is a helpful indicator of a response to therapy. However, measuring plasma erythropoietin concentration is not usually recommended.

Detection of concomitant iron deficiency may be compromised by the fact that patients with chronic kidney disease may have associated inflammation; therefore ferritin levels may be difficult to interpret. In general, serum ferritin level less than 100 mg/L (in nondialyzed patients) or transferrin saturation less than 20%, or both, is considered compatible with iron deficiency in patients with chronic kidney disease. Even more challenging is the detection of the functional iron deficiency that may compromise the effectiveness of ESA therapy. The small numbers of hypochromic RBCs produced in this circumstance will not be detected reliably by the MCV or MCHC. Parameters to detect functional iron deficiency include percentage of hypochromic

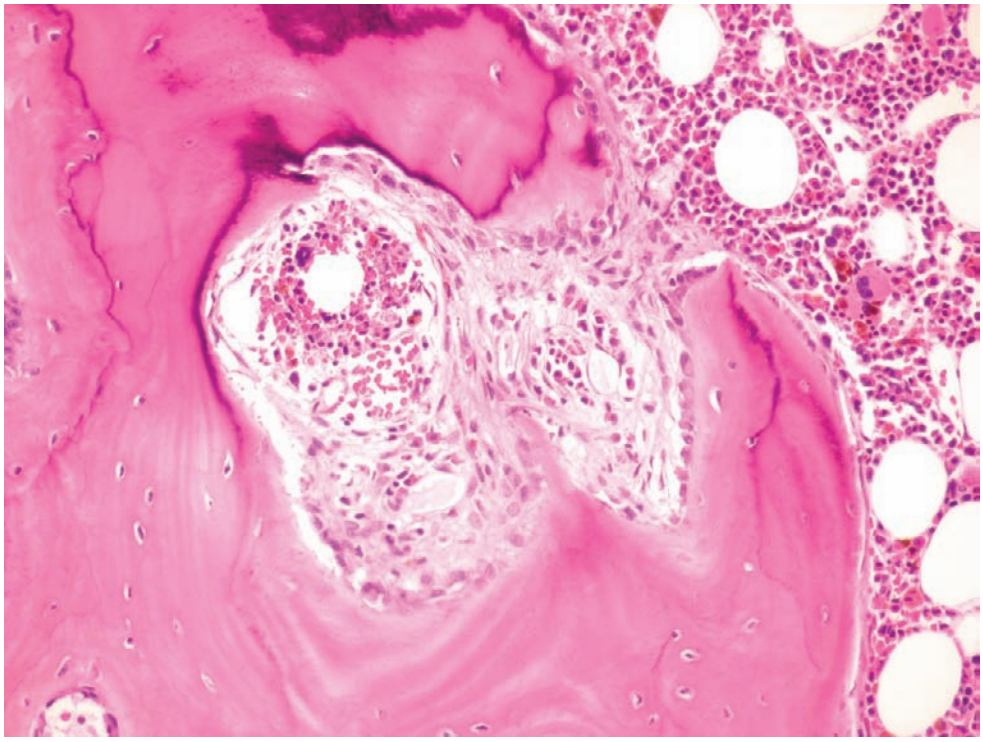

**FIGURE 1-12**

Renal osteodystrophy, bone marrow biopsy findings. The bone in this marrow biopsy specimen from a patient with renal osteodystrophy demonstrates an irregular erosive lesion with fibrosis, prominent osteoblastic rimming, and osteoid deposition.

red cells and hemoglobin content of reticulocytes. These parameters are now available on many but not all hematology analyzers and thus are not available in all laboratories.

## Differential Diagnosis

Nutritional deficiencies, infections, and other causes of inflammation commonly have a role in the development of anemia in patients with chronic kidney disease. In addition to malnutrition, blood loss associated with hemodialysis can contribute to iron deficiency. Patients receiving dialysis are also at increased risk of megaloblastic anemia, because Cbl and folate are water soluble and dialyzable.

Elevated markers of inflammation, such as C-reactive protein or erythrocyte sedimentation rate, indicate that chronic infection or inflammation may contribute to anemia. Patients with high levels of inflammatory cytokines are more resistant to ESA therapy.

Hypothyroidism and secondary hyperparathyroidism can also contribute to anemia and resistance to ESA therapy among patients with chronic kidney disease. Similar to ACKD, anemia related to hypothyroidism manifests with an inappropriately low reticulocyte response and is usually normocytic and normochromic. Aluminum toxicity associated with dialysis is another occasional cause of anemia in patients with renal failure; when chronic, this anemia becomes hypochromic and microcytic. A sudden lack of response to erythropoietin therapy could herald pure red cell aplasia caused by the development of antierythropoietin antibodies, but this is rare and mostly related to a formulation that had been used outside of the United States.

## Prognosis and Therapy

Appropriate use of ESAs and supplemental iron can improve the quality of life of patients with chronic kidney disease and reduce dependence on transfusions. A poor response to this therapy should prompt an evaluation to exclude other causes for anemia.

An excessive Hb target level when using this therapy appears to be associated with increased cardiovascular complications and, thus, the optimal Hb target values for various groups of patients with ACKD is under investigation. The U.S. Kidney Disease Outcomes Quality Initiative, the U.S. Food and Drug Administration, and the National Institute for Health and Clinical Excellence (Royal College of Physicians, London) offer therapeutic recommendations.

## ANEMIA OF CHRONIC DISEASE (ANEMIA OF INFLAMMATION)

ACD, also known as *anemia of inflammation,* is a mild to moderate anemia that is associated with inflammatory disorders, infection, and malignancy. ACD is characterized by reduced transport of iron to erythroid precursors, reduced erythropoiesis, ineffective erythropoiesis, and normal or increased iron stores. The underlying disease has usually existed more than 1 or 2 months before development of ACD, but anemia can also develop rapidly during critical illness, largely because of the same mechanisms.

Increased levels of interleukin-6 upregulate hepatic production of hepcidin, the major iron regulatory hormone, which binds to the iron exporter ferroportin on macrophages and enterocytes. Subsequent internalization and lysosomal degradation of ferroportin results in reduced release of iron into plasma. Other inflammatory cytokines (e.g., tumor necrosis factor α, interleukin-1, and the interferons) also likely contribute to the development of ACD by reducing mature red cell survival, suppressing erythropoietin production, and diminishing the response of erythroid precursors to erythropoietin.

ACD is considered to be the most frequent cause of anemia overall in hospitalized patients, and it has been estimated to affect 2.5% of individuals 65 years and older within the community-dwelling population in the United States, according to the third National Health and Nutrition Examination Survey (1988 to 1994). For persons 65 years and older who were surveyed, there is a significantly higher proportion of non-Hispanic black persons among those with ACD versus those without anemia.

**ANEMIA OF CHRONIC DISEASE (ANEMIA OF INFLAMMATION)–FACT SHEET**

**Definition**
- Anemia due to inflammatory cytokines associated with infection, inflammatory disorders, and cancer

**Incidence and Location**
- Most frequent contributing cause of anemia among hospitalized patients
- Affects about 2.5% of community-dwelling population 65 years and older in the United States

**Morbidity and Mortality**
- The effect on morbidity and mortality has not been systematically evaluated for many chronic diseases
- Amelioration of anemia contributes to improved quality of life and reduced need for red blood cell transfusions in patients with inflammatory disorders and cancer

**Gender, Race, or Age Distribution**
- More common among debilitated patients and the elderly
- Possible increased risk in blacks among patients older than 65 years of age

**Clinical Features**
- Signs and symptoms are the same as those for chronic anemia in general
- Consequences of anemia on quality of life can be appreciated only in retrospect after response to therapy

**Prognosis and Therapy**
- Treat underlying disorder
- Erythropoiesis-stimulating agents and iron supplementation may be of benefit in some cases
- Transfusions for severe anemia with hemodynamic instability

### Clinical Features

The severity of ACD is related to the level of disease activity of the chronic inflammatory disorder or the extent of tumor burden in malignancy. However, the anemia is generally mild or moderate, with hemoglobin levels usually not lower than 9 g/dL in uncomplicated cases. The symptoms and signs of ACD have an insidious onset and are similar to mild or moderate chronic anemia due to any cause. Because patients with chronic diseases tend to adjust to the effects of anemia and, in general, a lower quality of life, the consequences of ACD may be appreciated in retrospect only after response to therapy.

### Pathologic Features

Because understimulation of erythropoiesis is characteristic of ACD, the peripheral blood film shows a mild to moderate anemia without noticeable polychromasia or increased anisocytosis (Figure 1-13). There may be rouleaux formation, correlating with an elevated erythrocyte sedimentation rate. The RBCs are generally normochromic and normocytic. However, iron transport to the erythroid precursors is characteristically diminished in ACD, resulting in functionally iron-deficient conditions even in light of the already reduced stimulation of erythropoiesis associated with ACD. As a result, anemia becomes hypochromic and microcytic in approximately 20% of cases.

The bone marrow is usually normocellular but may reveal diminished erythropoiesis. Other nonspecific findings associated with inflammatory conditions may be observed, such as increased plasma cells. A Prussian blue iron stain performed on an aspirate smear with sufficient marrow spicules will demonstrate features that can confirm a diagnosis of ACD. Iron stored as hemosiderin within macrophages will be normal or, more commonly, increased (Figure 1-14), whereas

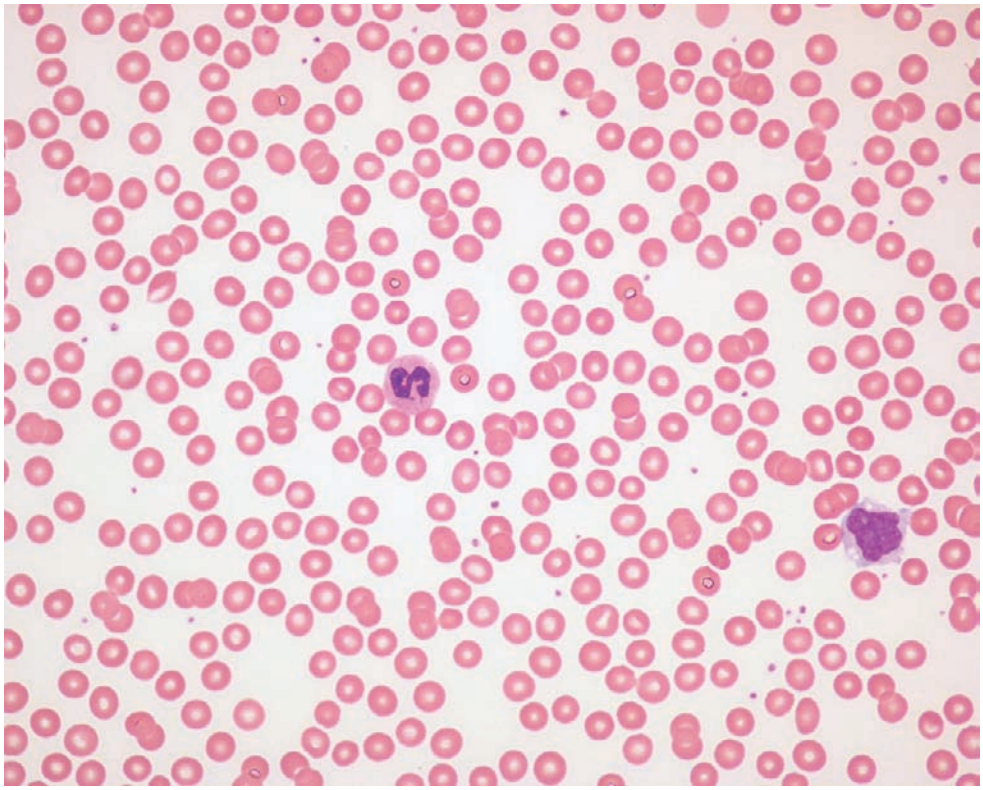

**FIGURE 1-13**

Anemia of chronic disease, peripheral blood findings. This anemia of chronic disease is characterized by a bland peripheral blood appearance with minimal anisopoikilocytosis.

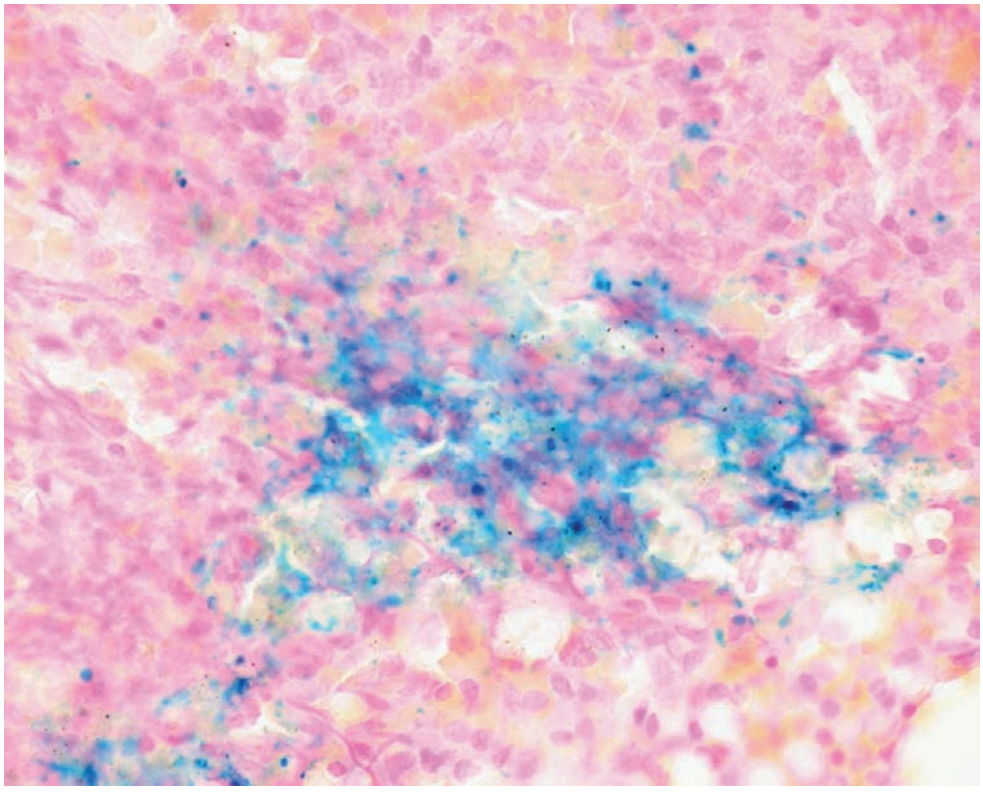

**FIGURE 1-14**

Anemia of chronic disease, marrow iron stain findings. This Prussian blue stain of a bone marrow aspirate smear particle demonstrates increased storage iron (blue staining), characteristic of anemia of chronic disease. Close examination of this stain would reveal absence or near absence of siderotic granules in erythroid precursors.

**ANEMIA OF CHRONIC DISEASE (ANEMIA OF INFLAMMATION)–PATHOLOGIC FEATURES**

**Peripheral Blood**

- Mild to moderate anemia that may be normochromic and normocytic or hypochromic and microcytic
- Lack of prominent anisocytosis or polychromasia

**Bone Marrow**

- Normocellular to slightly hypocellular marrow with normal to slightly reduced number of erythroid precursors
- Increased stainable iron on a Prussian blue stain with marked reduction or absence of sideroblasts

**Differential Diagnosis**

- Iron deficiency anemia
- Thalassemia minor or trait
- Anemia of chronic kidney disease
- Drug effects
- Hypothyroidism
- Marrow infiltration
- Hematopoietic stem cell defects, including myelodysplastic syndromes

nucleated red cells with iron-containing granules (i.e., sideroblasts) will be absent. The lack of sideroblasts indirectly reflects diminished delivery of iron to erythroid precursors, which is typical of ACD.

### Ancillary Studies

Because ferritin is a positive acute-phase reactant, serum ferritin is often elevated in ACD. Reduced serum iron concentration, despite adequate or increased storage iron, is a hallmark of ACD, which consequently can result in low transferrin saturation. However, the transferrin saturation is not reduced to the same extent as seen in pure IDA, because serum transferrin concentration and total iron binding capacity are not elevated in ACD. sTfR levels are normal or only slightly increased in uncomplicated ACD, which is consistent with understimulation of erythropoiesis.

### Differential Diagnosis

Other causes for anemia associated with reduced erythropoiesis include ACKD, drug effects, hypothyroidism, marrow infiltration, myelodysplastic syndromes, other stem cell defects, and IDA. Most can be ruled out based on correlation of clinical and pathologic data (sometimes including a bone marrow examination) and appropriate laboratory testing. The distinction between ACKD and ACD requires correlation with other evidence of renal failure and inflammation.

Uncomplicated IDA is easily distinguished from ACD (see Table 1-3); however, exclusion of IDA becomes more complicated in the presence of infection, inflammation, or cancer. A low serum ferritin level signifies iron deficiency, but would not rule out concurrent ACD. On the other hand, intermediate ferritin levels do not rule out IDA when there is coexisting inflammation. When serum ferritin levels are equivocal, sTfR levels may be helpful; elevated sTfR in this context strongly suggests the presence of iron deficiency. In difficult cases, a bone marrow examination with a Prussian blue stain will generally allow definitive assessment of iron status.

### Prognosis and Therapy

The best therapy for ACD is to identify and treat the underlying infection, inflammatory disease, or cancer. In some situations, the underlying disorder cannot be alleviated, and therapy with an ESA and iron supplementation if needed may improve a patient's quality of life. RBC transfusions are generally reserved for patients who have a poor response to therapy and for whom hemodynamic stability is compromised.

## HEMOLYTIC ANEMIAS

*Hemolysis* is the term given to an increased rate of peripheral destruction of RBCs. When mild, the bone marrow is able to compensate, and no anemia results. When more severe, or when the ability of the marrow to compensate is compromised, anemia develops. Hemolytic anemias can be divided broadly into those caused by intrinsic red cell abnormalities, nearly all of which are inherited, and those caused by extrinsic factors, nearly all of which are acquired. An exception to this rule is paroxysmal nocturnal hemoglobinuria, an acquired intrinsic red cell disorder resulting in hemolysis. This disorder, which is closely related to aplastic anemia, is discussed in Chapter 5 in the context of bone marrow failure syndromes.

A generic feature of all forms of hemolytic anemia is a compensatory erythroid hyperplasia in the bone marrow; therefore bone marrow findings are not discussed further for individual disorders. The erythroid expansion may be accompanied by a shift toward immaturity, mild nuclear to cytoplasmic dyssynchrony and dyserythropoiesis, and increased binucleated erythroid precursors (Figure 1-15).

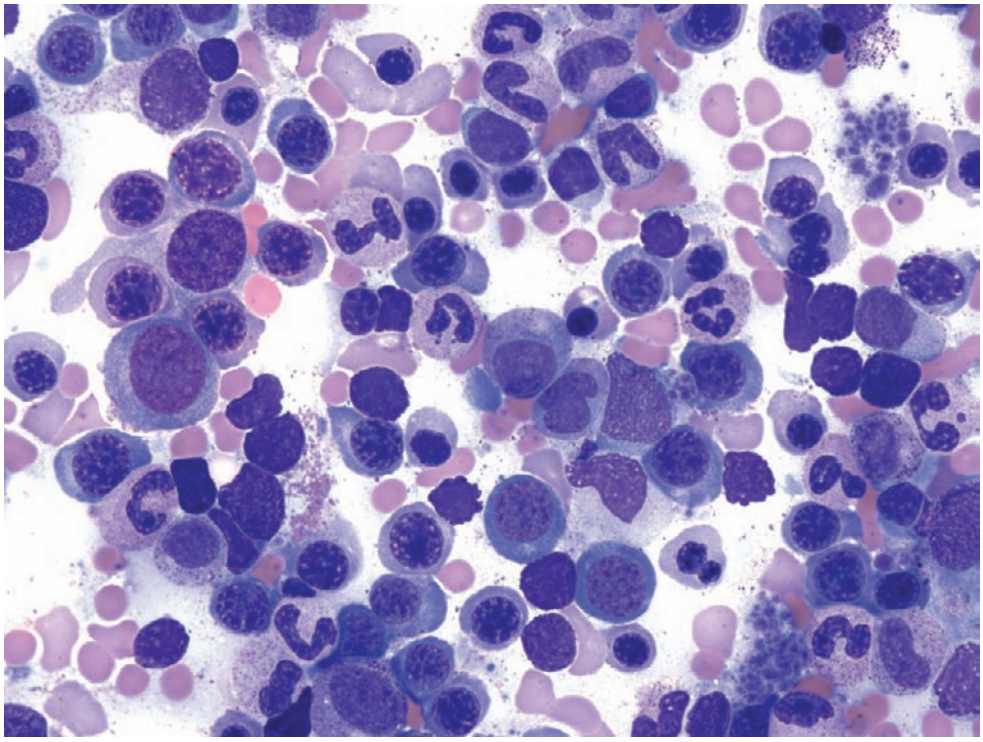

**FIGURE 1-15**

This bone marrow aspirate smear from a patient with warm autoimmune hemolytic anemia demonstrates erythroid hyperplasia, shift toward immaturity, mild nuclear:cytoplasmic dyssynchrony, increased binucleation, and mild terminal dyserythropoiesis in the form of nuclear budding. Such changes are common to states in which there is increased red blood cell production owing to hemolysis or bleeding.

## INTRINSIC RED CELL DEFECTS

### HEMOGLOBIN DISORDERS

#### Structural

##### Sickle Cell Disease

Sickle cell disease (SCD) is a disorder of chronic hemolytic anemia and microvascular occlusion involving the Hb S mutation in the β-globin gene on chromosome 11. This mutation results in a substitution of valine for glutamic acid at the sixth position of the β-globin chain, an alteration that causes abnormal Hb polymerization at low oxygen tensions. This abnormal polymerization produces rigid, deformed RBCs, resulting in chronic hemolysis and vasoocclusive phenomena. SCD most commonly results from homozygous Hb S mutations (SS disease), but also is a consequence of compound heterozygosity for Hb S and Hb C (SC disease), β-thalassemia (S/β-thalassemia), or rarely other β-chain variants.

##### Clinical Features

SCD ranges from an asymptomatic disorder to a severe, debilitating disease. It occurs most frequently in patients of African descent. The incidence of SS disease, SC disease, and S/β-thalassemia is approximately 1 per 600, 1 per 1000, and 1 per 1500 African American births, respectively. The disease occurs at a lower frequency in patients in Mediterranean populations. Infants are clinically unaffected until 4 to 6 months of age when Hb F production is largely replaced by Hb S production. Clinical manifestations result from both the chronic hemolysis and the microvascular occlusion.

Baseline Hb levels in SS disease range from 6 to 10 g/dL (average, 8 g/dL). The clinical consequences of the hemolysis are similar to those in other types of chronic hemolytic anemia: fatigue, exercise intolerance, jaundice, gallstones, and leg ulcerations. Aplastic crisis resulting from parvovirus B19 infection is a potentially devastating complication because of the markedly decreased RBC life span. Clinical manifestations resulting from vasoocclusion are protean. A uniform finding in SS disease is repeated splenic infarction resulting in functional asplenia by adulthood, predisposing patients to infectious complications. Patients also almost always have recurrent pain crises characterized by severe skeletal pain. Other manifestations include acute chest syndrome, stroke, renal dysfunction, priapism, aseptic necrosis of the femoral head, retinal pathology, and acute splenic sequestration. The last manifestation represents acute, massive pooling of blood in the spleen and can be fatal.

### SICKLE CELL DISEASE–FACT SHEET

**Definition**
- Disorder caused by abnormal polymerization of deoxygenated hemoglobin S, resulting in chronic hemolysis and microvascular occlusion
- Mutation results in valine to glutamic acid substitution at amino acid 6 of β globin

**Incidence and Location**
- Approximately 1500 infants with SCD are born annually in the United States

**Morbidity and Mortality**
- SS disease: often a severely debilitating disease with increased early mortality (2.9% between ages 1 and 3 years), 15% dead by age 29 years, median life span of 40 to 50 years
- SC disease: median life expectancy of 60 to 70 years

**Gender, Race, or Age Distribution**
- Equal gender incidence
- Most common in individuals of African descent; SS disease occurs in one per approximately 600, SC disease in one per approximately 1000, and S/β-thalassemia in one per approximately 1500 African American births; also seen in Mediterranean populations

**Clinical Features**
- Widely variable, including recurrent painful crises, chronic hemolytic anemia, gallstones, leg ulcers, stunted growth, bony abnormalities, susceptibility to infection, acute chest syndrome, stroke

**Prognosis and Therapy**
- Symptomatic therapy for crises
- Transfusion for acute exacerbations of anemia and in preparation for surgery
- Regular transfusion for severely affected patients
- Hydroxyurea therapy to increase levels of Hb F
- Good prognostic features include high levels of Hb F, low frequency of pain crises, no severe anemia before the age of 2 years, low white blood cell count, low frequency of acute chest syndrome, and low blood pressure

SC disease is a milder disorder than SS disease. Although nearly all the complications of SS disease may be seen, they are less frequent and less severe in SC disease. In addition, patients do not demonstrate autosplenectomy, but instead often have splenomegaly. S/$\beta^{\circ}$-thalassemia (in which there is no normal β-chain production from the thalassemic allele) is a slightly milder disorder than SS disease. S/$\beta^{\circ}$-thalassemia differs from SS disease in that there is less severe hemolysis and often splenomegaly. S/$\beta^{+}$-thalassemia (in which some normal β-globin chains are produced) is a milder disease and, in fact, is asymptomatic in many cases.

## Pathologic Features

The peripheral blood smear in patients with SS disease demonstrates anemia with prominent anisopoikilocytosis (Figure 1-16). Most prominently, one sees sickled cells (drepanocytes). Classic sickled cells are elongate, tapered cells with pointed ends and no central pallor (Figure 1-17). Some, however, are broader with more blunted ends (see Figures 1-16 and 1-17). Variant forms such as boat-shaped (Figure 1-18) or holly leaf–shaped cells are also seen. In addition, a small population of blister cells is usually seen (Figure 1-19). In these cells, the Hb is pushed to one side with a thin strip of membrane remaining on the other side. The number of sickled cells on blood smears is highly variable and correlates with the severity of hemolysis. A small number of spherocytes are generally seen, and manifestations of hyposplenism are nearly always evident in adult patients, including Howell-Jolly bodies (see Figure 1-17), Pappenheimer bodies, and target cells (see Figure 1-16). Circulating nucleated red blood cells are usually present as well. The white blood cell count is often modestly increased, even in the absence of acute complications.

In SC disease, classic sickled cells are rare or absent. Generally there are prominent target cells and scattered Hb C crystals (Figures 1-20 and 1-21). The C crystals tend to be misshapen, bent, or abortive appearing and tend to produce RBCs with odd spikes and hooks (Figure 1-22). Occasionally there are classic Hb C crystals (see

### SICKLE CELL DISEASE–PATHOLOGIC FEATURES

**Microscopic Findings**
- SS disease
  - Sickled cells and variants (boat-shaped and holly leaf–shaped)
  - Occasional blister cells and spherocytes
  - Evidence of hyposplenism in adults (target cells, Howell-Jolly bodies, Pappenheimer bodies)
  - Mild leukocytosis
- SC disease
  - Rare sickled cells
  - Many target cells
  - Malformed hemoglobin C crystals
  - Bizarre red blood cell forms, including cells with spikes, hooks, and irregular condensation of hemoglobin
- S/β-thalassemia
  - Similar to SS disease, but fewer sickled cells, more target cells, absence of hyposplenic features

**Differential Diagnosis**
- Other forms of inherited hemolytic anemia

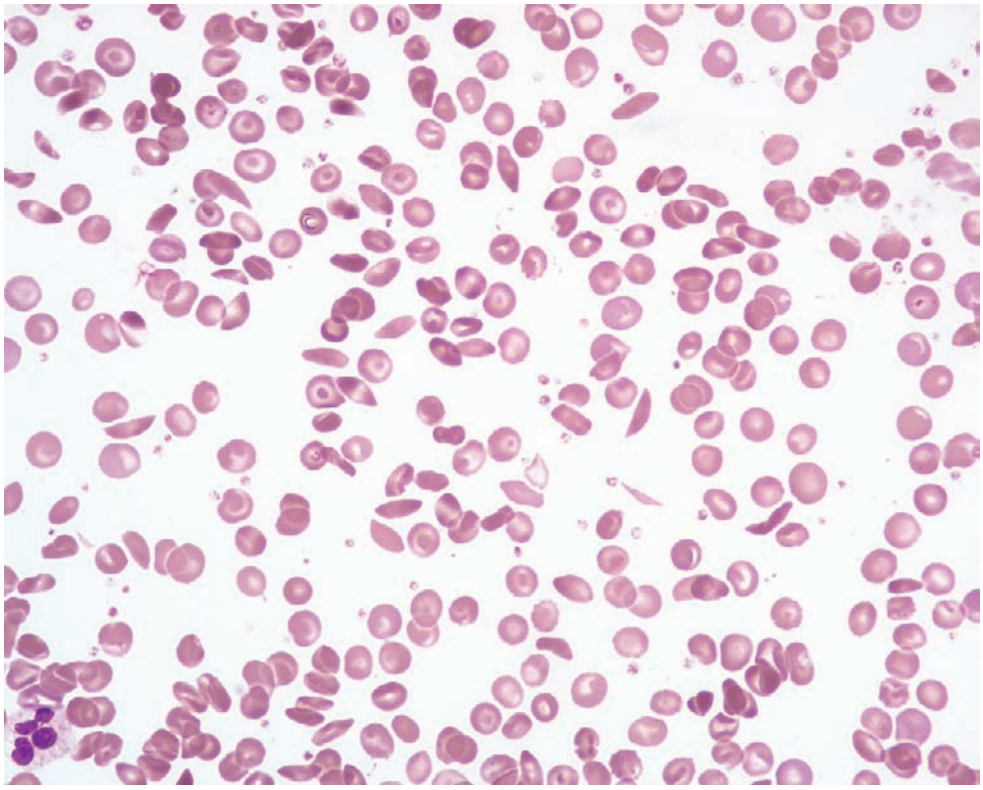

**FIGURE 1-16**

Hemoglobin SS disease, peripheral blood findings. This blood smear from a patient with hemoglobin SS disease demonstrates prominent anisopoikilocytosis. The most prominent poikilocytes are elongate, sickled cells (drepanocytes) and cells with a droplet of hemoglobin within the area of central pallor (target cells or dacrocytes).

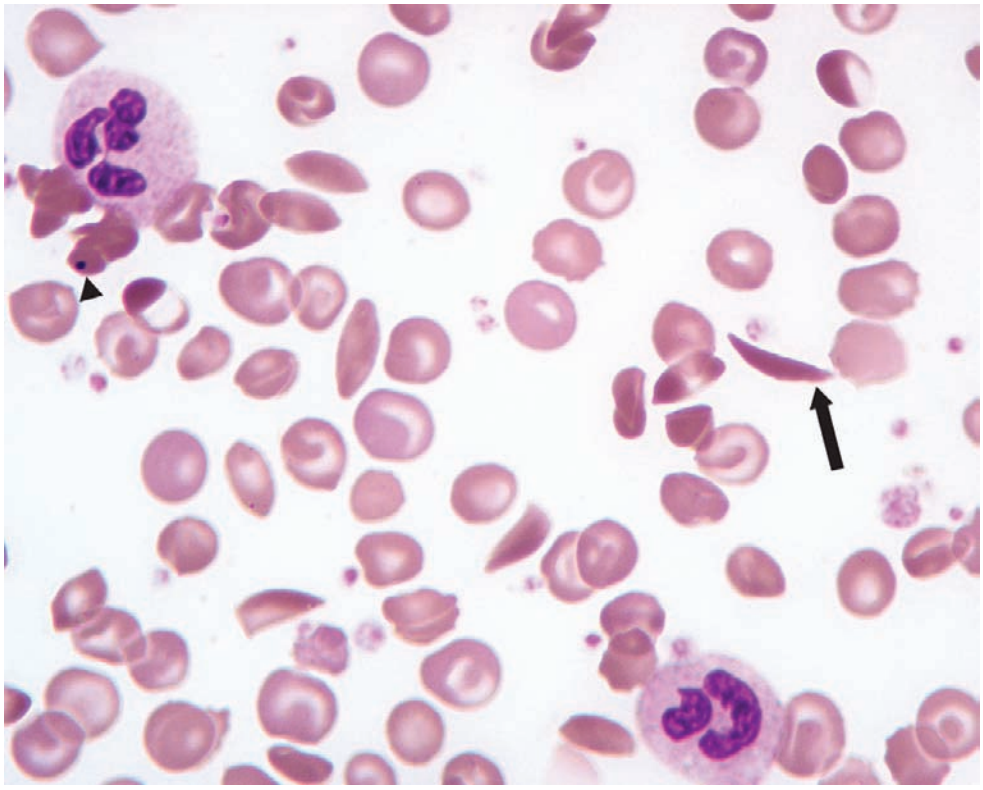

**FIGURE 1-17**

Hemoglobin SS disease, peripheral blood findings. This high-power image of hemoglobin SS disease demonstrates a classic, slender sickled cell *(arrow)* with a concave–convex appearance, lack of central pallor, and pointed ends. Variant, broader sickled cells are also evident. Note also the Howell-Jolly body *(arrowhead)*.

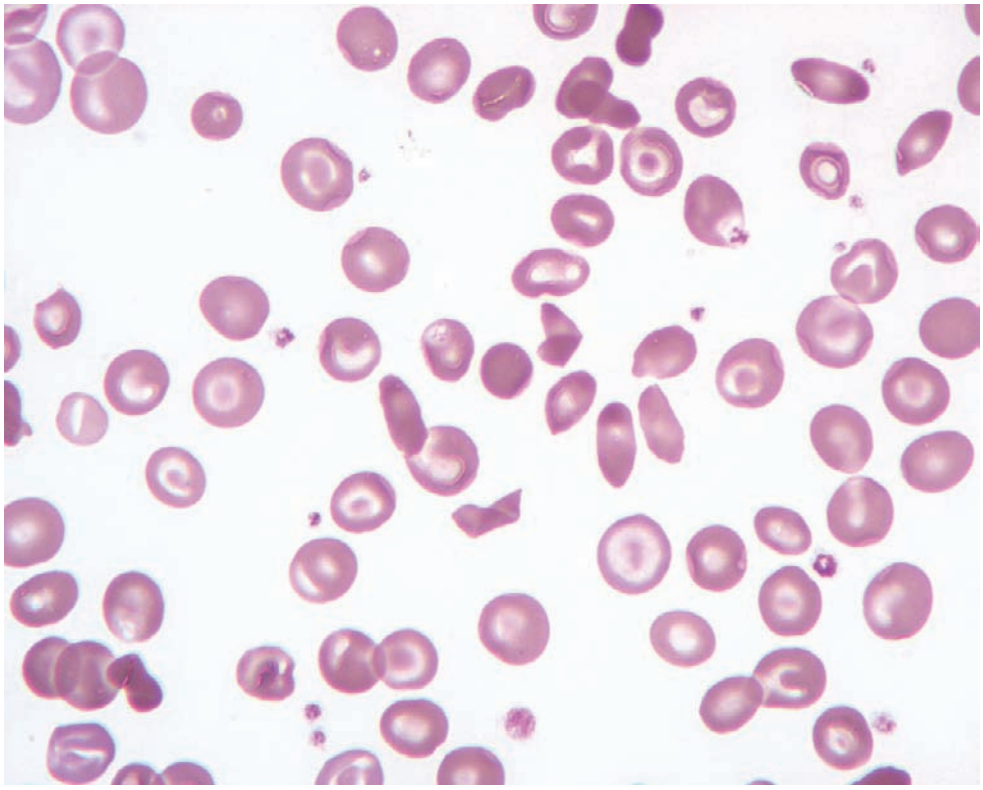

**FIGURE 1-18**

Hemoglobin SS disease, peripheral blood findings. This field from a patient with hemoglobin SS disease features two variant sickled cells with a folded, boat-shaped appearance. Broad sickled cells are also present.

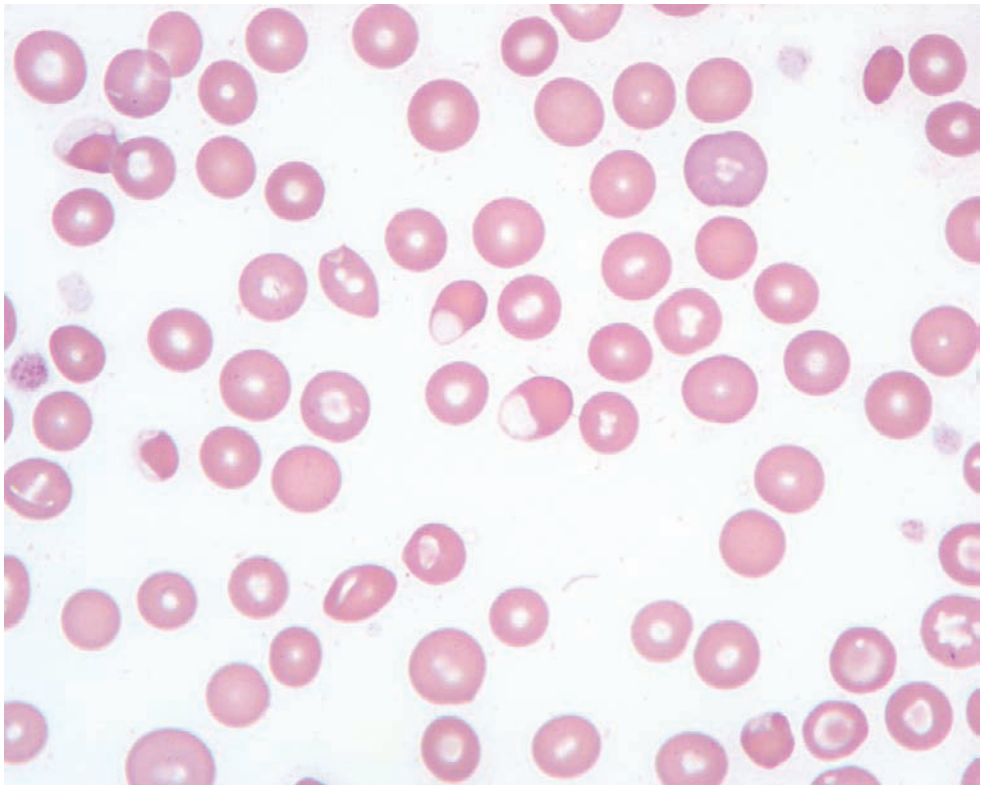

**FIGURE 1-19**

Hemoglobin SS, peripheral blood findings. Blister cells are usually present in small numbers in smears from patients with hemoglobin SS disease. The hemoglobin mass is pushed to one side in these cells, with only a thin rim of membrane remaining on the other side.

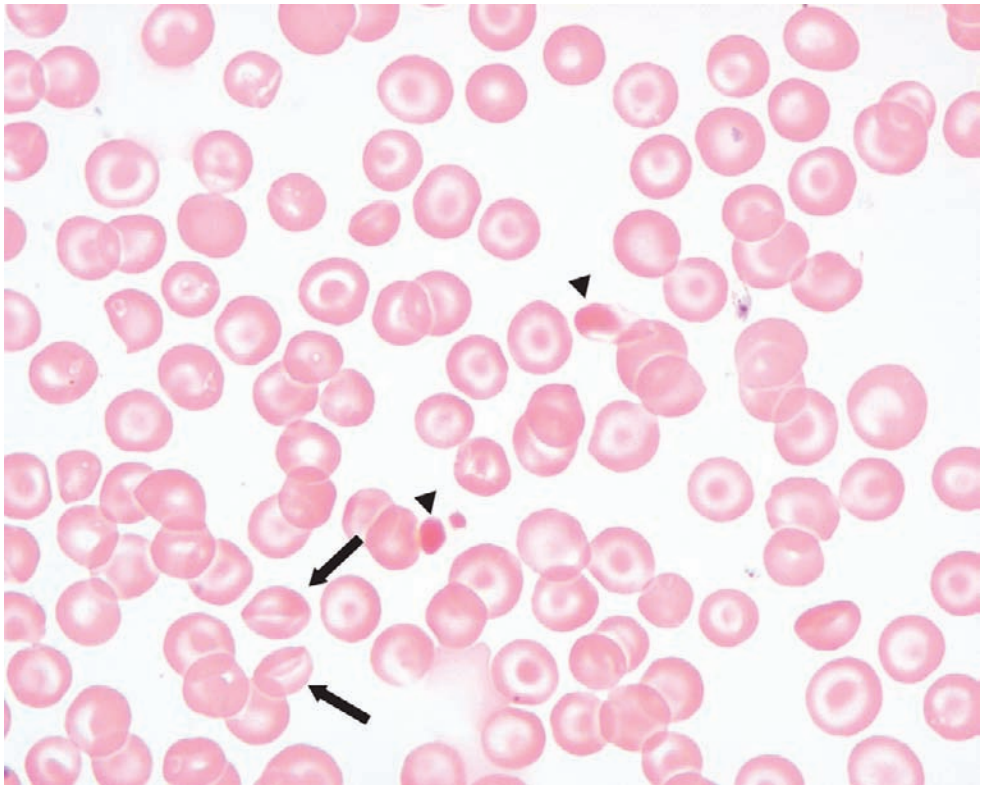

**FIGURE 1-20**

Hemoglobin SC disease, peripheral blood findings. This smear from a patient with hemoglobin SC disease demonstrates prominent target cells, folded cells *(arrows),* and poorly formed Hb C crystals *(arrowheads).*

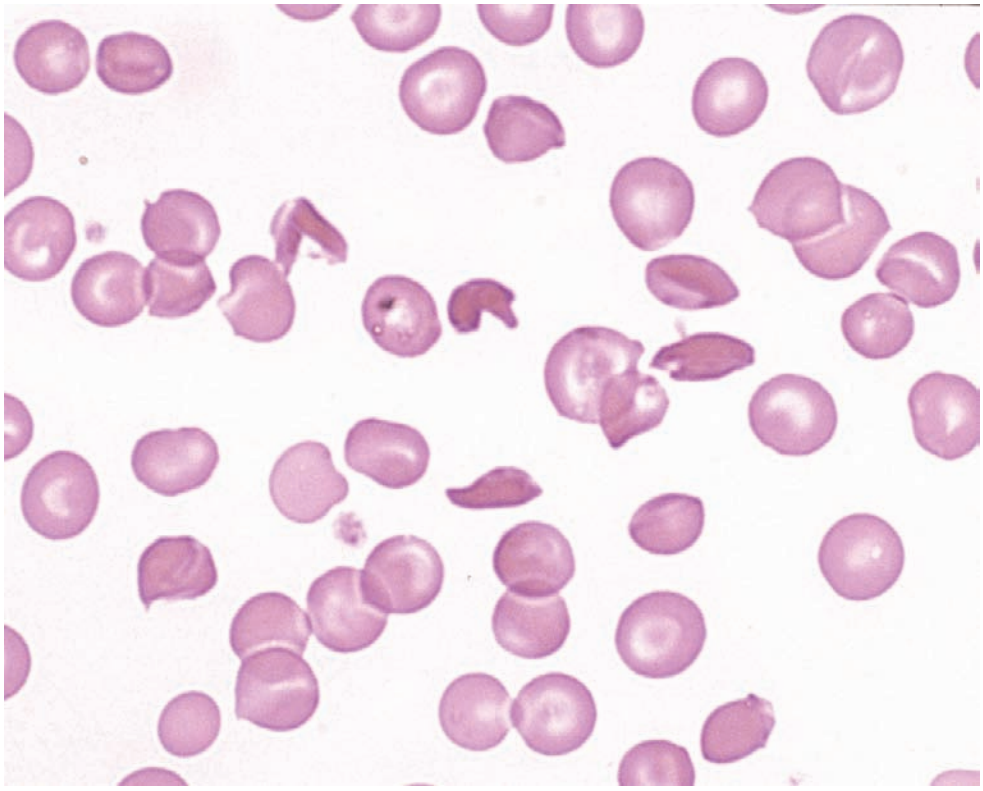

**FIGURE 1-21**

Hemoglobin SC, peripheral blood findings. Crystals in hemoglobin SC disease may be irregular, somewhat amorphous, or bent.

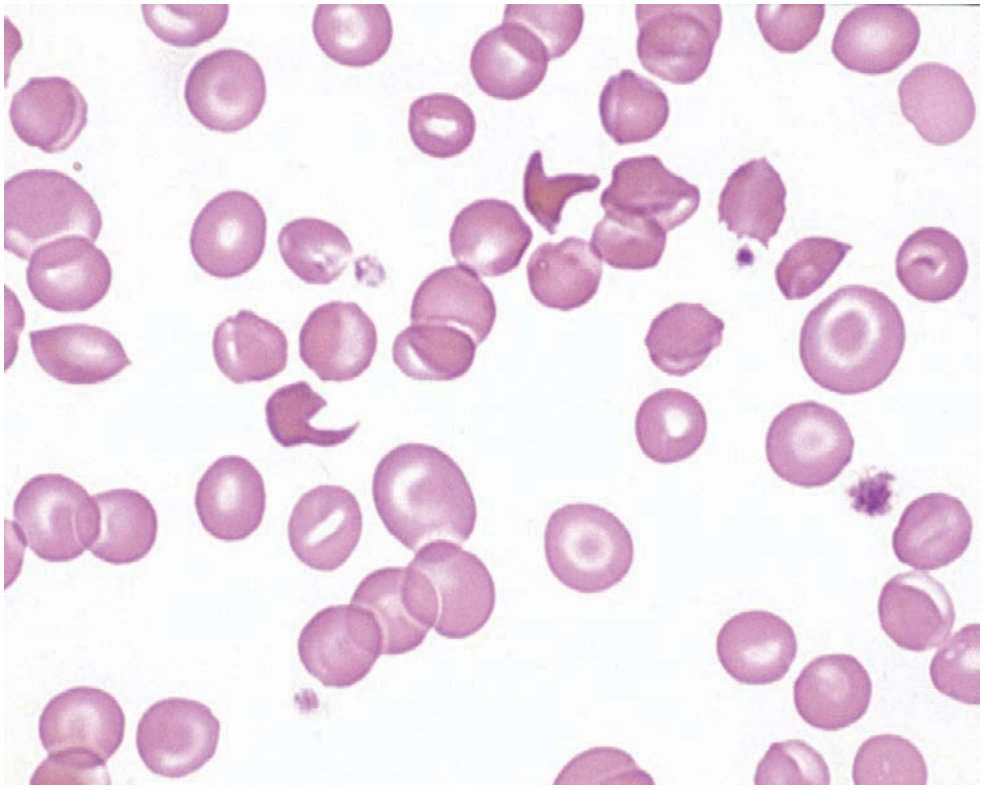

**FIGURE 1-22**

Hemoglobin SC disease, peripheral blood findings. Red blood cells in hemoglobin SC disease may show peculiar spikelike or hooklike projections.

discussion under Hemoglobin CC Disease). Some cells show irregularly concentrated hemoglobin. S/β-Thalassemia differs from SS disease in that there is microcytosis, fewer sickled cells, and more target cells.

## Ancillary Studies

Hb electrophoresis, isoelectric focusing, capillary electrophoresis, and high-performance liquid chromatography in SS disease will all demonstrate a predominance of Hb S, variable Hb F (1% to 20%), and no Hb A. SC disease results in approximately equal amounts of Hb S and Hb C. S/$\beta^0$-thalassemia appears essentially identical to SS disease electrophoretically. S/$\beta^+$-Thalassemia is characterized by a mixture of Hb S and Hb A, with more Hb S (in contrast to sickle trait, in which there is more Hb A than Hb S). All forms of SCD, as well as sickle trait, result in a positive sickle solubility test; therefore this procedure will not discriminate between these disorders. The Hb S mutation can also be detected by molecular methods.

## Differential Diagnosis

The differential diagnosis of SCD includes other forms of chronic hemolytic anemia. However, only SCD is associated with sickled RBCs and vasoocclusive phenomena. Hb SC disease can be confused with Hb CC disease because of the presence of Hb C crystals. However, the crystals in CC disease are straight, in contrast to the irregular, bent, or malformed crystals of SC disease. Rare cases of SS disease will have few sickled cells but frequent blister cells. Such cases may be confused with oxidant hemolysis. Hemoglobin studies will be definitive.

## Prognosis and Therapy

Children with SS disease have increased early mortality: 2.9% between the ages of 1 and 3 years, and 15% die by the age of 29 years. Patients have a significantly decreased average life span, with a median age at death of 42 years in men and 48 years in women. A variety of prognostic factors for SS disease have been identified in a number of studies. One important factor is the level of Hb F. High levels of Hb F inhibit the polymerization of Hb S, ameliorate the clinical symptoms, and prolong the life span. Other good prognostic factors that have been identified include low frequencies of pain crises and acute chest syndrome, lack of severe anemia in early childhood, low baseline white blood cell counts, and low blood pressure.

Patients with SC disease have significantly better life expectancy than those with SS disease, with median

ages at death of 60 years for men and 68 for women. Patients with S/β-thalassemia have variable prognoses depending on the severity of the clinical phenotype.

Therapy for SCD depends on the severity of clinical manifestations. Acute vasoocclusive events such as pain crises, acute chest syndrome, and infection may be treated conservatively or, when severe, may require exchange transfusion. Patients generally receive prophylactic transfusion before surgery, and patients with particularly severe disease manifestations can be treated with regular transfusions. Hydroxyurea has been used in some patients to elevate levels of Hb F within RBCs and thus diminish the severity and frequency of complications. Prolonged use of this agent has been shown to dramatically improve morbidity and mortality in SCD.

### Hemoglobin CC Disease

Hb CC disease is due to homozygous inheritance of an abnormal β-globin chain, and, as in the S mutation, the C mutation occurs at the sixth amino acid of the β-globin molecule. However, rather than a valine, the substituted amino acid is a lysine. Hb CC RBCs have abnormal membrane ion transport, causing them to be dehydrated. This dehydration is thought to be the primary cause of hemolysis in this disorder.

## Clinical Features

Hb CC disease is a relatively innocuous clinical syndrome consisting of mild hemolytic anemia. Hb CC is most prevalent in individuals of African descent, occurring at a frequency of one in 6000 African Americans. Infants are well at birth and develop hemolysis only after Hb F production is replaced by Hb C. Patients generally have Hb levels in the range of 9 to 12 g/dL with microcytosis. Clinical symptoms are largely absent, whereas most adults have splenomegaly that is generally asymptomatic.

**HEMOGLOBIN CC DISEASE–FACT SHEET**

**Definition**
- Disorder caused by homozygous inheritance of the hemoglobin C mutation of the β-globin gene, resulting in red blood cell dehydration and mild chronic hemolysis

**Morbidity and Mortality**
- Mild anemia, occasional symptoms referable to splenomegaly
- No increased mortality

**Gender, Race, or Age Distribution**
- Equal gender incidence
- Common in individuals of African descent, 1 in 6000 African Americans

**Clinical Features**
- Mild hemolytic anemia
- Splenomegaly, usually asymptomatic

**Prognosis and Therapy**
- No therapy required

**HEMOLOBIN CC DISEASE–PATHOLOGIC FEATURES**

**Microscopic Findings**
- Numerous uniform target cells
- Scattered folded cells and spherocytes
- Hemoglobin C crystals

**Differential Diagnosis**
- Thalassemia minor
- SC disease

## Pathologic Features

Blood smears from patients with Hb CC disease are notable for numerous target cells that are relatively uniform in appearance (Figure 1-23). Minor subsets of folded cells and scattered spherocytes are present as well. Hb C crystals are present in varying numbers in most patients, although occasionally they may be rare or absent. Hb C crystals are classically elongate, straight, octahedral crystals and are most often within residual RBC membranes (Figures 1-23 and 1-24). However, they may be shorter and blunter and occasionally are multiple within an individual cell (see Figure 1-24).

## Ancillary Studies

Hb electrophoresis, capillary electrophoresis, isoelectric focusing, and high-performance liquid chromatography in Hb CC disease will each demonstrate nearly all Hb C with small amounts of Hb F and Hb $A_2$. Hb C is a relatively slow Hb variant on conventional alkaline electrophoresis, migrating in the same position as Hb $A_2$, Hb E, and Hb O.

## Differential Diagnosis

Because of the microcytosis and target cell formation, Hb CC disease can be mistaken for mild forms of

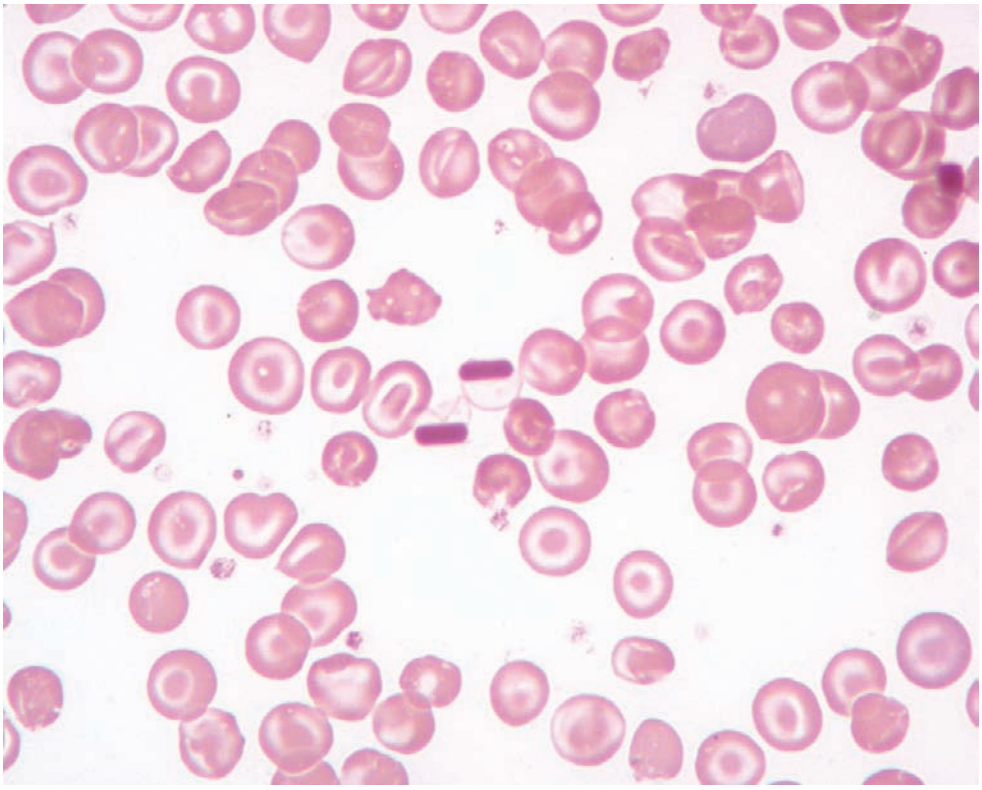

**FIGURE 1-23**

Hemoglobin (Hb) CC disease, peripheral blood findings. This image from a patient with Hb CC disease demonstrates two classic Hb C crystals. These elongate, uniformly dense structures are present within a ghostlike, residual red blood cell membrane. Hb C crystals are diagnostic of the presence of Hb C, although they do not strictly distinguish between CC and SC diseases. Many target cells and a few folded cells are also present.

thalassemia. However, the target cells are much more numerous in Hb CC disease than in mild thalassemia, and the presence of C crystals will establish the diagnosis. SC disease can also be confused with Hb CC disease. However, the crystals in SC disease are usually not straight and well formed, and the irregular spiked or hooked cells of SC disease are not a feature of CC disease.

### Prognosis and Therapy

CC disease is a clinically benign disorder with a normal life expectancy. No therapy is required.

## Thalassemias

The thalassemias are a group of disorders characterized by decreased production of either α- or β-globin chains. The diseases are designated according to which chain is underproduced, that is, β-thalassemias involves decreased β-chain production and α-thalassemia decreased α-chain production. In pure thalassemic syndromes, the globin chains that are produced are structurally normal, distinguishing them from the structural Hb disorders discussed previously. However, several structural Hb abnormalities may produce syndromes closely mimicking the thalassemias, because of underproduction of structurally abnormal globin chains. The most common of these is Hb E.

The decreased Hb production in the thalassemias results in hypochromic, microcytic RBCs. In addition, the decreased globin chain synthesis results in an excess of the chain that is produced in normal amounts. These excess chains precipitate in the more severe forms of these diseases and cause intramedullary death of RBC precursors (in α-thalassemia), decreased RBC lifespan (in both α- and β-thalassemias), or both. Therefore the clinically significant thalassemias can be considered to be primarily hemolytic anemias.

### Clinical Features

The thalassemias are extremely heterogeneous, both genetically and clinically. The vast majority of the heterozygous forms of β-thalassemia, two gene deletion forms of α-thalassemia, and heterozygous and homozygous Hb E are clinically innocuous, manifesting as RBC microcytosis with mild or no anemia. One-gene deletion α-thalassemia is clinically and hematologically silent.

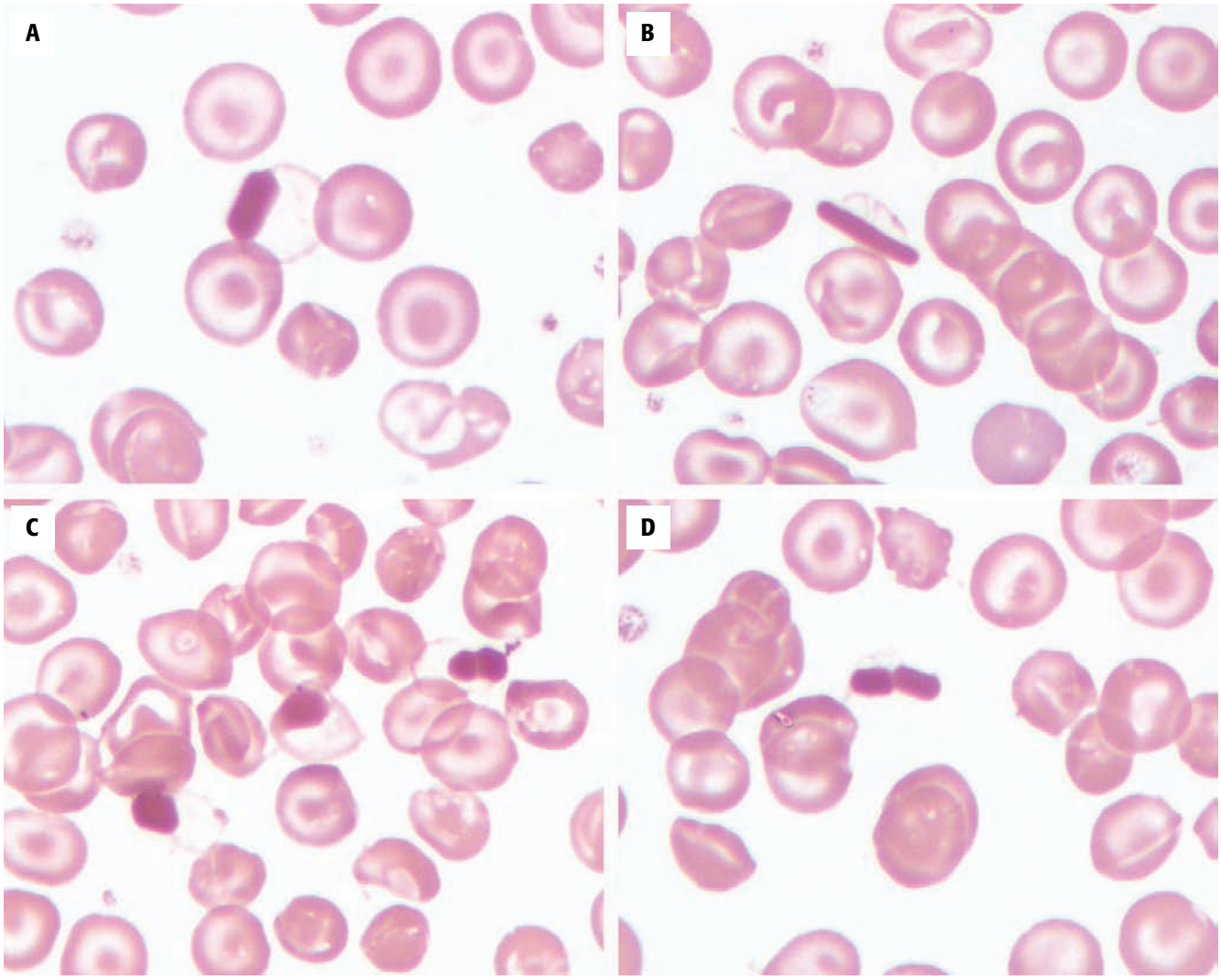


**FIGURE 1-24**

Hemoglobin (Hb) CC disease, peripheral blood findings. This composite image shows multiple C crystals in a patient with Hb CC disease. **A,** The polyhedral crystalline nature of these structures is evident in this Hb C crystal. **B,** A long, slender crystal. **C,** Several blunt crystals and a double crystal. **D,** Two well-formed crystals within a single cell.

However, the homozygous or compound heterozygous β-thalassemias, the three- and four-gene deletion α-thalassemias, and the compound heterozygous state for Hb E and β-thalassemia range from moderate to profound hemolytic anemias. The thalassemias show a wide geographic distribution in Mediterranean countries, the Middle East, parts of India and Pakistan, and Southeast Asia. The incidence is highly variable in different regions and populations.

The most severe form of thalassemia is four-gene deletion α-thalassemia, or Hb Bart's hydrops fetalis. The total absence of α-chain production results in a profound defect in Hb production. Hb levels average 6.2 g/dL in affected fetuses; however, this value is deceptive because the majority of this is Hb Bart's (gamma-tetramers), which is ineffective for delivering oxygen to the tissues. Only the small amounts of embryonic Hb present are effective at delivering oxygen, and these infants suffer profound tissue hypoxia. As with other forms of severe intrauterine anemia, this condition results in the syndrome of hydrops fetalis, with high-output cardiac failure, generalized edema, and organomegaly.

β-Thalassemia major (Cooley anemia) results from a complete absence or profound decrease in β-chain production from both β-globin alleles. Infants are well at birth but develop severe anemia over the first few months of life as γ-chain production is largely replaced by β-chain production. By definition, the anemia is transfusion dependent, with steady-state Hb levels of 2 to 3 g/dL. If transfusion is not performed, children develop a wide spectrum of clinical manifestations caused by the severe hemolytic anemia itself and the body's attempts to compensate for it. These manifestations include stunted growth, wasting, fever, hyperuricemia, infections, organomegaly, frontal bossing, spontaneous fractures, and other bony abnormalities. Patients with β-thalassemia major who receive an

**THALASSEMIAS–FACT SHEET**

**Definition**

- A group of hypochromic, microcytic anemias characterized by underproduction of normal α- or β-globin chains

**Morbidity and Mortality**

- α-Thalassemia
  - Hemoglobin (Hb) Bart's hydrops fetalis: infants stillborn or die within first hours of life
  - Hb H disease: mild morbidity, probably near-normal or normal life expectancy
  - One- and two-gene deletion α-thalassemia: no increased morbidity or mortality
- β-Thalassemia
  - Major: severe, chronic complications; death in childhood without long-term transfusion; death in second to fourth decades with regular transfusion, depending on adequacy of chelation
  - Intermedia: highly variable, ranging from similar to β-thalassemia major to no increased morbidity and mortality
  - Minor: no increased morbidity or mortality

**Gender, Race, or Age Distribution**

- Equal gender incidence
- Wide geographic distribution with highly variable incidence: Mediterranean, Middle East, India and Pakistan, Southeast Asia

**Clinical Features**

- α-Thalassemia
  - Hb Bart's hydrops fetalis: severe anemia, generalized edema, organomegaly, heart failure
  - Hb H disease: jaundice, splenomegaly, gallstones, rare leg ulcers
  - Two-gene deletion alpha-thalassemia: mild microcytic anemia
- β-Thalassemia
  - Major: profound hemolytic anemia with stunted growth, wasting, fever, hyperuricemia, infections, organomegaly, frontal bossing, spontaneous fractures, and other bony abnormalities; if transfused, endocrine, cardiac, and liver dysfunction due to iron deposition
  - Intermedia: highly variable, ranging from similar to β-thalassemia major to asymptomatic
  - Minor: mild microcytic anemia

**Prognosis and Therapy**

- Transfusion support for severe thalassemias with iron chelation
- Supportive care of chronic hemolytic complications
- Prognosis in severe thalassemias dependent on adequacy of transfusion program and chelation

adequate transfusion program have essentially normal early development, but ultimately manifest complications resulting from iron overload. These manifestations include endocrine problems, such as absent menarche and pubertal growth spurt, diabetes mellitus, and adrenal insufficiency. Cardiac dysfunction resulting from iron overload ultimately is the most prominent cause of morbidity and mortality in these patients.

Moderately severe forms of thalassemia include β-thalassemia intermedia and Hb H disease. β-Thalassemia intermedia covers a wide spectrum, ranging from disorders nearly as severe as β-thalassemia major (but lacking transfusion dependence) to mild disorders with few clinical manifestations. The complications depend on the severity of the hemolysis and are largely those seen in other forms of hemolytic anemia, including jaundice, organomegaly, gallstones, susceptibility to infections, and leg ulcers. Hb H disease, a result of three α-gene deletions, is a moderate hemolytic anemia with Hb levels in the range of 7 to 10 g/dL. Hb E/β-thalassemia generally manifests as β-thalassemia intermedia.

## Radiologic Features

β-thalassemia major patients who have not received transfusions manifest characteristic radiologic abnormalities, including radiating striations in the subperiosteal bone of the skull, producing a "hair on end" appearance, cortical thinning, rarefaction of long bones and small bones of the hands and feet, and pathologic fractures.

## Pathologic Features

The pathologic features of the thalassemias are as variable as the clinical findings. The peripheral blood smears in Hb Bart's hydrops fetalis and severe forms of β-thalassemia are similar, characterized by prominent erythroblastosis and pronounced anisopoikilocytosis, including prominent hypochromia and target cells, as well as fragments, teardrop cells, and other bizarre poikilocytes (Figure 1-25). Differences between these two disorders include generally more bizarre anisopoikilocytosis in severe β-thalassemia compared with Hb Bart's hydrops fetalis as well as less polychromasia. The latter is due to the prominent intramedullary cell death that is a feature of severe β-thalassemia but not α-thalassemia. Occasional elongate cells closely resembling sickled cells have been described in Hb Bart's hydrops fetalis. Finally, whereas the MCV is low in severe β-thalassemia, it tends to be normal or high in Hb Bart's hydrops fetalis, likely due in part to the prominent reticulocytosis in this disorder.

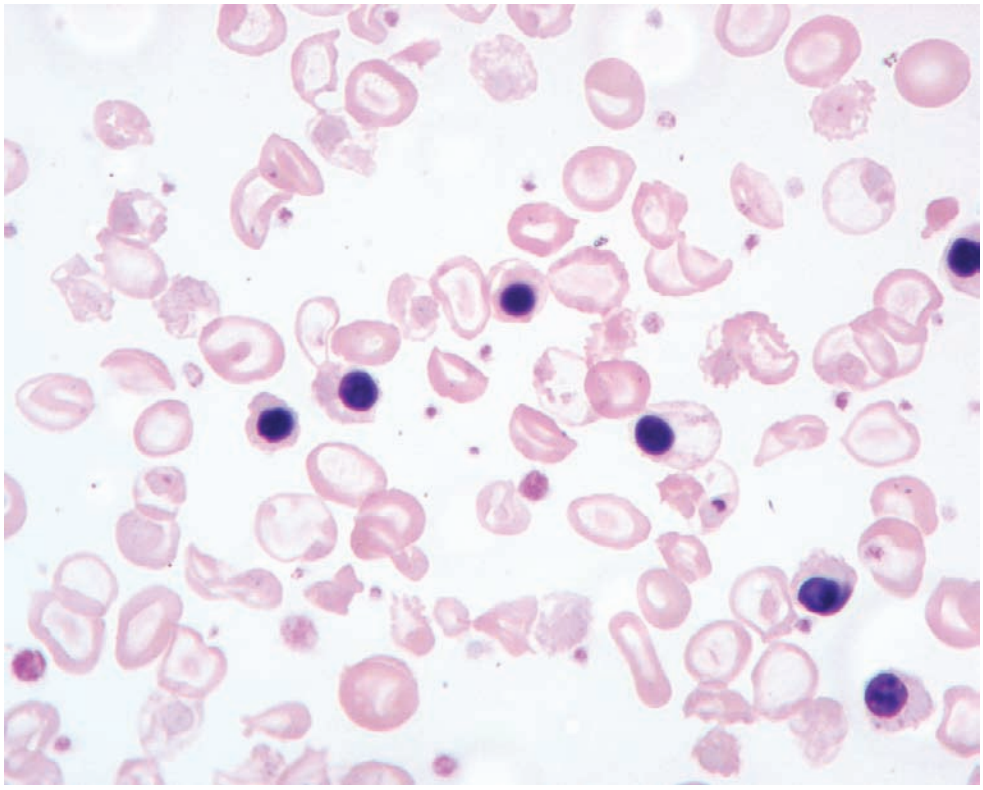

**FIGURE 1-25**

Severe β-thalassemia, peripheral blood findings. This smear from a patient with severe β-thalassemia demonstrates erythroblastosis, prominent hypochromia, target cells, and other bizarre poikilocytes.

**THALASSEMIAS–PATHOLOGIC FEATURES**

**Microscopic Findings**

- Severe β-thalassemia and hemoglobin (Hb) Bart's hydrops fetalis
  - Pronounced, bizarre anisopoikilocytosis
  - Target cells, teardrop cells, fragments
  - Hypochromia
  - Prominent erythroblastosis
- Hb H disease
  - Moderate anisopoikilocytosis
  - Target cells
  - Hypochromia
  - Numerous Hb H inclusions with brilliant cresyl blue staining

**Differential Diagnosis**

- Hb Bart's hydrops fetalis: other causes of fetal anemia or hydrops (e.g., Rhesus disease, parvovirus B19, fetomaternal hemorrhage)
- Hb H disease: iron deficiency, Hb C disease
- β-Thalassemia minor and two-gene–deletion α-thalassemia: iron deficiency, ACD

The blood smear in Hb H disease is characterized by hypochromia, microcytosis, moderate anisopoikilocytosis dominated by target cells, and moderate polychromasia (Figure 1-26). Erythroblastosis is not a feature of this disorder.

β-Thalassemia minor and two-gene deletion α-thalassemia are characterized by a fairly uniform population of microcytic RBCs with occasional target cells (Figure 1-27). Coarse basophilic stippling is a classical (although fairly insensitive) feature of β-thalassemia minor, but is not a feature of mild α-thalassemia.

## Ancillary Studies

### Hemoglobin Analysis

Because of the absence of α-chain production, Hb electrophoresis or HPLC will demonstrate no Hb A, Hb F, or Hb A2 in Hb Bart's hydrops fetalis. Instead, most of the Hb present will be Hb Bart's, which represents γ-chain tetramers. Small amounts (5% to 20%) of the embryonic Hbs Gower-1 ($\zeta_2\varepsilon_2$) and Portland ($\zeta_2\gamma_2$) are also present. Hb Bart's and the embryonic Hbs are all "fast" Hbs, migrating anodal to Hb A on alkaline electrophoresis. Hb H disease in adults is characterized by the presence of Hb H (β-chain tetramers), which, along with Hb I, is the fastest moving of all Hb variants on alkaline electrophoresis. In adults, there may also be traces of Hb Bart's migrating slightly cathodal to Hb H. At birth, Hb Bart's constitutes 20% to 40% of total Hb in Hb H disease.

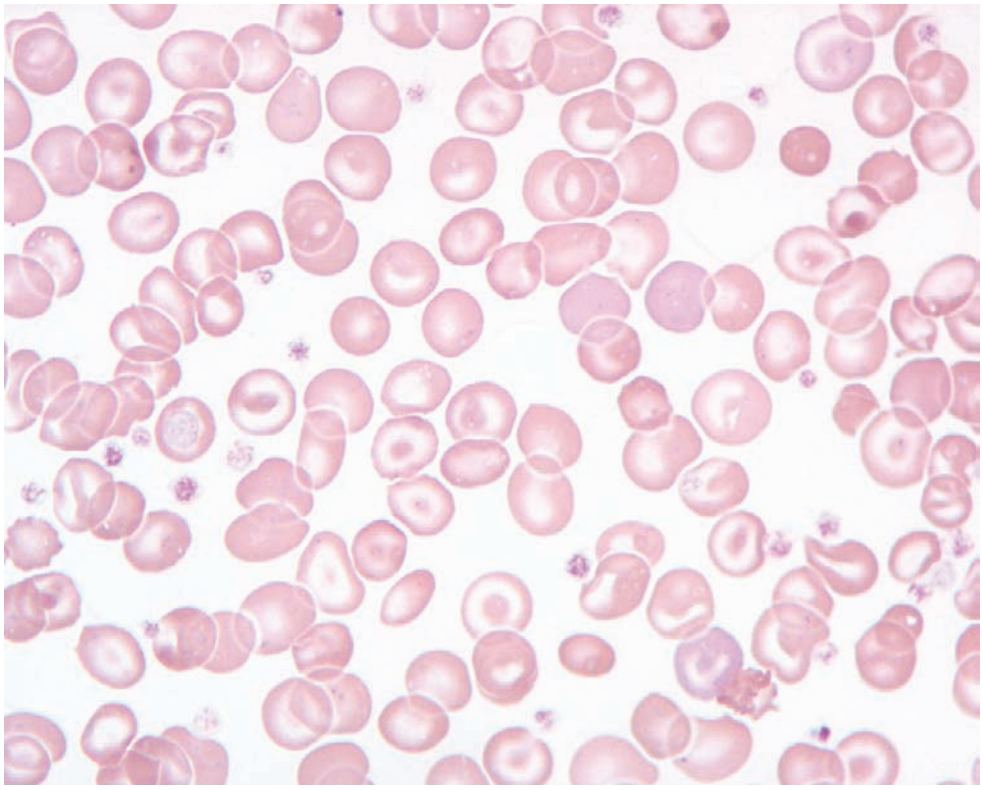

**FIGURE 1-26**

Hemoglobin H disease, peripheral blood findings. Compared to severe beta-thalassemias, the blood smear findings in hemoglobin H disease are relatively bland, with hypochromia and target cells.

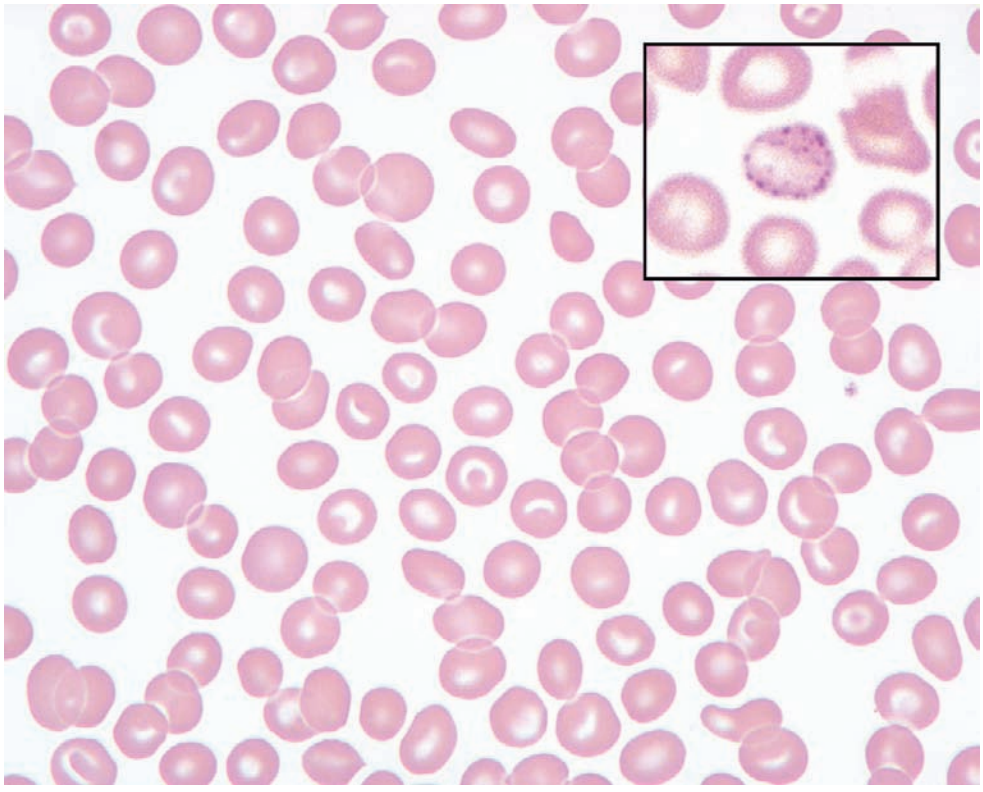

**FIGURE 1-27**

β-Thalassemia minor, peripheral blood findings. This smear from a patient with β-thalassemia minor demonstrates a fairly uniform population of microcytic cells with scattered target cells. Coarse basophilic stippling *(inset)* may be seen in β-thalassemia minor, helping to distinguish it from iron deficiency anemia and anemia of chronic disease, in which this is an uncommon finding.

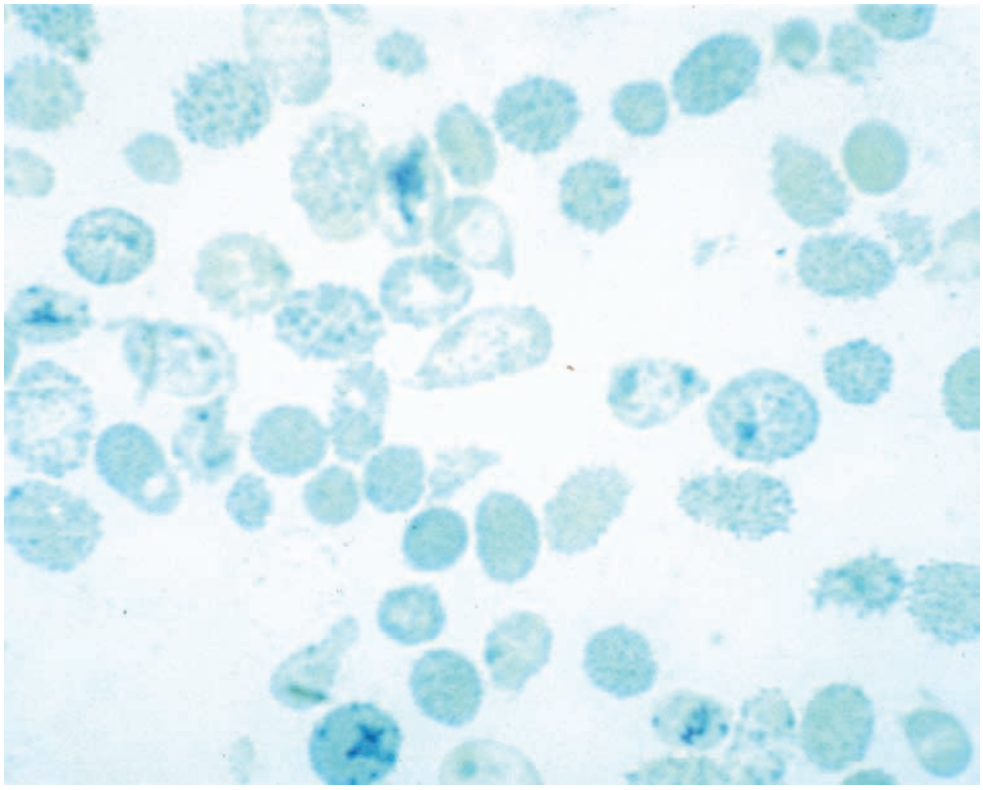

**FIGURE 1-28**

Hemoglobin H disease, peripheral blood findings. This brilliant cresyl blue stain reveals numerous pale blue inclusions in most of the red cells, producing a golf ball–like appearance.

β-Thalassemia major manifests as all or nearly all Hb F on electrophoresis or HPLC. Hb A may be present in small amounts, and Hb $A_2$ may be normal or increased. β-Thalassemia intermedia shows varying proportions of Hb A and Hb F, depending on the severity of the disease and may or may not show elevated Hb $A_2$. The hallmark of β-thalassemia minor is an increase in Hb $A_2$ above approximately 3.5%.

### Other Studies

Incubation of red cells with brilliant cresyl blue in patients with Hb H disease will produce numerous pale blue inclusions in RBCs, producing a "golf ball" appearance (Figure 1-28). Molecular methods that will easily detect common thalassemia mutations are also available (see Chapter 24).

## Differential Diagnosis

The clinical differential diagnosis of Hb Bart's hydrops fetalis includes other causes of hydrops fetalis associated with anemia, including Rh hemolytic disease, intrauterine parvovirus B19 infection, severe fetomaternal hemorrhage, twin-twin transfusion, and cystic hygroma. Whereas erythroblastosis will be a feature of any form of hydrops in which marrow RBC production is not compromised, the morphologic changes of the RBCs in Hb Bart's hydrops fetalis will serve to distinguish it from other causes of fetal anemia. Hb studies will be diagnostic. Hb H disease may potentially be confused with other forms of moderate microcytic anemia. Because of the hypochromia, IDA might be considered; however, target cells are much more prominent in Hb H disease, and reticulocytosis is not a feature of untreated iron deficiency. Because of the prominence of target cells, Hb CC disease may also be a consideration. However, hypochromia is not a prominent feature of Hb CC disease, and Hb C crystals will be evident. Hb studies demonstrating a fast moving Hb H band will be diagnostic.

The clinical differential diagnosis of the severe β-thalassemias includes other forms of moderate to severe chronic hemolytic anemia. However, the bizarre morphologic changes in the RBCs and the degree of erythroblastosis in severe β-thalassemia are essentially diagnostic. Hb analysis, which will show large amounts of Hb F without other abnormal Hb species, will confirm the diagnosis. From the electrophoretic standpoint, severe β-thalassemia may be confused with some forms of hereditary persistence of fetal Hb. However, patients with hereditary persistence of fetal Hb either have normal Hb levels or polycythemia, permitting ready distinction.

The differential diagnosis of β-thalassemia minor and two-gene deletion α-thalassemia are IDA and ACD (see Table 1-3). Elevated Hb $A_2$ levels and normal iron studies will confirm a diagnosis of β-thalassemia minor. However, as noted earlier, Hb $A_2$ levels may be in the normal range in patients with concomitant β-thalassemia minor and iron deficiency. Two-gene deletion α-thalassemia is largely a diagnosis of exclusion in adults, although the presence of small amounts of Hb Bart's may be detected in newborns. If necessary, molecular analysis of the α-globin genes can be obtained to confirm the diagnosis of α-thalassemia (see Chapter 24).

### Prognosis and Therapy

Without therapy, fetuses with Hb Bart's hydrops fetalis are stillborn between 30 and 40 weeks' gestation or die shortly after birth. Fetuses can occasionally be rescued with intrauterine transfusion, but subsequently must receive lifelong transfusion and chelation therapy. Patients with β-thalassemia die in childhood if untreated. Without adequate transfusion, but not chelation, patients die of cardiac disease because of iron overload at a median age of 17 years. With aggressive chelation, however, patients have a median survival of 31 years. Allogeneic stem cell transplantation is a consideration in patients with an available donor and is potentially curative. The prognosis after transplantation depends in part on the amount of end organ damage incurred prior to transplantation.

## MEMBRANE DISORDERS

### Hereditary Spherocytosis

Hereditary spherocytosis (HS) is the most common inherited hemolytic anemia, with a frequency of 1 in 5000 in the United States and 1 in 2000 in individuals of Northern European ancestry. It is a genetically and clinically heterogeneous disorder of the RBC membrane characterized by defective vertical interactions between the RBC membrane and the underlying cytoskeleton, resulting in destabilized membrane with a tendency toward vesiculation and loss of RBC membrane. Progressive loss of membrane results in rigid spherocytic cells that show delayed egress from the splenic cords. Prolonged exposure to the splenic cordal environment causes further membrane damage, and ultimately these cells are prematurely removed from circulation by the spleen. In three fourths of cases, HS is inherited in an autosomal dominant manner. Of the remaining cases, approximately half are autosomal recessive and half are new mutations.

**HEREDITARY SPHEROCYTOSIS–FACT SHEET**

**Definition**
- Disorder caused by defective vertical interactions between the RBC membrane and cytoskeleton, resulting in vesiculation and loss of membrane, spherocyte formation, and hemolysis

**Incidence and Location**
- One in 5000 in the United States

**Morbidity and Mortality**
- Mild morbidity owing to complications of chronic hemolysis
- Aplastic crises owing to parvovirus B19
- Megaloblastic anemia
- Slightly increased mortality, usually a result of postsplenectomy sepsis

**Gender, Race, or Age Distribution**
- Equal gender incidence
- More common in patients of Northern European descent (1 in 2000)

**Clinical Features**
- Variably severe hemolytic anemia
- Jaundice
- Splenomegaly

**Prognosis and Therapy**
- Supportive therapy
- Splenectomy for more severe variants

### Clinical Features

HS ranges from an asymptomatic disorder to a severe, lifelong hemolytic anemia. Approximately one fourth of patients have mild, completely compensated hemolysis with no anemia. Most of the remainder have only mild to moderate anemia, with Hb levels ranging from 8 to 12 g/dL. Only a small minority (5% to 10%) have more severe anemia. In symptomatic forms, anemia is typically absent at birth but develops over several weeks. A common presentation of HS is unexplained neonatal hyperbilirubinemia, which appears in the first 2 days of life and may then increase rapidly. Splenomegaly is common in patients with HS, seen in 50% of infants and 75% to 95% of older children and adults. Jaundice is seen in half of patients. Other complications include gallstones, megaloblastic anemia, and aplastic crisis owing to parvovirus B19 infection. The latter is a catastrophic, potentially fatal complication.

### Pathologic Features

The blood smear in patients with HS shows variable spherocytosis and polychromasia (Figure 1-29).

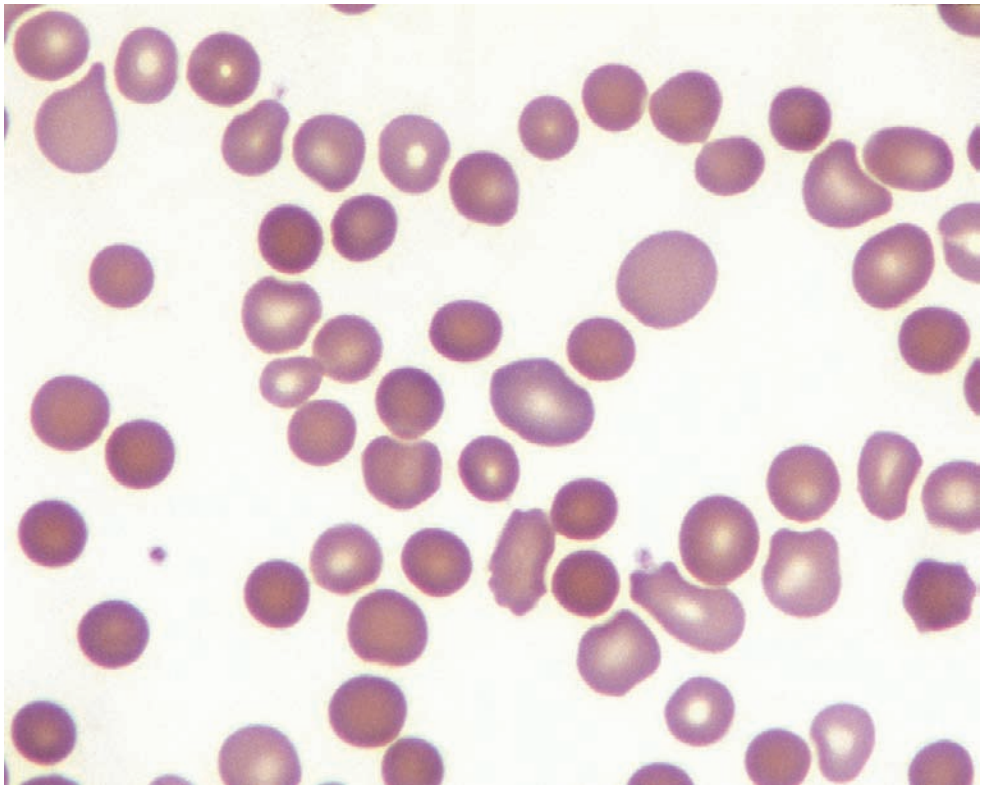

**FIGURE 1-29**

Hereditary spherocytosis (HS), peripheral blood findings. This blood smear from a patient with HS shows spherocytes and polychromasia. Spherocytes are round cells with a dense staining quality, lack of central pallor, and reduced diameter compared to other red cells. Polychromatophilic cells represent young reticulocytes and are larger than mature red cells, have a slightly bluish staining quality, and may lack central pallor.

**HEREDITARY SPHEROCYTOSIS–PATHOLOGIC FEATURES**

**Microscopic Findings**

- Spherocytes
- Polychromasia

**Differential Diagnosis**

- Spherocytic immune hemolysis

Spherocytes are round RBCs that are smaller in diameter than normal RBCs, lack central pallor, and have a denser (hyperchromic) staining quality. They vary in number considerably, depending on the severity of the disease. In the mildest forms of HS, spherocytes may be rare or absent. Postsplenectomy, spherocytes are still present, in addition to postsplenectomy RBC changes such as target cells, Howell-Jolly bodies, and Pappenheimer bodies. Therapeutic splenectomy is sometimes performed in patients with HS. Histologic examination of the spleen typically demonstrates congestion of the splenic cords because of entrapped spherocytes as opposed to the typical sinusoidal pattern in passive congestion.

## Ancillary Studies

### Osmotic Fragility Test

Because of their decreased surface area to volume ratio, spherocytes are more susceptible to osmotic lysis in hypotonic media than normal RBCs. This feature is the basis of the osmotic fragility test, in which RBCs are suspended in hypotonic saline of varying concentrations. The amount of hemolysis at each concentration is measured, and these values are plotted on a graph and compared with a normal control. A shift of the osmotic fragility curve toward higher concentrations of saline, either immediately or after 24 hours of incubation at 37° C, constitutes a positive result.

### Other Studies

Although not widely available, analysis of membrane protein composition and molecular analysis of cytoskeletal protein genes may be useful to specifically characterize the defect in individual cases of HS. Flow cytometric analysis of red cells using the dye eosin-5'-maleimide, which binds specifically to the anion transport protein (band-3) at lysine-430, has proven useful in screening for red cell membrane disorders with

decreased fluorescence seen in cases of membrane disorders such as HS, hereditary elliptocytosis, and pyropoikilocytosis.

## Differential Diagnosis

The major differential diagnosis of HS is spherocytic immune hemolytic anemia, because of either alloantibodies or warm autoantibodies. The blood smear findings will be essentially identical in these disorders. Furthermore, the osmotic fragility test will also be positive in spherocytic immune hemolysis. In addition to the fact that HS is a lifelong condition, demonstration of RBC antibodies, typically with a direct antiglobulin test, will generally easily distinguish between these disorders. Evaluating family members of the patient can also be helpful in difficult cases because, in the majority of cases there will be affected first-degree relatives.

Spherocytes are also seen in small numbers in most other forms of hemolytic anemia. However, in these disorders, they are not the dominant abnormal RBC form and are generally seen in small numbers.

## Prognosis and Therapy

HS in most cases requires no specific therapy other than treatment for complications, such as gallstones. Patients with more severe symptomatic disease may be considered for splenectomy, which corrects the anemia in most cases. Splenectomized patients are at risk of postsplenectomy sepsis, which can be fatal.

## Hereditary Elliptocytosis and Hereditary Pyropoikilocytosis

Hereditary elliptocytosis (HE) and hereditary pyropoikilocytosis (HPP) are related disorders caused by defects in the horizontal interactions between membrane cytoskeletal proteins, specifically the association of spectrin heterodimers into heterotetramers. Like HS, these are genetically and clinically heterogeneous. HE results from a heterozygous gene defect, whereas HPP results from homozygosity for a particular HE mutation, double heterozygosity for two different HE mutations, or double heterozygosity for an HE mutation and a low-expression α-spectrin allele. HE is usually inherited in an autosomal-dominant fashion. Patients with HPP either have two parents with HE or one parent with HE and one without apparent hematologic abnormalities (carriers of the low-expression α-spectrin allele). HE is common, with an incidence in the United States of 1 in 2000 to 4000, and it is more common in individuals of African or Mediterranean heritage. HPP is rare.

## Clinical Features

The most common form of HE is a very mild, completely compensated hemolysis that does not produce anemia and is completely asymptomatic; therefore HE is most commonly discovered incidentally. More severe forms of HE occur in which the hemolysis is incompletely compensated, with Hb levels in the range of 9 to 12 g/dL. These patients commonly have jaundice, splenomegaly, and gallstones. Patients with HPP have Hb levels in the range of 4 to 8 g/dL and experience clinical complications similar to those with more severe HE. In addition, patients with the most severe forms of HPP may have clinical manifestations similar to those seen in β-thalassemia major, such as growth retardation and frontal bossing.

**HEREDITARY ELLIPTOCYTOSIS AND HEREDITARY PYROPOIKILOCYTOSIS–FACT SHEET**

**Definition**
- A group of disorders caused by defective horizontal interactions between spectrin heterodimers in the red blood cell cytoskeleton

**Incidence and Location**
- One in 2000 to 4000 in the United States

**Morbidity and Mortality**
- Most asymptomatic patients with hereditary elliptocytosis (HE) have no significant morbidity or mortality
- Complications of chronic hemolysis in severe HE and hereditary pyropoikilocytosis (HPP)

**Gender, Race, or Age Distribution**
- Equal gender incidence
- HE more common in patients of African or Mediterranean descent
- Most HPP cases seen in black patients

**Clinical Features**
- Common HE
  - None
- Severe HE and HPP
  - Moderate to severe hemolytic anemia
  - Jaundice
  - Splenomegaly
  - Gallstones

**Prognosis and Therapy**
- No therapy required in most patients with HE
- Supportive therapy
- Splenectomy for HPP and severe HE

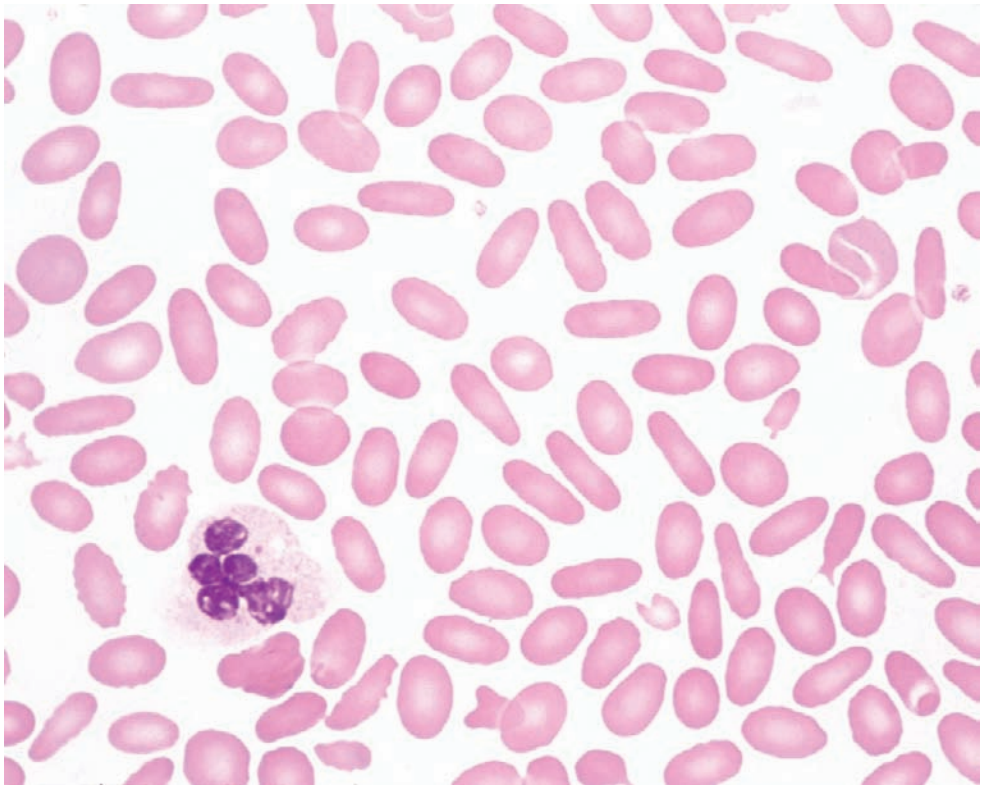

**FIGURE 1-30**

Hereditary elliptocytosis (HE), peripheral blood findings. Patients with HE have 25% to 100% elliptocytes-elongate cells with rounded ends and nearly parallel sides. A few fragments are also present in this case.

## Pathologic Features

Blood smears in patients with HE demonstrate 25% to nearly 100% elliptocytes (Figure 1-30). These cells are rod-shaped with nearly parallel sides and rounded ends. In the common form of HE, these cells are almost the only poikilocyte present. In more severe forms, there is usually a subpopulation of fragments and bizarre poikilocytes. In addition, some newborn infants with mild common HE display prominent RBC fragmentation with moderate hemolysis, closely mimicking HPP; however, this resolves over time.

HPP is characterized by striking RBC fragmentation and bizarre poikilocytosis, usually, but not always, in a background of elliptocytosis (Figure 1-31). Because of the extreme RBC fragmentation, the MCV in HPP may be as low as 25 fL.

## Ancillary Studies

RBCs from patients with HPP and some with HE demonstrate abnormal sensitivity to heat-induced fragmentation. Normal RBCs fragment after 10 minutes at 49° C, whereas HPP RBCs fragment between 44° C and 46° C, and some HE RBCs between 47° C and 48° C.

**HEREDITARY ELLIPTOCYTOSIS AND HEREDITARY PYROPOIKILOCYTOSIS–PATHOLOGIC FEATURES**

**Microscopic Findings**

- Hereditary elliptocytosis (HE)
  - 25% to 100% elliptocytes
  - Variable polychromasia
  - Red blood cell (RBC) fragmentation in more severe forms
- Hereditary Pyropoikilocytosis (HPP)
  - Severe RBC fragmentation
  - Bizarre poikilocytosis
  - Low mean corpuscular volume
  - Variable elliptocytosis

**Differential Diagnosis**

- HE
  - Disorders with increased elliptocytes, including iron deficiency, megaloblastic anemia, myelofibrosis, myelodysplasia, and thalassemia
- HPP
  - Severe burns
  - Clostridial hemolysis
  - Accidental heating of specimen

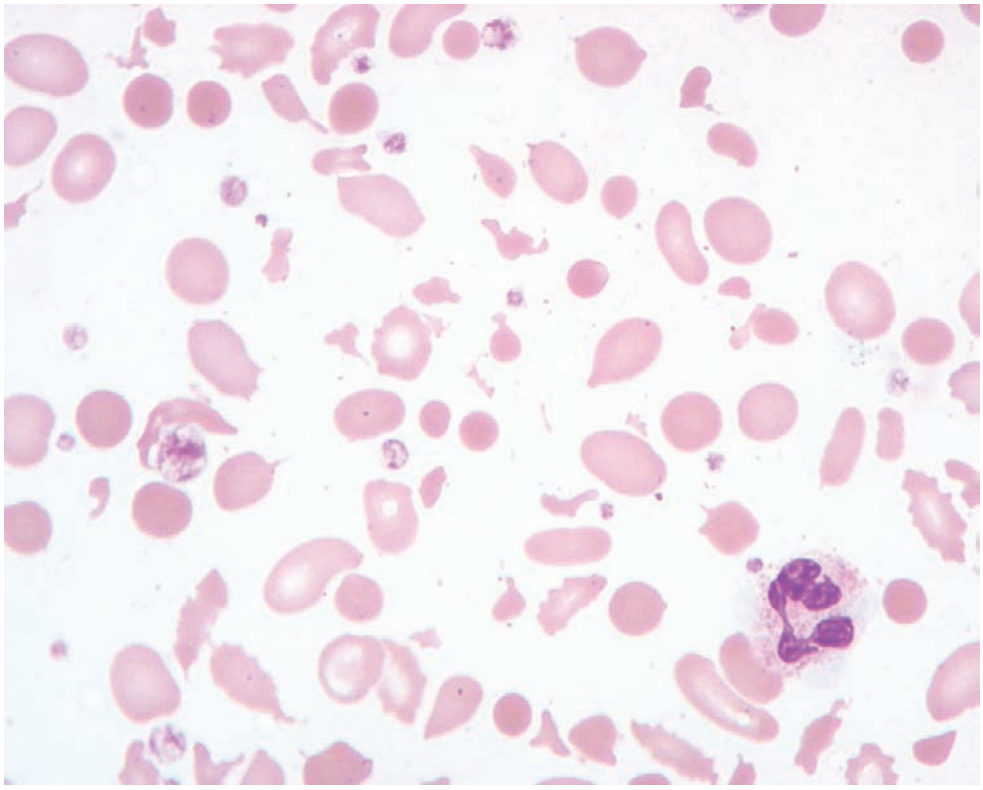

**FIGURE 1-31**

Hereditary pyropoikilocytosis, peripheral blood findings. In this case there are fragmented red cells of varying shape and size, microspherocytes, elliptocytes, teardrop cells, and other bizarre poikilocytes.

### Differential Diagnosis

A variety of disorders may be accompanied by increased elliptocytes, such as iron deficiency, megaloblastic anemia, myelofibrosis, myelodysplasia, and thalassemia. However, the number of elliptocytes is usually less than 25% and should not exceed 35%. Other features of these disorders will usually allow their distinction from HE.

The differential diagnosis of HPP includes severe burns, hemolysis resulting from *Clostridium perfringens* sepsis, and artifactual RBC fragmentation resulting from accidental heating of the blood tube. These disorders are usually easily distinguished clinically from HPP.

### Prognosis and Therapy

Most patients with HE require no specific therapy. Other than supportive care, splenectomy is the main therapy for HPP and more severe forms of HE, and it generally results in an improvement in Hb levels and a decrease in clinical symptoms, although the anemia is not typically completely eliminated.

## ENZYME DISORDERS

Although deficiencies of a number of RBC enzymes in the glycolytic and hexose monophosphate pathways may result in hemolytic anemia, the only one that is sufficiently common to warrant discussion here is glucose-6-phosphate dehydrogenase (G6PD) deficiency. The interested reader may refer to the references listed for other, rarer enzyme disorders.

### Glucose-6-Phosphate Dehydrogenase Deficiency

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common RBC metabolic disorder, with 400 million people affected worldwide. Because the gene for G6PD is located on the X chromosome, clinical disease is largely limited to males. However, heterozygous females may manifest clinical disease, particularly in cases of extreme lyonization. More than 160 mutant G6PD alleles have been characterized. Commonly affected populations include Africans, Greeks, Sardinians, Sephardic and Kurdish Jews, and Southeast Asians. As many as 11% of African American males in the United States are affected.

G6PD is an enzyme in the hexose monophosphate pathway, a biochemical pathway for metabolizing glucose that bypasses the glycolytic pathway. The hexose

monophosphate shunt is necessary for the generation of NADPH, which in turn is required to regenerate reduced glutathione from its oxidized form. Decreased reduced glutathione levels render RBCs more susceptible to oxidant stress. The severity of the deficiency of G6PD correlates well with the severity of the clinical phenotype in this disorder.

## Clinical Features

The most commonly encountered form of G6PD deficiency in the United States is the A-variant seen in blacks. In this form of the disease, there is no hemolysis under normal physiologic conditions. However, 2 to 4 days after exposure to an oxidant stress—most commonly in the form of drugs, toxins, and infections—there is an acute, primarily intravascular hemolysis manifested clinically as jaundice, pallor, dark urine, and sometimes abdominal or back pain. In patients with G6PDA–, there is a drop in Hb (3 to 4 g/dL) that self-terminates within 1 week, even without removal of the offending agent. The self-limited nature of the hemolytic episode results because the young RBCs in patients with G6PDA– have sufficient amounts of G6PD to withstand oxidant stress. More severe variants of G6PD deficiency, such as the Mediterranean and Mahidol variants, can manifest more severe drops in Hb and also may be sensitive to less severe oxidant stresses. For example, patients with G6PD Mediterranean variant may have acute hemolytic episodes in response to fava bean ingestion (i.e., favism). Rare G6PD variants manifest as chronic, ongoing hemolysis, even in the absence of oxidant stress.

**GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY–FACT SHEET**

**Definition**
- An inherited RBC enzyme deficiency resulting in episodic hemolysis in response to oxidant stress

**Incidence and Location**
- Highly variable geographically

**Morbidity and Mortality**
- Clinical effects related to acute anemia
- Fatalities rare

**Gender, Race, and Age Distribution**
- Men most commonly affected; female heterozygotes generally clinically unaffected
- Prevalent in blacks, Greeks, Sardinians, Sephardic and Kurdish Jews, and Southeast Asians

**Clinical Features**
- Acute hemolytic anemia in response to oxidant drugs, toxins, infections, and fava beans

**Prognosis and Therapy**
- Transfusion support if necessary
- Avoidance of oxidant drugs

## Pathologic Features

The characteristic poikilocyte seen in G6PD deficiency, and in other uncommon forms of oxidant-induced hemolysis, is the bite cell. Bite cells are characterized by peripheral defects in the RBC contour that resemble bites. These defects can be smoothly contoured or somewhat irregular, small or large, single or multiple (Figures 1-32 and 1-33). A characteristic form seen in oxidant hemolysis is the "apple core" cell, in which two large bites are present on opposite sides of the cell (see Figure 1-33). Bite cells are often slightly dense and appear contracted relative to normal RBCs. A second poikilocyte related to the bite cell seen in some cases of oxidant hemolysis, sometimes representing the predominant form, is the eccentrocyte (Figure 1-34). This cell is similar to the blister cells seen in SCD, with Hb pushed to one side and a residual thin blister. However, in contrast to the smooth contour seen in blister cells (see Figure 1-19), the hemoglobin mass in eccentrocytes typically has an irregular edge adjacent to the vacuole. Finally, small numbers of spherocytes are usually seen as well. Because of the acute nature of the hemolysis in G6PD deficiency, little polychromasia is expected because it takes several days before the development of a reticulocyte response to the anemia.

## Ancillary Studies

An easily performed, reliable fluorescent screening ("spot") test for G6PD deficiency is available in many clinical laboratories. However, this test may give a

**GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY–PATHOLOGIC FEATURES**

**Microscopic Findings**
- Bite cells, eccentrocytes, or both
- Scattered spherocytes
- Little polychromasia during acute episode

**Differential Diagnosis**
- Other causes of oxidant hemolysis
- Microangiopathic hemolytic anemia

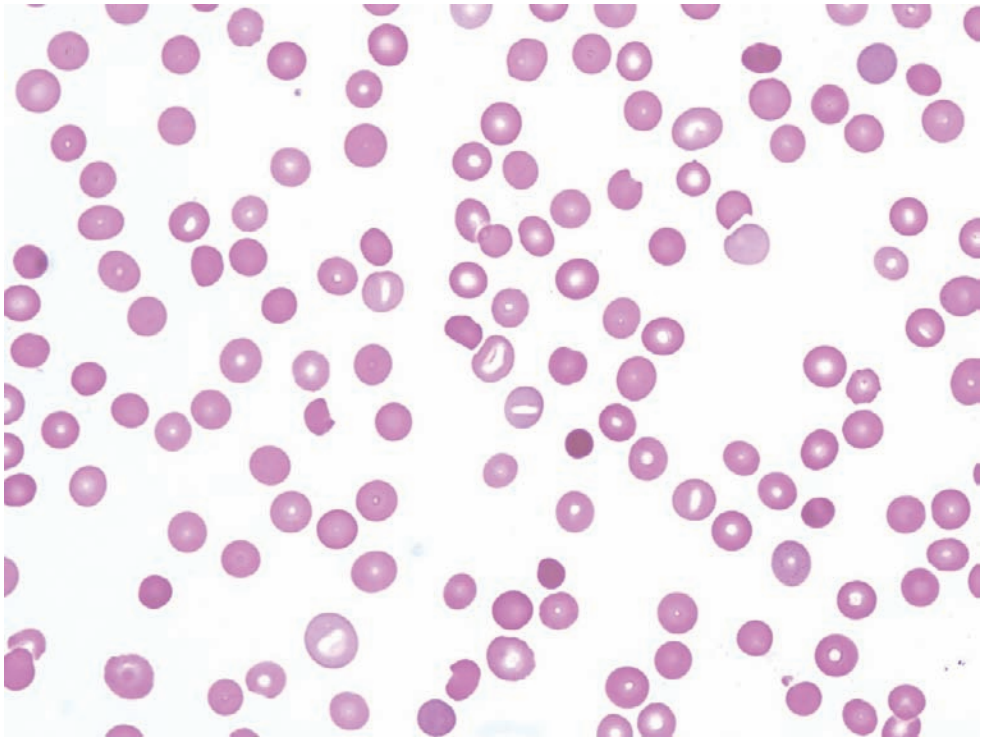

**FIGURE 1-32**

Oxidant hemolysis, peripheral blood findings. A case of oxidant hemolysis due to glucose-6-phosphate dehydrogenase deficiency with multiple bite cells. These cells have variably sized, peripheral "bitelike" defects. Note also the presence of occasional spherocytes.

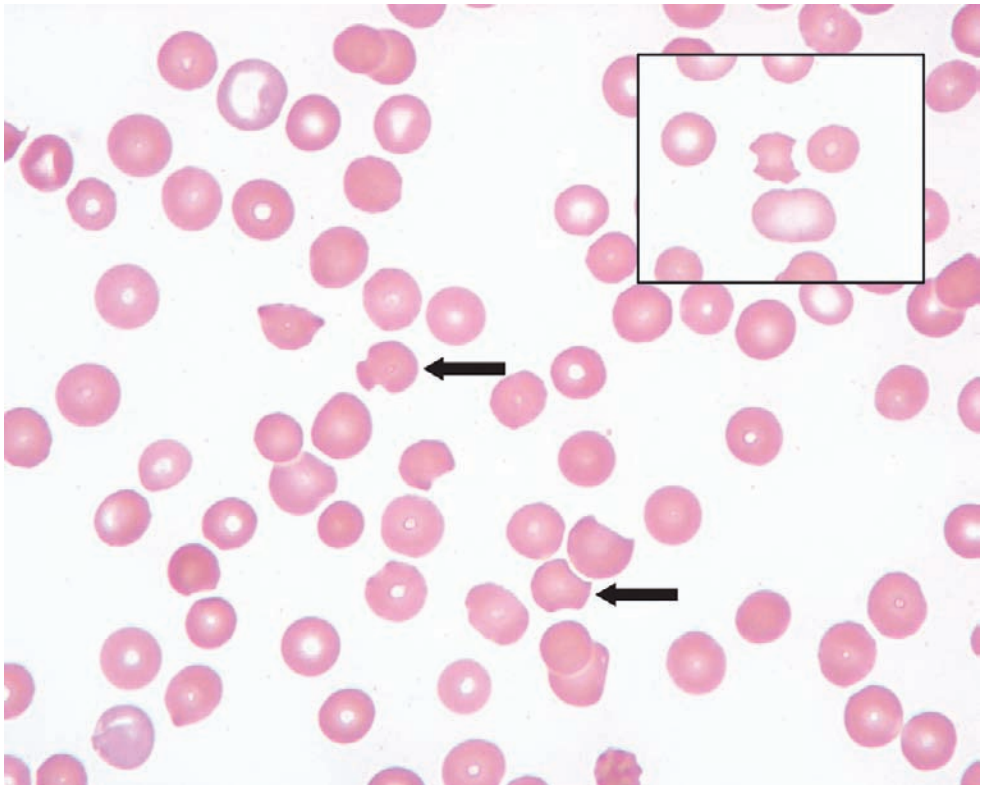

**FIGURE 1-33**

Oxidant hemolysis, peripheral blood findings. This field, from the same patient as in Figure 1-32, reveals two "double-bite" cells *(arrows)*. An "apple core" cell is illustrated in the *inset*.

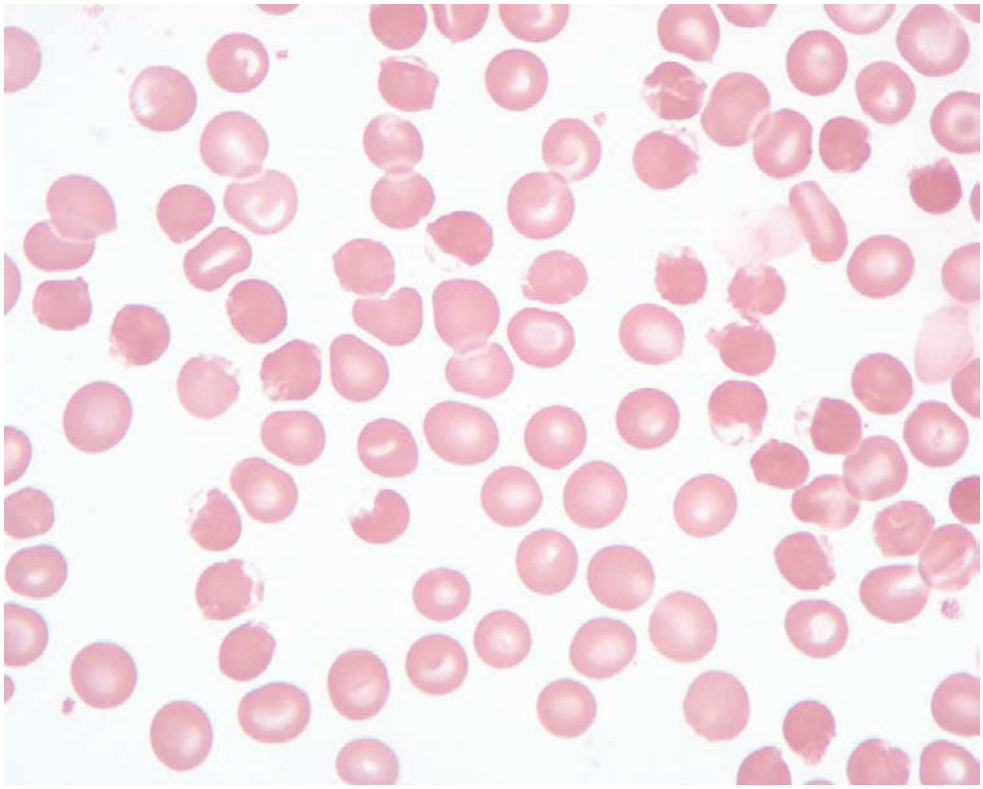

**FIGURE 1-34**

Oxidant hemolysis, peripheral blood findings. Multiple eccentrocytes in a patient with severe oxidant hemolysis owing to glucose-6-phosphate dehydrogenase deficiency. These cells resemble the blister cells of sickle cell disease, with a peripheral defect covered by a thin residual membrane.

false-negative result during or soon after the acute hemolytic episode because the older, more deficient RBCs are hemolyzed and what remains are younger cells with higher levels of G6PD. Ideally, the test should be performed 2 to 3 months after resolution of the acute hemolytic episode to avoid this problem. Alternatively, a quantitative, spectrophotometric assay for G6PD can be performed, even during the acute episode, but this test is generally available only in specialized laboratories.

The oxidant damage in G6PD deficiency causes denaturation of Hb within the RBCs to form Heinz bodies. These inclusions are usually not visible on Wright-stained preparations, but can be visualized in some cases of oxidant hemolysis as dark blue, often peripheral masses on staining with supravital stains such as methyl violet.

## Differential Diagnosis

Oxidant hemolysis resulting from other causes can produce the same morphologic features as in G6PD deficiency. These features include unstable Hbs, other RBC enzyme deficiencies, and severe oxidant stress resulting from exposure to oxidant chemicals or drugs. For example, dapsone, an antibiotic commonly used for prophylaxis against *Pneumocystis jirovecii* pneumonia frequently causes mild oxidant hemolysis, even in the absence of G6PD deficiency. Correlation with clinical history and laboratory results should serve to distinguish these causes.

Microangiopathic hemolytic anemias can also be confused with oxidant hemolysis because of similarities between bite cells and RBC fragments (Figure 1-35). In fact, on a cell-by-cell basis, bite cells can be impossible to distinguish from helmet cells. However, careful attention to the spectrum of poikilocytes present will usually be helpful in this discrimination. Specifically, the finding of double bite cells and cells with very small defects (i.e., "nibble cells") is much more indicative of oxidant hemolysis. However, helmet cells in microangiopathic states will be accompanied by other types of fragments (e.g., triangular fragments and schistocytes). Finally, thrombocytopenia is not a feature of oxidant hemolysis, but will be present in common forms of microangiopathic hemolytic anemia.

## Prognosis and Therapy

The therapy for acute hemolysis resulting from G6PD deficiency is withdrawal of the offending agent or treatment of the precipitating condition. Some patients may

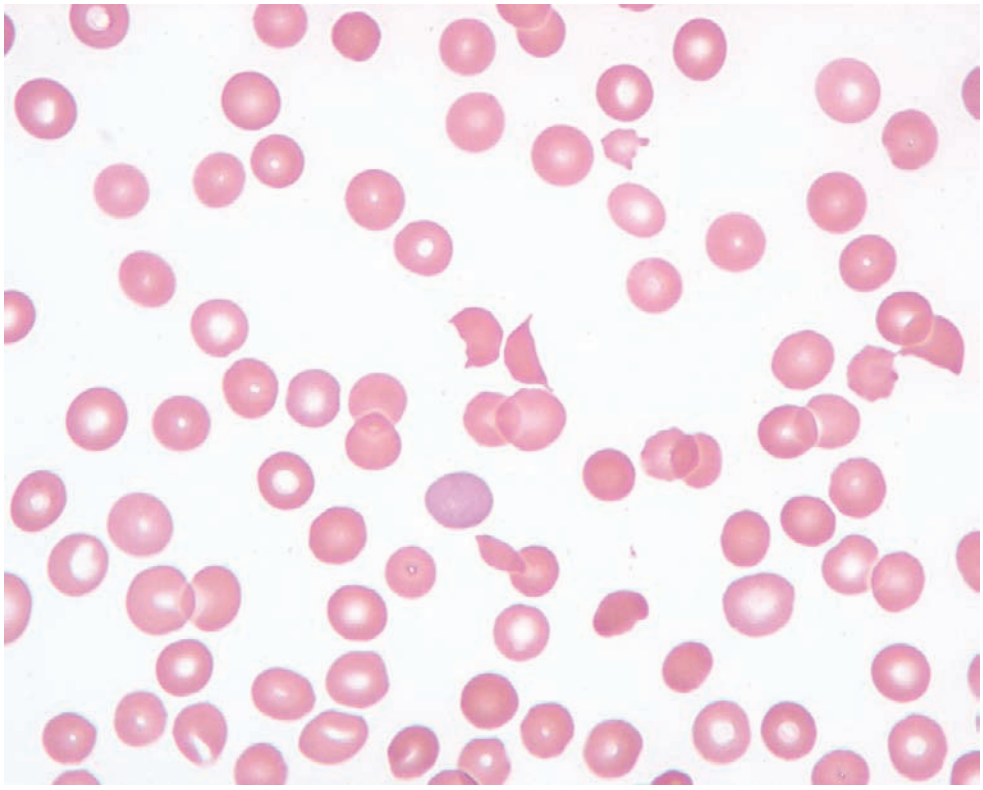

**FIGURE 1-35**

Oxidant hemolysis, peripheral blood findings. Two cells resembling helmet cells *(center)* in a patient with oxidant hemolysis owing to glucose-6-phosphate dehydrogenase deficiency.

require transfusion support. Drugs known to cause oxidant hemolysis should be avoided. Patients with more severe deficiency variants (e.g., the Mediterranean variant) are at higher risk of severe anemia requiring therapy.

## EXTRINSIC DEFECTS

### AUTOIMMUNE HEMOLYTIC ANEMIA

Autoimmune hemolytic anemia (AIHA) can be divided into warm and cold forms. These two forms differ in their pathophysiology and in their clinical and pathologic features. Warm AIHA is usually caused by immunoglobulin (Ig) G antibodies that are most active at 37° C and may or may not bind complement. These antibodies sensitize RBCs, which are then removed from circulation by the spleen. Cold AIHA is usually caused by IgM antibodies that have maximal affinity at lower temperatures. They bind complement and lead to either intravascular RBC destruction or removal of red cells in the liver.

#### Clinical Features

Warm AIHA has an incidence of approximately 10 per 1 million. It occurs at any age and affects women twice as often as men. Warm AIHA can be primary (idiopathic) or associated with a wide variety of underlying conditions, including autoimmune disorders, immunodeficiency syndromes, lymphoproliferative disorders, other malignancies, and certain drugs. The hemolysis in this disorder ranges from a compensated, subclinical increase in RBC destruction to severe hemolysis. The onset may be insidious or acute. Symptoms are those generically associated with anemia, including weakness, dizziness, and dyspnea. The severity of symptoms correlates with the severity of the anemia. Other symptoms may be seen because of associated underlying disorders. The majority of patients with warm AIHA have splenomegaly and approximately have hepatomegaly. Jaundice may be seen in more severe cases.

Cold AIHA, also known as *cold agglutinin disease* (CAD), is less than half as common as warm AIHA. As with warm AIHA, CAD can be primary or secondary. Primary CAD is a chronic disorder generally seen in older patients, with a female predominance. Secondary CAD can be associated with B cell lymphoproliferative disorders, such as Waldenström macroglobulinemia or chronic lymphocytic leukemia. When associated with B cell neoplasms, the pathologic IgM antibody is a product of the monoclonal B cells. CAD can also be associated with nonlymphoid malignancies, but this is relatively rare. Another important cause of CAD, seen most often in younger patients, is infection. The most common agents associated with infectious CAD are *Mycoplasma pneumoniae* infection and infectious mononucleosis

### AUTOIMMUNE HEMOLYTIC ANEMIA–FACT SHEET

**Definition**
- Hemolytic anemia caused by abnormal production of antibodies against red blood cell antigens

**Incidence and Location**
- Warm autoimmune hemolytic anemia (AIHA): 10 per 1,000,000
- Cold agglutinin disease (CAD): approximately 4 per 1,000,000

**Morbidity and Mortality**
- Warm AIHA
  - Complications of chronic hemolysis
  - Life-threatening anemia in a small number of cases
- CAD
  - Generally mild with no major morbidity

**Gender, Race, or Age Distribution**
- Warm AIHA: twice as common in women as in men
- CAD: women affected more often than men

**Clinical Features**
- Warm AIHA
  - Possible antecedent viral infection
  - Underlying lymphoproliferative disorder in some cases
  - Chronic hemolysis of variable severity
  - Jaundice and splenomegaly in more severe cases
- CAD
  - Mycoplasma or Epstein-Barr virus infection may be precipitating cause
  - Underlying lymphoproliferative disorder in some cases
  - Mild hemolysis with exacerbations upon exposure to cold
  - Acrocyanosis

**Prognosis and Therapy**
- Warm AIHA
  - Steroids
  - Cytotoxic agents
  - Splenectomy in severe cases
- CAD
  - No treatment required in most cases
  - Cytotoxic agents or rituximab in more severe cases

caused by Epstein-Barr virus. Hemolysis resulting from chronic CAD is usually mild, with exacerbations upon cold exposure. However, rare cases are associated with severe ongoing hemolysis. Acrocyanosis upon exposure to cold may be seen because of agglutination of RBCs in relatively cool extremities. Only a minority of patients have organomegaly. Acute CAD caused by infection usually resolves within several weeks.

## Pathologic Features

The blood smear in warm AIHA demonstrates variable spherocytosis and polychromasia (Figure 1-36). Agglutination is only rarely seen, because of the inability of most IgGs to effectively cross-link RBCs. Occasionally there will be erythrophagocytosis by monocytes.

CAD is characterized by RBC agglutination in unwarmed (and occasionally warmed) blood smears (Figure 1-37). Agglutination of RBCs is visible as clumps of several to many RBCs. Spherocytosis is usually not a feature of CAD.

### AUTOIMMUNE HEMOLYTIC ANEMIA–PATHOLOGIC FEATURES

**Microscopic Findings**
- Warm autoimmune hemolytic anemia (AIHA)
  - Spherocytes
  - Polychromasia
  - Rare erythrophagocytosis
- Cold agglutinin disease (CAD)
  - Red blood cell agglutination (clumping)
  - Polychromasia
  - Lack of spherocytosis

**Differential Diagnosis**
- Warm AIHA
  - Hereditary spherocytosis
- CAD
  - Rouleaux
  - Warm AIHA

## Ancillary Studies

The most important ancillary study in the evaluation of AIHA is the direct antiglobulin test, which is positive with polyspecific sera (a mixture of anti-IgG and anti-complement) in the vast majority of patients with both warm AIHA and CAD. Patients with warm AIHA will have IgG, complement, or both detectable on the surface of RBCs, whereas patients with CAD will have complement detectable on the RBC surface but not IgM, which has dissociated from the cells.

## Differential Diagnosis

The differential diagnosis of warm AIHA is largely restricted to HS, and these two disorders are not distinguishable morphologically. However, a direct antiglobulin test will discriminate in the vast majority of cases. Clinical and family histories may also be helpful in the distinction of these disorders.

The agglutination of CAD is sometimes confused with rouleaux, a phenomenon associated with increased levels of plasma proteins. However, agglutination differs from rouleaux in that the RBCs are arranged in clumps rather than the single-file, "stack of coins"

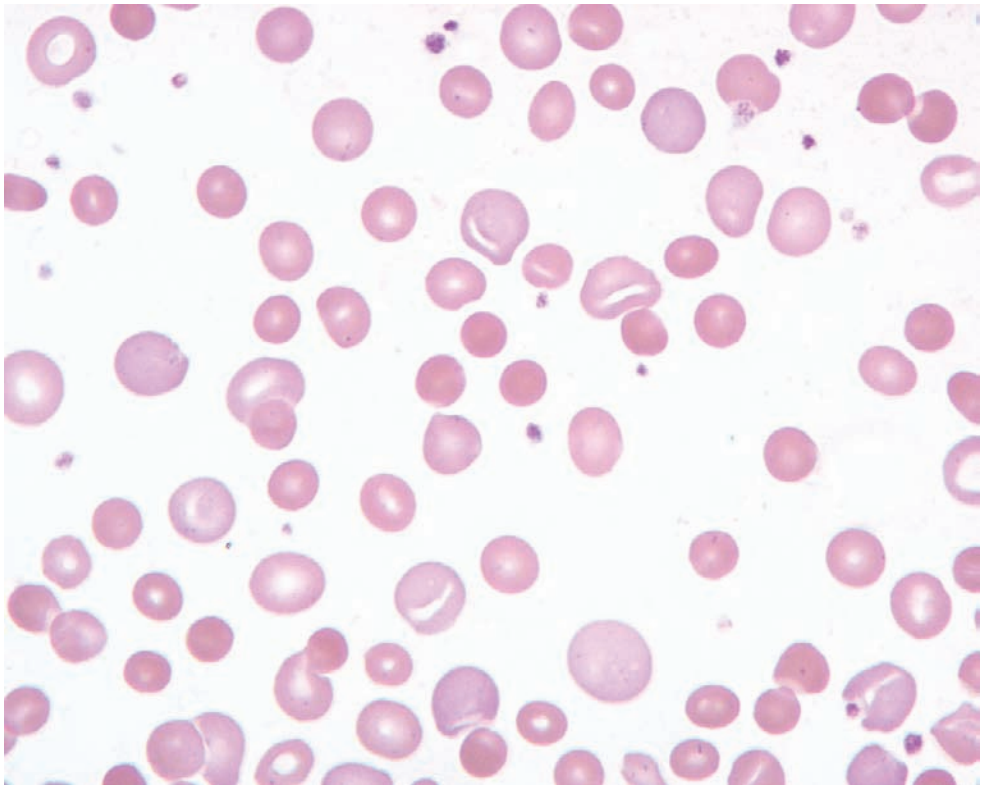

**FIGURE 1-36**

Warm autoimmune hemolytic anemia (AIHA), peripheral blood findings. In this patient with severe warm AIHA, there are prominent spherocytes and polychromatophilic cells. Compare with hereditary spherocytosis, as seen in Figure 1-29.

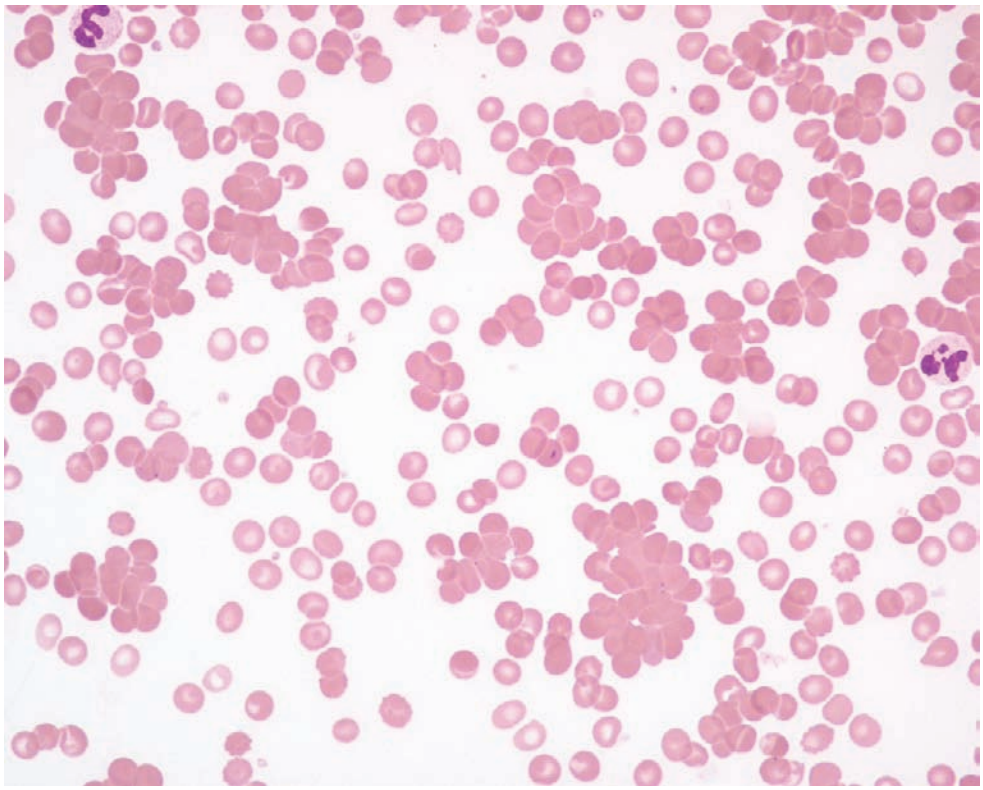

**FIGURE 1-37**

Cold agglutinin disease, peripheral blood findings. In this patient with cold agglutinin disease, there are prominent clumps of red blood cells. These differ from the linear, "stack of coins" formations that characterize rouleaux.

arrangement that is characteristic of rouleaux. Occasionally warm AIHA produces agglutination; the direct antiglobulin test results should distinguish this phenomenon from CAD.

### Prognosis and Therapy

The therapy for patients with warm AIHA depends on the severity of the hemolysis. In patients with completely compensated hemolysis, no therapy is required other than folate supplementation to prevent the development of megaloblastic anemia. For more severe cases, steroids are the mainstay of therapy. In steroid-refractory cases, more potent immunosuppression may be effective. Patients with severe, refractory disease may benefit from splenectomy. Severe warm AIHA can be life threatening and require aggressive therapy.

In general, CAD is clinically mild and requires no specific therapy other than avoiding the cold as much as possible. In more severe cases, immunosuppressive therapy with cytotoxic agents such as chlorambucil or cyclophosphamide may be helpful. Rituximab (anti-CD20) therapy has shown promise in patients with refractory CAD. If necessary, packed red cell transfusions should be administered with in-line blood warmers, if available. Steroids and splenectomy have little role in CAD.

## MICROANGIOPATHIC HEMOLYTIC ANEMIA

Microangiopathic hemolytic anemias (MAHAs) are characterized by mechanical disruption and fragmentation of RBCs because of occlusion of the microvasculature. The most well-known forms of MAHA are thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), and disseminated intravascular coagulation (DIC). However, MAHA may also be seen in association with other disorders affecting the microvasculature, such as malignant hypertension, preeclampsia–hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome, widely metastatic carcinoma, and autoimmune disorders. Certain drugs are also known to cause MAHA.

Although the precise classification and diagnostic criteria for TTP and HUS remain somewhat controversial, recent investigation has dramatically advanced our understanding of these disorders. For practical purposes, there are three distinct mechanisms that account for most cases of these two overlapping clinical syndromes. The large majority of cases satisfying traditional criteria for TTP are known to be associated with either acquired (in most cases) or inherited (rare) deficiencies in ADAMTS13, a metalloprotease responsible for cleaving von Willebrand factor when it is unfolded by shear stress within blood vessels, thus preventing excess platelet aggregation. When deficient, platelet thrombi form in small blood vessels, with resultant mechanical shearing of red cells during passage. The acquired form of this deficiency is due to inhibitory autoantibodies directed at ADAMTS13. The second major group of disorders, corresponding largely to classical childhood HUS, are associated with gastrointestinal infection with Shiga toxin–producing organisms, most often the O157:H7 serotype of *Escherichia coli*. It is believed that these toxins produce direct endothelial damage with resultant small vessel thrombosis. The third group, termed *atypical HUS,* is not associated with enteric infections, and has been associated in up to half of cases with mutations in genes for one of several proteins regulating complement activation. Additional details on MAHA can also be found in the chapter on platelet disorders.

### Clinical Features

The clinical features of MAHA depend on the associated disorder. Classical TTP associated with ADAMTS13 deficiency is a disorder of adolescents and adults with a female predominance and an incidence of 3.7 per 1 million per year. It is characterized by acute or insidious onset of hemolytic anemia, thrombocytopenic purpura, neurologic symptoms, variable (usually not severe) renal dysfunction, and fever. Abdominal pain, elevated pancreatic enzymes, and cardiac conduction defects may be seen as well. Shiga toxin–associated HUS has an incidence rate of about 0.7 per 1 million. It is predominantly a disease of children, but it shows overlapping clinical features with TTP. After a prodrome of bloody diarrhea, patients develop signs of intravascular hemolysis, variable thrombocytopenia, and renal failure, sometimes in an acute and dramatic fashion. In general, HUS is characterized by more severe renal impairment, whereas TTP has more prominent neurologic manifestations. Atypical HUS is characterized by acute renal failure and MAHA, but lacks a diarrheal prodrome or other identifiable cause. DIC is a final common pathway of systemic activation of the clotting system characterized mainly by both tissue infarction due to occlusive microthrombi and hemorrhage due to depletion of clotting factors. Associated disorders are myriad and include sepsis, burns, severe trauma, malignancies, obstetric events, hemorrhagic shock, and heat stroke.

### Pathologic Features

The blood smear findings in MAHA are similar, no matter what the underlying disorder. The dominant

**MICROANGIOPATHIC HEMOLYTIC ANEMIA–FACT SHEET**

**Definition**
- Hemolytic anemia resulting from mechanical disruption and fragmentation of red blood cells because of microvascular occlusion

**Incidence and Location**
- Thrombotic thrombocytopenic purpura (TTP): 3.7 per 1,000,000
- Hemolytic uremic syndrome (HUS): 0.7 per 1,000,000

**Morbidity and Mortality**
- TTP
  - Fatal in 10% to 20% cases
  - Chronic renal insufficiency in minority of surviving patients
- Shiga toxin–associated HUS
  - Fatal in approximately 5%
  - Chronic renal insufficiency in up to 50% of patients

**Gender, Race, or Age Distribution**
- TTP
  - Generally adults
  - Male-to-female ratio of 2:3
- Shiga toxin–associated HUS
  - Generally children
  - No gender predilection

**Clinical Features**
- TTP
  - Antecedent viral infection in minority of cases
  - Hemolytic anemia
  - Thrombocytopenic purpura
  - Neurologic symptoms
  - Fever
  - Renal abnormalities
- HUS
  - Bloody diarrheal syndrome several days before onset
  - Acute renal failure
  - Hypertension
  - Mental status changes

**Prognosis and Therapy**
- TTP: plasmapheresis
- HUS: supportive care

feature is RBC fragmentation. These RBC fragments take several forms, including helmet cells, triangulocytes, and schistocytes. Helmet cells are the largest form of RBC fragment and are characterized by a large, smooth, variably concave defect interrupting an otherwise normal RBC contour (Figure 1-38). Triangulocytes are small fragments with a distinctly triangular shape (Figure 1-39). Other RBC fragments that do not fall into one of these categories are best termed *schistocytes* (see Figure 1-38). Strictly speaking, RBC fragments should lack central pallor. A cell related to fragments that may be seen in MAHA is the keratocyte. This type of cell has two projections or horns protruding from one side of the cell and retains central pallor. Small irregular cells with retained central pallor are best considered nonspecific poikilocytes. Scattered spherocytes are also a constant feature of MAHA. As with any hemolytic anemia of at least several days' duration, MAHA will be accompanied by polychromasia.

**MICROANGIOPATHIC HEMOLYTIC ANEMIA–PATHOLOGIC FEATURES**

**Microscopic Findings**
- Red blood cell fragmentation (helmet cells, triangulocytes, and schistocytes)
- Spherocytes
- Thrombocytopenia
- Polychromasia

**Differential Diagnosis**
- Oxidant hemolysis
- Macroangiopathic hemolytic anemia

## Ancillary Studies

TTP and HUS are notable for a lack of disturbance of routine coagulation tests (prothrombin time and partial thromboplastin time), although D-dimers may be elevated in Shiga toxin–associated HUS. Disturbed coagulation parameters and evidence of thrombolysis (increased D-dimers or fibrin-fibrinogen split products) are hallmarks of DIC. Assays for ADAMTS13 activity will show a dramatic reduction in the activity of this enzyme in TTP (<10%), with normal or mildly decreased levels in other forms of MAHA. In addition, inhibitory IgG antibodies to ADAMTS13 are detectable by enzyme-linked immunosorbent assay in the vast majority of acquired TTP cases. Culture of enteropathogenic *E. coli* or other Shiga toxin–producing organisms from stool can help to establish a diagnosis of HUS.

## Differential Diagnosis

The differential diagnosis of MAHA includes oxidant hemolysis. As noted in the discussion of G6PD deficiency, bite cells and helmet cells may be indistinguishable on a cell-by-cell basis (see Figure 1-35). However,

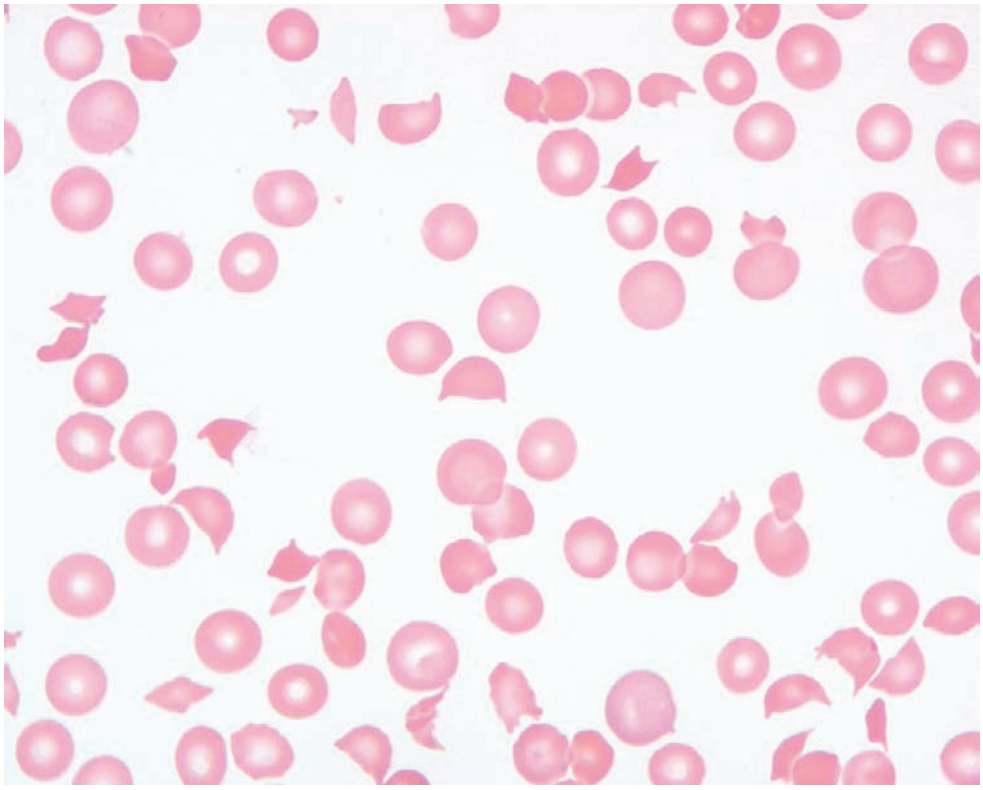

**FIGURE 1-38**

Thrombotic thrombocytopenic purpura (TTP), peripheral blood findings. This smear from a patient with florid TTP demonstrates a classic helmet cell *(center)* and a variety of nonspecific schistocytes of varying size and shape.

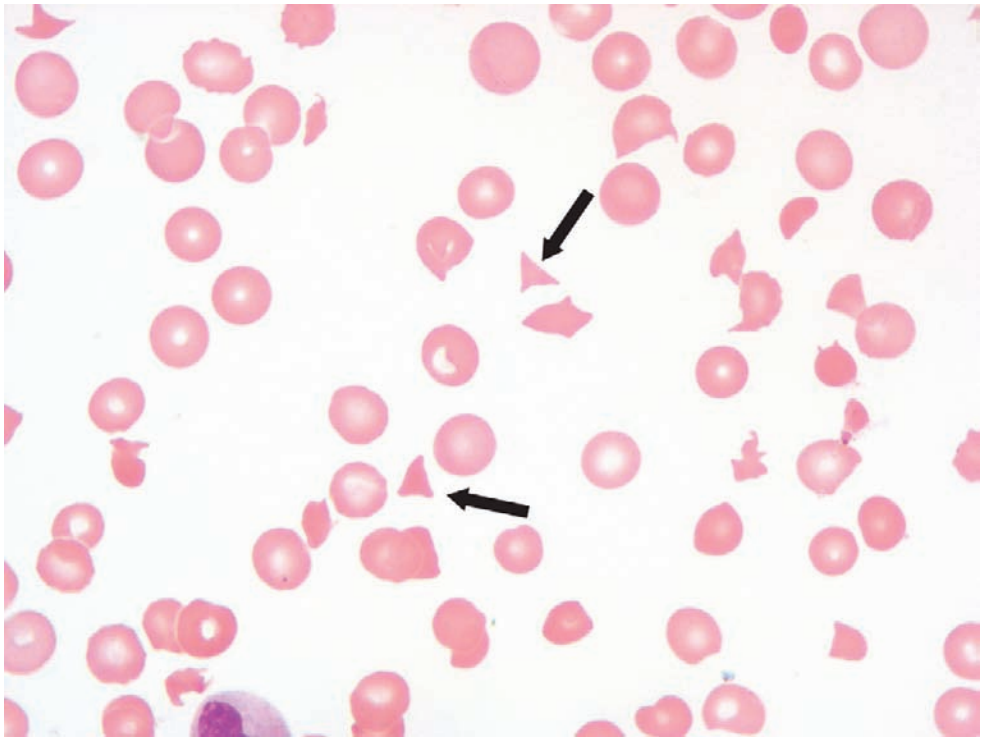

**FIGURE 1-39**

Thrombotic thrombocytopenic purpura (TTP), peripheral blood findings. This image from a patient with TTP contains two triangulocytes *(arrows)* and various schistocytes.

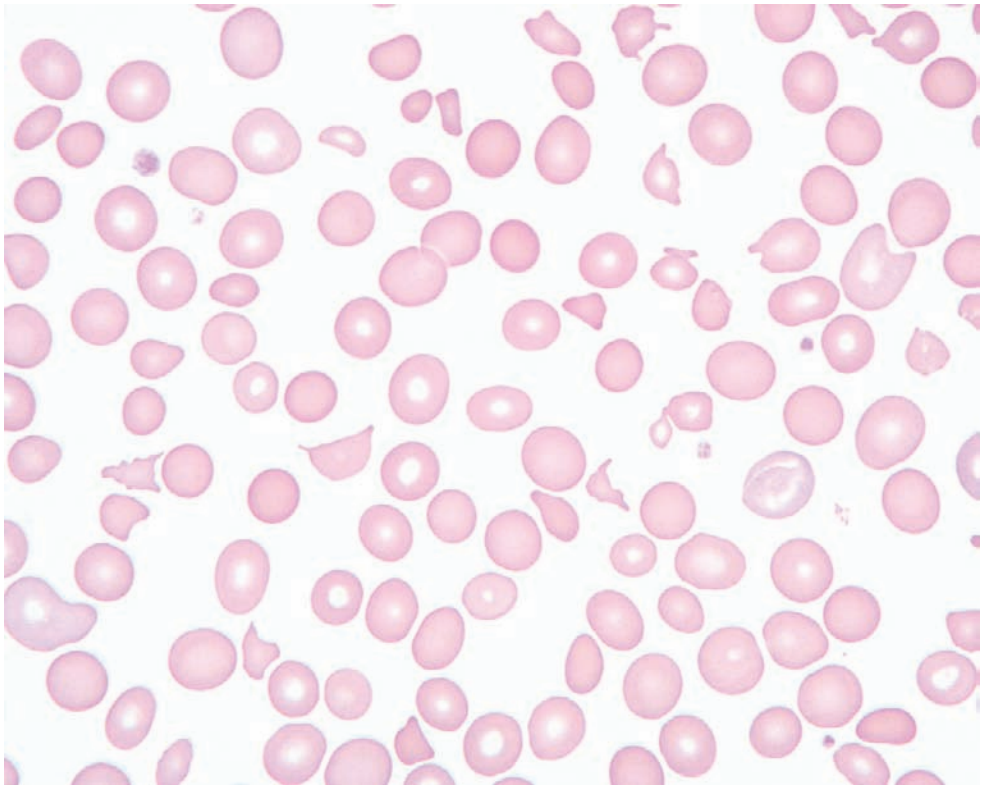

**FIGURE 1-40**

Valve hemolysis, peripheral blood findings. Red cell fragmentation in a patient with a defective cardiac valve. The morphologic features are similar to those seen in microangiopathic hemolytic anemia.

the full spectrum of RBC fragments seen in MAHA, in particular the presence of triangulocytes, should serve to distinguish it from oxidant hemolysis. Thrombocytopenia is also not a feature of oxidant hemolysis. Occasionally RBC fragmentation occurs as a result of so-called macroangiopathic hemolytic anemia (Figure 1-40); this results from jet lesions or turbulent flow resulting from gross lesions of large vessels or cardiac valves. In current practice, this is most often encountered in patients with malfunctioning replacement valves.

### Prognosis and Therapy

Without therapy, TTP is a universally fatal disorder. However, since the advent of plasma exchange therapy, most patients (approximately 80%) achieve complete remissions, although approximately one third of patients will ultimately relapse. Rituximab may be of value in some patients with refractory or recurrent disease. Long-term survival in TTP is 80% to 90%. Shiga toxin–associated HUS is typically a self-limited disorder requiring only supportive care with a current mortality rate of approximately 5%. Some patients develop chronic renal insufficiency following resolution of the acute HUS episode. Atypical HUS is generally managed supportively; plasma therapy may be beneficial in some patients. Relapses are frequent in this disorder, and some cases may progress to end stage renal failure. The therapy for DIC includes replacing clotting factors and platelets with component therapy; however, treatment of the underlying disorder causing the DIC is of paramount importance. The prognosis of DIC depends largely on the nature of the underlying condition and whether it can be treated rapidly and effectively.

## PARASITIC HEMOLYSIS

A variety of microorganisms may produce hemolytic anemia through a variety of mechanisms; however, this section focuses on hemolysis caused by intracellular parasites, which are disorders that can be diagnosed based on morphologic findings in blood smear. Belonging to this group are *Plasmodium* species, which produce malaria, and *Babesia* species. These organisms mature and proliferate, ultimately resulting in RBC destruction.

### Clinical Features

Malaria is an important worldwide public health problem, with 300 to 500 million people afflicted

**PARASITIC HEMOLYSIS–FACT SHEET**

**Definition**
- Hemolytic anemia resulting from proliferation of parasitic organisms within red blood cells

**Incidence and Location**
- Malaria: approximately 1800 cases per year in the United States
- Babesiosis: approximately 233 cases seen in the United States per year

**Morbidity and Mortality**
- Malaria
  - *Plasmodium falciparum* may pursue fulminant course if untreated or if drug resistant
  - Considerable morbidity and mortality in children in hyperendemic regions
- Babesiosis
  - Usually self-limited
  - 5% mortality in U.S. cases, usually elderly, immunosuppressed, or debilitated

**Clinical Features**
- Malaria
  - Cyclic fevers, sometimes preceded by flulike syndrome
  - Moderately severe hemolytic anemia in 20% of *P. falciparum* infection
  - Rare fulminant intravascular hemolysis (blackwater fever)
- Babesiosis
  - Similar to malaria, but fevers not cyclic

**Prognosis and Therapy**
- Malaria
  - Various antimalarial agents
  - Excellent prognosis if treated promptly
- Babesiosis
  - No therapy necessary in most cases
  - Clindamycin or quinine in severe cases

annually; 1.5 to 3 million people die of this disease every year. In the United States, there are approximately 1800 cases annually. Malaria caused by *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae,* or *Plasmodium ovale* is characterized by periodic episodes of fever, sometimes preceded by a flulike prodrome. Splenomegaly is common. Anemia is also common and correlates with species and organism load. Approximately 20% of patients with the most severe type of malaria, due to *P. falciparum*, demonstrate moderately severe anemia associated with the acute infection. Rarely, patients with fulminant *P. falciparum* infection develop acute intravascular hemolysis, a syndrome known as blackwater fever. Patients with malaria typically have a history of travel to Africa, Asia, or Central or South America.

The U.S. form of babesiosis, most commonly caused by *Babesia microti,* is a mild, self-limited disorder usually seen in the northeast part of the country. There are approximately 230 cases of babesiosis diagnosed annually in the United States, but it is thought that it often goes clinically undetected. Babesiosis is associated with a malaria-like syndrome of fever, chills, sweating, and headache, but the fever in babesiosis lacks the regular periodicity of that seen in malaria. Anemia, if present, is usually mild; however, occasional cases of babesiosis produce severe hemolytic anemia, usually in patients with prior splenectomy. A more severe European form of babesiosis is due to *Babesia divergens*.

**PARASITIC HEMOLYSIS–PATHOLOGIC FEATURES**

**Microscopic Findings**
- Malaria
  - Range of maturational forms seen in erythrocytes: immature trophozoites (rings), mature trophozoites, schizonts (undivided and divided), gametocytes
  - Only rings and banana-shaped gametocytes in *Plasmodium falciparum*
- Babesiosis
  - Mainly delicate rings, resembling those in *P. falciparum* (1 to 12 per cell)
  - No maturational forms as seen in malaria

## Pathologic Features

Malarial parasites take a variety of morphologic forms that can be visualized on routine, Wright-stained blood smears. Early ring-trophozoites are delicate, pale blue rings, 2 mm or less in diameter, with one red chromatin dot (Figure 1-41). Occasionally, ring forms can contain a double chromatin dot, or two ring trophozoites may be seen in the same RBC. Both of these findings are most common with *P. falciparum*. More mature trophozoites are larger with more abundant cytoplasm and a single chromatin mass (Figure 1-42). The shape of the parasite at this stage varies depending on the species and the stage of maturation. Mature trophozoites are commonly associated with hemozoin pigment because of the breakdown of Hb. Schizonts, the dividing form of the organism, are characterized by two or more chromatin masses and undivided (early; Figure 1-43) or divided cytoplasmic masses (Figure 1-44). Finally, the gametocyte forms of the organism are round or elongate (i.e., banana shaped, in the case of *P. falciparum*) structures with a compact or dispersed chromatin mass (Figures 1-41 and 1-45). Infections with *P. vivax, P. ovale,* and *P. malariae* are associated with parasites at various stages of maturation, whereas *P. falciparum* manifests only ring trophozoites and gametocytes in the blood. A more detailed description of the maturational forms in various types of malaria can be found in many textbooks.

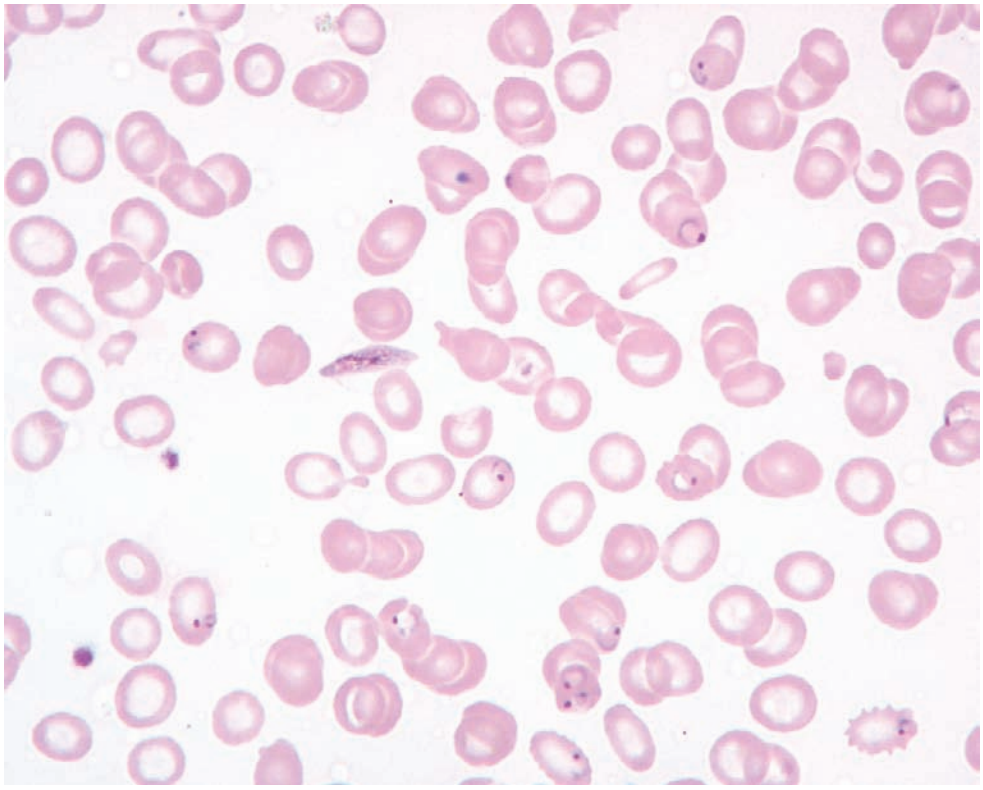

**FIGURE 1-41**

Malaria, peripheral blood findings. This example of *Plasmodium falciparum* malaria with a high level of parasitemia demonstrates multiple red blood cells containing early trophozoites (rings). These have a pale blue, annular cytoplasmic body and a red chromatin dot. Also present is an elongate (banana-shaped) gametocyte, diagnostic of *P. falciparum*.

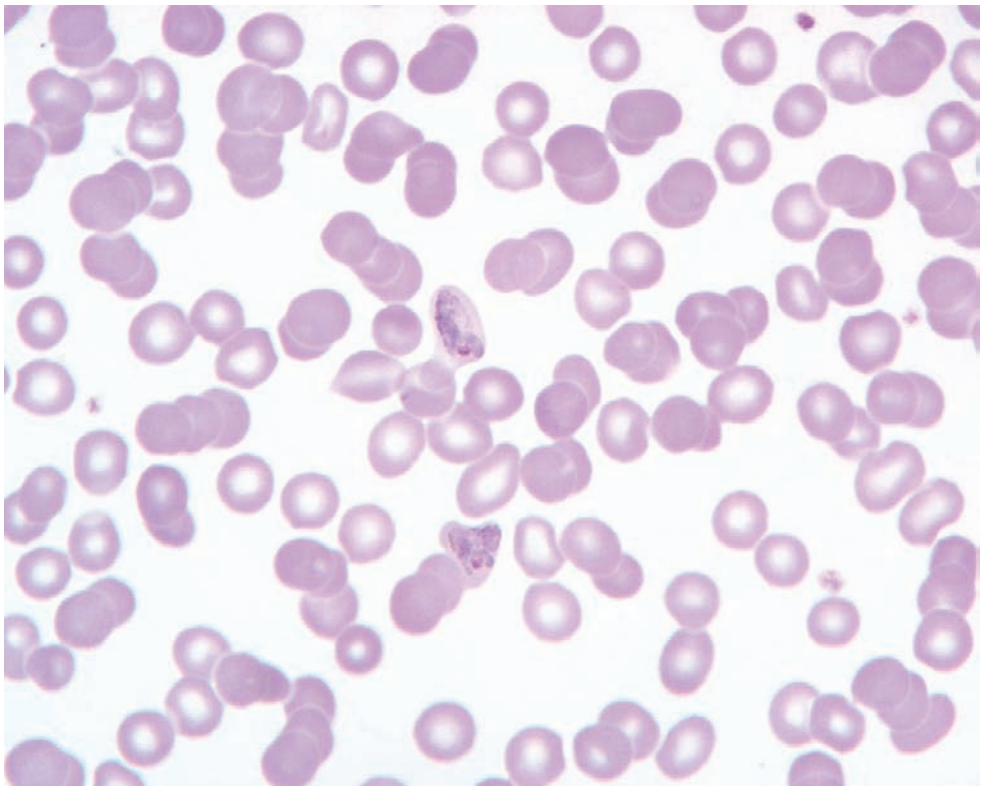

**FIGURE 1-42**

Malaria, peripheral blood findings. Two mature, "ameboid" trophozoites of *Plasmodium vivax*. These have a large, irregular cytoplasmic mass and a distinct red chromatin mass.

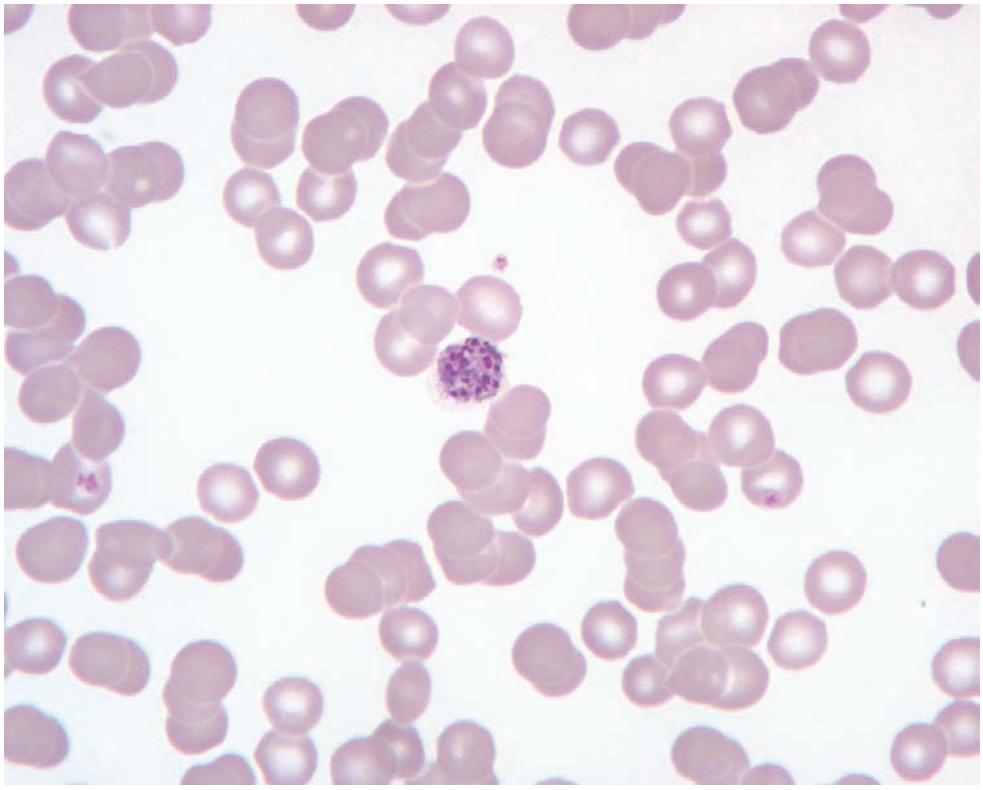

**FIGURE 1-43**

Malaria, peripheral blood findings. This early schizont (dividing form of the organism) of *Plasmodium vivax* has a single cytoplasmic body with multiple chromatin masses.

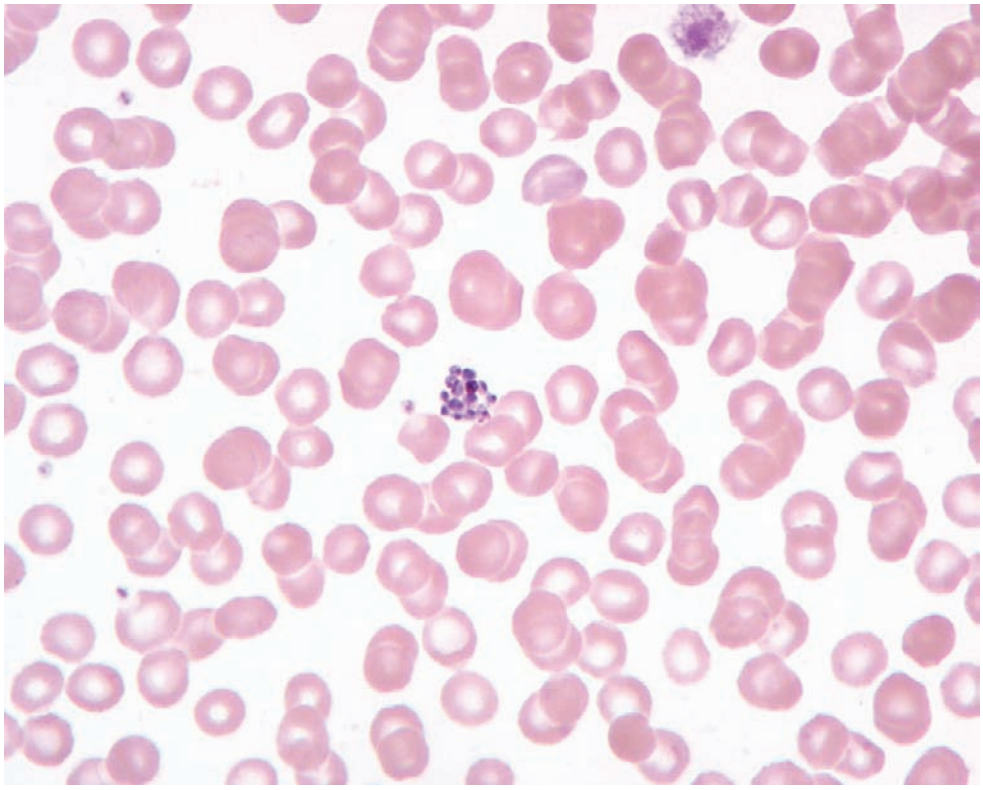

**FIGURE 1-44**

Malaria, peripheral blood findings. This late (divided) schizont of *Plasmodium vivax* is a cluster of small round cytoplasmic masses, each containing a chromatin dot.

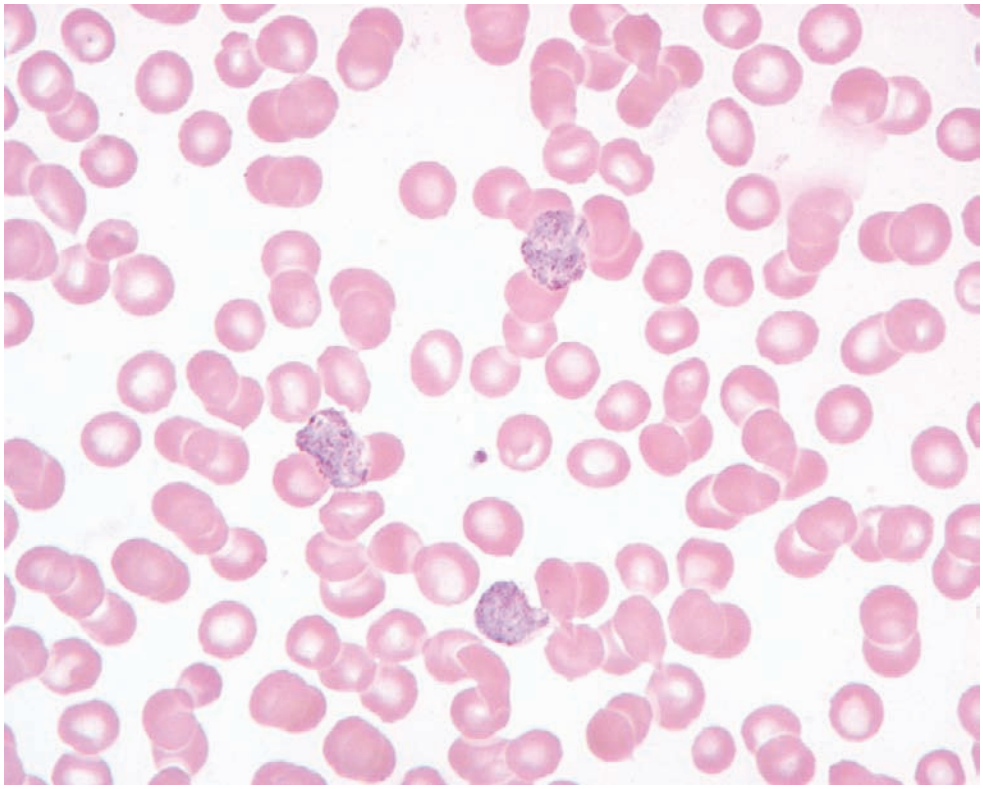

**FIGURE 1-45**

Malaria, peripheral blood findings. This field contains three macrogametocytes of *Plasmodium vivax*. The chromatin masses are visible as compact, wedge-shaped pink masses compressed to one edge of the organism.

The predominant form of *Babesia* spp. seen in the blood is a small ring form, closely resembling *P. falciparum* (Figure 1-46). However, *Babesia* spp. trophozoites may show wide morphologic variability, ranging from tiny forms composed of a minute cytoplasmic body with a barely visible nuclear dot to larger amoeboid, pear-shaped, or elongate forms. As many as 12 *Babesia* spp. trophozoites may be seen in a single RBC (Figure 1-47). Tetrads of *Babesia* spp. rings are rare but diagnostic.

## Ancillary Studies

Thick films or cytospins are helpful in identifying and speciating malarial parasites, particularly in cases with a low level of parasitization. Species-specific serologic tests are available, but fail to adequately differentiate past from current infection. Rapid test strips that use a drop of blood from a finger stick are also available. Finally, species-specific polymerase chain reaction (PCR) tests are now available for definitive speciation of confirmed infections. Serologic and PCR tests are also available for the diagnosis of babesiosis.

## Differential Diagnosis

The differential diagnosis of malaria and babesiosis is largely with each other. Travel history and clinical manifestations (e.g., periodicity of fever) may be helpful. Morphologically, the presence of schizonts and gametocytes rules out babesiosis. Other distinguishing features include the generally smaller size of rings, absence of hemozoin, lack of synchronization of parasite forms, many rings per RBC, and the occasional presence of extraerythrocytic ring forms in *Babesia* spp. infections. Finally, serologic or molecular tests will help to definitively distinguish these infections.

## Prognosis and Therapy

Treatment of malaria involves a variety of agents, including quinine, chloroquine, sulfones, sulfonamides, and pyrimethamine. The prognosis is excellent if therapy is initiated promptly. *P. falciparum* may pursue a fulminant course if treatment is delayed or if the organisms are drug resistant. In areas of the world where malaria

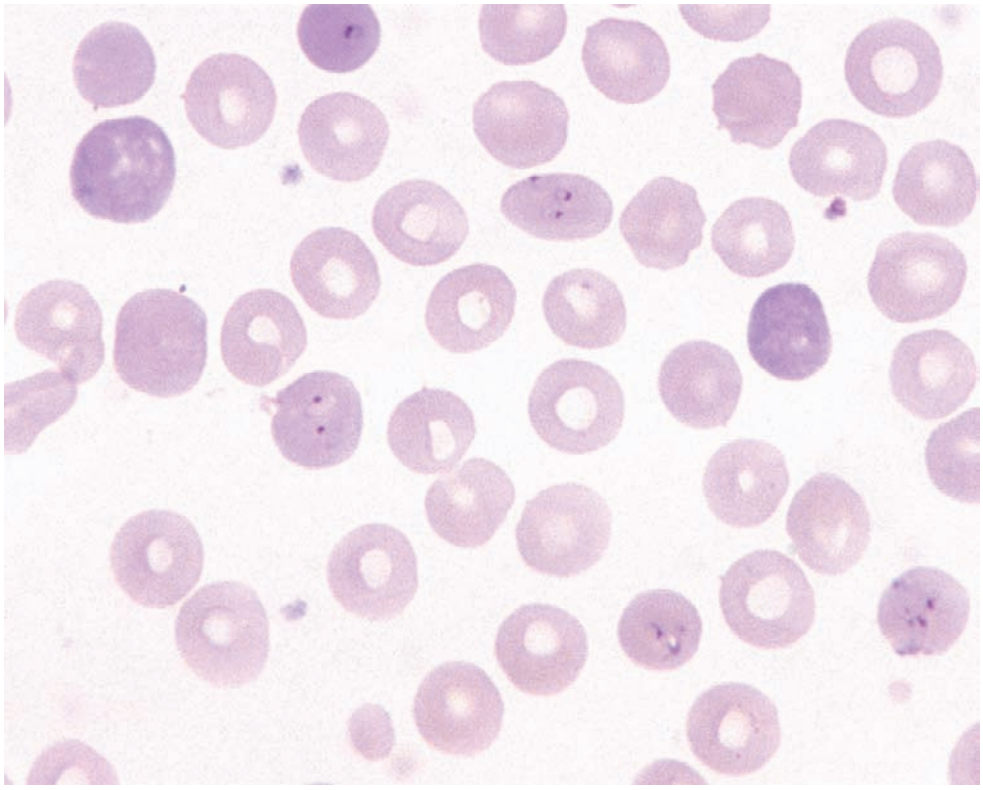

**FIGURE 1-46**

Babesiosis, peripheral blood findings. Five erythrocytes in this field contain one to several delicate ring forms with a barely visible, pale blue cytoplasmic body and a red chromatin dot. These are similar to the ring forms of *Plasmodium falciparum*.

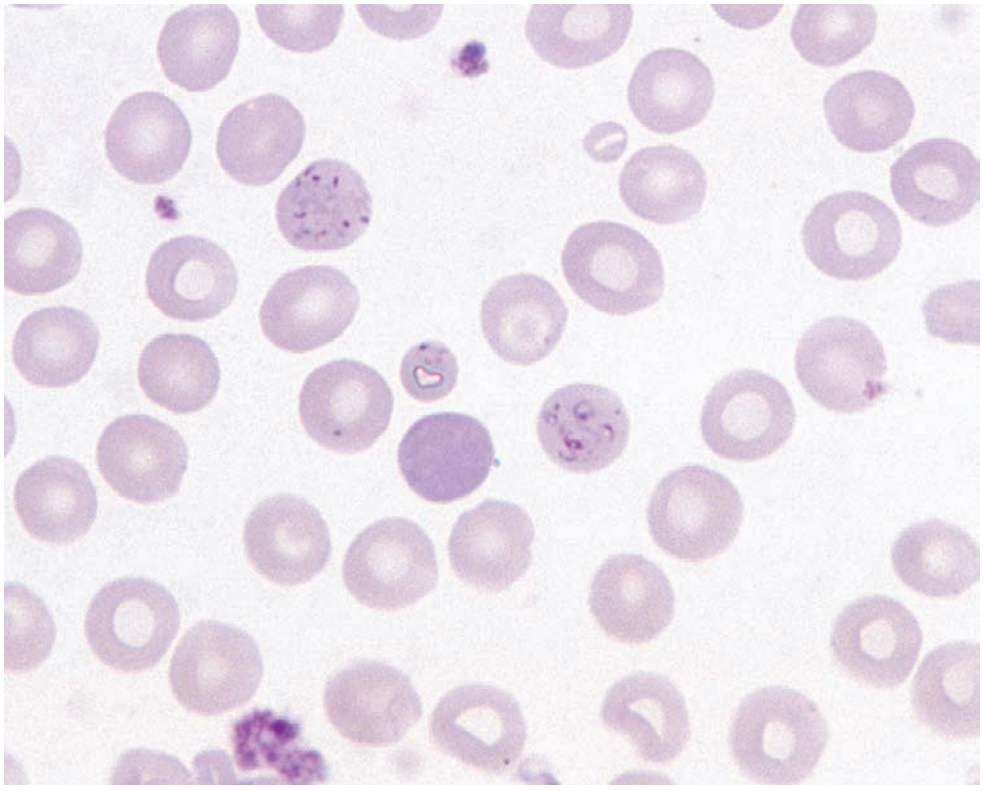

**FIGURE 1-47**

Babesiosis, peripheral blood findings. Two infected erythrocytes in this field contain numerous ring forms. As many as 12 rings per erythrocyte can be seen in babesial infections.

is hyperendemic, there is considerable morbidity and mortality, particularly in children.

Babesiosis generally is self-limited, but treatment with clindamycin or quinine may be helpful in more severe cases. Among clinically evident cases in the United States, babesiosis carries a mortality rate of 5%, with those succumbing usually being elderly, immunosuppressed, or asplenic. European babesiosis has a mortality rate of 42%.

## ERYTHROCYTOSIS

Erythrocytosis, an increase in the concentration of RBCs and Hb in the blood, can result from either an absolute increase in body red cell mass (polycythemia) or from a decrease in plasma volume (relative erythrocytosis). Erythrocytosis in which the concentration of RBCs is increased with little or no anemia can also occur in microcytic RBC states, most notably thalassemia minor or trait. However, this type of erythrocytosis is a consequence of the microcytosis and is not associated with an increased Hb concentration and is not discussed further in this text.

Relative polycythemia generally results from hemoconcentration because of a variety of pathophysiologic processes. A contraction of plasma volume produces the appearance of erythrocytosis without any actual increase in the body red cell mass. A chronic form of relative polycythemia (Gaisböck's disease) is an obscure syndrome of uncertain cause.

Absolute erythrocytosis or polycythemia can be either primary or secondary. Primary erythrocytosis can be either congenital or acquired. The congenital form, primary familial polycythemia, is a consequence of mutations in the erythropoietin receptor that confer hypersensitivity to erythropoietin. Acquired absolute erythrocytosis, termed *polycythemia vera,* is a clonal stem cell disorder that belongs to the group of chronic myeloproliferative neoplasms. This disease is associated with an increase in all myeloid cell lines, but its primary clinical manifestations relate to erythrocytosis. This disorder is discussed in detail in Chapter 17. Secondary erythrocytosis is a consequence of increased erythropoietin resulting from one of several causes. Chronic hypoxia, caused by cardiac or pulmonary disease or residence at high altitude, results in a compensatory erythrocytosis. Certain abnormal Hbs, such as inherited high oxygen affinity variants or increased carboxyhemoglobin in smokers, fail to deliver oxygen to the tissues effectively, resulting in increased erythropoietin production by the kidneys. Finally, some patients may have aberrant erythropoietin production by neoplasms.

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The complete reference list is available online at www.expertconsult.com.

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# 2

# Platelet Disorders

**Kandice Kottke-Marchant, MD, PhD**

## PLATELETS: PRODUCTION AND HEMOSTATIC FUNCTION

Platelets are small (2-μm diameter), nonnucleated blood cells that have a vital role in hemostasis and are produced in the bone marrow from megakaryocytes (Figure 2-1). Megakaryocytes are descended from pluripotent hematopoietic progenitors through a bipotential erythroid–megakaryocytic cell. Megakaryoblasts express CD41, the thrombopoietin receptor (c-Mpl) and the α chemokine receptor CXCR4. After this stage, megakaryoblasts undergo maturation to megakaryocytes, stimulated by thrombopoietin, stem cell factor, stromal cell-derived factor (SDF-1/CXCL12) and other cytokines (Figure 2-2). Megakaryocytes are exquisitely responsive to thrombopoietin; engagement of only 25 to 100 c-Mpl receptors leads to downstream biochemical signals, including JAK2 kinase phosphorylation, activation of the mitogen-activated protein kinase and extracellular signal-regulated kinase 1 and 2 pathways. Maturation is dependent on transcription factors GATA1 and GATA2 together with the cofactor FOG1. This maturation is accompanied by endomitosis, whereby the megakaryocyte progenitor DNA increases up to 256-fold the normal chromosome complement through repetitive abortive mitosis yielding a highly lobated, polypoid nucleus. During a terminal phase of megakaryocyte differentiation, platelets are produced from cytoplasmic projections known as *protoplatelets* (see *arrow,* Figure 2-1). Transverse bands of microtubules form along longitudinal bundles of microtubules in the protoplatelets, facilitating trafficking of granules and proteins to the distal end, with breakage at constriction zones and liberation of newly formed platelets from the tip of the protoplatelet.

Megakaryocytes release platelets into the sinusoids of the bone marrow. After release, platelets circulate in a quiescent state, largely because of inhibition by endothelial-derived nitrous oxide and prostacyclin, but are activated rapidly upon blood vessel injury and have a crucial role in the primary hemostatic response. In their inactivated state, platelets are roughly discoid in shape with numerous intrinsic glycoproteins (GPs) embedded in the outer surface of the plasma membrane that are receptors for adhesive ligands ranging from fibrinogen (GP IIb/IIIa or $\alpha_{IIb}\beta_3$), collagen (GP Ia/IIa and GP VI), and thrombospondin (GP IV) to von Willebrand factor (VWF; GP Ib/V/IX), fibronectin (GP Ic/IIa), and vitronectin ($\alpha_v\beta_3$). Platelets also have numerous membrane receptors involved in signal transduction and biochemical activation, such as adenosine diphosphate (ADP) receptors ($P2Y_1$ and $P2Y_{12}$), thrombin receptors (PAR-1 and PAR-4), thromboxane receptors (TPα and TPβ), serotonin 5HT-2A receptors, and adrenergic receptors. There is an important cytoskeletal system, composed of actin, spectrin, and filamin A, along with a circumferential band of microtubules and microfilaments. The cytoskeleton is responsible for maintaining the discoid shape of resting platelets and is involved in the development of pseudopods and lamellipodia during platelet adhesion and activation. The platelet cytosol contains typical cellular organelles such as mitochondria, lysosomes, and glycogen; it also contains platelet-specific granules, called *α-granules* and *dense granules,* that have a major role in amplification of platelet activation and interaction with coagulation (Figure 2-3). There is an invaginating open-canalicular membrane system that facilitates platelet granule release; it reaches from the outer plasma membrane to the depths of the platelet cytosol and gives the platelet a spongelike structure. In addition, there is a dense tubular system that stores metabolic enzymes and calcium.

Platelets promote hemostasis by four interconnected mechanisms: (1) adhesion to sites of vascular injury; (2) cellular activation and release of granule contents; (3) aggregation, recruitment, and amplification to form a hemostatic platelet plug; and (4) providing a procoagulant phospholipid surface (Figure 2-4). Vascular injury and endothelial denudation is rapidly followed by platelet adhesion to the subendothelium. The subendothelium is composed of extracellular matrix proteins, such as collagen, fibronectin, vitronectin, VWF, thrombospondin, and laminin; their exposure leads to binding to platelet surface receptors. Because of the large number of extracellular matrix proteins and a high density of

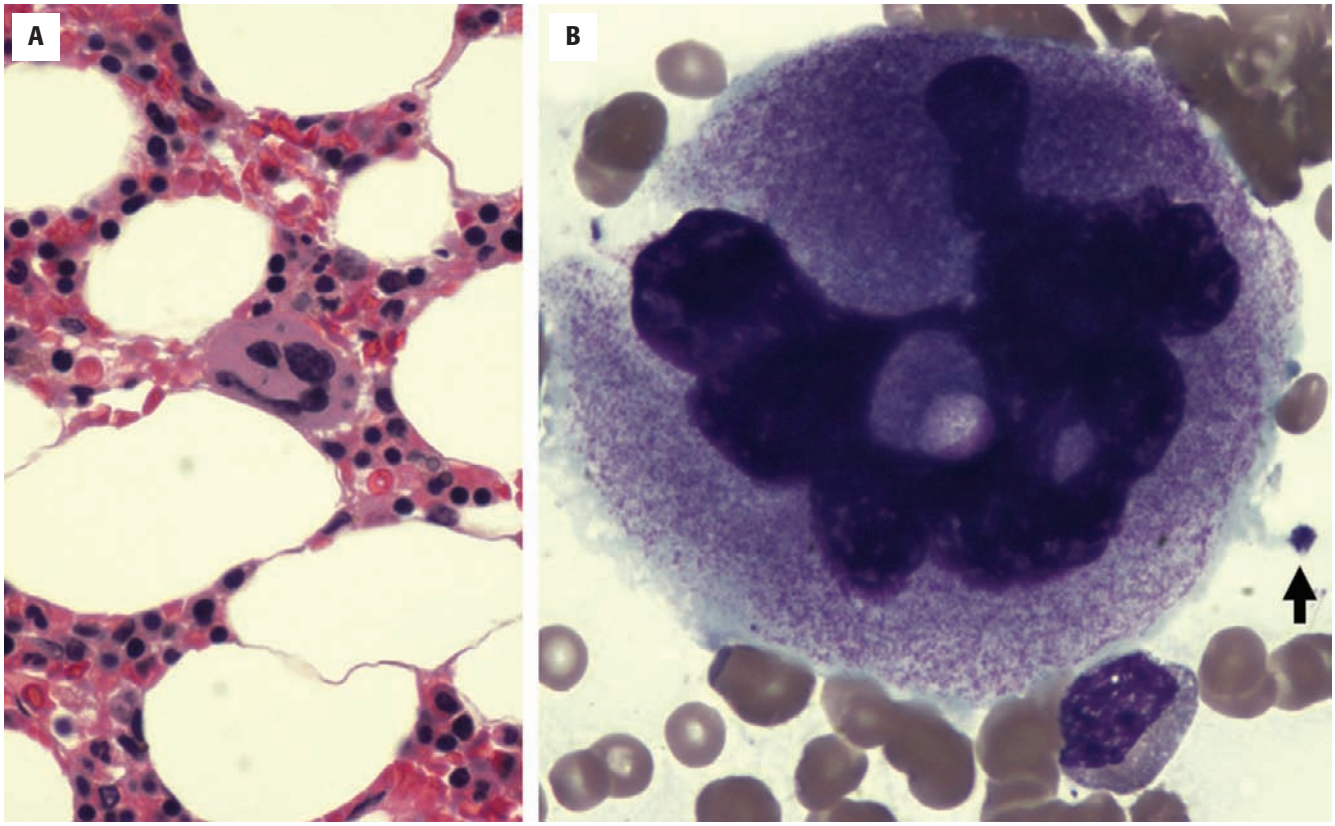


**FIGURE 2-1**

Photomicrographs of normal megakaryocytes. **A,** Bone marrow aspirate smear (Wright stain, original magnification ×100). **B,** Bone marrow biopsy specimen (hematoxylin and eosin, original magnification ×40). Notice the granular appearance of the megakaryocyte cytoplasm. The *arrow* points to protoplatelet formation. The convoluted nucleus is aneuploid.

platelet surface receptors, platelet adhesion to areas of vascular injury, even in flowing blood, is extremely rapid. At high shear rates VWF, a large, multimeric protein secreted abluminally from endothelial cells, facilitates platelet rolling and adhesion by binding to platelet surface GP Ib/IX/V. This binding is followed by binding of GP Ia/IIa and GP VI to collagen, platelet activation via GP VI, leading to inside-out signaling and firm adhesion via vascular wall–associated fibrin or fibrinogen through interaction with GP IIb/IIIa.

After adhering to the subendothelium, platelets initially are activated by thrombin and collagen via binding to distinct receptors. Thrombin, produced locally by the activated coagulation system, converts fibrinogen to fibrin and activates platelets through the G (Gq and G12/G13) protein-coupled receptors, PAR-1 and PAR-4. Other G-coupled receptors involved in platelet activation include the Thromboxane/PGH2 receptor (Gq and G13), serotonin receptor 5HT2A (Gq), and ADP receptors P2Y1 (Gq) and P2Y12 (Gi). GPVI activation requires coupling to the Fc receptor γ chain (FcRγ) and CD148, with inhibition by platelet-endothelial cell adhesion molecule-1 (PECAM-1). Binding of ligands to the various receptors leads to activation of many signaling pathways, including the Src family kinase, phosphoinositide 3-kinase, and the immunoreceptor tyrosine-based activation motif pathways. Convergence of these pathways leads to activation of phospholipase Cγ 2, with cleavage of polyphosphoinositides to diacylglycerol and inositol 1,4,5-trisphosphate (IP3), resulting in activation of protein kinase C and increased cytosolic calcium because of mobilization of calcium from intracellular depots and by entry of calcium from the plasma. The increased cytoplasmic calcium activates phospholipase $A_2$ to liberate arachidonic acid from the platelet membrane, leading to thromboxane $A_2$ production by cyclooxygenase and thromboxane synthase. Thromboxane $A_2$, together with phosphorylated pleckstrin and increased cytoplasmic calcium, leads to degranulation of α- and dense granules, mediated by fusion of granule and plasma membranes by SNARE protein complexes (vesicle associated membrane protein, VAMP-8, and syntaxin and synaptosome-associated protein-23; Figure 2-5). Degranulation releases the contents of α-granules (platelet factor 4, β-thromboglobulin, thrombospondin, platelet-derived growth factor, fibrinogen, VWF, among others) and dense granules (ADP, adenosine triphosphate [ATP], serotonin, calcium) into the surrounding milieu. The granule membranes contain many integral glycoproteins on their inner

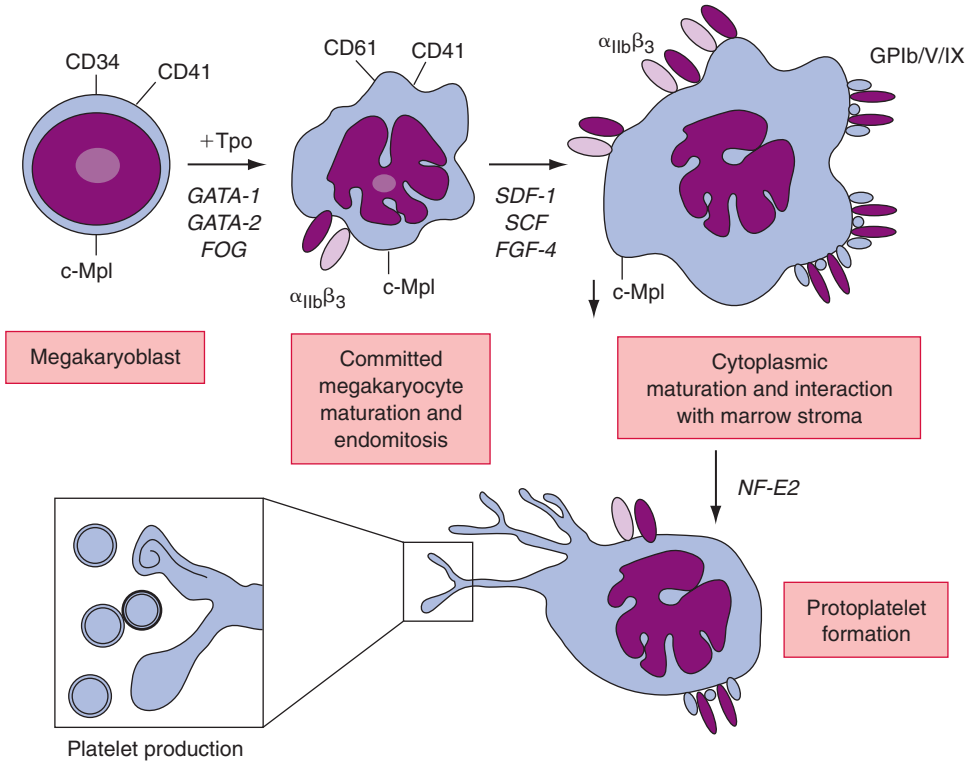


**FIGURE 2-2**

Platelet maturation process. Megakaryoblasts express CD34, CD41, and the thrombopoietin receptor (c-Mpl). After this stage, the megakaryoblasts undergo committed maturation to megakaryocytes with expression of the β3 integrin CD61 and increased levels of $\alpha_{IIb}\beta_3$ (GPIIb/IIIa), dependent on transcription factors GATA1 and GATA2 together with the cofactor, FOG1. This maturation is accompanied by endomitosis, resulting in an increase in DNA content accompanied by expression of the von Willebrand factor receptor, GPIb/V/IX. The cytoplasm enlarges (up to 100 to 150 μm) and becomes filled with platelet-specific organelles, proteins, and membrane systems. During a terminal phase of megakaryocyte differentiation, platelets are produced from cytoplasmic projections known as *protoplatelets* that form along one pole of the megakaryocyte cytoplasm under the guidance of transcription factor NF-E2. Boluses of platelet granules and membrane systems travel from the megakaryocyte cytoplasm down the tubules, yielding bulbous projections at tubule ends and along their length, giving a beaded appearance to the developing tubules. Longitudinal microtubule bundles composed of tubulin αβ-dimers form along, and move down, the tubule. The microtubule bundles reorganize in the protoplatelet tip to form a microtubule coil that envelopes and defines a newly matured protoplatelet.

leaflet, such as P-selectin (CD62p) in the α-granule and lysosome-associated membrane protein (CD63) in the lysosome, which become expressed on the outer platelet membrane after the exocytosis-based release reaction.

Increased cytosolic calcium also leads to gelsolin activation, resulting in cytoskeletal actin remodeling and polymerization that leads to a shape change with the development of cytoplasmic projections termed filopodia. Further spreading of the platelet by lamellipodia follows the formation of large networks of actin filaments (Figure 2-6).

The release of ADP from the dense granules, together with calcium mobilization, leads to phosphorylation of pleckstrin and molecular reorganization of GP IIb/IIIa (integrin $\alpha_{IIb}\beta_3$; Figure 2-7), with activation of the receptor and subsequent platelet aggregation, whereby a GP IIb/IIIa receptor on one platelet is bound in a homotypic fashion to the same receptor on adjacent platelets via a central fibrinogen molecular bridge. In addition to ADP, other agonists such as epinephrine, thrombin, collagen, and platelet-activating factor can initiate platelet aggregation by interaction with membrane receptors. The release of ADP, TxA2, and serotonin leads to the recruitment of many other platelets to the vessel wall with activation, amplification, and formation of a hemostatic platelet plug (Figure 2-8).

Activated platelets also have a vital procoagulant role that serves as a link between platelet function and coagulation activation. Platelet membrane phospholipids undergo a rearrangement during activation with a transfer of phosphatidyl serine from the inner table to the outer table of the platelet membrane, providing a binding site for phospholipid-dependent coagulation complexes that activate both factor X and prothrombin.

## EVALUATION OF PLATELET FUNCTION

### CLINICAL HISTORY FOR PATIENTS WITH A BLEEDING DIATHESIS

In a patient with a bleeding diathesis, a detailed personal and family bleeding history should be obtained before beginning a laboratory evaluation of platelet

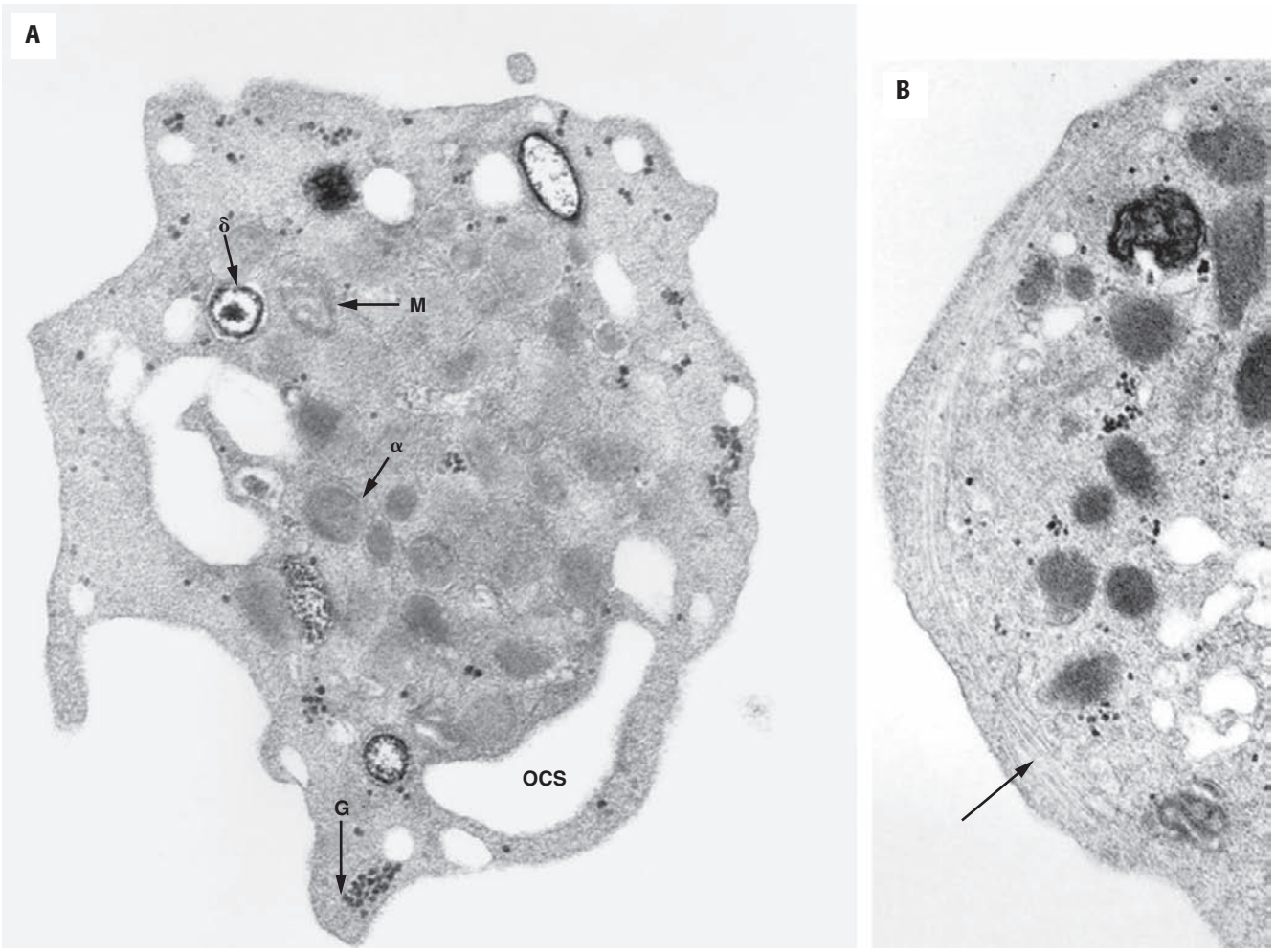


**FIGURE 2-3**

Electron micrographs of normal platelets. **A,** Note the abundant dark α-granules (α), the surface-connected open canalicular system (OCS), the dense granules with electron-dense central core (δ), the mitochondria (M), and glycogen (G; original magnification, ×22,000). **B,** Note the circumferential band of microtubules and microfilaments just under the plasma membrane (*arrow;* original magnification, ×40,500).

function. In addition, bleeding disorders caused by coagulation proteins, fibrinolysis, or the vascular system should be excluded. The history should include an assessment of the duration, pattern, and severity of bleeding problems, including whether the bleeding is spontaneous or associated with trauma or surgery. A lifelong bleeding diathesis suggests a congenital thrombocytopenia or platelet dysfunction, but an onset in adulthood does not necessarily exclude a congenital problem. It is often helpful to assess whether the bleeding is out of proportion to the degree of trauma or whether blood transfusions were required for relatively minor surgical procedures, such as tooth extractions.

Platelet-mediated bleeding disorders typically manifest with a mucocutaneous bleeding pattern involving small vessels. Ecchymosis, petechiae, purpura, epistaxis, and gingival bleeding are commonly observed. This pattern is in contrast to that observed with coagulation protein disorders in which deep-tissue bleeding and hemarthroses are more common. Von Willebrand disease (VWD), an abnormality of VWF, has bleeding symptoms similar to platelet dysfunction, and evaluation for VWD should be included in the initial evaluation of a possible platelet disorder. Bleeding diatheses resulting from vascular malformations may give a bleeding pattern similar to platelet disorders, but the pattern is often more focal than diffuse.

Many drugs and foods, such as caffeine and garlic, can affect platelet function; therefore a complete drug and dietary history should be obtained. It is important to remember that aspirin, an irreversible inhibitor of platelet function, is an ingredient in many over-the-counter and prescription medications, such as cold and flu remedies. The clinical history should include investigation of systemic disorders, such as renal disease, hepatic failure, connective tissue disorders, myeloproliferative disorders, myelodysplastic disorders, malignancy, and cardiovascular disease, as platelet dysfunction is associated with many of these diseases. In addition, some specific clinical features such as albinism,

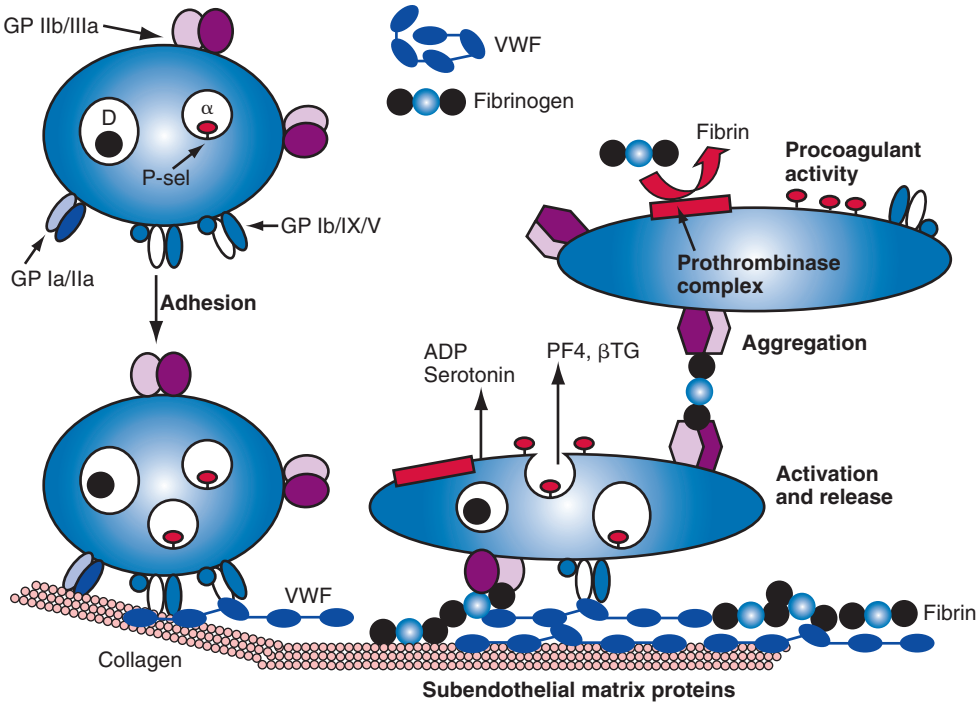


**FIGURE 2-4**

Schematic diagram of the role of platelets in hemostasis. Platelet activation is stimulated by vascular injury through exposure of platelets to extracellular matrix proteins and adsorbed plasma proteins, including von Willebrand factor (VWF) and fibrinogen. Platelets adhere to VWF through the surface glycoprotein (GP) Ib/IX/V and to fibrinogen through GP IIb/IIIa. Platelet adhesion stimulates intracellular signaling, leading to degranulation of α-granules (platelet factor 4 [PF4] and β-thromboglobulin [βTG]), and phospholipid reorganization with formation of coagulation complexes and fibrin formation. Adenosine diphosphate (ADP) release from dense granules and activation of the GP IIb/IIIa receptor also occur, leading to aggregation of platelets to the adherent layer.

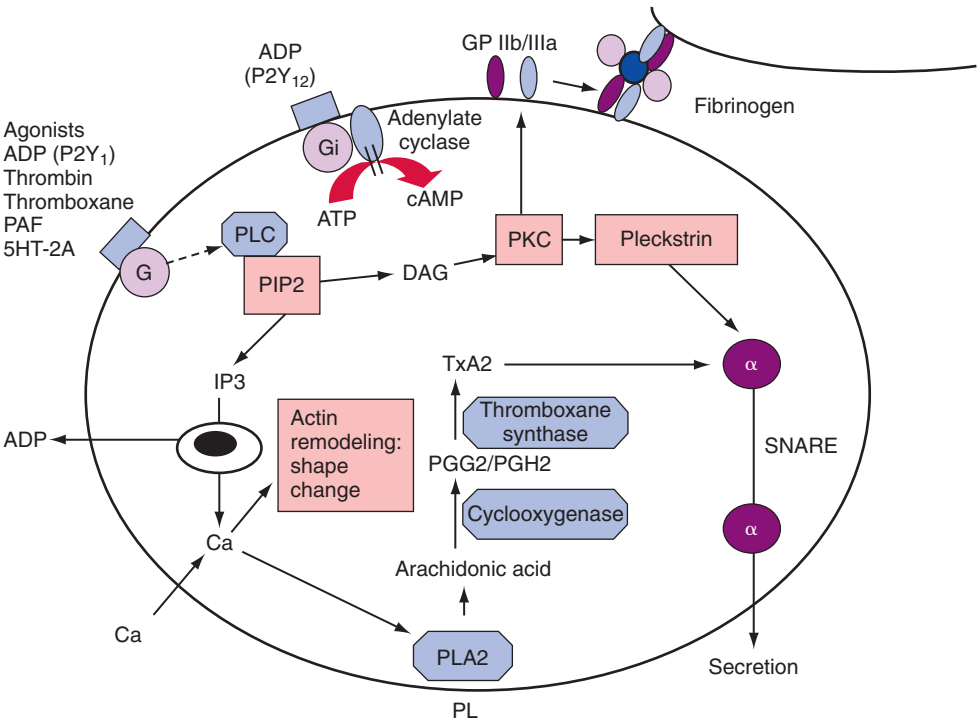


**FIGURE 2-5**

Schematic diagram of platelet activation. Stimulation of platelet receptors by agonists such as adenosine diphosphate (ADP), thrombin, thromboxane, and platelet-activating factor (PAF) signal through guanidine triphosphate–binding proteins (Gq or Gi), leading to activation of phospholipase C (PLC). PLC converts phosphatidylinositol bisphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). This leads to activation of protein kinase C (PKC) and phosphorylation of pleckstrin and molecular reorganization of glycoprotein (GP) IIb/IIIa, with activation of the receptor, allowing binding to fibrinogen and subsequent platelet aggregation. IP3 production leads to the release of calcium from dense granules and calcium flux into the cytoplasm. The increased cytoplasmic calcium activates phospholipase A2 (PLA2) to liberate arachidonic acid from the platelet membrane. Arachidonic acid is acted on by cyclooxygenase and thromboxane synthase to produce thromboxane A2 (TxA2). Thromboxane A2, together with phosphorylated pleckstrin and increased cytoplasmic calcium, leads to degranulation of α- and dense granules. *PGG2,* Prostaglandin G2; *PGH2,* prostaglandin H2; *SNARE,* soluble NSF attachment protein receptor.

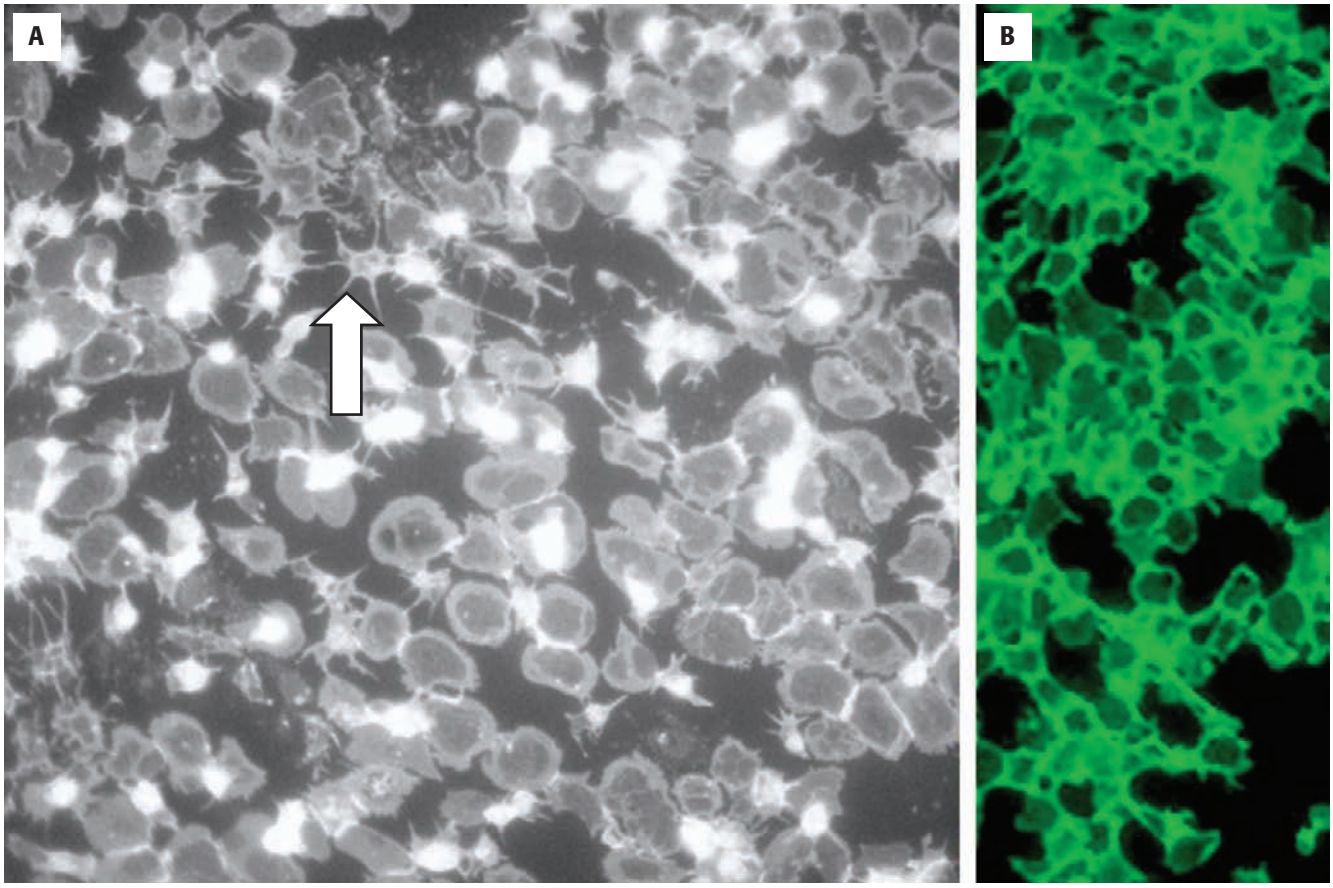


**FIGURE 2-6**

Photomicrograph of human platelets adherent to a polycarbonate substrate. **A,** Note the different stages of platelet adhesion, from pseudopods *(arrow)* to fully spread platelets. **B,** Prominent surface staining by glycoprotein IIb/IIIa on the adherent platelets that are in close proximity to adjacent platelets. (CD41 fluorescein isothiocyanate immunostain, original magnification ×40.)

deafness, nephritis, and susceptibility to infections may help in the differential diagnosis of the inherited platelet disorders.

## PLATELET COUNT AND PERIPHERAL BLOOD SMEAR

Investigation of platelet disorders should start with measurement of the platelet count and review of peripheral smear morphology. On a properly prepared Wright-stained blood smear, the platelets are of roughly uniform size (approximately 2-μm diameter) with abundant purple-staining granules (Figure 2-9). Degranulated pale gray platelets, doublets, or larger platelets are unusual. The presence of more than a few large platelets suggests increased platelet turnover, myeloproliferative disorder, or a congenital macrothrombocytopenia.

The accepted normal range of the platelet count is generally 150 to 400 × $10^3$/μL of blood, although values much lower than this can be adequate for hemostasis. The mean platelet volume (MPV) is an indication of platelet size. Normal MPV ranges are approximately 7 to 11 fL. The platelet distribution width (PDW) is a measure of the dispersion of the platelet sizes and is analogous to the red cell distribution width. The MPV can be an indication of platelet turnover, because platelets newly released from the bone marrow are larger and tend to decrease in size with age in the circulation. In patients with rapid turnover, the platelets will be larger in general because of the larger size of newly produced platelets, and their PDW will be increased because of a mixture of large and small platelets. True congenital macrothrombocytopenias usually have uniformly large platelets with a high MPV and normal PDW; often the platelets are at least twice normal size and may be as large as erythrocytes. This can result in falsely low platelet counts, because the large platelets may be counted as leukocytes by automated cell counters. Newer techniques based on messenger RNA (mRNA) detection in platelets (reticulated platelets and immature platelet fraction) may also be helpful to indicate the rate of thrombopoiesis, because mRNA levels are high in newly formed platelets and decline progressively during blood circulation time.

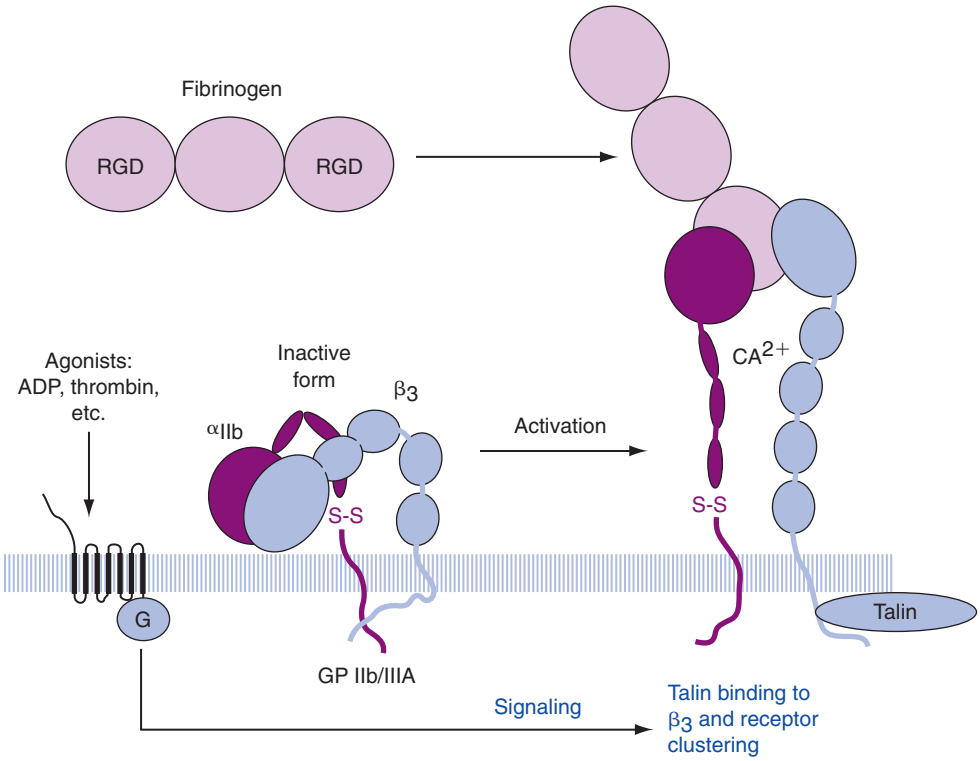


**FIGURE 2-7**

Activation of glycoprotein (GP) IIb/IIIa (αIIb/β3 integrin), the receptor for fibrinogen. During platelet activation, an agonist binds to a surface receptor and initiates inside-out signaling. In the resting state, the GP IIb/IIIa receptor is in an inactive conformation and cannot bind to fibrinogen. After signaling, there is a molecular conformational change that allows binding of the ligand (RGD peptide from the fibrinogen α-chain), which is followed further by cytoskeletal rearrangement and receptor clustering. *ADP,* Adenosine diphosphate.

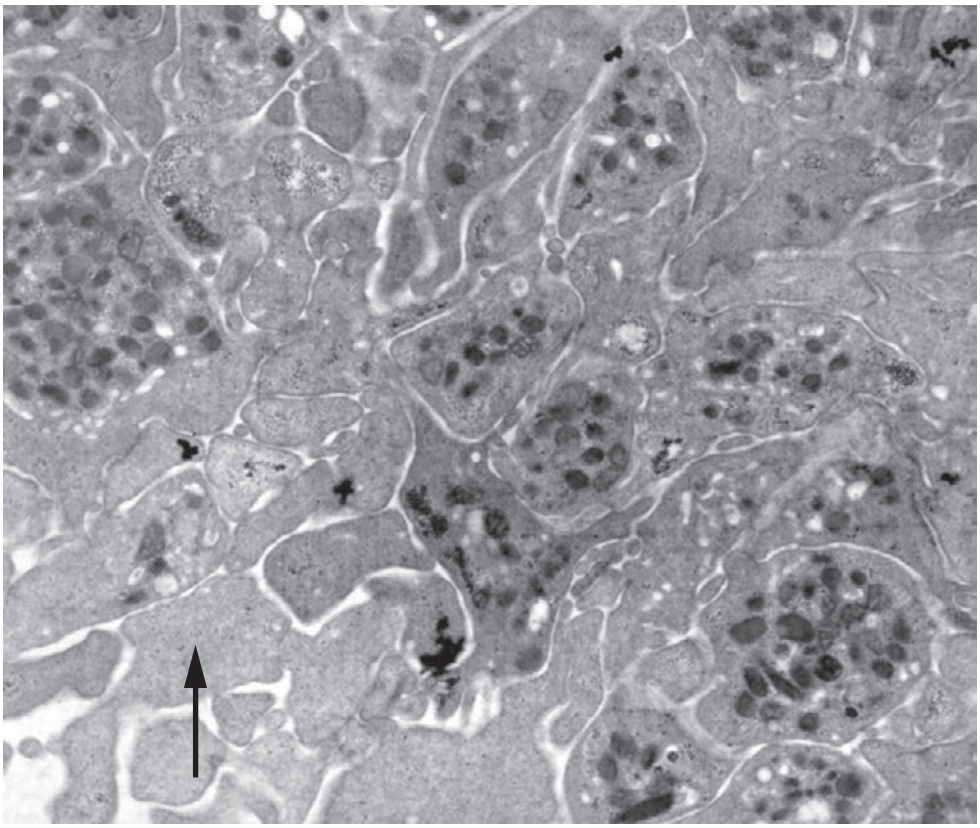

**FIGURE 2-8**

Electron micrograph of a platelet aggregate. Note the closely adherent platelets with abundant cytoplasmic projection. Some platelets have completely released their cytoplasmic granules *(arrow),* while others at the edge of the aggregate still maintain the majority of their granules (original magnification ×18,900).

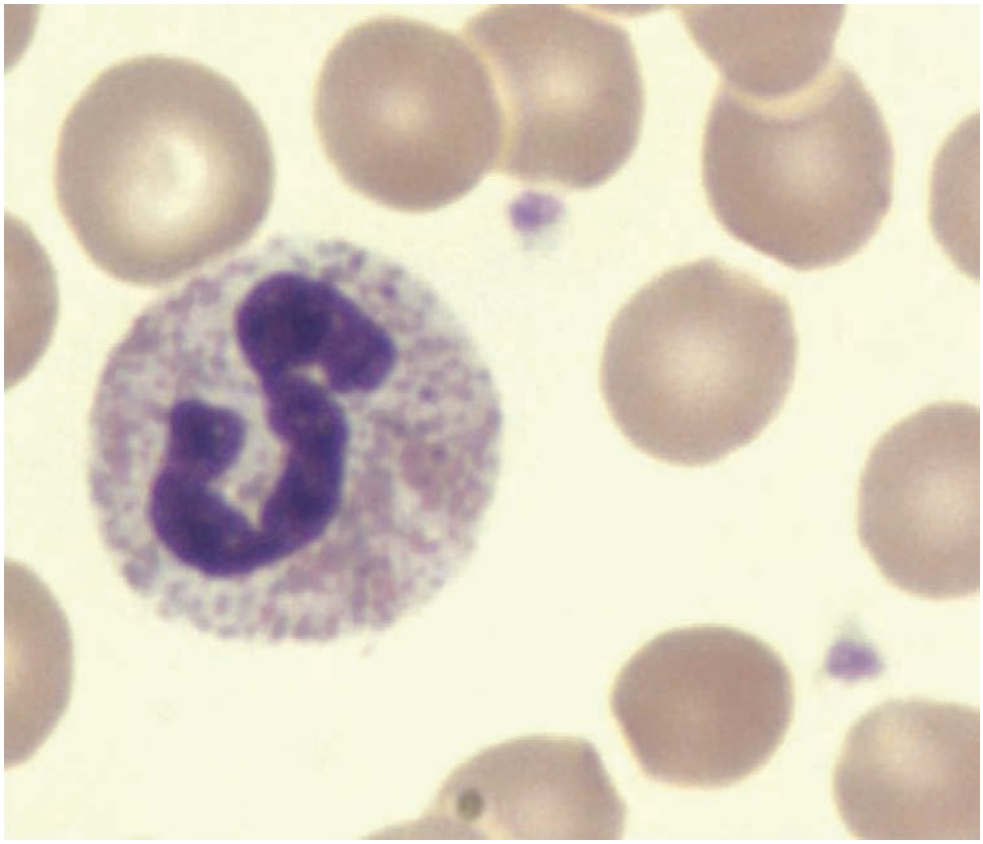

**FIGURE 2-9**

Normal platelet morphology. Normal platelets on a Wright-stained peripheral blood smear. Note the small size of the platelets (approximately 2-μm diameter) compared to the large erythrocytes. The purple granular staining corresponds to α-granules in the platelet cytoplasm (Wright stain, original magnification ×100).

True thrombocytopenia must be distinguished from pseudothrombocytopenia or spurious in vitro platelet clumping. Pseudothrombocytopenia is often caused by cold-reacting platelet agglutinins that may be seen in patients with high immunoglobulin levels or infections; they usually bind platelets only when calcium is chelated, such as in an ethylenediamine tetraacetic acid blood collection tube. Pseudothrombocytopenia associated with the GP IIb/IIIa antagonist drug abciximab has also been reported. Pseudothrombocytopenia can be diagnosed by examining a peripheral smear, where large aggregates of platelets are observed, often around the feathered edge (Figure 2-10). Pseudothrombocytopenia is often accompanied by platelet satellitism, in which platelets are bound to the cytoplasmic membranes of neutrophils (Figure 2-11). A more accurate platelet count can be established by collecting the blood sample in either citrate or heparin anticoagulants or by collecting blood directly from a finger stick into a diluent.

Some platelet disorders can be associated with unique platelet and/or leukocyte morphology. Giant platelets are seen in Bernard-Soulier syndrome; other macrothrombocytopenia syndromes associated with myosin heavy chain gene defects (MYH9) additionally may have Döhle body–like neutrophil inclusions. Immunostains for nonmuscle myosin heavy chain α may be useful to show abnormal subcellular localization in neutrophils from patients with *MYH9* mutations. In patients with Wiskott-Aldrich syndrome, the platelets may be small. Platelets in the gray platelet syndrome, an α-granule deficit, are characteristic for being pale, gray, and hypogranular on a Wright-stained blood smear. Some platelet storage pool disorders (SPDs) may have morphologically normal platelets by light microscopy, but may have decreased α-granules or dense granules, or both, shown by electron microscopy.

## BONE MARROW EXAMINATION

Examination of the bone marrow can be performed to evaluate the cause of both thrombocytopenia and thrombocytosis, but it has little role in the evaluation of platelet dysfunction with a normal platelet count. A bone marrow aspirate and biopsy may be helpful to ascertain whether thrombocytosis is due to reactive or myeloproliferative disorders. Megakaryocytes are located in the central areas of the biopsy specimen between bony trabeculae adjacent to sinusoids. Increased and clustered megakaryocytes are seen in myeloproliferative disorders, whereas micromegakaryocytes or hypolobate megakaryocytes suggest a

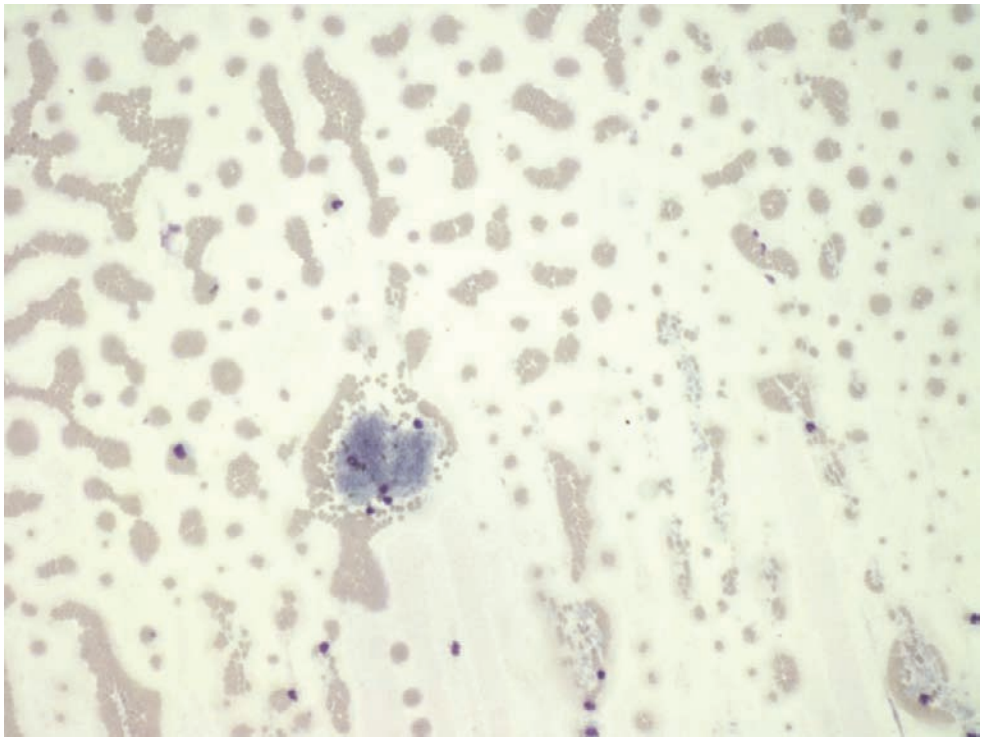

**FIGURE 2-10**

Pseudothrombocytopenia. The peripheral smear with pseudothrombocytopenia usually shows large clumps of platelets along the feathered edge or sides of the smear (Wright stain, original magnification ×10).

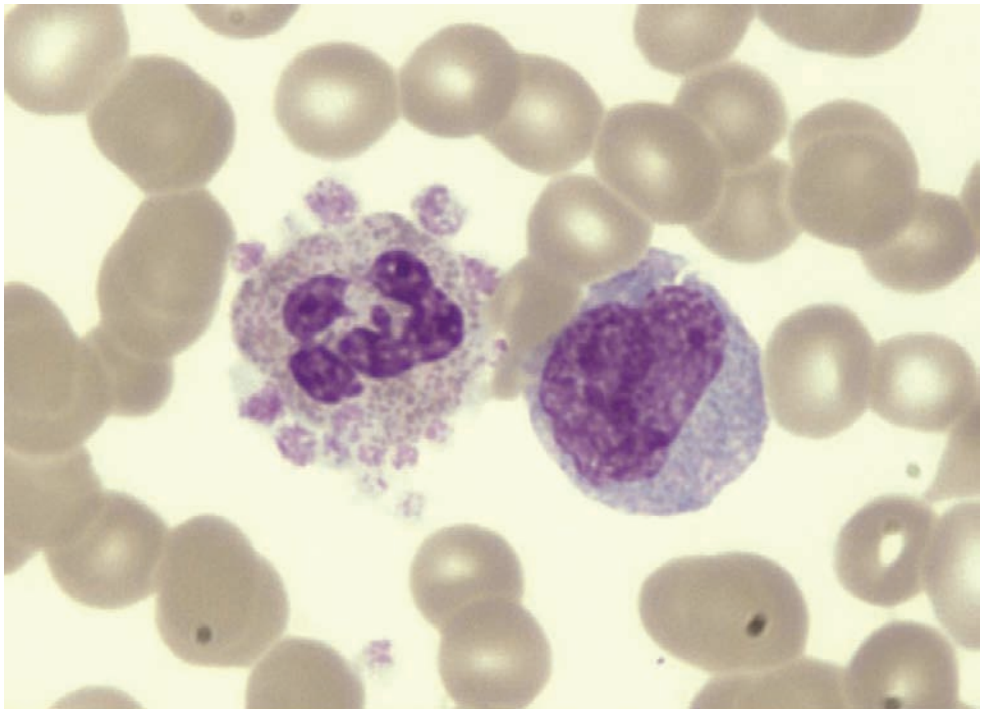

**FIGURE 2-11**

Platelet satellitism. This peripheral smear shows prominent decoration of neutrophil membranes by platelets (Wright stain, original magnification ×100).

myelodysplastic process. Because of their large size, the full thickness of megakaryocytes not represented in the thin biopsy sections, so care should be taken before diagnosing nuclear hyposegmentation based on the biopsy alone.

In a patient with thrombocytopenia, when no other reason for low platelet counts can be determined, examination of the bone marrow biopsy is useful for determining the presence or absence of megakaryocytes. Absence indicates dysfunctional marrow and increased numbers suggest peripheral destruction with attempted bone marrow compensation. Bone marrow examination can also detect myelophthisic disorders, such as acute leukemia, lymphoma, or metastatic malignancy as a cause for thrombocytopenia.

## PLATELET FUNCTION SCREENING TESTS OR BLEEDING TIME

In the initial evaluation of platelets, it is desirable to perform a screening test to evaluate platelet function. However, caution must be exercised in interpreting platelet function testing because abnormal results are often observed in thrombocytopenic patients; distinction of an abnormal result caused by intrinsic platelet dysfunction from thrombocytopenia alone may not be possible.

For nearly a century, the bleeding time test was the only platelet function screening test available. The bleeding time is a test that involves the creation of a cut in the skin and measurement of the time it takes for bleeding to stop, but is fraught with variability. The bleeding time test result depends not only on platelet number and function, but also on fibrinogen concentration, adequate vascular function, orientation, size of the incision, site of the incision, skin quality, skin temperature, operator technique, and patient cooperation. The bleeding time test has been demonstrated to be poorly reproducible with poor sensitivity and specificity for detection of the milder platelet dysfunctions and lack of ability to predict intraoperative hemorrhage. For these reasons, many laboratories have stopped performing the bleeding time test.

Newer, semiautomated whole blood platelet function screening assays are gaining popularity as an initial screen for platelet function. Most of these assays are small, stand-alone devices that measure platelet function in whole blood and can be used in laboratories that otherwise could not perform platelet function studies. These include the PFA-100 (Platelet Function Analyzer; Siemens USA, Washington DC), the VerifyNow (Accumetrics, San Diego, Calif.), the Plateletworks (Helena, Beaumont, Tex.), and the IMPACT (Diamed, Yokneaam, Israel). The thromboelastograph (TEG) (Haemonetics, Braintree, Mass.), while not a new technology, has been adapted recently for use in the clinical coagulation laboratory; it measures a combination of coagulation, platelet function, and fibrinolysis.

The PFA-100 is a device that measures shear-induced, platelet-related primary hemostasis in a citrated whole blood specimen (Figure 2-12). The disposable cartridges contain a membrane coated with aggregation agonists (collagen/epinephrine or collagen/ADP); the membrane has a central aperture (147 μm). Blood is sampled from a reservoir through the aperture at high shear rates (5000 to 6000 $s^{-1}$). The instrument measures the closure time required for platelets to adhere to the membrane, activate, aggregate, and occlude the aperture. Recent Clinical and Laboratory Standards Institute (CLSI) guidelines for platelet testing have been published that include the PFA-100. The test is sensitive to hematocrit, platelet count, and VWF; it is insensitive to coagulation disorders. VWD, intrinsic platelet dysfunction, and nonaspirin drugs may give an abnormal closure time with both cartridges. In distinction, aspirin-like drugs give a normal closure time with the collagen–ADP cartridge because of a high ADP concentration in the cartridge. Severe platelet disorders, such as Glanzmann thrombasthenia and Bernard-Soulier syndrome, give markedly prolonged closure times (typically greater than 300 seconds), but the device is not as sensitive to milder disorders, such as storage pool disorders or signaling defects. The PFA-100 results can be affected by low platelet counts and low hematocrits, but they are not affected by heparin.

The VerifyNow, a rapid platelet function assay, is an automated turbidimetric whole blood assay designed to assess platelet aggregation, based on the ability of activated platelets to bind fibrinogen. Agonist-activated platelets and fibrinogen-coated polystyrene microparticles agglutinate in whole blood in proportion to the number of available platelet GP IIb/IIIa receptors, making this device dedicated to the measurement of anti-platelet drug effect rather than screening for intrinsic platelet dysfunction. The VerifyNow IIb/IIIa is designed to measure specifically the effect of GP IIb/IIIa antagonist drugs. Use of cartridges with different agonists allows distinction of antiplatelet drug effects, such as those of aspirin and clopidogrel. The VerifyNow Aspirin is a cartridge designed to aid in detecting platelet dysfunction owing to aspirin therapy using an arachidonic acid agonist, with results reported in aspirin reaction units. A similar cartridge, VerifyNow P2Y12, is designed to detect the inhibition of platelet function by thienopyridines, such clopidogrel and prasugrel, using an ADP agonist.

Plateletworks is a new technology designed to determine platelet aggregation in fresh whole blood samples collected into agonist-containing blood tubes. It uses a cell counter to measure the change in the platelet count caused by ADP or collagen-induced aggregation of functional platelets in the blood sample. This is the first

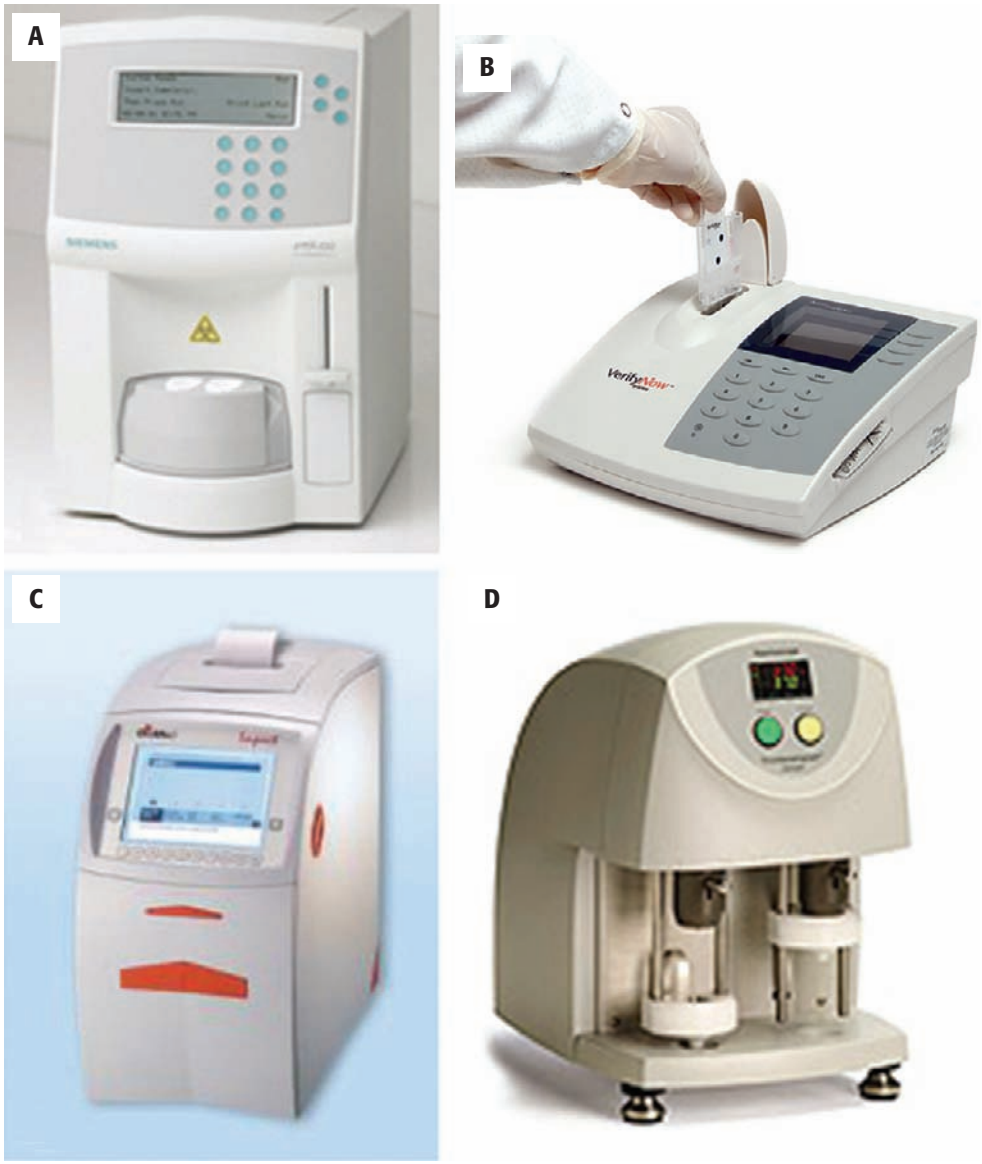


**FIGURE 2-12**

Platelet testing devices. **A,** Platelet Function Analyzer (PFA)-100 (Siemens USA, Washington DC). **B,** VerifyNow (Accumetrics, San Diego, Calif.). **C,** IMPACT (Diamed, Yokneaam, Israel). **D,** Image of the TEG® hemostasis analyzer is used by permission of Haemoscope Corporation.

test to simultaneously measure both platelet count and platelet aggregation. The results are highly time dependent, requiring testing within 10 to 30 minutes. Plateletworks has been studied mostly for the measurement of antiplatelet drug effects; there is little information to assess the utility of this technique in the diagnosis of platelet dysfunction.

The IMPACT, a modified cone and plate viscometer, measures shear-induced platelet adhesion and aggregation to a rotating polystyrene cup via a charge-coupled device camera. Image analysis can measure parameters such as surface coverage and a distribution histogram of adherent platelets. Platelet adhesion in this system is dependent on GP Ib, GP IIb/IIIa, VWF, and fibrinogen.

The TEG is a whole blood device that measures the viscosity of blood clotting and is sensitive to coagulation proteins, platelets, and fibrinolysis. The maximal amplitude (MA) is a parameter dependent on platelet number, function, and fibrinogen concentration, but has limited sensitivity to antiplatelet drugs. The platelet mapping TEG is a modification of the thromboelastograph that measures the viscoelastic effect of platelet function by comparing MA performed with kaolin activation and MA performed with an agonist to MA activated with reptilase and factor XIIIa. The platelet mapping TEG has been studied more thoroughly for measurement of antiplatelet drug effect than detection of intrinsic platelet dysfunction.

## PLATELET AGGREGATION

Platelet aggregation measures the ability of agonists to cause in vitro platelet activation and platelet–platelet binding. As such, platelet aggregation is often useful to distinguish intrinsic platelet disorders involving surface glycoproteins, signal transduction, and platelet granules. Platelet aggregation studies can be performed in whole blood by an impedance technique or in platelet-rich plasma by a turbidimetric technique, often called *light transmission aggregometry*. Platelet aggregation can be combined with studies of dense granule ATP release with a lumiaggregometer. Many factors can affect the platelet aggregation results, such as thrombocytopenia, thrombocytosis, processing temperature, stirring rate, and processing time (testing should be completed within 4 hours of phlebotomy). In addition, clinicians ordering the tests should advise patients to discontinue, if possible, any medication, such as aspirin or nonsteroidal antiinflammatory agents that might interfere with assessment of the test results.

In the light transmission aggregometry assay, whole blood is centrifuged to yield platelet-rich plasma, which is stirred in a cuvette at 37° C. Platelet aggregation is measured spectrophotometrically by the increase in light transmission after addition of an aggregation agonist. The agonists typically used include ADP, collagen, arachidonic acid, epinephrine, and occasionally thrombin receptor antagonist peptide or the thromboxane analog U46619 (Figure 2-13, A). The laboratory performance of platelet aggregation has been highly varied, and recent CLSI and North American guidelines have been published in an attempt to standardize testing methodologies and interpretation. Optimal platelet aggregation shows a biphasic pattern for the agonists ADP and epinephrine; the initial increase in aggregation is due to primary aggregation in response to activation of the GP IIb/IIIa platelet membrane receptor, whereas the second wave of aggregation is the result of platelet degranulation with recruitment of additional platelet aggregates. At higher agonist concentrations, the two waves merge into one wave of aggregation. Other agonists, such as arachidonic acid, thrombin receptor agonists, and collagen, usually show only a single wave of aggregation. Collagen characteristically shows an initial shape change before the wave of aggregation; this is seen as a transient increase in turbidity.

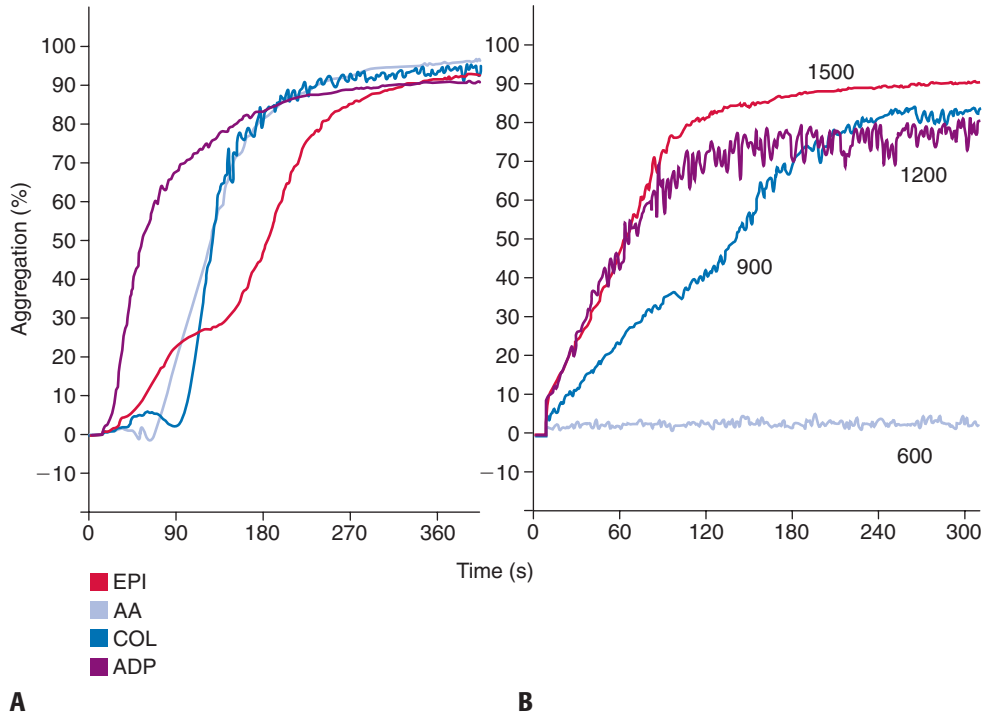


**FIGURE 2-13**

Normal light transmission platelet aggregation tracings. **A,** Platelet aggregation with 5 μmol/L adenosine diphosphate (ADP; *magenta*), 100 μmol/L epinephrine (EPI; *red*), 2 mg/mL collagen (COL; *blue*), and 0.5 mg/mL arachidonic acid (AA; *lavender*). At this concentration, ADP shows two waves of aggregation. After the initial wave of aggregation, platelet ADP release from dense granules stimulates the final and irreversible wave of aggregation. Collagen aggregation characteristically shows an initial shape change. Normal aggregation for all agonists is more than 70% aggregation. **B,** Platelet aggregation with several concentrations of ristocetin. Ristocetin stimulates a conformational change in von Willebrand factor, leading to aggregation through the glycoprotein Ib/IX/V complex. Note that aggregation is virtually absent at low ristocetin concentration (600 μg/mL), becoming progressively stronger until complete aggregation is reached somewhere between 1200 and 1500 μg/mL (optical platelet aggregation using a Helena PACKS-4 aggregometer).

Ristocetin-induced platelet aggregation (RIPA) utilized the antibiotic ristocetin, which facilitates the binding of VWF to the GP Ib/IX/V complex (see Figure 2-13, B). Addition of escalating concentrations of ristocetin to platelet-rich plasma allows detection of both increased and decreased sensitivity to ristocetin. A normal result requires the presence of both functional VWF and normal GP Ib/IX/V, so that RIPA can detect both VWD and some platelet dysfunctions, such as Bernard-Soulier syndrome.

## COAGULATION TESTING AND VON WILLEBRAND ASSAYS

Most types of intrinsic platelet dysfunction do not directly affect coagulation proteins; however, the laboratory evaluation of platelet dysfunction should also include some basic coagulation assays, such as the prothrombin time (PT) and activated partial thromboplastin time (APTT), to exclude a coagulopathy as the reason for bleeding.

VWD is due to decreased levels or defective function of VWF. Because VWF is a protein involved in platelet adhesion, it is often considered in the differential diagnosis of bleeding disorders with abnormal platelet function screening tests. One subtype, platelet-type VWD, is actually a platelet defect caused by an abnormality of GP Ib leading to increased binding to VWF. VWD is grouped into three general categories: types 1, 2, and 3. Type 1 is a quantitative defect of VWF, type 2 is a qualitative defect, and type 3 is a severe, recessive defect with absent VWF. The pattern of laboratory testing that suggests VWD is a normal or increased APTT, an abnormal platelet function screen, a normal platelet count, and variably decreased (depending upon the subtype) VWF antigen, decreased ristocetin cofactor activity, decreased RIPA, and decreased factor VIII.

## FLOW CYTOMETRY

Flow cytometry is used to study platelet structure and function, but this technique is used in only specialized centers. Flow cytometric analysis is based on the detection of cell surface proteins with fluorescently labeled antibodies. It has been used in the detection of platelet activation by using antibodies to proteins newly expressed on the platelet surface during activation, such as P-selectin and thrombospondin, or by detecting new epitopes on GP IIb/IIIa induced by binding fibrinogen (ligand-induced binding sites). Platelet flow cytometry can be used to diagnose deficiencies of platelet surface glycoproteins, such as GP IIb/IIIa in patients with Glanzmann thrombasthenia; GP Ib, GP V, and GP IX in patients with Bernard-Soulier syndrome; GP Ia, GP IIa, and GP VI in collagen receptor deficiencies; and P2Y1 and P2Y12 in ADP receptor deficiency. In some centers, quantitative flow cytometry is performed to quantify receptor numbers on the platelet surface. Flow cytometric methods can be combined with the use of platelet agonists to measure dense granules (mepacrine uptake or release), α-granule release, aggregation, microparticle formation, and platelet procoagulant activity.

Another use of flow cytometry is in the detection of platelet-associated immunoglobulins. These immunoglobulins are seen in patients with inhibitors to various surface glycoproteins and in patients with immune thrombocytopenic purpura (ITP) and drug-induced thrombocytopenias. Simple measurement of platelet-associated immunoglobulin is sensitive but nonspecific. The test can be made more specific for drug-induced antibodies by incubating the platelets in the presence of the drugs in question or by using activation-dependent tests, such as $^{51}Cr$ release or $^{14}C$ serotonin release. Antigen-capture assays, such as monoclonal antibody immobilization of platelet antigens, have improved specificity further by being able to detect antibody binding to specific platelet surface glycoproteins.

Platelets with increased RNA content (reticulated platelets) can be measured by flow cytometry using the dye thiazole orange, which binds to RNA and DNA. This technique is gaining acceptance as a diagnostic tool to distinguish thrombocytopenia caused by increased platelet destruction from decreased platelet production, because platelets newly released from bone marrow have increased RNA content. This principle has been adapted to some automated cell counters, with reporting of an immature platelet fraction.

## ELECTRON MICROSCOPY

Electron microscopy can be used for the ultrastructural evaluation of platelets, particularly in patients with suspected SPDs in which a decrease or absence of dense bodies is observed (see Figure 2-3). Giant platelet disorders also have characteristic electron microscopic findings.

## OTHER DIAGNOSTIC TECHNIQUES

An additional technique to study the presence of storage and release defects is measurement of the ratio of ADP to ATP within platelets by high-pressure liquid chromatography. Dense granule defects can also be studied by lumiaggregometry, where ATP released from dense granules is measured by a firefly luciferin-luciferase reagent simultaneously with aggregation. The Québec platelet disorder is due to a cis regulatory defect linked to the urokinase plasminogen activator (uPA) gene

**TABLE 2-1**
**Identified Genetic Disorders Associated with Platelet Disorders**

| Syndrome | Affected Gene And Chromosome Location |
|---|---|
| ***Defects of platelet production*** | |
| GATA1-related thrombocytopenia and erythroid defects | *GATA1*, Xp11.23 |
| Paris-Trousseau and Jacobsen syndromes | *FLI-1*, 11q23 |
| Familial platelet disorder with acute myelogenous leukemia | *RUNX1*, 21q22 |
| Amegakaryocytic thrombocytopenia with radioulnar synostosis | *HOXA11*, 7p15-14 |
| Congenital amegakaryocytic thrombocytopenia | *c-MPL*, 1p34 |
| Thrombocytopenia and absent radii | Large deletion of 1q21.1 |
| ***Defects of platelet surface receptors*** | |
| Bernard-Soulier syndrome | *GPIBA* (17p13), *GPIBB* (22q11), *GP9* (3q21) |
| Platelet-type von Willebrand disease | *GPIBA*, 17p13 |
| Mediterranean macrothrombocytopenia | *GPIBA*, 17p13 |
| Glanzmann thrombasthenia | *ITGA2B, ITGB3* |
| Scott syndrome | *ABCA1* |
| Collage receptor disorders | *ITGA2, GP6* |
| ADP receptor disorders | *P2Y12* |
| ***Secretion and organelle biogenesis*** | |
| Hermansky-Pudlak syndrome | *HPS1 to HPS8* |
| Chediak Higachi syndrome | *CHS* |
| ***Cytoskeletal and signaling pathways*** | |
| Myosin heavy chain (MYH9) disorders | *MYH9*, 22q12-13 |
| Wiskott-Aldrich syndrome (and X-linked thrombocytopenia) | *WAS*, Xp11.23-p11.22 |
| Others | *GSN* (gelsolin), *TBXAS1* (thromboxane synthase), *COX1* (cyclooxygenase1) |

leading to increased uPA. Western blot analysis showing abnormal expression of uPA and secondary proteolysis of platelet α-granule proteins can be detected by this technique. Future platelet studies will likely include investigation of platelet signaling pathways, because many platelet disorders appear to involve dysregulation of signaling pathway. Genomic approaches will also be used to identify specific inherited platelet defects (Table 2-1).

## DIAGNOSTIC CATEGORIES OF PLATELET BLEEDING DISORDERS

The initial evaluation of platelet bleeding disorders entails performing a complete blood cell count (CBC) and evaluating a peripheral smear; therefore this review divides platelet-mediated bleeding disorders into several categories, based on the typical approach by a pathologist investigating the nature of the platelet disorder. They have been grouped into disorders associated with an increased, decreased, or normal platelet count. Some categories are then further subdivided by platelet morphology with giant platelets, normal-size platelets, and small platelets.

## PLATELET DISORDERS WITH THROMBOCYTOSIS

Patients with elevated platelet counts may have clinical bleeding, but may also be asymptomatic or have thrombosis. Laboratory evaluation should be aimed at elucidating the cause of the thrombocytosis and should include a CBC, peripheral blood smear, bone marrow evaluation, cytogenetic study, and platelet aggregation study. The differential diagnosis is between a primary thrombocytosis or a secondary (reactive) thrombocytosis. Primary thrombocytosis can be seen in familial disorders and with the myeloproliferative neoplasms (essential thrombocythemia [ET], chronic

myelogenous leukemia (CML), polycythemia vera, and primary myelofibrosis, or some myelodysplastic processes (5q-).

## PRIMARY THROMBOCYTOSIS

### Clinical Features

Primary familial thrombocytosis has been described in association with mutations in *TPO*, the thrombopoietin gene, and in MPL, the thrombopoietin receptor. These patients have thrombocytosis with few clinical symptoms and do not progress to acute leukemia. Myeloproliferative neoplasms (MPNs) associated with thrombocytosis include CML, ET, primary myelofibrosis, and polycythemia vera. A large proportion of patients with non-CML MPNs have a mutation of the Jak2 tyrosine kinase, JAK2-V617F, through which the Epo-R, Mpl, and Gcf-R receptors signal. The distinction between primary familial thrombocytosis and MPN is becoming less distinct, because MPL mutations can be seen in some cases of MPN and a hereditary component may be a contributing factor in patients with sporadic MPN. MPNs are associated with thrombocytosis, accompanied by thrombosis or bleeding, or both, with progression to acute leukemia or marrow failure, or both, and fibrosis. See Chapter 17 for further discussion of the clinical features of these disorders.

### Pathologic Features

In general, patients with primary thrombocytosis often have platelet counts greater than $1000 \times 10^3/\mu L$, and patients with secondary thrombocytoses have counts less than this; however, there is a great deal of overlap, and platelet count alone cannot distinguish between these categories. In general, the peripheral smear in MPNs will show a mixture of platelet size and platelet morphology. Occasional degranulated, giant platelets or megakaryocyte fragments are observed. The morphologic features of MPNs are covered in detail in Chapter 17, but the platelet features of the myeloproliferative neoplasms are discussed here.

### Ancillary Studies

Platelet abnormalities have been described in all MPNs, but they are best studied in ET. Platelet testing is not needed for diagnosis, which is based on bone marrow and cytogenetic studies with demonstration of JAK2-V617F in the majority of patients. Aggregation studies in patients with MPNs are often abnormal, but the patterns observed are neither specific nor diagnostic. ET can be associated with an isolated defect in epinephrine-induced aggregation (Table 2-2). The decreased epinephrine-induced aggregation is thought to be due to downregulation of α2-adrenergic receptors. Other patterns of platelet dysfunction with MPNs include decreased platelet aggregation to ADP or collagen, dense granule storage pool pattern, abnormalities of the arachidonic acid pathway, and decreased receptors for fibrinogen, VWF, or prostaglandin D2. In the clinical evaluation of patients with MPNs, it is important to remember that both bleeding and thrombosis can be observed and that the results of the platelet function tests will not necessarily distinguish whether the patient is at risk of bleeding or thrombosis. Bleeding complications are more frequently observed in patients with a platelet count greater than $10 \times 10^3/\mu L$, and this may be due to an acquired deficiency of VWF by adsorption to the platelets.

### Differential Diagnosis

Abnormal platelet aggregation, when observed, may be helpful in distinguishing myeloproliferative from familial or reactive thrombocytosis, but it is neither diagnostic nor specific. Family studies demonstrating thrombocytosis in other first-degree relatives should prompt a search for familial thrombocytosis. A decreased aggregation response to epinephrine is also observed in patients with a congenital defect of the α2-adrenergic receptors, but these patients usually have a normal platelet count. In general, patients with MPNs have a higher PDW than do patients with reactive thrombocytosis, but there is a great deal of overlap. The MPV is usually unhelpful in distinguishing these two classes of disorders. The presence of the *JAK2-V617F* mutation indicates an MPN, whereas mutations of TPO or MPL suggest a familial thrombocytosis, although there is some overlap.

## SECONDARY THROMBOCYTOSIS

### Clinical Features

A reactive, or secondary, thrombocytosis is not a single disease entity and can be associated with many clinical disorders, such as iron deficiency and inflammatory and infectious diseases, malignancies, such as carcinomas or lymphomas, smoking, or exercise. It can also be observed as a rebound thrombocytosis after splenectomy, or during treatment for ITP or pernicious anemia, or after cessation of myelosuppressive drugs.

**TABLE 2-2**
**Aggregation Characteristics**

| Disorder | ADP 1° | ADP 2° | AA | EPI | Collagen | Ristocetin | Other Studies |
|---|---|---|---|---|---|---|---|
| ***Surface glycoprotein disorders*** | | | | | | | |
| Bernard Soulier syndrome | N | N | N | N | N | ↓ or absent | Macrothrombocytopenia<br>Deficiency of GP Ib/IX/V (one or more) by flow cytometry, mutation of *GPIBA, GPIBB, GP9* |
| Glanzmann thrombasthenia | ↓ or absent | ↓ or absent | ↓ or absent | ↓ or absent | ↓ or absent | N | Deficiency of GB IIb and/or GP IIIa by flow cytometry, mutation of *ITGA2B, ITGB3* |
| Platelet-type VWD | N | N | N | N | N | ↑ | Loss of HMW VWF multimers, gain of function mutation of *GPIBA* |
| VWD, other | N | N | N | N | N | ↓, N, or ↑ | FVIII:C, VWF:Ag, VWF:RiCOf, VWF multimers |
| ADP receptor | ↓ | ↓ | N | N or ↓ | N or ↓ | N | Gene defects of *P2Y12* |
| GP Ia/IIa or GP VI disorder | N | N | N | N | ↓ | N | Mutations of GP Ia/IIa or GP VI genes *(ITGA2, GP6)*<br>Decreased adhesion to collagen |
| ***Storage pool disorders*** | | | | | | | |
| Dense granule platelet storage pool disorder (δ-SPD) | N | ↓ | N | N or ↓ | N or ↓ | N | Decreased ATP release by lumiaggregometry<br>↓ dense granule mepacrine uptake and release by flow cytometry<br>Decreased dense granules by TEM<br>Abnormally high ATP:ADP ratio<br>Acquired SPD exhausted platelets (CPB, DIC, TTP, HUS, MPN)<br>Albinism in Hermansky-Pudlak and Chédiak-Higashi syndrome<br>Infections, small platelets seen with Wiskott-Aldrich syndrome |
| Alpha (α)-SPD | Var | Var | N | N | Var ↓ | N | Pale platelets on smear, ↓ α-granules by TEM, ↓ P-selectin |
| ***Activation disorders*** | | | | | | | |
| Signal transduction disorders | N | ↓ | Var ↓ | Var ↓ | Var ↓ | N | Decreased granule release with normal numbers of granules<br>↓ G-protein activation, phospholipase C activation, calcium mobilization, pleckstrin or tyrosine phosphorylation |
| Scott syndrome | N | N | N | N | N | N | Defective platelet procoagulant activity<br>Defective microparticle formation |
| Defects of thromboxane synthesis or aspirin-like drug | N | ↓↓ | ↓ or absent | ↓ | ↓ or absent | N | Normal aggregation with prostaglandin G2 seen with aspirin or cyclooxygenase deficiency<br>Decreased prostaglandin G2 aggregation with thromboxane synthetase deficiency |
| ***Other disorders with platelet dysfunction*** | | | | | | | |
| Myeloproliferative neoplasm | N | N | N | ↓ or absent | N | N | Other abnormalities: α- or D-SPD, cyclooxygenase abnormality, other surface GP derangements, ↓ or ↑ aggregation to ADP, collagen, spontaneous aggregation |
| Uremia | N | ↓ | ↓ | N/↓ | N/↓ | N | Abnormal creatinine, BUN<br>Decreased PF3 |
| ***Drug effects*** | | | | | | | |
| Thienopyridines | ↓ | Absent | N/↓ | N/↓ | N/↓ | N | Clopidogrel or prasugrel therapy |
| GP IIb/IIIa antagonists | ↓ or absent | ↓ or absent | | ↓ or absent | ↓ or absent | N | History of abciximab, tirofiban, or eptifibatide |
| Aspirin | N/↓ | ↓↓ | ↓↓ or absent | ↓ | ↓ | N | |

*AA,* Arachidonic acid; *ADP,* adenosine diphosphate; *ATP,* adenosine triphosphate; *BUN,* blood urea nitrogen; *CPB,* cardiopulmonary bypass; *DIC,* disseminated intravascular coagulation; *EPI,* epinephrine; *GP,* glycoprotein; *HMW,* high-molecular-weight; *HUS,* hemolytic uremic syndrome; *MPN,* myeloproliferative neoplasm; *N,* normal; *SPD,* storage pool disease; *TEM,* transmission electron microscopy; *TTP,* thrombotic thrombocytopenic purpura; *VWD,* von Willebrand disease; *VWF,* von Willebrand factor.

### Pathologic Features and Laboratory Studies

Patients with reactive thrombocytosis have an elevated platelet count, but the degree of elevation is variable and the count may be greater than $10 \times 10^3/\mu L$. A peripheral smear usually shows an increased number of platelets with normal morphology, although the MPV may be increased. The remainder of the CBC is usually normal. The degree of thrombocytosis may often increase or decrease in conjunction with the leukocyte count in inflammatory disorders and malignancy, signaling that it is an acute-phase reactant. Bone marrow examination may show increased numbers of megakaryocytes, but specific features associated with myeloproliferative or myelodysplastic disorders are not identified.

Reactive thrombocytoses are usually associated with normal platelet aggregation studies. In contrast to myeloproliferative disorders, cytogenetic studies do not show a clonal process; there is absence of the *BCR-ABL* translocation and *JAK2 V617F, MPL,* and *TPO* mutations.

### Differential Diagnosis

Reactive thrombocytosis is a diagnosis of exclusion, and other neoplastic and familial disorders should be ruled out before making this diagnosis. There is some suggestion that patients with reactive thrombocytosis have elevated levels of interleukin-6 or C-reactive protein, whereas patients with MPN have normal levels. Thrombocytosis can often accompany iron deficiency; evaluation of serum iron studies together with the characteristic microcytic, hypochromic erythrocyte indices on the CBC can usually diagnose iron deficiency and obviate the need for a bone marrow evaluation.

## PLATELET DISORDERS WITH THROMBOCYTOPENIA

Disorders in which the platelet count is decreased can be congenital or acquired, but they have been grouped by platelet size in this discussion, because platelet size is one of the first distinguishing features appreciated by the evaluating pathologist.

## THROMBOCYTOPENIA WITH INCREASED PLATELET SIZE (MACROTHROMBOCYOPENIAS)

The rare macrothrombocytopenia disorders are all congenital in nature, and most are inherited in an autosomal dominant fashion. They are usually due to congenital defects in platelet production by megakaryocyte or demarcation membrane systems. Some patients with acquired platelet destruction and turnover, such as ITP, may have high MPVs because of the rapid release of new platelets, but in general the macrothrombocytopenia syndrome platelets are much larger and more uniform in size.

## MACROTHROMBOCYTOPENIAS WITH NEUTROPHILIC INCLUSIONS (MYH9 DISORDERS)

### Clinical Features

Mutations in the *MYH9* gene encoding for the nonmuscle myosin heavy chain IIA result in a spectrum of macrothrombocytopenic disorders with neutrophil inclusions that are now referred to as *MYH9* disorders, formerly called May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome. Clinically, these disorders are characterized by a mild bleeding diathesis with large platelets and neutrophils

**MACROTHROMBOCYTOPENIAS WITH NEUTROPHILIC INCLUSIONS (*MYH9* DISORDERS)—FACT SHEET**

**Definition**
- Macrothrombocytopenia owing to a congenital disorder of myosin heavy chain IIA gene
- Disorder includes previously described May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome (now called *MYH9* disorder)

**Incidence**
- 1 per 500,000

**Morbidity and Mortality**
- Associated with mild bleeding diathesis and nephritis, deafness, cataracts

**Gender, Race, or Age Distribution**
- Autosomal dominant congenital disorder

**Clinical Features**
- Mutations in motor head region of myosin heavy chain II associated with more severe thrombocytopenia, nephritis, sensorineural hearing loss, ocular abnormalities
- Mutations in tail region associated with less severe macrothrombocytopenia and fewer nonhematologic complications

**Prognosis and Therapy**
- The mild bleeding disorder is not often associated with significant morbidity
- Appropriate treatment is required for the nephritis, deafness, and cataracts

with Dohle-like body inclusions, a ribonucleoprotein complex composed of aggregates of myosin IIA protein MYH9 mRNA and clusters of ribosomes. There are variable clinical findings of nephritis, sensorineural hearing loss, and cataracts. There is a genotype-phenotype correlation, with *MYH9* mutations in the motor head domain of myosin IIA having severe macrothrombocytopenia and deafness, nephritis and cataracts, whereas mutations in the tail domain have mild macrothrombocytopenia and low risk of nonhematologic complications.

## Pathologic Features and Laboratory Studies

*MYH9*-related macrothrombocytopenia disorders are characterized by a decreased platelet count with large platelets and the presence of neutrophilic inclusions, which can be observed on a peripheral smear (Figure 2-14). The Dohle-like bodies are blue, spindle-shaped inclusions in the periphery of the neutrophil cytoplasm. They may also be seen in monocytes, eosinophils, and basophils, unlike true Döhle bodies. These inclusions become gradually less stained after blood sampling, so there should be no delay when the peripheral smears are made. The thrombocytopenia is usually moderate, with platelet counts of 60 to $10 \times 10^3/\mu L$ being common, and the mean MPV is approximately 12.5 fL but is often much larger. Some caution should be exercised in interpreting CBCs performed on automated instruments in these patients, because some of the platelets are so large that they are outside of the platelet gate and could be counted as leukocytes. In general, smear evaluation will show a uniform population of large, normally granulated platelets. Bone marrow analysis is not generally indicated but will show increased megakaryocytes often surrounded by the production of large platelets.

Immunostains of the peripheral smear with an antibody against human nonmuscle myosin heavy chain IIA will show abnormal subcellular localization in leukocytes. In normal individuals, nonmuscle myosin heavy chain IIA is distributed diffusely, while in the *MYH9* disorders, nonmuscle myosin heavy chain IIA is associated with the neutrophil inclusions. Three patterns of inclusions have been described: types I, II, and III. In type I, there are one or two large, intensely stained granules. In type II, there are up to 20 small granules. In type III, there is speckled staining. The granule pattern correlates with the site of the *MYH9* mutation, with deletion, insertion, and nonsense mutations associated with a type I pattern and missense mutations associated with a type II pattern. Electron microscopic study of the platelets typically shows very large platelets with abundant α-granules (Figure 2-15).

Platelet aggregation and bleeding time studies are normal, attesting to the increased functionality of the larger platelets. Because of the large platelet size, centrifugation speeds for the preparation of platelet-rich plasma may have to be modified or many platelets may sediment with the leukocytes or erythrocytes. Platelet surface GPs are usually normal. The *MYH9* gene comprises 40 exons, so genetic analysis is complex and not routinely available. A genetic strategy with targeted mutational analysis of certain exons based on the immunofluorescent staining pattern is usually used. More

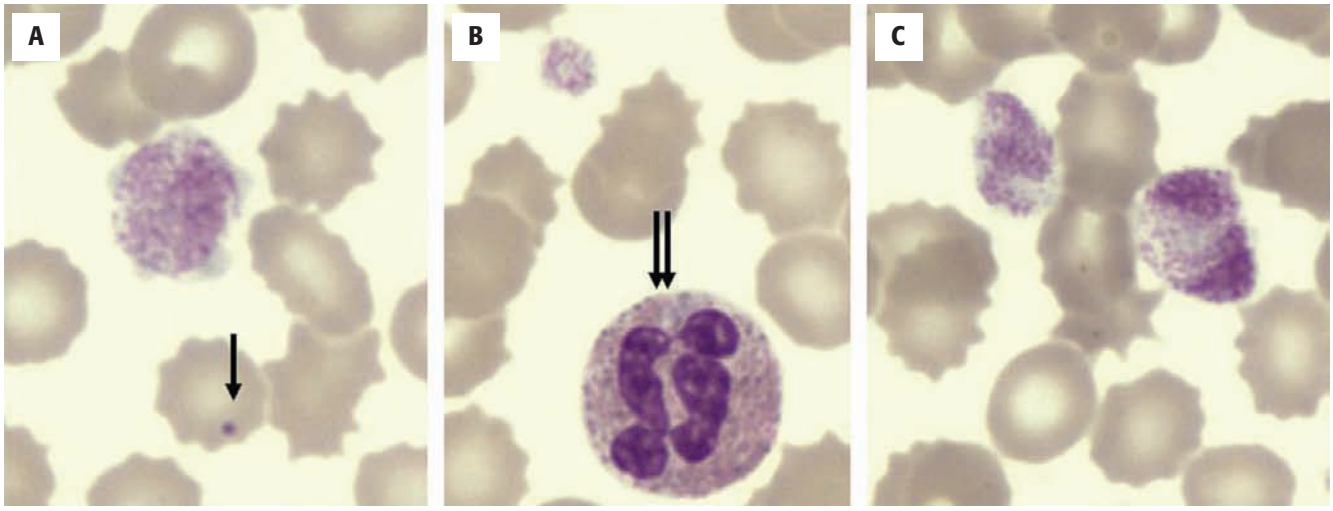


**FIGURE 2-14**

Peripheral smear in MYH9-associated macrothrombocytopenia. Note the large granular platelets that are nearly the size of erythrocytes. **A,** In this patient, Howell-Jolly bodies were observed in the erythrocytes, a consequence of a splenectomy at age 4 years for suspected immune thrombocytopenic purpura (ITP; *arrow*). **B,** The neutrophils show faint blue Döhle bodies *(double arrow).* **C,** Patients with these rare macrothrombocytopenias, associated with a genetic disorder of the myosin heavy chain gene *(MYH9),* may be thought to have ITP if the familial nature of the thrombocytopenia is not detected. (Wright stain, original magnification ×100.)

## MACROTHROMBOCYTOPENIAS WITH NEUTROPHILIC INCLUSIONS (MYH9 DISORDERS)—PATHOLOGIC FEATURES

### Complete Blood Cell Count and Peripheral Smear Morphology

- Moderate thrombocytopenia (60 to 100 × $10^3/\mu L$) with giant, normally granulated platelets
- Cytoplasmic inclusions in neutrophils

### Bone Marrow Findings

- Increased megakaryocytes with otherwise normal hematopoiesis
- The megakaryocytes are surrounded by production of large platelets

### Platelet Screening Tests

- Usually normal

### Platelet Aggregation

- Platelet aggregation is usually normal

### Ancillary Studies

- Abnormal subcellular localization of nonmuscle myosin heavy chain IIA in neutrophils; type I (few large granules), type II (up to 20 smaller granules), and type III (speckled) immunofluorescent staining patterns
- By electron microscopy, inclusions consist of an amorphous cluster of ribosomes oriented along parallel microfilaments and lack a limiting membrane

### Genetic Studies

- More than 40 *MYH9* mutations have been described, most (80%) being point mutations, such as exon 1 (Ser96), exon 16 (Arg 702), exon 26 (Arg1165), exon 30 (Asp1424), exon 38 (Glu1841), and exon 40 (Arg1933)

### Differential Diagnosis

- MYH9 disorders can be distinguished from other macrothrombocytopenias by the presence of mutations in *MYH9,* neutrophilic inclusions and abnormal localization of nonmuscle myosin heavy chain IIA in polymorphonuclear neutrophils

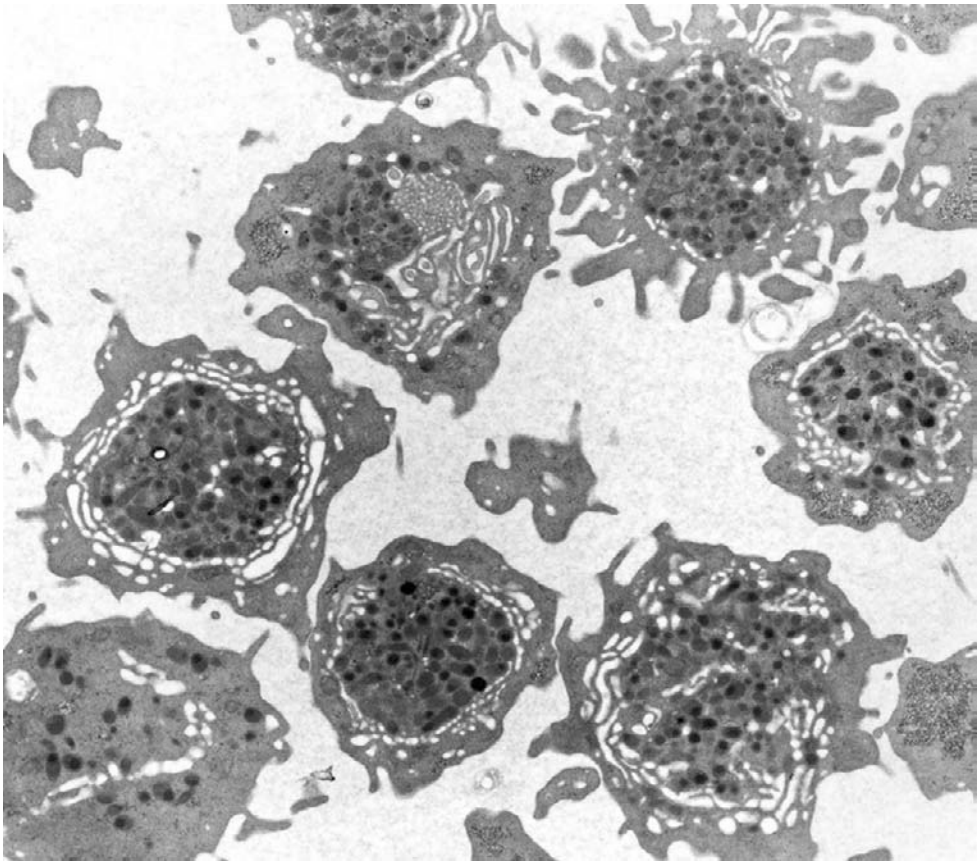

**FIGURE 2-15**

Electron micrograph of the large platelets in a patient with an MYH9 disorder. Note the large platelets with abundant granules and expanded open canalicular system. Compare the ultrastructural morphology here to the peripheral smear morphology in Figure 2-14 (original magnification ×5000).

than 40 distinct mutations have been described, with most (80%) being point mutations, such as exon 1 (Ser96), exon 16 (Arg 702), exon 26 (Arg1165), exon 30 (Asp1424), exon 38 (Glu1841), and exon 40 (Arg1933).

### Differential Diagnosis

The *MYH9* disorders can be distinguished from other macrothrombocytopenias by genetic abnormalities in *MYH9,* the presence of neutrophilic inclusions, and abnormal neutrophil localization of nonmuscle myosin heavy chain IIA.

### Prognosis and Therapy

The bleeding symptoms in the *MYH9* disorders are usually mild and are not often a source of significant morbidity or mortality. The thrombocytopenia may develop in utero and is a rare cause of fetal or neonatal intracranial hemorrhage. The morbidity in these disorders is more related to the severity of the renal abnormalities.

**BERNARD-SOULIER SYNDROME—FACT SHEET**

**Definition**
- Macrothrombocytopenia with a congenital deficiency of the platelet glycoprotein Iba/Ibb/IX/V receptor

**Incidence**
- Rare, less than 1 per 1 million

**Morbidity and Mortality**
- Moderate to severe bleeding disorder

**Gender, Race, or Age Distribution**
- Autosomal recessive congenital disorder; both males and females affected

**Clinical Features**
- Incompletely recessive autosomal trait
- Moderate to severe mucocutaneous bleeding
- May be associated with DiGeorge syndrome (deletion 22q11.2)

**Prognosis and Therapy**
- Routine therapy is not required.
- There is an increased bleeding risk during surgical procedures.
- Desmopressin platelet transfusions, or both, may help to prevent bleeding intraoperatively.

## BERNARD-SOULIER SYNDROME

### Clinical Features

Bernard-Soulier syndrome is a rare congenital deficiency of the platelet GP Ibα/Ibβ/IX/V receptor, the surface receptor for VWF-mediated platelet aggregation. It is usually classified as a macrothrombocytopenia disorder, with clinical findings of early onset bleeding symptoms, thrombocytopenia, decreased platelet adhesion, reduced platelet survival, and giant platelets. Most of the Bernard-Soulier genetic defects are due to mutations of *GPIBA* the GP Ibα gene, but they may also be due to defects of *GPIBB* and *GP9,* the GP Ibβ and GP IX genes, respectively. GP Ib is expressed on the demarcation membrane system in the megakaryocytes that are responsible for platelet fragmentation. The large platelets are thought to be due to the loss of interaction between actin-binding proteins in the platelet cytoskeleton and the GPIbα cytoplasmic domain. The disorder is inherited as an incompletely autosomal recessive trait with severe, lifelong mucocutaneous bleeding that is often out of proportion to the mild thrombocytopenia.

Defects in *GPIBB,* located on chromosome 22, are also associated with the more extensive genetic defects observed in the velocardiofacial or DiGeorge syndrome associated with a deletion in chromosome 22 (22q11.2). However, the *GPIBB* defect in these patients is usually heterozygous, and bleeding symptoms are uncommon. These patients may have associated velopharyngeal insufficiency, conotruncal heart disease, immunodeficiency, and learning disabilities.

### Pathologic Features and Laboratory Studies

Individuals with Bernard-Soulier syndrome typically have moderately severe thrombocytopenia (30 to 200 × $10^3$/μl), with uniformly large, granulated platelets. Platelets occasionally are the size of erythrocytes or lymphocytes, so automated platelet counts are often inaccurate. No neutrophil inclusions are present. This disorder has diagnostic surface GP abnormalities, so bone marrow analysis is not usually indicated.

Normal platelet aggregation is noted with exposure to ADP, collagen, epinephrine, and arachidonic acid, but aggregation is characteristically absent with the addition of ristocetin or botrocetin (Figure 2-16; see Table 2-2). Adhesion of platelets to subendothelium or immobilized VWF is markedly reduced at all shear rates; this finding

**BERNARD-SOULIER SYNDROME—PATHOLOGIC FEATURES**

**Complete Blood Cell Count and Peripheral Smear Morphology**
- Thrombocytopenia (30 to 100 × $10^3/\mu L$) with large, normally granulated platelets

**Bone Marrow Findings**
- Not indicated for diagnosis
- Normal megakaryocyte morphology

**Platelet Screening Tests**
- Markedly abnormal PFA-100

**Platelet Aggregation**
- Absent aggregation response to ristocetin and botrocetin
- Normal aggregation to ADP, epinephrine, collagen, and arachidonic acid
- Aggregation by α-thrombin may be abnormal

**Ancillary Studies**
- Abnormal shear-induced platelet adhesion
- Flow cytometry shows lack of platelet expression of GP Ib/IX
- Variant Bernard-Soulier syndrome may show partial expression
- Genetic mutations of *GPIBA, GPIBB,* and/or *GP9*

**Differential Diagnosis**
- The characteristic glycoprotein abnormalities are usually diagnostic
- Lack of neutrophil inclusions distinguishes Bernard-Soulier syndrome from MYH9 disorders
- Normal VWF studies exclude VWD

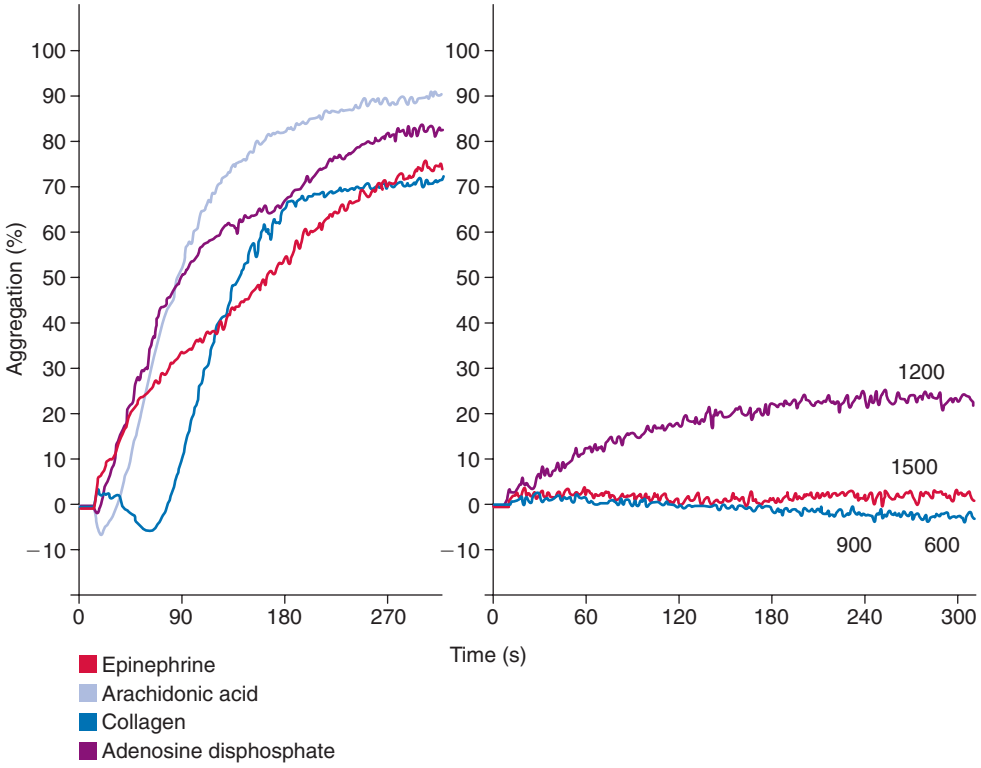


**FIGURE 2-16**

Platelet aggregation in Bernard-Soulier syndrome. The aggregation response to adenosine diphosphate, epinephrine, collagen, and arachidonic acid is preserved. However, the aggregation response to ristocetin (right panel), which facilitates aggregation through von Willebrand factor binding to glycoprotein Ib, is nearly absent (optical platelet aggregation using a Helena PACKS-4 aggregometer).

may have direct clinical consequences. Patients with Bernard-Soulier syndrome will also have abnormal PFA-100 results.

The genes *GPIBA* and *GPIBB* map to chromosomes 17 (17q12) and 22 (22q11.2), respectively. The genes *GP9* and *GP5* are both on chromosome 3, 3q21, and 3q29, respectively. More than 47 genetic defects in Bernard-Soulier syndrome have been described; most are due to mutations of *GPIBA* and *GPIBB* with truncation, frameshift, or nonsense mutations leading to loss of the extracellular protein domain. More rarely, mutations of *GP9* have been described. Although the genetic

defect usually affects only one protein, the entire GP Ib/IX/V complex is not expressed on the platelet surface if there is a deficiency of the GP Ibα, GP Ibβ, or GP IX chains. Variant Bernard-Soulier syndrome is due to genetic defects with a nonfunctional GP Ib/IX/V complex. The GP abnormality can be confirmed with flow cytometry or crossed immunoelectrophoresis in which a combined lack of GP Ibα, GP Ibβ, and GP IX is identified.

### Differential Diagnosis

Bernard-Soulier syndrome, although rare, has characteristic surface GP abnormalities and is not usually confused with other platelet disorders. However, the thrombocytopenia in Bernard-Soulier syndrome presenting in childhood can often be mistaken for ITP, and patients with Bernard-Soulier syndrome have been known to undergo unnecessary splenectomy. The lack of neutrophil inclusions distinguishes Bernard-Soulier syndrome from the *MYH9* disorders. Additional laboratory studies show normal VWF antigen and ristocetin cofactor activity, distinguishing Bernard-Soulier syndrome from VWD.

### Prognosis and Therapy

Patients with Bernard-Soulier syndrome are subject to a lifelong bleeding diathesis and can be at increased risk of bleeding during surgical procedures. Patients do not usually require therapy on a routine basis, but preoperative therapy is prudent. There is no uniform therapeutic approach to these patients, but desmopressin (DDAVP) and platelet transfusions are the most common. Because of the risk of development of platelet refractoriness, the use of leukocyte-reduced platelets is advised. There is little experience with the use of recombinant factor VIIa.

## GRAY PLATELET SYNDROME

### Clinical Findings

Gray platelet syndrome (GPS) is a congenital α-granule SPD (α-SPD) that is also considered a macrothrombocytopenia disorder. Some patients may have marrow fibrosis, pulmonary fibrosis, and splenomegaly. The disorder is characterized by mild, lifelong bleeding symptoms. Other SPDs involving dense granules are discussed in the section Platelet Dysfunction with Normal Platelet Count.

**GRAY PLATELET SYNDROME—FACT SHEET**

**Definition**
- Macrothrombocytopenia syndrome and a deficiency of platelet α-granules (α-SPD)

**Incidence**
- Rare

**Morbidity and Mortality**
- Mild bleeding disorder

**Gender, Race, or Age Distribution**
- Autosomal recessive or dominant congenital disorder

**Clinical Features**
- Mild, lifelong mucocutaneous bleeding disorder
- There may also be bone marrow failure in some individuals with marrow fibrosis

**Prognosis and Therapy**
- Routine treatment is not necessary

### Pathologic Features and Laboratory Studies

Individuals with GPS have a variable thrombocytopenia and large (mean 13 fL), gray-appearing platelets on the peripheral blood smear because of decreased α-granules. Platelets may appear vacuolated. Bone marrow examination often shows reticulin fibrosis of the bone marrow, thought to be due to the inability of megakaryocytes to store platelet-derived growth factor.

Platelet aggregation studies may be normal for ADP and epinephrine, but they are often abnormal for thrombin and collagen (see Table 2-2). Gray platelets are principally deficient in soluble proteins normally contained in α-granules, such as platelet factor 4, β-thromboglobulin, VWF, thrombospondin, and platelet-derived growth factor. In contrast, the α-granule membrane proteins are normal, suggesting that the disorder is primarily one of defective targeting and packaging of proteins into platelet α-granules. Flow cytometry studies have shown increased surface P-selectin but decreased α-granule P-selectin. By electron microscopy, platelets are enlarged and α-granules are not present; however, dense granules, mitochondria, and lysosomes appear normal. Immunogold staining by electron microscopy may show P-selectin localization in the open canalicular system. The precise genetic defect is not known, but the gene has been localized to a 9.4-Mb interval on 3p21.1-3p22.1.

**GRAY PLATELET SYNDROME—PATHOLOGIC FEATURES**

**Complete Blood Cell Count and Peripheral Smear Morphology**
- Variable thrombocytopenia (30 to 100 × $10^3/\mu L$)
- Uniformly large, pale, agranular platelets

**Bone Marrow Findings**
- Reticulin fibrosis

**Platelet Screening Tests**
- PFA-100 may be abnormal

**Platelet Aggregation**
- Sometimes normal; may be decreased with thrombin and collagen

**Ancillary Studies**
- Flow cytometry shows increased surface P-selectin but decreased α-granule P-selectin
- Electron microscopy: platelets are enlarged and α-granules are not observed
- Immunogold stain shows P-selectin localization in the open canalicular system

**Differential Diagnosis**
- Pale platelets can also be observed with ongoing platelet activation and "exhausted" platelets in DIC or with implanted cardiac devices; however, these patients usually have a mixture of normal and degranulated platelets, as well as red blood cell fragments and coagulation abnormalities

In one GPS family, a missense mutation in GATA1, including a Arg216Gln mutation was associated with an X-linked disorder with gray platelets and erythroid abnormalities, but this has been considered X-linked thrombocytopenia with thalassemia or an X-linked GPS-like disease.

## Differential Diagnosis

Pale platelets can also be seen with ongoing platelet activation and circulating "exhausted" platelets in patients with disseminated intravascular coagulation (DIC) or with implanted cardiac devices, but in these patients, there will be a mixture of normal and pale platelets and routine coagulation test results will be abnormal. A related disorder is the Québec platelet syndrome, which is caused by tandem duplication of *PLAU,* the urokinase plasminogen activator gene, leading to degradation of α-granule proteins. Aggregation studies in Québec platelet syndrome show a decreased response to epinephrine. Platelets have normal α-granule ultrastructural morphology, but demonstrate protease-mediated degradation of α-granule proteins.

## OTHER MACROTHROMBOCYTOPENIA DISORDERS

Mediterranean macrothrombocytopenia is an inherited macrothrombocytopenia caused by a defect in the demarcation membrane system leading to the production of large platelets and increased megakaryocyte apoptosis. Other than thrombocytopenia with large platelets, the platelet survival and ultrastructure are normal. Platelet aggregation is abnormal to ristocetin, with variable responses to other agonists. This disorder has been associated with stomatocytic hemolysis and phytosterolemia, the unselective and unrestricted gut absorption of cholesterol. Some Mediterranean macrothrombocytopenia family pedigrees with elevated blood phytosterols have been reported with mutations in *ABCG5* and *ABCG8*. Mediterranean macrothrombocytopenia in Italy has been reportedly associated with heterozygous mutations in *GP1BA* (most commonly Val156Ala) or *GP1BB,* making these kindred a heterozygous form of Bernard Soulier syndrome.

Maturation of megakaryocytic and erythroid cell lines is dependent on transcription factors GATA1 and GATA2, together with the cofactor FOG1. GATA1 is involved in both megakaryopoiesis and erythropoiesis, while GATA2 stimulates megakaryopoiesis. Genetic defects in GATA1 that prevent its interaction with FOG1 have been associated with X-linked thrombocytopenia with dyserythropoiesis, a syndrome with severe anemia, abnormal erythrocyte morphology, and red blood cell hemolysis. Other *GATA1* mutations that prevent its reaction with DNA are associated with X-linked thrombocytopenia with thalassemia. Neonates with Down syndrome (trisomy 21) and transient abnormal myelopoiesis (TAM), where increased myeloblasts and thrombocytopenia are observed, have been shown to have mutations of *GATA1*. Other macrothrombocytopenia disorders are being identified as the megakaryocytic transcription factor pathways are being elucidated. Examples are the Paris-Trousseau type thrombocytopenia and Jacobsen syndrome, both due to a deletion of 11q23.3, a chromosomal region that includes the genes for the transcription factors Ets-1 and Fli-1.

Montreal platelet syndrome is a rare, autosomal dominant syndrome with moderate to severe thrombocytopenia and large platelets. Aggregation studies show spontaneous agglutination and decreased response to thrombin; reduced calpain activity has been identified. This disorder is now classified as a type 2B VWD with a VWF *V1316M* mutation. Altered megakaryopoiesis appears to result from the enhanced interaction between 2B VWF and megakaryocyte GPIbα resulting in decreased platelet production.

# THROMBOCYTOPENIA WITH DECREASED PLATELET SIZE

## WISKOTT-ALDRICH SYNDROME AND X-LINKED THROMBOCYTOPENIA

### Clinical Features

Wiskott-Aldrich syndrome and X-linked thrombocytopenia are related X-linked recessive disorders because of a defect in the *WAS* gene characterized by immunodeficiency, recurrent infections, eczema, and thrombocytopenia with small platelets and a platelet SPD. The *WAS* gene is expressed exclusively in hematopoietic stem cells, and the WAS protein (WASp) is involved in signal transduction and adapter protein function and also is known to regulate actin filament assembly. Deficiency of WASp induces premature protoplatelet formation. These individuals also will have absent immunologic responses to polysaccharide antigens and progressive decline in T-lymphocyte function. A subset of patients will develop malignancies, primarily lymphomas and leukemias. More than 300 mutations of the *WAS* gene on the X chromosome have been identified, with the majority being missense mutations in exons 1 to 4. The XLT form involves defects in exon 2 of the *WAS* gene and is accompanied by a minimal immunodeficiency. These patients have an increased risk of bleeding, even at modestly low platelet counts because of small platelet mass and platelet dysfunction. The thrombocytopenia often responds to splenectomy with a return in both platelet count and size, but postsplenectomy sepsis is a risk. Female carriers are asymptomatic.

### Pathologic Features and Laboratory Studies

The peripheral smear shows a decreased number of uniformly small platelets, and the MPV is often low (approximately half normal size). Bone marrow evaluation shows normal megakaryocyte number and morphology.

Platelet dysfunction is severe. The platelets are unable to aggregate to ADP, epinephrine, and collagen. Platelets have decreased dense granules and a storage pool pattern. Lymphocytes are deficient in CD43 (sialophorin).

**WISKOTT-ALDRICH SYNDROME AND X-LINKED THROMBOCYTOPENIA—FACT SHEET**

**Definition**
- Defect in the *WAS* gene characterized by immunodeficiency, recurrent infections, eczema, thrombocytopenia, and development of malignancy, with small platelets, and a platelet SPD
- The milder XLT form involves defects in exon 2 of the *WAS* gene

**Incidence and Location**
- Rare (4 per 250,000 male births in Europe)

**Morbidity and Mortality**
- Mild to severe mucocutaneous bleeding disorder

**Gender, Race, or Age Distribution**
- Congenital, X-linked recessive disorder
- Only males are affected
- Female carriers are asymptomatic

**Clinical Features**
- Eczema, immune deficiency, infections, and mucocutaneous bleeding disorder
- Development of lymphoma and leukemia in a subset

**Prognosis and Therapy**
- Desmopressin or platelet transfusions, or both, for severe bleeding or operative prophylaxis

**WISKOTT-ALDRICH SYNDROME AND X-LINKED THROMBOCYTOPENIA—PATHOLOGIC FEATURES**

**Complete Blood Cell Count and Peripheral Smear Morphology**
- Thrombocytopenia (10 to 100 × $10^3/\mu L$) with uniformly small, normally granulated platelets
- Decreased MPV

**Bone Marrow Findings**
- Normal megakaryocyte number and morphology

**Platelet Screening Tests**
- Abnormal

**Platelet Aggregation**
- Abnormal aggregation to many agonists, including ADP, epinephrine, and collagen

**Ancillary Studies**
- Decreased dense granules by electron microscopy or mepacrine uptake
- Decreased platelet adenosine triphosphate release by lumiaggregometry

**Differential Diagnosis**
- Patients with thrombocytopenia resulting from marrow aplasia may also have small platelets, but the MPV is usually low normal, not decreased
- Patients with TORCH (toxoplasma-rubella-cytomegalovirus-herpes) infection may also have small platelets, but clinical and serologic features are distinctive

### Differential Diagnosis

Patients with thrombocytopenia owing to marrow aplasia may also have small platelets, but the MPV is usually low normal, not decreased. Patients with toxoplasma-rubella-cytomegalovirus-herpes infection may also have small platelets, but clinical and serologic features are distinctive. The mild thrombocytopenia seen in XLT may be misdiagnosed as ITP.

## THROMBOCYTOPENIA WITH NORMAL PLATELET SIZE

Bone marrow examination can be helpful in differentiating the underlying causes in thrombocytopenic platelet disorders with normal platelet morphology and size. This group of disorders includes both congenital and acquired thrombocytopenias that are usually caused by either decreased platelet production or increased platelet destruction. The number of megakaryocytes on the bone marrow can help to distinguish between these causes, but analysis of platelet turnover by mRNA analysis or immature platelet fraction may also be helpful.

The finding of adequate or increased megakaryocytes on the bone marrow or increased reticulated platelets suggests peripheral platelet destruction. Platelet function tests are usually not helpful in differentiating between the entities in this class of disorders, because most functional studies will give abnormal results simply because of the low platelet number. The overall MPV is usually normal with destructive thrombocytopenia, but there is typically a range of platelet size with increased PDW and many large platelets, indicating the rapid platelet turnover. These disorders are invariably acquired, and an underlying abnormality should be sought. In general, the clinical scenario is the most helpful in classifying these disorders.

## PERIPHERAL PLATELET DESTRUCTION

### IMMUNE DESTRUCTIVE THROMBOCYTOPENIAS

#### Immune Thrombocytopenic Purpura

### Clinical Features

ITP is a disorder characterized by thrombocytopenia owing to platelet sensitization with autoantibodies leading to platelet destruction in the reticuloendothelial system. However, the recent success of treatment with thrombopoietin receptor agonists also suggests that platelet production may also be insufficient in ITP. It is defined clinically as thrombocytopenia in the presence of a normal hemoglobin level and a normal white blood cell count and differential, and in the absence of hepatosplenomegaly, lymphadenopathy, and abnormalities of the radii or other underlying disease. Patients usually have acute onset of bleeding symptoms and marked thrombocytopenia. *Chronic ITP* refers to patients who have had the illness for 12 months or more, and *refractory ITP* is defined as patients with severe symptoms who have failed previous therapies, including splenectomy.

Adult ITP has an incidence of 1/100,000 with a 2:1 to 3:1 female-to-male predominance. Childhood ITP has a peak age incidence of 2 to 4 years, with girls and boys being affected equally. ITP is often associated with viral infections and may have a genetic predisposition.

**IMMUNE THROMBOCYTOPENIC PURPURA—FACT SHEET**

**Definition**
- Immune destructive thrombocytopenia owing to platelet autoantibodies and decreased platelet production

**Incidence**
- 1 per 100,000

**Morbidity and Mortality**
- Mild to severe mucocutaneous bleeding disorder

**Gender, Race, or Age Distribution**
- 2:1 to 3:1 female-to-male predominance
- Childhood ITP has a peak age incidence of 2 to 4 years with 1:1 female-to-male predominance

**Clinical Features**
- Acquired autoimmune thrombocytopenia disorder with acquired bleeding disorder

**Prognosis and Therapy**
- Prognosis is usually good, but severe bleeding can be seen when the platelet count is less than 10 × 103/μL
- Acute treatment is usually aimed at increasing the platelet count by immunoglobulin therapy (intravenous immunoglobulin or anti-D), steroids, vincristine, or plasmapheresis; recent trials with rituximab (monoclonal antibody against CD20) and thrombopoietin receptor agonists have shown success
- Long-term therapy, if necessary, may entail splenectomy

### Pathologic Features and Laboratory Studies

The platelet count is decreased and may be markedly decreased in cases of acute ITP. The pathologic features of ITP on the peripheral smear are nondiagnostic, showing a mixture of normal and larger platelets, with the MPV being normal to slightly increased. Erythrocyte and granulocyte counts and indices should be normal.

**IMMUNE THROMBOCYTOPENIC PURPURA—PATHOLOGIC FEATURES**

**Complete Blood Cell Count and Peripheral Smear Morphology**

- Thrombocytopenia, often severe, with an otherwise normal CBC
- Mixture of normal and large platelets; the MPV is normal to slightly increased

**Bone Marrow Findings**

- Normal to increased megakaryocytes with occasional megakaryocyte clustering
- Megakaryocyte morphology is normal; hematopoiesis is normal

**Platelet Screening Tests**

- Abnormal PFA-100, usually in proportion to the degree of thrombocytopenia

**Platelet Aggregation**

- Not usually helpful because the degree of thrombocytopenia alone can result in aggregation abnormalities

**Ancillary Studies**

- Autoantibodies to GPs are present, but this finding is neither sensitive nor specific
- Immunoassays may help to identify the specific platelet surface GP target, such as GP IIb/IIIa and GP Ib
- There are also elevated thrombopoietin and increased reticulated platelets

**Differential Diagnosis**

- ITP is a diagnosis of exclusion.
- Exclude congenital thrombocytopenias and other destructive thrombocytopenias, such as hypersplenism, HIT, TTP, and DIC

Bone marrow evaluation may be helpful in cases in which there are other abnormalities of the peripheral smear or complicated clinical features. In uncomplicated ITP, the bone marrow usually demonstrates normal to increased numbers of morphologically normal megakaryocytes, with occasional megakaryocyte clustering. Other pathologic changes in granulopoiesis or erythropoiesis should not be observed.

Autoantibodies to surface GPs can be detected by flow cytometry or immunoassay, although these findings may be neither sensitive nor specific. Immunoassays are available to identify the specific platelet surface GP target, such as GP IIb/IIIa or GP Ib, but these assays cannot reliably distinguish ITP from nonimmune thrombocytopenia or myelodysplasia. Although not specific for ITP, elevated levels of thrombopoietin and increased reticulated platelets can indicate destructive thrombocytopenia with increased platelet turnover. Platelet functional tests are usually unhelpful in ITP because the low platelet count alone can give abnormal functional test results. Rarely, patients with ITP owing to anti-GP IIb/IIIa antibodies may have a Glanzmann thrombasthenia–like phenotype by platelet flow cytometry.

## Differential Diagnosis

ITP is a diagnosis of exclusion because specific diagnostic tests are not available. The diagnostic approach usually involves excluding other causes of thrombocytopenia, such as congenital thrombocytopenias and other causes of destructive thrombocytopenia, such as hypersplenism, heparin-induced thrombocytopenia (HIT), thrombotic thrombocytopenic purpura (TTP), and DIC. A family history of thrombocytopenia and the presence of uniformly large platelets on the peripheral smear suggest a congenital thrombocytopenia. Previous exposure to heparin with or without thrombosis and positive HIT tests, such as anti-platelet factor 4 enzyme-linked immunosorbent assay (ELISA) or serotonin release studies, are helpful to exclude HIT as a diagnosis. TTP is associated with a characteristic clinical pentad of renal failure, mental status changes, thrombosis, and hemolytic anemia in addition to the thrombocytopenia. Assays for VWF-cleaving metalloprotease (ADAMTS13) may be helpful in excluding TTP. A normal red blood cell reticulocyte count in ITP may also be helpful to exclude TTP. DIC is an intravascular consumptive coagulopathy usually accompanied by changes in coagulation assays, such as elevation of the PT, APTT, and D-dimer and a decrease in fibrinogen, antithrombin, and protein C. Examination of the peripheral smear in both TTP and DIC should show schistocytes (red blood cell fragments) in addition to the thrombocytopenia. Testing for antinuclear antibody and anti-DNA antibody may be helpful to investigate systemic lupus erythematosus, which could indicate secondary ITP.

## Prognosis and Therapy

Patient prognosis in ITP is usually good, but severe bleeding complications can be seen when the platelet count is less than 10 to 20 $\times$ $10^3/\mu L$. Acute treatment is aimed at increasing the platelet count through immunoglobulin therapy (intravenous immunoglobulin or anti-D), steroids, vincristine, or plasmapheresis. Approximately 50% of children and 10% to 30% of adults respond to initial therapy. Long-term therapy, if necessary, may entail splenectomy. Recent clinical trials have shown therapeutic success with rituximab, a CD20 monoclonal antibody, and thrombopoietin (TPO) receptor agonists. Two TPO receptor agonists, romiplostim and eltrombopag have been approved for use in ITP.

## ALLOIMMUNE THROMBOCYTOPENIAS

Alloimmune thrombocytopenias include posttransfusion purpura and neonatal alloimmune thrombocytopenia. Posttransfusion purpura is a rare alloimmune disorder in which immune destruction of both transfused and recipient platelets is seen, usually beginning 5 to 12 days after transfusion. Patients often have low-frequency platelet antigens and are frequently human platelet antigen (HPA)-1a negative (HPA-1b homozygous). Patients are often multiparous women who have been exposed to HPA-1a during pregnancy and often are HLA-DR3-positive. Anti–HPA-1a antibodies can be detected both in plasma and attached to the platelet surface.

Neonatal alloimmune thrombocytopenia (NAIT) is a severe thrombocytopenia that occurs shortly after birth. This syndrome is due to maternal immunization against fetal platelet antigens and occurs when the mother lacks a platelet antigen present on the baby's platelets; it is the platelet equivalent of hemolytic disease of the newborn. Maternal antibodies cross the placenta and cause the severe thrombocytopenia, which can result in intracranial hemorrhage. Sixteen HPAs have been described with NAIT, but only three (HPA-1a, HPA-5b, and GHP-15b) cause 95% of cases in white populations, with 75% of cases attributable to HPA-1a. The diagnosis is usually made by platelet surface GP phenotyping by ELISA or by molecular genetic studies. HPA-1a incompatibility occurs in 1:350 pregnancies, but NAIT is seen in only 1:1000 to 1:1500 pregnancies, with HLA-DRB3*0101–positive women being more likely to produce anti–HPA-1a antibodies. Therapy is by transfusion with HPA-compatible platelets, but initial therapy can be started with HPA-1a and 5b negative platelets or washed maternal platelets while laboratory confirmation is being performed.

## DRUG-INDUCED THROMBOCYTOPENIAS

Drug-induced thrombocytopenias caused by immunologic and nonimmunologic platelet destruction can be seen with many drugs.

## IMMUNE DRUG-INDUCED THROMBOCYTOPENIAS

### Clinical Features

Autoimmune thrombocytopenia can be seen with many drugs, but the most common ones are quinidine, quinine, heparin, sulfonamide drugs, and gold salts. Various mechanisms are involved, with the drug acting as either a direct or an indirect hapten. For example, drugs such as penicillin can bind directly to platelet surface GPs and act as a direct hapten. On the other hand, drugs such as quinidine, quinine, and sulfonamides bind to platelet surface glycoproteins and induce a conformational change or compound epitope that is immunogenic. The implicated target GPs include GP IIb/IIIa and GP Ib/IX. The development of immune complex formation and thrombocytopenia is characteristic of HIT, which is discussed later. Binding of drugs to the GP IIb/IIIa fibrinogen receptor, such as eptifibatide and tirofiban, can create a neoepitope (ligand-induced binding site) that is immunogenic. Other GP IIb/IIIa antagonists, such as abciximab, a chimeric monoclonal antibody to the fibrinogen receptor, can induce thrombocytopenia by an alloimmune mechanism. However, acute thrombocytopenia with abciximab can occur at the first exposure to the drug, and preexisting antibodies and nonimmunologic mechanisms have been postulated. Pseudothrombocytopenia has also been described with abciximab resulting from in vitro platelet clumping associated with calcium-chelating ethylenediamine tetraacetic acid anticoagulants.

The clinical features of drug-induced thrombocytopenia include acute thrombocytopenia within a few hours of drug ingestion, accompanied by flushing, chills, fever, and occasionally syncope. The thrombocytopenia, if severe, can lead to a bleeding diathesis characterized by mucosal bleeding, petechial hemorrhage, and purpura.

### Pathologic Features and Laboratory Studies

Other than thrombocytopenia on the peripheral smear, there are no characteristic pathologic features of this disorder. Bone marrow analysis is helpful only to exclude other causes of thrombocytopenia. Drug-induced thrombocytopenias can be diagnosed by detecting the presence of platelet-associated antibody by flow cytometry or immunoassay, although this is a nonspecific finding that can also be seen with infections and autoimmune disorders, such as ITP. The drug dependence of the antibody binding can be demonstrated by incubating platelets with patient plasma in the presence of the drug. Conversely, patients with drug-induced thrombocytopenia may have negative test results and a presumptive diagnosis based on the drug history.

### Differential Diagnosis

The diagnosis of immune drug-associated thrombocytopenia is usually made by the history of documented drug therapy with drugs known to cause thrombocytopenia, the time course of the thrombocytopenia, and the

presence of drug-associated platelet antibodies. In the absence of diagnostic features, other causes of destructive thrombocytopenia, such as ITP, DIC, TTP, and alloimmune thrombocytopenia, should be excluded as detailed in previous sections.

### Prognosis and Therapy

Bleeding complications are related to the severity of thrombocytopenia, which usually resolves with discontinuation of the inciting drug.

## HEPARIN-INDUCED THROMBOCYTOPENIA

### Clinical Features

HIT is a distinctive drug-induced thrombocytopenia associated with heparin therapy in which antibodies are formed to heparin–platelet factor 4 complexes that persist for 2 to 3 months, with immunoglobulin (Ig) G being the pathogenic isotype. The immune complexes bind to and cross-link the platelet FcγIIa receptor, leading to platelet aggregation, platelet microparticle formation, platelet procoagulant activity, and endothelial and monocyte activation, leading to extensive thrombin generation and paradoxical thrombosis (Figure 2-17). Antibodies to other heparin-binding proteins (interleukin-8 and neutrophil activating protein [NAP]-2) have been described in association with HIT.

The thrombotic complications can be either venous or arterial and may be devastating, with death, limb loss, and pulmonary thrombosis. Bleeding secondary to thrombocytopenia is rare in HIT. HIT is unique among drug-induced thrombocytopenias for the association with thrombosis rather than bleeding. In HIT, there is usually more than a 50% decrease in platelet count or development of absolute thrombocytopenia (less than $150 \times 10^3/\mu L$). In typical HIT, the thrombocytopenia develops 5 to 10 days after starting heparin therapy, with a nadir at 7 to 14 days. Rapid onset HIT, with thrombocytopenia developing in 24 hours, may be associated with reexposure to heparin within 100 days of initial therapy. Delayed HIT is infrequent, but these patients develop thrombocytopenia only several days

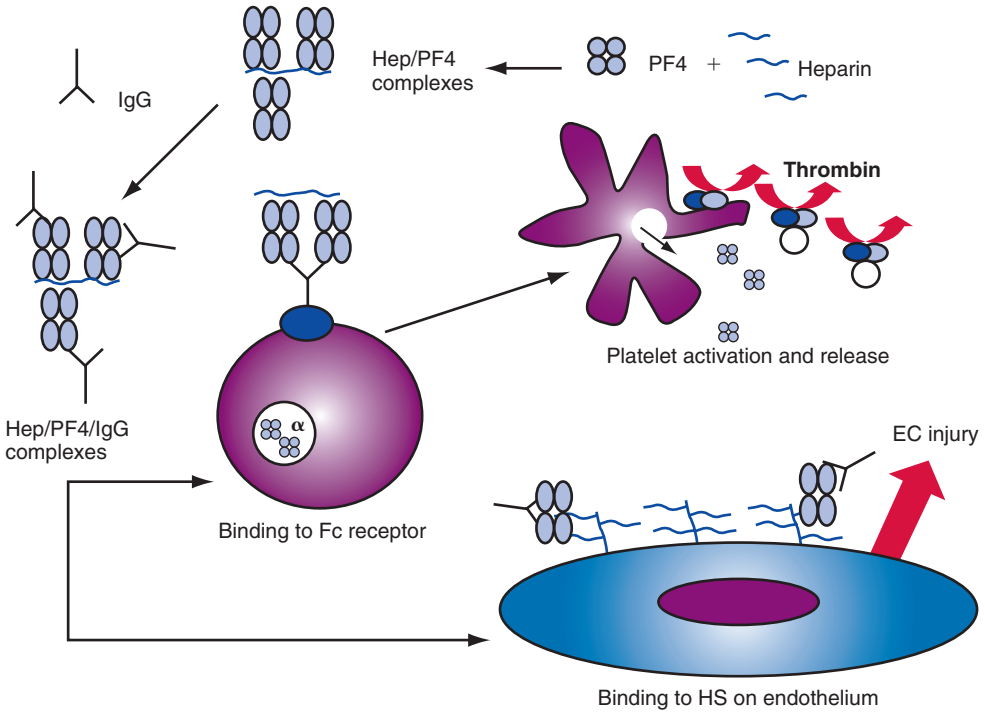


**FIGURE 2-17**

Schematic diagram of the pathophysiology of heparin (Hep)-induced thrombocytopenia. Platelet factor 4 (PF4) is a heparin-binding protein released from platelet α-granules (α) during platelet activation. The formation of heparin–platelet factor 4 complexes induces a conformational change in platelet factor 4 that is antigenic. Elicitation of an immune response leads to formation of heparin–platelet factor 4–immunoglobulin G (IgG) complexes, which bind to the Fc receptor on the platelet membrane. This binding triggers platelet activation, with more release of platelet factor 4 and development of procoagulant activity on the platelet surface leading to thrombin formation and thrombosis. Simultaneously, the platelet factor 4 binds to heparin sulfate (HS) on the luminal membrane of endothelial cells (EC), again forming immune complexes that lead to endothelial injury and thrombosis.

**HEPARIN-INDUCED THROMBOCYTOPENIA—FACT SHEET**

**Definition**
- Drug-induced thrombocytopenia owing to heparin therapy in which formation of antiplatelet factor 4–heparin–antibody (IgG) complexes lead to platelet activation and procoagulant activity

**Prevalence**
- 1% to 5% of postoperative patients treated with heparin; 0.1% to 1% of medical patients treated with heparin

**Morbidity and Mortality**
- Approximately 25% to 50% will develop arterial or venous thrombosis
- May be associated with death, limb loss, and pulmonary thrombosis
- Bleeding is rare

**Gender, Race, or Age Distribution**
- Males and females affected
- Described in all age groups

**Clinical Features**
- Thrombocytopenia starts 5 to 10 days after commencement of heparin therapy, with a nadir at 7 to 14 days
- The thrombocytopenia is defined as a decrease in platelet count of 50% or more or development of absolute thrombocytopenia (less than $150 \times 10^3/\mu L$, but more than $20 \times 10^3/\mu l$)
- Rapid-onset HIT, with thrombocytopenia developing in 24 hours, may be associated with reexposure to heparin within 30 days of initial therapy
- 4T Score (see Table 2-3) should be assessed for pretest probability

**Prognosis and Therapy**
- Discontinue heparin and substitute a direct thrombin inhibitor, such as argatroban or bivalirudin or lepirudin
- There is a high risk of thrombosis if heparin is not discontinued
- Because of the risk of warfarin-associated venous limb gangrene, use of warfarin is not indicated

after cessation of heparin therapy. In all types of HIT, the thrombocytopenia usually resolves after heparin therapy is stopped.

HIT can be observed with exposure to all types of heparin, including intravenous or subcutaneous administrations, but is most common with intravenous unfractionated heparin. HIT is most prevalent with bovine lung unfractionated heparin, followed by porcine mucosal unfractionated heparin, and least prevalent with low molecular weight heparin. The pentasaccharide fondaparinux, although based on a heparin structure, is rarely associated with development of HIT. HIT is more commonly seen in postoperative patients treated with heparin (1% to 5%) than in medical patients (0.1% to 1%) or in pregnancy. In patients who develop HIT, approximately 25% to 50% will develop thrombosis.

## Pathologic Features and Laboratory Studies

The thrombocytopenia just described is usually not associated with other CBC abnormalities. Some platelets may appear degranulated on the peripheral smear if extensive in vivo platelet activation is occurring. Bone marrow evaluation is usually not necessary because of the characteristic clinical scenario, drug association, and specific laboratory testing.

Thrombectomy, if performed, often shows "white clot" because of the platelet-rich nature of the clot. In HIT, the initial thrombotic nidus is usually a platelet-rich thrombus, but this may propagate as a typical thrombus intravascularly and the characteristic white clot may be overlooked. Autopsy evaluation of thrombosis usually reveals multiple thrombi of the venous and arterial system, usually involving large vessels. The

**HEPARIN-INDUCED THROMBOCYTOPENIA—PATHOLOGIC FEATURES**

**Complete Blood Cell Count and Peripheral Smear Morphology**
- Isolated thrombocytopenia without other smear abnormalities
- The thrombocytopenia is either absolute (less than $150 \times 10^3/\mu L$ and more than $20 \times 10^3/\mu L$) or more than a 50% drop from baseline

**Bone Marrow and Other Pathologic Findings**
- Bone marrow examination is usually not indicated; no characteristic morphologic findings
- Thrombectomy of large-vessel thrombi often shows a "white clot" because of platelet-rich thrombi
- Autopsy usually shows multiple thrombi of the arterial and venous system, usually involving large vessels

**Platelet Function Tests**
- The PFA-100 may be abnormal in proportion to the degree of thrombocytopenia
- Platelet aggregation is not usually helpful

**Ancillary Studies**
- Testing not indicated if 4T score is less than 4
- Immunologic assays detect anti-platelet factor 4 antibody; assays for IgG have only increased specificity
- Functional HIT assays demonstrate platelet activation in the presence of the antibody; they include a serotonin release assay (sensitivity 80% to 90% and positive predictive value more than 95%) and heparin-induced platelet aggregation (sensitivity <60% and specificity 95%)

**Differential Diagnosis**
- Positive anti-PF4 or functional assays can diagnose HIT in the correct clinical setting with a high 4T score
- Other causes of destructive thrombocytopenia should be excluded; these include ITP, DIC, TTP, and other autoimmune drug–induced thrombocytopenias

**TABLE 2-3**

**Pretest Scoring System for Heparin-Induced Thrombocytopenia (4T)**

| 4 *Ts* | 2 Points | 1 Point | 0 Points |
|---|---|---|---|
| Thrombocytopenia | Platelet count fall >50% and platelet nadir >20 × $10^3$/μL | Platelet count fall 30%-50% or platelet nadir 10 to 19 ×$10^3$/μL | Platelet count fall <30% or platelet nadir <10 × $10^3$/μl |
| Timing of platelet count fall | Clear onset between days 5 and 10 or platelet fall ≤1 day (prior heparin exposure within 30 days) | Consistent with days 5-10 fall, but not clear; onset after day 10 or fall ≤1 day (prior heparin exposure 30-100 days ago) | Platelet count fall <4 days without recent heparin exposure |
| Thrombosis or other sequelae | New thrombosis (confirmed); skin necrosis; acute systemic reaction postintravenous UFH bolus | Progressive or recurrent thrombosis; nonnecrotizing skin lesions; suspected thrombosis (not proven) | None |
| Other causes for thrombocytopenia | None apparent | Possible | Definite |

From Greinacher A, Althaus K, Krauel K, Selleng S: Heparin-induced thrombocytopenia, *Haemostaseologie* 30:17–28, 2010.
*UFH,* Unfractionated heparin.

thrombi are often variegated matrices of platelet aggregates, interspersed by small islands of fibrin and erythrocytes. Microvascular thrombosis, more typical of DIC and TTP, is not commonly observed.

HIT is a clinicopathologic syndrome, and pretest probability using a 4-T score should be assessed before ordering laboratory testing (Table 2-3). According to the score, the clinical probability of HIT can be low (0 to 3 points), intermediate (4 to 6 points) and high (7 to 8 points). Laboratory testing is not recommended for patients with a 4-T score less than 4 points. Specific laboratory tests either are immunologic, aimed at detection of heparin–platelet factor 4 antibodies, or functional, designed to detect heparin-induced platelet activation. Several immunologic assays are available, with ELISAs designed to detect antiplatelet factor 4 antibody binding to heparin or polyvinylamine-coated microtiter plates. Another variation of the assay uses heparin-coated surfaces to which a platelet-leukocyte lysate is added, allowing attachment of not only heparin–PF4 complexes, but complexes of heparin with other chemokines such as IL-8 or NAP-2. The assay specificity can be improved by using a secondary antibody to detect IgG and a second step using soluble heparin to block specific heparin–platelet factor 4 antibody complex binding to the plate. The sensitivity of these assays is typically more than 95%, but the specificity is approximately 80%. The immunoassay may be positive in up to 30% of patients after cardiopulmonary bypass and open heart surgery and may be positive in patients who never develop thrombocytopenia. A rapid particle gel immunoassay method (PaGIA) is based on a Micro Typing System (DiaMed, Biorad, Switzerland) gel system and uses red-dyed polymer particles coated with human heparin–platelet factor 4 to detect anti-heparin–platelet factor 4 antibody complexes. The patient's serum is incubated with the polymer particles and applied to the top of a gel column, which is then centrifuged. If the antibody (IgG, IgM, or IgA) is present, the immune complexes bind to the polymer particles and are trapped at the top of the gel. Otherwise, the polymer particles are forced to the bottom of the gel.

Functional HIT assays are based on demonstrating some aspect of platelet activation in the presence of heparin. Because patients with HIT usually have thrombocytopenia, donor platelets are used. The serotonin release assay uses fresh, washed donor platelets loaded with radiolabeled serotonin, heat-inactivated patient serum, and two heparin concentrations. After incubation in the presence of antibody complexes in the patient serum, platelet degranulation is observed in the low heparin concentration (usually 0.1 U/mL), but not with the high heparin concentration (usually approximately 100 U/mL) or in the absence of heparin. This assay has a reported sensitivity of 80% to 90% and a positive predictive value more than 95%.

Other functional assays include heparin-induced platelet aggregation, functional flow cytometric assays (microparticle release, P-selectin, annexin V, procoagulant activity), luminescent ATP release, and serotonin release by enzyme immunoassay. The heparin-induced platelet aggregation has a low sensitivity of <60% with a high specificity of 95%. Problems with the heparin-induced platelet aggregation that make it difficult to perform in the laboratory are that some donors are unresponsive and that potential donors should be screened with known positive plasma or platelets from three donors should be pooled. There may be some donor reactivity variation because of Fc receptor

phenotype and some advocate using platelets from donors with blood type O to avoid ABO antibody response. Occasionally, a positive aggregation response is seen without added heparin in patients with alloantibodies to the donor platelets. Conversely, aggregation can be falsely negative in patients receiving intravenous GP IIb/IIIa antagonists because of inhibition of donor platelets.

### Differential Diagnosis

The diagnosis of HIT is straightforward in patients being treated with heparin for uncomplicated thrombosis in whom the laboratory tests are positive and the timing and degree of thrombocytopenia are characteristic (i.e., high 4-T score). However, many hospitalized patients receiving heparin have various underlying medical and surgical disorders and are concomitantly treated with numerous drugs, making the diagnosis problematic. Complicating the matter further are the relatively low specificity of the anti-platelet factor 4 ELISA and the relatively low sensitivity of the heparin-induced platelet aggregation. The serotonin release assay, with a higher sensitivity and specificity, is unfortunately not a widely available assay. Diagnostic sensitivity and specificity can be improved by measuring both immunologic and functional assays or by following serial assay results. The diagnosis of HIT is thus often a clinical diagnosis, with the exclusion of other causes of thrombocytopenia.

### Prognosis and Therapy

The major morbidity in HIT is due to the risk of thrombosis, which is seen in 25% to 50% of patients. Arterial and venous thrombosis can lead to organ malfunction, limb loss, myocardial infarction, and death. Discontinuation of heparin is insufficient because of the marked thrombin generation and procoagulant state that characterizes HIT; switching to an alternate anticoagulant, such as a direct thrombin inhibitor (lepirudin, bivalirudin, or argatroban), is recommended. Because of the persistent procoagulant state, starting warfarin is not recommended as a therapy in acute HIT. Early implementation of warfarin has been associated with venous limb gangrene.

## NON–IMMUNE DRUG-INDUCED THROMBOCYTOPENIAS

Nonimmune platelet destruction may be seen with hematopoietic growth factors, tumor necrosis factor α, interferon-γ, and interleukin-2. Desmopressin, which causes rapid release of high-molecular-weight VWF from endothelial cells, is used as a treatment for some types of VWD. Its use has been occasionally associated with a thrombocytopenia in type 2b and platelet-type VWD because of binding of high-affinity VWF or platelet GP Ib. Thrombocytopenia can also be associated with use of porcine factor VIII in patients with factor VIII inhibitors.

Drug-induced thrombocytopenia can also be caused by inhibition of megakaryocyte production, but in most instances all cell lines are affected and pancytopenia is usually seen. Drugs that can specifically affect megakaryopoiesis include anagrelide, alcohol, interferons, and valproic acid.

## NONIMMUNE DESTRUCTIVE THROMBOCYTOPENIAS

### DISSEMINATED INTRAVASCULAR COAGULATION

#### Clinical Features

DIC occurs with an intravascular stimulation of the coagulation and fibrinolytic systems, leading to intravascular coagulation activation, fibrin formation, platelet activation, thrombocytopenia, fibrinolytic activation, and clotting factor consumption. DIC is not a discrete disease entity, but it is initiated by other disease processes because of production of coagulation-activating or fibrinolysis-activating factors. Common associations include infections and sepsis as well as malignancy and pregnancy complications. Bacterial products, such as lipopolysaccharide, can activate tissue factor and factor VIIa, generating thrombin.

Individuals with DIC may have a variety of clinical symptoms, from a bleeding diathesis to thrombosis. DIC can be classified as acute, chronic, or low-grade, although the terms *overt* and *nonovert* have also been suggested. Acute DIC often manifests as a fulminant intravascular activation of coagulation and fibrinolysis, leading to a diffuse coagulopathy. Concomitant thrombosis is usually microvascular and can lead to end-organ dysfunction, particularly renal failure, pulmonary insufficiency, or mental status changes. Large-vessel thrombosis can be observed but is less common. Low-grade DIC is characterized by a coagulation activation that is usually mild. The body often counterbalances the factor consumption by increased synthesis of coagulation factors so that bleeding symptoms are minor. This type of DIC is often called *compensated DIC* because coagulation factor levels are usually normal. The primary clinical presentation with low-grade DIC may be an increased risk of thrombosis.

### Pathologic Features and Laboratory Studies

Acute DIC can be recognized on a peripheral smear by the presence of thrombocytopenia and erythrocyte fragments. Bone marrow evaluation is usually not necessary for diagnosis, but may show vascular thrombi and increased megakaryocytes. Pathologic evaluation of other tissues usually shows microvascular platelet-fibrin thrombi, especially in renal glomeruli and pulmonary capillaries. Chronic or low-grade DIC may have a mild thrombocytopenia on the peripheral smear. Erythrocyte fragments are usually present but may be more inconspicuous.

Because DIC is due to coagulation and fibrinolysis activation, multiple hemostasis laboratory test results are typically abnormal. With low-grade DIC, the PT is often prolonged with an elevation of D-dimer, a marker of fibrin degradation. There may be variable mild decreases in fibrinogen, antithrombin, and other coagulation proteins. In acute or overt DIC, both the PT and APTT are prolonged, with a marked elevation of D-dimer. This elevation is accompanied by decreased fibrinogen, with often marked decreases in antithrombin, protein C, plasminogen, and other coagulation factors such as factor VIII. The relative decrease of fibrinogen versus plasminogen may indicate the relative activation of the coagulation versus fibrinolysis system.

### Differential Diagnosis

DIC should be distinguishable from other consumptive platelet disorders by the presence of coagulation and fibrinolysis activation in addition to the thrombocytopenia. The abnormal coagulation test results, discussed previously, are not observed in ITP or TTP. However, many of the abnormal coagulation test results observed in DIC are not specific for DIC and can be observed in patients with acute thrombosis, massive hemorrhage, or liver disease. Patterns that help to distinguish DIC from acute thrombosis include the more severe thrombocytopenia and more marked decrease in fibrinogen in DIC. Clinical features and imaging studies with large focal vessel deep vein thrombosis, pulmonary embolism, or arterial thrombosis may also be helpful to diagnose acute thrombosis. Patients with massive hemorrhage may have low factor levels, thrombocytopenia, and elevated PT or APTT, but the D-dimer is not usually elevated initially. Liver disease can be difficult to distinguish from DIC because end-stage liver disease is complicated by thrombocytopenia and leads to decreased synthesis of many coagulation factors. However, levels of factor VIII are typically normal to elevated in liver disease, because factor VIII is synthesized in the endothelial cells, not the liver. D-Dimer levels, while elevated in liver disease, are usually only modestly increased.

### Prognosis and Therapy

Because DIC is an intermediary mechanism of many different diseases, the prognosis is mainly related to the underlying disease. Nonetheless, the development of DIC, in addition to the inciting disease, often bodes a worse prognosis. Low-grade DIC may be clinically silent, but development of thrombosis can lead to morbidity and death; however, acute DIC with rapid progression of a consumptive coagulopathy can lead to a significant hemorrhagic diathesis. Therapy for DIC is usually aimed at treating the underlying disorder, and mere replacement of clotting factors or platelets via transfusion can accelerate the process. Decreasing coagulation and fibrinolysis activation via the use of anticoagulant and antifibrinolytic drugs, while theoretically attractive, is controversial. Recently, drotrecogin alfa, a form of recombinant activated protein C, has been introduced for patients with DIC associated with sepsis.

## THROMBOTIC THROMBOCYTOPENIC PURPURA

### Clinical Features

The thrombocytopenia of TTP is thought to be secondary to deficiency of or an autoimmune response against a VWF-cleaving metalloproteinase (a disintegrin and metalloproteinase with thrombospondin-1-like domains [ADAMTS13]) in many patients, leading to diffuse thrombus formation in small vessels and a decrease in circulating platelets. These patients will show characteristic clinical symptoms with fever, thrombocytopenia, and microangiopathic hemolytic anemia accompanied by multiorgan failure, often manifest as renal failure and mental status changes. The erythrocyte fragmentation likely occurs as red blood cells are sheared by fluid turbulence in areas of platelet aggregates.

Rare individuals with deficiency of ADAMTS13 have a familial form of TTP that often manifests in infancy or childhood and can recur as chronic relapsing TTP or the Upshaw-Schulman syndrome. More than 70 genetic mutations of the ADAMTS13 gene on chromosome 9q34 have been identified. A more common idiopathic form of TTP that is seen in adulthood is thought to be due to autoantibodies directed against ADAMTS13. Pathogenic antibodies that have been identified are typically directed against the cysteine-rich–spacer domain of ADAMTS13.

**THROMBOTIC THROMBOCYTOPENIC PURPURA—FACT SHEET**

**Definition**
- Thrombotic microangiopathy owing to a deficiency of or an autoimmune response against a VWF cleaving metalloproteinase (ADAMTS13), leading to diffuse thrombus formation in small vessels and a thrombocytopenia

**Incidence**
- 3.7 per 1 million

**Morbidity and Mortality**
- Death rare at younger than 20 years of age; mortality increases with age older than 20 years

**Gender, Race, or Age Distribution**
- Typically seen in childhood (younger than 5 years old) or in adulthood (20 to 60 years old)

**Clinical Features**
- Fever, thrombocytopenia, and microangiopathic hemolytic anemia accompanied by multiorgan failure such as renal failure and mental status changes
- Congenital deficiency of ADAMTS13 leads to chronic relapsing TTP

**Prognosis and Therapy**
- High mortality due to multiorgan failure
- Supportive therapy employs daily plasma exchange with fresh frozen plasma. Newer therapies include rituximab

A clinically similar thrombotic microangiopathy can be observed with drugs such as cyclosporine, tacrolimus (FK506), quinine, ticlopidine, and clopidogrel and after allogeneic organ transplantation. Hemolytic-uremic syndrome is a related disorder, typically seen in children with thrombocytopenia, microangiopathic hemolytic anemia, and renal failure. Two types of HUS have been identified: (1) diarrhea-associated HUS caused by a Vero toxin–producing strain of *Escherichia coli* (serotype O157:H7 or O104:H4) and (2) diarrhea-negative familial types of atypical HUS that may be due to a defect in a complement regulatory protein (complement factors H, I, B, C3, membrane-cofactor protein CD46, or thrombomodulin).

VWF is a large, multimeric protein produced by endothelial cells that is involved in platelet adhesion to sites of vascular injury. VWF typically exists in plasma in a range of molecular sizes, but it is initially released from endothelial cells and tethered to the luminal cell membrane as unusually large VWF (ULVWF) via binding to the transmembrane protein P-selectin. A region in the A2 domain of VWF is responsible for binding to platelet GP Ib/IX/V, and the larger VWF multimers bind more avidly to platelets because of the presence of multiple binding sites. Tethered VWF is rapidly processed into smaller fragments released into the plasma because of cleavage of Tyr842-843Met bonds by the ADAMTS13 metalloprotease. In individuals with a deficiency of ADAMTS13, the ULVWF remains attached to endothelial cells, leading to platelet adhesion, aggregation, and microvascular thrombosis. It is thought that these long VWF–platelet aggregates can detach and embolize, further facilitating microvascular thrombosis.

## Pathologic Features and Laboratory Studies

A review of the CBC highlights a normocytic anemia with profound thrombocytopenia; platelet counts are frequently less than $20 \times 10^3/\mu L$. The reticulocyte count, red blood cell distribution width, and MPV are often increased, indicating the increased turnover of both erythrocytes and platelets. Morphologic evaluation of

**THROMBOTIC THROMBOCYTOPENIC PURPURA—PATHOLOGIC FEATURES**

**Complete Blood Cell Count and Peripheral Smear Morphology**
- Normocytic anemia with profound thrombocytopenia (less than $20 \times 10^3/\mu L$)
- Increased reticulocyte count, red cell distribution width, and MPV
- Red blood cell morphology shows anisocytosis and prominent schistocytes (see Figure 2-18)

**Bone Marrow Findings**
- Bone marrow findings are nonspecific, but small vessels may show platelet thrombi
- Autopsy shows obstructive platelet thrombi in the microvasculature of the brain, heart, pancreas, spleen, adrenal gland, and kidneys, but rarely the lungs

**Platelet Function Tests**
- Usually unhelpful because of the degree of thrombocytopenia

**Ancillary Studies**
- ADAMTS13 assays show a decreased amount or decreased function
- Inhibitors may be detected
- Patients with acute TTP usually have activity less than 5%
- VWF multimer analysis may show an increase in ultrahigh molecular weight multimers, but other coagulation assays (PT, APTT, VWF antigen, factor VIII, ristocetin cofactor) are usually normal

**Differential Diagnosis**
- Exclude other consumptive thrombocytopenic disorders
- DIC may also show schistocytes on the peripheral smear but can be distinguished by characteristic coagulation abnormalities, including elevated PT, APTT, D-dimer, and decreased clotting factor levels
- Hemolytic uremic syndrome is distinguishable by serology for *E. coli* O157:H7 or O104:H4, lack of ADAMTS13 abnormalities, and more severe renal dysfunction
- HIT should have characteristic positive laboratory tests and a history of heparin exposure

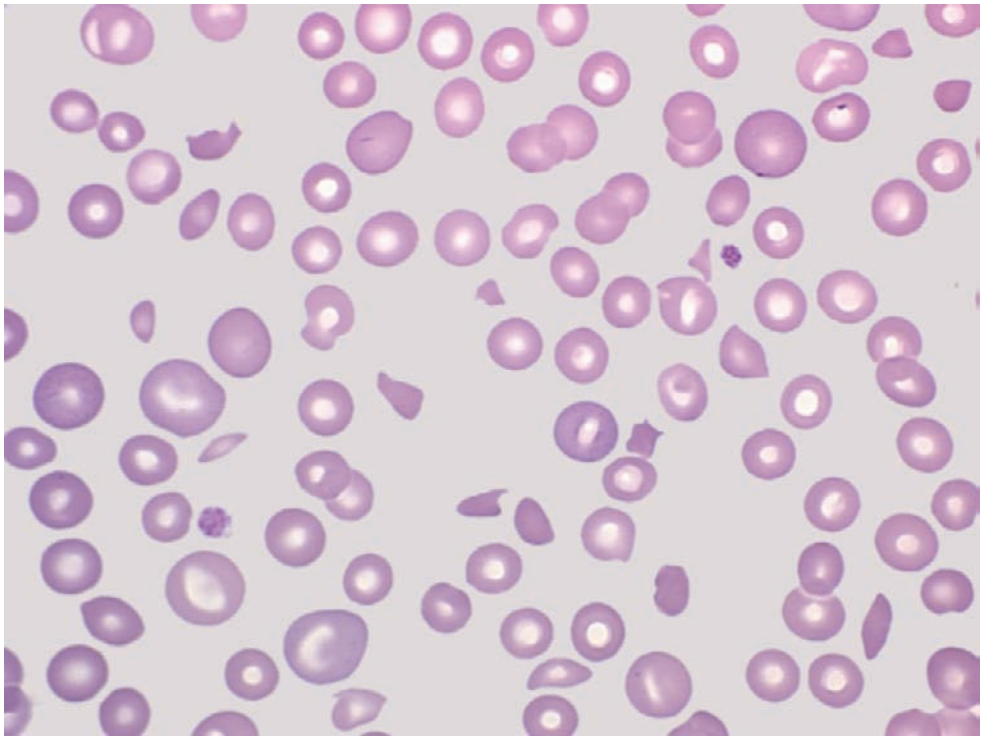

**FIGURE 2-18**

Peripheral smear in thrombotic thrombocytopenia purpura. Notice the typical morphologic features of a microangiopathic hemolysis: schistocytes (erythrocyte fragments), anisocytosis, and polychromasia with decreased numbers of platelets (original magnification × 40).

the peripheral blood smear reveals erythrocyte polychromasia and anisocytosis with prominent schistocytes (Figure 2-18). Bone marrow evaluation is typically not necessary in establishing a diagnosis of TTP, but it may be helpful to exclude other causes of thrombocytopenia. Bone marrow morphology is typically nonspecific and shows normal to increased numbers of megakaryocytes. Small vessels may show platelet thrombi, but these are not always observed. Autopsy reflects the pathophysiology of the disease, with platelet-rich thrombi plugging the microvasculature of the brain, heart, pancreas, spleen, adrenal gland, and kidneys but rarely involving the liver or lungs, in distinction from DIC. On immunohistochemical analysis, these thrombi are also rich in VWF, in contrast to the platelet-fibrin thrombin in DIC.

Several different assays for the VWF-cleaving metalloproteinase are available, including assays of ADAMTS13 activity, antigen, neutralizing or nonneutralizing autoantibodies, and genetic characterization of the *ADAMTS13* gene for mutations. The functional assays are based on the degradation of VWF or VWF-derived peptides and detection of the cleavage products. The initial assay based on degradation of intact VWF measured the enzyme activity by following a progressive decrease in VWF multimer size, but this relied on a VWF multimer Western blot assay that took as long as 2 days to perform and was not readily available in most laboratories. More recent modifications of the assay based on intact VWF measure the metalloprotease-induced decrease in VWF multimer size by either immunoradiometric assay in microtiter plates or tracking the ability of VWF to bind to collagen or ristocetin, an activity that is directly proportional to VWF molecular weight.

More recently developed functional assays are based on the degradation of VWF peptides with direct detection of metalloprotease activity. These assays have used recombinant ADAMTS13 substrate peptides from the VWF A2 domain, such as L1591-R1668 or D15960R1668. Peptide cleavage is often facilitated by a denaturing agent (i.e., guanidine HCl or urea). Peptide cleavage products (labeled, unlabeled, or both) are detected by various techniques, including ELISA, Western blot, and fluorescence resonance energy transfer.

Immunoassays for quantitative measurement of ADAMTS13 antigen are available. The results may be normal in patients with TTP resulting from neutralizing autoantibodies, where immune complexes are formed. Alternatively, the antigen levels may be decreased if nonneutralizing autoantibodies lead to ADAMTS-13 clearance. Neutralizing antibodies can be detected using a mixing study of heat-inactivated patient and normal plasma (typically a 1:1 mix) combined with either an immunoassay or functional assay.

Genetic studies of ADAMTS13 by sequence analysis can be performed. Published studies have shown the majority of mutations to be missense (59%), with 13%

nonsense, 13% deletions, 6% insertions, and 9% splice site mutations.

Plasma ADAMTS13 activity in normal individuals typically ranges from 50% to 180%. Patients with acute TTP usually have activity less than 5%. Variably decreased ADAMTS13 activity can also be observed with liver disease, disseminated malignancy, and inflammatory disorders and during pregnancy. Infants may also have a lower activity than do adults.

Traditional screening coagulation studies, such as PT and APTT, are normal in patients with TTP. Results of assays for VWF, such as VWF antigen, ristocetin cofactor, and factor VIII, are typically normal. However, VWF multimer analysis may show an increase in ULVWF multimers during the acute disease presentation.

### Differential Diagnosis

TTP can be diagnosed definitively in many patients based on the distinct clinical features and markedly decreased ADAMTS13 activity. However, ADAMTS13 assays are not widely available, and rapid diagnosis often rests on excluding other consumptive thrombocytopenic disorders. In addition, many patients with clinical symptoms of TTP have only modestly decreased levels of ADAMTS13, whereas other individuals with markedly decreased ADAMTS13 levels might not have clinical symptoms of TTP.

TTP and DIC are both thrombotic microangiopathies with schistocytes on the peripheral smear, but these two disorders can be distinguished by the characteristic coagulation test abnormalities found in DIC, as indicated previously. Hemolytic-uremic syndrome is distinguishable by serology for *E. coli* O157:H7 or O104:H4, a lack of abnormalities in ADAMTS13, and the more severe renal dysfunction. Patients with ITP typically lack hemolytic anemia and multiorgan failure and do not have schistocytes on the peripheral smear. TTP can be distinguished from posttransfusion purpura resulting from a lack of association with transfusions or platelet HPA-1a/1b genotype. Distinction of TTP from drug-induced thrombocytopenia can be difficult in patients treated with multiple drugs, but these disorders usually lack microangiopathic thrombosis or organ failure. HIT is one drug-induced thrombocytopenia that is complicated by thrombosis, but HIT is preceded by heparin exposure 5 to 14 days prior and is characterized by large-vessel thrombosis and the presence of anti-platelet factor 4 antibodies. Paroxysmal nocturnal hemoglobinuria occasionally manifests with thrombocytopenia and microangiopathic hemolysis, but it is usually distinguishable by a lack of renal dysfunction and a flow cytometric analysis demonstrating decreased expression of glycophosphatidylinositol-linked proteins such as CD55 and CD59 on red blood cells or neutrophils.

### Prognosis and Therapy

TTP is associated with a high mortality because of multiorgan failure. Supportive therapy has typically used daily plasma exchange with fresh frozen plasma, and the majority of patients now survive the initial episode. However, relapse of TTP may be seen in 30% to 60% of patients, with relapse most frequent in the first month. Newer therapies based on infusion of recombinant ADAMTS13 or rituximab to suppress the immune response have shown promise.

## HYPERTENSIVE DISORDERS OF PREGNANCY, INCLUDING PREECLAMPSIA

Thrombocytopenia complicating pregnancy has many causes, including incidental thrombocytopenia of pregnancy, ITP, antiphospholipid antibody syndrome, and hypertensive disorders of pregnancy. Incidental thrombocytopenia of pregnancy is the most common cause of thrombocytopenia during pregnancy (up to 75% of cases) and is usually asymptomatic. The antiphospholipid antibody syndrome, lupus anticoagulant, and systemic lupus erythematosus are uncommon but are associated with significant pregnancy complications, including venous thrombosis and spontaneous abortion.

Hypertensive disorders of pregnancy account for approximately 20% of pregnancy-associated thrombocytopenia. Preeclampsia and eclampsia are associated with graded degrees of hypertension and proteinuria, headache, abdominal pain, and thrombocytopenia. Laboratory studies will show increased MPV and increased reticulated platelets associated with increased platelet activation. The disorder is associated with deregulation of the coagulation cascade and hypofibrinolysis; there is a reported weak association with *PAI1* gene polymorphisms. Subsets of these patients have a syndrome called *hemolysis, elevated liver enzymes, and low platelets* (HELLP). There may be some overlap between patients with HELLP syndrome and gestational TTP or hemolytic-uremic syndrome.

## OTHERS

Other causes of a destructive thrombocytopenia include the Kasabach-Merritt syndrome, hypersplenism, human immunodeficiency virus–associated thrombocytopenia, malignancy, and connective tissue disorders. Kasabach-Merritt is an infrequent disorder with cavernous hemangiomas associated with a microangiopathy hemolytic anemia, consumptive coagulopathy, and

thrombocytopenia. Hypersplenism is associated with a destructive thrombocytopenia resulting from platelet sequestration in an enlarged spleen.

## DECREASED PLATELET PRODUCTION

Thrombocytopenias resulting from decreased platelet production include both rare congenital and more common acquired causes. Acquired causes of thrombocytopenia are generally due to disorders associated with marrow failure, such as aplastic anemia and myelodysplasia, and are covered in other chapters. Fanconi's anemia is a congenital thrombocytopenia, but is distinguished by erythroid hypoplasia and DNA instability and is covered in Chapter 5. Other hereditary thrombocytopenias associated with large platelets are covered in the section Thrombocytopenia with Increased Platelet Size.

## HEREDITARY THROMBOCYTOPENIAS WITH DECREASED PLATELET PRODUCTION AND NORMAL PLATELET SIZE

### Familial Thrombocytopenia–Leukemia

Patients with this autosomal dominant syndrome have a relatively mild thrombocytopenia (80 to $100 \times 10^3/\mu L$) but often have a bleeding diathesis symptomatically out of proportion to the degree of thrombocytopenia, consistent with clinical platelet dysfunction. Platelet function testing usually reveals an SPD. Approximately half of the patients develop a malignancy, usually acute myeloid leukemia or solid tumors. Mutations in the transcription factor RUNX1 (CBFA2, AML1) and decreased expression of myosin light chain (MYL9) have been identified in families with this disorder.

### Congenital Amegakaryocytic Thrombocytopenia

These patients have a genetic defect in the *MPL* (1p34) gene, leading to a deficiency or dysfunction of the thrombopoietin receptor. They have neonatal thrombocytopenia with normal platelet size and a lack of skeletal abnormalities associated with marrow megakaryocytic hypoplasia that evolves into marrow aplasia. It should be differentiated from Fanconi anemia, thrombocytopenia with absent radii and congenital amegakaryocytic thrombocytopenia with radioulnar synostosis by the lack of other skeletal abnormalities, hypoplasia of other cell lines, and by the presence of *MPL* mutations and high plasma levels of TPO. Two types of mutations have been described: type I, with a stop codon or frameshift eliminating transcription of the intracellular domain, and Type II, with missense mutations resulting in a dysfunctional receptor with some residual signaling. It is worth mentioning that the mutations of MPL associated with congenital amegakaryocytic thrombocytopenia are distinct from the *MPL* mutations leading to transmembrane abnormalities and persistent receptor activation, which are associated with congenital thrombocytosis and some myeloproliferative neoplasms.

### Congenital Amegakaryocytic Thrombocytopenia with Radioulnar Synostosis

Patients with this autosomal dominant syndrome have a mutation in the *HOXA11* gene (7p15-14), which is involved in the development of both the forelimbs and megakaryocytes. The patients have thrombocytopenia of normal platelet size with a general aplastic anemia. Other associated abnormalities include radioulnar synostosis and sensorineural hearing loss, together with other skeletal malformations. Bone marrow evaluation reveals reduced to absent megakaryocytes.

### Thrombocytopenia with Absent Radii

Thrombocytopenia with absent radii is an autosomal recessive disorder that is characterized by severe thrombocytopenia of normal platelet size presenting within the first year of life. Bone marrow evaluation during this period shows reduced megakaryocytes. The thrombocytopenia ameliorates during infancy and there is lack of marrow failure. The thrombocytopenia progressively improves, with normal platelet count during adulthood. The white blood cell count is often elevated. The syndrome is accompanied by bilateral radial aplasia and other abnormalities, including cardiac and renal defects. The genetic defect has not been characterized. However, TPO levels are increased and no abnormalities of the TPO receptor have been identified, implicating defects in the TPO signaling pathway.

### Acquired Thrombocytopenias with Decreased Platelet Production

These disorders are due to ineffective megakaryopoiesis and are usually associated with many disorders of bone marrow failure, such as aplastic anemia, drug-induced aplasia, toxic chemicals, viral infection, radiation, myelophthisic disorders, paroxysmal nocturnal hemoglobinuria, and myelodysplasia. A few rare disorders, such as acquired amegakaryocytic thrombocytopenic purpura and cyclic thrombocytopenia, affect platelet production only.

## PLATELET DYSFUNCTION WITH NORMAL PLATELET COUNT

Platelet dysfunction with a normal platelet count usually indicates a qualitative platelet disorder. These disorders would be sought in a patient with a bleeding

diathesis despite a normal PT, APTT, and platelet count. A platelet function screening test result would be abnormal and test results for VWD would be normal. Platelet aggregation studies would then be used to distinguish the following disorders, followed by more specific tests, if required. Most drug-induced platelet dysfunction will also demonstrate platelet dysfunction with a normal platelet count, so it is extremely important to take a careful drug history. Platelet aggregation abnormalities typically found with antiplatelet drugs such as aspirin, GP IIb/IIIa antagonists, and the thienopyridines can be found in Table 2-2.

## GLYCOPROTEIN DISORDERS

### GLANZMANN THROMBASTHENIA

#### Clinical Features

Glanzmann thrombasthenia is a congenital deficiency or dysfunction of GP IIb/IIIa (αIIb/β3 integrin), the fibrinogen receptor responsible for mediating platelet aggregation. It is an autosomal recessive disorder that manifests in lifelong mucocutaneous bleeding, with purpura, epistaxis, gingival bleeding, and menorrhagia as common features. The GP IIb/IIIa deficiency leads to a virtual absence of platelet aggregation to most agonists, but there is also decreased intracellular signaling, decreased procoagulant activity, and decreased microparticle production. Glanzmann thrombasthenia can be classified according to the amount of GP IIb/IIIa: type I, 0% to 5% of normal; type II, 6% to 25%; and variant disease, 50% to 100% with abnormal fibrinogen binding.

The genes for both αIIb and β3 are located at chromosome 17q21-23, with the αIIb gene *(ITGA2B)* having 30 exons and the β3 gene *(ITGB3)* having 15 exons. Many different mutations and partial deletions of both the GP IIb and GP IIIa have been described; updates can be accessed at http://sinaicentral.mssm.edu/intranet/research/glanzmann. The database currently lists 103 mutations of *ITGA2B* and 68 mutations of *ITGB3* (accessed March 9, 2011). Because of the large number of mutations identified, many patients with Glanzmann thrombasthenia are compound heterozygotes. Often, mutations of one subunit prevent the formation of the entire complex on the platelet surface by inhibiting transport of the αIIbβ3 complexes from the endoplasmic reticulum to the Golgi, and it is common to detect neither GP IIb nor GP IIIa on the surface of the platelet. The β3 integrin chain is also a part of the vitronectin receptor (αvβ3), which is present on vascular endothelial cells and other cell types. Individuals with β3 mutations do not have a distinctive phenotype, and vascular development or angiogenesis does not seem to be affected.

**GLANZMANN THROMBASTHENIA—FACT SHEET**

**Definition**
- Congenital deficiency or dysfunction of GP IIb/IIIa (αIIb/β3 integrin), the fibrinogen receptor responsible for mediating platelet aggregation
- Type I, 0% to 5% GP IIb/IIIa; type II, 6% to 25%; variant, 50% to 100% with abnormal fibrinogen binding
- The *ITGA2B* and *ITGB3* genes are located at 17q21-23, and more than 160 mutations or deletions have been described

**Incidence**
- Rare

**Morbidity and Mortality**
- Moderately severe bleeding disorder

**Gender, Race, or Age Distribution**
- Autosomal recessive congenital disorder; many patients are compound heterozygotes

**Clinical Features**
- Lifelong mucocutaneous bleeding disorder

**Prognosis and Treatment**
- Routine treatment is not usually necessary
- Prophylactic therapy with desmopressin or platelet transfusions is usually required before surgical procedures

#### Pathologic Features and Laboratory Studies

The CBC in individuals with Glanzmann thrombasthenia is usually normal, with normal platelet morphology on the peripheral smear. This disorder has distinctive laboratory features, so bone marrow analysis is not indicated.

In patients with Glanzmann thrombasthenia, the bleeding time and PFA-100 result will be abnormal. No aggregation response will be seen upon addition of ADP-, collagen-, epinephrine-, and arachidonic acid–aggregating agents, whereas the ristocetin-induced aggregation is normal (Figure 2-19). This finding is virtually diagnostic of Glanzmann thrombasthenia, but the disorder can be confirmed by platelet flow cytometry or crossed immunoelectrophoresis of platelet membrane proteins (see Table 2-2). Most patients with type I Glanzmann thrombasthenia will have a deficiency of both GP IIb and GP IIIa (Figure 2-20). Patients with type II Glanzmann thrombasthenia may have decreased, but detectable, surface expression of both proteins. Distinguishing patients with variant Glanzmann thrombasthenia from heterozygous carriers may be difficult, because both may have slightly

## GLANZMANN THROMBASTHENIA—PATHOLOGIC FEATURES

### Complete Blood Cell Count and Peripheral Smear Morphology
- Usually normal
- The platelet count and morphology are normal

### Bone Marrow Findings
- Not helpful

### Platelet Screening Tests
- Abnormal PFA-100

### Platelet Aggregation
- Characteristic abnormality; no aggregation response to ADP, collagen, epinephrine, and arachidonic acid with normal dose response to ristocetin

### Ancillary Studies
- Flow cytometry or crossed immunoelectrophoresis will show a lack of expression of both GP IIb and GP IIIa
- Patients with type II Glanzmann thrombasthenia may have decreased but detectable surface expression of both proteins
- Genetic diagnosis can be done by sequencing

### Differential Diagnosis
- The distinctive laboratory features usually allow definitive diagnosis
- Afibrinogenemia, which can give similar aggregation findings, has undetectable fibrinogen levels and normal expression of GP IIb/IIIa
- Inhibitors to GP IIb/IIIa can be detected by mixing the patient's plasma with normal platelets

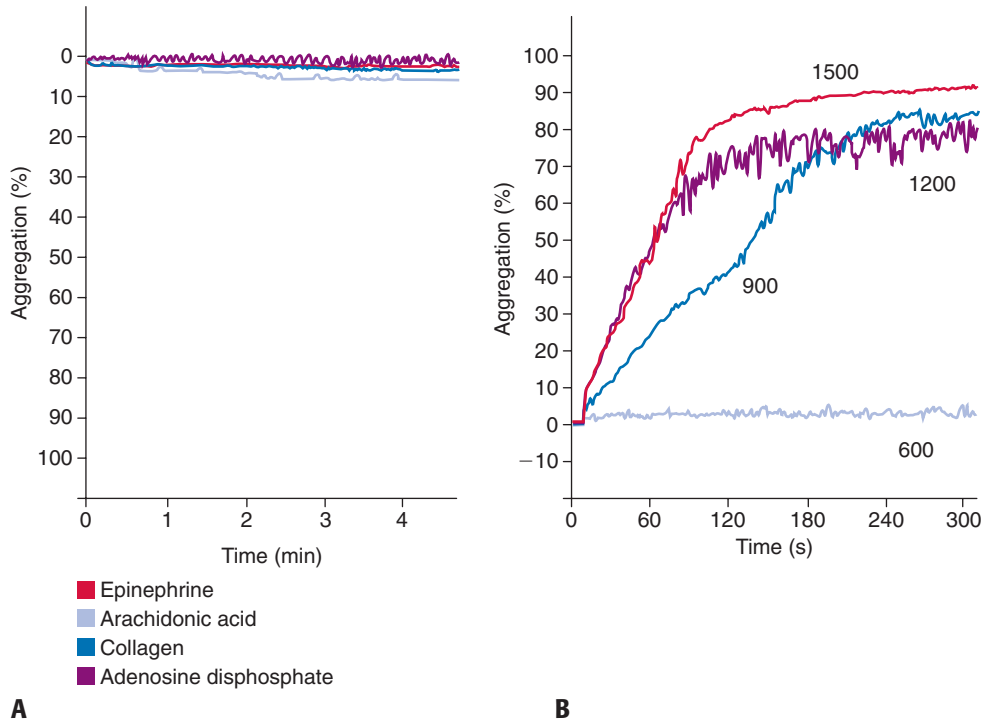


**FIGURE 2-19**

Light transmission platelet aggregation in Glanzmann thrombasthenia. **A,** Note the absent aggregation response to adenosine diphosphate (ADP), epinephrine (EPI), collagen (COL), and arachidonic acid (AA; optical platelet aggregation using a Biodata PAPS-4 aggregometer). **B,** The ristocetin dose response is normal (optical platelet aggregation using a Helena PACKS-4 aggregometer).

decreased levels of the GP IIb/IIIa complex on the platelet surface. Flow cytometric analysis does not usually provide a quantitative measure of surface protein expression, but some quantitative assays are now available that use calibration beads. Additional laboratory studies in patients with Glanzmann thrombasthenia will show decreased platelet-associated fibrinogen, defective fibrinogen binding to platelets, and decreased clot retraction. Genetic diagnosis of Glanzmann thrombasthenia can be done by direct sequencing. However, this sequencing is not widely available, even in research laboratories, because of the complexity of the genes and the large number of known mutations.

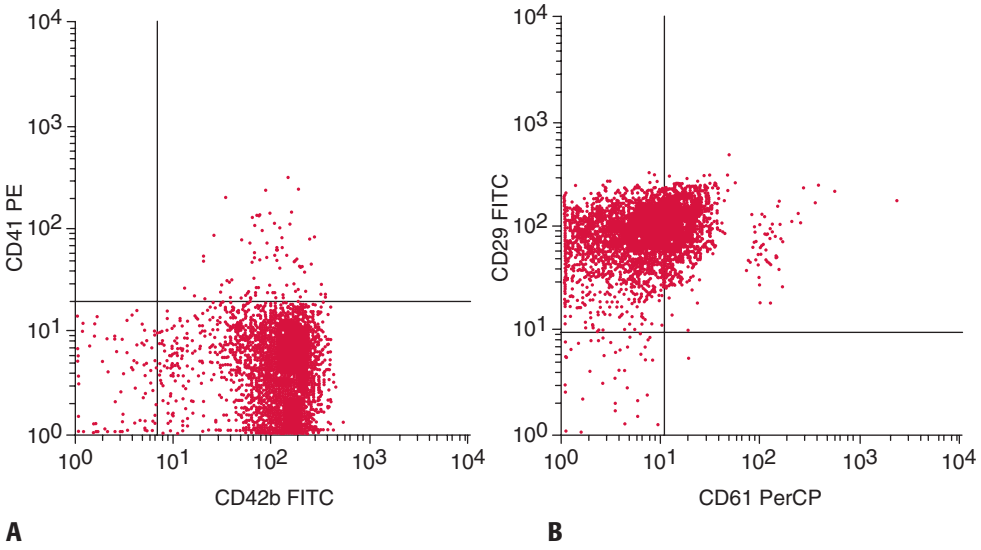


**FIGURE 2-20**

Flow cytometric immunophenotyping in Glanzmann thrombasthenia. The scattergrams show glycoprotein surface expression in a gated platelet population from a whole blood specimen in a patient with Glanzmann thrombasthenia. **A,** The platelet population shows normal surface expression of CD42b (glycoprotein [GP] Ib) with no expression of CD41 (GP IIb/IIIa). **B,** The platelet population shows markedly decreased surface expression of CD61 (GP IIIa) with normal surface expression of CD29 (GP IIa; BD FACScan, Becton Dickenson, San Jose, CA). *FITC,* Fluorescein isothiocyanate.

## Differential Diagnosis

Glanzmann thrombasthenia has distinctive laboratory features and usually can be diagnosed definitively. However, afibrinogenemia, a rare deficiency of fibrinogen, can manifest with similar initial platelet aggregation results with no response to ADP, epinephrine, collagen, and arachidonic acid. However, the aggregation defect in afibrinogenemia is restored with the addition of fibrinogen to the specimen. In addition, patients with afibrinogenemia have immeasurably long fibrinogen-dependent clotting tests, such as PT and APTT, and normal platelet flow cytometry. The GP IIb/IIIa antagonist drugs induce a Glanzmann-like inhibition of platelet function as part of their potent antiplatelet mechanism, but these are easily distinguished by the history of intravenous medication therapy.

## Prognosis and Therapy

The bleeding symptoms in Glanzmann thrombasthenia are significant, and prophylactic therapy with platelet transfusions or desmopressin is recommended before major surgical procedures. Desmopressin leads to increased blood levels of VWF and does not directly affect the platelets, but it has been shown to lessen bleeding complications in Glanzmann thrombasthenia.

## BERNARD-SOULIER SYNDROME

Bernard-Soulier syndrome is discussed under Thrombocytopenia with Increased Platelet Size.

## PLATELET-TYPE VON WILLEBRAND DISEASE

Most types of VWD are due to abnormalities of the VWF protein, but platelet-type VWD (also called *pseudo-VWD*) is due to *GPIBA* mutations leading to a molecular abnormality of the GP Ib/IX/V VWF receptor on platelet surfaces. In this autosomal dominant inherited disorder, unusual gain-of-function abnormality of GP Ib leads to increased binding of platelet GP Ib/IX/V to VWF, with removal of high molecular weight VWF multimers from the plasma, resulting in a bleeding diathesis. Increased ADAMTS13 cleavage of platelet-bound VWF may also contribute to the bleeding phenotype.

Platelet-type VWD is distinct from Bernard-Soulier syndrome in that the platelet morphology is normal and molecular defects of GP Ib (Gly233Val and Met239Val) lead to increased binding of GP Ib to soluble VWF, not decreased binding, as typical of Bernard-Soulier syndrome. Platelet-type VWD closely resembles type 2b VWD, which is due to a gain-of-function mutation in the A2 domain of VWF, also leading to clearance of high-molecular-weight multimers from plasma. The two disorders can be distinguished by DNA analysis of exon 28 of the VWF gene, which is available at some large reference laboratories. VWF analysis is similar in the

two disorders, with the findings of low-normal VWF and factor VIII levels and decreased functional ristocetin cofactor activity. Ristocetin aggregation shows an increased dose response in both disorders. However, the addition of cryoprecipitate with its high-molecular-weight VWF forms causes spontaneous aggregation in platelets from individuals with platelet-type VWD, but not with type 2b VWD.

## GLYCOPROTEIN IV DISORDERS

GP IV, also known as CD36, is a membrane glycoprotein expressed on platelets, monocytes, macrophages, and several other cell types that was recently demonstrated to be involved in platelet activation in response to oxidized phospholipids, including oxidized LDL. It also may have a role as an adhesive receptor for collagen and thrombospondin. It is deficient in approximately 4% to 7% of healthy donors in the Japanese population and 7% to 10% of normal individuals in a Sub-Saharan African population. The deficiency state has been described to be associated with hypoglycemia. One reported molecular basis of GP IV deficiency is a polymorphism in codon 90, leading to a Ser132Pro shift. A clinical association with GP IV deficiency has not been elucidated specifically.

## COLLAGEN RECEPTOR DISORDERS

### Glycoproteins Ia/IIa

The integrin $\alpha_2\beta_1$ (GP Ia/IIa) is a platelet surface receptor for collagen. Rare patients with deficiency of this receptor and selective absence of platelet aggregation to collagen have been described. These patients have decreased adhesion to vascular subendothelium, poor activation, and decreased spreading. Interestingly, the collagen-aggregation defect was reported to correct after menopause. An acquired deficiency of $\alpha_2\beta_1$ has been described in isolated cases of myeloproliferative disorders. More commonly, polymorphisms of $\alpha_2\beta_1$ leading to dysfunction or decreased platelet surface density of the receptor can lead to decreased platelet adhesion to collagen and an increased risk in association with VWD.

### Glycoprotein VI

GP VI is a member of the immunoglobulin superfamily of cell membrane receptors that participates with $\alpha_2\beta_1$ and FcR$\gamma$ in platelet adhesion under shear. Deficiency of GP VI has been described in Japanese subjects in conjunction with a mild bleeding diathesis and lack of collagen-induced platelet aggregation. Defects in GP VI can occur because of receptor loss by proteolysis involving the ADAM10 metalloprotease.

## ADENOSINE DIPHOSPHATE RECEPTOR ABNORMALITIES

As the primary receptor for ADP, genetic variations in the *P2Y12* ADP receptor gene at chromosome 3q24-25 could have a marked effect on platelet function. Rare patients with congenital platelet defect characterized by abnormalities of *P2Y12* and severe impairment of platelet response to ADP have been reported. One, in an Italian family, was associated with a frame-shift leading to premature truncation of the protein. Another was associated with a significant bleeding disorder and 2-bp deletion (CA) at codon 240 of the *P2Y12* gene, introducing a premature stop codon. A rare mutation of the *P2X1* gene has been identified that is associated with bleeding symptoms.

## OTHER SURFACE GLYCOPROTEIN DEFECTS

Rare defects of the platelet receptors for thromboxane A2 and epinephrine have been described. These defects can be distinguished because usually they demonstrate a selective defect in aggregation to a single agonist. These disorders can be confirmed by flow cytometry in which a deficiency of a surface GP is identified, but this testing is likely only available in a research setting.

## PLATELET RELEASE DEFECTS

## ALPHA AND DENSE GRANULE STORAGE POOL DISORDERS

### Clinical Features

Abnormalities of platelet secretion can be due to either deficiency of platelet granules or defects in the signal transduction events that regulate secretion or aggregation. Platelet SPDs can be congenital or acquired. They are the result of either a deficiency of granules ($\alpha$- or dense granules, or both) or defective granule release upon platelet activation. The molecular defect underlying most types of storage pool deficiency is unknown. Dense granule SPDs ($\delta$-SPDs) can be seen as a singular clinical entity or as part of other hereditary disorders, such as Chédiak-Higashi syndrome, Hermansky-Pudlak syndrome, thrombocytopenia with absent radii, or Wiskott-Aldrich syndrome. Between 10% and 18% of patients with congenital platelet dysfunction have SPDs. The $\alpha$-SPD leading to the gray platelet syndrome has been discussed previously under Macrothrombocytopenias with Neutrophilic Inclusions, because this disorder leads to the production of decreased numbers of large platelets devoid of $\alpha$-granules, giving them a ghostly gray color on the peripheral smear. Another type of $\alpha$-granule

**DENSE GRANULE STORAGE POOL DISORDER—FACT SHEET**

**Definition**
- Deficiency of platelet dense granules
- Can be seen as a singular entity or in association with other hereditary disorders such as Chédiak-Higashi syndrome, Hermansky-Pudlak syndrome, thrombocytopenia with absent radii, or Wiskott Aldrich Syndrome

**Incidence**
- Rare (estimated 10% to 18% patients with congenital platelet dysfunction)

**Morbidity and Mortality**
- Mucocutaneous bleeding of variable severity

**Gender, Race, or Age Distribution**
- Autosomal dominant congenital disorder

**Clinical Features**
- Mucocutaneous bleeding of variable severity

**Prognosis and Therapy**
- Prophylactic therapy with platelet transfusions or desmopressin may be helpful before surgical procedures

disorder, the Québec Platelet syndrome, is due to duplication of the *PLAU* gene, leading to increased urokinase plasminogen activator in α-granules leading to the degradation of α-granule proteins. A rare α/δ-SPD has been described that has features of both disorders.

Patients with δ-SPD have a bleeding diathesis of variable severity, usually characterized by mucocutaneous bleeding. In more severely affected patients, postsurgical bleeding is also observed. There have been rare familial associations with primary pulmonary hypertension and development of acute myelogenous leukemia. In patients with Chédiak-Higashi syndrome, there is neutropenia with recurrent infections in addition to both the SPD and oculocutaneous albinism. Neutrophils in patients with Chédiak-Higashi syndrome have characteristic peroxidase-positive cytoplasmic granules.

Hermansky-Pudlak syndrome is a well-defined autosomal recessive syndrome with oculocutaneous albinism and variable hypopigmentation of the hair, skin, and irises. The syndrome is prevalent in Puerto Rico. Hermansky-Pudlak syndrome (HPS) is a disorder of subcellular organelles in many tissues, including dense granules of platelets. The HPS proteins interact together in complexes called *BLOCS* that, when disrupted by genetic mutations, lead to defective trafficking and sorting of dense granule membrane proteins. Seven different subtypes of the syndrome have been described to be caused by genetic mutations of eight genes (*HPS1* through *HPS8*). Type I Hermansky-Pudlak syndrome is the most severe form and is due to a 16-bp duplication mutation of the *HPS1* gene located at 10q23.1-13.3. This form of the syndrome is most commonly observed in northwest Puerto Rico, where it has been described in one in 1800 individuals and in 5 of 6 albinos. The other seven subtypes of Hermansky-Pudlak syndrome have been described in relatively few individuals, but the gene defects in these disorders have recently been elucidated because of studies of associated SPDs in mice.

## Pathologic Features and Laboratory Studies

In patients with δ-SPD, the CBC indices are normal and the peripheral smear shows normal numbers of platelets with apparently normal granule staining on the Wright-stained preparation, as the purple granules usually

**DENSE GRANULE STORAGE POOL DISORDER—PATHOLOGIC FEATURES**

**Complete Blood Cell Count and Peripheral Smear Morphology**
- CBC indices are normal, and the peripheral smear shows normal numbers of platelets with normal granule staining on the Wright-stained preparation

**Bone Marrow Findings**
- Megakaryocytes are present with normal morphology

**Platelet Screening Tests**
- The PFA-100 may be abnormal in some but not all patients

**Platelet Aggregation**
- Decreased aggregation is a response to ADP, epinephrine, and collagen, with normal aggregation to arachidonic acid and ristocetin
- Aggregation studies with ADP and epinephrine characteristically show only a primary wave of aggregation with an absent to decreased secondary wave, but aggregation studies with these agonists may be normal in as many as 25% of patients

**Ancillary Studies**
- Decreased lumiaggregometry ATP release and decreased mepacrine dense granule uptake are observed by flow cytometry
- Ultrastructural abnormalities in these disorders usually show decreased dense granules, although dense granules are not usually absent
- Often the dense granules lack their characteristic electron-dense core and appear empty
- An increased platelet ATP-to-ADP ratio is often seen with δ-SPD

**Differential Diagnosis**
- α-SPD (gray platelet syndrome) has decreased α-granules
- Acquired platelet SPDs can be seen with underlying myeloproliferative disorders, DIC, cardiac devices, and TTP in which the platelet degranulation is defective as a result of the disease

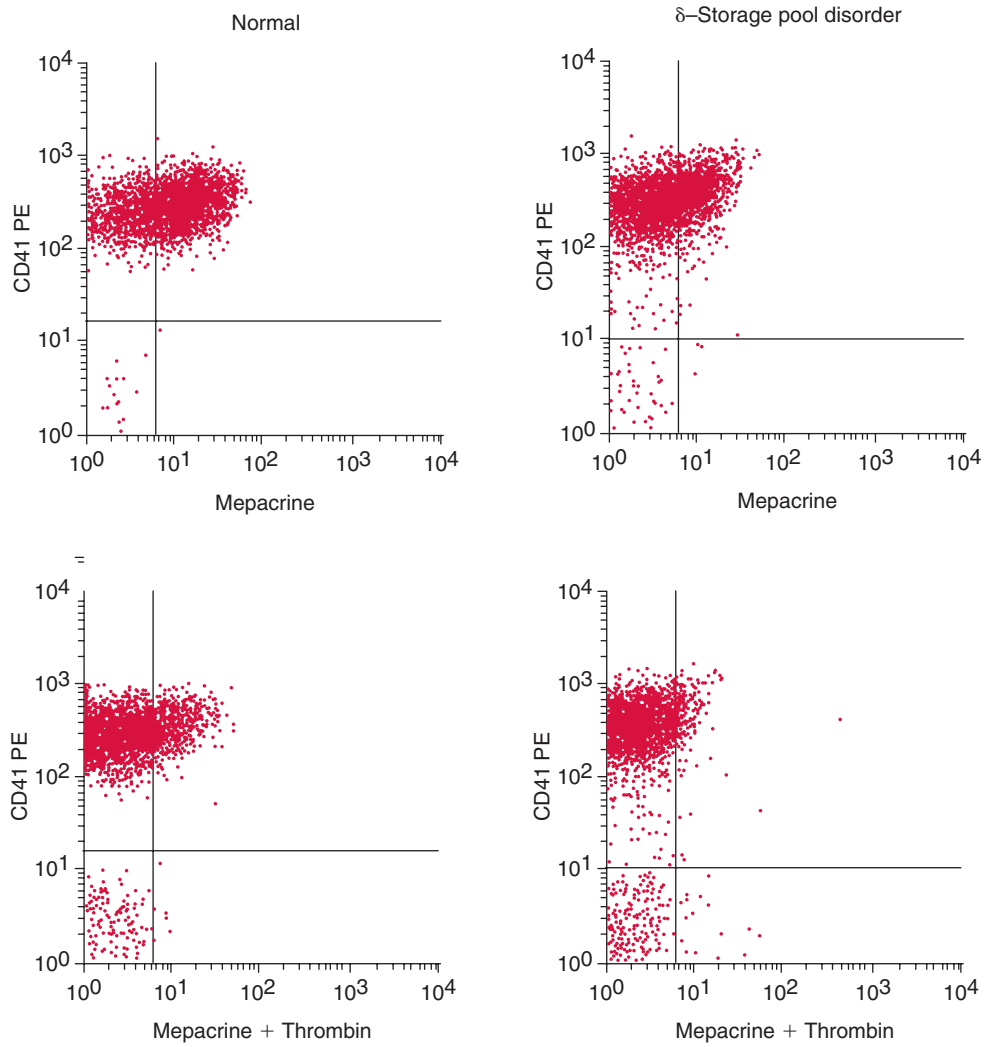


**FIGURE 2-21**

Decreased mepacrine uptake in dense granule storage pool disorder (SPD) by flow cytometry. In dense granule SPDs, there is a decrease in dense granules. This technique loads platelets with the fluorescent dye mepacrine, which is taken up specifically into dense granules. In patients with SPD, there is decreased uptake of mepacrine. This patient had 33% mepacrine uptake *(right panels)* compared with a normal control (70% uptake; *left panels*). Patients with SPD have a preserved granule release mechanism, despite the decreased granule numbers. In the lower panels, platelets have been treated with thrombin to stimulate granule release. The percent of release is calculated as the difference between the mepacrine uptake *(top)* and the thrombin–stimulated platelets *(bottom)*. The patient with an SPD had 65% release, compared to normal with 68% release.

visualized on the Wright stain are largely α-granules, which are present in normal numbers in this disorder. Bone marrow evaluation is not usually helpful because megakaryocytes are present in normal number with normal morphology.

A decreased aggregation response to ADP, epinephrine, and collagen, with normal aggregation to arachidonic acid and ristocetin, is often seen with δ-SPD. Aggregation studies with ADP and epinephrine characteristically show only a primary wave of aggregation with an absent to decreased secondary wave, but aggregation studies with these agonists may be normal in as many as 25% of patients. The PFA-100 test result may be abnormal in some but not all patients. Decreased lumiaggregometry ATP release and decreased mepacrine dense granule uptake are observed by flow cytometry (Figure 2-21). Agonist-stimulated mepacrine release from dense granules is usually not impaired. Ultrastructural abnormalities in these disorders show decreased dense granules, although dense granules are not usually absent. Often the dense granules lack their characteristic electron dense core and appear empty. The dense granule membrane protein granulophysin is usually present in normal amounts in isolated δ-SPD, but is deficient in δ-SPD associated with Hermansky-Pudlak syndrome and Chédiak-Higashi syndrome. This fact suggests that the latter two disorders have defective granule packaging, whereas isolated δ-SPDs are due to an inability to effectively store dense granule contents. An increased platelet ATP-to-ADP ratio is often seen with δ-SPD. Acquired platelet SPDs can be seen with underlying myeloproliferative disorders in which the platelet degranulation is defective as a result of the disease.

### Differential Diagnosis

Circulating exhausted platelets simulating SPD can be observed in clinical scenarios in which there is ongoing in vivo platelet activation, such as with cardiopulmonary bypass, implantation of ventricular assist devices or total artificial hearts, DIC, and TTP–hemolytic uremic syndrome. The isolated δ-SPD can be distinguished from these disorders by the lack of specific clinical associations, coagulation test abnormalities, or thrombosis.

Hermansky-Pudlak syndrome can be distinguished from isolated δ-SPD by the associated oculocutaneous albinism and its prevalence in the Puerto Rican population. Platelets in Hermansky-Pudlak syndrome have very low levels of the δ-granule membrane protein granulophysin, in contrast with normal levels in isolated δ-SPD. However, this assay is done only in the research setting. Chédiak-Higashi syndrome and Hermansky-Pudlak syndrome are both associated with oculocutaneous albinism, but Chédiak-Higashi syndrome is distinguishable by the presence of large peroxidase-positive cytoplasmic granules in neutrophils and recurrent infections. The gene responsible for Chédiak-Higashi syndrome is located at 1q42-44, and several mutations have been described, but definitive genetic diagnosis is not widely available.

## SIGNAL TRANSDUCTION DISORDERS

In addition to the SPDs, platelet release defects can be seen with disorders of platelet signal transduction. In general, these disorders compose a poorly defined group, but they may constitute a significant percentage of patients with abnormal secondary wave of aggregation and decreased granule release in which α- and δ-granules are not deficient. These disorders include defects of the platelet cyclooxygenase and phospholipase C pathways. Defects of the receptors for platelet agonists, such as ADP, collagen, thromboxane A2, and epinephrine, have been included in the earlier discussion of surface GP abnormalities.

Defects of thromboxane A2 synthesis have been described, including defective liberation of arachidonic acid from the platelet membrane by phospholipase A2, cyclooxygenase enzyme deficiency, or thromboxane synthase deficiency. Individuals with defective liberation of arachidonic acid will have normal aggregation with exogenous arachidonic acid, but decreased aggregation and thromboxane B2 production with other agonists, such as ADP. The rare individual with cyclooxygenase or thromboxane synthase deficiency will display an aspirin-like defect in aggregation despite no history of aspirin therapy. Platelet aggregation studies show a marked inhibition of platelet aggregation with arachidonic acid, depressed aggregation with collagen, and slightly decreased aggregation to ADP; aggregation with thromboxane analogues and ristocetin is usually normal. They also have defective production of thromboxane B2. Patients with cyclooxygenase deficiency also have defective production of prostacyclin (prostaglandin I2) by endothelium, whereas this activity is normal with thromboxane synthase defects. True cyclooxygenase deficiency can be diagnosed by demonstrating decreased cyclooxygenase levels on a Western blot of a platelet lysate. Distinguishing these disorders from surreptitious aspirin or salicylate use requires a careful history, because many over-the-counter drug preparations contain salicylates. Serum salicylate levels may be helpful, but they have a short plasma half-life because they are rapidly cleared from the plasma. The finding of acetylated platelet cyclooxygenase could definitively identify salicylate use, but this is a research assay only performed in specialized centers.

Defects of the signaling pathways, including G-protein activation, phospholipase C activation, calcium mobilization, pleckstrin phosphorylation, and tyrosine phosphorylation, have also been described. Abnormalities of the guanidine triphosphate–binding proteins that link surface receptors and intracellular enzymes, such as Gaq, Gai2, and Gas, have been described. In general, patients with signaling defects show normal thromboxane B2 production, decreased primary aggregation, and decreased granule release without granule, deficiency. In contrast to patients with dense granule storage pool disorder (δ-SPD), flow cytometry studies will show normal mepacrine uptake into dense granules but decreased agonist-induced release. Identification of the exact defect requires detailed biochemical and genetic studies, which are not available in most laboratories.

## PLATELET PROCOAGULANT DISORDERS

Platelets have an important procoagulant role with assembly of coagulation complexes on activated platelet membranes that are rich in phosphatidyl serine. A rare congenital platelet functional disorder is Scott syndrome, which is caused by a defective "flip" of phosphatidyl serine to the outer table of the platelet membrane and is a bleeding disorder resulting from a lack of thrombin generation. These patients will have normal platelet aggregation study results but have abnormal platelet procoagulant activity (platelet factor 3) and decreased microparticle formation. A missense R1925Q mutation of the ATP-binding cassette transporter A1 gene *(ABCA1)* leading to abnormal regulation of phosphatidyl serine translocase regulation, was found in a patient with Scott syndrome.

## PLATELET DYSFUNCTION ASSOCIATED WITH OTHER ILLNESSES

Other significant disorders of platelet function with platelet counts in the normal range are usually acquired with the presence of another disease or drug therapy. These disorders are much more common than the disorders described previously. Platelet dysfunction is often observed with chronic renal failure or liver disease and in patients with a variety of myeloproliferative and lymphoproliferative disorders. Platelet dysfunction also may be associated with a variety of clinical scenarios such as cardiopulmonary bypass, implantation of vascular grafts, prosthetic heart valves, and ventricular assistive devices. Platelet dysfunction in these disorders is usually difficult to characterize, because nonspecific defects of platelet aggregation are usually observed.

## SUMMARY

Multiple causes exist for platelet-derived disorders. The laboratory evaluation of these disorders can range from simple to complex. However, the laboratory evaluation of all these disorders should start with measurement of the platelet count and evaluation of platelet morphology on a well-prepared blood smear. A careful and thorough patient history is essential, especially in excluding drugs or other substances that can interfere with platelet function. It is hoped that the pathologist and clinician may find the discussion and morphology-based approach presented here to be helpful in elucidating the cause of platelet disorders.

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# 3

# Non-Neoplastic Morphologic Abnormalities of White Blood Cells and Macrophages

■ **Dennis P. O'Malley, MD** ■ **Eric D. Hsi, MD**

## ■ INTRODUCTION

A variety of morphologic changes can occur in non-neoplastic white blood cells (WBCs). Some reflect non-specific reactive changes, whereas others are specific and are associated with clinically significant disorders. In this chapter we will review non-neoplastic changes representing a spectrum of morphologic abnormalities of leukocytes and macrophages seen in congenital or acquired disorders. The standard organization seen in other chapters will not be followed here, because these disorders may not necessarily represent distinct biologic entities and do not lend themselves easily to such a format.

## ■ CONGENITAL MORPHOLOGIC ABNORMALITIES OF WHITE BLOOD CELLS

Congenital morphologic changes in WBCs are rare. In many cases there is a known history of a genetic disorder and the corresponding WBC changes are identified subsequent to an established diagnosis. On rare occasions, an astute morphologist may suggest a diagnosis, based on the morphologic changes seen in either the peripheral blood smear or in a bone marrow sample.

### PELGER-HUET ANOMALY

The Pelger-Huet anomaly is characterized by granulocytes that are either bilobed or completely unsegmented (Figure 3-1). These bilobed neutrophils are referred to as *pince-nez* ("nose pinch") *forms,* referring to an old style of eyeglass that sat on the bridge of the nose. The chromatin is mature with heavy condensation, which is the same as seen in normal mature neutrophils. All cells in a peripheral blood smear will be affected. The cells are typically found only on manual review of peripheral blood smears, because automated hematology analyzers do not detect the neutrophils as abnormal in patients with Pelger-Huet anomaly. Inheritance of Pelger-Huet anomaly is usually autosomal dominant, although it is likely that new mutations are common. The prevalence is estimated at 0.01%. The unilobed form of Pelger-Huet cells are occasionally called *Stodtmeister forms*. These cells can be the predominant cell type in the exceedingly rare homozygous form of Pelger-Huet anomaly. Homozygous patients may have varying degrees of psychomotor retardation, abnormal body stature, macrocephalus, ventricular septal defect, polydactyly, developmental delay, epilepsy, and skeletal abnormalities. In heterozygous Pelger-Huet anomaly, neutrophil function is normal, and there are no other clinical abnormalities. The Pelger-Huet anomaly appears to be due to abnormalities in laminin beta-receptor *(LBR)* gene that has been discovered in several kindreds. The gene maps to chromosome 1q41-q43.

Morphologic mimics of Pelger-Huet anomaly are more common than the true disorder (Table 3-1). The changes of hypolobation can be seen primarily in three circumstances: as a reactive condition in association with infections (typically severe), in association with medications or drugs such as mycophenolate or valproate, or in cases of myelodysplastic syndromes or other myeloid stem cell disorders. When this hypolobation is seen in these circumstances, it is referred to as *pseudo–Pelger-Huet* or *Pelgeroid change*. Distinguishing the mimics and true Pelger-Huet anomaly is usually straightforward. In the case of myelodysplastic syndrome (MDS) or other myeloid neoplasms, the hypolobation is often accompanied by hypogranulation of neutrophils, as well as other dysplastic changes seen in erythroid of platelet and megakaryocytic cells. In particular, isochromosome 17q or 17p minus syndrome are cytogenetic abnormalities specifically associated with these changes. In both reactive conditions and dysplastic type changes, only a

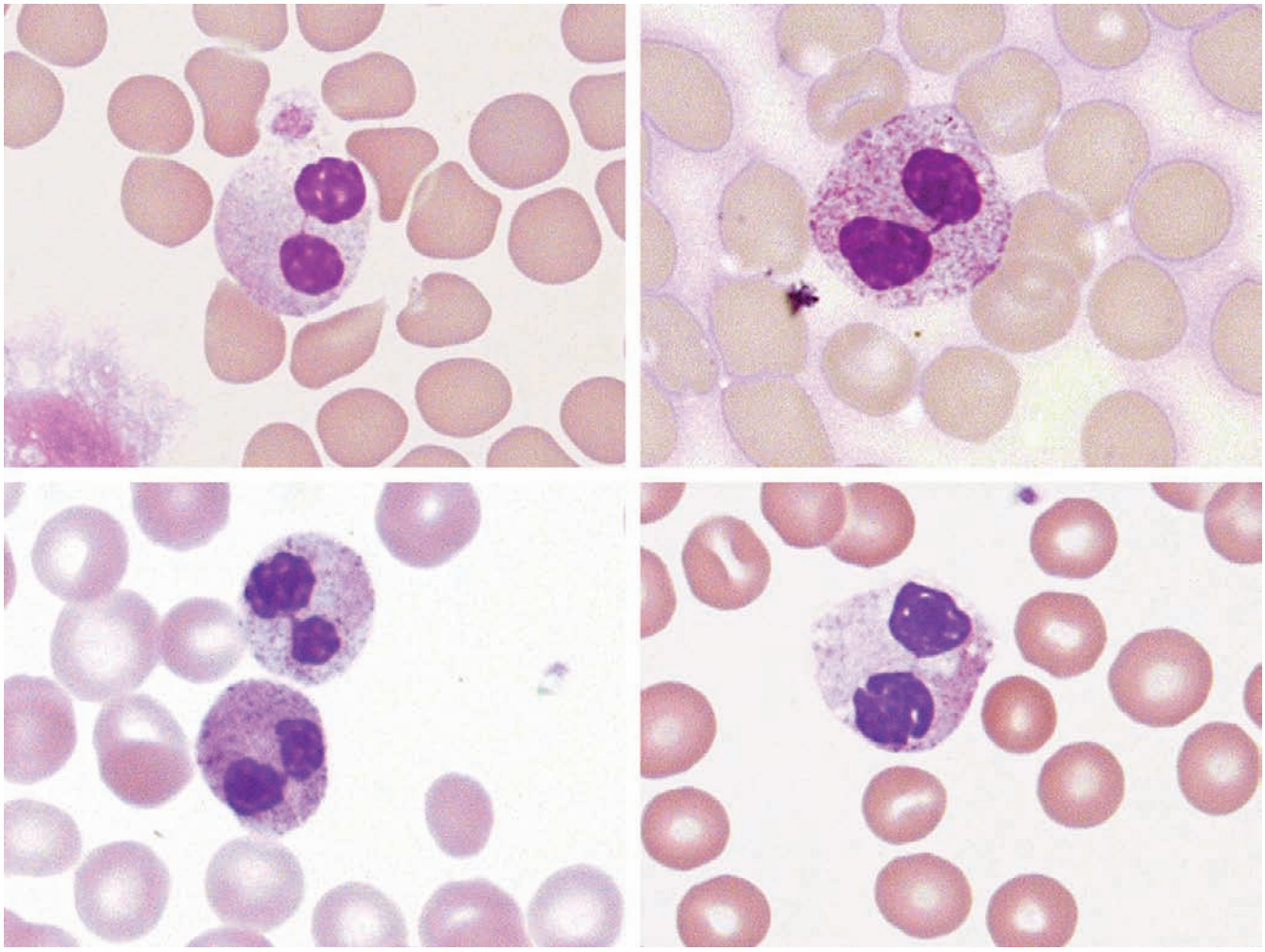

**FIGURE 3-1**

Multiple neutrophils with Pelger-Huet anomaly showing the classic, bilobed appearance of the nuclei. Note the presence of normal granulation of the cytoplasm. In myelodysplastic syndrome, the neutrophils are often hypogranular and hypolobated.

**TABLE 3-1**

**Potential Causes of Pelger-Huet–Like Changes in Granulocytes**

| Medications | Chemicals | Hematologic Disorders | Systemic Disorders | Infectious Disorders |
|---|---|---|---|---|
| Colchicine | Benzene | Acute myeloid leukemia | Enteritis | Influenza |
| Docetaxel | Urethane | Chronic myelogenous leukemia | Muscular dystrophy | HIV |
| Fluconazole | | Erythroleukemia | Myxedema | Malaria |
| Ganciclovir | | Fanconi anemia | Systemic lupus | Mycoplasma pneumonia |
| Ibuprofen | | Granulocytosis | Trisomy 21 | Tuberculosis |
| Melphalan | | Hodgkin lymphoma | | |
| Mycophenolate mofetil | | Immune thrombocytopenia purpura | | |
| Paclitaxel | | Leukemoid reaction | | |
| Sulfisoxazole | | Megaloblastic anemia | | |
| Sulfonamide | | Myelodysplastic syndrome | | |
| Tacrolimus | | Myelofibrosis | | |
| Valproic acid | | Non-Hodgkin lymphoma | | |
| | | Plasma cell myeloma | | |
| | | Polycythemia vera | | |

Data from Speeckaert MM, Verhelst C, Koch A, Speeckaert R, Lacquet F: Pelger-Huët anomaly: A critical review of the literature, *Acta Haematol* 121:202–206, 2009.

subset of neutrophils is usually affected, in contrast to the entire population in Pelger-Huet anomaly. Toxic changes of granulocytes may also be seen in cases associated with infection. Identification of drug-related cases requires knowledge of the clinical history and a high index of suspicion.

## MYELOKATHEXIS

Myelokathexis is an exceedingly rare disorder of neutrophil development and function. Patients exhibit severe neutropenia, evident in infancy, that results in recurrent bacterial or fungal infections. The inheritance pattern seems to be autosomal dominant. Neutrophil precursors and especially mature neutrophils are retained in the bone marrow, leading to hypercellularity and increased myeloid:erythroid (M:E) ratio (*kathexis* = retention). Morphologically, the neutrophil and neutrophilic marrow precursors have marked abnormalities of nuclear shape and lobation (Figure 3-2). Often, the chromatin appears fragmented and broken, with pyknotic chromatin lobes connected by a long strand of chromatin. Cytoplasmic vacuoles may be present. There are significant functional abnormalities of neutrophils and other marrow lineages, leading to abnormal innate immunity. Other abnormalities that have been associated with myelokathexis include skeletal abnormalities and growth retardation. Treatment with growth factors has been successful in some patients. Although the molecular basis for this disorder is unknown, investigators have shown increased apoptosis in neutrophil precursors in the bone marrow associated with decreased expression of BCL-X.

WHIM syndrome is an autosomal dominant immunodeficiency disease characterized by: *w*arts, *h*ypogammaglobulinemia, *i*mmunodeficiency, and *m*yelokathexis. These patients have increased susceptibility to human papilloma virus infection and increased upper respiratory infections. WHIM syndrome appears to be due to abnormalities of *CXCR4* located on 2q21.

## CHÉDIAK-HIGASHI SYNDROME

Chédiak-Higashi syndrome (CHS) is an autosomal recessive disorder that manifests in early childhood. It is characterized by immunodeficiency, recurrent infection, oculocutaneous albinism, bleeding tendency, and multiple neurologic abnormalities. The molecular defect has been defined as mutation in the *CHS1 (LYST)* gene located on chromosome 1q42.1-1q42.2. This gene product is involved in lysosomal trafficking. Abnormal endosomal-lysosomal fusion occurs in these patients and results in large, abnormal granules within granulocytes, monocytes, and lymphocytes (Figure 3-3). These abnormal granules result from fusion of normal secondary granules of neutrophils, eosinophils, basophils, or

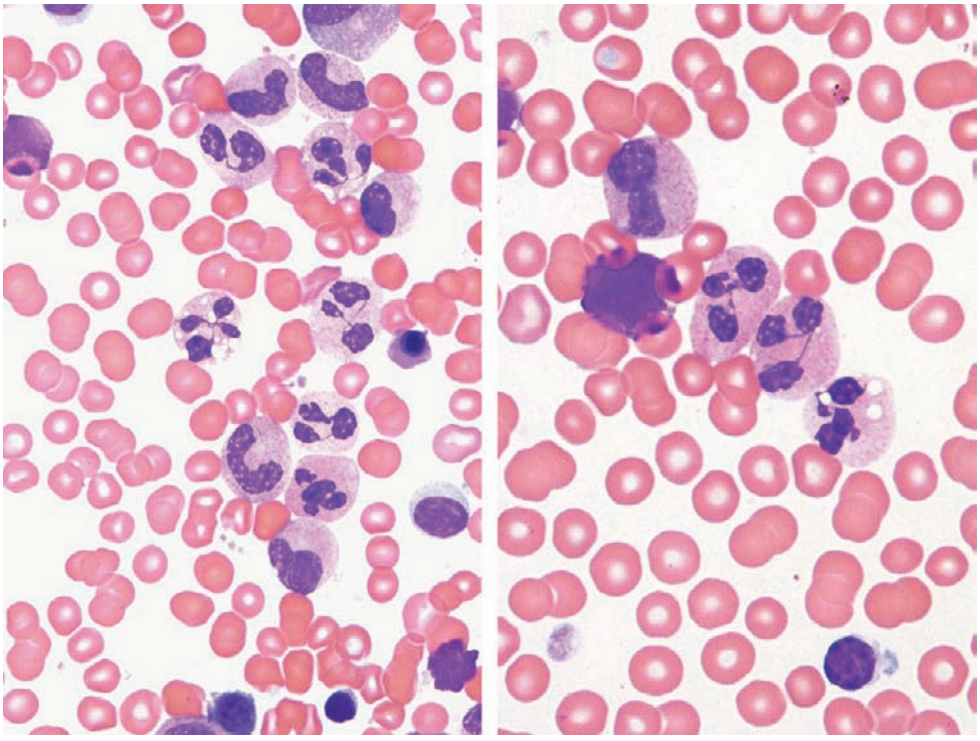

**FIGURE 3-2**

Bone marrow aspirate in myelokathexis. Note the abnormal nuclear segmentation and connecting strands of nuclear material. *(Courtesy S. Kroft, Medical College of Wisconsin.)*

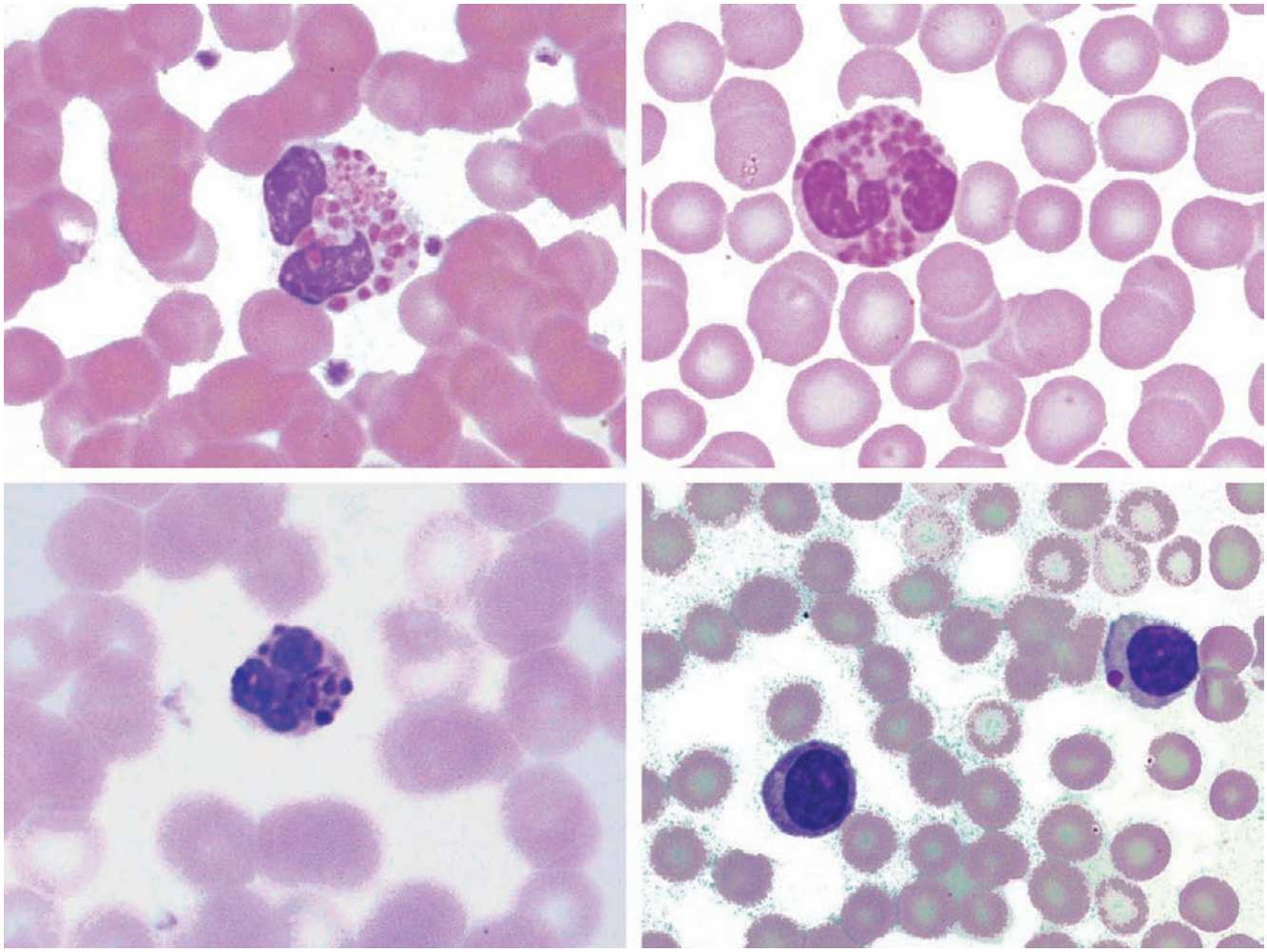

**FIGURE 3-3**
Abnormal large granules of Chédiak-Higashi syndrome seen in a neutrophil, eosinophil, basophil, and lymphocyte.

the cytotoxic granules of monocytes or lymphocytes. Peripheral blood smears show abnormal giant cytoplasmic granules in leukocytes.

The neutrophils do not function appropriately, leading to increased susceptibility to bacterial infections. Natural killer (NK) cell function is also impaired. Deficiencies of pigmentation are due to abnormal, giant melanosomes.

Patients who do not succumb to infection may eventually develop an unusual lymphoproliferative syndrome, the so-called accelerated phase of the disorder, characterized by generalized lymphohistiocytic infiltrates, fever, jaundice, hepatosplenomegaly, lymphadenopathy, pancytopenia, and bleeding. Allogeneic bone marrow transplantation has been successful in some cases and improves the hematologic and immunologic defects, but does not affect the neurologic complications.

Morphologic changes similar to CHS occur rarely in acute myeloid leukemia (AML) or MDS, including acute promyelocytic leukemia. In these instances, there may be abnormal large granules; however, they are not found in the context of a pediatric patient with other genetic anomalies, but usually in adult patients with underlying myeloid disorders. Furthermore, the abnormal granules are usually limited to a subset of cells, are most commonly seen in a population of myeloid blasts, and are not seen in other cell lines such as granular lymphocytes.

## ALDER-REILLY ANOMALY

The morphologic changes in myeloid cells termed *Alder-Reilly anomaly* are seen in patients with mucopolysaccharidoses (MPS). Seen predominantly in granulocytes, although also variably present in monocytes, there are large numbers of relatively coarse, azurophilic granules in the cytoplasm resembling toxic granulation (Figure 3-4). Patients with MPS have either X-linked or autosomal recessive abnormalities in the metabolism of mucopolysaccharides. Historically, eponymous names (e.g., Hunter syndrome, Hurler syndrome, Morquio syndrome, Sanfilippo syndrome, Scheie syndrome) and the term *gargoylism,* referring to the coarse facies seen in these patients, have been used to refer to this group of diseases;

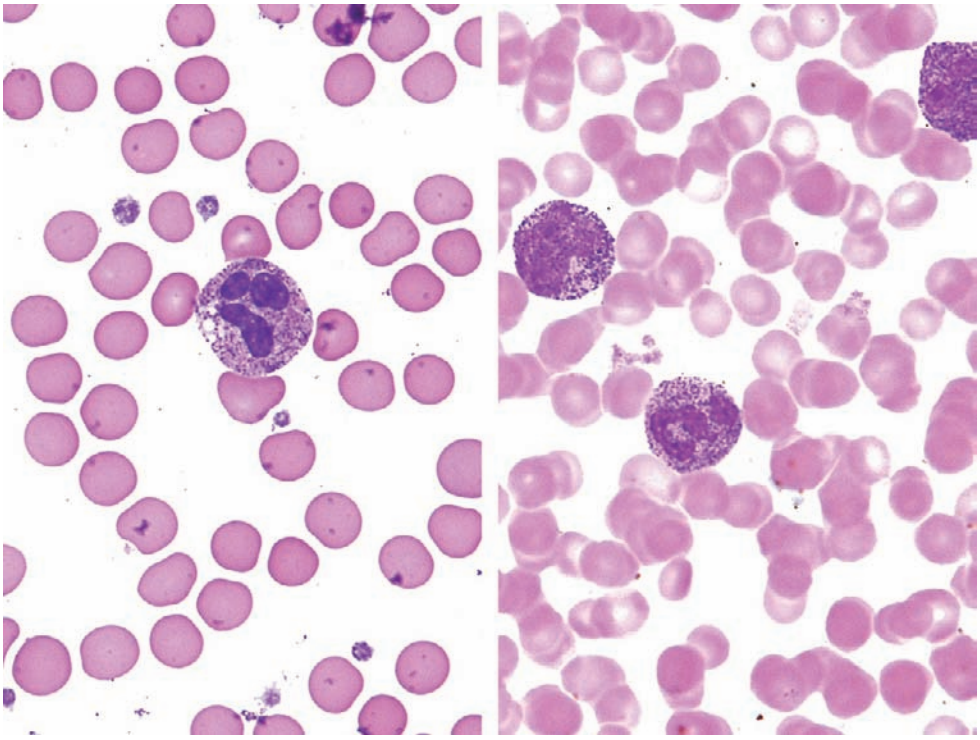

**FIGURE 3-4**

Two examples of Alder-Reilly anomaly. The degree of granulation can vary in individual cases. The coarse, azurophilic granules most resemble toxic changes in neutrophils.

**TABLE 3-2**

**Features of Autosomal Dominant Macrothrombocytopenias with *MYH9* Mutation**

| Clinical Feature | May-Hegglin Anomaly | Sebastian Syndrome | Fechtner Syndrome | Epstein Syndrome |
|---|---|---|---|---|
| Macrothrombo-cytopenia | + | + | + | + |
| Döhle-like bodies | + | + | + | – |
| Hearing impairment | – | – | + | + |
| Cataract | – | – | + | – |
| Nephritis | – | – | + | + |

currently they are classified according to the underlying enzymatic defects, and then named according by type (e.g., MPS type I, type II). All these disorders are associated with numerous other physical abnormalities with varying degrees of severity, which are beyond the scope of this chapter. The Alder-Reilly anomaly should not be mistaken for typical toxic granulation. A mimic has also been described in association with myelodysplasia.

## MAY-HEGGLIN ANOMALY

The group of May-Hegglin, Fechtner, Sebastian, and Epstein syndromes are collectively referred to as *autosomal dominant giant-platelet disorders*. Characteristics are shown in Table 3-2. All these disorders have mutations in the gene encoding nonmuscle myosin heavy chain-9 *(MYH9)* located at chromosome 22q12.3-q13.2. May-Hegglin is characterized by the triad of thrombocytopenia, giant platelets, and inclusion bodies in leukocytes (Figure 3-5). Most patients are asymptomatic, although some may have a mild bleeding tendency. Patients are discovered because of thrombocytopenia. The main complications are misdiagnosis as another disorder, such as idiopathic thrombocytopenic purpura, and undue therapy.

The characteristic white blood cell abnormality in May-Hegglin anomaly is the presence of large blue cytoplasmic inclusions that resemble Döhle bodies. The Döhle-like bodies are composed of paracrystalline arrays

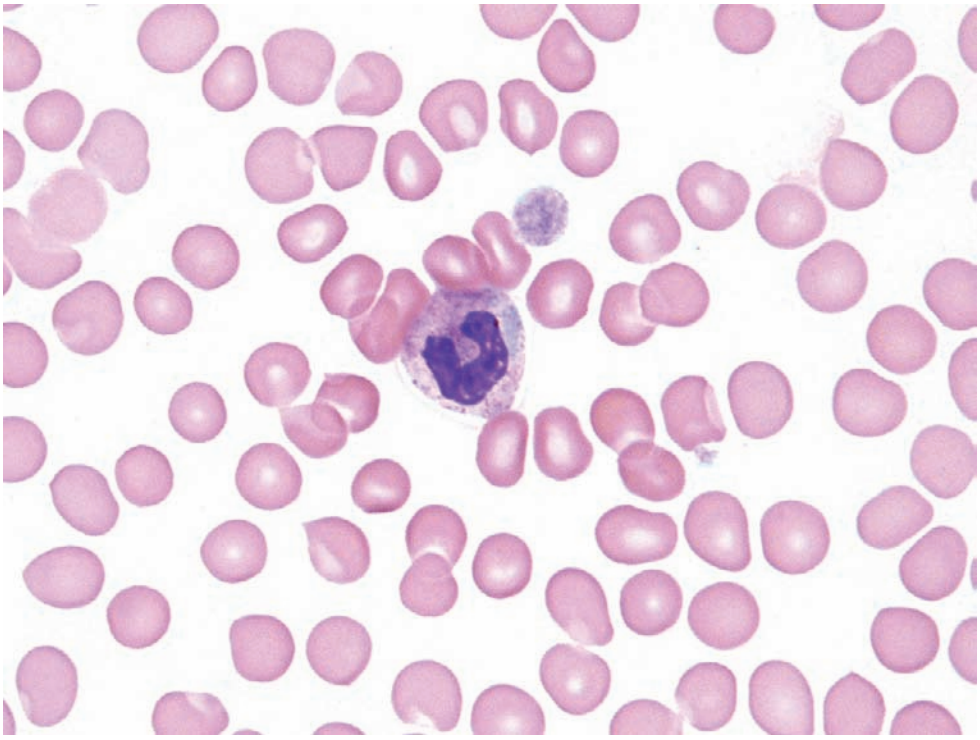

**FIGURE 3-5**

Peripheral blood smear in May-Hegglin anomaly. Note the pale blue inclusion in the neutrophil and the large platelet, which are typical findings in May-Hegglin anomaly.

of ribosomes and RNA and are larger (2 to 4 μm) than true Döhle bodies. Furthermore, Döhle-like bodies are seen in the absence of toxic granulation and are not limited to neutrophils. In May-Hegglin anomaly, the inclusions can be seen within all types of granulocytes (neutrophils, basophils, eosinophils) and monocytes. Large platelets are also present and are a characteristic feature. Mean platelet volume is increased but often underestimated because of inclusion as erythrocytes or leukocytes by automated cell counters.

The granulocytes appear to have normal function. The results of platelet function studies are typically normal, but there still may be an increased tendency for bleeding. Defects in expression of GP Ib/IX/V complex have been reported in May-Hegglin anomaly, which may account for bleeding tendencies.

**TABLE 3-3**

**Disorders with Vacuolated Lymphocytes**

| |
|---|
| Mannosidosis |
| Batten disease (Spielmyer-Vogt) |
| GM1 gangliosidosis |
| Neuraminidase deficiency |
| Galactosidosis |
| Mucolipidosis II |
| Aspartylglycosaminuria |
| Salla disease |
| Infantile free sialic acid storage |
| Wolman disease |
| Niemann-Pick disease type A |
| Aspartylglycosaminuria |
| Glycogenosis type II, Pompe disease |
| Fucosidosis |
| Mucolipidosis III |

## VACUOLATED LYMPHOCYTES

Lymphocyte vacuoles are relatively rare in reactive conditions. In a wide variety of inherited genetic disorders, otherwise normal peripheral blood lymphocytes may be vacuolated. In some cases, the vacuoles will stain positively with the periodic acid Schiff (PAS) stain. The disorders in which these vacuoles are seen are shown in Table 3-3. Although rarely seen in reactive conditions or infections, the vacuoles are present in more than 20% of lymphocytes when seen in association with genetic disorders.

## ACQUIRED ABNORMALITIES OF WHITE BLOOD CELLS

Several abnormalities are seen in normal leukocytes in response to acquired conditions. These abnormalities can occasionally mimic the findings seen in genetic

disorders or may be similar to those seen in neoplastic cells. Familiarity with these changes can prevent misinterpretation.

## "TOXIC" CHANGES IN LEUKOCYTES

Increased WBC counts (e.g., leukocytosis) can be seen in a wide variety of conditions. One common cause is as a response to infections, particularly bacterial. In these cases, a left shift of granulocytes is also seen. The term *toxic changes* refers to a constellation of alterations that occur in the cytoplasm of granulocytes, including excessive granulation (toxic granules), vacuolation, or Döhle bodies (Figure 3-6). These changes can be seen either individually or in combination. Toxic granulation is a persistence of the deep blue-purple color of the primary granules of the neutrophil; they are azurophilic and appear most prominent in neutrophils and band forms, but are also present in myelocytes and metamyelocytes. These granules are thought to be due to abnormal, accelerated maturation and exit from the marrow space. Toxic vacuoles may be seen as circular, clear areas in the cytoplasm of neutrophils. They are typically less than 1 to 2 μm in diameter and represent sites of phagocytized material. They are most often seen in septicemia, but can also be seen in cases of acute ethanolism and certain medications (e.g., chloramphenicol). Döhle bodies are pale blue or blue-gray inclusions, 1 to 3 μm in diameter, and are seen in the cytoplasm of neutrophils and precursors; they are composed of aggregates of denatured ribosomes.

In the neutrophils of patients being treated for HIV/AIDS, intracytoplasmic inclusions of DNA material can be seen (*termed Howell-Jolly body–like inclusions;* see Figure 3-6). Like true Howell-Jolly bodies, these bodies appear to be fragments of nuclear chromatin separated from the main body of the nucleus. They have the same dark purple color of nuclear material and are round in shape. These inclusions have also been described in patients without HIV but being treated with immunosuppressive drugs (e.g., organ transplant patients) and

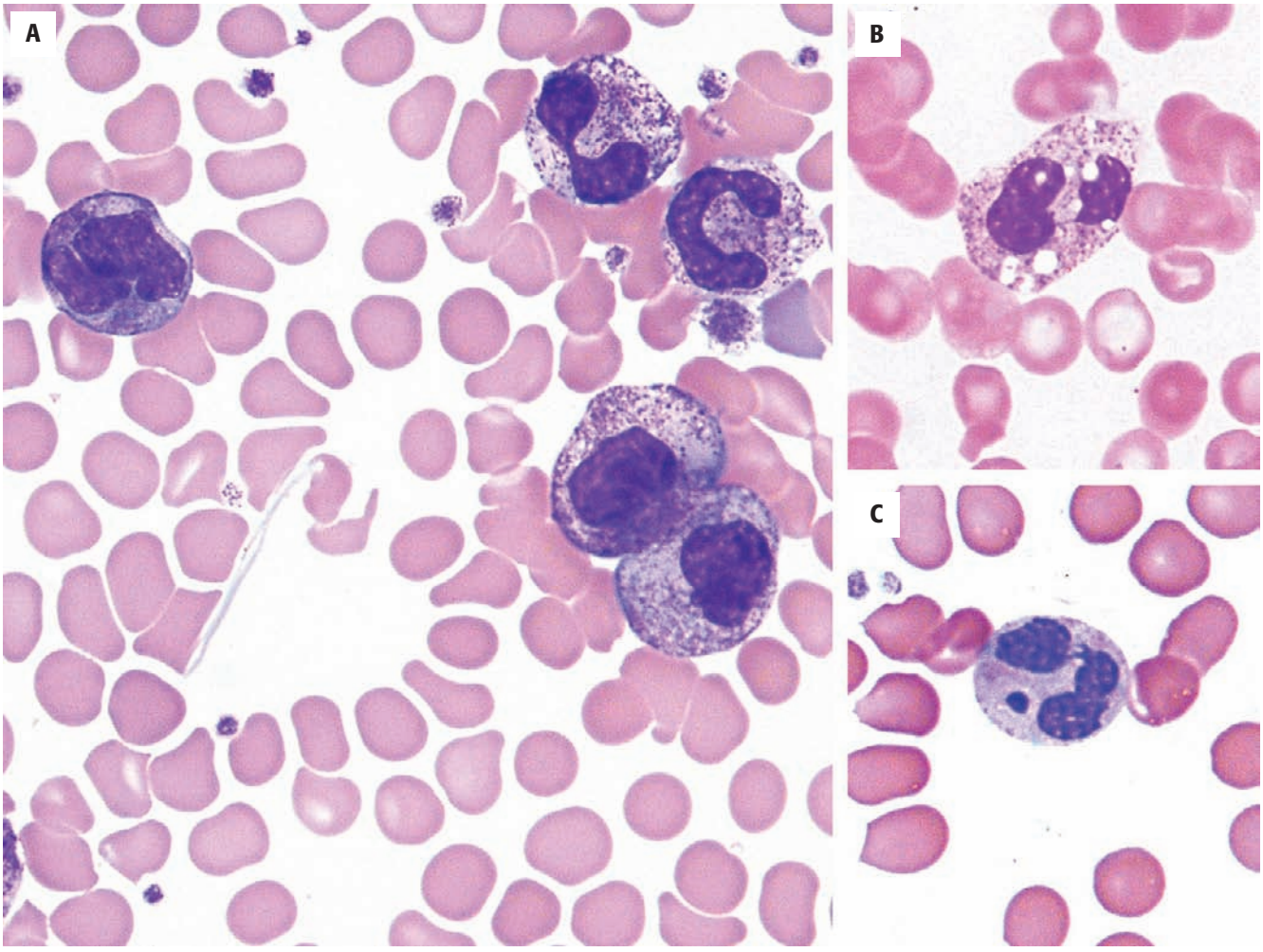


**FIGURE 3-6**

**A,** Peripheral blood changes in a case of neonatal sepsis, which include prominent toxic granulation and a left shift in the neutrophils. **B,** Toxic vacuoles in a neutrophil. **C,** A Howell-Jolly–like body (e.g., nuclear fragment) in the neutrophils of a patient with HIV/AIDS.

chemotherapy. The most common differential diagnosis of these inclusions are intracytoplasmic infectious agents, such as bacteria or parasites.

Apoptotic neutrophils, also termed *necrobiosis,* appear as several round hyperchromatic nodules of nuclear chromatin material. These cells can be seen in a variety of conditions, including infections and medication-related conditions. Comparable changes can be seen in MDS or AML. Apoptosis of neutrophils is also a common finding in aged samples and should not be overinterpreted as evidence of dysplasia.

## CYTOKINE EFFECTS

Cytokine therapy has become commonplace. Some of these cytokines can produce significant bone marrow and peripheral blood changes in WBCs. The most commonly seen effect is that of granulocyte-colony stimulating factor (G-CSF). Commonly G-CSF is used to increase the number of neutrophils in the peripheral blood, or as part of therapy to increase the number of peripheral blood stem cells. The effects of G-CSF on peripheral blood neutrophils include "toxic" granulation, a left shift, an overall increase in granulocytes and precursors, a transient increase in circulating blasts, vacuolation, Döhle bodies, variable nuclear-cytoplasmic dyssynchrony, and abnormalities of nuclear segmentation. Marrow changes include similar findings, with prominent granulation being especially frequent, and an increase in myeloid elements (e.g., increased M:E ratio; Figure 3-7). Other less common features include binucleate promyelocytes and myelocytes, giant myeloid precursors, and rarely the development of bone marrow fibrosis (typically mild). Granulocyte-macrophage colony-stimulating factor has similar changes to G-CSF, with the addition of significant monocytosis and eosinophilia.

Cytokine effects may occasionally simulate the findings seen in acute leukemias, especially acute promyelocytic leukemia. Rare cases of myeloid stem cell disorders (acute myeloid leukemia, myelodysplastic syndromes) may respond to G-CSF, leading to dramatic increases in circulating blasts. Careful attention to the morphologic appearance of the cells, evaluation of the clinical history, and in some circumstances flow cytometry or bone marrow examination may aid in distinguishing cytokine effect from leukemia. Reassessment after waiting several days to allow cytokine effects to subside is often a prudent course of action.

## MEGALOBLASTIC AND DYSPLASTIC CHANGES

Megaloblastic changes in WBCs are typically described in bone marrow as a result of $B_{12}$ or folate deficiencies, although the changes may be seen as a result of medications or other medical disorders. These changes manifest as nuclear and cytoplasmic dyssynchrony with nuclear development lagging behind that of the

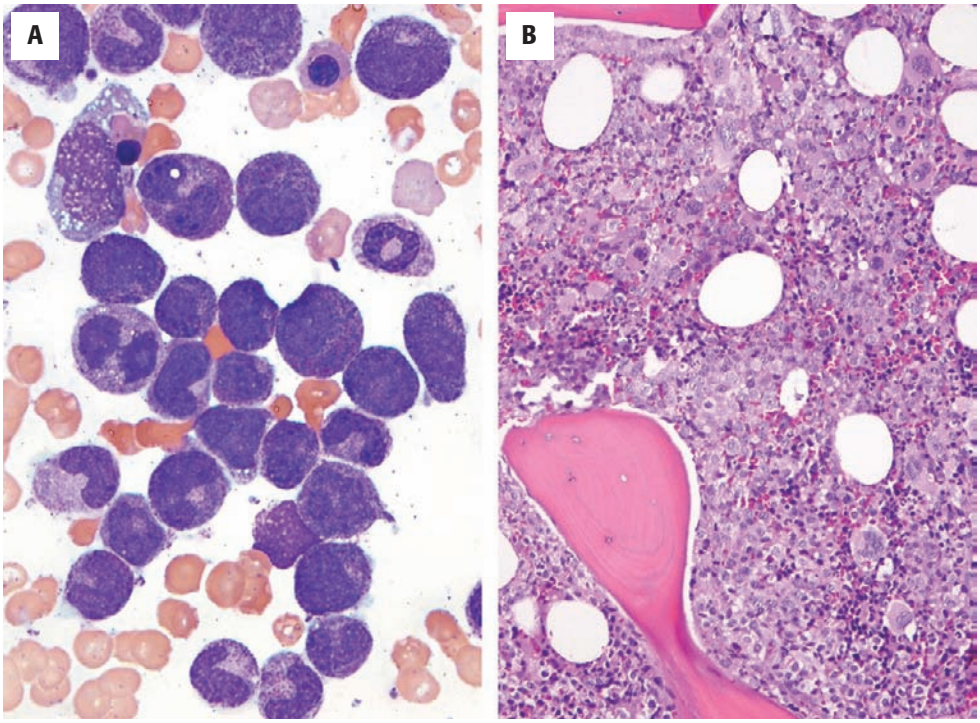


**FIGURE 3-7**

Bone marrow changes associated with granulocyte colony-stimulating factor administration. The aspirate smear **(A)** may show extensive, heavy toxic granulation. In the core biopsy **(B),** there is an increase in immature myeloid elements, expanding away from the bone trabeculae into the intertrabecular space.

cytoplasm (Figure 3-8). As a result, WBCs in the bone marrow may show giant precursors (giant myelocytes, giant bands) and hypersegmentation of granulocytes. In the peripheral blood, hypersegmented neutrophils (more than four lobes, with six lobes or more being diagnostic) suggest megaloblastic anemia, but should be correlated with red blood cell indices (especially mean corpuscular volume [MCV]), medication history, and clinical findings (see Figure 3-8).

One common issue that hematopathologists face is distinguishing secondary causes of dyspoiesis from primary myelodysplastic syndromes. This may not be possible by evaluating only the morphology. Familiarity with potential clinical situations in which dysplastic features (not limited with leukocytes) might arise can be invaluable. Drug or toxin exposures represent one of the most significant challenges. Common scenarios that could produce cellular dyspoiesis include: recent cytotoxic therapy with any number of chemotherapeutic agents, arsenic (either as a toxic or therapeutic exposure) exposure, heavy metal exposure, copper deficiency (zinc induced), alcohol toxicity, antituberculous drugs (isoniazid) therapy, trimethoprim therapy, valproic acid therapy, and HIV/AIDS.

## EOSINOPHILS

Eosinophils can occasionally be associated with the formation of Charcot-Leyden crystals, which are large, orange-red hexagonal or diamond-shaped forms within the cytoplasm of eosinophils, within macrophages, or in tissues infiltrated by eosinophils. The crystals are formed by fusion of eosinophil granules that contain large amounts of Charcot-Leyden protein or lysophospholipase. Charcot-Leyden crystals are seen in conditions associated with eosinophilia. Vacuolated eosinophils can be seen in reactive conditions with eosinophilia. This change has been associated with hypereosinophilic syndrome, but in our experience it is nonspecific.

## REACTIVE LYMPHOCYTES AND LYMPHOCYTOSIS

### SMUDGE CELLS

Smudge cells, sometimes referred to as *basket* or *Gumprecht cells,* are fragments of cells that are damaged and broken apart during the process of preparing a blood smear. It is presumed that the cells are more fragile than their intact counterparts. Most commonly these cells are lymphocytes, but monocytes, blasts, or any cell type can fragment during the process of smear preparation. In the appropriate clinical context (i.e., an older patient), the presence of numerous smudge cells with lymphocytosis is suggestive of a diagnosis of a lymphoproliferative disorder, most commonly chronic lymphocytic leukemia (Figure 3-9). However, smudge cells can also be seen in acute lymphoblastic leukemia and in infectious

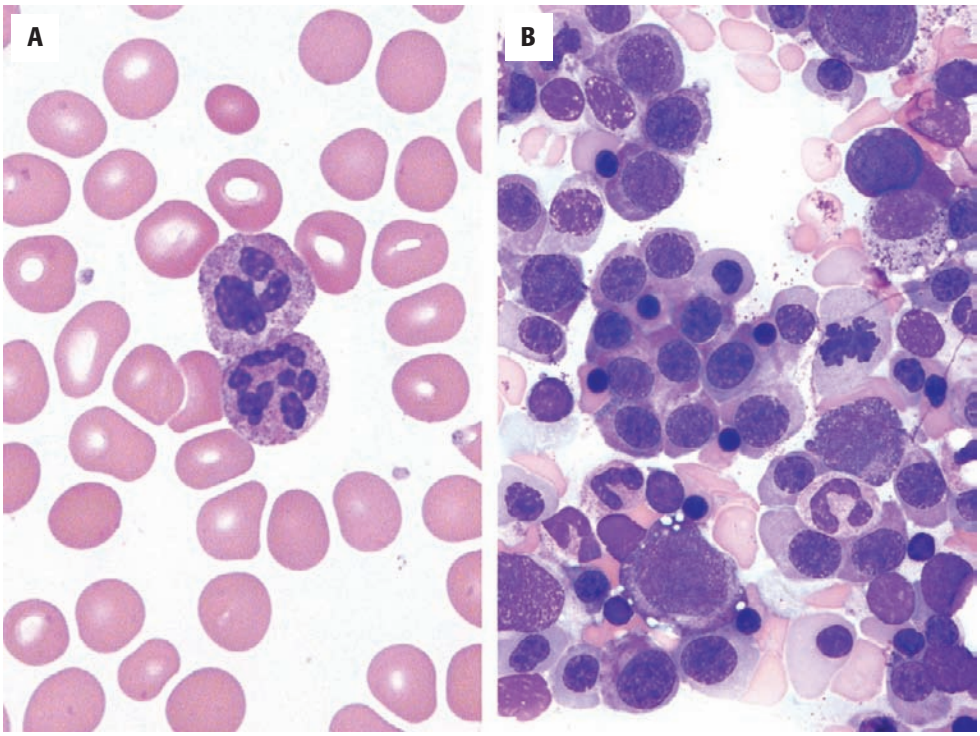


**FIGURE 3-8**

Megaloblastic anemia caused by folate deficiency. A hypersegmented neutrophil **(A)** and a bone marrow aspirate showing megaloblastic changes, including erythroid hyperplasia, nuclear/cytoplasmic dyssynchrony, and dyserythropoiesis **(B).**

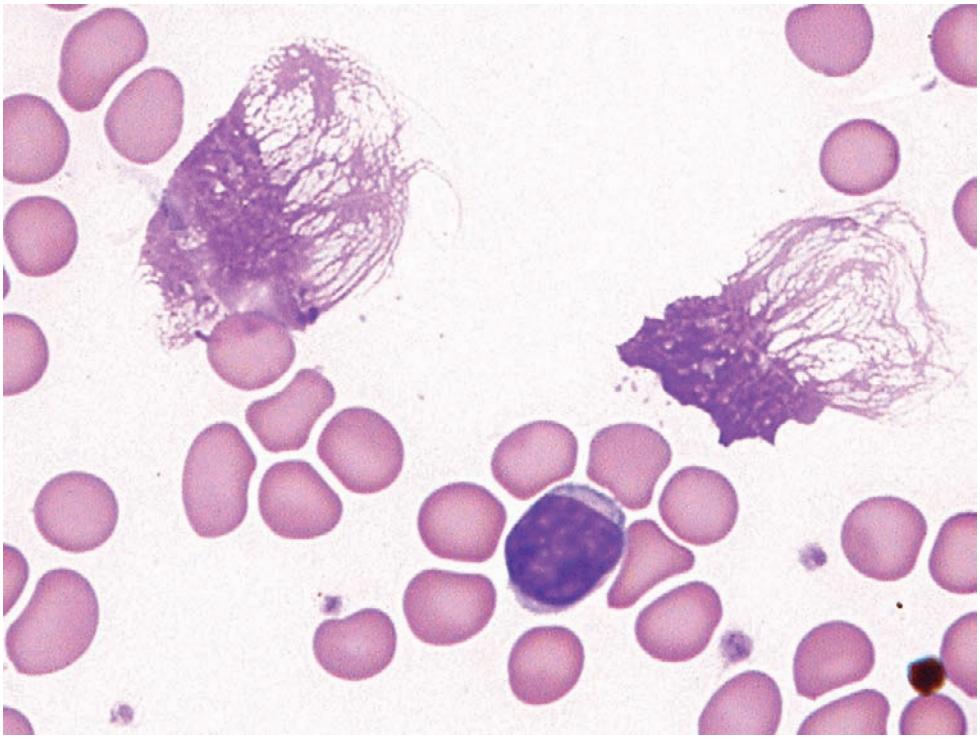

**FIGURE 3-9**
A peripheral blood smear with two smudge cells and an intact lymphocyte.

mononucleosis. The addition of albumin to the blood droplet before smearing usually stabilizes the cells and prevents the formation of smudge cells. Careful morphologic examination of the intact cells on the smear usually provides enough information to infer the identification of the smudge cell population.

## VIRAL LYMPHOCYTOSIS

Infectious mononucleosis resulting from Epstein-Barr virus infection is the prototypical condition for transient reactive (viral) lymphocytosis. Morphologic heterogeneity of lymphocyte appearance is the rule. Compared to normal lymphocytes, reactive lymphocytes contain abundant basophilic cytoplasm. Cytotoxic granules may be present. The nuclei are slightly enlarged (1.5 to 2 times normal), have loosely clumped nuclear chromatin, and have one or two nucleoli. These cells are mostly cytotoxic T cells responding to the infection. Occasional immunoblasts and plasma cells may be seen. Depending on the changes seen, reactive lymphocytes can mimic a variety of lymphoproliferative disorders, including both acute and chronic lymphoid leukemias. Careful morphologic examination (particularly noting the heterogeneity of lymphocyte morphology), knowledge of the clinical findings, and occasionally immunophenotypic or molecular testing may be necessary to appropriately identify the process. Other viral infections including but not limited to cytomegalovirus, hepatitis, HIV, and Hantavirus can also cause reactive lymphocytosis.

## OTHER REACTIVE LYMPHOCYTE CHANGES

*Bordetella pertussis* infection can result in a peculiar lymphocytosis composed of cleaved or notched lymphocytes mimicking the buttock cells of leukemic follicular lymphoma (Figure 3-10). Infection occurs in children, and the situation should be monitored closely to avoid a costly workup.

An underrecognized lymphocytosis in adults (so-called stress lymphocytosis) can also be seen in patients in the emergency department. Lymphocytosis is usually mild and does not persist. Thus, a reevaluation in 2 to 4 weeks time will allow distinction from a neoplastic condition since the transient stress lymphocytosis resolves.

Persistent polyclonal B cell lymphocytosis is an intriguing reactive cause of lymphocytosis and should be noted as a mimic of lymphoid leukemic. Affected individuals are usually women who smoke and have an HLA-DR7 phenotype. There is a persistent lymphocytosis that, when investigated by immunophenotyping, is polyclonal and B cell in nature. The lymphocytes are small and often have deep nuclear clefts. Interestingly, some studies have shown *BCL2/IGH* rearrangements (polyclonal) in lymphocytes from these patients.

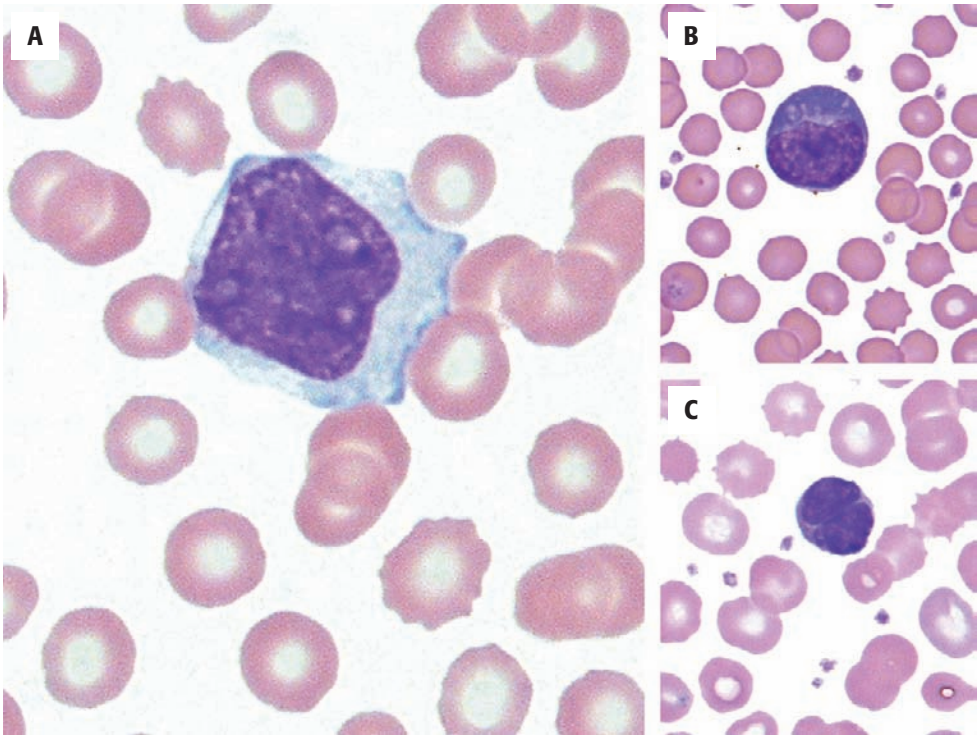


**FIGURE 3-10**

**A,** A classic, atypical or reactive lymphocyte seen in a case of infectious mononucleosis. **B,** An atypical lymphocyte seen in nonspecific viral infection. **C,** A cleaved lymphocyte in the peripheral blood of a patient with pertussis. *(**C,** Courtesy M. Drachenberg, Long Beach Memorial Hospital.)*

Isochromosome 3q has also been reported in 34% of patients. The vast majority of patients have a stable lymphocyte count and indolent course. One large series of 111 patients reported 89% of patients to be free of complications at a median of 53 months. B-cell lymphomas have been reported in rare patients, but whether persistent polyclonal B-cell lymphocytosis can progress to lymphoma is an open question and patients should receive clinical follow-up.

## MORPHOLOGIC ABNORMALITIES IN MACROPHAGE CHANGES (BONE MARROW)

Macrophages, also referred to as *histiocytes,* are phagocytic cells of the marrow that are derived from monocytes. When the cytoplasmic contents of macrophages have a distinctive appearance, they may be useful in suggesting a diagnosis or may mimic other disorders. One distinctive macrophage change is that seen in Gaucher disease, an inherited (autosomal recessive) enzyme defect of β-glucocerebrosidase. Macrophages in Gaucher disease characteristically have increased amounts of blue cytoplasm on Wright stain, with a fibrillar or "folded tissue paper" appearance (Figure 3-11).

Pseudo-Gaucher cells are difficult to distinguish from true Gaucher cells by morphology. Pseudo-Gaucher cells are caused by the rapid turnover of cells and ingestion of the cellular products by macrophages. Pseudo-Gaucher cells are seen most frequently in chronic myeloproliferative disorders, especially chronic myelogenous leukemia, and less commonly in other marrow neoplasms. True Gaucher disease is suggested when numerous Gaucher cells are present and other clinical features, such as hepatosplenomegaly, are present. Confirmation of Gaucher disease should be done in all suspected cases by appropriate biochemical testing.

Foamy macrophages have multiple clear cytoplasmic vacuoles and can be seen in a variety of conditions, including genetic disorders of lipid metabolism or excess marrow accumulation of lipids. One specific type of foamy macrophage is the Niemann-Pick cell. Niemann-Pick disorder is an autosomal recessive inherited disorder; a lack of sphingomyelinase leads to accumulation of the abnormal metabolic product in a variety of tissues. The macrophages in this disorder are typically large with numerous small, uniform lipid-filled vacuoles (see Figure 3-11) and can be found in large numbers. The macrophages are typically seen in the bone marrow, liver, and spleen. As in any suspected storage or metabolic disorder, confirmatory testing (enzyme activity or genetic) should be performed.

Sea-blue histiocytes are macrophages with cytoplasm that appears blue or blue-green after a standard Wright stain. The staining pattern is due to accumulation of ceroid, a metabolic product of cell membrane digestion. As an acquired phenomenon, sea-blue histiocytes can be

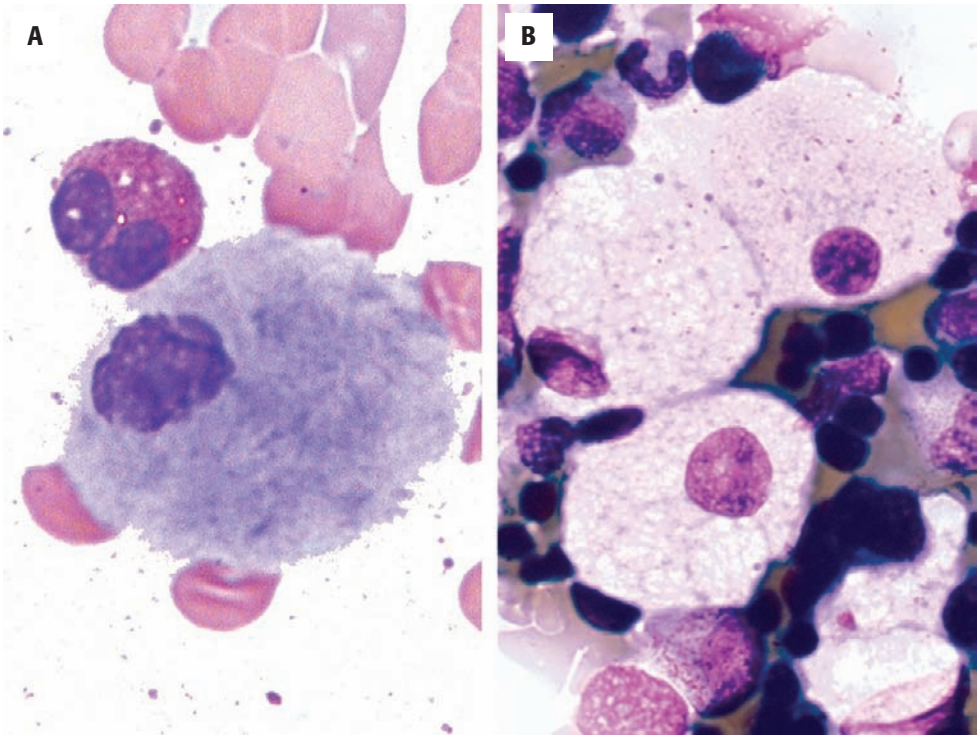


**FIGURE 3-11**

**A,** A classic Gaucher cell, with pale blue, "folded tissue paper" cytoplasm. **B,** A Niemann-Pick cell with numerous cytoplasmic vacuoles filled with lipid.

found in disorders with rapid cell turnover, such as chronic myelogenous leukemia, where they are also commonly found, albeit in small numbers. They can also be seen in other hematologic disorders such as myelodysplastic syndromes. An unusual scenario in which sea-blue histiocytes are seen is in the setting of prolonged total parenteral nutrition. Bone marrow smears from these patients may demonstrate large numbers of sea blue histiocytes, believed to be related to the lipid emulsion component of the total parenteral nutrition. Idiopathic sea-blue histiocytosis was described in 1970; it is a rare syndrome of sea-blue histiocytes in bone marrow, hepatosplenomegaly, thrombocytopenia, purpura, pulmonary infiltrates and has a benign clinical course. Some authors consider this sea-blue histiocytosis to be part of the spectrum of Niemann-Pick disease, because a partial sphingomyelinase deficiency may be the cause. A rare genetic disorder, mutation in the *APOE* (apolipoprotein E) gene (19q13.2) may also be associated with splenomegaly, sea-blue histiocytes, thrombocytopenia, and hypertriglyceridemia.

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4

# Reactive Lymph Nodes and Castleman Disease

**Judith A. Ferry, MD**

## INTRODUCTION

Lymph nodes and lymphoid tissue in certain extranodal sites can be involved by a variety of reactive processes. In many cases, the histologic changes are nonspecific and a particular cause cannot be assigned. In other cases, the findings are suggestive or diagnostic of a certain entity. This discussion focuses on histologically distinctive lymphoid hyperplasias and lymphadenitides and on their differential diagnosis. A number of types of reactive lymphoid hyperplasias can have histologic features that are atypical, distorting the nodal architecture and potentially mimicking a lymphoproliferative disorder. Familiarity with the range of changes that may be seen in reactive lymph nodes can help to prevent a misdiagnosis of lymphoma.

## NORMAL LYMPH NODE ANATOMY

Normal lymph nodes are typically bean shaped. They have, beginning at their periphery and moving centrally, a fibrous capsule, a cortex, a paracortex, a medulla, and a hilus. The cortex, a B cell area, contains follicles. When inactive, follicles are composed of a uniform population of small lymphoid cells (primary follicles). Hyperplastic follicles have a germinal center surrounded by a mantle of small cells (secondary follicle). Follicle centers are composed of centrocytes (small cleaved cells) and centroblasts (large noncleaved cells) of B cell lineage. There are also T cells admixed with follicle center cells, and a narrow layer of T cells is typically seen around the periphery of a hyperplastic follicle center. In peripheral lymph nodes, a marginal zone is not usually conspicuous, but in certain sites, such as Peyer's patches, mesenteric lymph nodes, and the spleen, follicles often have a distinct marginal zone composed of small lymphoid cells with a moderate quantity of clear cytoplasm surrounding the mantle zone.

The paracortex, primarily a T cell area, contains lymphocytes, antigen-presenting cells, and varying numbers of immunoblasts, depending on the degree of activation of the lymph node. High endothelial venules lined by cuboidal (high) endothelial cells are found in the paracortex; they have an important role in lymphocyte trafficking.

The medulla contains cords occupied by a varying admixture of lymphocytes, plasma cells, and immunoblasts. A mixture of B and T cells is usually found in medullary cords. A network of sinuses traverses the lymph node. The network begins with the subcapsular sinus, which then feeds into sinuses that cross the node from cortex to medulla. The sinuses in the medulla delineate the medullary cords (Figure 4-1).

There are a number of nonspecific changes that may be found in reactive lymph nodes. One of these changes is follicle lysis, in which follicles and their underlying dendritic networks are disrupted, imparting a fragmented appearance to the follicle center. This phenomenon is usually found in the setting of florid follicular hyperplasia.

Progressive transformation of germinal centers is characterized by infiltration of germinal centers by small lymphocytes of mantle zone type, with enlargement of the follicles. The follicles eventually acquire a dark, monotonous appearance. When multiple follicles show progressive transformation of germinal centers, the appearance can raise the question of nodular lymphocyte-predominant Hodgkin lymphoma.

Monocytoid B cells are not usually a conspicuous feature of nonspecific reactive hyperplasia, but are prominent in certain reactive conditions, including cytomegaloviral lymphadenitis and toxoplasmic lymphadenitis. Monocytoid B cells are lymphoid cells with small to medium-sized, oval or indented nuclei and abundant pale cytoplasm. They are found in bands along sinuses.

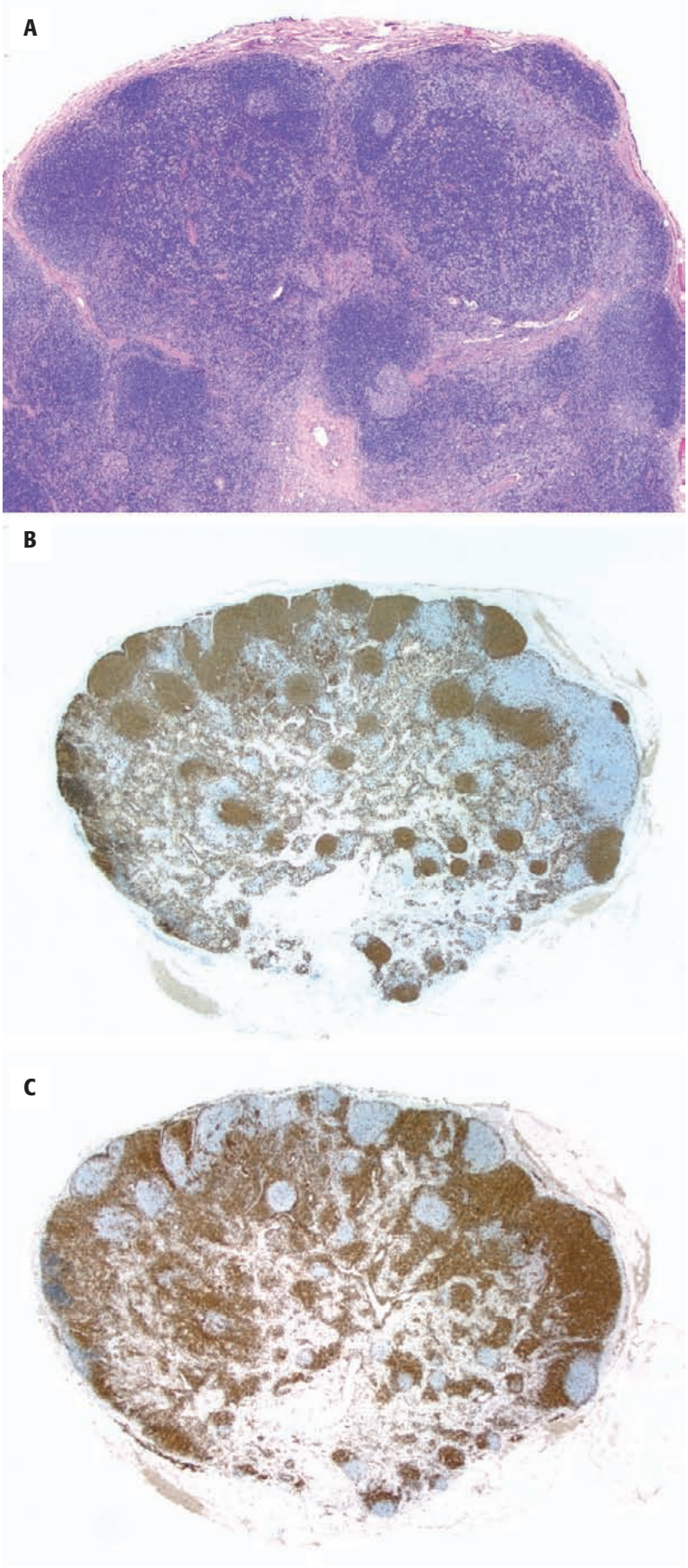


**FIGURE 4-1**

Lymph node with nonspecific reactive hyperplasia. **A,** Low power shows a lymph node with intact architecture, with several reactive follicles and a hyperplastic paracortex. **B,** B cells ($CD20^+$) are found mainly in follicles and distributed in a thin layer along sinuses. **C,** T cells ($CD3^+$) occupy the paracortex.

## VIRAL INFECTIONS

# INFECTIOUS MONONUCLEOSIS

### Clinical Features

The diagnosis of symptomatic Epstein-Barr virus (EBV) infection, infectious mononucleosis (IM), is usually established on clinical grounds, but when involved lymph nodes or tonsils are biopsied, they frequently cause problems in differential diagnosis. IM most often affects adolescents and young adults, although cases of IM in young children and in adults as old as 80 years have been reported. Manifestations include fever, pharyngitis, cervical lymphadenopathy, splenomegaly, rash, atypical peripheral blood lymphocytosis, and a positive heterophile antibody (monospot) test. However, the heterophile antibody test result might not be positive throughout the illness, sometimes requiring repeated monospot or serologic studies to establish a diagnosis. In some cases, particularly among young children, a positive heterophile antibody test result may never be obtained. In more severe cases, there may be generalized lymphadenopathy, upper airway obstruction related to lymphoid hyperplasia, hepatomegaly with hepatic dysfunction, peripheral cytopenia, neurologic complications such as aseptic meningitis and meningoencephalitis, and even a hemophagocytic syndrome (hemophagocytic lymphohistiocytosis). Most patients with IM who receive a β-lactam antibiotic agent develop a morbilliform rash. In most cases the illness is self limited, but rarely intercurrent infection, Guillain-Barré syndrome, or splenic rupture with hemorrhage results in death. Young children with genetic defects, such as perforin deficiency, are at increased risk for hemophagocytic lymphohistiocytosis. Boys with the X-linked lymphoproliferative disorder are at risk for severe IM. Those who survive the acute infection often develop lymphoma or have persistent immunologic abnormalities, or both.

**INFECTIOUS MONONUCLEOSIS—FACT SHEET**

**Definition**
- Symptomatic infection by EBV

**Age, Race, and Gender Distribution**
- Most patients are teenagers or young adults, but individuals of any age may be affected
- There is no known gender or racial predisposition

**Risk Factors**
- Close contact with an EBV-infected person

**Clinical Features**
- Fever, pharyngitis, cervical lymphadenopathy, splenomegaly, fatigue, and an atypical blood lymphocytosis are common
- Some patients have more widespread lymphadenopathy, hepatomegaly, hepatic dysfunction, or splenic rupture

**Morphology**
- Lymph nodes, tonsils: interfollicular or diffuse polymorphous infiltrate of EBV-positive immunoblasts that are sometimes Reed-Sternberg-like in a mixed background of plasma cells, histiocytes, and lymphocytes, with apoptotic debris with and without zonal necrosis
- Peripheral blood: lymphocytosis, usually with >50% lymphocytes and >10% atypical lymphocytes

**Diagnosis**
- Monospot (heterophile antibody), serology, biopsy of affected tissue, PCR assay for EBV

**Prognosis and Therapy**
- Supportive therapy is sufficient, and spontaneous recovery is the rule in nearly all cases, except for those with serious complications

### Pathologic Features

The lymph nodal architecture is typically distorted but not effaced by an expanded paracortex containing a polymorphous population of lymphoid cells, including small lymphocytes, intermediate-sized lymphoid cells, immunoblasts, tingible body macrophages, and sometimes plasma cells. The immunoblasts may be atypical, with pleomorphic or lobated nuclei; binucleated cells resembling Reed-Sternberg cells may be identified. Mitotic figures may be numerous. Apoptosis is common, and zonal necrosis may be present. Large, EBV-infected cells may be most numerous surrounding the necrosis. Reactive follicles may be present, but follicular hyperplasia is usually inconspicuous. Sinuses are patent in at least some areas, and frequently they are dilated. Sinuses contain histiocytes and a polymorphous population of lymphoid cells, including immunoblasts. Lymphoid cells sometimes infiltrate the capsule and extend into perinodal fat. Similar histologic features are found in the tonsils of patients with IM. In tonsils, crypts are usually present, although the epithelium lining them may be necrotic (Figure 4-2, A-C).

The small and intermediate-sized cells in the paracortex are predominantly T cells, including many that are activated in response to the presence of the virus. The CD4:CD8 ratio is decreased. Most paracortical immunoblasts are $CD20^+$ B cells. The blasts typically show focal staining for the activation antigen CD30; they are $CD15^-$. Using in situ hybridization with probes for EBV-encoded RNA (EBER), EBV can be detected in the paracortical immunoblasts (see Figure 4-2, D, E).

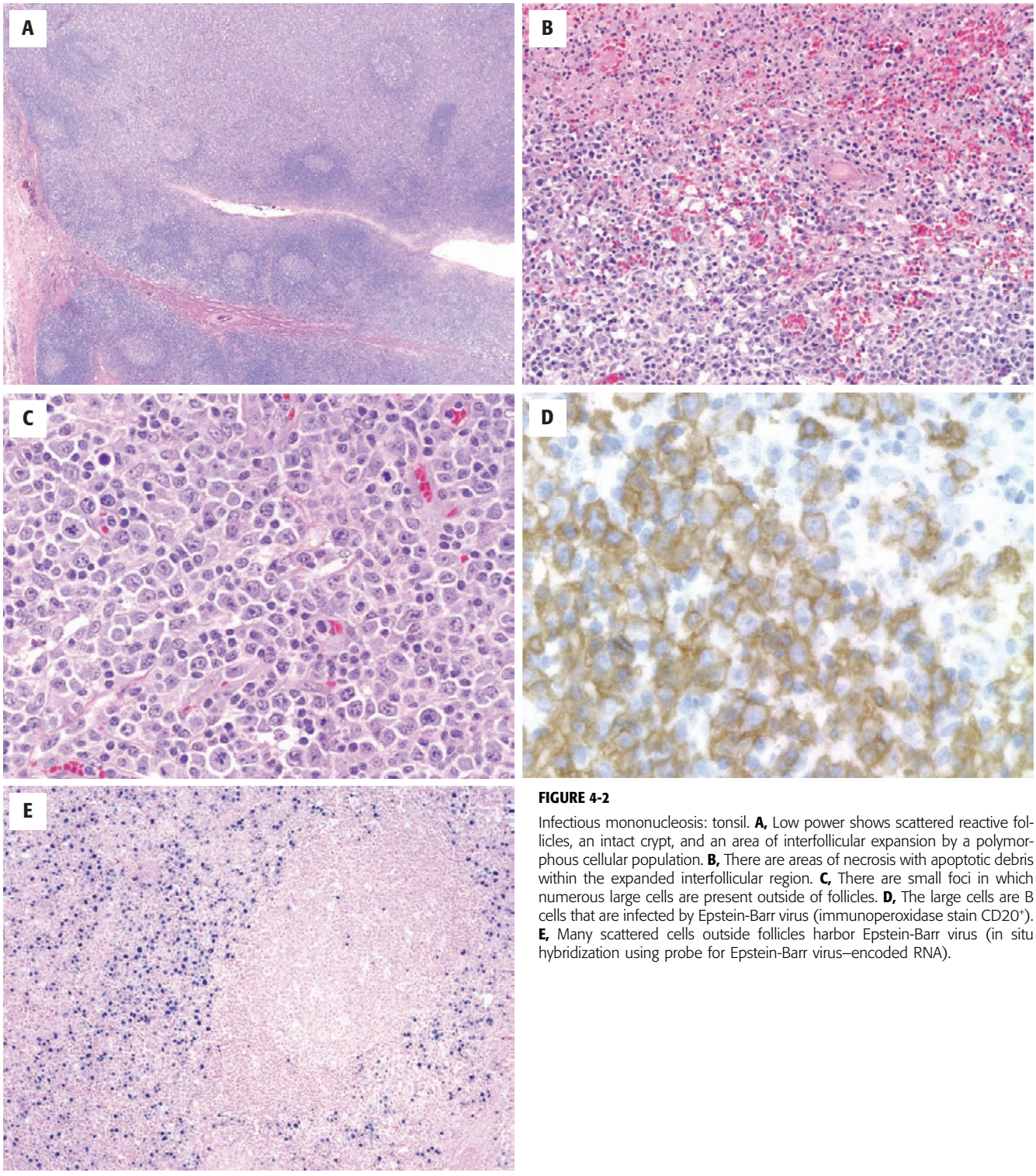


**FIGURE 4-2**

Infectious mononucleosis: tonsil. **A,** Low power shows scattered reactive follicles, an intact crypt, and an area of interfollicular expansion by a polymorphous cellular population. **B,** There are areas of necrosis with apoptotic debris within the expanded interfollicular region. **C,** There are small foci in which numerous large cells are present outside of follicles. **D,** The large cells are B cells that are infected by Epstein-Barr virus (immunoperoxidase stain $CD20^+$). **E,** Many scattered cells outside follicles harbor Epstein-Barr virus (in situ hybridization using probe for Epstein-Barr virus–encoded RNA).

## Differential Diagnosis

The differential diagnosis of IM includes a variety of reactive and neoplastic conditions. Obtaining adequate clinical information is helpful in making the correct diagnosis, whether the differential is with lymphoma or other types of reactive hyperplasia. Since the early years of the twentieth century, the tendency of IM to mimic lymphoid malignancies has been recognized. If a patient is known to have clinical or laboratory evidence of IM, a diagnosis of Hodgkin lymphoma or non-Hodgkin lymphoma should be made with caution.

When immunoblasts and intermediate-sized lymphoid cells are numerous, the possibility of non-Hodgkin lymphoma is often a consideration. Histologic features

that favor IM are the lack of architectural effacement, presence of areas readily recognizable as reactive hyperplasia, a polymorphous background of lymphoid cells, patent sinuses containing lymphoid cells including immunoblasts, and lack of monotypic immunoglobulin (Ig) expression on immunophenotyping.

Hodgkin lymphoma is often included in the differential diagnosis, because Reed-Sternberg–like cells are often seen in IM; however, most of the large, EBV-infected cells resemble immunoblasts rather than Reed-Sternberg cells and variants. Hodgkin lymphoma is more often associated with obliteration of the lymph nodal architecture. The polymorphous background of lymphoid cells ranging from small to intermediate and large in the paracortex and sinuses seen in IM is helpful in excluding Hodgkin lymphoma, in which background lymphocytes are all typically small. Immunoblasts in IM are $CD15^-$, in contrast to the CD15 expression by Reed-Sternberg cells that is found in most cases of Hodgkin lymphoma.

Other viral infections, vaccination, certain drugs, and acute reaction to severe necrotizing processes can produce lymphadenopathy with histologic features similar to or indistinguishable from those of IM. Clinical information can be helpful in investigating the etiology of the lymphadenopathy. In addition, in IM, EBER-positive immunoblasts are typically present in large numbers, whereas EBER-positive cells are usually absent in other conditions.

**CYTOMEGALOVIRAL LYMPHADENITIS—FACT SHEET**

**Definition**
- Lymphadenopathy resulting from CMV infection

**Incidence**
- Uncommon

**Gender, Race, and Age Distribution**
- Can occur in patients of any age and either gender
- No known racial predisposition

**Risk Factors**
- Increased risk with congenital or acquired immunodeficiency
- Possible increased risk in lymphoma patients
- Also occurs in immunologically normal individuals

**Clinical Features**
- Patients may be asymptomatic or have an IM-like illness
- Lymphadenopathy may be localized or generalized

**Morphology**
- Florid follicular hyperplasia and monocytoid B cell hyperplasia are seen, with or without paracortical hyperplasia
- CMV-infected cells are most often found within monocytoid B-cell aggregates, although infected cells are probably histiocytes, not B cells
- Inclusions are also occasionally found in endothelial cells
- Infected cells harbor large red nuclear inclusions and sometimes also finely granular red cytoplasmic inclusions

**Diagnosis**
- Recognition of CMV-infected cells on routinely stained slides
- Perform immunostaining for CMV in cases with suspicious histology, but with no definite inclusions identified

**Prognosis and Therapy**
- Most patients have a self-limited illness and require no specific therapy
- Patients with associated lymphoproliferative disorder or immunodeficiency may have a more severe course

## CYTOMEGALOVIRAL LYMPHADENITIS

### Clinical Features

Cytomegalovirus (CMV) can cause localized or generalized lymphadenopathy. Lymph node enlargement caused by CMV infection may be found in patients who are otherwise asymptomatic. It may also be seen in the setting of a heterophile-negative, IM-like illness. CMV can infect lymphoid tissues in patients who have or have had Hodgkin lymphoma, non-Hodgkin lymphoma, or acquired or inherited immunodeficiency syndromes or in patients with a healthy immune system. CMV infection is common in patients with common variable immunodeficiency.

### Pathologic Features

On microscopic examination, involved lymph nodes most often show florid follicular hyperplasia and monocytoid B cell hyperplasia. Paracortical hyperplasia may also be prominent. Infected cells contain a large eosinophilic intranuclear inclusion (mean size, 9 μm) and often also contain multiple tiny eosinophilic to amphophilic cytoplasmic inclusions. Cells with inclusions are usually present focally in relatively small numbers, but occasionally they are numerous. Neutrophils and histiocytes are often scattered around the infected cells. Inclusions are typically found among monocytoid B cells, but the infected cells are most likely histiocytes rather than lymphocytes; finding inclusions in the paracortex has also been described. Occasionally endothelial cells are infected. Immunoperoxidase stains for CMV-associated antigens can be used to confirm the diagnosis when inclusions are found on routine sections and can help to identify CMV-infected cells when inclusions are difficult to find. Cells containing inclusions

express CD15, usually with a Golgi region or diffuse cytoplasmic pattern of staining, but membrane staining is uncommon (Figure 4-3).

### Differential Diagnosis

The differential diagnosis of CMV lymphadenitis includes reactive hyperplasia caused by something other than CMV, Hodgkin lymphoma, or non-Hodgkin lymphoma of the follicular or nodal marginal zone type. The overall appearance of the lymph node in CMV lymphadenitis is similar to that of toxoplasmic lymphadenitis, although in most cases the characteristic epithelioid cell aggregates of toxoplasmosis are not seen. Cells with nuclear viral inclusions may resemble Reed-Sternberg cells and variants on routinely stained sections, raising the possibility of Hodgkin lymphoma. In addition, CD15 expression by the virally infected cells heightens the resemblance to Hodgkin lymphoma; however, CMV lymphadenitis is much less likely to cause obliteration of the nodal architecture. Cells harboring virus may contain numerous granular cytoplasmic inclusions, in contrast to the agranular cytoplasm of Reed-Sternberg cells. Membrane staining by CD15 is more common in Reed-Sternberg cells than in CMV-infected cells. On occasion, the follicular and monocytoid B cell hyperplasia of CMV lymphadenitis can be so florid as to distort the nodal architecture, potentially suggesting follicular lymphoma or nodal marginal zone lymphoma. Immunostaining with CMV-specific antibodies provides a definitive diagnosis of CMV lymphadenitis.

## HERPES SIMPLEX VIRAL LYMPHADENITIS

### Clinical Features

Infection by herpes simplex virus (type 1 or 2) can cause localized or generalized lymphadenopathy or involve lymph nodes in the setting of widespread visceral infection. When localized, the lymphadenopathy most often affects inguinal nodes, with cervical nodes being the next most often affected. Lymphadenopathy is usually painful. Typical mucocutaneous herpetic lesions are found in some patients, but they may be inconspicuous or may not appear until after a lymph node biopsy has been performed. Many patients with herpes simplex lymphadenitis have an associated hematologic malignancy or an underlying immunodeficiency. The most common associated disorder is chronic lymphocytic leukemia, but B cell lymphomas and myeloid leukemias are also occasionally present. Although disseminated herpes simplex viral infection has a poor prognosis, isolated herpes simplex lymphadenitis is self-limited in most cases.

**HERPES SIMPLEX VIRAL LYMPHADENITIS—FACT SHEET**

**Definition**
- Lymphadenitis resulting from infection by herpes simplex virus type 1 or 2

**Incidence**
- Rare

**Gender, Race, and Age Distribution**
- Adults over a wide age range affected
- No gender predilection
- No known racial predisposition

**Risk Factors**
- In many reported cases, patients also have a hematologic malignancy or other cause of immunodeficiency

**Clinical Features**
- Tender lymphadenopathy, which may be localized, multifocal, or associated with widespread visceral involvement
- Skin or mucosal lesions possibly present

**Diagnosis**
- Biopsy of infected tissue, identification of cells with intranuclear inclusions on routine sections, and confirmation with immunostaining for herpes simplex viral antigens
- Electron microscopy or viral culture

**Prognosis and Therapy**
- Some patients have had no specific therapy, some have received acyclovir, and some have received therapy for the associated hematologic disorder
- Prognosis is related to extent of infection and to the prognosis of any underlying hematologic disorder or immunodeficiency

### Pathologic Features

Lymph nodes show prominent paracortical hyperplasia with areas of necrosis often with prominent extension into perinodal soft tissue. Paracortical immunoblasts may be numerous. The necrotic areas contain neutrophils, karyorrhectic or amorphous eosinophilic debris, and a variable number of cells with intranuclear viral inclusions, ranging from rare to abundant. Most of them are uninucleated, although a few multinucleated cells are seen. Intact neutrophils are most abundant in early lesions and may be absent in long-standing lymphadenitis. Histiocytes often surround the necrotic areas, but granulomas are absent. Similar changes are found in the tonsils in herpetic tonsillitis. Varicella zoster virus rarely causes lymphadenitis; the histologic features are similar to those of herpes simplex lymphadenitis. The diagnosis can be confirmed using immunohistochemical stains, in situ hybridization, electron microscopy, or viral culture (Figure 4-4).

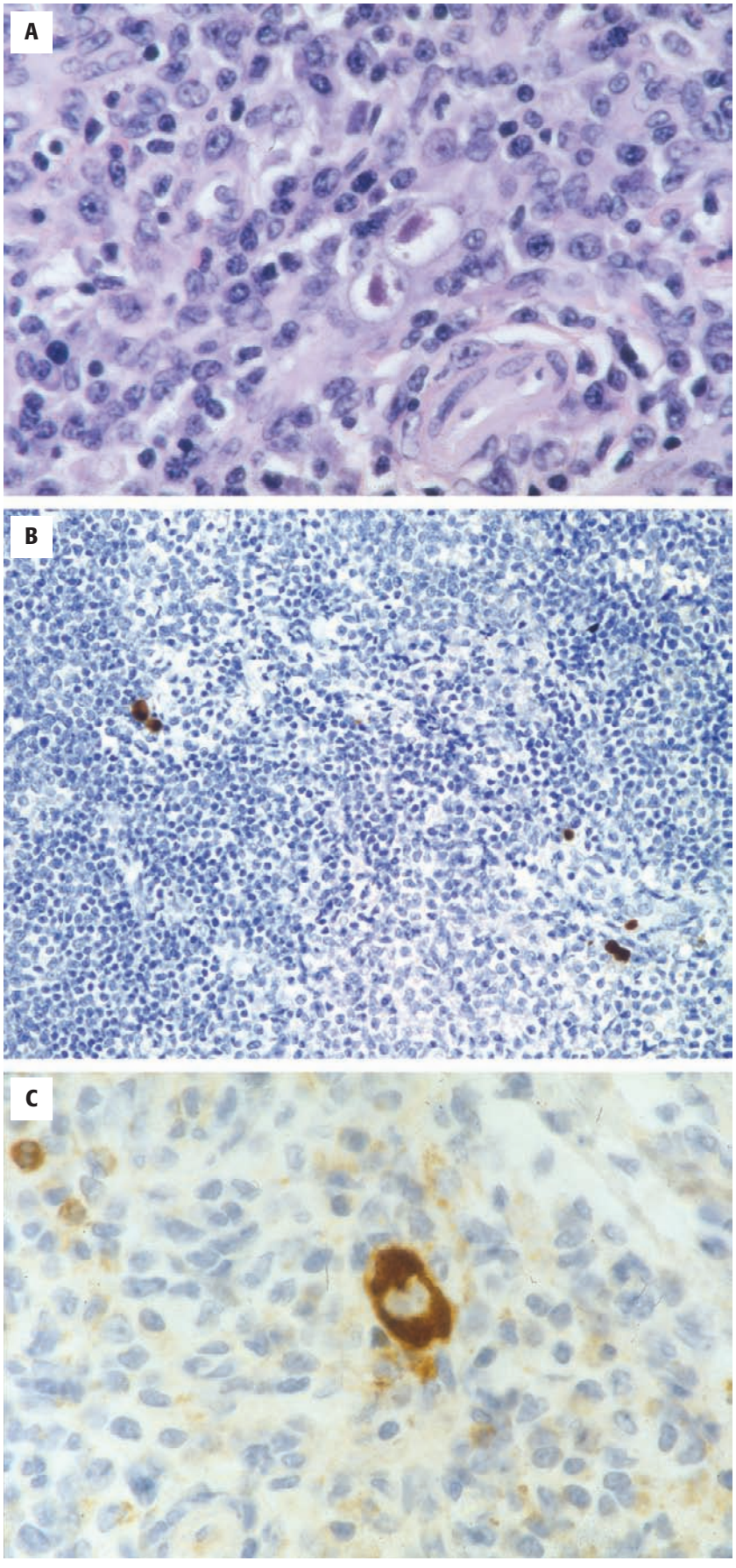


**FIGURE 4-3**

Cytomegaloviral lymphadenitis in an individual with human immunodeficiency virus. **A,** The large red inclusions give an appearance closely mimicking a binucleated Reed-Sternberg cell. **B,** Scattered cells are intensely stained using immunoperoxidase staining for cytomegalovirus. **C,** A cell infected by cytomegalovirus shows diffuse cytoplasmic staining for CD15, a finding that heightens the resemblance to a Reed-Sternberg cell.

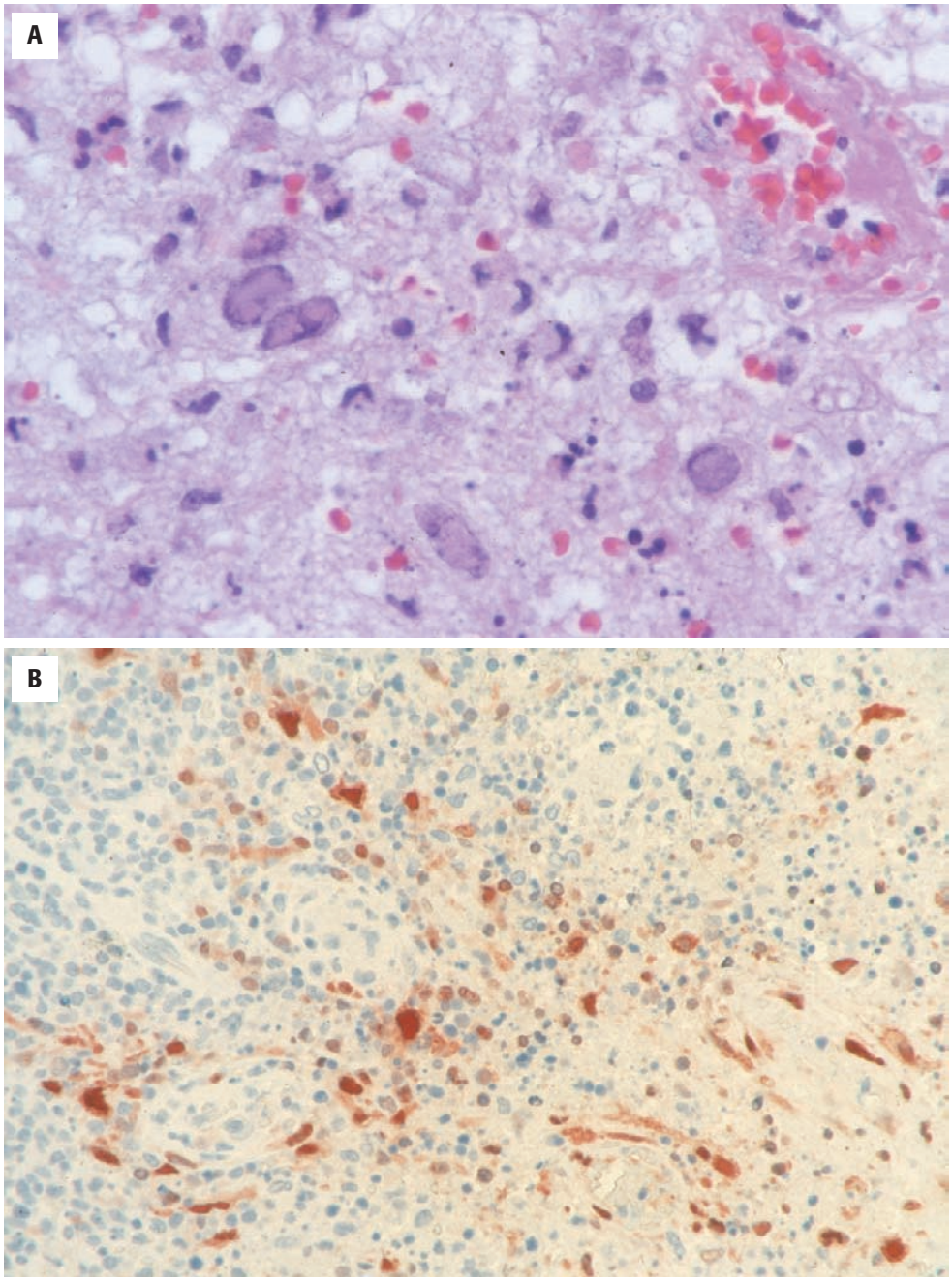


**FIGURE 4-4**

Herpes simplex viral lymphadenitis. **A,** Scattered virus-infected cells are present in a background of debris. The infected cells are usually uninucleated and contain glassy nuclear inclusions, with peripheral margination of chromatin. **B,** Many cells are stained using an immunostain for herpes simplex virus.

## Differential Diagnosis

The differential diagnosis based on histologic features includes other types of necrotizing lymphadenitis and, in some cases, lymphoma. The lack of granulomatous inflammation with epithelioid or palisading histiocytes provides evidence against infections caused by mycobacteria, fungi, yersinia, cat-scratch bacilli, and lymphogranuloma venereum. IM lacks viral inclusions. When there is pronounced paracortical expansion with numerous immunoblasts, the differential includes lymphoma. In herpes simplex lymphadenitis, the nodal architecture may be distorted, although it is not obliterated, in contrast to most nodes involved by lymphoma. Discrete foci of necrosis are less common in lymphoma, and herpes viral inclusions are absent in lymphoma, except in the very rare cases in which both herpes simplex virus and lymphoma involve the same lymph node. In instances in which patients have an established diagnosis of lymphoma and then develop rapidly enlarging lymphadenopathy related to viral infection, the clinical differential can include relapse or progression of the lymphoma.

## HIV-ASSOCIATED LYMPHADENOPATHY

The range of reactive changes in lymph nodes in HIV infection is described in this section. Lymphadenitis or lymphadenopathy in HIV-positive patients related to specific causes other than HIV are discussed in those sections.

### Clinical Features

Acute symptomatic HIV infection presents as an IM-like illness with fever, pharyngitis, and cervical lymphadenopathy in approximately 16% of cases. Persistent generalized lymphadenopathy, defined as extrainguinal lymphadenopathy persisting for at least 3 months, involving at least two noncontiguous node groups, is common among patients with HIV. Persistent generalized lymphadenopathy mainly affects adult males and is often accompanied by fever, weight loss, headaches, and malaise. A range of histologic and immunohistologic changes is found in lymphadenopathy associated with HIV infection; similar changes are found in organized extranodal lymphoid tissue.

### Pathologic Features

Early stages of immunodeficiency are characterized by florid follicular hyperplasia, with large, irregular germinal centers with a high mitotic rate, numerous blast cells, many tingible body macrophages, and ill-defined, attenuated, or effaced mantle zones. There is often follicle lysis. The interfollicular region contains a mixture of immunoblasts, plasma cells, lymphocytes, and histiocytes. Monocytoid B cells are often prominent. Sinus histiocytosis may be seen, sometimes with erythrophagocytosis, epithelioid histiocytes, and polykaryocytes (Warthin-Finkeldey–type giant cells).

More advanced stages of immunodeficiency are associated with lymphoid depletion. Reactive follicles are decreased in number or are absent. Residual follicle centers are "burnt out" or regressively transformed and contain a decreased number of B cells. Over time, follicles can become inconspicuous and difficult to identify. The interfollicular region contains scattered lymphocytes, immunoblasts, plasma cells, and many blood vessels. Amorphous eosinophilic material may be present, or there may be fibrosis, and the node may have a pale, depleted appearance. In some cases, lymph nodes show changes intermediate between florid follicular hyperplasia and lymphoid depletion. Plasmacytosis is

#### HIV-ASSOCIATED LYMPHADENOPATHY—FACT SHEET

**Definition**
- Reactive lymphoid changes related to HIV infection not resulting from another specific cause

**Incidence**
- Common among HIV-positive patients; in one study, 4% of cases of unexplained follicular hyperplasia were from HIV-positive individuals not known to be HIV-positive before the lymph node biopsy

**Gender, Race, or Age Distribution**
- Most patients are young to middle-aged adults (more males than females), but smaller numbers of older adults and children of either gender may be affected

**Clinical Features**
- There are a number of different clinical settings in which HIV-associated lymphoid hyperplasia can be found:
  - Lymphadenopathy, usually in the cervical area, occurring in the setting of an IM-like illness, precipitated by initial HIV infection
  - Persistent generalized or isolated lymphadenopathy that may be accompanied by constitutional symptoms
  - Cystic lymphoid hyperplasia, involving periparotid lymph nodes and causing multicystic, often bilateral, masses in the area of the parotid glands
  - Hyperplasia of organized extranodal lymphoid tissue, such as Waldeyer ring, with symptoms related to mass effect

**Morphology**
- In early stages, lymph nodes show florid follicular hyperplasia, often with mantle zone attenuation and follicle lysis; some follicles are highly irregular with geographic shapes
- In advanced stages of immunodeficiency, there is lymphoid depletion
- Follicles are small and burnt out and sometimes difficult to identify, and the paracortex appears hypocellular, often with prominent vascularity in late stages
- Plasma cells are often abundant in lymphoid tissues in early and late stages of disease

**Immunophenotype**
- B cells in follicles are polytypic; T cells show a decreased CD4:CD8 ratio
- Few to many scattered cells infected by EBV may be found

**Diagnosis**
- The morphologic changes are not completely specific, but if an individual has lymphadenopathy with the features described, and the patient is not known to be HIV positive, performing a test for HIV is warranted

**Prognosis and Therapy**
- Therapy is directed against the HIV infection itself
- Outcome is related to the prognosis of the underlying HIV infection, but with the availability of highly active antiretroviral therapy in recent years, the prognosis has significantly improved

frequent. Occasional polykaryocytes may be found. Cutaneous rashes are common among patients with HIV; these individuals often have a component of dermatopathic lymphadenopathy.

The interfollicular region contains decreased numbers of $CD4^+$ cells, and the CD4:CD8 ratio is usually reversed. It is frequent to find scattered cells harboring Epstein-Barr virus ($EBER^+$) consistent with poor T cell control of EBV-infected B cells.

Most patients who have been treated with highly active antiretroviral therapy show improvement in lymphoid architecture, as well as an increase in $CD4^+$ T cells and a decrease in $CD8^+$ T cells, within lymphoid tissue. However, HIV often is detectable in follicular dendritic cells in lymphoid tissue, even after a prolonged course of highly active antiretroviral therapy, and even in the absence of detectable virus in the peripheral blood.

## MEASLES LYMPHADENITIS

Lymphadenitis caused by measles virus, an RNA paramyxovirus, is seen rarely in the United States because of inoculation programs. The most distinctive feature is the presence of Warthin-Finkeldey giant cells within reactive germinal centers. Warthin-Finkeldey cells are found in the prodromal stage of infection and generally disappear as a rash develops (Figure 4-5).

## KAWASAKI DISEASE

Although Kawasaki disease is of uncertain cause, evidence strongly suggests that it is of infectious etiology. For this reason, it is included in the section on infectious lymphadenitis. Pathologic findings are not specific, but include multifocal necrosis and fibrin thrombi in the microvasculature (see Fact Sheet).

# BACTERIAL LYMPHADENITIS

## PYOGENIC BACTERIAL LYMPHADENITIS

Bacterial infections are a common cause of cervical lymphadenopathy. Lymphadenitis resulting from streptococcal or staphylococcal infection is probably the most common cause of acute cervical lymphadenopathy in children in the United States, although a biopsy is not often performed. Microscopic examination usually shows follicular hyperplasia and a variably dense paracortical neutrophilic infiltrate.

## CAT-SCRATCH DISEASE

### Clinical Features

Cat-scratch disease is among the most common causes of subacute or chronic benign lymphadenopathy in the United States, with approximately 24,000 cases occurring per year. For reasons that may be related to the breeding patterns of cats, it is more common between July and December than between January and June. The principal causal agent for cat-scratch disease is *Bartonella henselae*. Although cat-scratch disease can

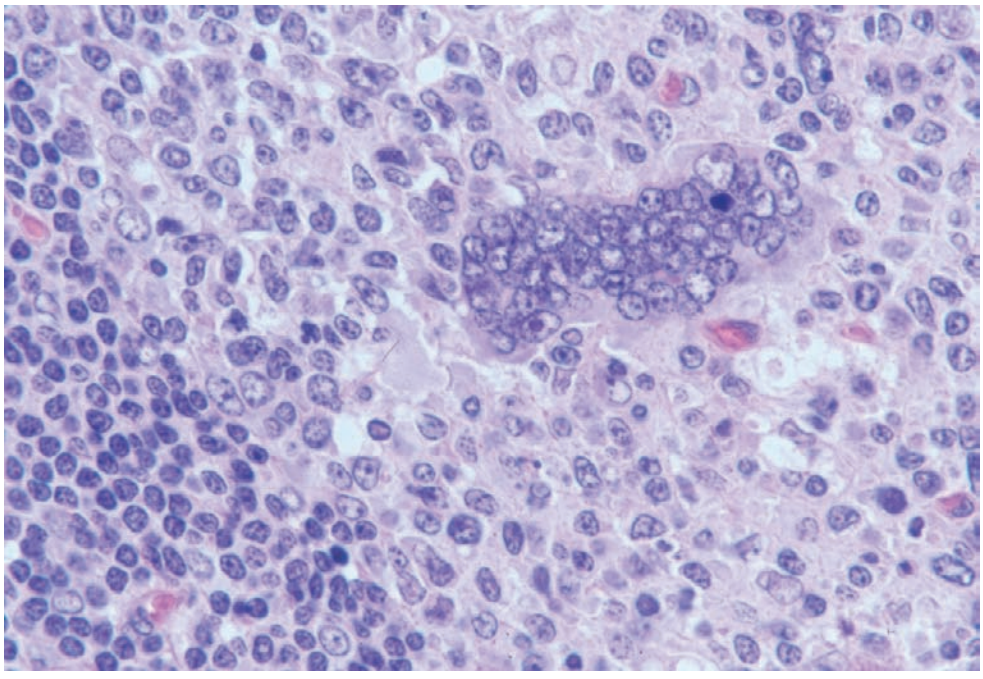

**FIGURE 4-5**

Measles: appendix. The lymphoid follicles in this appendix contain multinucleated Warthin-Finkeldey giant cells. The appendix was removed because of symptoms suggesting acute appendicitis. The patient developed a rash typical of measles immediately after appendectomy.

**KAWASAKI DISEASE—FACT SHEET**

**Definition**

- An acute febrile disease of young children of uncertain cause, with most evidence suggesting an infectious, possibly viral, cause
- Synonyms: mucocutaneous lymph node syndrome, infantile polyarteritis

**Incidence and Location**

- Approximately 17 cases per 100,000 children younger than 5 years per year in the United States
- Higher in Japan and in other Asian countries

**Gender, Race, and Age Distribution**

- 85% of patients are younger than 5 years
- Boys are more often affected than girls (male-to-female ratio, approximately 2 : 1)
- Prevalence is higher among Asians

**Risk Factors**

- Higher risk among family members of patients with Kawasaki disease

**Diagnosis**

- The Centers for Disease Control and Prevention requirements for the diagnosis of Kawasaki disease include finding fever of 5 or more days, unresponsiveness to antibiotics, and at least four of the following five features:
  8.664 Bilateral conjunctival congestion
  - Abnormalities of lips and oral cavity including diffuse erythema, dry fissured lips, and prominent lingual papillae (strawberry tongue)
  - Abnormalities of the skin of the distal extremities, including erythema of palms and soles with early edema and desquamation of fingertips later in the course of the disease
  - Polymorphous, nonvesicular, primarily truncal rash
  - Acute, nonsuppurative cervical lymphadenopathy not a result of any other identifiable cause

**Clinical Features**

- In addition to the diagnostic criteria noted, patients may have cardiac abnormalities (electrocardiographic changes, cardiomegaly, murmurs), diarrhea, arthritis or arthralgia, proteinuria, sterile pyuria, neutrophilic leukocytosis with a leftward shift, anemia, thrombocytosis, elevated sedimentation rate, aseptic meningitis, mild jaundice, and elevated transaminases
- Most patients recover after an illness of 3 to 4 weeks' duration, but without therapy, 20% to 25% develop complications of coronary arteritis (aneurysm, thrombosis), which can be fatal, sometimes years later
- Among patients with cervical lymphadenopathy the male-to-female ratio tends to be higher than among those without adenopathy. Patients with cervical lymphadenopathy are older on average. They tend to have more pronounced changes in markers of systemic inflammation, such as an elevated white blood cell count and C-reactive protein, and they tend to include a higher proportion of patients with coronary artery disease

**Pathologic Features**

- Limited information is available on findings in lymph nodes
- Findings described include:
  - Paracortical expansion by lymphocytes and a variable number of immunoblasts, plasma cells, and histiocytes
  - Small to large areas of necrosis containing karyorrhectic debris with or without neutrophils
  - Increased numbers of blood vessels lined by swollen endothelial cells and containing fibrin thrombi around the necrotic areas
- Underlying the changes is activation of the immune response, with important components being IgA, cytotoxic T cells, and monocytes–macrophages

**Prognosis and Therapy**

- Intravenous Ig and aspirin; steroids and other antiinflammatory agents can be used if symptoms persist
- Prompt administration of intravenous Ig reduces the risk of coronary arterial changes and thus the risk of serious or fatal complications of this disease

affect patients of any age, 85% are younger than the age of 18 years, and the majority are between 3 and 10 years old. A history of exposure to a cat (typically a kitten with fleas) can be found in most cases. The infectious agent has been found within the gastrointestinal tract of fleas, and it is possible that fleas act as vectors in the transmission of the disease. Three to 10 days after exposure, a papule develops in the skin at the inoculation site. The papule typically becomes vesiculated and then crusted over the next several days. Regional lymphadenopathy is usually found 1 to 2 weeks after the papule appears.

The lymphadenopathy, which is often tender, usually involves only one node or one group of nodes. Noncontiguous lymphadenopathy is occasionally found, probably because of more than one inoculation site or because the inoculation site is midline, allowing organisms to drain to lymph nodes bilaterally. The axilla is the single most common site for adenopathy (45%). When the inoculation site is the eye, patients may develop the oculoglandular syndrome of Parinaud (granulomatous conjunctivitis and preauricular lymphadenopathy). As many as half of patients have fever, but it is usually low grade (body temperature less than 39° C). Patients may also experience malaise, anorexia, or, rarely, nausea or abdominal pain. The disease is usually mild and self-limited, and some cases probably go unrecognized. Severe complications, occurring in as many as 2%, include involvement of the nervous system, bone, lung, liver, or spleen. Neurologic

manifestations include encephalopathy, encephalitis, meningitis, and involvement of cranial or peripheral nerves. The liver and spleen may be the sites of abscess formation. The skeleton is rarely involved by a necrotizing granulomatous osteomyelitis, sometimes via direct extension from an affected lymph node. Patients with lung involvement may have pneumonia or pleural effusions.

## Pathologic Features

The appearance of the infected lymph nodes changes over time. The earliest changes are follicular hyperplasia and a prominent proliferation of monocytoid B cells. Subsequently, within aggregates of monocytoid B cells, small foci of necrosis with a few neutrophils and scant fibrin and cellular debris appear. These foci are usually close to, or encroach on, germinal centers, or they may be adjacent to the subcapsular sinus. In some cases, the foci are found in the vicinity of a small blood vessel. The paracortex is hyperplastic. Sinuses contain immunoblasts, neutrophils, and histiocytes. With time, the necrotic foci enlarge, extending deeper into the node; they contain pus, fibrin, and abundant cellular debris and acquire a rim of macrophages. The foci continue to enlarge and coalesce and are surrounded by palisading histiocytes to produce the classic stellate microabscess or granuloma. The cat-scratch bacillus is a small, slender, pleomorphic, weakly gram-negative rod measuring as long as 3 μm; its small size makes it difficult to see. Using a Warthin-Starry stain or Steiner stain, the bacilli are coated with the black reaction product, making them appear larger and more readily visualized. Bacilli are present singly, in chains, or in large clumps; they may be found along the walls of blood vessels, in macrophages in necrotic areas, in sinus histiocytes, or admixed with necrotic debris. Bacilli are most numerous in the stages of early necrosis within clusters of monocytoid B cells. When well-developed stellate abscesses have formed, the organisms can be difficult to detect (Figure 4-6). In addition, *B. henselae* is a fastidious organism that is difficult to culture.

Serologic studies and skin testing may be helpful in establishing a diagnosis, although serologic testing can yield false-negative results and also might not distinguish between recent and past infections. An antibody to *B. henselae* has been developed and may help to identify the microorganisms. In addition, probes for *Bartonella*-specific genetic sequences are available that can be used to detect cat-scratch disease using the polymerase chain reaction (PCR). However establishing a diagnosis is often difficult because microorganisms may be present only focally and in small numbers, and because prior antibiotics may decrease the sensitivity of some of these techniques.

### CAT-SCRATCH DISEASE—FACT SHEET

**Definition**
- Lymphadenitis typically transmitted by cats, caused in nearly all cases by *B. henselae*

**Incidence and Location**
- Relatively common
- Approximately 24,000 cases per year in the United States

**Gender, Race, and Age Distribution**
- 85% of affected individuals younger than age 18; most between 3 and 10 years old
- No known gender or racial predilection

**Risk Factors**
- Contact with a cat, especially a kitten with fleas

**Clinical Features**
- A cutaneous lesion appears at the inoculation site (usually the site of a scratch) after 3 to 10 days
- Tender regional lymphadenopathy appears 1 to 2 weeks later
- Patients often have low-grade fever and malaise and infrequently have evidence of involvement of other sites, such as lungs, liver, spleen, bones, or central nervous system

**Morphology**
- Early stage: lymph nodes show follicular and monocytoid B cell hyperplasia, with small foci of acute inflammation and necrosis usually beginning within aggregates of monocytoid B cells
- Later stage: large stellate microabscesses and necrotizing granulomas with palisading histiocytes

**Diagnosis**
- Identification of cat-scratch bacilli with a silver stain or an immunostain
- PCR on affected tissues
- Serologic studies

**Prognosis and Therapy**
- Most cases have a mild, self-limited course and do not require specific therapy

## Differential Diagnosis

A variety of microorganisms cause necrotizing lymphadenitis that can be considered in the differential diagnosis of cat-scratch disease. Clinical features, including age, risk factors for different types of infection, anatomic distribution of lymphadenopathy, and severity of disease, special stains on tissue sections, serologic studies, and culture are helpful in establishing a definite diagnosis. Infection caused by certain bacteria, including *Chlamydia trachomatis* (lymphogranuloma venereum), *Francisella tularensis* (tularemia), *Hemophilus ducreyi* (chancroid), *Yersinia enterocolitica*

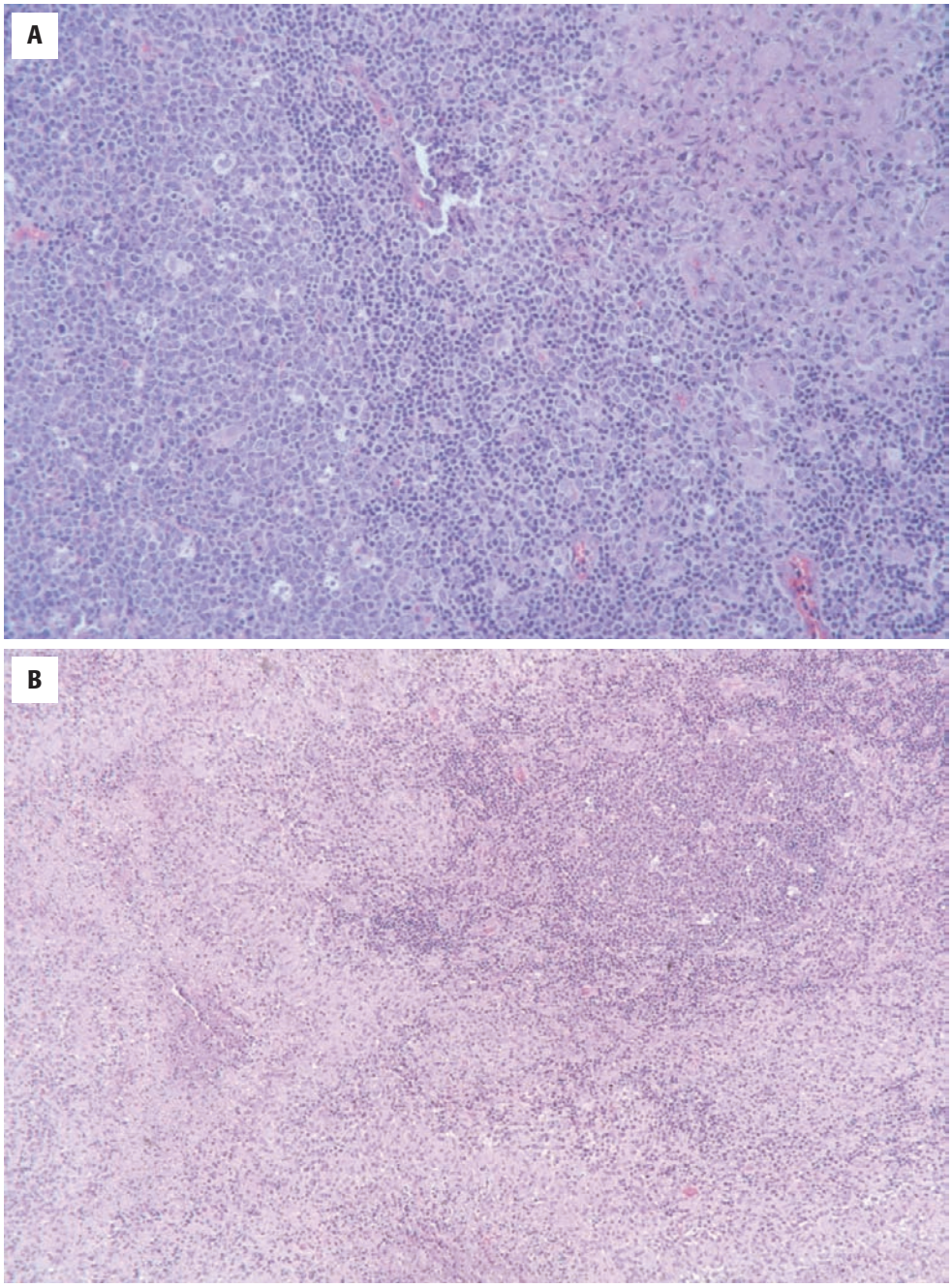


**FIGURE 4-6**

Cat-scratch disease (*Bartonella henselae*–associated lymphadenitis). **A,** In an example of an early stage of infection, there is a large reactive follicle; adjacent to it are an aggregate of histiocytes, fibrin, and debris. **B,** In this case, the inflammatory process is more advanced. One reactive follicle is seen, but most of the rest of the node is replaced by necrotizing granulomatous inflammation with palisading histiocytes.

(pseudotuberculous mesenteric lymphadenitis), *Listeria monocytogenes* (listeriosis), *Pseudomonas mallei* (glanders), and *Pseudomonas pseudomallei* (melioidosis), can be associated with a necrotizing lymphadenitis with histologic features that may be indistinguishable from those of cat-scratch disease. In nearly all cases, the diseases listed here are associated with significantly greater morbidity than cat-scratch disease. Early involvement by cat-scratch disease may also resemble toxoplasma lymphadenitis (see Toxoplasmosis).

As noted previously, infection by pyogenic cocci may be associated with an acute suppurative lymphadenitis with follicular hyperplasia that can suggest cat-scratch disease. Unlike cat-scratch disease, however, the pyogenic cocci typically do not elicit a rim of palisading histiocytes around areas of suppurative necrosis. One exception is in cases of chronic granulomatous disease of childhood, in which palisading granulomas with suppurative necrosis may be found in association with infection by *Staphylococcus aureus* and a variety of

gram-negative bacteria (see later section on this topic). The appearance can closely resemble that of cat-scratch disease.

Mycobacteria (*Mycobacterium tuberculosis* and atypical mycobacteria) and fungi can produce lymphadenitis with granulomatous microabscesses that may resemble those of cat-scratch disease, and the most important differential diagnosis may be between cat-scratch disease and atypical mycobacterial infection of cervical lymph nodes in children. Conversely, in some late-stage cases of cat-scratch disease, the necrotic material acquires a pink, amorphous quality closely resembling the caseation necrosis characteristic of tuberculosis. Special stains for microorganisms, culture, and other special techniques may be helpful in establishing a diagnosis. The distinction is clinically relevant because cat-scratch disease often does not require treatment, whereas infection by *M. tuberculosis* should be treated with antibiotics.

In immunocompromised hosts, *B. henselae* infection rarely takes the form of localized granulomatous lymphadenitis. Instead, it can result in widespread granulomatous inflammation, bacillary angiomatosis, bacillary peliosis, or bacteremia.

## BRUCELLOSIS

See Fact Sheet.

## SYPHILITIC LYMPHADENITIS

In primary and secondary syphilis, lymph nodes show marked follicular hyperplasia with numerous plasma cells. They may also contain nonnecrotizing or suppurative granulomas and show marked capsular and perinodal fibrosis and prominent vascular changes (endarteritis and phlebitis). Spirochetes can be found in blood vessels, germinal centers, and granulomas and are often within the cytoplasm of histiocytes. Involved lymph nodes may have the appearance of an inflammatory pseudotumor on routinely stained sections. The changes characteristic of syphilis are best seen in inguinal nodes. Although secondary syphilis may be associated with diffuse lymphadenopathy, nodes away from the inguinal region may show only nonspecific follicular hyperplasia.

## MYCOBACTERIAL LYMPHADENITIS

Mycobacterial lymphadenitis can occur in isolation or in conjunction with pulmonary tuberculosis or disseminated infection. Tuberculous lymphadenitis is the most common form of tuberculosis except for pulmonary

### BRUCELLOSIS—FACT SHEET

**Definition**
- An acute, intermittent, or chronic febrile illness resulting from infection by *Brucella* species
- *Brucella melitensis* is the most common cause of brucellosis in humans and is especially prevalent in developing countries
- Brucella are gram-negative, intracellular coccobacilli
- Synonym: undulant fever

**Incidence and Location**
- Highly variable
- Approximately 3.5 cases per 100,000 persons per year in an endemic area
- Rare in the United States

**Gender, Race, and Age Distribution**
- Children and adults and males and females affected, depending on exposure
- No known racial predisposition

**Risk Factors**
- Close animal contact (shepherds, goatherds), ingestion of unpasteurized milk, consumption of uncooked meat

**Clinical Features**
- The severity and type of symptoms present are highly variable from case to case
- Fever is virtually always present and nearly all patients complain of malaise and night sweats
- Arthralgia, myalgia, back pain, headache, abdominal pain, and gastrointestinal symptoms (diarrhea, nausea, vomiting) are common
- Splenomegaly is common
- Hepatomegaly and lymphadenopathy are slightly less common
- Lymphadenopathy appears to be more common in children than in adults and to be more conspicuous early in the course of the disease
- Some patients have evidence of cardiac and central nervous system involvement
- Laboratory abnormalities include anemia, leukopenia, monocytosis, and eosinophilia
- The presence of splenomegaly tends to correlate with the finding of leukopenia and with fever lasting more than 1 week

**Pathologic Features**
- Involved tissues show nonnecrotizing, sarcoidal granulomas, necrotizing granulomas, sometimes with suppurative necrosis, abscesses, or diffuse mononuclear infiltrates

**Diagnosis**
- Blood culture, biopsy of involved lymph node (or other tissue) with microscopic examination and culture, serologic studies, standard tube agglutination test, and Rose-Bengal slide agglutination test

**Prognosis and Therapy**
- Treatment consists of administration of appropriate antibiotics; prolonged use of antibiotics can help to prevent relapse
- Prognosis is good with appropriate therapy
- Animal testing and treatment could decrease the incidence of disease in humans

**TABLE 4-1**
**Tuberculous versus Nontuberculous Lymphadenitis in Immunocompetent Patients**

| Feature | Tuberculous Mycobacterial Infection | Nontuberculous Mycobacterial Infection |
|---|---|---|
| Definition | Lymphadenitis caused by tuberculous organisms (*Mycobacterium tuberculosis, Mycobacterium bovis)* | Lymphadenitis caused by atypical mycobacteria (*Mycobacterium avium-intracellulare* and others) |
| Age | Any age | Majority <5 years |
| Gender predilection | No gender predilection | M = F or M < F (varies by series) |
| Constitutional symptoms | Often present | Infrequent; mild if present |
| Sites | Cervical nodes, mediastinal nodes, lungs | Cervical nodes, unilateral, most often submandibular, jugulodigastric, or preauricular lymph nodes |
| Chest radiograph | Frequently abnormal | Negative, almost always |
| Contagious | Yes | No |
| Purified protein derivative test | Usually strongly positive | Negative or weakly positive |
| Treatment | Antibiotics | Excision or FNA followed by observation |

tuberculosis. The clinical picture varies with the type of mycobacteria and the age and immune status of the affected patients.

## TUBERCULOUS LYMPHADENITIS

### Clinical Features

Tuberculous lymphadenitis is due to infection by tuberculous mycobacteria, such as *M. tuberculosis* or *Mycobacterium bovis*. Infection by *M. tuberculosis* is a serious global health problem, with approximately one third of the world's population harboring *M. tuberculosis*. There are approximately $9 \times 10^6$ new cases of tuberculosis and approximately $2 \times 10^6$ deaths from tuberculosis each year. In Central Africa, tuberculous lymphadenitis is the most common finding among patients who undergo a superficial lymph node biopsy; nearly all of these African patients are HIV positive. *M. tuberculosis* was also the most common specific cause of cervical lymphadenopathy in children in a study from South Africa. Although tuberculosis is much less common among individuals in the United States than in other parts of the world, there have been recent increases in the number of cases resulting from homelessness and poor living conditions. In addition, administration of incomplete courses of antibiotics because of poor patient compliance has led to the emergence of increased numbers of drug-resistant strains.

Patients with lymphadenitis caused by *M. tuberculosis* infection are usually adults with nontender lymphadenopathy, constitutional symptoms (fever, fatigue, and weight loss), and associated pulmonary tuberculosis. When peripheral lymph nodes are involved, they tend to be in the supraclavicular fossa or posterior cervical triangle. Frequently, multiple lymph nodes are involved bilaterally, and there are draining sinuses. The purified protein derivative test is usually strongly positive (Table 4-1).

### Pathologic Features

Lymph nodes in immunocompetent patients show multiple well-formed granulomas composed of epithelioid histiocytes and Langhans-type giant cells; caseation necrosis is present to a variable extent in the centers of the granulomas. Dystrophic calcification may be seen. A Ziehl-Neelsen stain is used to identify the acid-fast bacilli, which are usually few in number in immunocompetent patients. Culture is needed to diagnose cases in which organisms cannot be identified in tissue sections and to distinguish definitively between infections by *M. tuberculosis* and atypical mycobacteria.

## NONTUBERCULOUS MYCOBACTERIAL LYMPHADENITIS

Nontuberculous mycobacterial lymphadenitis is due to infection by the so-called atypical mycobacteria, most often *Myzzcobacterium avium-intracellulare* and less often *Mycobacterium scrofulaceum, Mycobacterium hemophilum, Mycobacterium malmoense,* or *Mycobacterium kansasii*.

## Clinical Features

Most immunocompetent individuals who develop nontuberculous mycobacterial lymphadenitis are children, most of whom are younger than 5 years. Some studies have described an equal sex ratio; others report a higher proportion of female patients. It is said to be the most common cause of chronic lymphadenitis among previously healthy children, although it should be noted that in some developing countries, tuberculous lymphadenitis is more prevalent than nontuberculous mycobacterial lymphadenitis. The annual incidence is estimated to be 1.2 to 2.1 cases per 100,000 persons. The incidence appears to have increased in recent years.

Children exhibit firm, nontender cervical lymphadenopathy that is almost always unilateral. Constitutional symptoms are typically absent. Lymph nodes away from the cervical area are rarely affected. The microorganisms are believed to infect the patient via the oropharynx, skin, or conjunctiva, and involved lymph nodes correspond to those with lymphatic drainage from these sites. Over time, necrosis develops within the infected nodes, and they become fluctuant. The overlying skin may become discolored, and sinus tracts may form. The purified protein derivative test is usually weakly positive or negative. The chest radiograph is almost always normal.

Patients do not usually respond to antibiotics. Surgical excision of the abnormal lymph nodes is the traditionally accepted optimal therapy, associated with a low rate of recurrence. If incomplete removal of the lymph nodes by incision and drainage or curettage is performed, the infection often persists, resulting in sinus tract formation and additional surgery may be required. Recently however it has been suggested that diagnosis based on fine needle aspiration with culture followed by observation only may be considered as an alternative to excision. Although patients treated in this way experience purulent drainage for a period of weeks eventually nearly all have a flat, skin-colored area of scarring that is considered cosmetically acceptable.

## Pathologic Features

The children have enlarged, sometimes matted lymph nodes with necrotizing and nonnecrotizing granulomas. The necrosis often forms a stellate pattern and may be caseous or noncaseous. Necrotic areas may contain neutrophils and nuclear debris focally or diffusely. Langhans-type giant cells can often be found. The diagnosis can be suggested based on clinical features. It can be confirmed and the infection can be treated by complete excision of affected lymph nodes. Special stains for microorganisms and culture will demonstrate the organisms. Ziehl-Neelsen stain typically reveals small numbers of acid-fast bacilli in some cases, particularly at the periphery of the necrotic area, but in other cases, organisms cannot be identified without culture. In selected instances, PCR may be used to subclassify the organisms (see Table 4-1).

## MYCOBACTERIAL LYMPHADENITIS IN IMMUNOCOMPROMISED PATIENTS

In contrast to atypical mycobacterial infection in immunocompetent patients, atypical mycobacteria in HIV-positive patients, especially *M. avium-intracellulare,* can be associated with disseminated infection. Immunosuppressed patients sometimes have lymphadenopathy with necrotizing or nonnecrotizing granulomas; however, sometimes they fail to develop well-formed granulomas. In such cases, involved tissues may contain only loose aggregates and sheets of foamy histiocytes containing numerous microorganisms. Rarely patients develop so-called mycobacterial pseudotumors, composed of rounded and elongate histiocytes containing numerous acid-fast bacilli. The appearance on routinely stained sections can occasionally suggest a mesenchymal neoplasm (Figure 4-7). The microorganisms can be seen with the Grocott methenamine silver stain as well as with the Ziehl-Neelsen stain. When the acid-fast bacilli have been phagocytosed and are intracellular they may be periodic acid-Schiff (PAS)-positive.

HIV-positive patients with subclinical *M. avium-intracellulare* infection who receive highly active antiretroviral therapy may develop immune restoration disease in the form of symptomatic *M. avium-intracellulare* lymphadenitis. A similar phenomenon can occur with other types of opportunistic infections.

## Differential Diagnosis

The differential diagnosis of mycobacterial lymphadenitis is broad. Cases with little or no necrosis resemble sarcoidosis. A Ziehl-Neelsen stain and culture are helpful in establishing a diagnosis; however, the Ziehl-Neelsen stain is often negative in cases of mycobacterial infection unless there is necrosis. A wide variety of microorganisms, including fungi, cat-scratch bacilli, *Brucella* species, spirochetes, and leishmania, can be associated with granulomatous lymphadenitis. Caseation necrosis is more common in mycobacterial infections, although necrosis resembling caseation may be seen in fungal infections and, less often, with other infections. In cases of primary or secondary syphilis, lymph nodes may show nonnecrotizing or suppurative granulomas, but they are found in the setting of marked follicular hyperplasia and plasmacytosis. Cat-scratch disease, a common cause of necrotizing granulomatous

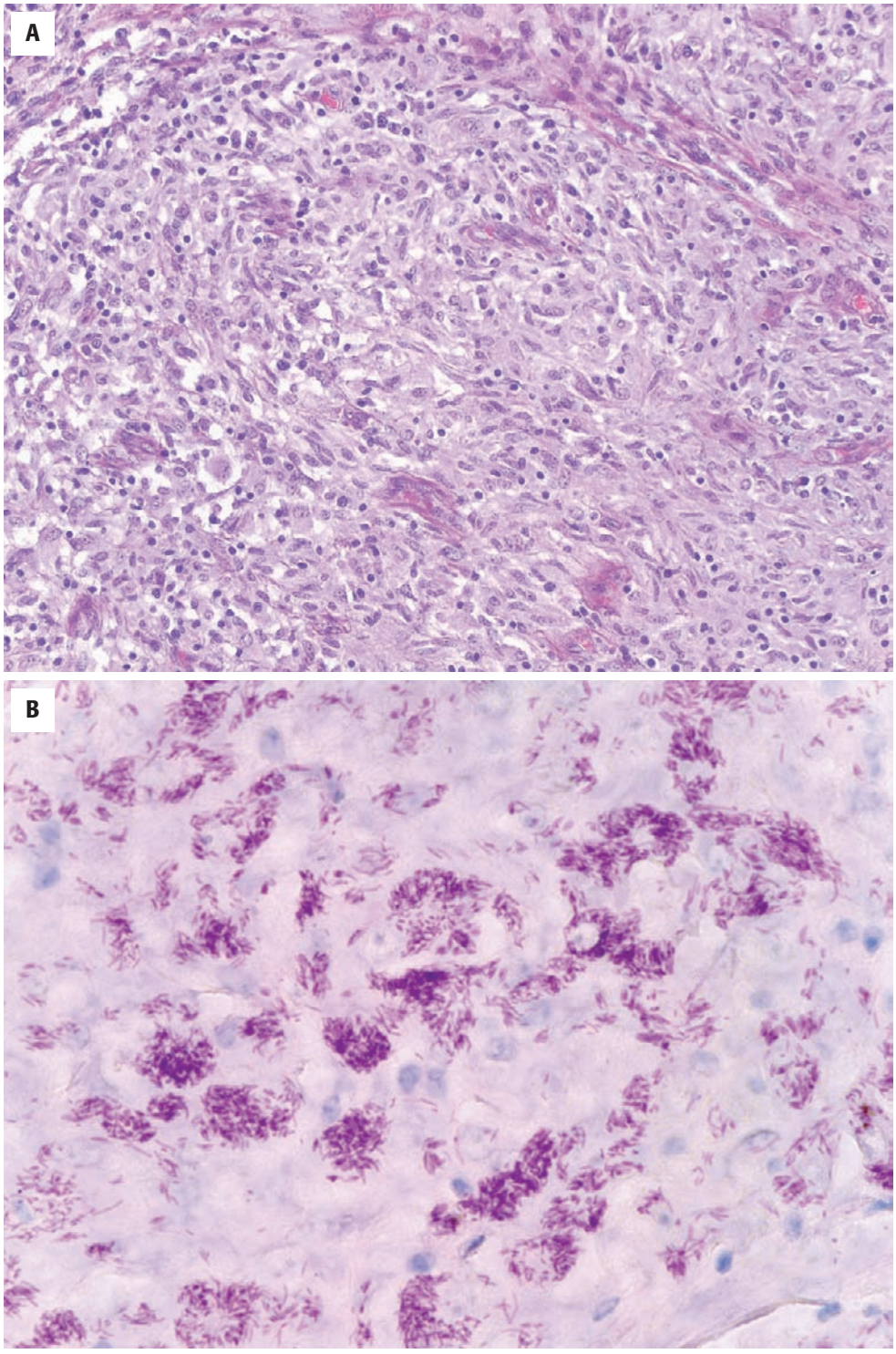


**FIGURE 4-7**

Mycobacterial pseudotumor in a patient with human immunodeficiency virus. **A,** The lymph node is replaced by histiocytes and spindle cells with finely granular pink cytoplasm. **B,** A Ziehl-Neelsen stain discloses numerous intracellular acid-fast bacilli.

lymphadenitis in children, is more likely to be associated with exposure to a cat, a cutaneous inoculation site, systemic symptoms, and tender lymphadenopathy compared with atypical mycobacterial lymphadenitis in immunocompetent children. Uncommon entities that can be considered in the differential diagnosis include leprosy, particularly the tuberculoid form, and chronic granulomatous disease of childhood. Atypical mycobacterial infection in HIV-positive patients in the form of numerous foamy histiocytes can mimic lepromatous leprosy. Because intracellular mycobacteria may be PAS positive, cases with foamy histiocytes may mimic

Whipple disease, although the clinical features of Whipple disease differ from those of mycobacterial infection, and Whipple bacilli are not expected to be acid-fast. A few cases of Wegener granulomatosis have been described in which there is necrotizing lymphadenitis with granulomatous vasculitis, and such cases could potentially enter the differential diagnosis of mycobacterial lymphadenitis. Results of stains for microorganisms, culture, and clinical information can be helpful in establishing a diagnosis.

Granulomatous inflammation, often accompanied by extensive necrosis, may be found in tissues involved by Hodgkin lymphoma. Identification of Reed-Sternberg cells, which tend to be found in greatest numbers at the periphery of necrotic areas, confirms the diagnosis of Hodgkin lymphoma. The presence of a polymorphous inflammatory cell infiltrate, with eosinophils and plasma cells, is more common in Hodgkin lymphoma than in mycobacterial infection. Non-Hodgkin lymphomas may also occasionally be associated with granulomas, but this is much less common than in Hodgkin lymphoma.

Metastatic carcinoma can be associated with granulomas in lymph nodes. In cases of nasopharyngeal carcinoma metastatic to lymph nodes, there may be a marked necrotizing granulomatous lymphadenitis. The granulomatous inflammation may obscure the neoplastic population, and the carcinoma may be overlooked. Granulomas can also be found in lymph nodes draining carcinoma that are themselves free of metastases.

## BACILLE CALMETTE-GUÉRIN LYMPHADENITIS

Bacille Calmette-Guérin (BCG) vaccine has been administered in some developing countries to decrease the risk of developing tuberculosis. Ipsilateral regional lymphadenopathy occurs after BCG vaccination in approximately 1% of individuals who are vaccinated. Other infrequent complications of BCG vaccination include abscesses at the vaccination site and inflammation involving bones, joints, and other sites.

Lymphadenopathy usually develops within 6 months of vaccination. Immunocompetent patients have nontender lymphadenopathy without constitutional symptoms. There are two pathologic forms: suppurative and nonsuppurative. Pathologic examination reveals epithelioid granulomas with or without necrosis. Acid-fast bacilli are present in small numbers. The nonsuppurative form usually resolves spontaneously. The suppurative form is accompanied by erythema and edema of overlying skin and sometimes by ulceration and sinus tract formation. Drainage by needle aspiration can help to prevent sinus tracts and to expedite healing.

Vaccination with BCG is contraindicated in patients who are immunodeficient. When BCG is administered to individuals with an underlying immunodeficiency, such as severe combined immunodeficiency, chronic granulomatous disease, or HIV infection, they are at risk of the development of disseminated BCG infection with generalized lymphadenopathy. Depending on the type of immunodeficiency, there may be epithelioid granulomas with few acid-fast bacilli or diffuse infiltrates of histiocytes laden with numerous acid-fast bacilli. This serious complication of the vaccination may be fatal and requires antituberculous therapy and treatment of the immunodeficiency.

## WHIPPLE DISEASE

### Clinical Features

Whipple disease predominantly affects middle-aged to older white men and is commonly characterized by diarrhea, weight loss, abdominal pain, and arthritis. Peripheral lymphadenopathy is common; intraabdominal and mediastinal nodes may also be enlarged. Skin may become hyperpigmented. The disease can also affect the heart and lungs, with inflammation or fibrosis of endocardium, myocardium, or pericardium; thickened, distorted cardiac valves; chronic cough; or pleuritic pain. Whipple disease can produce a wide variety of manifestations among patients with central nervous system

**BACILLE CALMETTE-GUÉRIN LYMPHADENITIS—FACT SHEET**

**Definition**
- Ipsilateral regional lymphadenopathy occurring after BCG vaccination

**Incidence**
- Approximately 1% of vaccinated patients

**Risk Factors**
- Immunodeficiency predisposes to severe BCG infection

**Clinical Features**
- Immunocompetent individuals develop lymphadenopathy that is usually not tender within 6 months of vaccination
- Immunodeficient individuals may develop generalized lymphadenopathy and disseminated BCG infection

**Morphology**
- Lymph nodes show granulomas with or without necrosis.
- When present, necrosis is usually suppurative
- Immunodeficient patients have histiocytic infiltrates with or without granuloma formation

**Prognosis and Therapy**
- Nonsuppurative lymphadenitis usually resolves spontaneously
- Drainage of suppurative lymphadenitis can help to expedite healing and prevent development of sinus tracts
- Immunodeficient individuals require prompt treatment with antibiotics

involvement. Ocular involvement, almost always found in patients who also have central nervous system disease, can result in retinitis or uveitis and loss of vision. Although symptoms related to gastrointestinal involvement are generally the most prominent, some patients have exhibited lymphadenopathy without diarrhea or malabsorption. In some of these unusual cases, the diagnosis was delayed by years or was made only at autopsy.

Whipple disease usually responds to antibiotics, but the treatment duration must be long to prevent relapse. The antibiotics should cross the blood-brain barrier to treat any central nervous system disease and to prevent relapse in the central nervous system, which is associated with a high mortality rate.

Electron microscopy reveals that the PAS-positive histiocytes are filled with intact and degenerated bacilli. The Whipple bacilli are bacteria with an unusual trilaminar membrane and a cell wall similar to that of gram-positive bacteria.

PCR has been used on fluids, such as cerebrospinal fluid and blood, and on biopsy specimens to detect the presence of Whipple bacilli. However, *Tropheryma whipplei* DNA has reportedly been found by PCR in healthy individuals; therefore PCR should not be used in isolation to establish a diagnosis of Whipple disease. PCR is useful in confirming a diagnosis in patients with clinical or pathologic features suggestive of Whipple disease. PCR may also have a role in monitoring the efficacy of therapy.

## Pathologic Features

In lymph nodes, the distinctive finding is the presence of lipogranulomas with clusters of foamy histiocytes and epithelioid cells and sinuses distended by cystic appearing, lipid-filled spaces surrounded by foreign body giant cells. The histiocytes have granular, PAS-positive, diastase-resistant cytoplasm. However, in some cases, especially when peripheral lymph nodes have been examined, nonnecrotizing granulomas are identified and are similar to those seen in sarcoidosis. In such cases, the PAS stain may be negative or only focally positive, and bacilli may be rare or absent.

## Differential Diagnosis

Based on the pathologic features, Whipple disease has a wide differential diagnosis. Clinical features are of great use in narrowing the differential. Lymph nodes in Whipple disease with a lipogranulomatous appearance can resemble lymph nodes in the portal circulation with lipogranulomas related to dietary lipid or bile metabolites, or both. Correlation with the anatomic site is helpful. Sarcoidosis, Crohn's disease, and mycobacterial infections are included in the differential diagnosis when nonnecrotizing granulomas are found. Special stains for acid-fast bacilli and culture are useful in

### WHIPPLE DISEASE—FACT SHEET

**Definition**
- An infectious disease caused by *Tropheryma whipplei*
- Synonym: intestinal lipodystrophy (older nomenclature)

**Incidence**
- Rare; estimated annual incidence of less than 1 per 1 million persons

**Gender, Race, and Age Distribution**
- Male predominance (male-to-female ratio: 5:1 to 8:1)
- Age ranges from childhood to old age; mean age at diagnosis is approximately 50 years
- Most patients are white

**Risk Factors**
- Possible increased risk for farmers
- Transmission from person to person has not been described

**Clinical Features**
- Symptoms are variable
- Lymphadenopathy (peripheral, internal, or both) is found in 50% of cases
- Weight loss, malabsorption, diarrhea, abdominal pain, arthralgias, anemia, cutaneous hyperpigmentation, and fever occur in 50% or more of patients
- Cardiac disease (endocarditis, pericarditis, myocardial fibrosis, valvular abnormalities), chronic cough, central nervous system manifestations (cognitive changes, motor and sensory deficits, altered consciousness, hypothalamic dysfunction, and visual changes) are found in 50% or less of patients

**Morphology**
- Lymph nodes are often enlarged and may show lipogranulomas, with many histiocytes with PAS-positive cytoplasm, or may show sarcoidal granulomas

**Diagnosis**
- Biopsy of affected tissue, especially small intestine for light microscopy, including PAS stain, and for electron microscopy
- PCR on tissue or fluid, such as blood or cerebrospinal fluid

**Prognosis and Therapy**
- Chronic disease is slowly progressive; if untreated, it can be fatal
- Treatment consists of long-term administration of antibiotics, preferably with the ability to cross the blood-brain barrier

excluding a mycobacterial infection. Intestinal biopsies are helpful in establishing a diagnosis in cases of Crohn's disease. PAS staining and electron microscopy may be helpful in differentiating between Whipple disease and sarcoidosis, but the findings are indistinguishable from those of sarcoidosis in some lymph node biopsy specimens in Whipple disease. *M. avium-intracellulare* infection and lepromatous leprosy can be associated with prominent infiltrates of histiocytes with finely granular or vacuolated cytoplasm and can enter the differential diagnosis of Whipple disease. Special stains for microorganisms establish a diagnosis. If clinical features suggest the possibility of Whipple disease, obtaining additional tissue, especially from the small bowel, should be strongly considered to establish a diagnosis.

## CHRONIC GRANULOMATOUS DISEASE

Chronic granulomatous disease is a genetically heterogeneous disorder characterized by impaired killing of phagocytosed microorganisms, leading to recurrent infections beginning in early childhood. Phagocytes are able to ingest microbes normally, but microbicidal activity is reduced because of abnormal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme complex found in granulocytes, monocytes, and macrophages that normally generates the superoxide ions and hydrogen peroxide that are toxic to microbes. NADPH oxidase has membrane-bound components (gp91-phox, p22-phox, and rap1a, together composing cytochrome b558) and cytosolic components (p47-phox, p40-phox, p67-phox, Rac2). Defects in the gp91-phox of cytochrome b558 account for the majority of cases; these have X-linked inheritance. A defect with autosomal recessive inheritance accounts for the remaining cases. Among autosomally recessively inherited defects, an abnormal p47-phox is most common; abnormal p67-phox and abnormal p22-phox each account for a small proportion of cases. The severity of the disease is dependent on the underlying genetic defect; X-linked inheritance with absence of cytochrome b558 expression is associated with more severe disease than other defects.

### Clinical Features

Lymphadenopathy is common in chronic granulomatous disease and is the most common manifestation of chronic granulomatous disease in some series. Prophylactic use of antibiotics can provide a significant decrease in number of bacterial infections; prophylaxis for fungal infections and interferon-γ are administered to some patients. Bone marrow transplantation and gene therapy have also been proposed as possible modes of therapy. The most common cause of death is infection by *Aspergillus* species. Mortality is estimated to be 2% to 5% per year.

### Differential Diagnosis

Based on morphology in an involved lymph node (Figure 4-8), the differential diagnosis includes a variety of

**CHRONIC GRANULOMATOUS DISEASE—FACT SHEET**

**Definition**
- A disorder characterized by leukocytes with impaired intracellular killing of microorganisms after phagocytosis, leading to recurrent, severe infections by bacteria and less often by fungi

**Frequency**
- Prevalence is approximately 1 in 1.2 million individuals
- Incidence is approximately 1 in 250,000 live births
- Chronic granulomatous disease accounts for less than 10% of primary immunodeficiencies, but is the most common inherited disorder causing phagocytic dysfunction

**Gender, Race, and Age Distribution**
- Age at onset is usually younger than 1 year
- Boys are affected more often than girls (male-to-female ratio: 2:1 to 4:1)
- There is no known racial predisposition

**Risk Factors**
- Consanguinity, for the autosomal recessive form

**Clinical Features**
- Recurrent suppurative infections, with lymphadenitis, pulmonary infection, upper respiratory infection, cutaneous abscesses, gastrointestinal infections, hepatic abscesses, sepsis, and osteomyelitis caused by bacteria, most often *S. aureus*, *Klebsiella* species, *Salmonella* species, *Burkholderia* species, or *M. tuberculosis*, or caused by fungi such as *Aspergillus* or *Candida* species
- Noninfectious manifestations: poor wound healing, overly exuberant inflammatory reactions, autoimmune-type phenomena

**Morphology**
- Microscopic examination reveals necrotizing granulomas with suppurative necrosis and abscesses (see Figure 4-8)

**Diagnosis**
- Nitroblue tetrazolium reduction test and others

**Prognosis and Therapy**
- Treatment consists of antibiotics to treat established infections
- Antibiotics are often also given prophylactically to prevent infection
- Prognosis has improved over time
- Median survival varies in different series, but many patients reach young adulthood or middle age
- Prognosis is worse with complete absence of cytochrome $b_{558}$

other types of necrotizing lymphadenitides, including cat-scratch disease, atypical mycobacteria, brucellosis, and fungal infection. The lack of specificity of the pathologic findings and the rarity of the disease often result in delay, sometimes for years, in establishing a diagnosis. Chronic granulomatous disease is distinctive in that there are recurrent, often severe, pyogenic infections usually beginning in the first year of life and almost always present by the age of 2 years.

Diagnosis can be established using the traditional nitroblue tetrazolium reduction test, with chemiluminescent or fluorescent assays of oxidase activity, or with

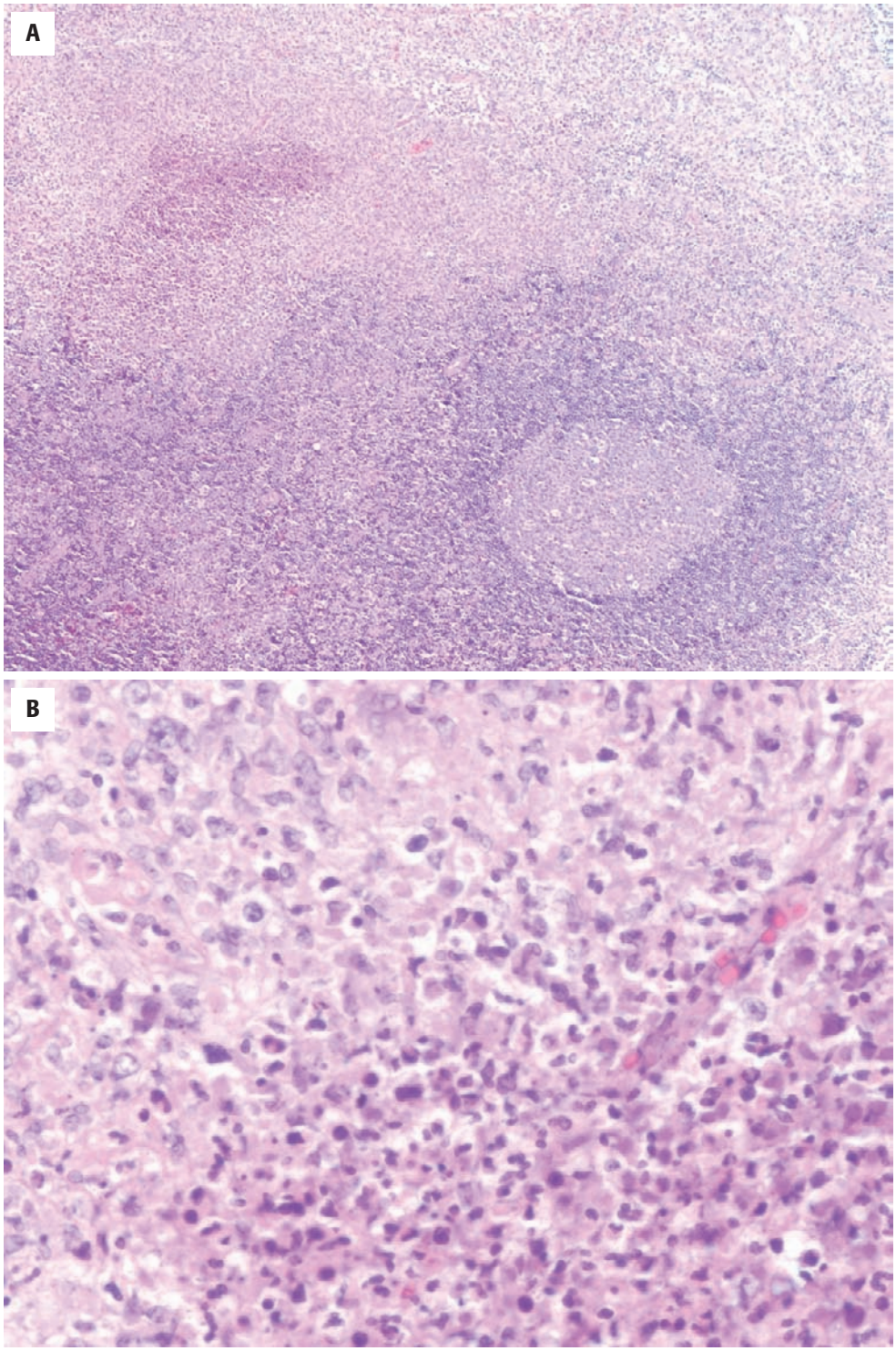


**FIGURE 4-8** Chronic granulomatous disease. **A,** This lymph node shows necrotizing granulomatous inflammation adjacent to a reactive follicle. **B,** Higher power shows palisading histiocytes surrounding suppurative necrosis.

assays that detect generation of peroxide. When the specific genetic defect is known, a prenatal diagnosis can be made.

## FUNGAL LYMPHADENITIS

Fungal lymphadenitis is uncommon. Patients with fungal lymphadenitis are often immunosuppressed because of HIV infection, malignancy (most often hematolymphoid neoplasia), or iatrogenic immunosuppression; occasionally patients with normal immunologic function are affected. Lymphadenitis can be caused by a variety of fungi, but *Histoplasma capsulatum* and *Cryptococcus neoformans* are among the most common. *Penicillium marneffei* is an uncommon cause of opportunistic infection, but when it does occur it is often associated with lymphadenopathy. Symptomatic fungal lymphadenitis is uncommonly an isolated finding; patients often have involvement of the lungs, liver, spleen, bone marrow, or other sites. Patients with normal immunologic function who develop fungal pulmonary infection may also have hilar lymph node involvement. Lymphadenitis in this setting may be asymptomatic; however, histoplasmosis involving mediastinal nodes can be associated with inflammation and severe scarring of the surrounding tissue, producing sclerosing mediastinitis.

Infection is typically associated with granulomatous inflammation, which may be necrotizing. In immunodeficient individuals, granulomas may be poorly formed or absent. Fibrosis and even calcification may develop in old lesions, especially in cases of histoplasmosis. The differential diagnosis is similar to that of *M. tuberculosis* and atypical mycobacteria. During institution of highly active antiretroviral therapy in HIV-positive patients harboring fungi, symptomatic fungal lymphadenitis may emerge as an immune reaction to the fungi is mounted.

## PROTOZOAL LYMPHADENITIS

### TOXOPLASMOSIS

#### Clinical Features

Although lymphadenitis caused by other types of protozoal infection has been reported, infection by *Toxoplasma gondii* is the only one encountered with any frequency in the United States. Toxoplasmosis is a common cause of lymphadenopathy. This type of lymphadenopathy was first described by Piringer-Kuchinka in 1952 and is sometimes called *Piringer-Kuchinka lymphadenitis*. The five modes of presentation of toxoplasmosis are lymphadenopathy, encephalitis, chorioretinitis, disseminated infection, and congenital infection. The most frequent clinical manifestation of *T. gondii* infection is cervical lymphadenopathy. Transmission to humans is thought to be through exposure to cat feces containing oocysts or exposure to uncooked meat. Individuals with toxoplasma lymphadenitis are usually children or young adults with unilateral or bilateral cervical lymphadenopathy. They may have malaise or they may be asymptomatic. An atypical lymphocytosis may be seen. The disease is typically self-limited. Rarely, immunocompetent patients develop serious complications, such as myocarditis or encephalitis, which may be fatal. Immunodeficient patients may develop lymphadenopathy, but they are at high risk of severe

**TOXOPLASMOSIS—FACT SHEET**

**Definition**
- Infectious disease due to *T. gondii*

**Incidence and Anatomic Location**
- Relatively common
- *Toxoplasma* lymphadenitis is the most common manifestation of toxoplasmosis

**Gender, Race, or Age Distribution**
- Most patients are children and young adults; toxoplasma lymphadenitis is more common in females than males

**Risk Factors**
- Immunodeficient patients are at risk of more severe manifestations of toxoplasmosis

**Clinical Findings**
- Patients with *Toxoplasma* lymphadenitis typically have unilateral or bilateral cervical lymphadenopathy, especially in the posterior cervical area; they may have malaise or be asymptomatic
- Other forms of toxoplasmosis include encephalitis, chorioretinitis, disseminated disease, and congenital infection

**Morphology**
- Lymph nodes are slightly to moderately enlarged and show florid follicular hyperplasia, monocytoid B cell hyperplasia, and clusters of epithelioid histiocytes surrounding and encroaching on follicle centers
- The organisms are not usually identifiable in lymph nodes

**Diagnosis**
- Lymph node biopsy specimen shows characteristic changes
- Serologic studies confirm the diagnosis

**Prognosis and Therapy**
- Patients with *Toxoplasma* lymphadenitis typically have a self-limited illness and need no specific therapy
- Adults with more severe manifestations may respond to drug therapy
- Congenital infection can cause irreversible damage

manifestations, especially encephalitis. Infection during pregnancy may be associated with fetal morbidity or mortality.

### Pathologic Features

Lymph nodes show (1) florid follicular hyperplasia; (2) prominent bands of parasinusoidal and parafollicular monocytoid B cells; and (3) many small, irregular parafollicular clusters of histiocytes, some of which encroach on germinal centers (Figure 4-9). The microorganisms are identified in lymph nodes only rarely as parasitic cysts, although finding *Toxoplasma* species DNA using the PCR reaction may be helpful in establishing a diagnosis. The diagnosis can be confirmed using serologic studies; in 95% of cases with the triad of histologic findings described, results of serologic testing demonstrate *T. gondii* infection.

### Differential Diagnosis

The differential diagnosis of toxoplasma lymphadenitis includes other types of lymphadenitis associated with follicular hyperplasia or clusters of histiocytes, or both. Early or partial nodal involvement by cat-scratch disease, sarcoidosis, mycobacteria, or primary or secondary syphilis can result in an appearance that resembles toxoplasmic lymphadenitis, except that in none of these disorders is there a tendency for epithelioid histiocytes to encroach on lymphoid follicles. Significant areas of necrosis and true granuloma formation are unusual in toxoplasmosis and should lead to consideration of other infections. *Leishmania* species infection can produce a lymphadenitis closely resembling that of *Toxoplasma* species infection; it can also be associated with a necrotizing or nonnecrotizing granulomatous lymphadenitis. With *Leishmania* species infection, however, microorganisms are usually identifiable in epithelioid histiocytes (see the following section).

## LEISHMANIAL LYMPHADENITIS

Leishmaniasis is a protozoal infection that is transmitted by sandflies (*Phlebotomus* species). Leishmania species can infect lymph nodes in two settings: (1) localized lymphadenopathy, often draining a focus of cutaneous infection; and (2) widespread lymphadenopathy in the setting of visceral leishmaniasis (kala-azar).

The localized lymphadenitis associated with cutaneous leishmaniasis can show follicular and paracortical hyperplasia with necrotizing or nonnecrotizing granulomatous inflammation, sometimes with numerous plasma cells. The necrosis may be suppurative or caseating. The granulomas may resemble those seen in tuberculosis or can mimic the granulomas of cat-scratch disease. In visceral leishmaniasis, there is marked increase in histiocytes throughout the reticuloendothelial system. Organisms reside in histiocytes, but follicular hyperplasia and paracortical hyperplasia may not be prominent, and granulomas are typically absent.

Leishmaniasis is typically seen in the Middle East; in parts of Africa, South America, and Asia; and in Central America. Information about the patient's origin or travel history gives a clue to the diagnosis. The organisms can be seen on routinely stained sections, on Giemsa stain, and on reticulum stains, although in some cases they are present in only small numbers. In human tissue, the microorganisms are found mainly within histiocytes, but they are sometimes found in the extracellular space, and their presence in neutrophils has been reported. The organisms multiply within histiocytes. Individual organisms are 1.5 to 3 μm in diameter, round to oval in shape with a delicate cellular membrane, a nucleus, and a rod-shaped kinetoplast. In some instances the diagnosis can be established or suggested based on a fine needle aspiration biopsy, so that some patients might not require a more invasive procedure to establish the diagnosis.

## PNEUMOCYSTIS CARINII LYMPHADENITIS

See Fact Sheet.

## ■ FILARIAL LYMPHADENITIS

Among helminthic infections, only the filarial worms involve lymph nodes and lymphatics with any frequency. See Fact Sheet.

## ■ LYMPHOID HYPERPLASIA AND LYMPHADENITIS IN IMMUNOLOGIC DISORDERS

## RHEUMATOID ARTHRITIS

### Clinical Features

Rheumatoid arthritis is a chronic systemic illness characterized by a destructive arthritis and a variety of extraarticular manifestations, including hematologic, cardiovascular, neurologic, and pulmonary abnormalities. As many as approximately 75% of patients develop lymphadenopathy because of reactive hyperplasia during periods of active disease.

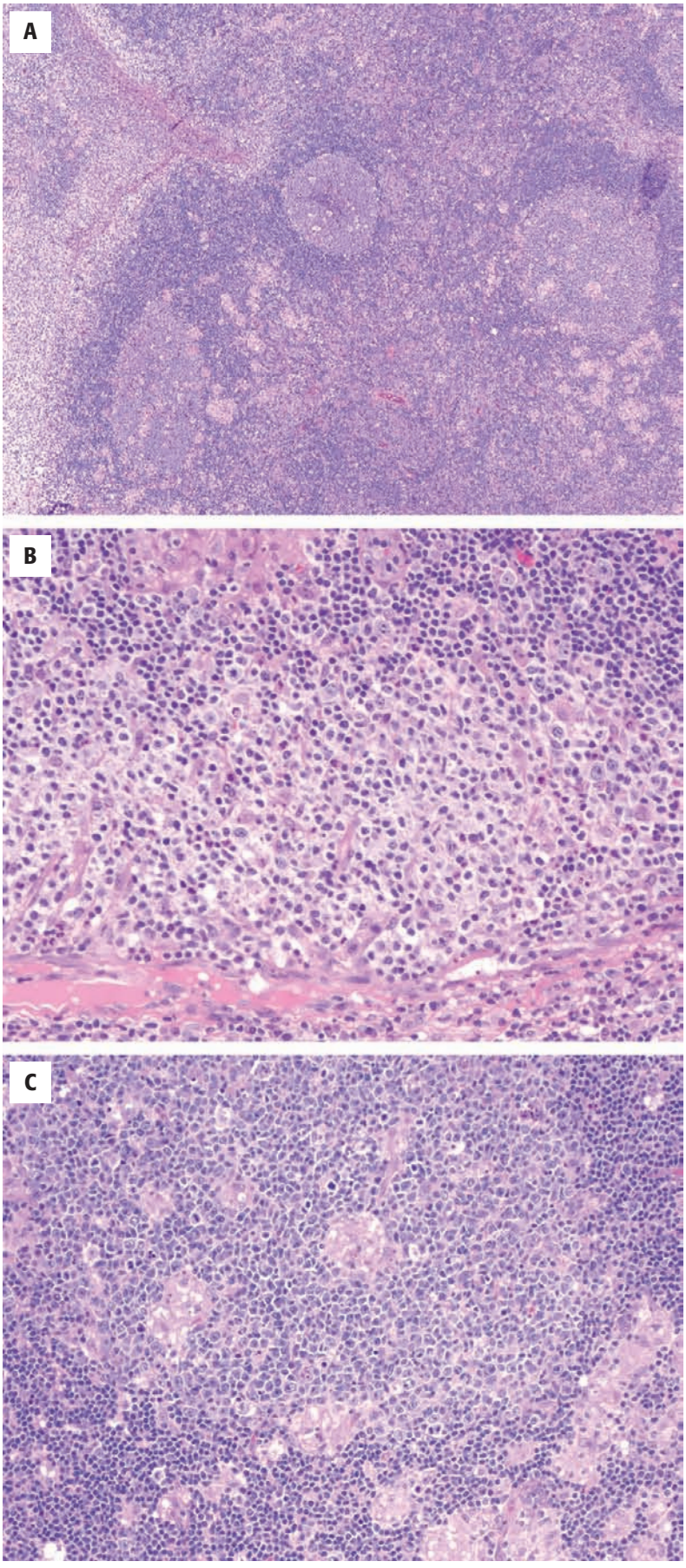


**FIGURE 4-9**

Toxoplasma lymphadenitis. **A,** Low power shows a lymph node with follicular and monocytoid B cell hyperplasia. **B,** A prominent band of monocytoid B cells with abundant clear cytoplasm. **C,** Small clusters of epithelioid histiocytes surround and invade a reactive follicle.

## *PNEUMOCYSTIS CARINII* LYMPHADENITIS—FACT SHEET

### Incidence and Anatomic Location

- Incidence is rare
- Less than 1% of all cases of pneumocystosis affect extrapulmonary sites
- Among extrapulmonary sites, lymph nodes are the site most often affected

### Gender, Race, and Age Distribution

- Patients include HIV-positive individuals with male preponderance, children with congenital immunodeficiency, organ transplant recipients, and patients with malignancy
- There is no known racial predisposition

### Risk Factors

- Treatment with aerosolized pentamidine, which prevents *P. carinii* pneumonia but not extrapulmonary infections

### Clinical Features

- *P. carinii* lymphadenitis usually occurs in association with *P. carinii* pneumonia or in the setting of widespread extrapulmonary infection

### Morphology

- Tissue shows frothy material with scattered helmet-shaped organisms that can be detected with a Gomori methenamine silver stain

### Diagnosis

- Can be established with biopsy of affected tissue, although made at autopsy in many cases

### Prognosis and Therapy

- Treatment is with appropriate antibiotics
- Without therapy, mortality is high

## FILARIAL LYMPHADENITIS—FACT SHEET

### Definition

- Lymphadenitis due to infection by a filarial worm, usually *Wuchereria bancrofti* or *Brugia malayi*

### Incidence

- Overall, incidence is common but with a restricted geographic distribution; 120 million individuals are affected worldwide
- *W. bancrofti* affects patients in equatorial Africa, India, the Mediterranean coast, the Caribbean, coastal areas of South America and parts of Central America; the infection is referred to as *Bancroftian filariasis*
- *B. malayi* affects patients in coastal areas of Southeast Asia and India; the infection is referred to as *Malayan filariasis*
- Incidence is rare in the United States, although zoonotic *Brugia* species may rarely cause human infection outside endemic areas

### Age, Gender, Racial Distribution

- None known

### Risk Factors

- Risk factor is living in or traveling to endemic areas; the vector is the mosquito

### Clinical Features

- Severity of manifestations varies greatly from asymptomatic or mildly symptomatic infections to severe infections with lymphedema, lymphadenopathy, and elephantiasis of extremities because of lymphatic obstruction

### Morphology

- Adult worms are found in lymphatics and in lymph node sinuses. They have a thin, finely striated cuticle and a layer of somatic muscle. Cross section of the midbody in females reveals an intestine and two uteri. Brugia species have smaller maximum midbody diameters (females, approximately 160 μm; males, approximately 90 μm) than do *Wuchereria* species (females, 250 μm; males, 150 μm). Dead worms may undergo dystrophic calcification
- Lymph nodes show changes such as prominent follicular hyperplasia, monocytoid B cell hyperplasia, and a mixed infiltrate of lymphocytes, plasma cells, eosinophils, and neutrophils. Dead worms may elicit a striking infiltrate of eosinophils, sometimes with eosinophilic microabscesses and granuloma formation. Capsular and intranodal fibrosis may be prominent

### Treatment and Prognosis

- Infections by *W. bancrofti* and *B. malayi* require antihelminthic therapy. Prognosis is good with prompt diagnosis and treatment. Zoonotic infections can be cured by excision of the infected lymph node, because often infection consists of the presence of a single adult worm

**RHEUMATOID ARTHRITIS—FACT SHEET**

**Definition**
- Rheumatoid arthritis is a chronic, systemic autoimmune disease causing a destructive arthritis and a variety of extraarticular manifestations, including lymphadenopathy

**Incidence**
- Common

**Gender, Race, or Age Distribution**
- Rheumatoid arthritis affects women three times as often as men
- Nearly all cases appear in adults, from the third to the seventh decades of life

**Risk Factors**
- Possible genetic predisposition

**Clinical Features**
- Lymphadenopathy occurs in approximately 75% of patients and is especially common during periods of active disease
- Lymphadenopathy may be localized or generalized. When localized it is most frequent in the axillary area, because these nodes receive lymphatic drainage from inflamed joints of the hand

**Morphology**
- Lymph nodes show florid follicular hyperplasia, sinus histiocytosis, and plasmacytosis and may show areas of hyalinization, sinus neutrophils, and deposition of iron
- Patients with joint replacement may have lymphadenopathy showing a reaction to foreign material if the prosthesis deteriorates
- Patients receiving gold therapy may develop lymphadenopathy showing a foreign body reaction to gold

**Diagnosis**
- Diagnosis is usually established based on clinical and laboratory abnormalities

**Prognosis and Therapy**
- The lymphadenopathy itself does not require therapy
- The goal of therapy is to decrease inflammation and minimize joint damage
- Rheumatoid arthritis is a chronic, slowly progressive disease that can eventually result in significant morbidity

### Pathologic Features

Reactive hyperplasia in rheumatoid arthritis typically shows prominent follicular hyperplasia. The follicles are present in the cortex and also frequently extend into the medulla. Infiltration of the capsule and perinodal fat by lymphocytes is common. Plasmacytosis of the interfollicular region and medulla is often conspicuous; Russell bodies may be found. The interfollicular area may also show admixed small lymphocytes, immunoblasts, and neutrophils. Sinuses may be compressed by the follicular and interfollicular expansion, but patent and even dilated sinuses can be found. Sinuses show sinus histiocytosis, sometimes including erythrophagocytic histiocytes, neutrophils, and stainable iron (Figure 4-10). Lymph nodes may have areas of hyaline material, possibly because of long-standing nonspecific inflammation. Amyloid rarely is deposited in lymph nodes. Lymphadenopathy associated with gold therapy has been described; in such cases, gold is present in the form of crystalline material and is associated with a multinucleated giant cell reaction. Rarely, lymph nodal infarction has been attributed to gold therapy. Patients with Silastic joint prostheses can develop silicone lymphadenopathy. Patients with lymphadenopathy associated with juvenile rheumatoid arthritis can have reactive hyperplasia similar to that seen with rheumatoid arthritis.

### Differential Diagnosis

The differential diagnosis of rheumatoid arthritis includes follicular lymphoma and other reactive conditions. The extensive follicular proliferation characteristic of rheumatoid arthritis lymph nodes may raise the question of follicular lymphoma. Criteria conventionally used to distinguish follicular lymphoma and follicular hyperplasia can be used in this setting. In the differential diagnosis with other causes of reactive hyperplasia, clinical correlation is required to confirm a diagnosis of lymphadenopathy resulting from rheumatoid arthritis. Other causes of lymphadenopathy that can mimic changes seen in rheumatoid arthritis include syphilis, HIV infection, plasma cell Castleman disease, and nonspecific follicular hyperplasia.

## SYSTEMIC LUPUS ERYTHEMATOSUS

### Clinical Features

Systemic lupus erythematosus is an autoimmune disorder that predominantly affects adolescents and young adults, with a female preponderance. It can occur at any age, and males are occasionally affected. It is characterized by a broad spectrum of clinical manifestations, including arthritis or arthralgias, fever, rash, renal disease, anorexia, nausea, vomiting, serositis, and neurologic manifestations. As many as two thirds of patients with active disease develop lymphadenopathy (lupus lymphadenitis), the most common site of which is the cervical area (43% of cases), although generalized lymphadenopathy is also relatively common. Patients with lymphadenopathy tend to be slightly younger

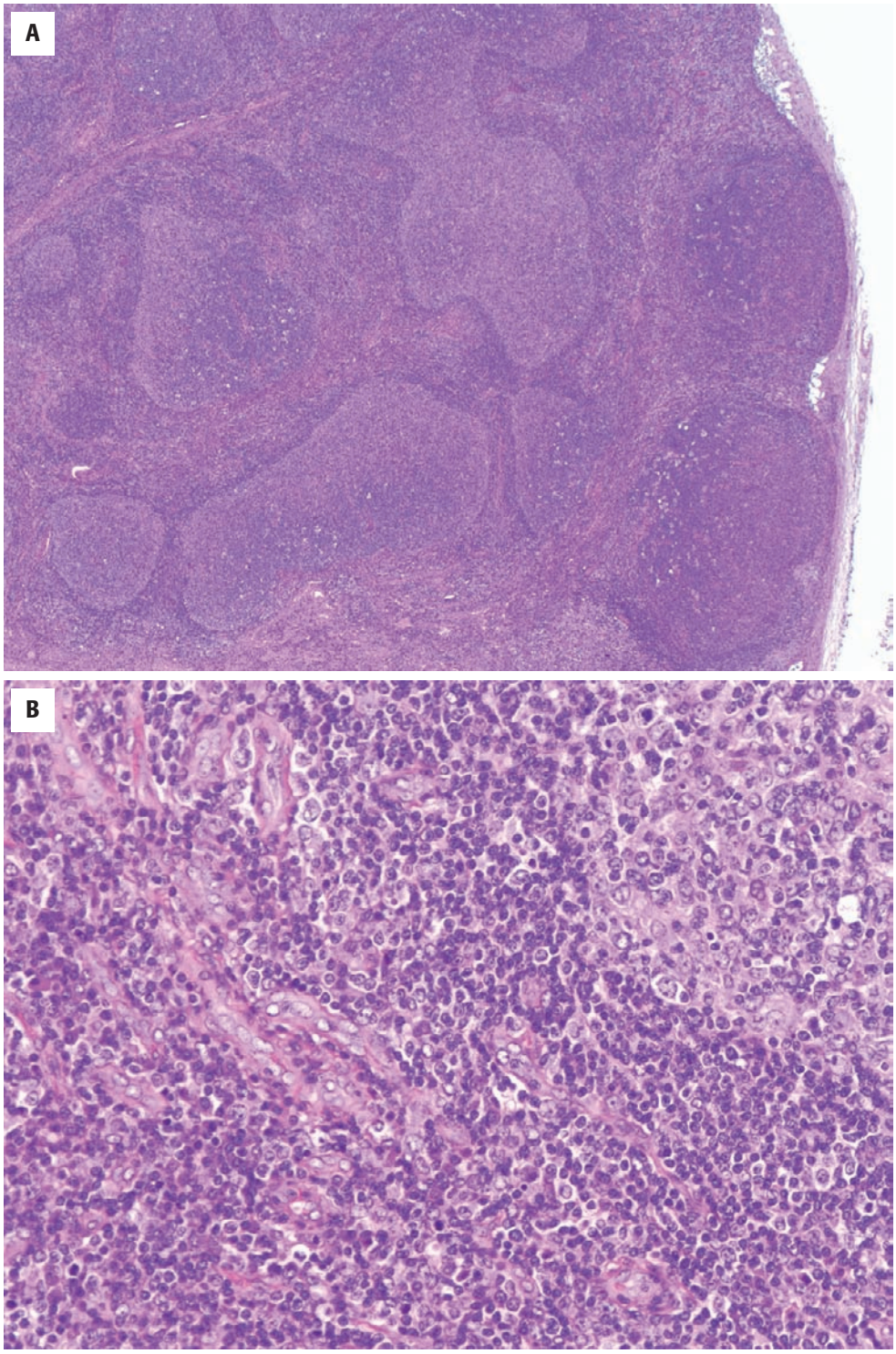


**FIGURE 4-10**

Rheumatoid arthritis–associated lymphadenopathy. **A,** Low power shows florid follicular hyperplasia, with large, sometimes irregularly shaped follicles. **B,** The interfollicular area shows numerous plasma cells.

(mean, 36 years with adenopathy versus 45 years without adenopathy) and to have more complaints of fatigue, fever, and weight loss; more cutaneous abnormalities; more frequent hepatomegaly and splenomegaly; and higher titers of anti-dsDNA antibodies than do those without lymphadenopathy.

## Pathologic Features

Lymph nodes in lupus lymphadenitis can be edematous with areas of hemorrhage. The lymph node architecture may be distorted, but it is not obliterated. The

**SYSTEMIC LUPUS ERYTHEMATOSUS—FACT SHEET**

**Definition**
- A systemic autoimmune disease characterized by a wide variety of clinical findings and laboratory abnormalities, such as autoantibodies, especially antinuclear antibodies

**Incidence**
- Relatively common

**Gender, Race, and Age Distribution**
- This disease mainly affects females (male-to-female ratio, approximately 1 : 10) and usually manifests during adolescence or young adulthood
- It is more frequent among blacks and Asians than among whites

**Risk Factors**
- Genetic susceptibility is well described
- Hormonal changes might have a role, and a role for certain environmental factors has been suggested in the development of lupus

**Clinical Features**
- Patients have manifestations that vary from case to case
- Abnormalities include fever, rash, anorexia, renal disease, serositis, neurologic findings, arthralgias, and positive antinuclear antibodies
- The majority of patients have lymphadenopathy at some time during the course of their disease
- Lymphadenopathy is most often cervical, but it can affect any site and can be generalized

**Morphology**
- Lymph nodes may show edema, hemorrhage, and paracortical areas of necrosis with amorphous pink debris surrounded by histiocytes and immunoblasts, but without granulomas
- Occasional cases show hematoxylin bodies or the Azzopardi phenomenon, features that are nearly pathognomonic of lupus
- Some cases show follicular hyperplasia with unusual features that could potentially mimic follicular lymphoma

**Diagnosis**
- Although the pathologic findings are distinctive, diagnosis is usually established mainly based on clinical findings and laboratory abnormalities

**Prognosis and Therapy**
- Treatment focuses on controlling inflammation and minimizing tissue damage
- The course is chronic, often remitting and relapsing, and the disease can result in shortening of the normal life span

paracortex often contains foci of necrosis, with a central zone of amorphous pink material. In early lesions, there may be little or no cellular reaction around the necrotic areas. In older lesions, large numbers of histiocytes, with admixed small and large lymphoid cells, surround the necrosis. Immunoblasts may form a prominent component of the infiltrate. Granulocytes are sparse or absent. Plasma cells are usually infrequent but are found in large numbers in occasional cases. Hematoxylin bodies, ill-defined violet structures thought to represent degenerated nuclei that have reacted with antinuclear antibodies, are sometimes found in areas of necrosis; they are virtually pathognomonic of lupus (Figure 4-11). Blood vessels in the necrotic foci may show the Azzopardi phenomenon, in which dark blue nuclear material is deposited on the basement membrane of blood vessels. Periarterial and periarteriolar fibrosis may also be seen. In some cases, lymph nodes lack necrosis and instead show mainly follicular hyperplasia, sometimes with unusual features. Rare patients with systemic lupus erythematosus have lymph nodes with the appearance of Castleman disease.

### Differential Diagnosis

Because there are numerous large lymphoid cells in some cases of lupus lymphadenitis, a diagnosis of non-Hodgkin lymphoma might be suggested. In favor of a diagnosis of lupus lymphadenitis are preservation of nodal architecture, characteristic necrotic areas, admixed histiocytes, and hematoxylin bodies. In difficult cases, immunophenotyping may be helpful in excluding lymphoma. Because of the extensive necrosis often found in lupus lymphadenitis, an infectious process may enter the differential diagnosis; however, neutrophils are sparse to absent in lupus and abundant in many different types of infectious lymphadenitides. The necrotic material in lupus lymphadenitis can resemble caseous necrosis, raising the possibility of tuberculous lymphadenitis, but epithelioid histiocytes, Langhans-type giant cells, and granulomas are not features of lupus lymphadenitis.

## KIKUCHI DISEASE

The cause of Kikuchi disease is uncertain, but in many cases the disease may represent a reaction to an infectious process and thus could be included in other sections of this chapter. It is included in the section on immunologic disorders because it is an important entity in the differential diagnosis of lupus lymphadenitis.

### Clinical Features

Kikuchi disease is also known as *histiocytic necrotizing lymphadenitis, Kikuchi lymphadenitis, Kikuchi-Fujimoto disease,* and *subacute necrotizing lymphadenitis*. It seems

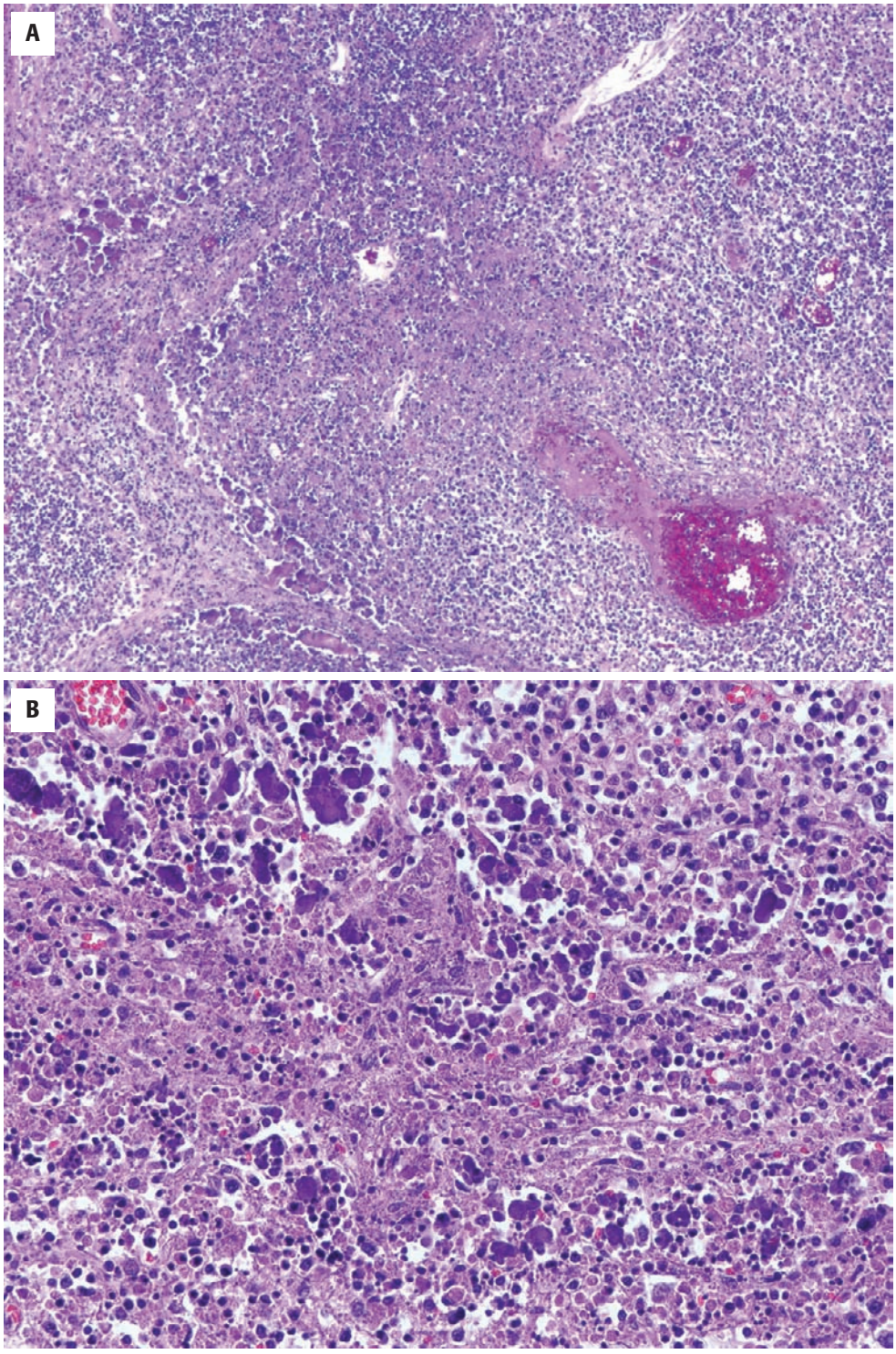


**FIGURE 4-11**

Systemic lupus erythematosus–associated lymphadenitis. The patient died of systemic lupus erythematosus, and the lymph node illustrated was obtained at autopsy. **A,** Low power shows extensive necrosis and vascular thrombosis. **B,** Higher power shows numerous violet-colored hematoxylin bodies.

to be more prevalent in Asian than Western populations. Most patients are young adults (mean age in the third decade), with a female preponderance in nearly all series. The typical presentation is with unilateral, frequently painful, cervical lymphadenopathy. Other lymph nodes are affected much less often. Infrequently, there is generalized lymphadenopathy or involvement of extranodal sites, the most common of which is the skin. Approximately half of all patients have fever at presentation, and some have flulike symptoms. Common peripheral blood abnormalities include anemia and neutropenia; an atypical lymphocytosis is found in 25% of

cases. Less than 5% of patients have a leukocytosis. Occasionally, patients have a rash. The disease is self-limited, and most patients recover without therapy. Less than 5% develop recurrent lymphadenopathy. In a few cases, usually in iatrogenically immunosuppressed organ transplant recipients or very young children, Kikuchi disease has resulted in widespread disease with multiorgan involvement and may be fatal.

A variety of viral and other types of infectious causes have been suggested to cause Kikuchi disease. The similarity of some the clinical and pathologic features of Kikuchi disease and systemic lupus erythematosus has raised the question of Kikuchi disease being a self-limited autoimmune disease. Some patients have developed Kikuchi disease in association with foreign material or other types of noninfectious conditions. It is possible that the histologic and immunohistologic features of Kikuchi disease can be produced by a variety of stimuli in susceptible individuals.

**KIKUCHI DISEASE—FACT SHEET**

**Definition**
- An illness of uncertain cause characterized by lymphadenopathy with distinctive histologic features

**Incidence**
- Uncommon

**Gender, Race, and Age Distribution**
- Most patients are young women, with a higher incidence among Asians

**Risk Factors**
- Rarely severe cases of Kikuchi disease occur in immunocompromised patients

**Clinical Features**
- Patients usually exhibit unilateral tender cervical lymphadenopathy
- Other common findings include fever, rash, anemia, and neutropenia
- Some patients have an atypical lymphocytosis

**Morphology**
- Lymph nodes show small or large foci of necrosis containing amorphous eosinophilic and apoptotic debris
- Necrotic areas are surrounded by numerous histiocytes and by variable numbers of immunoblasts
- Granulocytes and granulomas are absent. Plasma cells are sparse or absent

**Immunophenotype**
- Lymphoid cells are T cells, often with numerous cytotoxic ($CD8^+$) cells
- Histiocytes may express myeloperoxidase
- B cells are rare to absent in and around the necrotic foci

**Diagnosis**
- Lymph node biopsy

**Prognosis and Therapy**
- Nearly all patients have a self-limited illness requiring no specific therapy
- A few patients develop recurrent lymphadenopathy
- Rarely, usually with an underlying immunodeficiency, patients have a severe illness with a poor outcome
- A few patients thought to have Kikuchi disease later develop systemic lupus erythematosus; this may be coincidental, or the patient may have had lupus mimicking Kikuchi disease from the outset

## Pathologic Features

Lymph nodes show prominent paracortical hyperplasia with one or more round or irregular, discrete, or confluent eosinophilic areas in the cortex or paracortex containing histiocytes, lymphocytes, immunoblasts, plasmacytoid dendritic cells, and karyorrhectic and eosinophilic granular debris. The necrotic debris is most abundant in the centers of these areas, whereas immunoblasts are most numerous at the periphery. The histiocytes are of a variety of types, including phagocytic, nonphagocytic, and foamy histiocytes. Nonphagocytic histiocytes with eccentric sickle-shaped nuclei have been called *crescentic histiocytes*. Plasmacytoid dendritic cells are medium-sized cells with round nuclei, dispersed chromatin, small nucleoli, and a moderate amount of faintly amphophilic cytoplasm. In Kikuchi lymphadenitis, aggregates of plasmacytoid dendritic cells are frequently seen in nonnecrotizing areas; early foci of necrosis appear to begin within clusters of plasmacytoid dendritic cells. The necrosis is apoptotic rather than suppurative. Thrombosed vessels may be seen around necrotic foci. Epithelioid histiocytes, plasma cells, eosinophils, and neutrophils are virtually absent. The characteristic foci may be relatively small and confined to the paracortex or may occupy the majority of the lymph node. The infiltrate may extend beyond the capsule into perinodal soft tissue. Follicular hyperplasia may be seen, but it is not a constant or prominent feature.

Kikuchi disease can be subclassified into three histologic subtypes that may represent different stages in the evolution of the disease. In the proliferative type, microscopic examination reveals the mixture of cells described, with apoptosis but without coagulative necrosis (Figure 4-12). In the necrotizing type, lymph nodes show large areas of necrosis in addition to the changes characteristic of the proliferative type. In the xanthomatous type, there is a predominance of foamy histiocytes, and necrosis can be present or absent.

Immunohistochemical analysis confirms the presence of a mixture of lymphoid cells, histiocytes, and plasmacytoid dendritic cells. The lymphoid cells,

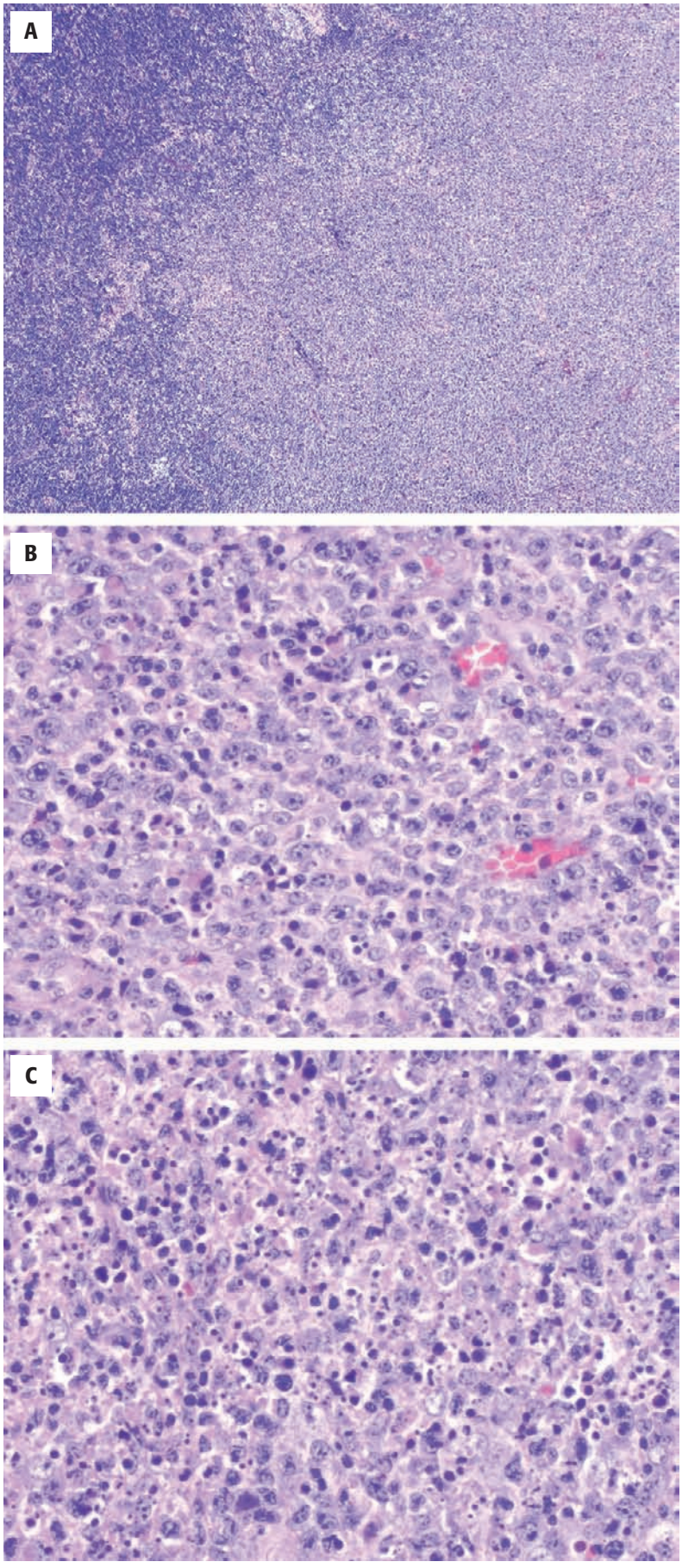


**FIGURE 4-12**

Kikuchi disease. **A,** Low power shows a rounded, slightly pale area within hyperplastic paracortex. **B,** High power in areas shows numerous immunoblasts, admixed with variable numbers of histiocytes and some apoptotic debris. **C,** In other areas, immunoblasts are less numerous, and apoptotic and eosinophilic cellular debris and histiocytes predominate.

including the immunoblasts, express pan–T cell antigens. There is a varying admixture of $CD4^+$ and $CD8^+$ T cells, but cytotoxic T cells are often abundant. $CD8^+$ T cells coexpress cytotoxic granule proteins, such as TIA-1 and perforin. $CD56^+$ natural killer cells are infrequent. The histiocytes are $lysozyme^+$ and $CD68^+$, and they are reported to coexpress myeloperoxidase, an unusual feature also reported in lupus lymphadenitis. The plasmacytoid dendritic cells express CD2, CD4, CD43, CD68, and CD123, but not CD3. Although B cells are found if reactive follicles are present, B cells are scarce in the areas of the node with histiocytes and cellular debris.

### Differential Diagnosis

The most important entity in the differential diagnosis is non-Hodgkin lymphoma. The distinction may be difficult when immunoblasts are abundant. Familiarity with the spectrum of changes found in Kikuchi disease and identification of areas with the characteristic polymorphous infiltrate are essential to rendering the correct diagnosis. In addition, Kikuchi disease more often shows partial nodal involvement than lymphoma. The vast majority of lymphomas in Western countries are B cell lymphomas. Only a minority of lymphomas in the United States are T cell lymphomas, but most of those arising in lymph nodes are $CD4^+$. The predominance of $CD8^+$ T cells in Kikuchi disease is an important clue in avoiding a misdiagnosis of lymphoma.

Because necrosis is a prominent component of Kikuchi disease, infectious lymphadenitis is often a consideration. However, neutrophils, suppurative necrosis, granulomas, and viral inclusions are characteristic of various types of infectious lymphadenitis, but are not found in Kikuchi disease. When crescentic histiocytes are prominent, metastatic carcinoma of the signet ring cell type can be considered in the differential diagnosis. However, histiocytes in Kikuchi disease lack nuclear atypicality, may contain cellular debris but not mucin, and express histiocyte-related antigens rather than keratins.

Lymphadenitis in patients with systemic lupus erythematosus can closely resemble, or even be indistinguishable from, the lymphadenitis of Kikuchi disease. Finding hematoxylin bodies, plasma cells, or deposition of nuclear material on blood vessels (the Azzopardi phenomenon) supports a diagnosis of lupus lymphadenitis over Kikuchi disease, but these features might not be found in every case of lupus lymphadenitis. Clinical features are sometimes helpful in making a definitive distinction. Because of the difficulty in definitively excluding lupus on microscopic examination and because a minority of patients diagnosed with Kikuchi disease have developed lupus later, clinicians may consider performing a workup for autoimmune disease in cases showing histologic features of Kikuchi disease.

## KIMURA DISEASE

### Clinical Features

Kimura disease is a chronic inflammatory disorder that mainly affects the area of the head and neck; it is associated with one or more relatively large, painless, slowly enlarging lesions (typically 2 to 5 cm, but with a range of 1 to 20 cm and an average of approximately 3 cm) in subcutaneous tissue and deep soft tissue, and lymphadenopathy. Lymphadenopathy usually affects the cervical area; it can be bilateral and is usually painless. In 40%

**KIMURA DISEASE—FACT SHEET**

**Definition**
- A chronic inflammatory disorder with distinctive histologic features mainly affecting the head and neck that appears to represent an aberrant immunologic reaction

**Incidence**
- Uncommon

**Gender, Race, and Age Distribution**
- Most patients are young adult males; it is more common in Asians than in whites or blacks

**Clinical Features**
- Patients typically have cervical lymphadenopathy or tumor-like lesions of salivary glands or soft tissue, or both
- Patients also have peripheral blood eosinophilia, elevated serum IgE, and occasionally asthma, suggesting an allergic process

**Morphology**
- Lymph nodes and other involved sites show an infiltrate of lymphocytes, eosinophils, plasma cells, mast cells, reactive follicles, and fibrosis
- There may be eosinophilic abscesses and polykaryocytes, especially in follicles

**Immunophenotype**
- IgE-positive follicular dendritic networks in follicles

**Diagnosis**
- Biopsy of involved tissue

**Prognosis and Therapy**
- The lymphadenopathy and other mass lesions do not require specific therapy, although they can be removed if they are large and persistent

of cases, there is involvement of major salivary glands. Kimura disease affecting the lacrimal gland has been described. Rarely, there are soft-tissue lesions and lymphadenopathy away from the head and neck, in sites that include the groin, axilla, forearm, and popliteal area. Kimura disease affects patients from childhood to middle age; most patients are young adults, with a significant male preponderance (6:1 in a recent series). The disease appears to be more common among Asians, but may also be seen in whites and blacks. Patients nearly always have peripheral eosinophilia and elevated serum IgE levels. Some patients also have asthma, proteinuria, or nephrotic syndrome. Lesions can persist or recur over a period of months or years. Findings on physical examination often suggest a diagnosis of a salivary gland tumor or lymphoma, so that a neoplastic process is often in the clinical differential diagnosis. Kimura disease is of uncertain etiology but may represent an aberrant immune reaction to an unknown stimulus.

### Pathologic Features

Lesions in salivary glands and subcutaneous tissue are poorly circumscribed and consist of a dense infiltrate of lymphocytes, eosinophils, mast cells, and plasma cells with many small blood vessels. Numerous reactive follicles are present; their germinal centers may be pierced by blood vessels or may be invaded and disrupted by eosinophils. In approximately half of all cases, there is deposition of homogeneous eosinophilic material in the interstitium of the germinal centers. Polykaryocytes may be present, most often in the follicles. Fibrosis is common, but its extent is variable. Collagen deposition can be perivascular or periductal in salivary glands, but over time it can progress to diffuse, extensive hyalinization of the involved tissues with only scattered lymphocytes, plasma cells, eosinophils, and mast cells. Involved lymph nodes show follicular hyperplasia with increased numbers of eosinophils in the paracortex, sinuses, and perinodal soft tissue, and within follicles, sometimes with the formation of eosinophilic microabscesses. Paracortical postcapillary venules are increased (Figure 4-13). Fibrosis tends to be less severe in lymph nodes than in subcutis or salivary glands.

Kimura disease has the distinctive finding of IgE-positive dendritic networks in germinal centers. The prominence of eosinophils, the presence of mast cells, and the characteristic IgE deposition in germinal centers reinforce the suggestion that Kimura disease is a manifestation of an abnormal immunologic response. An increased number of $IgG4^+$ plasma cells has been described in a few cases of Kimura disease (see IgG4-Related Lymphadenopathy) raising the question of a relation between these two disorders.

### Differential Diagnosis

The differential diagnosis of Kimura disease includes a variety of neoplastic and reactive conditions, such as Hodgkin lymphoma, angioimmunoblastic T cell lymphoma, Castleman disease, parasitic infection, nonspecific florid follicular hyperplasia, and angiolymphoid hyperplasia with eosinophilia. In many early reports, Kimura disease and angiolymphoid hyperplasia with eosinophilia were grouped together as one disease. Angiolymphoid hyperplasia with eosinophilia and Kimura disease tend to produce lesions with prominent blood vessels and eosinophils in the region of the head and neck. However, angiolymphoid hyperplasia with eosinophilia does not show any special predilection for Asians or for males and is associated with smaller lesions (average, 1 cm and typically less than 2 cm) that are better circumscribed and more superficially located (i.e., in the dermis and subcutaneous tissue), especially in the area around the ear. Lymphoid follicles and fibrosis are not always seen in angiolymphoid hyperplasia with eosinophilia. Angiolymphoid hyperplasia with eosinophilia may be associated with regional lymphadenopathy, but salivary gland involvement is distinctly unusual. Peripheral blood eosinophilia, elevated IgE, asthma, and proteinuria are unusual. A characteristic feature of angiolymphoid hyperplasia with eosinophilia is prominent epithelioid or histiocytoid endothelial cells, which are not seen in Kimura disease, whereas eosinophils are not always abundant, and eosinophilic abscesses are unusual in angiolymphoid hyperplasia with eosinophilia. Angiolymphoid hyperplasia with eosinophilia is considered to be a benign vascular tumor rather than a type of lymphoid hyperplasia. *Epithelioid hemangioma* is a synonymous term. This type of lesion has also been called *cutaneous histiocytoid angioma*.

## DRUG-RELATED LYMPHADENOPATHY

Infrequently patients receiving medication, usually anticonvulsant therapy, develop a severe hypersensitivity reaction with manifestations that commonly include a skin rash, fever, lymphadenopathy, elevated liver function tests, leukocytosis, peripheral eosinophilia, and elevated C-reactive protein and lactate dehydrogenase. The rash is usually maculopapular or erythrodermic, but a minority of patients has a bullous eruption (toxic epidermal necrolysis or Stevens-Johnson syndrome). The lymphadenopathy is more often multifocal than unifocal (Figure 4-14). Generally the triad of fever, rash, and multiorgan involvement are suggested to establish a diagnosis of drug-induced hypersensitivity syndrome, also called *drug rash with eosinophilia and systemic symptoms (DRESS)*.

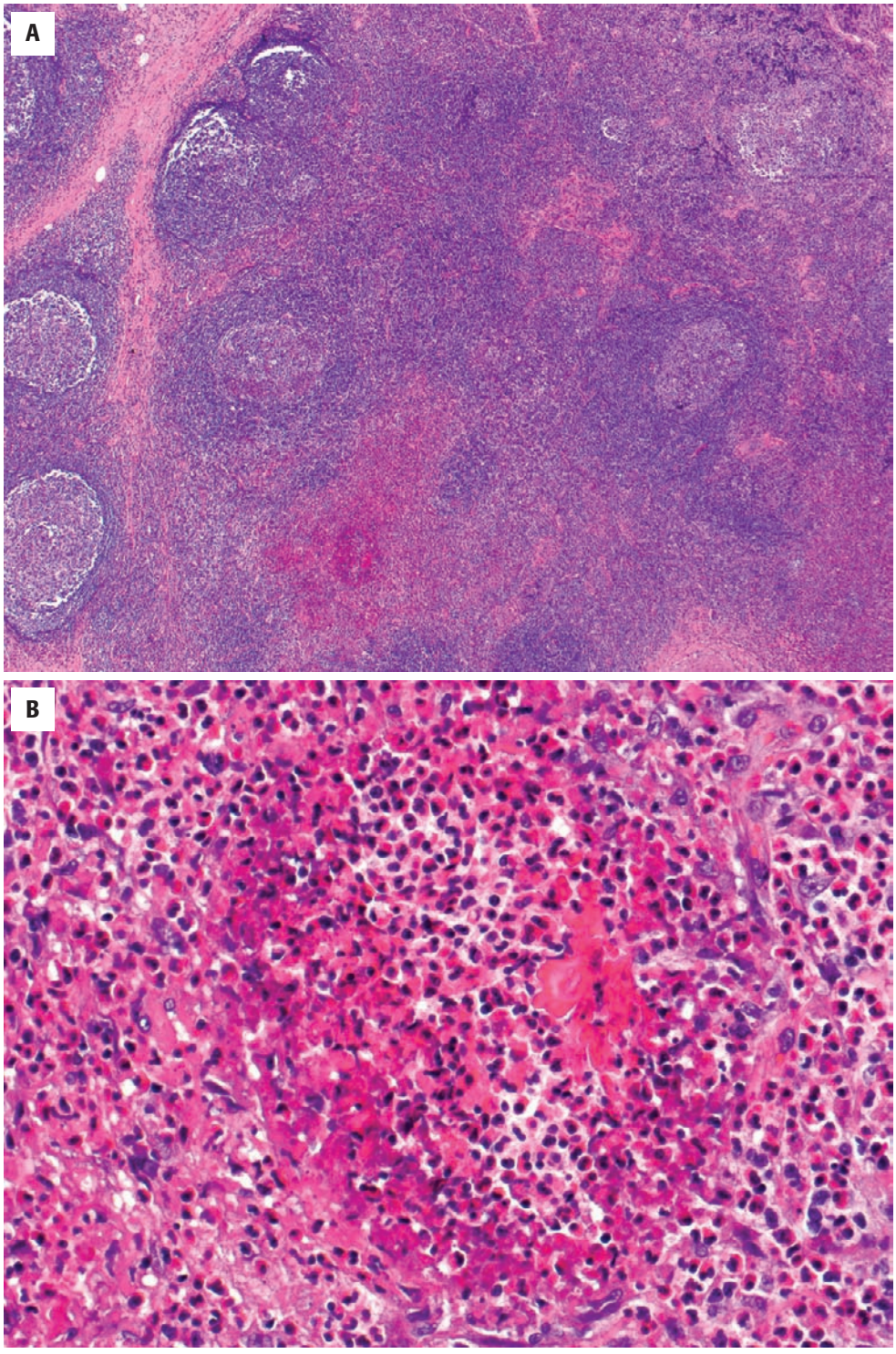


**FIGURE 4-13**

Kimura disease. **A,** Low power shows a nodule of lymphoid tissue with prominent follicular hyperplasia and focal fibrosis. **B,** Higher power shows an eosinophilic microabscess. IgE+ dendritic mesh works and IgE+ plasma cells were also present (not illustrated).

**DRUG-RELATED LYMPHADENOPATHY—FACT SHEET**

**Definition**
- Lymphadenopathy occurring as a reaction, typically a hypersensitivity reaction, to medication
- Antiseizure medication, especially phenytoin and carbamazepine, is most often associated with this type of lymphadenopathy

**Incidence**
- One in 1000 to 1 in 10,000 exposures to anticonvulsant therapy; more frequent in immunocompetent patients

**Gender, Race, or Age Distribution**
- Patients can be young or old, male or female

**Risk Factors**
- Possible link to a defect in the detoxifying enzyme epoxide hydrolase; can be familial; dark skin, vitamin D deficiency, winter months

**Clinical Features**
- The classic presentation is with a hypersensitivity reaction, with fever, malaise, rash, and lymphadenopathy 2 to 8 weeks after starting therapy, but lymphadenopathy has been reported after years of treatment with phenytoin
- Involved nodes are most often cervical, but other nodes may be involved and lymphadenopathy may be generalized

**Pathologic Features**
- The typical picture is a paracortical immunoblastic proliferation, with or without follicular hyperplasia, sometimes with eosinophils
- The immunoblastic reaction can be so striking in some cases as to suggest lymphoma; such cases have been called *pseudolymphoma* (see Figure 4-14)
- Rare patients have had lymphadenopathy with an immunoblastic reaction and later developed lymphoma, but the medications have not been proved to cause lymphoma
- Some individuals have had nonspecific reactive hyperplasia, necrotizing lymphadenitis, and dermatopathic lymphadenopathy, but these may be coincidental rather than caused by the medication
- Rare cases may be monoclonal; clinical history is important in making the correct diagnosis

**Prognosis and Therapy**
- Treatment consists of immediate discontinuation of the drug, with the addition of steroids or other antiinflammatory agents, if needed
- Many patients do well if treated promptly; the drug-induced hypersensitivity reaction is occasionally fatal

## IgG4–RELATED LYMPHADENOPATHY

IgG4 is the least common of all the IgG subclasses. IgG4-related sclerosing disease, also known as *IgG4-related autoimmune disease,* is a systemic disease characterized by the presence of tumor-like sclerosing lesions in extranodal sites, elevated serum IgG4 level, frequent presence of autoantibodies and often, a good response to steroid therapy. Among the more commonly involved extranodal sites are the pancreas (autoimmune pancreatitis), biliary tract (sclerosing cholangitis), submandibular gland (Küttner tumor) and lacrimal gland (sclerosing dacryoadenitis). On microscopic examination the sclerosing lesions typically show variably prominent lymphoplasmacytic infiltrates, sclerosis and obliterative phlebitis, with increased numbers and increased proportion of IgG4+ plasma cells. In addition to the elevated serum IgG4, common laboratory abnormalities include polyclonal hypergammaglobulinemia, elevated ESR and/or C-reactive protein, rheumatoid factor,

**IGG4-RELATED LYMPHADENOPATHY—FACT SHEET**

**Definition**
- Lymphadenopathy occurring in the setting of IgG4-related sclerosing disease

**Incidence**
- Unknown

**Gender, Race, or Age Distribution**
- Data are somewhat limited, but males appear to be affected more often than females; most patients are middle-aged to older, with a median of approximately 70 years; and most reported cases are from Asia

**Risk Factors**
- None known

**Clinical Features**
- Lymphadenopathy in the setting of IgG4-related sclerosing disease

**Pathologic Features**
- Three main histologic patterns are described, each showing increased numbers of IgG4+ plasma cells, and increased proportion of IgG4+ plasma cells compared with IgG+ plasma cells (>40% suggested as cut-off, usually higher; see Figure 4-15):
  - Castleman disease-like: the follicles show changes similar to those of hyaline-vascular Castleman disease, whereas the interfollicular area contains increased numbers of plasma cells
  - Follicular hyperplasia: the lymphoid follicles are hyperplastic, with increased numbers of intrafollicular plasma cells
  - Interfollicular expansion: in this pattern, there are a few follicles widely separated by an expanded interfollicular area occupied by plasma cells, including some immature forms, occasional immunoblasts, small numbers of lymphoid cells with clear cytoplasm, and arborizing high endothelial venules. The differential diagnosis could include angioimmunoblastic T-cell lymphoma, but the absence of $CD10^+$ T cells and lack of expanded follicular dendritic meshworks tend to exclude this possibility

**Prognosis and Therapy**
- Treatment with corticosteroids is often beneficial; prognosis is good

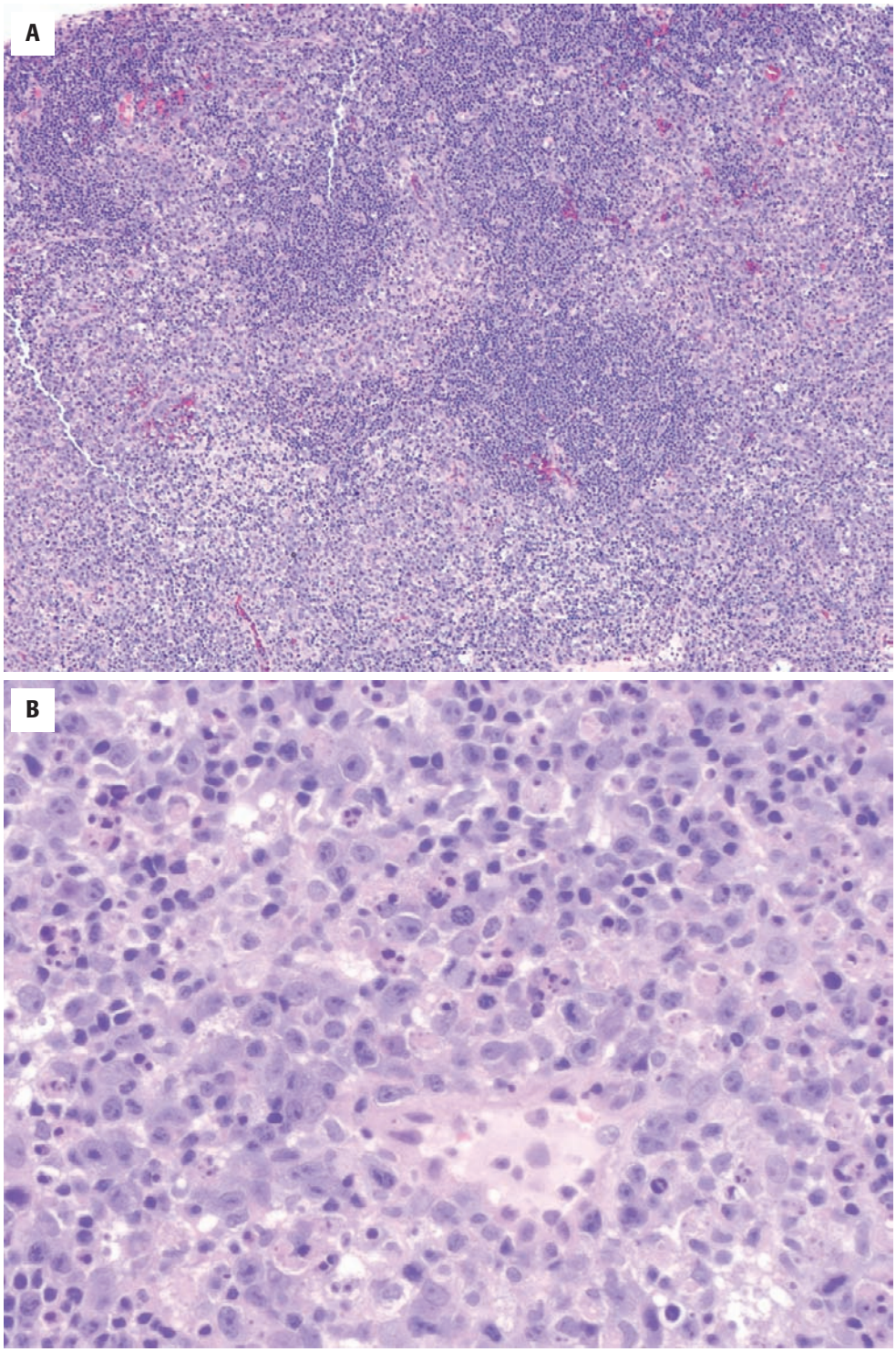


**FIGURE 4-14**

Phenytoin-associated lymphadenopathy. **A,** The lymph node was slightly enlarged. It shows scattered small, dark primary follicles and an expanded paracortex. **B,** High-power examination of the paracortex shows numerous immunoblasts, scattered lymphocytes, and apoptotic debris.

anti-nuclear antibodies and anemia, which may be a hemolytic anemia. Patients with IgG4-related sclerosing disease often have concurrent lymphadenopathy, which may be in the region of or distant from, extranodal sclerosing lesion(s). Lymphadenopathy with similar features has occasionally been identified in individuals without extranodal sclerosing lesions; some of them have subsequently developed extranodal sclerosing lesions. The lymphadenopathy has features that are distinctive but not entirely specific (Figure 4-15); evaluation for other features that are found in IgG4-related sclerosing disease is required to establish a diagnosis.

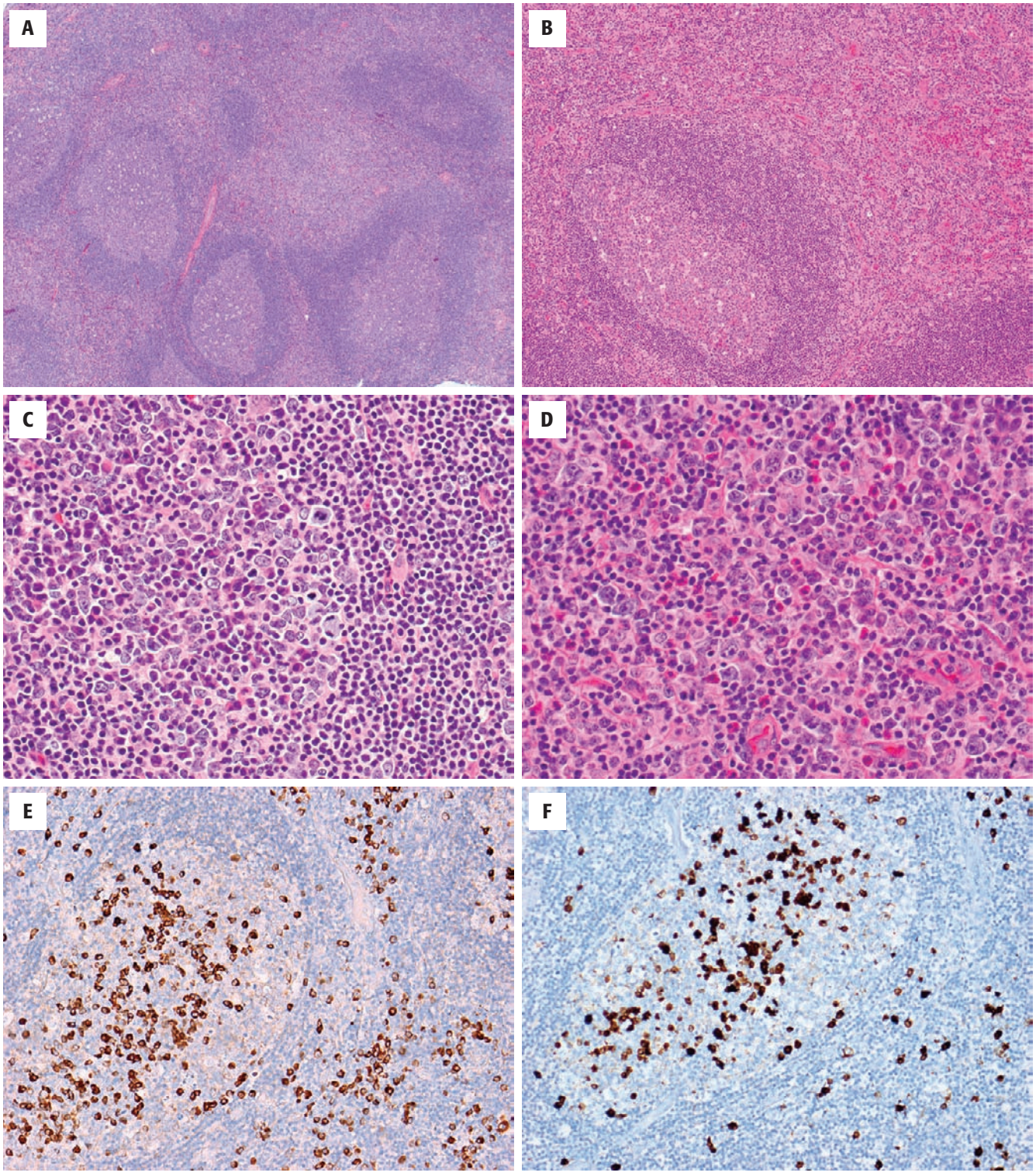


**FIGURE 4-15**

Immunoglobulin (Ig) G4-associated lymphadenopathy. **A,** Low power shows follicular hyperplasia and focal expansion of the interfollicular area. **B,** Medium power shows one follicle with an active follicle center and a discrete mantle. Cells in the interfollicular area are polymorphous. **C,** High power of a follicle shows many plasma cells admixed with follicle center cells. **D,** The interfollicular area contains small lymphocytes, plasma cells, eosinophils, and immunoblasts. With antibody to IgG **(E)** and IgG4 **(F),** most IgG+ plasma cells are positive for IgG4; this is most pronounced within follicles. This case shows a mixture of features of patterns 2 and 3 (see Fact Sheet).

## LYMPHADENOPATHY RELATED TO EXOGENOUS, EXCESS ENDOGENOUS, OR PIGMENTED MATERIAL

### DERMATOPATHIC LYMPHADENOPATHY

Dermatopathic lymphadenopathy is found in patients with a variety of chronic dermatoses or with mycosis fungoides. The affected lymph nodes are those draining the abnormal skin. In some cases, patients do not have appreciable skin disease. Lymph nodes show marked expansion of the paracortex, with formation of large, pale nodules containing lymphocytes, occasional immunoblasts, and numerous histiocytes with abundant pale cytoplasm including interdigitating dendritic cells, Langerhans cells, and phagocytic histiocytes containing melanin, lipid, and occasionally iron (Figure 4-16). Small numbers of lymphocytes with irregular nuclei, reminiscent of the cerebriform cells that are characteristic of mycosis fungoides, may be found, even in patients with no evidence of lymphoma. Follicular hyperplasia is usually inconspicuous. The paracortical T cells are predominantly T helper cells, with a CD4:CD8 ratio that is, on average, higher than that of nonspecific lymphoid hyperplasia. The interdigitating dendritic cells and Langerhans cells are S100 positive. Findings in patients with and without mycosis fungoides are similar. The majority of cases of dermatopathic lymphadenopathy in patients with mycosis fungoides show clonal T cell receptor gene rearrangement, suggesting that nodal involvement by mycosis fungoides is frequent and may be subtle and difficult to appreciate on routine sections.

### MISCELLANEOUS LYMPHADENOPATHIES WITH HISTIOCYTIC INFILTRATION

Hereditary storage diseases, such as Niemann-Pick disease and Gaucher disease, can be associated with nodal infiltration by histiocytes distended by storage products. The appearance of the histiocytes is similar to their appearance in other tissues. Lymphangiography, previously used for the staging of Hodgkin lymphoma, produces lipogranulomas with sinusoidal distention by foamy histiocytes, multinucleated giant cell, and large and small vacuoles of contrast material. Lipogranulomas are commonly found in lymph nodes related to the portal circulation, in the absence of a history of lymphangiography, and are probably the result of deposition of lipids related to the diet and bile metabolites. Hyperlipidemia is a rare cause of lymphadenopathy. Severe hyperlipidemia has been associated with droplets of lipid in nodal sinuses and large numbers of lipid-laden macrophages.

Prosthetic joint replacements can deteriorate over time; occasionally, this process is associated with a reaction to foreign material in regional lymph nodes. The histiocytic reaction can be confined to distended sinuses or extend to involve the paracortex. Prosthetic devices containing metal, polyethylene, and cement (polymethylmethacrylate) are associated with sheets of histiocytes with granular eosinophilic cytoplasm; metal and refractile foreign material are seen. Reaction to Silastic joint prostheses (silicone lymphadenopathy) consists of granulomas with multinucleated giant cells containing pale yellow, refractile particles of silicone.

Mammary prostheses (bag-gel prostheses or silicone injection) can also result in adenopathy. Silicone lymphadenopathy in this setting tends to show diffuse infiltrates of histiocytes with marked cytoplasmic vacuolization and extracellular deposits of pale foreign material forming prominent cystic spaces (Figure 4-17).

Patients with respiratory exposure to silica can develop lymphadenopathy, often in association with lung disease, although occasionally lymphadenopathy is present in isolation. Microscopic examination shows sclerotic nodules associated with a histiocytic and fibroblastic reaction and birefringent crystals on examination with polarized light. These lymph nodes often also show anthracosis (Figure 4-18).

Anthracosis is a common finding in mediastinal lymph nodes. Involved nodes typically show black discoloration on gross examination and numerous histiocytes with carbon particles on microscopic examination. Lymph nodes draining skin with tattoos may contain histiocytes with pigment that is usually black. Infrequently, this phenomenon produces clinically significant lymphadenopathy (Figure 4-19).

### SARCOIDOSIS

#### Clinical Features

Sarcoidosis is an uncommon granulomatous disorder of unknown cause; blacks are affected more often than whites, and women are affected more often than men. Involvement of a wide variety of tissues can occur, but the lymph nodes and lungs are the most common. Mediastinal and pulmonary hilar nodes are the most frequently involved, but any lymph node, as well as a wide variety of extranodal sites, may show changes of sarcoidosis. The clinical course is highly variable; some patients are asymptomatic and some develop significant complications, the most common of which is progressive pulmonary fibrosis.

#### Pathologic Features

Lymph nodes show multiple compact granulomas composed of epithelioid histiocytes and multinucleated giant

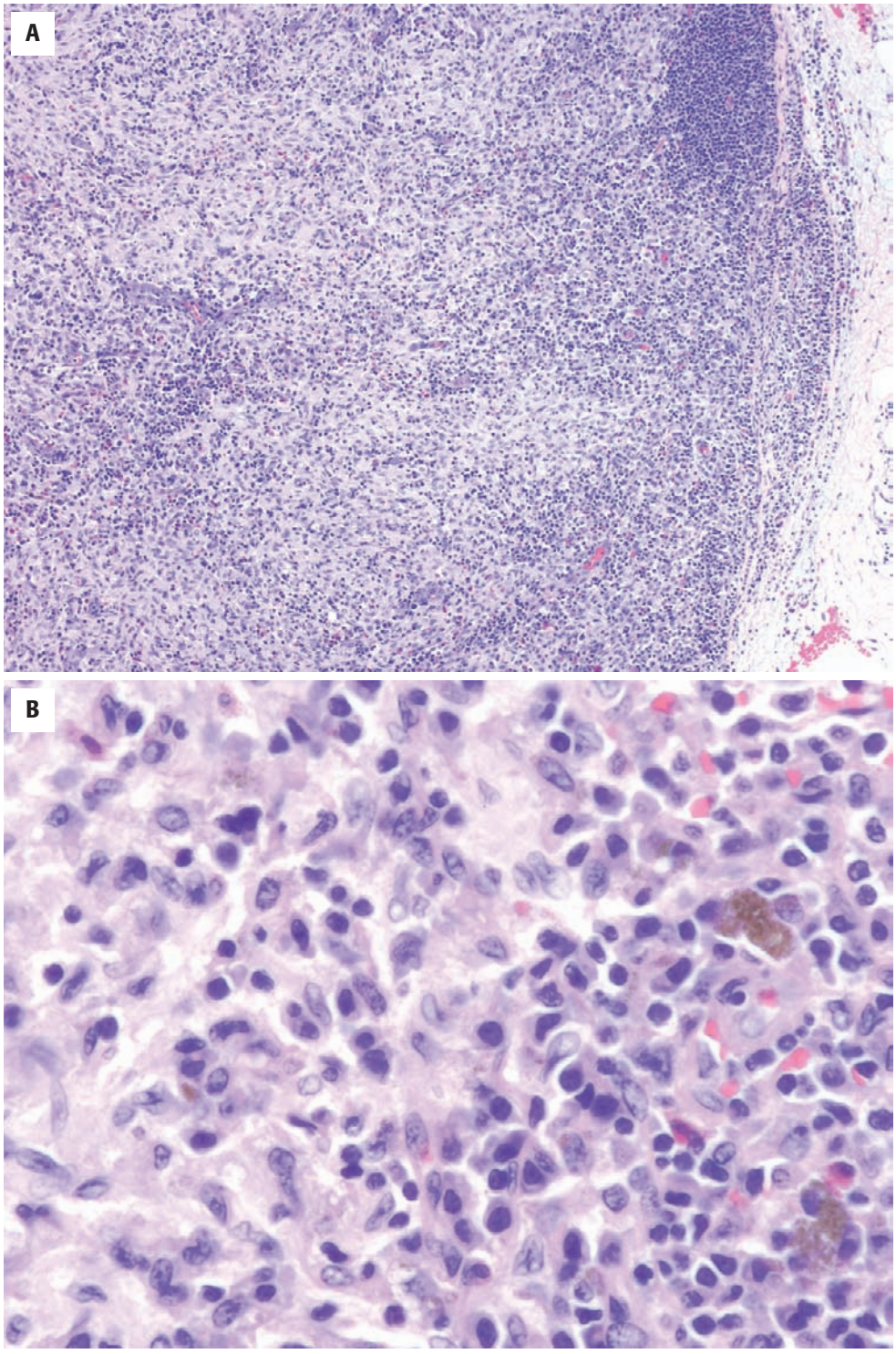


**FIGURE 4-16**

Dermatopathic lymphadenopathy. **A,** Low power shows vaguely nodular pale areas in the paracortex. A small dark primary follicle is present beneath the nodal capsule. **B,** The pale areas correspond to numerous antigen-presenting cells, including Langerhans cells with pale, folded, and grooved nuclei. Histiocytes containing pigment are also present.

cells with a narrow cuff of small lymphocytes. The granulomas are often confluent, replacing nearly the entire node; when the node is partially involved, the granulomas are usually in the paracortex. A small amount of fibrin and cellular debris may be seen in the centers of some granulomas, but necrosis is not prominent and caseation is absent. Over time, the granulomas may become hyalinized.

## Differential Diagnosis

Sarcoidosis is a diagnosis of exclusion; its differential is broad and includes tuberculosis, fungal infection, tuberculoid leprosy, brucellosis, Whipple disease, syphilis, and reaction to foreign material. Hodgkin lymphoma and certain types of non-Hodgkin lymphoma may be

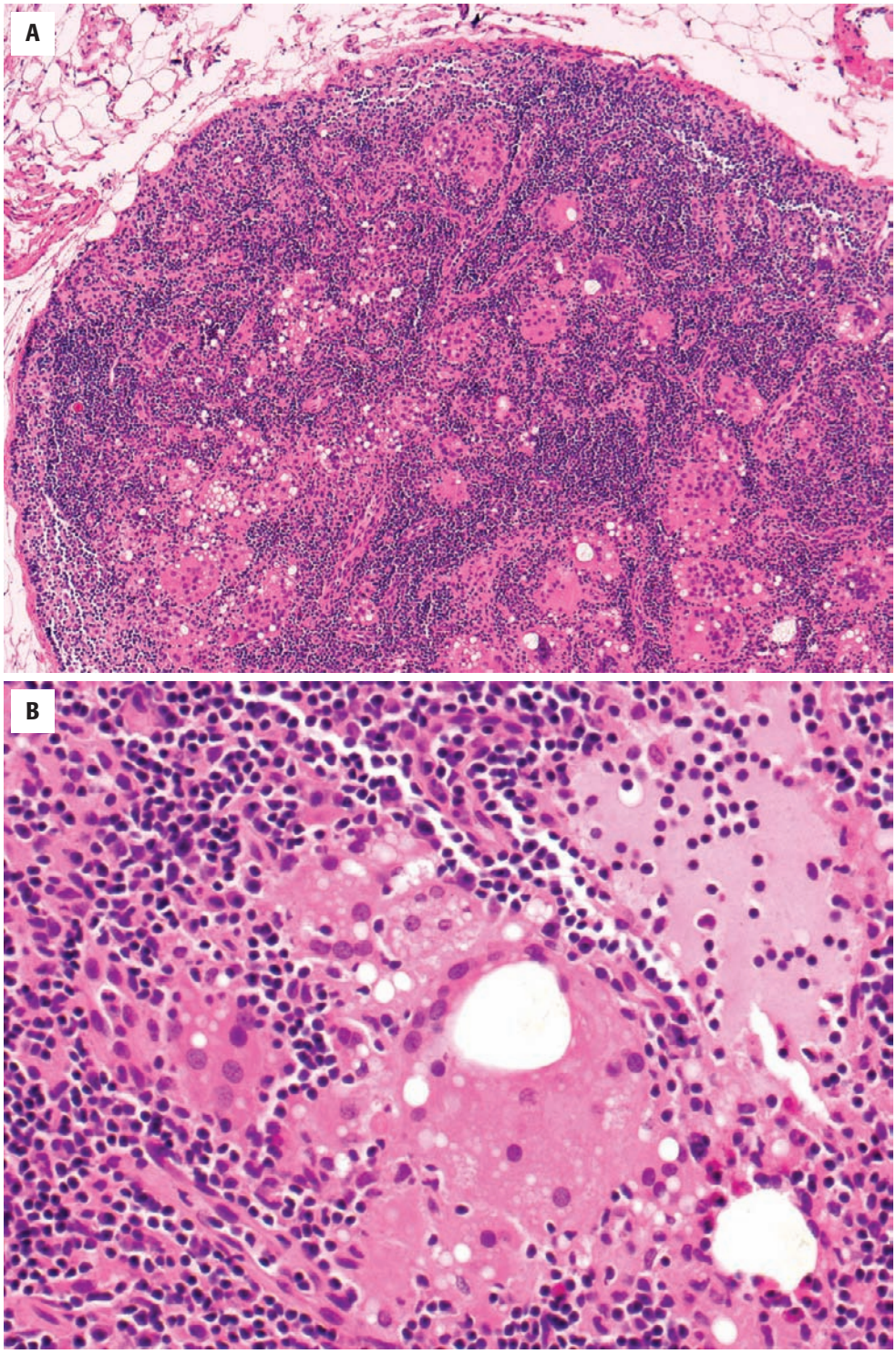


**FIGURE 4-17**

Silicone lymphadenopathy, related to a breast implant. **A,** Low power shows increased numbers of histiocytes, with multinucleated histiocytes and many histiocytes with prominently vacuolated cytoplasm. **B,** Higher power shows an aggregate of vacuolated histiocytes; some vacuoles contain pale material consistent with silicone.

associated with a granulomatous reaction resembling sarcoidosis. Examination of the areas between granulomas in these cases reveals an atypical lymphoid infiltrate. Patients with Hodgkin lymphoma may have sarcoidal granulomas in multiple tissues, including liver, spleen, and lymph nodes, in the absence of involvement by Hodgkin lymphoma. Sarcoidal granulomas may be found in lymph nodes draining tissues involved by carcinoma.

## CASTLEMAN DISEASE

Castleman disease has been subclassified based on pathologic features into hyaline-vascular and plasma cell variants. Occasional cases with features intermediate between the two types (transitional or mixed type) may be seen. An additional subtype of Castleman disease has

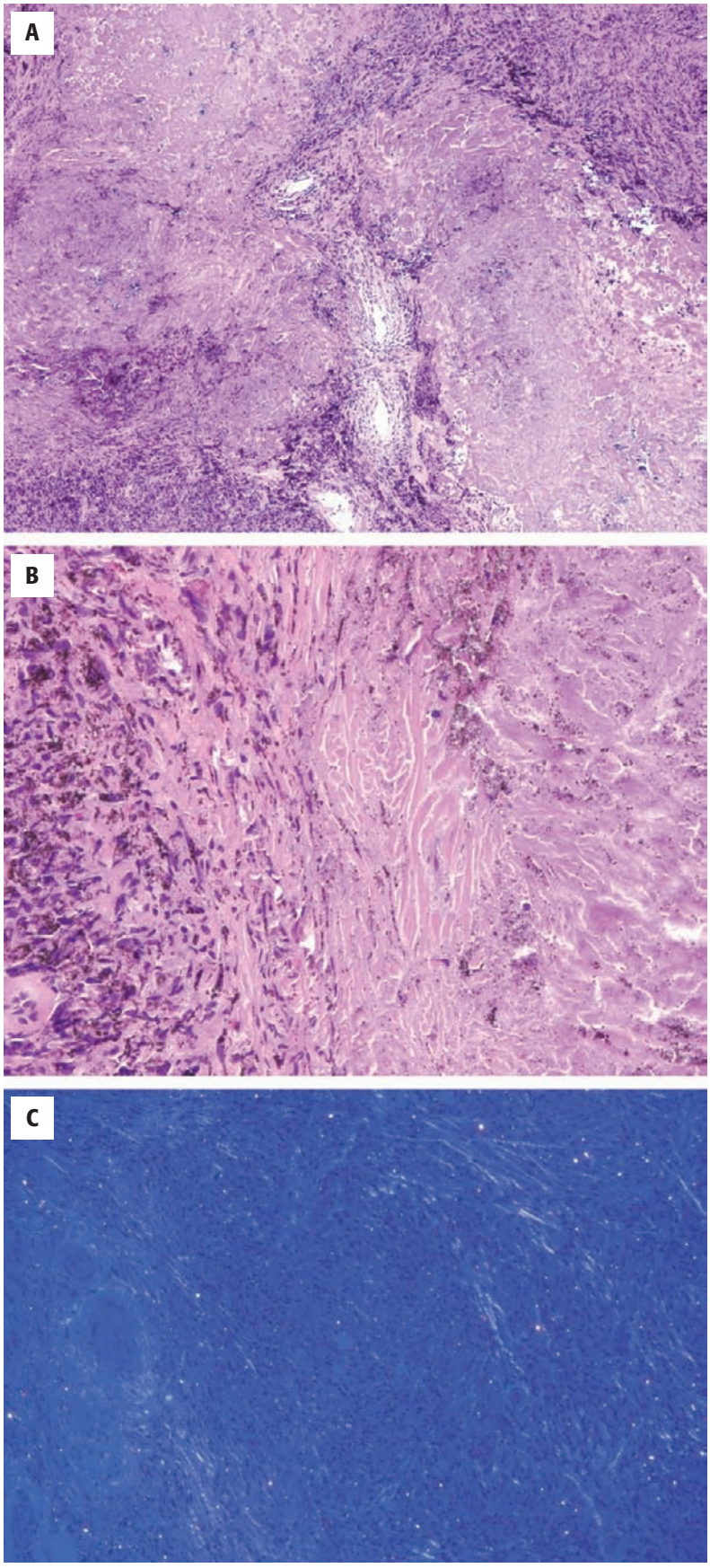


**FIGURE 4-18**

Lymph nodes with sclerotic nodules related to silica. **A,** Nodular sclerotic areas occupy much of the lymph node. The lymph node also shows anthracosis. **B,** Higher power shows hyalinized lymphoid tissue. **C,** Birefringent particles of silica are seen with polarized light.

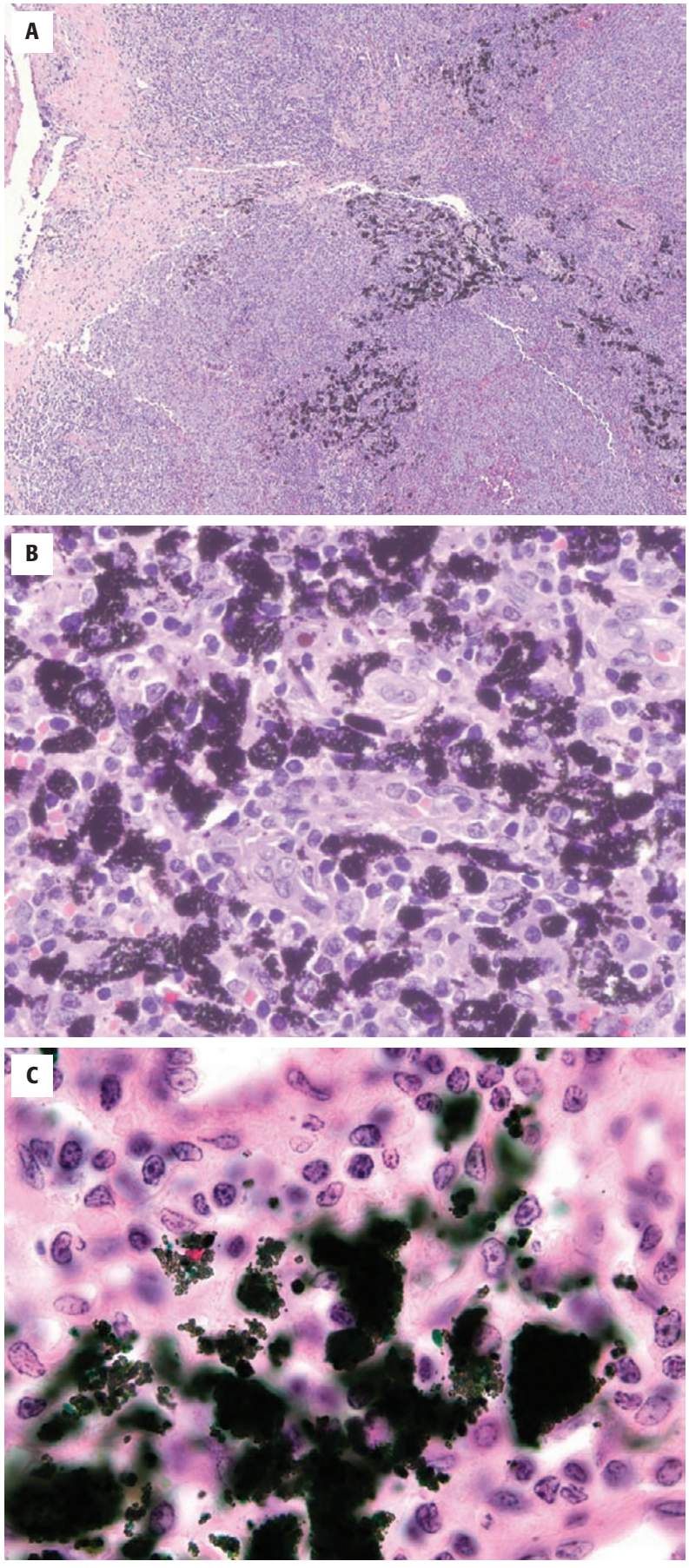


**FIGURE 4-19**

Lymph node with tattoo pigment. **A,** This axillary lymph node contains numerous histiocytes with black pigment. The patient had multiple tattoos. **B,** Higher power shows histiocytes with black particulate material. **C,** In another example, the pigment has a distinct green color.

been described more recently: HHV8-associated multicentric Castleman disease. Castleman disease occurs in two clinical forms: localized and multicentric.

## LOCALIZED CASTLEMAN DISEASE

Nearly all cases of hyaline-vascular Castleman disease, and a minority of plasma cell Castleman disease, are localized—that is, the disorder affects a single anatomic site or a single group of lymph nodes. Approximately 80% of localized Castleman disease is of the hyaline-vascular type, whereas approximately 20% is of the plasma cell type, with occasional cases of mixed type. Excision of the lesion is almost always curative.

## MULTICENTRIC CASTLEMAN DISEASE

Multicentric Castleman disease is a systemic disease with a relatively poor prognosis. Patients have lesions of Castleman disease in two or more separate anatomic sites. Most patients are middle-aged to older adults. Patients exhibit peripheral or internal lymphadenopathy, or both, and often with hepatomegaly and splenomegaly. Patients usually have systemic symptoms such as fatigue, fever, weight loss, and night sweats. Such symptoms tend to be more severe than those in localized plasma cell Castleman disease. Laboratory abnormalities usually include anemia and elevated sedimentation rate. Manifestations may be related to elevated levels of human or viral IL-6, likely produced in the affected tissues. The levels of IL-6 appear to correlate with the systemic inflammatory manifestations. Some patients respond to treatment with chemotherapy, steroids, radiation, or anti–IL-6 antibodies, but the best therapy for this disorder is unknown.

Cases of multicentric Castleman disease may have the histologic features of plasma cell Castleman disease or of mixed-type Castleman disease, but only rarely of the hyaline-vascular type. A subset of cases is related to human herpesvirus 8 infection (HHV-8, see Human Herpesvirus 8–Associated Multicentric Castleman Disease). A subset of patients has a monotypic plasma cell proliferation (almost always lambda+) that may be associated with POEMS syndrome (discussed under POEMS Syndrome). Other cases are of uncertain cause.

## HYALINE-VASCULAR VARIANT

### Clinical Features

Hyaline-vascular Castleman disease can affect patients of any age, but most are young adults. Females may be slightly more often affected than males. Hyaline-vascular Castleman disease is often an incidental finding on radiographic examination, but patients may also exhibit symptoms related to compression of adjacent structures or rarely with pain or a palpable mass. Constitutional symptoms and hematologic abnormalities are unusual. Patients have lymphadenopathy in a single site in nearly all cases. In rare cases, the lesion involves an extranodal site. There may be a main mass lesion with enlargement of adjacent lymph nodes, sometimes also showing changes of Castleman disease. Rarely, more than one site is involved. The mediastinum is most commonly affected, followed by lymph nodes in the abdomen, neck, and other sites. Hyaline-vascular Castleman disease almost never recurs after excision. The cause is uncertain but is most likely related to abnormal follicular dendritic cells.

### Pathologic Features

Involved lymph nodes take the form of rounded masses 1.5 to 16 cm in diameter (median, approximately 6 cm). Hyaline-vascular Castleman disease is characterized by lymphoid follicles with small, hyalinized germinal centers and broad mantle zones. Each follicle may contain one or more than one germinal center; some follicles contain no recognizable germinal center. The germinal centers contain an increased proportion of follicular dendritic cells and endothelial cells. Mantle zone lymphocytes are arranged in concentric rings (onion skin pattern) around the germinal center. Large, hyperchromatic, occasionally bizarre, dystrophic cells with scant cytoplasm, consistent with abnormal follicular dendritic cells, are sometimes found in the follicles, usually in the atrophic follicle centers and less often in the mantles. Follicles are often radially penetrated by a blood vessel (called *lollipop follicle*). The interfollicular region shows increased numbers of high endothelial venules and of small vessels with flat endothelium and hyalinized walls (Figure 4-20). Lymphocytes are fewer in number than in a normal lymph node, and there may be an admixture of scattered plasma cells, few immunoblasts, few eosinophils, and clusters of plasmacytoid dendritic cells. Sheets of plasma cells are absent. Epithelioid histiocytes and granulomas are inconspicuous or absent. In rare cases, the interfollicular region predominates with only rare follicles (so-called stroma rich variants). In some cases, patent sinuses may be found in residual normal lymphoid tissue at the periphery of the lesion, but in general they are absent within the lesion. Fibrosis is common peripherally and also in bands running through the lesion. There may be prominent perivascular fibrosis. Rarely, dystrophic calcification occurs in fibrotic areas. Rarely, hyaline-vascular Castleman disease is complicated by the development of a follicular dendritic cell

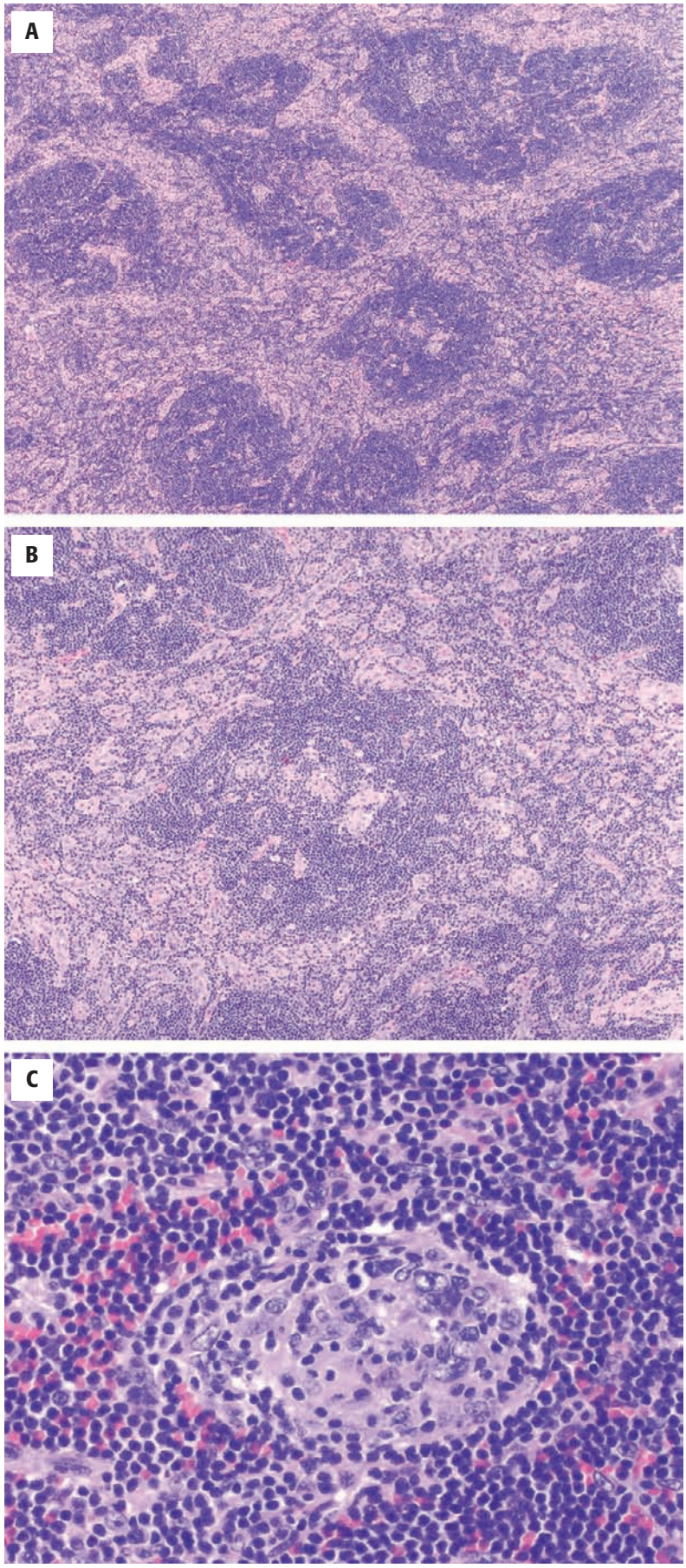


**FIGURE 4-20**

Hyaline-vascular Castleman disease. **A,** Low power shows a lymph node replaced by follicles with broad mantles and inconspicuous follicle centers and a hypervascular interfollicular area. Patent sinuses are absent. **B,** Higher power shows a follicle consisting mainly of small lymphocytes, with a suggestion of tiny sclerotic follicle centers, surrounded by numerous blood vessels. **C,** This follicle center contains few follicle-center B cells. Scattered large, oval, slightly atypical follicular dendritic cells are present.

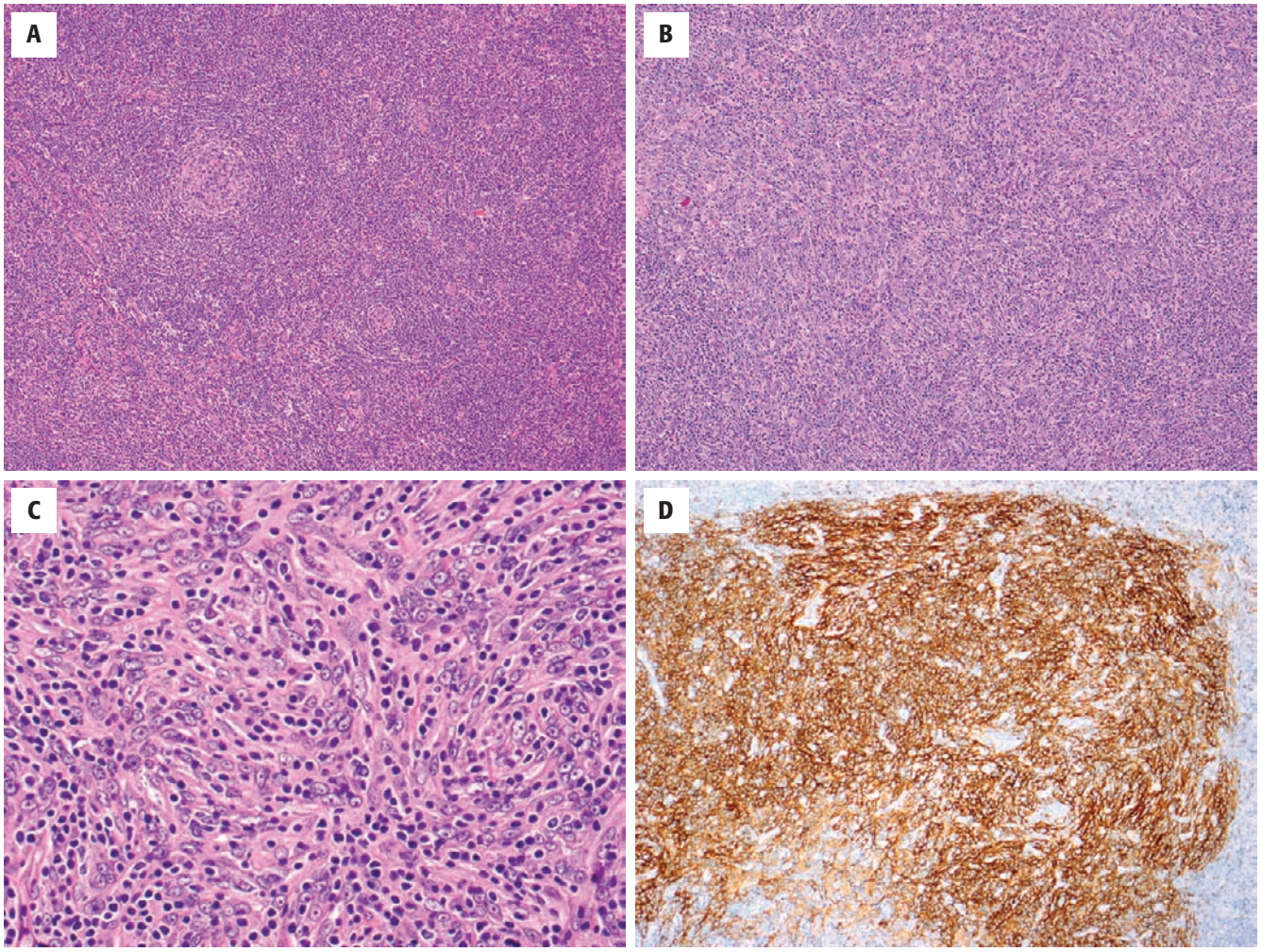


**FIGURE 4-21**

Follicular dendritic cell sarcoma, probably arising in association with hyaline-vascular Castleman disease. **A,** The enlarged lymph node shows areas with changes consistent with hyaline-vascular Castleman disease; two hyaline-vascular follicles are seen here. **B,** In other areas there is a prominent, disorderly proliferation of spindle cells with many admixed small lymphocytes. **C,** High power shows spindle cells with oval to slightly elongate nuclei, vesicular chromatin, small nucleoli, and indistinct cytoplasmic borders. **D,** Spindle cells are intensely positive for CD21, consistent with follicular dendritic cells.

tumor (Figure 4-21) or a vascular neoplasm that appears to be an outgrowth of the abnormal follicular dendritic cells or of the floridly proliferating blood vessels, respectively.

Follicles contain decreased numbers of B cells and relatively increased numbers of follicular dendritic cells ($CD21^+$, $CD23^+$). T cells ($CD3^+$, $CD45RO^+$) are present in smaller than normal numbers in follicles and interfollicular area. Many interfollicular blood vessels are factor VIII positive, $CD34^+$, HECA-452 positive, MECA-79 positive, which is consistent with high endothelial venules. The interfollicular area also contains $CD68^+$, $CD123^+$ plasmacytoid dendritic cells, and S-100–positive interdigitating dendritic cells. Follicular dendritic cells have aberrant expression of adhesion molecules and may show cytogenetic abnormalities.

## PLASMA CELL VARIANT

### Clinical Features

When plasma cell Castleman disease is localized, it affects a single anatomic site, usually mediastinal or intraabdominal lymph nodes. Patients of any age can be affected, but most are young adults. They may be asymptomatic, but systemic symptoms, including fever, weight loss, and fatigue, are common, and many have anemia, elevated erythrocyte sedimentation rate, and hypergammaglobulinemia. After excision of the mass, the symptoms and laboratory abnormalities disappear, and patients do well. Clinical features of multicentric plasma cell Castleman disease are described previously, under Multicentric Castleman Disease.

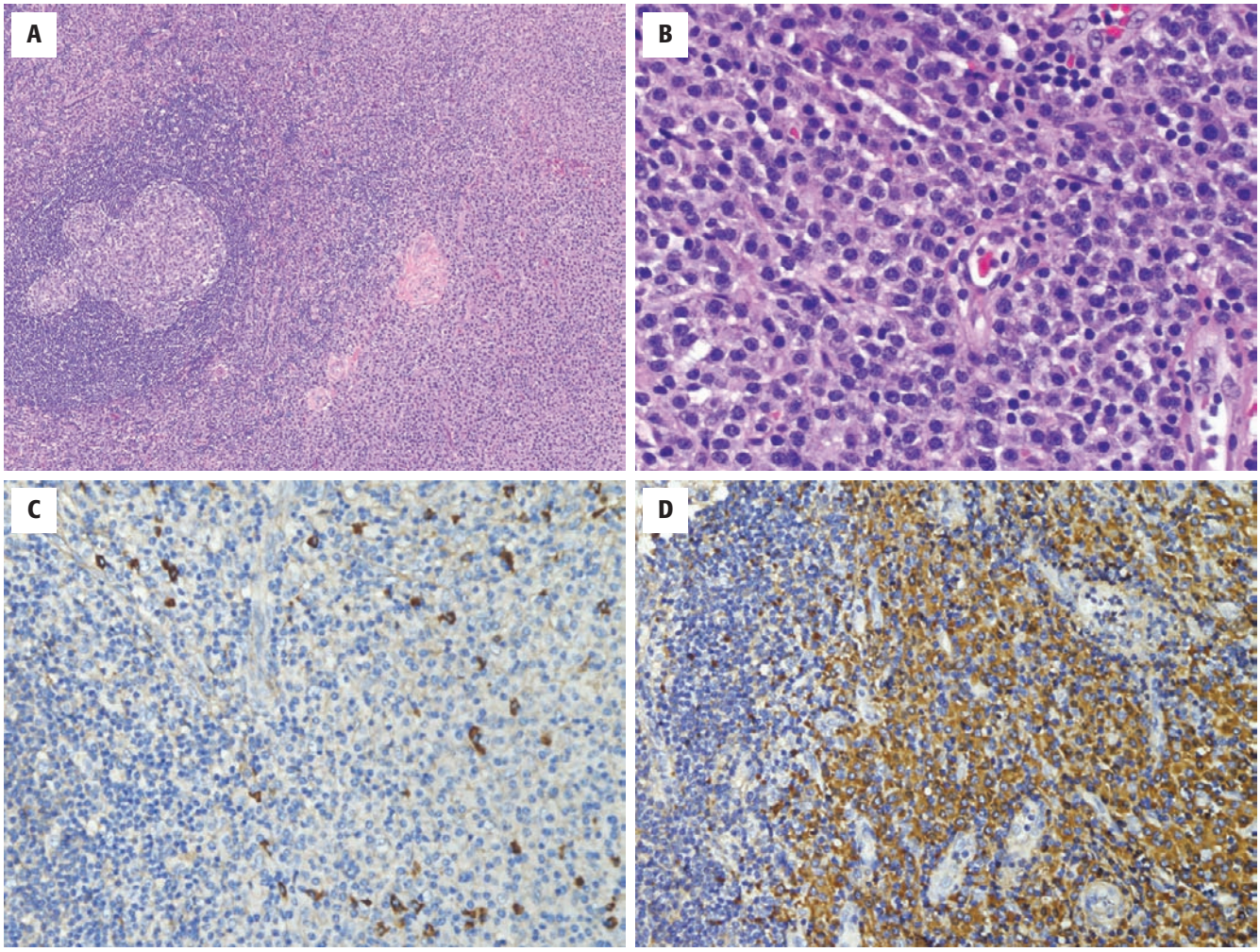


**FIGURE 4-22**

Plasma cell Castleman disease. **A,** The lymph node shows a reactive follicle and sheets of interfollicular plasma cells. **B,** Higher power of the interfollicular area shows a monotonous population of plasma cells. **C** and **D,** In this case, plasma cells express monotypic immunoglobulin (Ig), IgGλ type (**C,** κ; **D,** λ).

### Pathologic Features

Pathologic changes are similar in localized and multicentric plasma cell Castleman disease. The mass lesion is composed of one enlarged lymph node or an aggregate of enlarged nodes, measuring approximately 3 to 11 cm, with individual nodes measuring 2.5 to 7 cm. There are sheets of mature plasma cells in the interfollicular area, sometimes with Russell bodies; occasional immunoblasts may be seen. The follicles may be hyperplastic follicles of the usual type or hyaline-vascular follicles or a mixture of the two types of follicles. Blood vessels are less conspicuous than in hyaline-vascular Castleman disease. Sinuses are usually obliterated but may be patent focally. In some cases that may represent an early stage of disease, immunoblasts and high endothelial venules, in addition to plasma cells, are present in large numbers in the interfollicular region. In late stages of disease, the appearance can resemble hyaline-vascular Castleman disease. Plasma cells in as many as approximately half of cases express monotypic immunoglobulin, which is almost always IgGλ or IgAλ (Figure 4-22). In the remainder of cases, plasma cells express polytypic Ig. Some patients with monotypic plasma cells have POEMS syndrome.

## POEMS SYNDROME

POEMS syndrome (peripheral neuropathy, organomegaly, endocrinopathy, monoclonal plasma cell proliferation, and skin changes) is a rare systemic disease with paraneoplastic manifestations. All patients have peripheral neuropathy and a monoclonal plasma cell neoplasm, with a variable number of other manifestations of POEMS syndrome and commonly sclerotic bone lesions (osteosclerotic myeloma). The peak onset is in fifth to sixth decade of life; these patients are younger overall than in other cases of myeloma. Patients with POEMS syndrome have a chronic, sometimes slowly progressive course, with a median survival of approximately 14 years.

Plasma cell myeloma in the setting of POEMS syndrome is typically characterized by fewer than 5% plasma cells in the bone marrow, bony sclerosis, and $\lambda^+$ plasma cells. Anemia, hypercalcemia, and renal insufficiency are uncommon compared with myeloma occurring without associated POEMS syndrome. The plasma cells almost always express λ light chain (usually IgGλ or IgAλ). The light chain expressed is typically restricted to the Vλ1 subfamily with a pattern of somatic hypermutation suggesting antigen-driven selection. The plasma cells express vascular endothelial growth factor (VEGF). Serum VEGF levels may be elevated. The increased levels of VEGF and the specific λ light chain usage may contribute to the pathogenesis of POEMS. VEGF can promote organomegaly, edema, skin hemangiomas, and bony sclerosis resulting from osteoblastic differentiation. Elevated levels of VEGF could increase vascular permeability leading to endoneural edema and then nerve damage.

The neuropathy is typically demyelinating. The organomegaly consists of hepatosplenomegaly or lymphadenopathy. Lymphadenopathy in POEMS syndrome often has the appearance of Castleman disease, or if not diagnostic, has at least some histologic features of Castleman disease. Not all patients with POEMS syndrome have documented Castleman disease. Other manifestations include papilledema and evidence of extravascular volume overload.

The endocrinopathy in POEMS syndrome commonly consists of gonadal or thyroid dysfunction, abnormal glucose metabolism, or adrenal insufficiency. Although in most cases there is no evidence of a viral infection, POEMS syndrome has been reported rarely in association with $HHV8^+$ Castleman disease.

## HUMAN HERPESVIRUS 8–ASSOCIATED MULTICENTRIC CASTLEMAN DISEASE

In 1995, the finding of Kaposi sarcoma–associated herpesvirus-like sequences was reported in 100% of cases classified as multicentric Castleman disease in HIV-positive patients and in some cases of multicentric Castleman disease in HIV-negative patients. Subsequently, cases of multicentric Castleman disease in HIV-positive patients were described in which immunoblasts or plasmablasts in expanded mantle zones were positive for Kaposi sarcoma–associated herpesvirus (also known as *human herpesvirus 8* [HHV8]) and expressed monotypic IgMλ. Interfollicular plasma cells were polytypic. This entity has been named *HHV8-associated multicentric Castleman disease* (Figure 4-23). Additional studies showed that the plasmablasts could also proliferate to form solid aggregates (microlymphoma) or to form sheets of plasmablasts (referred to as *frank lymphoma, $HHV8^+$ plasmablastic Castleman disease,* or *large B cell lymphoma arising in $HHV8^+$ multicentric Castleman disease*). The $HHV8^+$ plasmablasts are $CD20^+$ or $CD20^-$, $CD138^-$, and monotypic $IgM\lambda^+$ (as noted previously) and are typically negative for EBV, although in rare cases the $HHV8^+$ cells are coinfected by EBV. Surprisingly, the monotypic plasmablasts in HHV8-associated Castleman disease and in the microlymphomas are usually polyclonal by molecular techniques, although the frank lymphomas are usually clonal. The $HHV8^+$ plasmablastic proliferations arising on the background of HHV8-associated Castleman disease must be distinguished from plasmablastic lymphoma of the oral cavity type, which is usually positive for EBV but is negative for HHV8.

## DIFFERENTIAL DIAGNOSIS

Castleman disease is a diagnosis of exclusion. A wide variety of specific diseases may be associated with morphologic features resembling those of Castleman disease, and these should be ruled out before making a diagnosis of Castleman disease.

### HYALINE-VASCULAR CASTLEMAN DISEASE

#### Mantle Cell Lymphoma

Because hyaline-vascular Castleman disease is characterized by prominent mantle zones, mantle cell lymphoma with a mantle zone pattern can be considered in the differential of hyaline-vascular Castleman disease. Residual follicle centers in mantle cell lymphoma do not show hyaline-vascular features, and the interfollicular region lacks the distinctive appearance seen in hyaline-vascular Castleman disease. Immunophenotyping is definitive in difficult cases.

#### Follicular Lymphoma

Some cases of follicular lymphoma have sclerotic neoplastic follicles. When there is early nodal involvement in such cases—that is, the architecture is not effaced—the appearance can resemble hyaline-vascular Castleman disease. In follicular lymphoma, mantle zones should not be prominent, and the interfollicular area should not be hypervascular. Immunophenotyping is definitive in difficult cases.

#### Thymoma

In the first reports of Castleman disease, the resemblance of hyaline-vascular follicles to Hassall corpuscles was noted; however, hyaline-vascular Castleman disease and thymoma do not otherwise resemble each other. Castleman disease arises in lymph nodes rather than the thymus, and patients with thymomas are usually older than those with hyaline-vascular Castleman disease.

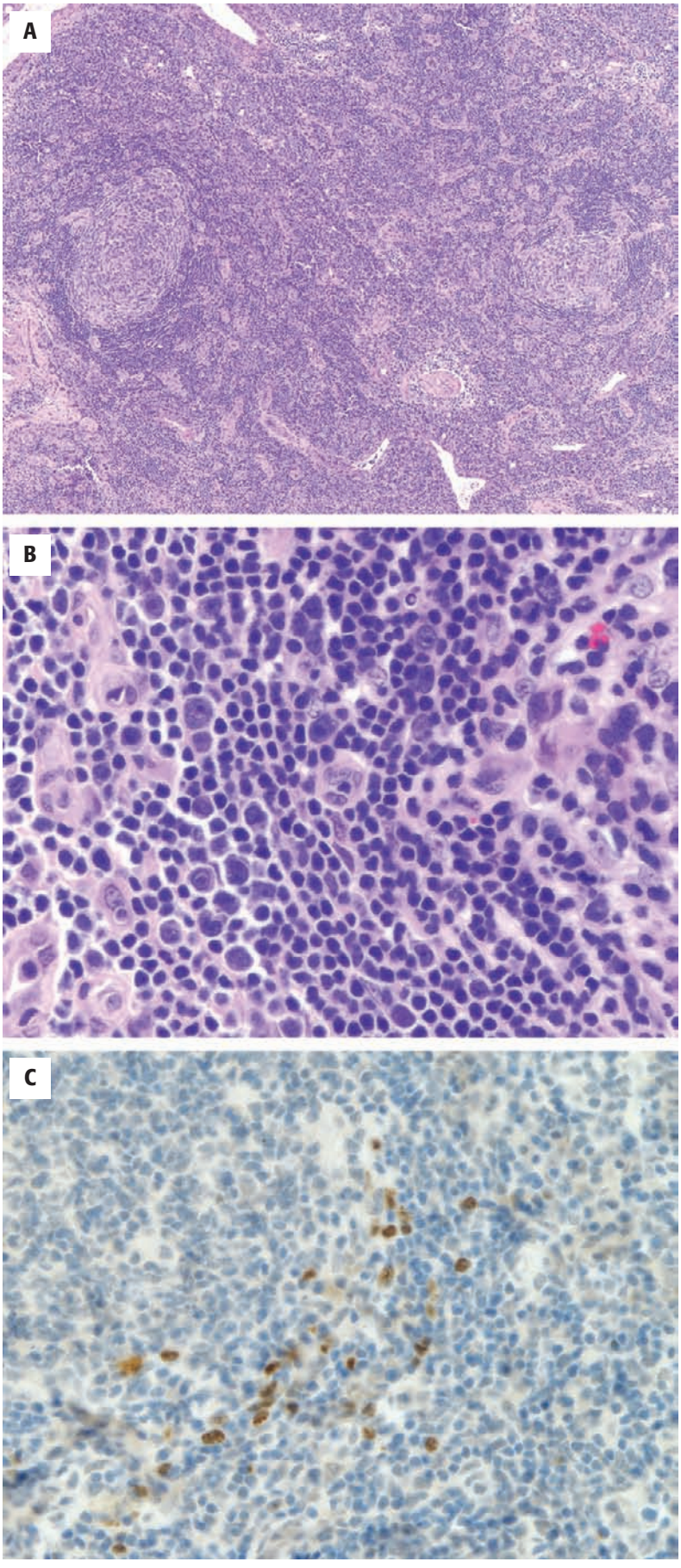


**FIGURE 4-23**

HHV8-associated multicentric Castleman disease in a human immunodeficiency virus-positive patient. **A,** Low power shows a lymph node with a few hyaline-vascular follicles and an interfollicular area with increased numbers of blood vessels. **B,** High power of a portion of a follicle shows a mantle zone composed of small lymphocytes with scattered large lymphoid cells. A small area of follicle center is seen within the mantle, and a few blood vessels are seen in the interfollicular area on the opposite side of the mantle. **C,** The large lymphoid cells in the mantle (plasmablasts) are human herpesvirus 8 positive.

### Plasma Cell Castleman Disease

#### Autoimmune Disease

Lymphadenopathy in patients with rheumatoid arthritis shows features that overlap those of plasma cell Castleman disease. Rare patients with systemic lupus erythematosus show lymphadenopathy with features of Castleman disease. Clinical correlation and strict use of morphologic criteria are helpful in distinguishing between the two.

#### Plasmacytoma

Nodal plasmacytomas are rare, and a proliferation of lymphoid follicles is not a feature. Polytypic plasma cells exclude plasmacytoma but are often seen in plasma cell Castleman disease. Finding monotypic κ-positive plasma cells is unusual in plasma cell Castleman disease. However, among cases with monotypic λ-positive plasma cells, the distinction may be to some extent a matter of terminology.

#### Lymphoplasmacytic Lymphoma

Lymphoplasmacytic lymphoma often partially involves nodes and can produce an interfollicular infiltrate of lymphocytes and plasma cells with sparing of follicles that resembles plasma cell Castleman disease. Demonstration of an interfollicular population of B lymphocytes is evidence against plasma cell Castleman disease. Unlike plasma cell Castleman disease, lymphoplasmacytic lymphoma usually expresses monotypic IgM and does not preferentially express λ light chain.

### Hyaline-Vascular and Plasma Cell Castleman Disease

#### Hodgkin Lymphoma

Occasional cases of Hodgkin lymphoma are assocta-ted with focal changes reminiscent of hyaline-vascular Castleman disease or plasma cell Castleman disease. The Hodgkin lymphoma can be in the interfollicular area of the tissue with the Castleman disease–like changes, or it can occupy an area adjacent to the Castleman disease–like area.

#### Miscellaneous

One or more features of Castleman disease can be found in lymph nodes showing reactive hyperplasia that is otherwise nonspecific. Such cases should not be interpreted as Castleman disease.

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The complete reference list is available online at www.expertconsult.com.

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# 5

# Bone Marrow Failure Syndromes

James R. Cook, MD, PhD

## INTRODUCTION

This chapter describes the peripheral blood and bone marrow findings in bone marrow failure syndromes leading to peripheral cytopenias. Two major entities seen primarily in adults lead to peripheral pancytopenia: acquired aplastic anemia and paroxysmal nocturnal hemoglobinuria. Fanconi anemia (FA) is also described as the paradigm of congenital bone marrow failure syndromes, with additional discussion of less common congenital disorders associated with peripheral cytopenias caused by bone marrow failure. Importantly, bone marrow failure may also be seen in the setting of myeloid neoplasms, such as myelodysplastic syndromes, or secondary to other malignancies such as the large granular lymphocyte leukemias. These disorders are discussed elsewhere in this book. Each of the diseases discussed is rare and diagnostically challenging. In addition, because the characteristic morphologic findings are generally nonspecific, these entities are often best defined as clinicopathologic syndromes. Establishing the most precise diagnosis requires the correlation of the clinical and morphologic findings as well as special ancillary testing. A close working relationship between the clinicians treating the patient and the pathologist interpreting the morphologic findings is essential for optimal patient care.

## ACQUIRED APLASTIC ANEMIA

Aplastic anemia (AA), broadly defined as peripheral blood pancytopenia secondary to bone marrow hypocellularity, can be either acquired or congenital. Acquired AA can be either primary (idiopathic) or secondary. This section focuses specifically on idiopathic acquired AA, whereas a subsequent section discusses FA, the paradigm of inherited AA.

### Clinical Features

In the United States, acquired AA is a rare disorder, with an approximate incidence of two cases per 1 million population per year. There is a large degree of geographic variation, however, with the incidence of AA being several times higher in Asia than in the United States or Western Europe. Although acquired AA can occur at any age, there are two characteristic peaks in the age at onset: one in childhood and young adulthood and a second peak after 60 years of age. Overall, the incidence in males and females is approximately equal. AA can occur secondary to a wide variety of toxic agents, including numerous drugs, viruses, environmental chemicals, and other agents (Table 5-1). In approximately 70% to 80% of cases, AA is idiopathic; therefore the remainder of this section discusses only idiopathic AA. The pathophysiology of idiopathic AA is complex, but in general idiopathic AA appears to be an autoimmune process, with both T cell– and B cell–mediated mechanisms implicated in the disease. The presenting clinical features are nonspecific but relate directly to the presence of peripheral blood cytopenia. Patients may exhibit bruising or mucocutaneous bleeding due to thrombocytopenia, fatigue due to anemia, or infections secondary to neutropenia.

### Pathologic Features

#### Peripheral Blood

The peripheral blood usually displays pancytopenia (Figure 5-1). By definition, at least two of the following criteria are present: hemoglobin less than 10 g/dL, platelet count less than $50 \times 10^9$/L, absolute neutrophil count less than $1.5 \times 10^9$/L. The residual neutrophils

**ACQUIRED APLASTIC ANEMIA—FACT SHEET**

**Definition**
- Peripheral blood pancytopenia caused by marrow hypoplasia

**Incidence**
- Rare (annual incidence of two new cases per 1 million population)
- Fewer than 1000 cases per year in the United States

**Morbidity and Mortality**
- Severe AA is rapidly fatal without treatment
- Cases that are not severe may have a prolonged, mild clinical course

**Gender, Race, and Age Distribution**
- No major gender or racial difference in the United States
- Most cases present at 15 to 25 years of age, with second peak in incidence after 60 years of age
- More common in Asia than in the United States and Western Europe

**Clinical Features**
- AA may be secondary to toxic agents, but most cases are idiopathic and likely autoimmune mediated
- Symptoms reflect peripheral cytopenia: fatigue (anemia), bruising or bleeding (thrombocytopenia), or infections (neutropenia)
- Some cases eventually develop features of paroxysmal nocturnal hemoglobinuria

**Prognosis and Therapy**
- Prognosis relates to degree of cytopenia
- Bone marrow transplantation can be curative in many cases
- Immunosuppressive treatment is effective but with risk of relapse
- There is a long-term risk of MDS or AML

**APLASTIC ANEMIA—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Peripheral blood pancytopenia without polychromasia, without myeloid left shift
- Markedly hypocellular aspirate and biopsy specimen, usually less than 10% cellular
- May have islands of residual hematopoiesis
- Dyspoietic changes, if any, limited to erythroid lineage

**Immunohistochemistry**
- $CD34^+$ blasts absent or rare and scattered

**Cytogenetics**
- Normal karyotype in most cases

**Differential Diagnosis**
- Hypocellular MDS
- Congenital aplastic syndrome (e.g., FA)
- PNH
- Large granular lymphocyte leukemias

**TABLE 5-1**
**Causes of Secondary Aplastic Anemia**

| |
|---|
| ***Drugs and Toxins*** |
| Chloramphenicol |
| Nonsteroidal antiinflammatory drugs |
| Antiepileptics |
| Gold |
| Chemotherapy agents |
| Benzene |
| Insecticides |
| ***Infectious Process*** |
| Epstein-Barr virus |
| Hepatitis |
| Influenza |
| Parvovirus |
| Human immunodeficiency virus (HIV) |
| ***Others*** |
| Radiation therapy |
| Autoimmune disease |

and platelets are morphologically unremarkable, and immature myeloid cells are not detected. The red blood cells are often macrocytic but are normocytic in some cases. There is reticulocytopenia. In occasional cases, there is initially unilineage cytopenia (often thrombocytopenia) with a progression to pancytopenia over time. The degree of the peripheral blood cytopenia has been used to separate patients into prognostically distinct groups. Cases are categorized as severe AA if two of the following are present: neutrophil count less than $0.5 \times 10^9$/L, platelet count less than $20 \times 10^9$/L, or reticulocyte count less than $20 \times 10^9$/L. Cases with an absolute neutrophil count of less than $0.2 \times 10^9$/L are categorized as very severe AA. Cases not fulfilling criteria for severe or very severe AA are classified as nonsevere AA.

### Bone Marrow

The bone marrow aspirate in AA is hypocellular and in many cases is markedly hypocellular. The spicules consist predominantly of fatty tissue. The scant cellularity present is typically composed of lymphocytes, plasma cells, histiocytes, and other stromal elements (Figure 5-2). Care should be taken not to misinterpret the residual lymphocytes and plasma cells as a lymphoproliferative disorder or plasma cell myeloma. Immunohistochemical stains or flow cytometric studies may be helpful. Some cases will display residual foci of hematopoiesis, but the overall cellularity remains greatly reduced. When residual hematopoietic elements are present, the erythroid elements may show dyspoietic

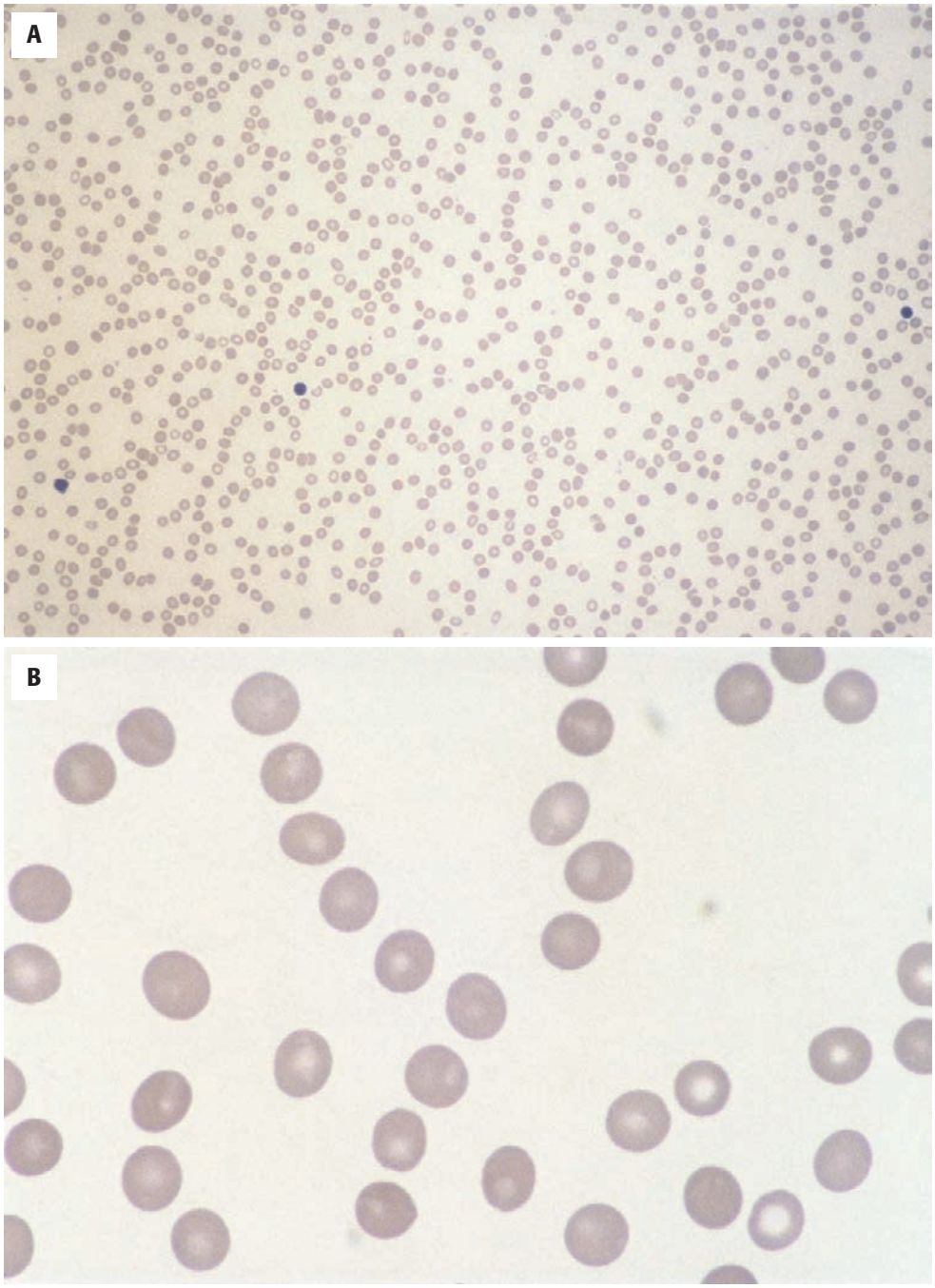


**FIGURE 5-1**

Acquired aplastic anemia: peripheral blood. **A,** At low power, there is pancytopenia. The few remaining white blood cells consist of unremarkable small lymphocytes. **B,** At higher power, the red blood cells are macrocytic or normocytic and show no polychromasia.

changes. However, granulocytic and megakaryocytic dysplasia are not detected. The bone marrow core biopsy is also hypocellular (<30%; Figure 5-3). Some cases may display residual islands of hematopoietic tissue, sometimes referred to as *hot pockets*. In most cases, however, the cellularity is markedly decreased, with less than 10% residual cellularity. The residual stromal elements and bone are normal.

## Ancillary Studies

### Cytogenetics

Classical cytogenetic studies are routinely performed on bone marrow aspirate specimens at most institutions. Cases of AA will generally display a normal karyotype. However, up to 12% of AA cases have been

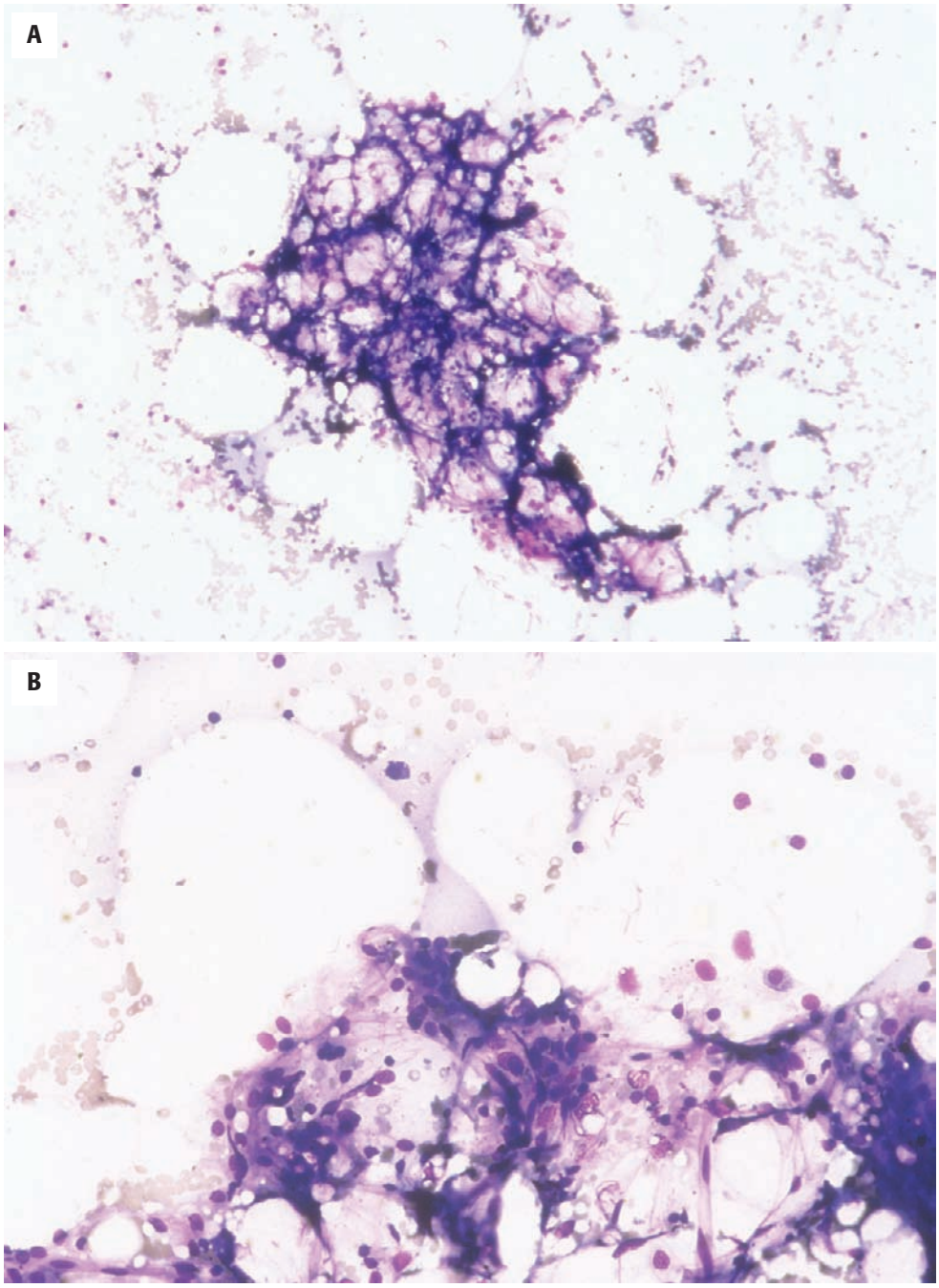


**FIGURE 5-2**

Acquired aplastic anemia: bone marrow aspirate. **A,** The aspirate smear contains numerous markedly hypocellular spicules. **B,** At high power, there is little to no residual hematopoietic activity. The scant cellularity present consists of small lymphocytes, plasma cells, histiocytes, and other stromal elements.

reported to show clonal cytogenetic abnormalities, including del(5q), trisomy 6, trisomy 8, and abnormalities of chromosomes 7 and 13. These abnormal clones tend to be present in only a small number of metaphases and are often transient or resolve with immunosuppressive therapy. In such cases, results should be interpreted very cautiously, and a definitive myelodysplastic syndrome should be diagnosed only in the presence of accompanying morphologic dysplasia. The identification of monosomy 7 may be particularly associated with progression to a morphologic myelodysplastic syndrome.

#### Flow Cytometry

Flow cytometric studies will demonstrate a normal phenotype in any residual hematopoietic elements. Such studies are useful primarily to exclude the presence

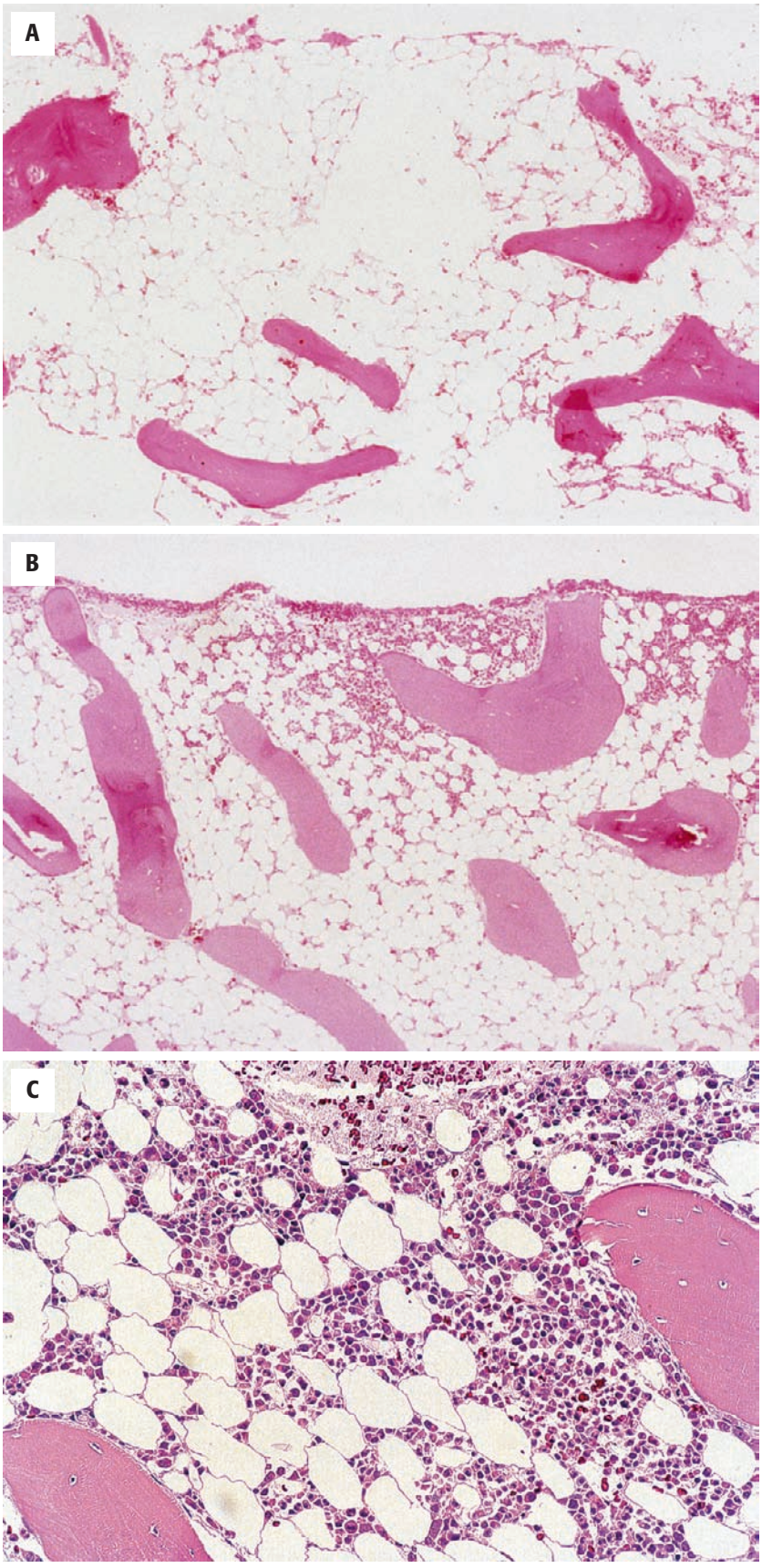


**FIGURE 5-3**

Acquired aplastic anemia: bone marrow biopsy. **A,** The bone marrow core biopsy is markedly hypocellular with little to no hematopoietic activity. **B,** In some cases, residual foci of hematopoiesis are seen, known as *hot pockets*. Overall, however, the bone marrow biopsy is markedly hypocellular. **C,** Higher power view of small, cellular focus. The residual hematopoietic activity is morphologically unremarkable.

of an abnormal myeloid population, such as in a hypocellular myelodysplastic syndrome, or the presence of an underlying lymphoproliferative disorder.

#### Immunohistochemistry

$CD34^+$ mononuclear cells, if present, are rare and scattered. The presence of increased numbers of $CD34^+$ blasts or clusters of $CD34^+$ cells is suggestive of a hypocellular myelodysplastic syndrome.

### Differential Diagnosis

The major differential diagnosis of acquired AA in adults is a hypocellular myelodysplastic syndrome (MDS). If significant dyspoietic changes are noted in the residual hematopoietic elements, the possibility of a hypocellular MDS should be considered. The lack of cytogenetic abnormalities and the presence of a normal immunophenotype favor a diagnosis of AA. Although cases of FA and other congenital aplasia syndromes typically present in childhood, some cases can manifest well into adulthood. As described later, chromosome breakage studies are essential for the recognition of FA. The presence of skeletal abnormalities, growth retardation, or other congenital abnormalities are also suggestive of a congenital disorder. Some cases of paroxysmal nocturnal hemoglobinuria will display marked hypocellularity. As discussed in the section on paroxysmal nocturnal hemoglobinuria (PNH), there is a fundamental interrelationship between AA and PNH. Flow cytometric analysis for glycosylphosphatidylinositol (GPI)-linked proteins is useful in distinguishing pure AA from PNH-AA overlap syndrome (see PNH). Last, marked hypocellularity may be apparent in some lymphoproliferative disorders, especially the large granular lymphocyte leukemias. Flow cytometric analysis in such cases will document the presence of increased numbers of cytotoxic lymphocytes (usually $CD57^+CD3^+$ T cells or $CD16/CD56^+CD3^-$ natural killer cells). Approximately 10% of patients with apparent idiopathic AA have been reported to show abnormally shortened telomeres and may represent part of the spectrum of dyskeratosis congenita. Assays for telomere length may also be helpful in evaluating apparent idiopathic AA.

### Prognosis and Therapy

The prognosis in AA depends on the degree of the patient's pancytopenia. In cases that are not severe, the clinical course is prolonged, and some patients, especially those with only minimal cytopenia, may have spontaneous recoveries. In severe cases, the untreated disease is generally fatal. Patients may be treated successfully with immunosuppression, in keeping with an autoimmune cause in most cases. Notably, patients found to have abnormally shortened telomeres have been reported to be generally unresponsive to immunosuppressive therapy. Allogeneic bone marrow transplantation is also curative for most patients. Patients who are treated successfully with either immunosuppression or transplantation, however, remain at risk for the development of secondary clonal disorders. Some cases will evolve into PNH, as discussed later in this chapter. Patients are also at long-term risk of MDS and overt acute myeloid leukemia (AML). In one large series, the risk of MDS or AML at 7 years of follow-up was 15%.

## PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

PNH is a syndrome of hemolysis, thrombosis, or bone marrow failure that is secondary to an acquired defect in the production of GPI-linked proteins on the cell membranes of red blood cells, platelets, and other hematopoietic cells. GPI anchors are required for the membrane-bound expression of a wide variety of proteins, including important complement regulatory proteins such as CD55 (DAF) and CD59 (MIRL). In cases of PNH, acquired somatic mutations occur in the *PIG-A* gene, an X-linked gene whose protein product is required for the synthesis of GPI anchors. The subsequent lack of CD55 and CD59 at the cell surface of hematopoietic elements is thought to lead to complement-mediated lysis of red blood cells and complement-mediated activation of platelets. The former leads to the hemolytic complications, and the latter leads to the thrombotic complications seen in PNH. The loss of other GPI-anchored proteins may also contribute to the manifestations of this disease.

### Clinical Features

PNH can occur at any age, although the median age at presentation is approximately 40 years. Males and females are equally affected. The clinical findings in PNH are related to the presence of hemolysis, thrombosis, or bone marrow failure. Patients with anemia may exhibit fatigue and lethargy, and jaundice may be present at times of ongoing hemolysis. In classic cases, there may be dark-colored urine secondary to hemoglobinuria, especially in the first morning urine; however, this supposedly classic presentation is actually uncommon. Venous thrombosis, which occurs in approximately 20% of patients, is an important source of morbidity and

**PAROXYSMAL NOCTURNAL HEMOGLOBINURIA—FACT SHEET**

**Definition**
- An acquired, clonal disorder of hemolytic anemia, thrombosis, or bone marrow failure secondary to loss of GPI-linked cell membrane proteins

**Incidence**
- Rare, estimated at 1 in 100,000

**Morbidity and Mortality**
- Major sources of mortality are thrombosis and bone marrow failure

**Gender, Race, and Age Distribution**
- Median age at onset: 40 years
- Males and females equally affected
- Many ethnic groups affected

**Clinical Features**
- Variable clinical presentation
- Anemia secondary to hemolysis, marrow failure, or both
- Increased thrombosis caused by platelet activation
- Risk of bleeding because of thrombocytopenia

**Prognosis and Therapy**
- Chronic clinical course
- Small risk of transformation to acute leukemia
- Treated by transfusion support, immunosuppression, and complement inhibiting monoclonal antibody (eculizumab)
- Bone marrow transplantation possibly curative

mortality in PNH. In patients with pancytopenia, thrombocytopenia may lead to petechia and bleeding. There is also an intriguing, although poorly understood, relationship to AA. In approximately one third of cases, patients carry a prior diagnosis of AA before development of symptoms of PNH. In another one third of PNH cases, patients will eventually meet criteria for severe AA. The term *PNH-AA overlap syndrome* has been used to describe these patients. The final third of PNH cases display primarily hemolytic features, without development of other significant cytopenia.

## Pathologic Features

### Peripheral Blood

The morphologic findings in the peripheral blood are highly variable. In some cases the predominant process is a hemolytic anemia. The red blood cells show polychromasia, and numbers of reticulocytes are increased (Figure 5-4). In other patients, however, the predominant clinical picture is one of bone marrow failure. In such cases, the anemia is often macrocytic, and there is a characteristic lack of polychromasia and reticulocytosis. Platelets are often mildly to moderately decreased. In patients with the full PNH-AA overlap syndrome, there will be pancytopenia.

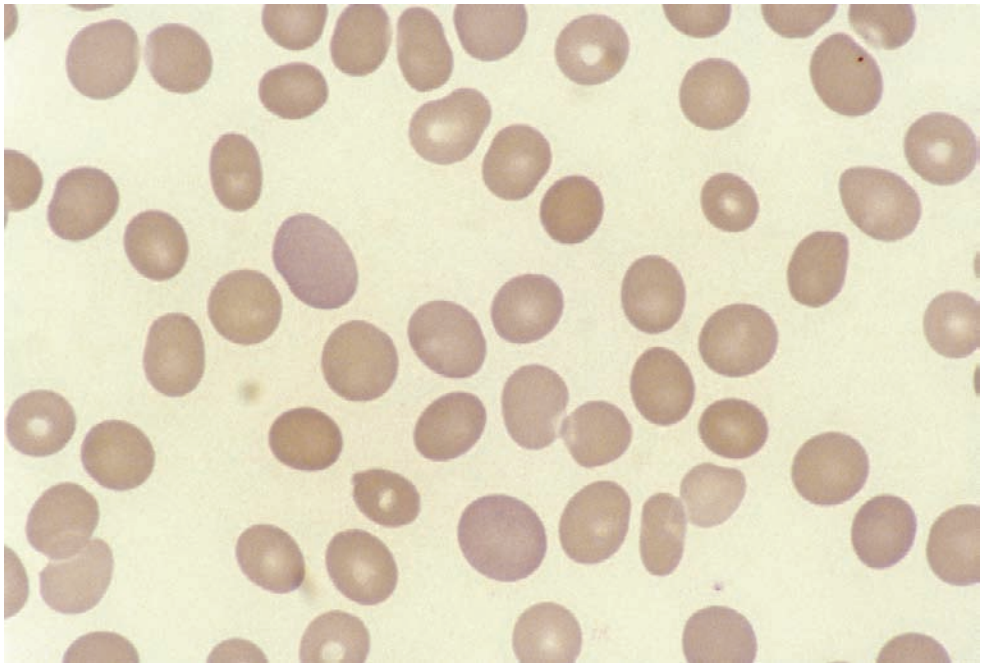

**FIGURE 5-4**

Paroxysmal nocturnal hemoglobinuria: peripheral blood. In this case of paroxysmal nocturnal hemoglobinuria with prominent hemolysis, the red blood cells are macrocytic with polychromasia. In cases with more prominent marrow failure, polychromasia and reticulocytosis may be absent.

**PAROXYSMAL NOCTURNAL HEMOGLOBINURIA—PATHOLOGIC FEATURES**

**Microscopic Findings**

- Variable peripheral blood and bone marrow morphology are seen
- Hemolytic cases display polychromasia and reticulocytosis with bone marrow erythroid hyperplasia
- Other cases show pancytopenia with marrow aplasia

**Ancillary Studies**

- Positive Ham's test
- Flow cytometry for GPI-linked proteins (e.g., CD55, CD59, FLAER) more sensitive than Ham's test
- Cytogenetic studies showing normal karyotype

**Differential Diagnosis**

- Autoimmune hemolytic anemia
- Aplastic anemia
- MDS

#### Bone Marrow

As with the peripheral blood, the morphologic findings in the bone marrow may also be highly variable in PNH (Figures 5-5 and 5-6). In patients with anemia that is mainly secondary to hemolysis, the bone marrow will generally be hypercellular with an erythroid predominance. In cases in which bone marrow failure is the predominant feature, there is hypocellularity that may be severe. Dyspoietic changes can be seen in those cases with florid erythroid hyperplasia; however, dyspoietic changes in the myeloid or megakaryocytic series are generally not seen.

## Ancillary Studies

#### Ham's Test

In Ham's test, complement is fixed to red blood cells in the setting of an acidic pH. Under these conditions, red cells in PNH are complement sensitive, whereas normal red blood cells are resistant to complement-mediated lysis. Although an abnormal Ham's test result is highly specific for PNH, the test is not very sensitive. For this reason, flow cytometric testing has recently replaced the traditional Ham's test as the technique of choice for diagnosing PNH.

#### Flow Cytometry

Flow cytometric studies can be used to detect the decreased expression of GPI-anchored proteins on granulocytes and red cells. Commonly used antibodies include those against CD55 and CD59 (Figure 5-7), although analysis of other GPI-linked proteins such as CD16 and CD66b on neutrophils or CD24 on lymphocytes may also be useful. More recently, many laboratories have adopted use of a fluorescently labeled aerolysin (FLAER), which binds specifically to GPI anchors. Flow cytometric studies have shown a greater sensitivity for detecting abnormal cells than the traditional Ham's acid lysis test. However, it should be noted that cells lacking GPI anchors can also be seen in other settings, especially when the percentage of abnormal cells is small. In particular, small numbers of PNH-type cells can be seen in cases of myelodysplasia or in patients with AA who lack any other features of PNH. The significance of these findings and the relationship of such cases to typical PNH are currently controversial.

## Differential Diagnosis

In cases of PNH in which the dominant feature is bone marrow failure, the differential diagnosis includes myelodysplasia and AA. The presence of dyspoietic changes in the myeloid or megakaryocytic series or the finding of clonal cytogenetic abnormalities suggests an MDS. Cases of pure AA lack significant populations of PNH-type cells in flow cytometric studies. A low percentage of PNH-type cells (usually less than 1%) can be seen in AA and myelodysplastic syndrome and does not imply a clinical diagnosis of PNH; however, such patients, particularly those with AA, may respond to immunosuppressive therapy. Serial measurements for PNH clone size is warranted because of the potential for subsequent clinical PNH. In cases dominated by the presence of hemolysis, the differential diagnosis includes other forms of intravascular hemolysis. A positive Coombs test result strongly suggests an autoimmune hemolytic anemia rather than PNH. Cases of drug-induced hemolysis or ABO-incompatible transfusion reactions are distinguished by the clinical history and the lack of PNH-type cells by flow cytometry.

## Prognosis and Therapy

A median survival of 10 to 15 years following a diagnosis of PNH has been reported. However, with the advent of flow cytometric testing, many cases of PNH are being diagnosed at an earlier stage of disease, and the survival of patients diagnosed with modern flow cytometric studies may therefore be significantly longer. The primary treatment approach in PNH has been to use immunosuppression to control hemolysis and increase bone marrow cellularity. Episodes of thrombosis are aggressively treated, because thrombotic complications

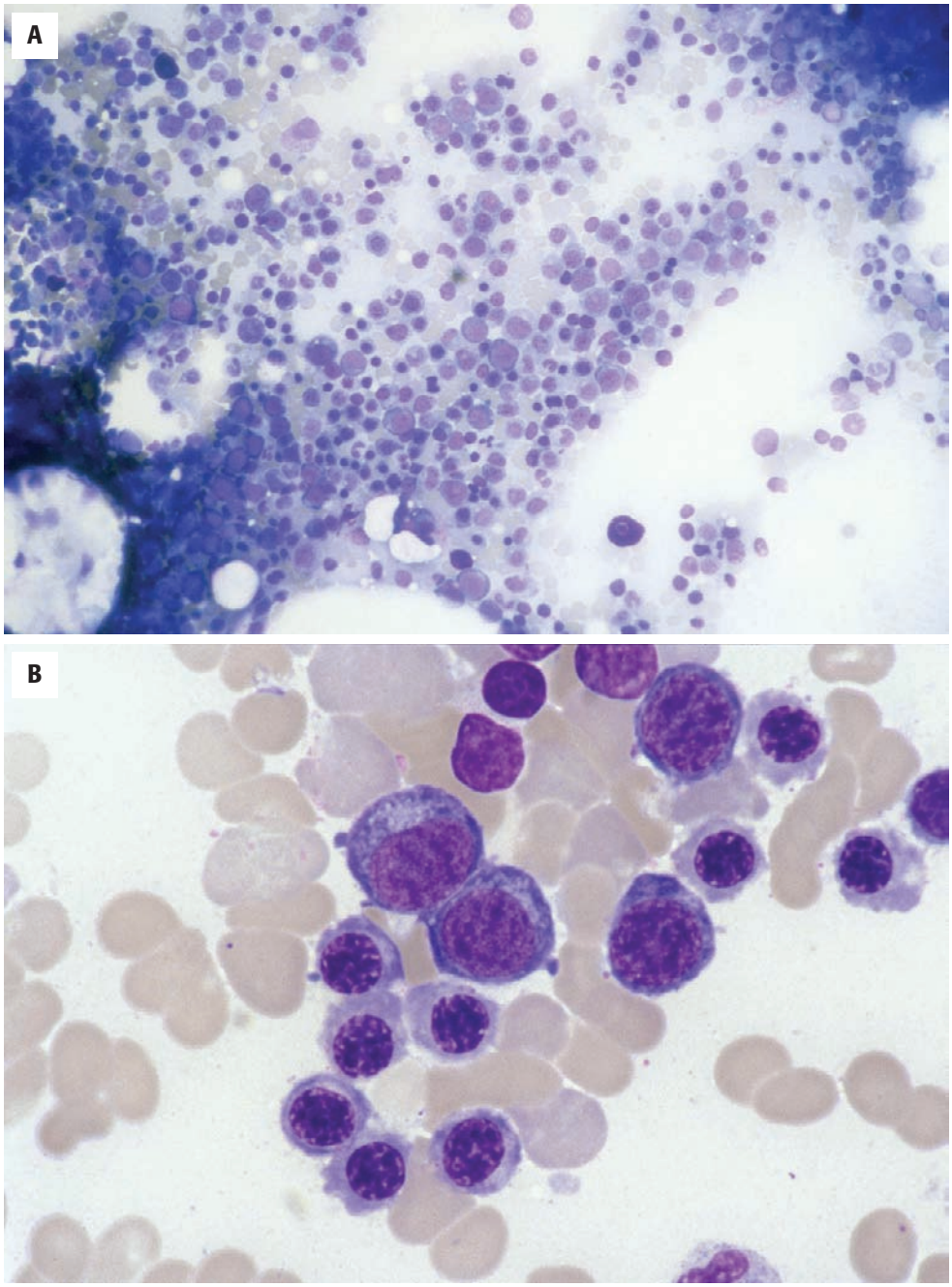


**FIGURE 5-5**

Paroxysmal nocturnal hemoglobinuria: bone marrow aspirate. **A,** In cases with prominent hemolysis, the bone marrow aspirate is cellular with an erythroid predominance. **B,** High-power view of erythroid elements with an essentially unremarkable morphology. Dyspoietic changes may be identified in other cases with florid erythroid hyperplasia.

appear to be a major source of mortality in PNH. More recently, the monoclonal antibody eculizumab, an anti-C5 complement inhibitor, has been shown to decrease hemolysis, the need for transfusion, and possibly thrombosis. Bone marrow transplantation may be curative, but currently is reserved primarily for cases with severe marrow failure or life-threatening thrombosis. Finally, there is a relatively low long-term risk of evolution to acute leukemia (less than 3% of cases overall).

## FANCONI ANEMIA

FA is a multigenic disease, with 13 different genes being identified to date *(FANCA-FANCN)*. All the mutated genes appear to have role in a common pathway of DNA repair. Twelve of the genes are inherited in an autosomal recessive fashion, whereas one (*FANCB* gene, 2% of patients) is X-linked. A detailed discussion of the molecular biology of FA is beyond the scope

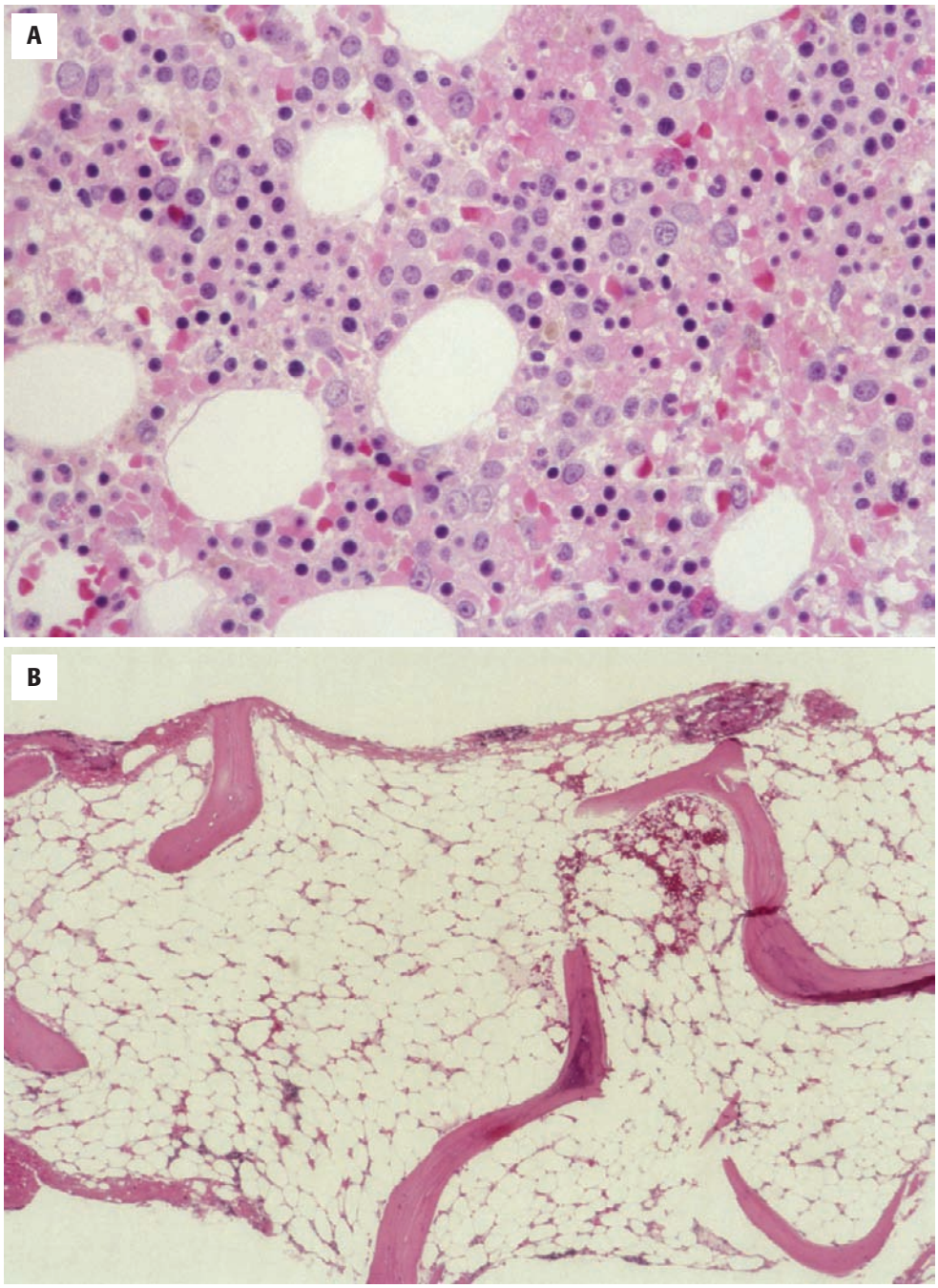


**FIGURE 5-6**

Paroxysmal nocturnal hemoglobinuria: bone marrow biopsy. **A,** In this case of paroxysmal nocturnal hemoglobinuria with prominent hemolysis, the bone marrow is hypercellular with an erythroid predominance. **B,** In cases with features of the paroxysmal nocturnal hemoglobinuria–aplastic anemia overlap syndrome, the bone marrow may be markedly hypocellular.

of this chapter, and detailed reviews are listed in the Suggested Reading, available on the ExpertConsult website.

## Clinical Features

The clinical features are diverse, but approximately 90% of FA cases will display hematologic abnormalities, usually manifest as pancytopenia secondary to bone marrow aplasia. Cytopenia typically will manifest in the first decade of life, although in some cases diagnosis may be delayed until early adulthood. The most common nonhematologic abnormalities include hyperpigmentation and hypopigmentation of the skin, skeletal defects (anomalies of the thumbs), renal abnormalities, and growth retardation. Many other types of abnormalities may occur at lower frequency. Patients with FA are also at increased risk of neoplasia, including MDS and AML, as well as squamous cell carcinomas.

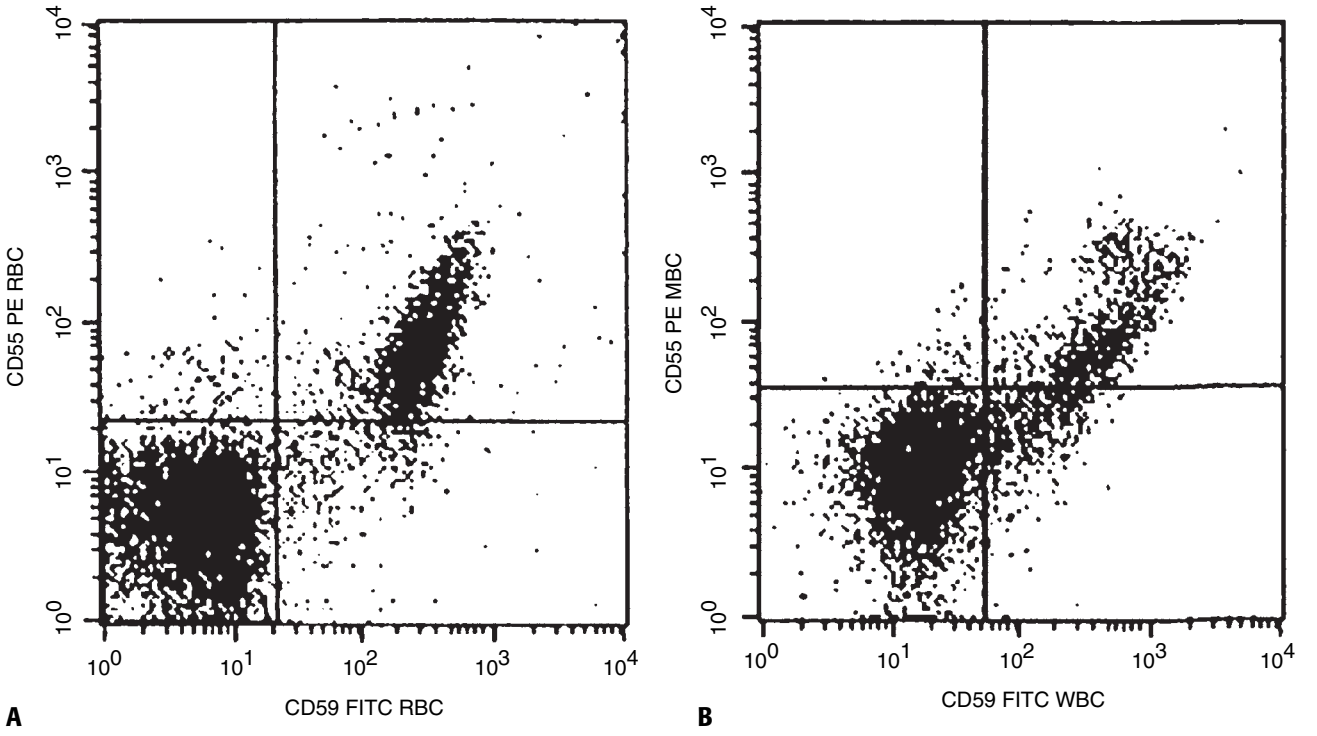


**FIGURE 5-7**

Paroxysmal nocturnal hemoglobinuria: flow cytometry testing. **A,** Analysis of red blood cells. Normal red blood cells show coexpression of CD55 and CD59 *(upper right),* whereas PNH-type red cells lack expression of both markers *(lower left).* **B,** Analysis of granulocytes. Normal granulocytes expressing CD55 and CD59 are present *(upper right)* as well as PNH-type granulocytes lacking both CD55 and CD59 *(lower left).*

## FANCONI ANEMIA—FACT SHEET

### Definition

- Multigenic disorder with susceptibility to chromosomal breakage
- Displays bone marrow failure with or without other congenital abnormalities

### Incidence

- Approximately 3 cases per 1 million population

### Morbidity and Mortality

- Bone marrow failure causes increased risk of infection (neutropenia), bleeding (thrombocytopenia), and symptoms of fatigue (anemia)
- There is an increased risk of malignancy, including both hematologic and solid tumors

### Gender, Race, and Age Distribution

- FA occurs equally in males and females and affects many ethnic groups
- Median age at onset of pancytopenia is 7 years, although there is substantial variation

### Clinical Features

- Clinical findings are heterogeneous
- Approximately 30% display only hematologic manifestations
- Seventy percent of patients have other abnormalities, including skin pigmentation anomalies, skeletal defects, renal malformations, and growth retardation

### Prognosis and Therapy

- There is a long-term risk of MDS and AML
- Bone marrow transplantation can be curative for hematologic disease, but an increased risk of solid tumors remains
- Survival is variable, but recent studies show median survival of 24 years

## Pathologic Features

### Peripheral Blood

The peripheral blood is typically normal at birth, and the first abnormality identified is usually a macrocytosis without anemia. As the child ages, cytopenia will eventually develop. In many cases, thrombocytopenia or leukopenia occurs first, followed by anemia and pancytopenia. The median age at onset of pancytopenia is 7 years. There is minimal, if any, morphologic evidence of dysplasia in the peripheral blood.

**FANCONI ANEMIA—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Peripheral blood pancytopenia
- Bone marrow hypocellularity
- Little to no dysplastic changes in residual hematopoiesis

**Cytogenetics**
- Susceptibility to chromosome breakage identified by diepoxybutane or mitomycin C testing
- Clonal karyotypic abnormalities generally absent

**Differential Diagnosis**
- Acquired AA
- Other congenital marrow failure syndromes

### Bone Marrow

The bone marrow features also vary with the course of disease progression. Early in childhood the results of a bone marrow examination may be morphologically unremarkable. By the time peripheral blood pancytopenia has developed, however, the bone marrow features mirror those seen in patients with acquired AA. The bone marrow aspirate is typically hypocellular with numerous fatty spicules (Figure 5-8). The residual cellularity often consists primarily of lymphocytes, plasma cells, and stromal cells. The bone marrow core biopsy will display marked hypocellularity. The aspirate and biopsy specimens, however, may each show foci of residual hematopoiesis, such that generous sampling of the bone marrow is needed for complete evaluation. Blasts are not increased, and there is little or no morphologic evidence of dysplasia. The presence of increased numbers of blasts or significant dysplastic changes in any lineage is suggestive of the development of an MDS.

## Ancillary Studies

### Chromosome Breakage Analysis

The defining feature of FA is an increased susceptibility to chromosomal breakage. When FA cells are treated with clastogenic agents such as mitomycin C or

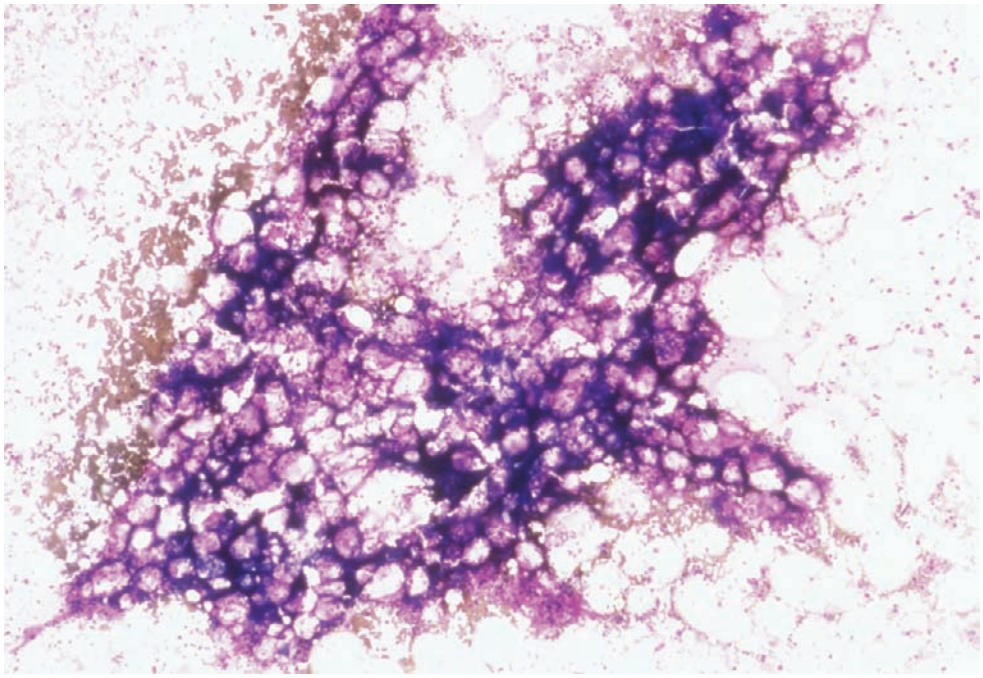

**FIGURE 5-8**

Fanconi anemia: bone marrow aspirate. The aspirate smear contains numerous markedly hypocellular spicules. The morphology is similar to that seen in acquired aplastic anemia. *(Courtesy Steven H. Swerdlow, University of Pittsburgh.)*

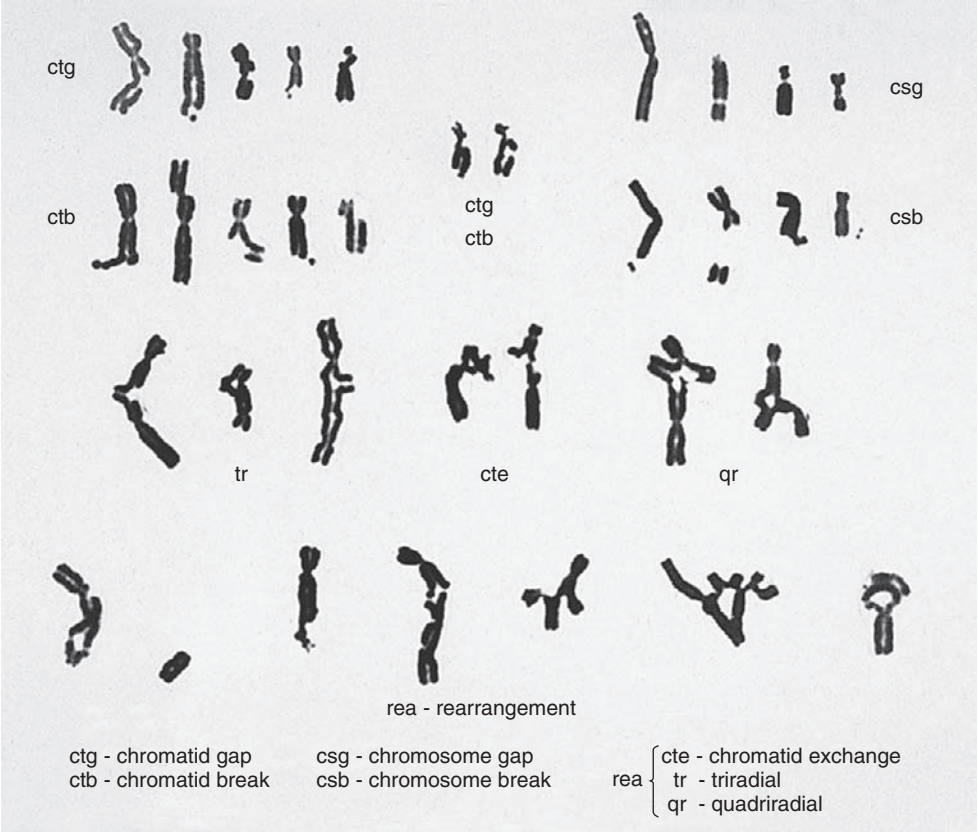


**FIGURE 5-9**

Fanconi anemia: chromosome breakage studies. Karyotype from a case of Fanconi anemia stimulated with clastogenic agents. Numerous chromosomal breaks and other anomalies are present. *(From Wickramasinghe SN, McCullough J*: Blood and Bone Marrow Pathology, *New York, 2003, Churchill Livingstone, p 259.)*

diepoxybutane, the resulting DNA damage is not properly repaired, and breaks in the chromosomes are visible in metaphase spreads (Figure 5-9). Testing for chromosomal breakage is therefore an essential component in the workup for children and young adults with hypocellular bone marrow.

### Metaphase Cytogenetic Studies

In addition to chromosome breakage studies, metaphase cytogenetic analysis is also important in the evaluation of suspected FA. Uncomplicated cases of FA are usually associated with a normal karyotype, although spontaneous chromosome breaks, similar to those induced by mitomycin C or diepoxybutane testing, can occur in some patients. Because patients with FA are at high risk of developing myelodysplasia, routine cytogenetic analysis is recommended. The presence of a clonal cytogenetic abnormality suggests development of an MDS. However, transient cytogenetic clones of uncertain clinical significance have been reported in some cases of FA. It is therefore important to carefully consider all the clinical, morphologic, and cytogenetic findings for final evaluation.

### Molecular Studies

Gene sequencing studies are available at some highly specialized laboratories or through international, disease-specific registries. Using lymphoblast complementation studies, in which lymphoblast cell lines derived from patient cells are hybridized with cell lines known for carrying defects, a complementation group can be defined that is associated with a particular genetic abnormality. Sequencing studies can then be used to confirm a precise mutation or to screen family members for carrier status.

## Differential Diagnosis

The differential diagnosis of FA includes acquired AA. Although the median age of onset of pancytopenia in FA is 7 years, onset can occur in some patients in early adulthood. There is therefore considerable overlap in the age groups of patients with FA and acquired AA. The morphologic findings in FA and AA are also highly similar. In some patients with FA, the correct diagnosis

is strongly suggested because of the presence of other congenital abnormalities. In other patients, however, only hematologic abnormalities are present, and chromosome breakage studies are required for definitive diagnosis. Other congenital syndromes associated with bone marrow failure must also be considered, as discussed later. FA can be distinguished from each of these other congenital disorders, however, by chromosomal breakage studies. Establishing a correct diagnosis of FA is extremely important, because conditioning regimens used in preparation for bone marrow transplant must be altered since patients with FA are highly sensitive to the DNA-damaging agents that are typically used.

### Prognosis and Therapy

Patients may be treated supportively with transfusions and growth factor therapy. However, the only definitive treatment for the hematologic features of FA is bone marrow transplantation. Importantly, successful transplantation does not ameliorate the increased risk of solid tumors in patients with FA. Patients undergoing bone marrow transplantation are also at risk of therapy-related malignancies, which can occur at a higher frequency in FA than in other conditions treated with bone marrow transplantation. A recent analysis of 20 years of data from the International Fanconi Anemia Registry demonstrated a median survival of 24 years. Approximately 5% to 10% of patients will eventually develop MDS or AML, with an actuarial risk as high as 50% by 40 years of age.

## OTHER CONGENITAL CYTOPENIA SYNDROMES

This section briefly summarizes the features of other rare inherited disorders leading to peripheral cytopenia resulting from bone marrow failure (Table 5-2). The bone marrow findings in some of these diseases have not been characterized as extensively as in FA. In many of these disorders, however, there is bone marrow hypocellularity that produces a differential diagnosis including FA and idiopathic AA. In many cases, the correct diagnosis may be suggested based on the constellation of congenital abnormalities. Confirmatory molecular studies are now available for several of these disorders.

**TABLE 5-2**
**Congenital Cytopenia Syndromes**

| |
|---|
| ***Anemia*** |
| Diamond-Blackfan anemia |
| Congenital dyserythropoietic anemias |
| Pearson syndrome |
| Cartilage-hair hypoplasia |
| ***Neutropenia*** |
| Severe congenital neutropenia (Kostmann syndrome) |
| Schwachman-Diamond syndrome |
| Cyclic neutropenia |
| Myelokathexis |
| Reticular dysgenesis |
| ***Thrombocytopenia*** |
| Congenital acquired megakaryocytopenia |
| Thrombocytopenia with absent radii |
| ***Pancytopenia*** |
| Fanconi anemia |
| Dyskeratosis congenita |
| Familial aplastic anemia |

### DIAMOND-BLACKFAN ANEMIA

Diamond-Blackfan anemia (DBA) is an autosomal dominant disorder characterized by macrocytic anemia and reticulocytopenia that manifests within the first year of age. Other somatic findings, including short stature, abnormal thumbs, craniofacial abnormalities, and cardiac or urogenital anomalies, are seen in approximately 25% of cases. The bone marrow shows a near complete absence of erythroid precursors. The neutrophilic and megakaryocytic series are generally unremarkable. The first gene to be identified in patients with DBA was *RPS19,* a ribosomal protein mutated in approximately 25% of patients. To date, eight additional genes have been identified in DBA, all encoding ribosomal proteins and accounting for approximately 50% of DBA cases in aggregate. These genetic findings have strongly suggested that DBA is due to ribosome haploinsufficiency, although it remains unclear why these abnormalities lead to selective loss of the erythroid lineage.

### CONGENITAL DYSERYTHROPOIETIC ANEMIAS

The congenital dyserythropoietic anemias (CDAs) are a heterogeneous group of disorders characterized by anemia manifesting in childhood with bone marrow erythroid hyperplasia and marked dyserythropoiesis. Three main types have been described (types I, II, and III), and other minor forms have also been reported in a small number of patients. In type I or type III CDA, anemia is typically macrocytic, whereas type II CDA manifests with normocytic anemia. The dyserythropoiesis noted on bone marrow biopsy is striking and highly characteristic, with frequent internuclear chromatin bridges noted in type I CDA, prominent binuclearity

noted in Type II, and numerous multinucleate erythroids found in type III CDA. Electron microscopic features specific for each type have also been described. In type II CDA there is abnormal glycosylation of membrane proteins, leading to positivity for the Ham's test (acidified serum lysis test). Recently, type I CDA has been shown to be secondary to mutations in the *CDAN1* gene, which encodes codanin, a protein of unknown function. Type 2 CDA has been shown to be due to mutations in the *SEC23B* gene, which encodes a component of the vesicle coat complex COP-II. The gene for CDA type III has been localized to a locus on chromosome 15q that has yet to be definitively characterized.

## PEARSON SYNDROME

Pearson syndrome is a rare congenital disorder of refractory anemia with ringed sideroblasts associated with pancreatic exocrine deficiency and metabolic acidosis. The syndrome is caused by variably sized deletions in mitochondrial DNA. Essentially all cases occur in sporadic fashion, although several cases have been reported in which the patient's mother had another mitochondrial disorder. Patients typically exhibit a macrocytic anemia during the first 6 months of life. Neutropenia and thrombocytopenia may also be present to varying degrees. Bone marrow examination reveals erythroid and myeloid precursors with cytoplasmic vacuoles. There is increased hemosiderin with ring sideroblasts. Bone marrow examination and confirmatory mitochondrial DNA testing establish the diagnosis. Survival is typically poor, with death generally attributed to metabolic acidosis or hematologic complications.

## CARTILAGE-HAIR HYPOPLASIA

Cartilage-hair hypoplasia is an autosomal-recessive form of dwarfism associated with metaphyseal dysostosis, hypoplasia of cartilage, and fine, lightly colored hair. Most patients display a macrocytic, reticulocytopenic anemia that may be accompanied by neutropenia in a subset of patients. Anemia is most severe during early childhood and in many cases resolves by adolescence. In vitro assays of the bone marrow demonstrate marked defects in formation of erythroid colonies and granulocyte-macrophage colonies, similar to other bone marrow failure syndromes. Cartilage-hair hypoplasia was initially described in Old Order Amish families and subsequently also found in families of Finnish background. The disorder is caused by mutations in the RNA component of the mitochondrial RNA-processing endoribonuclease (*RMRP* gene). There appears to be an increased risk of malignancy, including both hematologic and nonhematologic neoplasms.

## SEVERE CONGENITAL NEUTROPENIA (KOSTMANN SYNDROME)

Patients with severe congenital neutropenia (SCN), or Kostmann syndrome, develop recurrent infections secondary to absolute neutropenia in infancy. The peripheral red blood cells and platelets are generally normal. Bone marrow examination reveals a characteristic maturation arrest of the granulocytic series at the promyelocyte-myelocyte stage. Genetic studies have shown that SCN is molecularly heterogeneous. Approximately 50% of patients show an autosomal dominant inheritance with mutations in the *ELANE (ELA2)* gene, which encodes neutrophil elastase, a serine protease found in neutrophil primary granules. The same gene is mutated in cyclic neutropenia (see the following section), and these conditions must be distinguished by serial measurements of the absolute neutrophil counts. An autosomal recessive form of the disease is associated with a mutation in HAX1, a mitochondrial protein. Rare cases have been associated with mutations in *GFI1,* a transcriptional repressor that acts on the *ELANE (ELA2)* gene. Approximately 50% of cases have yet to be characterized at the molecular level. Patients with SCN have an increased risk of progression to MDS and AML.

## CYCLIC NEUTROPENIA

Cyclic neutropenia is an uncommon hereditary disorder that, like SCN, manifests in infancy with an isolated neutropenia. The two conditions are distinguished by the fact that in cyclic neutropenia the peripheral blood absolute neutrophil counts oscillate on an approximately 21-day cycle from within the normal range to markedly decreased. Serial measurements of the absolute neutrophil count two to three times per week for 6 to 8 weeks should demonstrate the typical periodicity. Bone marrow findings may vary with the course of the peripheral blood neutrophil counts, from a virtual absence of granulocytic cells beyond the promyelocyte stage at the nadir to granulocytic hyperplasia with complete maturation during the recovery phase. Cyclic neutropenia is caused by mutations in the *ELANE (ELA2)* gene, which is also mutated in severe congenital neutropenia. The molecular basis of the differing phenotypes in these two conditions remains uncertain, as overlapping mutations have been identified in some patients. In contrast to SCN, there does not appear to be a significantly increased risk of progression to MDS and AML.

## SHWACHMAN-DIAMOND SYNDROME

Shwachman-Diamond syndrome is a rare autosomal-recessive disorder characterized by pancreatic exocrine insufficiency and neutropenia. Other congenital abnormalities are also often present. Many patients develop anemia or thrombocytopenia, and pancytopenia is present in a smaller subset. Neutropenia is typically present at birth or develops in early infancy. When pancytopenia develops, the average age is approximately 3 years. Bone marrow examination typically reveals a hypoplastic marrow. Recent studies have shown that in more than 90% of patients, Shwachman-Diamond syndrome is caused by biallelic mutations in the *SBDS* gene, located on chromosome 7. The results of chromosome breakage studies are negative. The diagnosis can be confirmed by DNA analysis of the *SBDS* gene. There is an increased risk of development of myelodysplasia and AML.

## MYELOKATHEXIS

In myelokathexis, there is peripheral neutropenia because of prolonged retention of maturing granulocytes within the bone marrow. Bone marrow examination reveals granulocytic hyperplasia with numerous myeloid cells that display hypermature chromatin, frequently with irregular nuclear folding such as bilobed or bizarre forms. Myelokathexis usually manifests in infancy as part of the warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome. WHIM syndrome is caused by mutations in the *CXCR4* gene, which encodes a chemokine that regulates neutrophil migration.

## RETICULAR DYSGENESIS

Reticular dysgenesis is a rare, severe immunodeficiency syndrome of congenital agranulocytosis and lymphopenia. Both cytotoxic and humoral immune responses are absent, and there is little if any development of lymph nodes and thymus. Most cases also show anemia, and some patients will also have thrombocytopenia. Bone marrow examination shows a hypocellular marrow with a virtual absence of myeloid and lymphoid precursors. The presence of abnormalities involving both myeloid and lymphoid lineages suggests an abnormality of an early hematopoietic stem cell. Recent studies have identified mutations in the mitochondrial gene *AK2* as the cause of reticular dysgenesis in most patients. Fatal infection typically occurs in infancy. Bone marrow transplantation has been curative in some patients.

## CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA

Congenital amegakaryocytic thrombocytopenia is a rare disorder of marked thrombocytopenia and absent megakaryocytes. There are no other associated congenital abnormalities. Patients typically develop thrombocytopenia during infancy or early childhood, and bone marrow examination at this stage discloses normocellular bone marrow with absent or markedly decreased megakaryocytes. Approximately half of patients will develop pancytopenia with a mean age at onset of pancytopenia of 3 years. Bone marrow examination at this stage displays a hypocellular marrow similar to that seen in idiopathic AA. Most cases appear to be caused by mutations in the receptor for thrombopoietin, *C-MPL*. Without bone marrow transplantation, which can be curative, the average survival is reported to be 9 years.

## THROMBOCYTOPENIA WITH ABSENT RADII

Thrombocytopenia with absent radii (TAR) manifests neonatally with thrombocytopenia, bilateral absent radii, and other heterogeneous abnormalities. The condition can be distinguished from FA, which can also manifest with radial defects, by chromosome breakage studies. Bone marrow examination in TAR displays absent or decreased and abnormally small megakaryocytes. The genetic defect leading to TAR has not yet been identified, but most cases display an autosomal recessive pattern of inheritance.

## DYSKERATOSIS CONGENITA

Dyskeratosis congenita (DC) is a rare syndrome that is clinically defined by the triad of reticulated hyperpigmentation of the skin, dystrophic nails, and oral leukoplakia. A broad spectrum of other congenital abnormalities may also be present. In approximately 50% of cases, pancytopenia develops with a mean age at onset of 10 years. Examination of the bone marrow typically shows a hypocellular marrow consistent with AA. In recent years it has been shown that dyskeratosis congenita is secondary to the presence of abnormally shortened telomeres. Six genes have been identified, all of which appear to function in telomere maintenance. The most frequently mutated gene is the X-linked *DKC1*. Autosomal dominant forms of the disease are associated with mutations of *TINF2, TERC,* or *TERT,* and autosomal recessive forms have been rarely reported with mutations of *NOP10/NOLA3* and *NHP2/NOLA2*. Unlike in FA, the results of chromosomal breakage studies are negative. Screening for DC can be

accomplished through assays measuring telomere length. The finding of telomeres less than 1% of age-matched controls is highly sensitive and specific for DC. As with FA, patients with DC are at long-term risk of developing both hematologic and nonhematologic malignancies.

## FAMILIAL APLASTIC ANEMIA

A number of kindreds have been described with familial bone marrow failure syndromes that do not clearly correspond to one of those described previously. Cases have been reported with autosomal dominant, autosomal recessive, and X-linked inheritance patterns, with and without other associated congenital abnormalities. These cases are distinguished from FA by the absence of chromosomal breakage in clastogen-stimulated analysis. Bone marrow examination in such cases is reported to show variable degrees of bone marrow hypoplasia. Proper diagnosis and classification of such cases are difficult, and without knowledge of the familial inheritance pattern, many cases would likely be classified as idiopathic AA.

## NONHEMATOLOGIC SYNDROMES

Hematologic complications, including AA, have been reported in some patients with a variety of other syndromes that are conventionally considered nonhematologic, including Down syndrome, Dubowitz syndrome, Seckel syndrome, and Noonan syndrome. Correlation of the hematologic findings with the other clinical and constitutional cytogenetic findings is required for proper diagnosis.

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The complete reference list is available online at www.expertconsult.com.

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6

# Benign Causes of Bone Marrow Abnormalities Including Infections, Storage Diseases, Systemic Disorders, and Stromal Changes

**Dennis P. O'Malley, MD**

## INTRODUCTION

A wide variety of benign conditions can be encountered in the bone marrow, and their clinical and morphologic findings are quite broad. A bone marrow biopsy may provide diagnostic answers to hematologic or systemic problems that have not been diagnosed previously. Often the differential diagnosis of these conditions includes both primary hematopoietic and nonhematopoietic malignancies. This chapter gives the reader an overview of this wide array of disorders and important features for their diagnosis.

**INFECTIONS–FACT SHEET**

**Definition**

- A wide variety of infections can be identified in peripheral blood and bone marrow

**Clinical Features**

- Feature are variable, depending on the type and severity of infection
- Immunosuppressed individuals (after chemotherapy, after transplant, HIV/AIDS) have an increased incidence of infections

**Laboratory Features**

- If infectious etiology is suspected, appropriate microbiologic studies (culture, serology, polymerase chain reaction) should be performed on peripheral blood or bone marrow before initiating therapy

## INFECTIONS

### Clinical Features

The clinical features of infections of the hematopoietic system are broad. Exact incidences are difficult to estimate, but in general infections involving the bone marrow are rare. The frequency is increased in individuals that are immunosuppressed. There is no specific gender predilection for infection. The clinical signs and symptoms vary depending on the specific infection; however, when marrow involvement is present in a systemic infection, it is typically associated with a worse prognosis.

### Pathologic Features

The pathologic features of marrow infections are highly variable depending on the specific infectious agent. In general viral, bacterial, fungal, and parasitic or protozoal infections can all involve the marrow. These causative agents are discussed in the following sections. In addition, hemophagocytic syndromes, often associated with infections, are also discussed.

**INFECTIONS—PATHOLOGIC FEATURES**

**Peripheral Blood**

- Nonspecific changes: leukocytosis, eosinophilia, toxic changes (granulation, Döhle bodies), lymphocytosis with atypical forms, monocytosis, anemia, thrombocytopenia
- Circulating organisms rarely seen
- Notable intracellular organisms: bacteria (ingested by WBCs), malaria, histoplasmosis, *Candida* species, extracellular organisms: filaria, *Borrelia* species

**Aspirate Smear**

- Most often nonspecific changes including left shift, toxic changes, increased M:E ratio, lymphocytosis
- Rarely organisms may be seen within macrophages, such as leishmaniasis or histoplasmosis

**Core Biopsy and Clot Section**

- Nonspecific changes include granulomas, fibrosis, increased M:E ratio, lymphocytosis, plasmacytosis, eosinophilia
- Rarely organisms may be identified on H&E stain

**Special Stains**

- AFB/GMS/PAS stains are beneficial for mycobacterial or fungal infections
- Occasionally, tissue Gram stain or Warthin-Starry stain may be useful

**Flow Cytometry, Immunohistochemistry, Molecular, Cytogenetic**

- Many immunohistochemical stains are available for detection of viral agents
- Molecular methods for detection of organisms may be useful in confirming cases with equivocal morphologic findings

## VIRAL

Among the viruses, parvovirus B19, Epstein-Barr virus (EBV), HIV and other viruses can have significant peripheral blood and bone marrow effects. Because it is often associated with viral infections, hemophagocytic syndrome (HPS) will also be discussed in this section.

### EPSTEIN-BARR VIRUS

An acute EBV infection in an immunocompetent host (i.e., infectious mononucleosis [IM]), is typically characterized by the presence of atypical lymphocytosis in association with sore throat, fever, and lymphadenopathy. Laboratory findings include the presence of IgM antibody to EBV. More commonly the heterophile antibody, or monospot, test is performed to confirm acute infection. The peripheral blood usually has lymphocytosis with 10% or more large, atypical lymphocytes (Figure 6-1). These large lymphocytes appear approximately 3 to 4 days after the appearance of symptoms. The lymphocytosis may persist for several months.

The bone marrow is not typically examined in cases of IM. The changes in bone marrow associated with acute EBV infection are generally nonspecific and include: lymphoid aggregates, increased $CD8^+$ lymphocytes including atypical forms, increased myeloid to erythroid (M:E) ratio, and a left shift in myeloid maturation. Confirmation of the diagnosis on a bone marrow sample can be accomplished with various immunohistochemical stains or in situ techniques for EBV in acute infections.

Chronic active EBV infection is a systemic lymphoproliferative disorder occurring primarily in children and young adults of Asian or Latin American descent, although rare older individuals may be affected. Patients exhibit IM-like symptoms (fevers, lymphadenopathy and organomegaly) that occur at least 3 months after primary infection and with no known immunodeficiency. The bone marrow shows a subtle interstitial–sinusoidal infiltrate of small mature T or natural killer cells that are EBV positive by in situ hybridization. These cells might not be apparent on hematoxylin and eosin (H&E) staining and become apparent only upon staining for EBV; therefore a high index of suspicion is required. High-titer EBV viral capsid antigen IgG and early antigen IgG can be demonstrated in serum as can EBV DNA by polymerase chain reaction. By definition, T cell receptor gene rearrangement studies are polyclonal; if monoclonal, a diagnosis of EBV-positive lymphoproliferative disorder is warranted. This bona fide T cell malignancy is covered in the T cell lymphoma chapter. Prognosis is guarded with indolent behavior in some patients, whereas others may die of the disease. Evolution to monoclonal disease, overt T cell lymphoma, or hemophagocytic syndrome may occur.

## HEMOPHAGOCYTIC SYNDROME

Although rare, HPS in the marrow can both accompany malignancy and mimic it (Table 6-1). Clinically, HPS is often associated with severe anemia, other cytopenias, fever, and hepatosplenomegaly. There are four general

**TABLE 6-1**

**Etiologic Agents Associated with Hemophagocytic Syndrome**

| |
|---|
| Epstein-Barr virus |
| Hepatitis viruses |
| Cytomegalovirus |
| Other viruses |
| Congenital (±Epstein-Barr virus infection): familial hemophagocytic syndrome (perforin defect) |
| T cell lymphoma–associated |
| Germ cell tumor–associated |
| Other malignancies |
| Immunosuppression: probably infection-related |

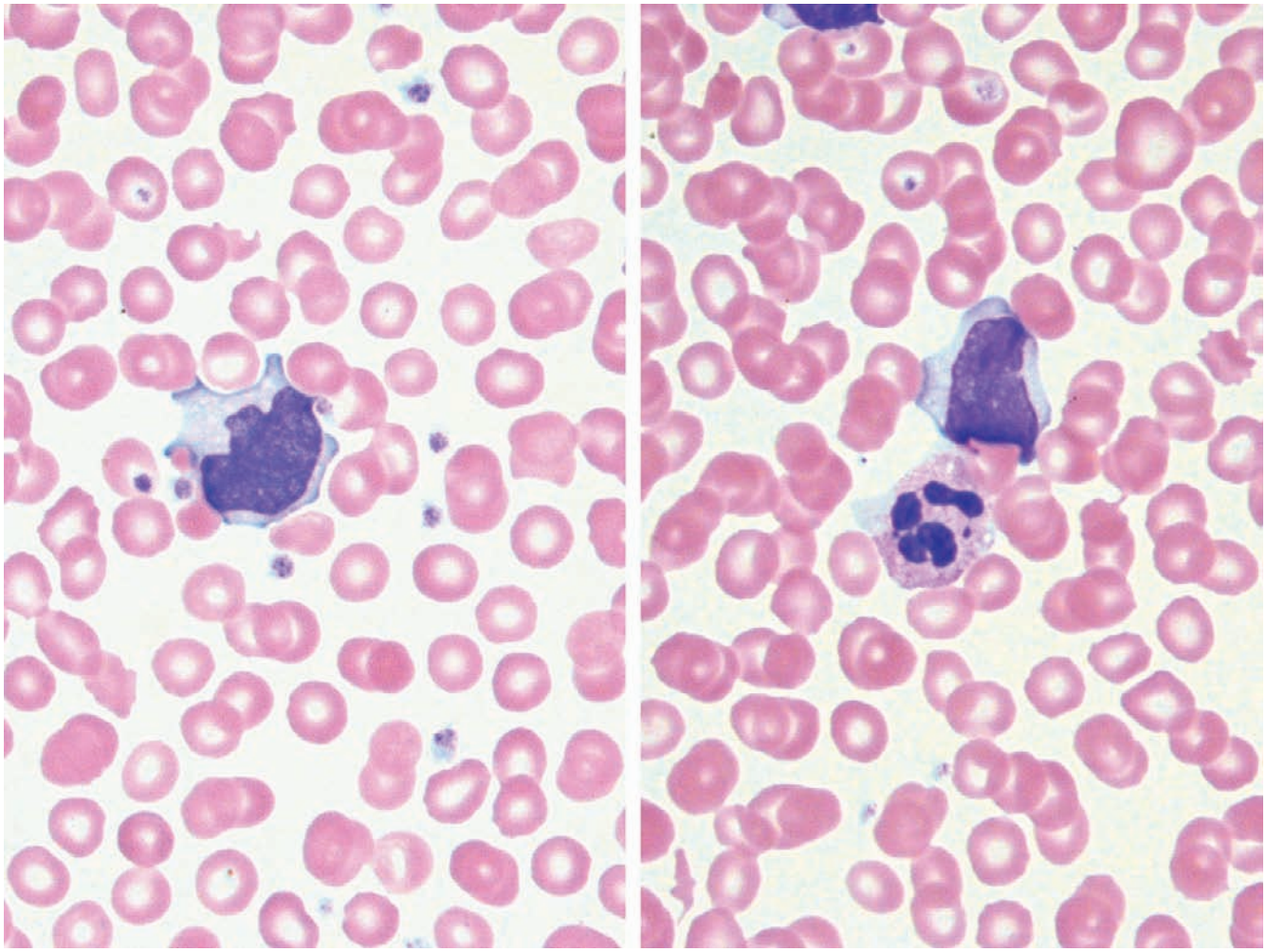

**FIGURE 6-1**

Examples of reactive lymphocytes seen in the peripheral blood in infectious mononucleosis. These findings can also be seen in other viral infections and are not specific to Epstein-Barr virus infection.

circumstances in which HPS is seen: (1) infection-associated (IAHPS), (2) malignancy-associated, (3) idiopathic, and (4) inherited or congenital. IAHPS can be induced by a variety of bacterial, fungal, and viral infections, but the most common association is with EBV infection. The underlying pathogenesis is thought to be immune dysregulation leading to a "cytokine storm" and unchecked macrophage and T-cell activation.

Malignancy-associated HPS can be seen in conjunction with peripheral T cell lymphomas, B cell lymphomas, Hodgkin lymphoma, epithelial malignancies, and leukemias, in roughly that order of frequency. In cases of T cell lymphomas, the malignant cells can be inconspicuous relative to the background of hemophagocytosis. One of the inherited forms of HPS, familial hemophagocytic lymphohistiocytosis, has been associated with mutations in the perforin gene. In the peripheral blood, atypical lymphocytosis may be seen, particularly if the HPS is associated with infection or a lymphoid malignancy.

**HEMOPHAGOCYTIC SYNDROME—FACT SHEET**

**Definition**

- A clinical syndrome associated with several etiologies characterized by prominent destruction of marrow elements within macrophages, resulting from an immune dysregulation with hypercytokinemia

**Incidence**

- Rare

**Clinical Features**

- Systemic symptoms (fever, night sweats, weight loss) common

The morphologic appearance of HPS of all types is similar. Core biopsy specimens and aspirate smears show macrophages with ingested cellular elements (Figure 6-2), most often erythrocytes; however, other hematopoietic cells can also be seen within the macrophages. The

## HEMOPHAGOCYTIC SYNDROME—PATHOLOGIC FEATURES

### Peripheral Blood
- Pancytopenia common

### Aspirate Smear
- Hypocellular aspirate smears, with prominent increase in macrophages with ingested cellular elements and debris

### Core Biopsy and Clot Section
- Reduction in cellularity and variably prominent macrophages with ingested cellular elements
- Lymphocytosis, fibrosis, and plasmacytosis common

### Flow Cytometry, Immunohistochemistry, Molecular, Cytogenetic
- Stains for EBV-LMP, Epstein-Barr nuclear antigens, or in situ hybridization for EBER may be informative
- Polymerase chain reaction for T cell receptor gene rearrangement may be beneficial in T cell lymphoma-associated types

### Differential Diagnosis
- Infection-associated (EBV most common); malignancy-associated (especially T cell lymphoma); familial (primary) hemophagocytic lymphohistiocytosis (*PFR1, Munc13D, STX11* mutations); idiopathic

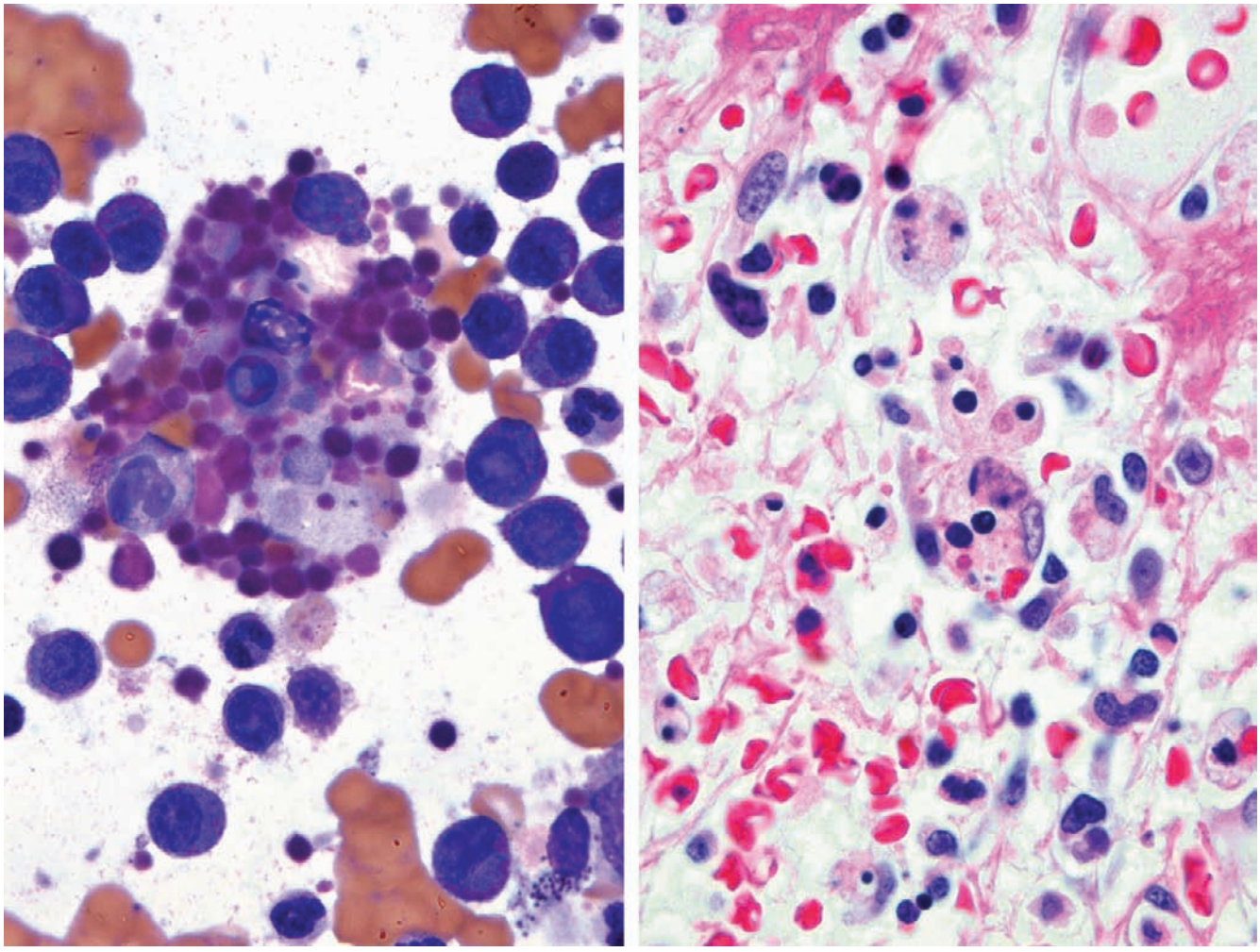

**FIGURE 6-2**

Example of aspirate smear and core biopsy findings in hemophagocytic syndrome. Although this case is due to Epstein-Barr virus infection, other causes would show similar morphologic findings.

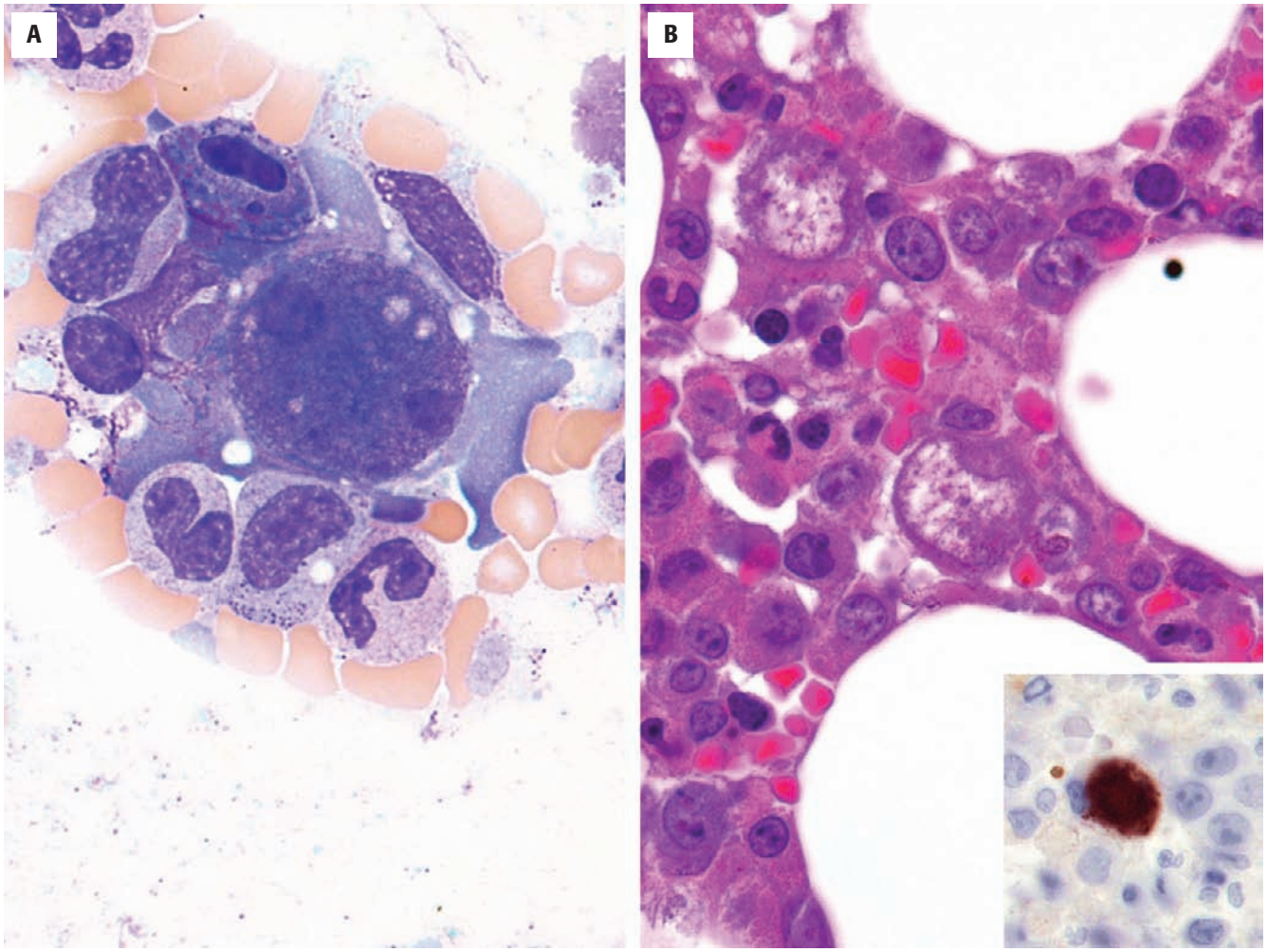


**FIGURE 6-3**

Parvovirus infection in bone marrow. **A,** Aspirate smear showing giant pronormoblasts characteristic of parvovirus infection. **B,** Biopsy showing infected erythroid elements of large size with open, cleared nuclei. *Inset,* An example of a parvoviral immunohistochemical stain.

macrophages may also contain cell fragments or amorphous debris, which are remnants of hematopoietic components. The macrophages are benign and lack cytologic atypia. In core biopsy specimens, the appearance of the marrow may have an unusual appearance. The intertrabecular space is filled with cells (hemophagocytic macrophages), but there is an overall decrease in hematopoiesis. In all cases of HPS the marrow should be assessed carefully for signs of viral inclusions and of atypical cells that may represent an associated malignancy. The differential diagnosis also includes true histiocytic malignancies that, although rare, may also phagocytize other cellular elements. More details on hemophagocytic lymphohistiocytosis are presented in Chapter 19.

## OTHER VIRUSES

Parvovirus B19 is a DNA viral infection seen predominantly in young or immunosuppressed patients, leading to severe anemias caused by red blood cell aplasia. In acute parvoviral infection the virus preferentially infects red blood cell precursors; in disorders with a shortened red blood cell life span (e.g., hereditary spherocytosis), this causes a severe symptomatic anemia. The marrow findings are characteristic (Figure 6-3). If the marrow is biopsied at the appropriate stage, giant pronormoblasts and intranuclear viral inclusions can be seen. Chronic infections by parvovirus, seen in immunocompromised patients, more often have pancytopenia. Occasionally these cases will have erythroid hyperplasia with numerous virally infected erythroid cells and prominent viral inclusions. An association with hemophagocytic syndrome has also been reported. An immunostain is available with approximately 80% sensitivity, but serologic testing for the virus is considered the gold standard.

HCV-infected patients can develop an atypical lymphoproliferative disorder in the setting of essential mixed (type II) cryoglobulinemia. This type of cryoglobulin is composed of a monoclonal immunoglobulin (Ig) M and polyclonal IgG components. Although patients do not manifest overt lymphoma, subtle

monoclonal lymphoid components may be detected in the bone marrow of some patients when detailed immunophenotyping is performed. Patients may then develop lymphoma (e.g., lymphoplasmacytic lymphoma) at some point, often many years later, in the disease course.

## HIV/AIDS

There are a wide variety of hematologic changes seen in patients with HIV/AIDS (Table 6-2). It is important to remember that while these findings are common in HIV/AIDS, they are also nonspecific.

Macrocytosis with or without anemia is a common finding in HIV/AIDS peripheral blood. These effects are multifactorial, and causes include direct effects of the virus, medications, and secondary dietary deficiencies. The macrocytosis is generally mild to moderate. Rouleaux resulting from hypergammaglobulinemia, hyperviscosity, and amyloidosis have been rarely associated with HIV infection.

The most common finding in peripheral blood is lymphopenia, as a result of reduced numbers of $CD4^+$ T cells. Occasionally there may be leukocytosis in HIV/AIDS patients. If lymphocytosis is present, it may be composed of large granular lymphocytes, which are either $CD8^+$ T cells or natural killer cells, or B cells with lymphoplasmacytic features. Increases in granulocytes, often with toxic changes (e.g., vacuolation, toxic granules, and Döhle bodies) may be due to bacterial infections. Nuclear fragmentation or other degenerative

**TABLE 6-2**
**Marrow Changes Associated with HIV/AIDS**

| | |
|---|---|
| Morphology | Cellularity changes (most often hypercellular)<br>Megakaryocyte changes (e.g., naked nuclei, clustering)<br>Fibrosis<br>Lymphoid aggregates<br>Plasmacytosis<br>Histiocyte: aggregates, hemophagocytosis<br>Dyspoiesis simulating myelodysplastic syndrome<br>Gelatinous transformation, serous fat atrophy |
| Infections | Fungal: cryptococcosis, histoplasmosis, coccidioidomycosis, blastomycosis, other fungi<br>Viral: direct HIV effects, Epstein-Barr virus–associated, human herpesvirus 8, cytomegalovirus, other viruses<br>Parasitic or protozoal: leishmania, toxoplasmosis, others<br>Bacterial: mycobacteria, rickettsia, others |
| Hematologic and autoimmune complications | Thrombotic thrombocytopenic purpura, immune thrombocytopenic purpura, autoimmune anemia, autoimmune neutropenia, coagulopathies |
| Malignancy | Hematologic: Burkitt lymphoma, diffuse large B cell lymphoma, large granular lymphocytosis, Hodgkin lymphoma<br>Nonhematologic: Kaposi sarcoma, other carcinomas, sarcoma |

**HIV/AIDS MYELOPATHY—FACT SHEET**

**Definition**
- *HIV myelopathy* refers to the constellation of bone marrow findings seen in association with HIV/AIDS

**Incidence**
- Essentially all patients with HIV/AIDS will experience hematologic and bone marrow changes throughout the course of their disease

**Morbidity and Mortality**
- Hematologic disease may contribute to morbidity in HIV/AIDS

**Clinical Features**
- Features are variable depending on severity of disease
- Clinical indication of severity is often based on $CD4^+$ lymphocyte count

**Prognosis and Therapy**
- Newer antiretroviral therapies improve quality of life and survival in HIV/AIDS. However, no cure is available and resistance to therapy is increasingly common

**HIV/AIDS MYELOPATHY—PATHOLOGIC FEATURES**

**Peripheral Blood**
- Macrocytosis (result of medication effect and nutritional effects), anemia (multifactorial), poikilocytosis, thrombocytopenia, lymphopenia, variable increases in large granular lymphocytes

**Aspirate Smear**
- Erythroid megaloblastic changes, increased lymphocytes, increased plasma cells, increased megakaryocytes including naked nuclei

**Core Biopsy and Clot Section**
- Hypercellularity common, atypical megakaryocytes with naked and hyperchromatic nuclei, lymphoid aggregates, granulomas, plasmacytosis (polyclonal), fibrosis

**Special Stains**
- Stains for fungal and mycobacterial infections (GMS, AFB) should be performed routinely
- Some patients with HIV/AIDS may have chronic parvoviral infection; immunohistochemistry for parvovirus may be of benefit

**Differential Diagnosis**
- HIV/AIDS myelopathy may mimic primary myelodysplastic syndromes or myeloproliferative neoplasms
- Increased incidence of infections and of malignancies can complicate diagnosis

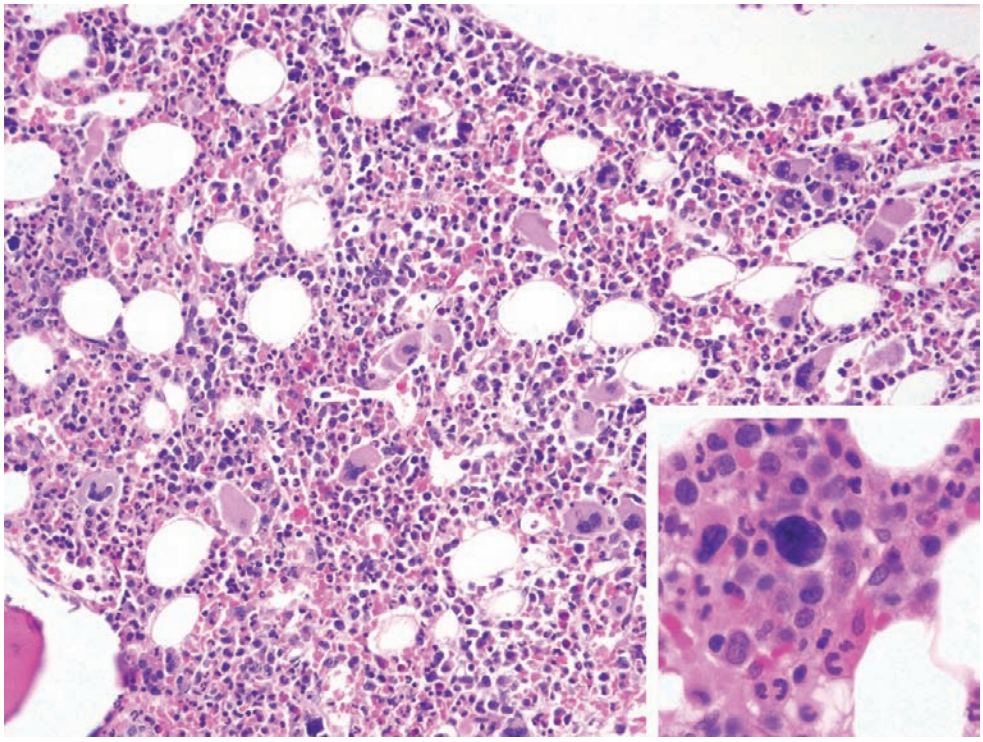

**FIGURE 6-4**

Bone marrow findings in HIV/AIDS. A variety of features can be seen, but common findings include hypercellularity, increases in megakaryocytes, lymphoid aggregates, and plasmacytosis. *Inset,* A naked megakaryocyte nucleus, a common and relatively specific bone marrow finding in HIV/AIDS.

changes of white blood cells (WBCs) can be seen and may be due to infections or drug effects. Eosinophilia is often seen in these patients.

Autoimmune cytopenias (i.e., immune thrombocytopenic purpura [ITP], autoimmune hemolytic anemia, autoimmune neutropenia) can be seen in association with HIV/AIDS infection and can have a significant effect on the peripheral blood findings. Thrombocytopenia may be one of the earliest findings in HIV infection. The two main causes of thrombocytopenia are: immune mediated destruction and decreased production. Hypersplenism and drug effects may also cause low platelets.

The bone marrow findings in HIV/AIDS are protean (Figure 6-4). There are a wide variety of relatively specific and numerous nonspecific findings in the bone marrow (see Table 6-2). The constellation of findings is referred to as *HIV myelopathy*. Some of the more common findings include: hypercellularity, granulomas, poorly-formed histiocytic aggregates, lymphoid aggregates, plasmacytosis (with occasional Russell or Dutcher bodies), focal serous fat atrophy (gelatinous transformation), dilated sinuses, increased iron, and marrow fibrosis. Some of the less common findings include: hypocellularity, infection without granuloma (low CD4 count), ring sideroblasts, hemorrhage, and diffuse serous fat atrophy or gelatinous transformation. Megakaryocytes can be infected directly by the HIV virus and consequently show several changes in HIV/AIDS. The megakaryocytes often have distinctive morphologic findings, the most pathognomonic of which is the presence of naked nuclei. Other changes include an absolute increase in number, clustering (mimicking myeloproliferative neoplasms), unilobate megakaryocytes (mimicking myelodysplastic syndromes), large forms, and abnormal nuclear chromatin.

Infectious agents of a variety of types may be seen in HIV/AIDS marrows; they may present confusing or unusual marrow findings that can mimic lymphomas, sarcomas, or epithelial malignancies. Clinical suspicion, culture results, and the use of organism stains can be beneficial. Relatively common infections in HIV/AIDS include: cryptococcosis, histoplasmosis, coccidioidomycosis, pneumocystosis, HHV-8, CMV, adenovirus, EBV, toxoplasmosis, and leishmaniasis.

Lymphomas (including Hodgkin and non-Hodgkin types), plasma cell dyscrasia, spindle cell lesions (including Kaposi sarcoma, inflammatory pseudotumor, and mast cell disease) can also be discovered initially in the bone marrow.

## BACTERIAL

Bone marrow findings in bacterial sepsis are nonspecific. In fact, most bacterial infections produce no significant marrow findings. Whipple disease *(Tropheryma whippelii),* which may be seen in the bone marrow, produces similar morphologic findings to those seen in

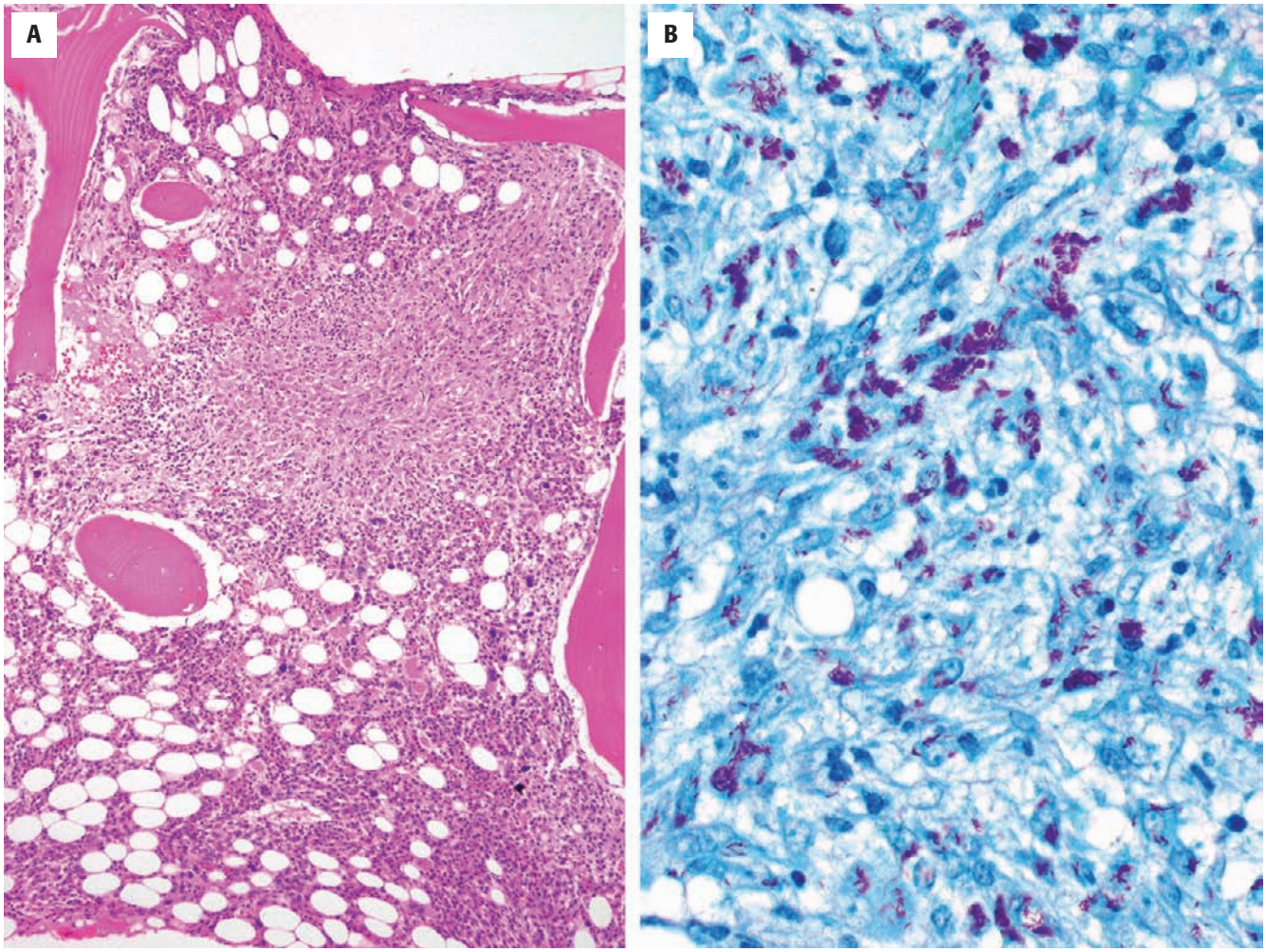


**FIGURE 6-5**

*Mycobacterium avium-intracellulare* in bone marrow. **A,** Biopsy showing large granuloma composed of expanded, pink macrophages. **B,** An acid-fast bacillus stain that highlights numerous positive elongated mycobacteria.

other sites. However, most cases manifest only as an increase in granulocytes, a left shift in maturation, toxic changes, or other nonspecific changes as outlined previously. Some of the organisms with identifiable changes are discussed in the following sections.

Rickettsial infections may show the presence of ring (or donut) granulomas. This finding is suggestive, but by no means specific, for Q fever *(Coxiella burnetii).*

## MYCOBACTERIAL

There are a number of mycobacterial species that can involve the bone marrow. Although not the most common in the United States, marrow involvement by *Mycobacterium tuberculosis* can be rarely associated with a diagnosis of myelophthisis (i.e., marrow failure resulting from replacement of hematopoietic marrow by an abnormal tissue causing a leukoerythroblastic blood picture). In typical cases of marrow involvement by tuberculosis, the marrow will show granulomatous inflammation, occasionally with central, caseous necrosis. The degree of involvement is variable, but immunocompetent hosts will typically form granulomas. In severely immunosuppressed patients, granulomas may be poorly formed or absent. Instead small, indistinct histiocytic aggregates may be seen. In either case, organisms are usually rare when visualized with an acid-fast stain.

*Mycobacterium avium-intracellulare* is more commonly seen in marrows of immunosuppressed patients. In the most obvious cases, there are aggregates of histiocytes filled with granular cytoplasm (Figure 6-5). Acid-fast staining usually reveals numerous organisms that, in contrast with *M. tuberculosis,* stain positively with periodic acid-Schiff (PAS).

## FUNGAL

A variety of fungi can be found in bone marrow. Marrow involvement is associated with disseminated infections and is most often seen in immunocompromised patients.

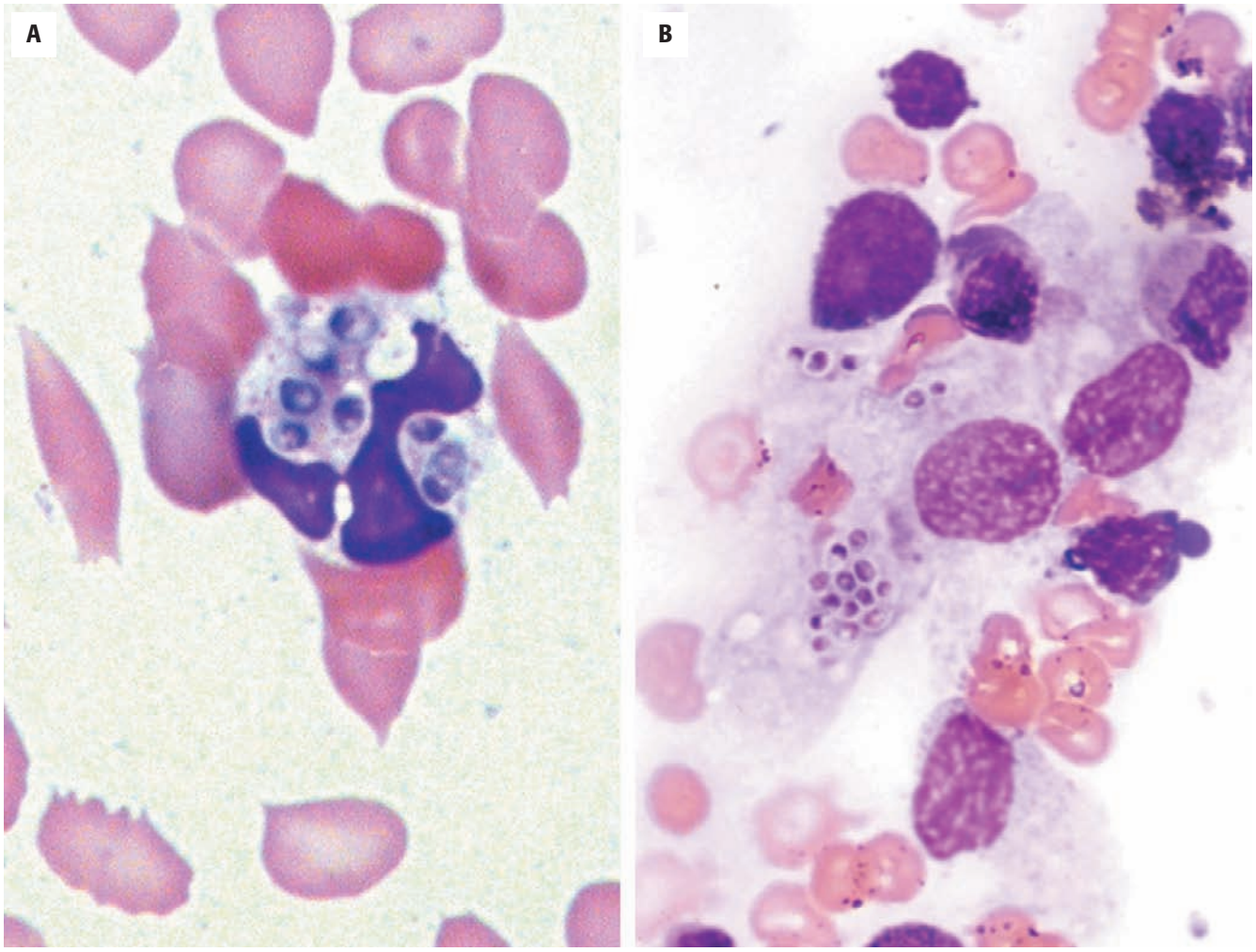


**FIGURE 6-6**

Histoplasmosis yeast forms in **(A)** peripheral blood and **(B)** bone marrow aspirate.

The nature of the infection will vary depending on the geographical location. The fungal infections which are more commonly seen in the United States include histoplasmosis, blastomycosis, and coccidioidomycosis. Other fungi that are worldwide in their distribution include *Cryptococcus* species, *Candida* species, and *Aspergillus* species. Other rare types of fungi have also been reported in the bone marrow.

Histoplasmosis *(Histoplasma capsulatum)* is a dimorphic fungus that is endemic in the central United States, particularly in the Ohio Valley region. It consists of small (2 to 4 μm) budding yeast forms usually seen within macrophages (Figure 6-6). They can be better visualized by Giemsa and Gomori methenamine silver (GMS) stains.

Blastomycosis *(Blastomyces dermatitidis)* is another dimorphic fungus (Figure 6-7). It typically infects the skin or respiratory tract. Disseminated infection can eventually lead to the presence of the organisms in the bone marrow. The yeast forms are generally spherical with thick walls, are up to 40 μm in diameter, and have characteristic broad-based budding of daughter forms. They are easily seen with a GMS stain.

Cryptococcus *(Cryptococcus neoformans)* is the most common systemic fungal infection occurring in patients with HIV (see Figure 6-7); however, marrow involvement is unusual. Typically the yeast forms are easy to see, with thick, gelatinous capsules. The thickness of the capsule depends on the strain and host conditions. Bone marrow biopsies can also occasionally have associated granulomatous inflammation or hemophagocytosis. Histochemical stains, most notably GMS and mucicarmine, are useful for identification.

Coccidioidomycosis *(Coccidioides immitis)* is a fungal infection that typically involves the lung. The most common geographic distribution is the Western United States and Mexico. Certain racial groups have an increased sensitivity to infection. Rare disseminated cases may have bone marrow involvement. The organisms are large spherules (10 to 80 μm), roughly the size of a megakaryocyte, packed with smaller (2 to 5 μm) endospores. Often the organisms are associated

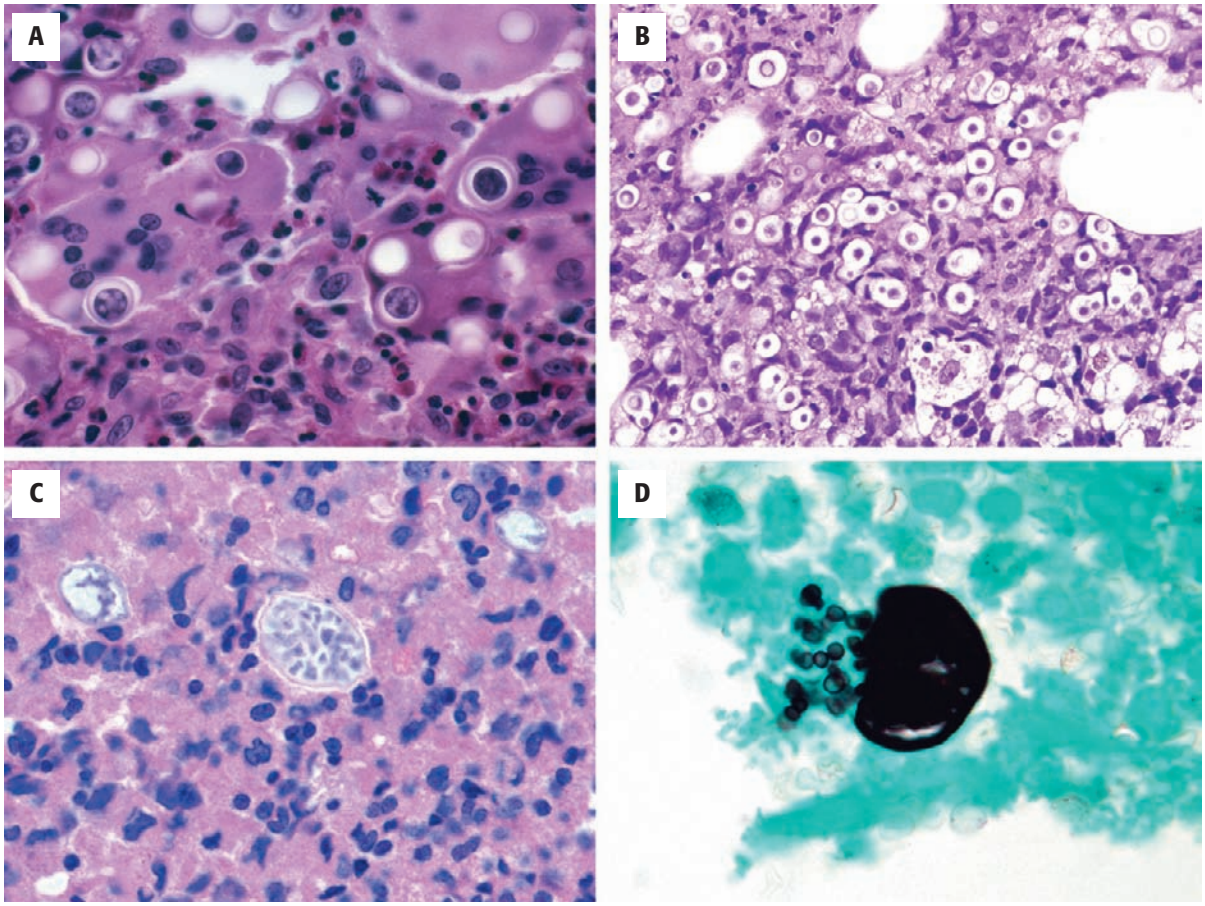


**FIGURE 6-7**

Fungi in bone marrow. **A,** Blastomycosis. **B,** Cryptococcus. **C,** Coccidioidomycosis in core biopsy. **D,** Rupturing endospores highlighted by Gomori methenamine silver stain.

with lymphoid aggregates and granulomas. They will stain well with fungal stains (GMS, PAS) (see Figure 6-7).

The classification of pneumocystis *(Pneumocystis carinii)* is uncertain, but currently it is classified as a fungus. It causes pneumonia in HIV-positive patients, but may also rarely involve the bone marrow in disseminated disease. The organisms may be seen in association with granulomas and histiocytic aggregates. The cysts are small and often intracellular within macrophages. Occasionally there is a frothy extracellular material, similar to that seen in lung (Figure 6-8). The organisms can be visualized with Giemsa, GMS, and immunohistochemical stains.

## PARASITIC AND PROTOZOAL

Most parasitic and protozoal infections are more frequent in peripheral blood (e.g., malaria, babesiosis, filaria); however, they can be seen rarely in bone marrow. In contrast, other protozoal infections may be seen more commonly in bone marrow.

Leishmaniasis is a relatively common protozoal infection worldwide. The most prevalent species causing disseminated leishmaniasis is *Leishmania donovani*. The organisms are small, intracellular, and typically within macrophages (Figure 6-9). They can sometimes be confused with histoplasmosis or, less commonly, *Cryptococcus* species. These organisms are PAS, GMS, and mucicarmine negative. They are best identified with a Giemsa stain, which allows better visualization of the nucleus and kinetoplast, a small rod-shaped structure seen opposite the nucleus.

In toxoplasmosis, bone marrow involvement is seen rarely. *Toxoplasma* species are obligate intracellular parasites, with cats as the typical host. Clinical presentation of disseminated disease includes pancytopenia and central nervous system infection with lung and heart involvement. The bone marrow may show only small histiocytic aggregates or well-formed granulomas less frequently. Less common findings include necrosis, edema, and fibrin deposition. Cysts with organisms are seen in histiocytes and megakaryocytes, or in extracellular locations; more rarely free trophozoites are seen in the interstitium.

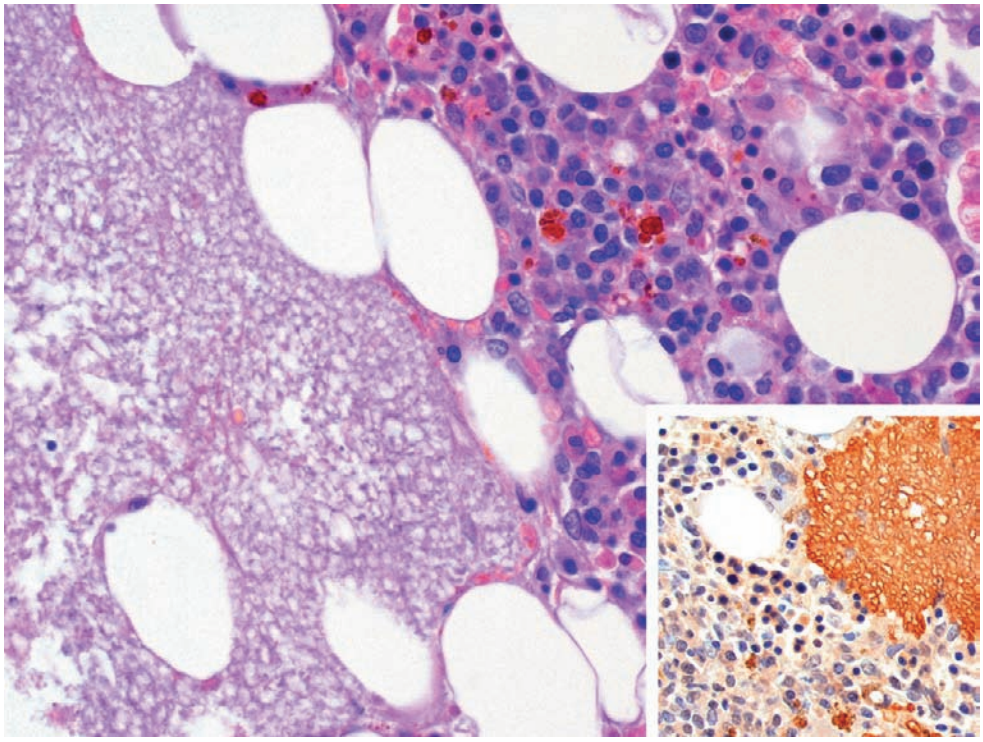

**FIGURE 6-8**

Pneumocystis involving bone marrow. Note the frothy material, similar to that seen in lung. *Inset,* Immunohistochemical staining for pneumocystis.

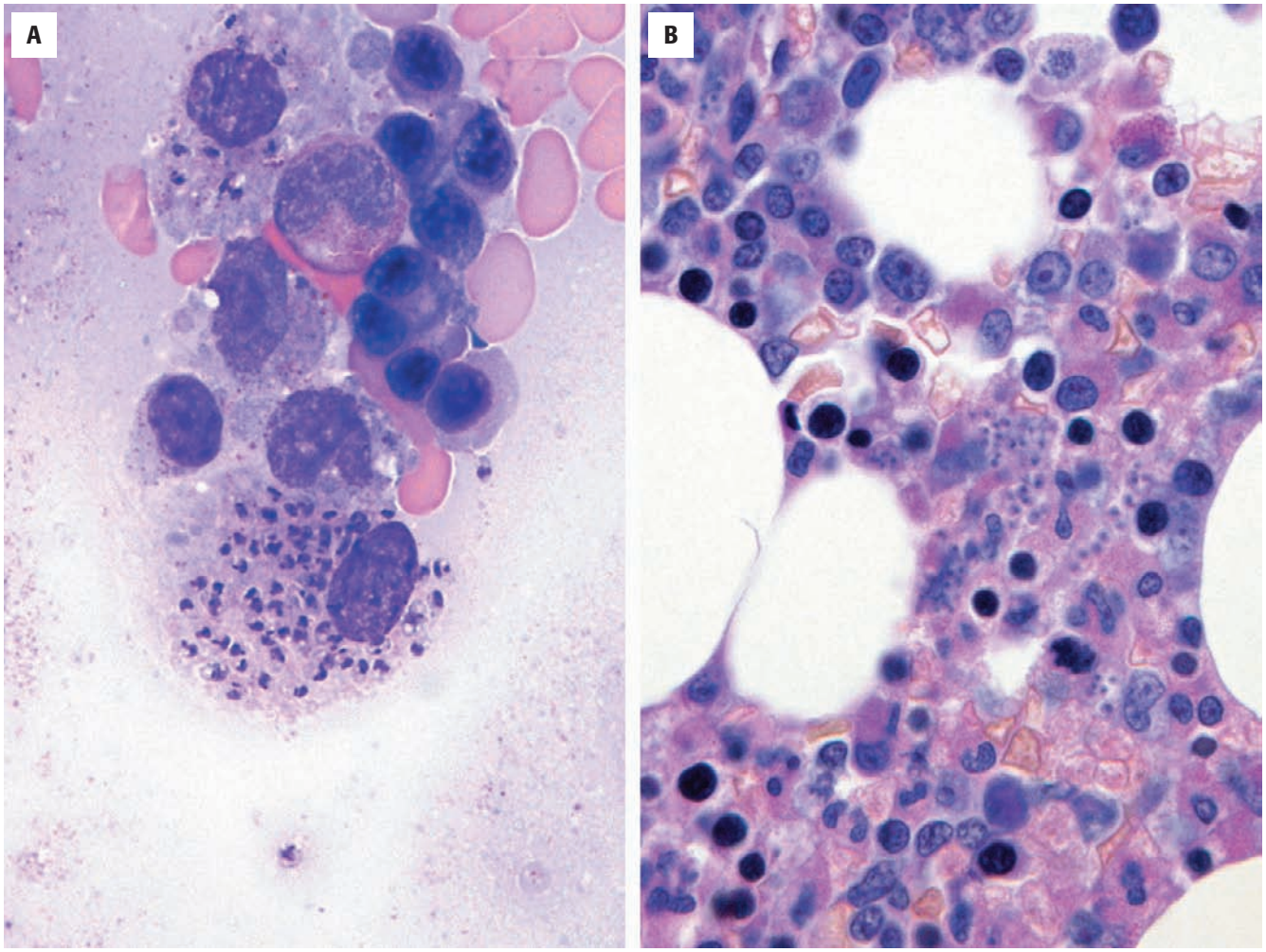


**FIGURE 6-9**

Leishmaniasis in **(A)** aspirate smear and **(B)** core biopsy specimen.

## GRANULOMAS

### Clinical Features

Bone marrow granulomas are rare and are reported in approximately 1% to 2% of all marrows. There are several types of granulomas seen in marrow, and these can be associated with specific etiologies, although no type of granuloma is entirely specific for a particular diagnosis. Granulomas have been associated with a variety of causes including: neoplasms, viral, bacterial and fungal infections, autoimmune disorders, medications, and sarcoidosis (Table 6-3; Figure 6-10).

**GRANULOMAS—FACT SHEET**

**Definition**
- Organized clusters of macrophages as a response to a variety of marrow insults

**Incidence**
- 1% to 2% of all marrows

**Clinical Features**
- Variable depending on underlying etiology

**GRANULOMAS—PATHOLOGIC FEATURES**

**Peripheral Blood**
- No specific findings seen

**Aspirate Smear**
- May be seen as cohesive aggregates or clusters of macrophages often associated with lymphocytes

**Core Biopsy and Clot Section**
- Varying-sized nodules of enlarged, pink macrophages (e.g., epithelioid) with varying numbers of infiltrating or surrounding lymphocytes

**Special Stains**
- AFB, GMS, PAS staining should be performed in all cases with granulomas to rule out mycobacterial and fungal organisms

**Differential Diagnosis**
- Infectious, malignancy-associated (e.g., Hodgkin lymphoma), sarcoid, numerous other etiologies

**TABLE 6-3**

**Causes of Granulomas in Bone Marrow**

***Infectious***

Viral infections
- HIV/AIDS
- Epstein-Barr virus, cytomegalovirus
- Hepatitis C virus

Fungal infections
- Histoplasmosis
- Cryptococcus
- Coccidioidomycosis
- Aspergillosis
- Blastomycosis

Bacterial, mycobacterial, rickettsial infections
- Tuberculosis
- *Mycobacterium avium-intracellulare*
- Tularemia
- Typhoid
- Brucellosis
- Borreliosis
- Q fever
- *Mycoplasma* species infections
- Bacillus Calmette-Guerin
- *Mycobacterium leprae* (leprosy)
- Syphilis

Other
- Toxoplasmosis

***Malignancies***

Hematologic
- Hodgkin lymphoma
- Non-Hodgkin lymphomas
- Myeloma
- Acute myeloid leukemia
- Acute lymphoblastic leukemia
- Chronic myelogenous leukemia
- Myelodysplastic syndromes

Nonhematopoietic
- Colon carcinoma
- Lung carcinoma
- Ovarian carcinoma
- Neuroblastoma
- Sarcomas

***Medication-Induced***

Nonsteroidal antiinflammatory drugs
Chemotherapy
Analgesics
Cytokines
Antibiotics
Antiepileptic
Antiarrhythmics

***Autoimmune Disorders***

Rheumatoid arthritis
Felty syndrome
Primary biliary cirrhosis
Sjögren syndrome
Pulmonary fibrosis
Systemic lupus erythematosus
Addison disease
Behçet's disease
Erythema nodosum
Temporal arteritis
Posttransplantation

***Foreign Bodies***

Pneumoconiosis, silicosis
Intravenous drug abuse (foreign body; e.g., talc)

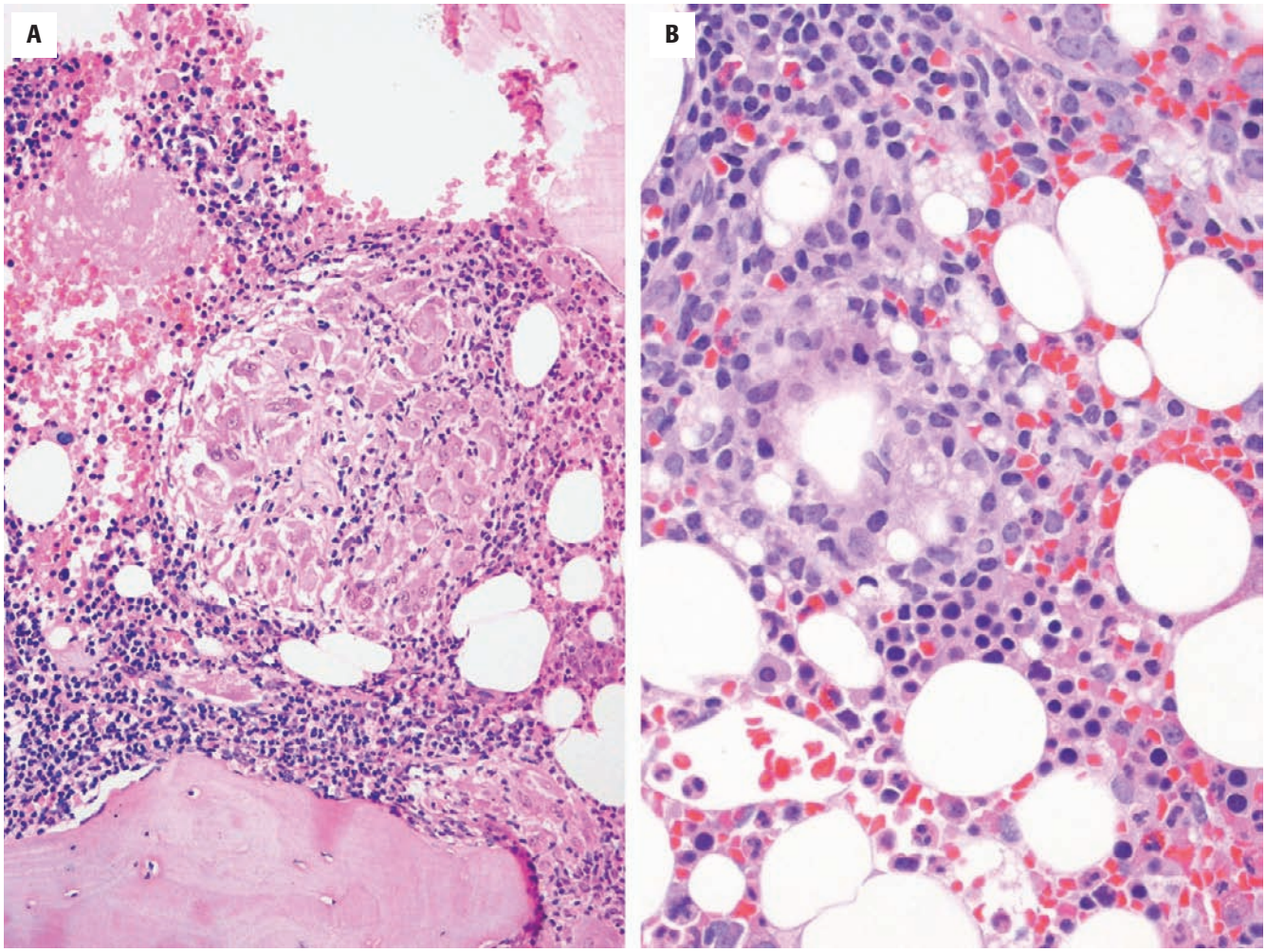


**FIGURE 6-10**

Granulomas. **A,** A sarcoidal granuloma in marrow. Note the well-defined nature of the granuloma, with sharp edges. **B,** A lipogranuloma, with central fat and associated lymphocytes.

## Pathologic Features

Granulomas are, at their most basic level, an aggregate of macrophages displaying variable degrees of epithelioid features (rounded cell morphology with abundant cytoplasm). There are often surrounding or intermixed lymphocytes and other inflammatory cells. Multinucleated giant histiocytic cells can be seen in almost any type of granuloma. The evaluation of granulomas in bone marrow, except perhaps lipogranulomas, should include special stains (i.e., acid fast bacilli [AFB], GMS, PAS) to evaluate for the presence of infectious microorganisms.

Lipogranulomas, which consist of small amounts of fat surrounded or ingested by macrophages and lymphocytes, are seen in approximately 10% of bone marrow biopsy specimens. A suggested cause in some cases is local damage to adipocytes, inciting macrophages and an immunologic response. Rarely, lipogranulomas may be caused by intravascular administration of fatty substances. Rarely, foreign body granulomas may simulate lipogranulomas because of the presence, in both conditions, of vacuolated macrophages. Examination under polarized light may make identification of the foreign material easier.

Necrotizing granulomas are most often associated with infectious etiologies. These are typically well-defined nodules of epithelioid macrophages surrounding an area of necrotic, fragmented, unstructured debris. Any necrotizing granuloma should be investigated for the presence of infectious organisms, such as tuberculosis or fungal infections (discussed previously). Rarely, systemic disorders associated with granulomas can manifest with central necrosis.

Several medications may be associated with granuloma formation. The most common of these are procainamide and sulfonamide. Other medications associated with marrow granulomas include: chlorpropamide, phenylbutazone, phenytoin, methyldopa, allopurinol, ibuprofen, penicillamine, and tolmetin sodium.

## SYSTEMIC DISORDERS

### Clinical Features

Many systemic disorders have effects on the blood and marrow that can be evaluated and correlated with clinical findings. The clinical features depend on the specific entity in question. Several groups of systemic diseases are discussed in the following sections, including: chronic alcoholism, gastrointestinal disorders, renal diseases, liver diseases, and autoimmune diseases.

### Pathologic Features

#### Chronic Alcoholism

One disorder associated with significant hematologic problems is chronic alcoholism. Ethanol has a direct deleterious effect on the development of hematopoietic cells; however, hematologic disorders seen in alcoholics can be caused by other mechanisms. Peripheral blood changes include: anemia, stomatocytosis, acanthocytosis, macrocytosis (secondary to folate deficiency), vacuolation of WBCs, hypersegmentation of granulocytes (secondary to folate deficiency). These changes are due to a variety of effects including liver disease, nutritional deficiencies, and direct toxic effects of ethanol on hematopoietic cell development. Marrow findings include: decreases in cellularity, ring sideroblasts, megaloblastic changes, mild dyspoiesis of erythroid precursors, excessive cytoplasmic granularity or vacuolation of myeloid cells (or both), and occasionally a left shift in WBC precursors. A mild polyclonal plasmacytosis of up to 10% often accompanies chronic alcoholism. A relatively specific but rare finding is the presence of cytoplasmic iron within plasma cells.

**CHRONIC ALCOHOLISM—FACT SHEET**

**Definition**
- Marrow changes are common in association with chronic ethanol abuse

**Clinical Features**
- Variably depending on severity and length of abuse

**Laboratory Features**
- Abnormal liver function tests (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase) commonly present

**CHRONIC ALCOHOLISM—PATHOLOGIC FEATURES**

**Peripheral Blood**
- Red blood cells: macrocytosis, stomatocytosis, acanthocytosis
- WBCs: hypersegmented neutrophils, toxic changes, vacuolated granulocytes
- Platelets: mild thrombocytopenia common

**Aspirate Smear**
- Cytoplasmic vacuoles may be seen, megaloblastic changes in erythroid series, toxic granulation of WBC precursors

**Core Biopsy and Clot Section**
- Often hypocellular
- Polyclonal plasmacytosis often present

**Special Stains**
- Iron stain may show ring sideroblasts
- Plasma cell iron is a rare finding

**Differential Diagnosis**
- Myelodysplastic syndromes (because of red blood cell changes and ringed sideroblasts)
- Monoclonal gammopathies of undetermined significance/myeloma (plasmacytosis)

#### Gastrointestinal Disorders

Most of the hematologic changes due to gastrointestinal disorders are due to specific nutritional deficiencies, such as B12, folate, or iron deficiencies. Malabsorption or liver disease can also lead to other hematologic problems, most specifically red blood cell disorders and hemolysis. Inflammatory bowel disorders such as Crohn disease may uncommonly be associated with the formation of bone marrow granulomas, with a lack of identifiable organisms. As mentioned previously, in Whipple disease the causative gram-positive organism can be found in the bone marrow in many cases.

#### Renal Diseases

Disorders of the kidneys are often associated with hematologic disorders. In chronic renal failure, there is a profound anemia resulting from a lack of erythropoietin production. Although the anemia is multifactorial, erythropoietin supplementation in these patients is often effective in ameliorating the anemia. Peripheral blood changes seen in chronic renal failure include increased numbers of echinocytes (burr cells). Bone marrow changes are those seen in anemia of chronic disease. A characteristic finding in renal disease includes the presence of diffuse changes of bone due to renal osteodystrophy. In these cases the intertrabecular spaces show myelofibrosis and increased vascularity. There is

also increased activity of osteoblasts and osteoclasts with abnormally mineralized bone trabeculae. Hematopoietic elements are often reduced in number. Hemosiderin laden macrophages can also be seen.

### Autoimmune Diseases

A variety of autoimmune disorders have nonspecific effects on the blood and marrow. In the peripheral blood, the presence of lymphocytosis and activated or plasmacytoid forms is common. Anemia and megaloblastic changes in the erythroid series are common to almost all autoimmune diseases. In the bone marrow, lymphocytosis, lymphoid aggregates, and plasmacytosis are also common. Occasionally reticulin fibrosis may be observed (see autoimmune myelofibrosis later in this section).

Autoimmune disorders associated with decreased bone marrow cellularity include systemic lupus erythematosus (SLE), Sjögren syndrome, and rheumatoid arthritis (RA). Another autoimmune disorder associated with marrow changes is Felty syndrome, which is the association of RA with neutropenia and splenomegaly. Both RA alone and Felty syndrome, and to a lesser extent other autoimmune diseases, have an association with large granular lymphocytosis and large granular lymphocytic leukemias. SLE is associated with a variety of hematologic complications including leukopenia, anemias (including rare cases of pure red blood cell aplasia), and thrombocytopenia. Almost all the phenomena in SLE are autoimmune-mediated destruction of cellular elements. Thyroid disease can be associated with anemia often resulting from erythroid hypoplasia. Macrocytosis and acanthocytes may be seen commonly in the peripheral blood. Rarely, in hypothyroidism, there may be serous atrophy in the bone marrow.

One of the more common autoimmune disorders seen in bone marrow evaluations is ITP. ITP is associated with in vivo destruction of platelets, most often caused by antibodies against common platelet antigens. There are few specific findings in the peripheral blood, other than thrombocytopenia, of varying severity. In the marrow, the most common finding is an increase in megakaryocytes, including smaller, more immature forms and senescent forms. Rarely, there are lymphocytes surrounding the megakaryocytes, in a rosette formation. Unfortunately, these findings are not specific for ITP and can be found in a variety of causes of peripheral platelet destruction including infections, medication-related platelet destruction, and hypersplenism.

Other autoimmune cytopenias can occur. In autoimmune hemolytic anemia, peripheral blood findings include reticulocytosis, with an increase in spherocytes and occasionally poikilocytosis. Laboratory findings include decreased haptoglobin and an increase in lactate dehydrogenase (LDH) and bilirubin. Bone marrow changes include a compensatory increase in erythroid elements. Autoimmune hemolytic anemia has several causes and is commonly associated with underlying lymphoproliferative disorders. Autoimmune neutropenia is considerably less common than ITP or autoimmune hemolytic anemia. As anticipated, the peripheral blood shows an absolute decrease in neutrophils. The marrow most commonly shows a compensatory increase in myeloid precursors, an increased M:E ratio, and a left shift in maturation.

One disorder that deserves special mention is autoimmune myelofibrosis. This rare disorder is usually found in young women with a history of other autoimmune phenomenon, such as SLE or RA. It can manifest with anemia or other cytopenias. The marrows are not aspirable. A review of the core biopsy morphology reveals relatively normal–appearing marrow, without significant dyspoiesis, except for general changes seen in autoimmune disorders (e.g., plasma cells, lymphocytes). However, reticulin staining of the marrow reveals moderate-to-severe fibrosis (Figure 6-11). The importance in recognizing this disorder is not to confuse it with other neoplastic causes of myelofibrosis, such as idiopathic myelofibrosis.

## STORAGE DISEASES

### Clinical Features

The diagnoses seen in bone marrow are most commonly secondary to a congenital metabolic disorder that causes accumulation of a biochemical intermediate substance (Table 6-4). Occasionally, similar findings can be seen in both abnormal physiologic states or in association with neoplasms. Most of the metabolic diseases manifest in pediatric age groups, although some disorders manifest in adulthood. Many of these storage disorders are of autosomal recessive inheritance. In the case of Gaucher and Niemann-Pick diseases, the incidence is increased in Ashkenazi Jews.

**TABLE 6-4**

**Storage Disorders with Marrow Involvement**

| |
|---|
| Neimann-Pick disease (types A, B, C) |
| Gaucher disease |
| Wolman disease |
| Cholesterol ester storage disease |
| $GM_1$ gangliosidoses |
| Tangier disease |
| Mannosidosis |
| Fucosidosis |
| Neuraminidase deficiency |

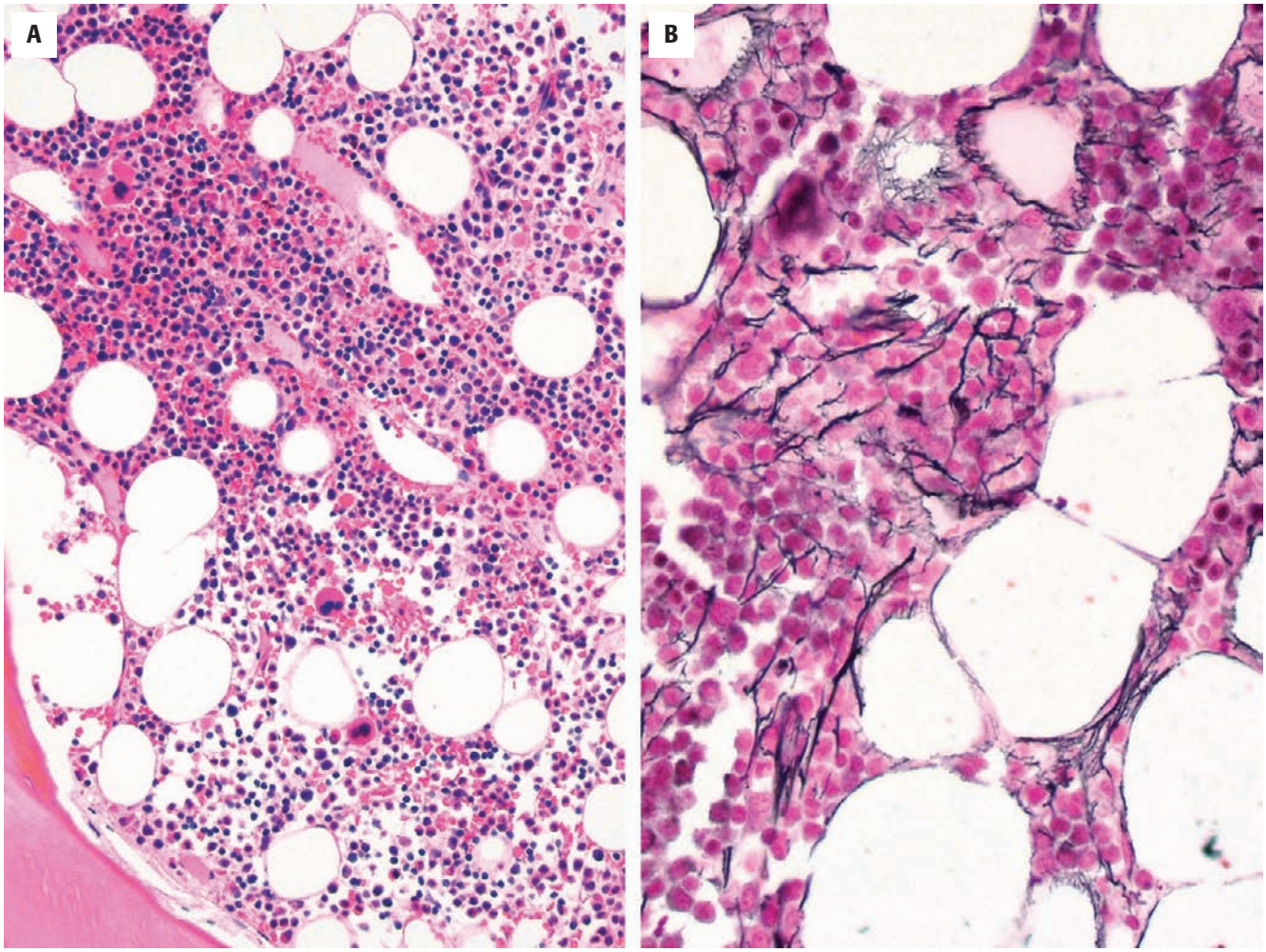


**FIGURE 6-11**

Autoimmune myelofibrosis. A relatively normal-appearing bone marrow that is not aspirable **(A)** may be due to reticulin fibrosis **(B),** as in this case. Autoimmune myelofibrosis is most often identified in women with other autoimmune manifestations.

**STORAGE DISEASES—FACT SHEET**

**Definition**

- Accumulation of metabolic product, typically because of inherited abnormality (often autosomal recessive), leading to varying physiologic dysfunctions

**Incidence**

- Rare

**Clinical Features**

- Variable depending on specific disorder, and presentation
- Many present in infancy and childhood and may be associated with hepatosplenomegaly

**Laboratory Features**

- Specific defects lead to metabolic derangements
- May have anemias or thrombocytopenia due to marrow involvement
- Liver involvement may lead to liver function abnormalities

## Pathologic Features

The two most commonly encountered storage disorders include Gaucher disease and Niemann-Pick disease. Gaucher disease is caused by an inborn error in metabolism, leading to accumulation of glucocerebroside in macrophages. There are several clinical variants of this disorder, with different degrees of clinical severity. In the bone marrow, there is an accumulation of macrophages engorged with the excess metabolic product (Figure 6-12). These macrophages have a distinctive appearance, with a pale blue cytoplasm in the standard Wright-Giemsa stain, and a delicate "folded tissue paper" appearance of the cytoplasm. In core biopsies with H&E stains, the macrophages are large and in clusters, with dense pink cytoplasm. The cells are strongly PAS positive and diastase resistant. Similar cells can be seen in other disorders with rapid cell turnover, such as chronic myelogenous leukemia. These cells

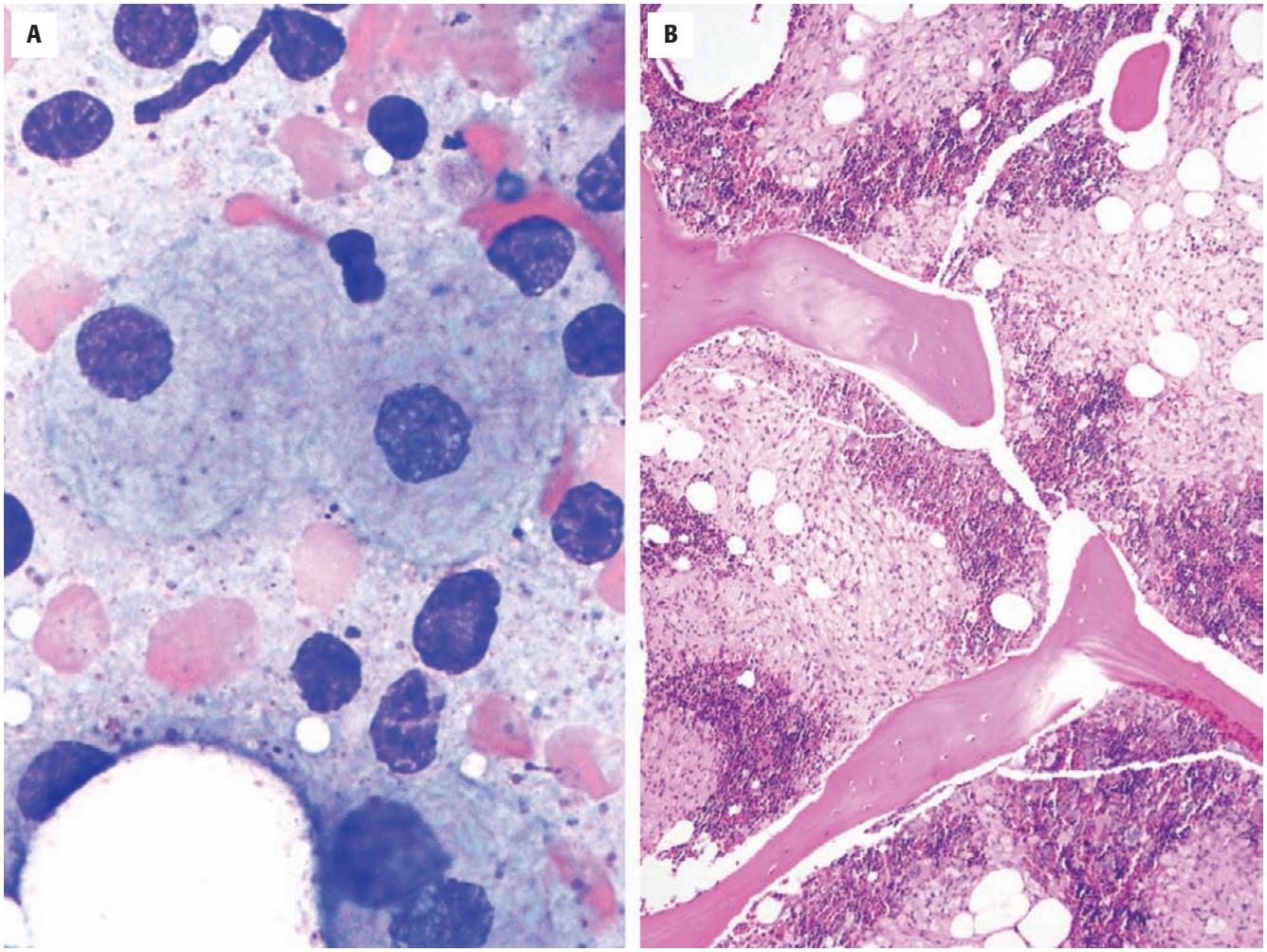


**FIGURE 6-12**

Gaucher disease in bone marrow. **A,** Macrophages filled with excess metabolic product with "folded tissue paper" appearance. **B,** The same macrophages in a marrow core biopsy, showing large clusters and aggregates.

**STORAGE DISEASES—PATHOLOGIC FEATURES**

**Peripheral Blood**

- Rare disorders may have prominent peripheral blood findings, such as prominent toxic-like granulation seen in mucopolysaccharidoses (e.g., Alder-Reilly anomaly)

**Aspirate Smear**

- Macrophages distended with abnormal metabolic product
- Gaucher disease: pale blue, folded tissue paper appearance
- Niemann-Pick disease: pale, with multiple small vacuoles

**Core Biopsy and Clot Section**

- Enlarged macrophages possibly present in clusters or sheets
- Often pale, granuloma-like appearance of H&E

**Flow Cytometry, Immunohistochemistry, Molecular, Cytogenetic**

- Molecular and genetic studies are available to evaluate many inherited storage diseases

**Differential Diagnosis**

- More common types: Gaucher disease (glucocerebrosidase deficiency) and Niemann-Pick disease (sphingomyelinase deficiency); occasionally mimic malignancy or infectious etiologies

are termed *pseudo-Gaucher cells* and have a similar appearance, although they are usually much less numerous than in the metabolic disorder.

Niemann-Pick is a disorder of sphingomyelin metabolism. The macrophages are filled with numerous small vacuoles that contain a fatty metabolic product (Figure 6-13). The cells usually stain only faintly with PAS, but may be positive with Sudan Black B stains. In aspirate smears and core biopsy specimens, these cells may be seen singly or in clusters.

Storage disorders can mimic neoplasms on occasion. Most commonly seen are forms of Niemann-Pick and Gaucher disease. These can mimic unusual lymphomas or, more rarely, true histiocytic malignancies. Histologically, these clusters of macrophages are benign. The cells lack atypical features and have large amounts of cytoplasm. Depending on the product and the type of staining, they may appear pale and vacuolated.

Fabry disease is an X-linked storage disorder caused by a mutation in α-galactosidase-A and build-up of globotriaosylceramide and related glycosphingolipids in involved tissues such as the heart, kidney, and nerves. Characteristic lamellar lysosomal inclusions are seen by electron microscopy. Macrophages in bone marrow contain globular inclusions in Romanowsky-stained smears. Sea blue histiocytes are macrophages containing blue-pigmented lipid on Romanowsky-stained smears and can be seen in several disorders including storage disease (Niemann-Pick), neoplastic processes such as chronic myelogenous leukemia or myelodysplastic syndrome, iatrogenic settings such as total parenteral nutrition, and reactive conditions such as ITP.

## BONE CHANGES

### Clinical Features

The clinical features of bone abnormalities are variable and depend on underlying pathologic processes.

### Pathologic Features

Evaluation for the presence of bone abnormalities is worthwhile and can lead to an unexpected diagnosis or support a clinical diagnosis. Changes such as thickening or thinning of bone trabeculae should be noted. Abnormal osteoid seams may indicate a bone disorder. Osteoblasts and osteoclasts are not prominent in adult bone, although they are common in pediatric specimens. An increase in osteoblastic or osteoclastic activity can be seen in diseases such as myeloma, metastases, or primary bone disorders such as Paget disease. Sites of previous biopsy and metabolic diseases can also cause significant bone changes. Osteoblasts are seen more frequently in children with active bone formation. These cells may be confused with plasma cells and have a similar size and cytoplasmic color (pale blue in the Wright-Giemsa stain). They also have a small round eccentric nucleus. One difference from plasma cells is the location of the Golgi. In osteoblasts the Golgi is located near the cytoplasm edge, as opposed to the paranuclear location in plasma cells. Osteoclasts can be confused with megakaryocytes. They

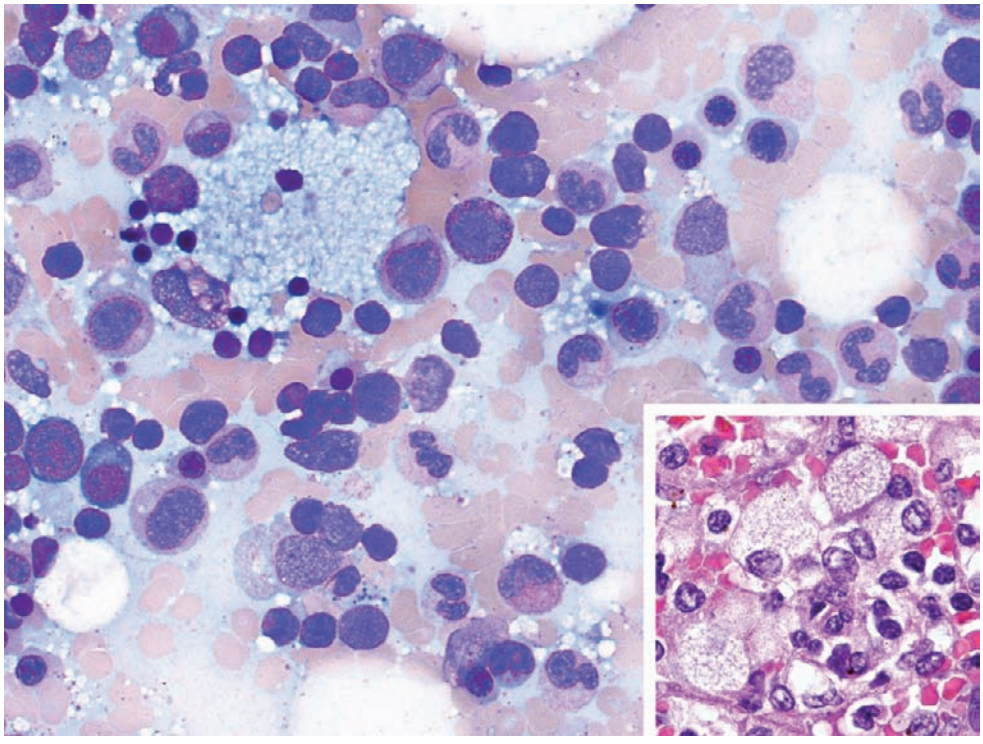

**FIGURE 6-13**

Niemann-Pick disease in bone marrow. Macrophages filled with excess metabolic product have a multivacuolated appearance, in contrast to the appearance of Gaucher disease. *Inset,* Hematoxylin and eosin stain of Niemann-Pick macrophages, in this case in a spleen.

**BONE MARROW FIBROSIS—FACT SHEET**

**Definition**
- Formation of collagen fibrosis induced by a variety of neoplastic and non-neoplastic etiologies

**Clinical Features**
- Severe marrow fibrosis may be associated with splenomegaly

**Prognosis**
- Variable and dependent on underlying cause of fibrosis

**BONE MARROW FIBROSIS—PATHOLOGIC FEATURES**

**Peripheral Blood**
- In severe fibrosis, leukoerythroblastic blood may be seen
- Teardrop red blood cells may be seen

**Aspirate Smear**
- In severe fibrosis, bone marrow aspiration may not be possible

**Core Biopsy and Clot Section**
- Core biopsy may show minimal changes or severe streaming of cellularity between bands of collagen fibrosis, depending on severity and etiology

**Special Stains**
- Reticulin stain should be performed for evaluation of fibrosis. Trichrome stain is positive in severe (mature collagen) fibrosis

**Differential Diagnosis**
- Chronic idiopathic myelofibrosis, other myeloproliferative neoplasms, acute megakaryoblastic leukemia, acute panmyelosis with myelofibrosis, HIV myelopathy, and autoimmune myelofibrosis

**BONE MARROW NECROSIS— FACT SHEET**

**Definition**
- Varying numbers of necrotic cells seen in bone marrow

**Incidence**
- Variable depending on the associated etiology

**Clinical Features**
- Features depend on underlying causes

**BONE MARROW NECROSIS— PATHOLOGIC FEATURES**

**Peripheral Blood**
- Often no abnormalities specific for bone marrow necrosis

**Aspirate Smear**
- Necrotic debris may be seen, with a lack of intact cells
- Debris-laden macrophages may be numerous

**Core Biopsy and Clot Section**
- Variably sized areas of nonviable cells or amorphous necrotic material are seen

**Differential Diagnosis**
- Etiology is variable and includes: hematopoietic malignancies (especially acute lymphoblastic leukemia), nonhematopoietic malignancies (neuroblastomas), coagulation disorders (antiphospholipid syndrome, disseminated intravascular coagulation), red blood cell disorders (sickle cell), medications or chemotherapy, and other causes

are large in size, but contrary to megakaryocytes, they have multiple nuclei rather than lobated nuclei.

One of the more commonly encountered primary bone disorders is osteoporosis. The finding typically encountered is thinning of the bone trabeculae. More rarely, disorders such as Paget disease are seen. Bone changes resulting from primary hyperparathyroidism include increased bone formation, fibrosis and cyst formation (osteitis fibrosa cystica). Chronic renal disease also results in PTH excess, and the bone marrow changes associated with renal disease are similar. (Figure 6-14). Thickened bone trabeculae with prominent osteoid seams from osteoblastic and osteoclastic activity are seen. Stromal fibrosis develops around the trabeculae.

## STROMAL DISORDERS

### Clinical Features

The clinical features of stromal abnormalities are variable and depend on underlying pathologic processes.

### Pathologic Features

Bone marrow stroma is often underappreciated in the evaluation of marrows. The stroma is a complex microenvironment that both nurtures and supports the development of hematopoietic cells. The stroma cells mostly consist of adventitial reticulum cells that are fibroblastic in nature and the precursors of myofibroblasts and smooth muscle cells that are found in the marrow. It is

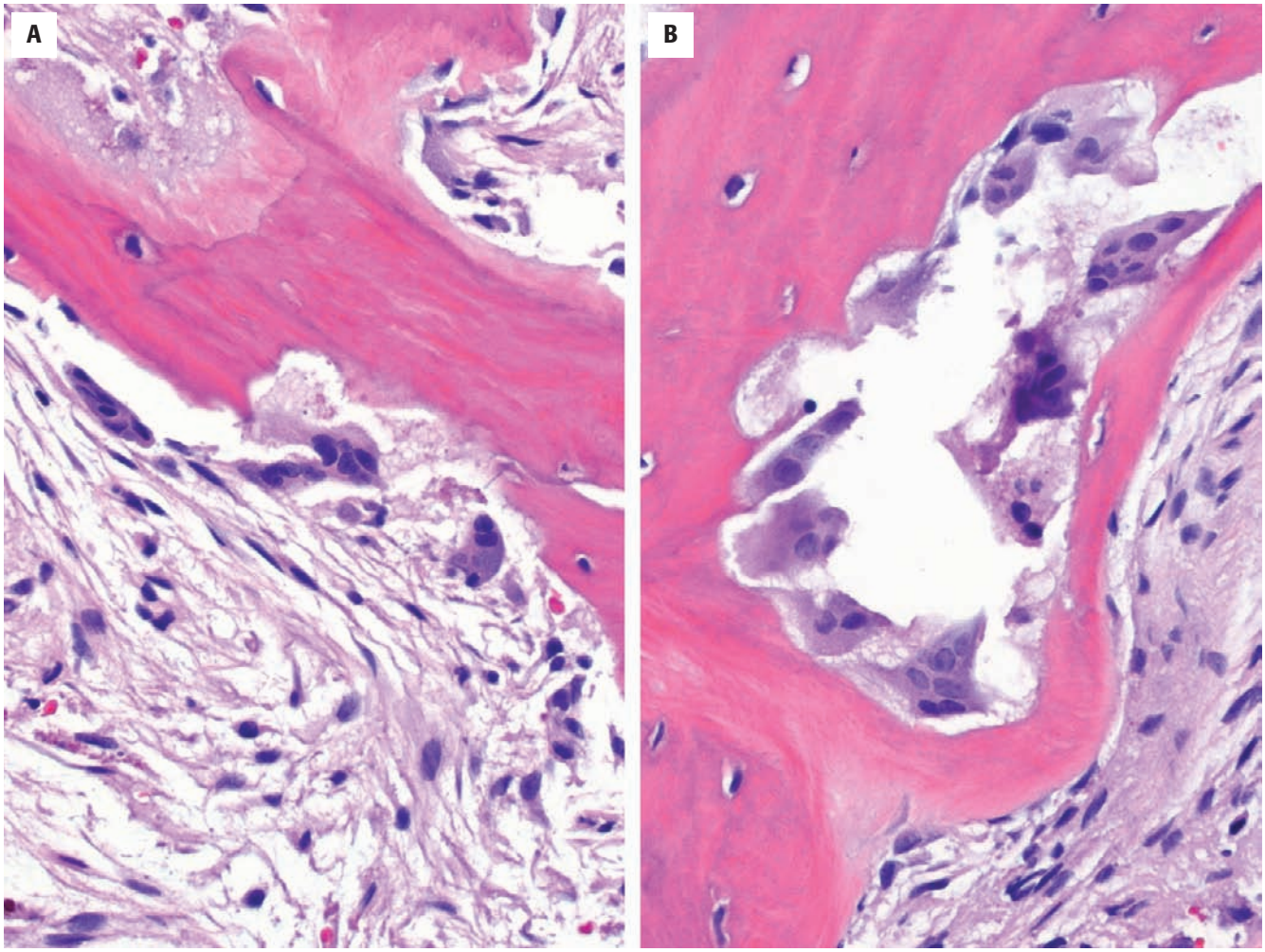


**FIGURE 6-14**

Bone and marrow changes seen in renal disease. Interstitial fibrosis and irregular bone trabeculae **(A)** are common, as well as increased osteoclastic and osteoblastic activity **(B).**

the adventitial reticulum cells and their derivatives that form the structural framework of the marrow. Other elements include fat cells, vascular elements, and bone. Additional components include undifferentiated stromal elements, which are typically inconspicuous cells in the marrow.

A variety of stromal changes can make these elements more apparent. Fibrosis is a common reactive change in marrows (Figure 6-15). Fibrosis in the marrow is typically secondary to other marrow changes. Reticulin, as assessed by the Gomori silver stain, is thought to represent largely immature collagen, presumably type III collagen; however, the stain identifies also collagen IV. Fibrosis of the marrow is routinely measured on a scale of 0 to 4. An important theoretical note is that fibrosis is not neoplastic; it is derived from nonneoplastic cells in response to a variety of conditions.

Changes in fat are only rarely significant in the marrow. Lipogranulomas (discussed previously) are probably due to localized disruptions of fat cells. Serous fat atrophy, also called *gelatinous transformation of bone marrow,* is characterized by a reduction in hematopoietic cells and the loss of marrow fat cells (Figure 6-16) replaced by adipocytes with a granular, pinkish cytoplasm. The hypocellular areas contain an amorphous extracellular gelatinous substance that is composed of mucopolysaccharides rich in hyaluronic acid. This change is seen most often in states of severe metabolic compromise, such as cachexia or anorexia. However, it can be seen uncommonly in a wide variety of settings including HIV infection, malignancies, alcoholism, acute febrile illnesses, and autoimmune disorders such as SLE.

Necrosis is an uncommon finding in the bone marrow and can be associated with a variety of causes (Table 6-5). Focal necrosis can be seen in a variety of infections and can be associated with granulomas. In addition, disorders of abnormal coagulation can be associated with ischemic marrow necrosis. These disorders include disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and antiphospholipid antibody syndrome. A relatively common cause of significant marrow necrosis is sickle

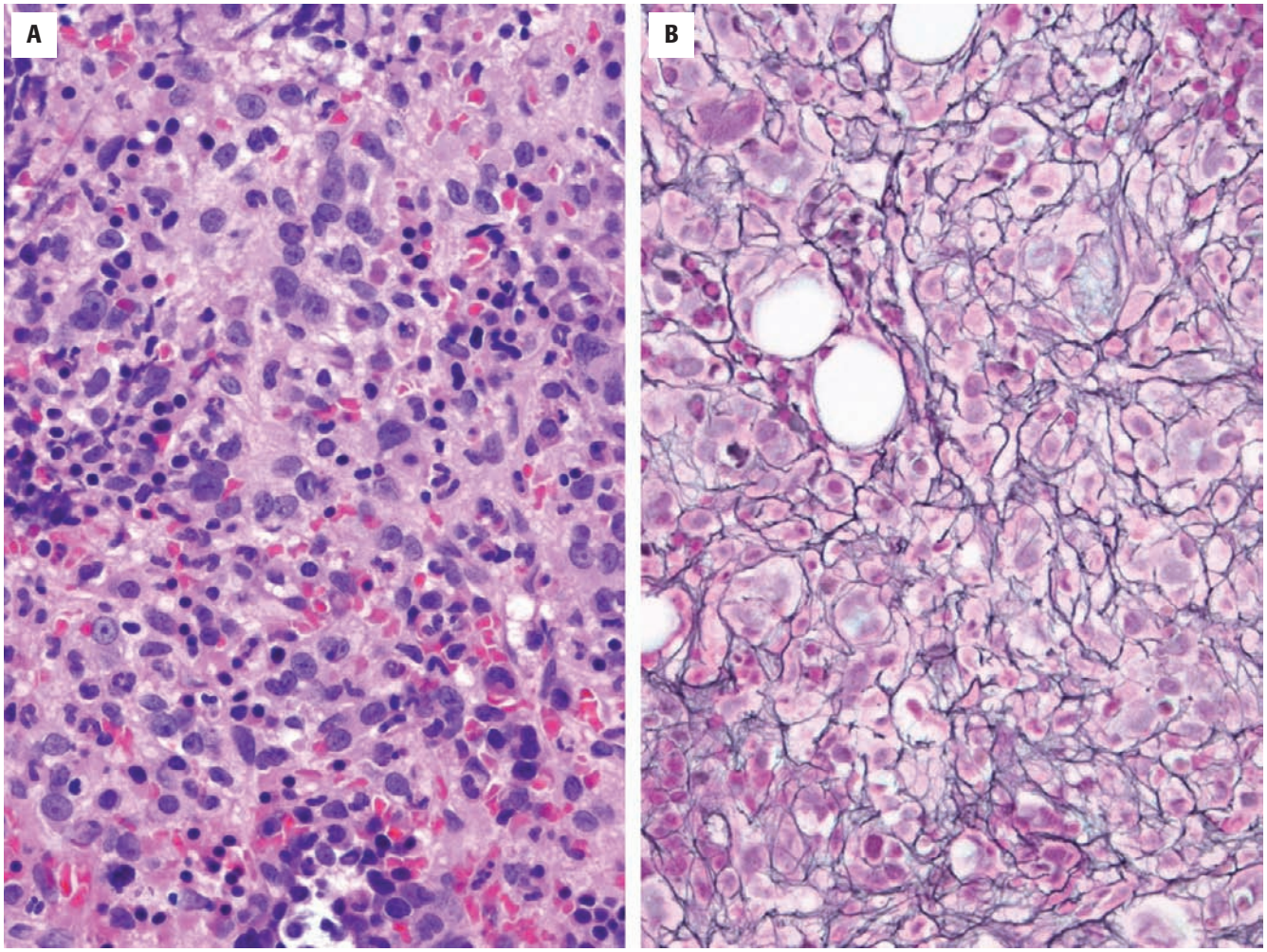


**FIGURE 6-15**

Bone marrow fibrosis. **A,** This case of acute myeloid leukemia developing from a previous myeloproliferative disorder has extensive reticulin fibrosis. **B,** The stromal elements that produce the fibrosis are not part of the neoplastic clone, but are in response to it.

**TABLE 6-5**

**Disorders Associated with Bone Marrow Necrosis**

| |
|---|
| Sickle cell disease |
| Severe infections |
| Disseminated intravascular coagulation |
| Thrombotic thrombocytopenic purpura |
| Hemolytic-uremic syndrome |
| Antiphospholipid syndrome |
| Acute graft-versus-host disease after bone marrow transplant |
| Megaloblastic/sideroblastic anemias |
| Malignancies |
| Hematologic (e.g., acute lymphoblastic leukemia, acute myeloid leukemia, Hodgkin lymphoma, chronic myelogenous leukemia, myeloma) |
| Carcinomas |
| Neuroblastoma and other "small blue cell" tumors |

cell disease. Subsequent embolization of the necrotic marrow is thought to contribute to the acute chest syndrome. Malignancies, both as initial presentations or after treatment, can manifest as areas of necrosis or sheets of "ghost" cells (Figure 6-17). In general, it is difficult to determine the etiology and cell type based on histologic findings alone. If there is viable marrow present, it will often provide clues to the diagnosis. Correlation with clinical history, and less commonly repeat biopsies, often yield a diagnosis.

## LYMPHOCYTES AND LYMPHOID AGGREGATES

### Clinical Features

Many different clinical conditions can be associated with the presence of an increased number of lymphocytes, lymphoid aggregates, and lymphoid precursors (hematogones) in the bone marrow. Lymphocytes represent

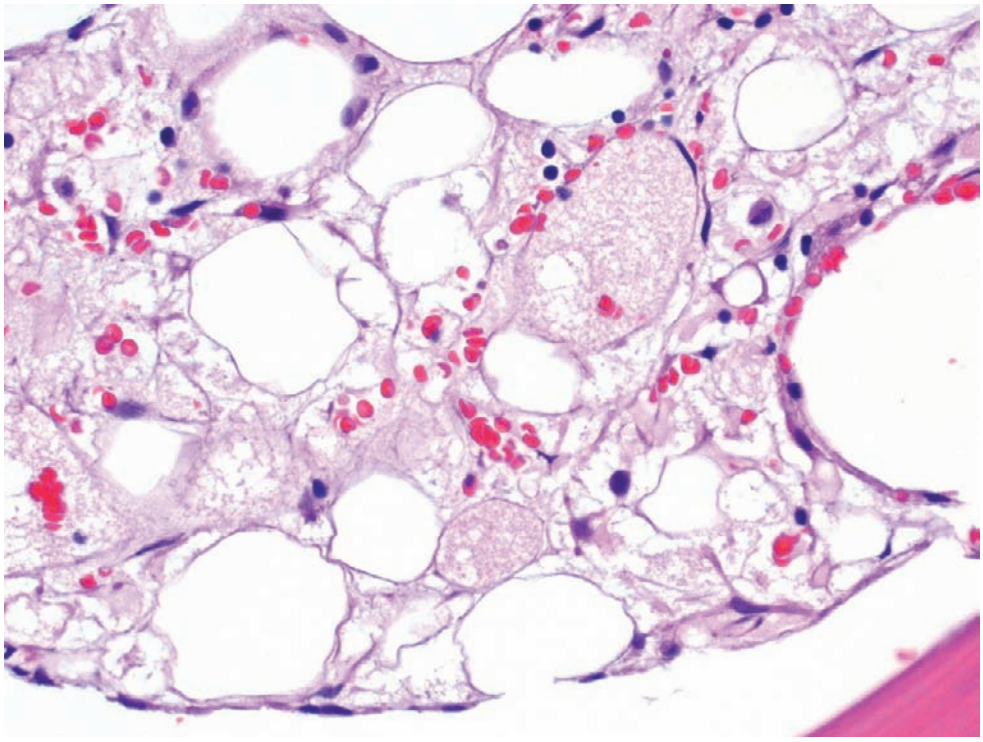

**FIGURE 6-16**

Serous atrophy, also known as *gelatinous transformation of bone marrow,* can be associated with a variety of causes, such as anorexia nervosa, chemotherapy, HIV/AIDS, and malnutrition states.

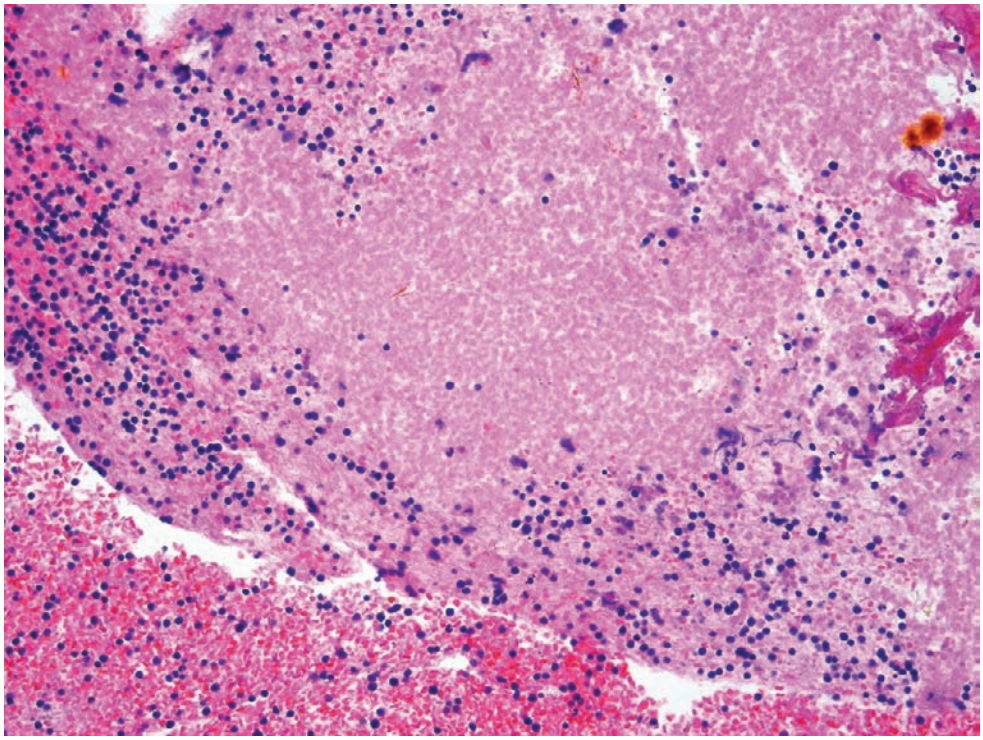

**FIGURE 6-17**

Bone marrow infarct can be associated with both benign and neoplastic causes. This case illustrates necrosis in a case of acute lymphoblastic leukemia, with rare viable tumor cells present.

approximately 20% of the normal marrow cellularity in adults and typically consist predominantly of T cells. B cells are more numerous in children, where the marrow is the site of formation and early development of B cell precursors, or hematogones. Increases in lymphocytes, hematogones, or lymphoid aggregates may be seen in both reactive and neoplastic conditions.

## Pathologic Features

### Hematogones

Hematogones are immature lymphoid precursor cells that are more numerous in the bone marrows of children. They are often increased after chemotherapy and in reactive marrow conditions. They are usually small, but can be of intermediate size, overlapping the size range of lymphoblasts (Figure 6-18); when increased, they can be confused with acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma. In contrast to leukemic blasts, hematogones typically have a more condensed, smudged chromatin, and only rarely have nucleoli. Hematogones tend to be dispersed throughout the marrow, with no tendency to cluster, in contrast to leukemic blasts. The immunophenotype largely overlaps that of blasts seen in precursor B-ALL. For example, hematogones are positive for CD19 and CD10. Careful flow cytometric analysis of hematogones can usually distinguish them from precursor B-ALL. Hematogone populations often express a spectrum of CD10, CD20, CD34, and surface immunoglobulin, reflecting their maturation from immature B cells to more mature stages. Leukemic populations tend to be more homogeneous in their expression pattern and often express abnormally high or low levels of one or more of these antigens. This fact is often reflected in the immunohistochemical staining patterns. Using immunohistochemistry for CD34 and TdT, markers of precursor cells, hematogones tend to be more variably positive, and scattered in distribution while B-ALL cells are more uniformly positive and show a tendency to cluster formation. CD20 usually stains hematogones more strongly and consistently than the lymphoblasts.

### Lymphoid Aggregates

Lymphoid aggregates (LAs) are frequently encountered in the bone marrow. They are not always pathologic, and their form, location, number and most importantly, the clinical setting in which they are observed, should be considered when evaluating them. LAs tend to occur with more frequency as patients age. In addition, they are more common in patients with chronic disease and autoimmune conditions. Numerous or large lymphoid aggregates should always raise a concern for lymphoma. A paratrabecular location of

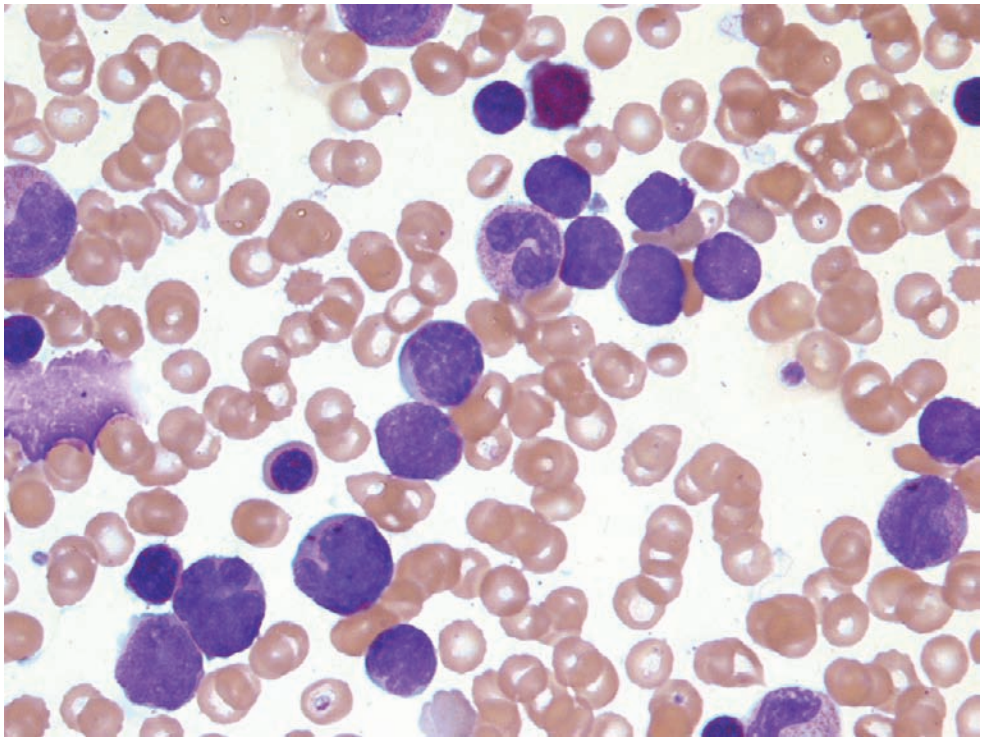

**FIGURE 6-18**

A bone marrow aspirate showing numerous hematogones or B lymphocyte precursors. Hematogones are more common in the bone marrow of children, especially in association with other reactive conditions and after chemotherapy.

lymphoid aggregates is suggestive of malignancy and should be evaluated by immunohistochemical stains. The composition of the LA is also important. Benign LAs are typically composed of predominantly small lymphocytes, with an intermixture of histiocytes and plasma cells with only rare larger lymphocytes. However, rare well-formed germinal centers can be seen and are more often benign than malignant. If the cellular composition of the aggregate is homogeneous or there are numerous large lymphocytes, the possibility of lymphoma should be considered. Benign lymphoid aggregates are typically located adjacent to or associated with blood vessels. They typically have sharper, more well-defined borders than do lymphoma aggregates in marrow.

A difficulty in the assessment of posttreatment marrow is associated with the recent use of monoclonal antibody-based treatments. Both rituximab (anti-CD20 antibody) and alemtuzumab (anti-CD52 antibody), which are used in the treatment of lymphoid malignancy, are associated with the development of large reactive T cell–rich LAs in posttreatment marrows that may be mistaken for residual lymphoma of leukemia. In the setting of B cell malignancy, immunostaining can assist in distinguishing between reactive T cell aggregates and B cell lymphoproliferative disorders. This evaluation is further complicated by the fact that B cells can underexpress CD20 after treatment with anti-CD20 antibodies. In the context of prior anti-CD20 antibody therapy, a back-up B cell stain (CD79a or PAX-5) should be used to evaluate the composition of the lymphoid aggregates.

## CYTOKINE EFFECTS

### Clinical Features

Cytokines are frequently used to treat a variety of cytopenic conditions. Most commonly, pathologists see marrows of patients who have received granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), or erythropoietin.

**CYTOKINE EFFECTS—FACT SHEET**

**Definition**
- Marrow changes associated with the pharmacologic use of cytokines for treatment
- Marrow changes may be predominant in individual cell lineages, depending on the cytokine used

**Incidence**
- Increases in use of cytokine therapies make these effects relatively common

**Prognosis and Therapy**
- G-CSF often used in stem cell mobilization
- Erythropoietin used in several types of chronic anemias

### Pathologic Features

With all types of cytokine treatments, the marrow may show an increase in immature cells and cellularity, which can mimic neoplastic conditions. The effects of G-CSF or GM-CSF can mimic acute myeloid leukemia (AML), but occasionally myelodysplastic syndromes or myeloproliferative neoplasms can be simulated. The single most important factor in dealing with these situations is obtaining an appropriate clinical and treatment history; this can resolve most difficulties. GM-CSF and G-CSF will cause increases in immature cells in the peripheral blood. These cells typically include left-shifted myeloid elements in a variety of stages of maturation. In particular, myeloid blasts can occasionally be seen. Neutrophils and precursors usually display toxic granulation (Figure 6-19). In the marrow, there will usually be an increase in the M:E ratio associated with left-shifted granulopoiesis with numerous promyelocytes and myelocytes. Again, toxic changes are characteristically present early in therapy. A slight increase in blasts may be seen. With GM-CSF, in addition to the effect on granulopoiesis, there are often increased monocytes, monocytic precursors, and variably increased numbers of eosinophils. On more than one occasion changes of growth factor (G-CSF or GM-CSF) therapy have been misinterpreted as acute leukemia or myeloproliferative neoplasms. Presence of only modestly elevated blast counts, a spectrum of maturation in the

**CYTOKINE EFFECTS—PATHOLOGIC FEATURES**

**Peripheral Blood**
- G-CSF: increased in granulocytes, left shift with increased blasts, increased coarse toxic granulation

**Aspirate Smear**
- G-CSF: increased M:E ratio, left shift but overall blast percentage not significantly increased
- GM-CSF: similar to G-CSF with increases in monocytic elements
- Erythropoietin: decreased M:E ratio, megaloblastic changes

**Core Biopsy and Clot Section**
- Often hypercellular; increases in individual cell lineages; often increase in immature elements

**Differential Diagnosis**
- Increased immature elements may be confused with leukemia
- Clinical history is important for evaluation
- Repeated biopsy may be necessary

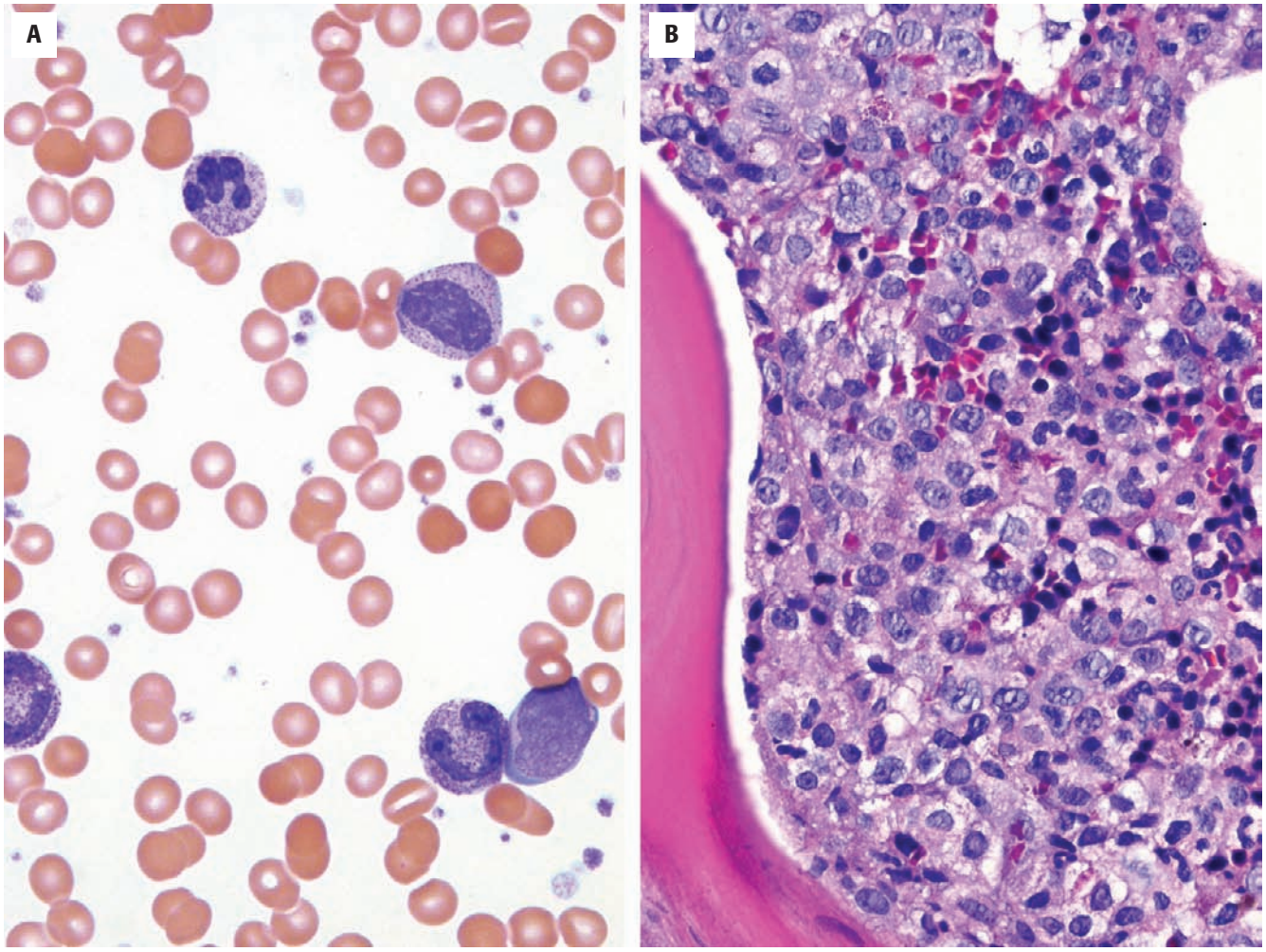


**FIGURE 6-19**

Examples of peripheral blood **(A)** and marrow findings **(B)** in a case of granulocyte colony-stimulating factor therapy. The blood shows circulating blasts, but toxic changes in the more mature granulocytic cells suggest a reactive process such as growth factor effect. In the biopsy specimen, the pattern of development of granulocytic maturation from the bone trabeculae outward also points to a reactive process.

granulocytic lineage (albeit concentrated in the early forms), toxic granulation, and a good history allow one to avoid mistaking growth factor effects for a myeloid malignancy. Patience will also clarify the matter because it is possible to follow the maturation of the granulocytic cells over the course of a few days. G-CSF and GM-CSF effects are also reviewed in the chapter on acute myeloid leukemias.

The effects of erythropoietin only rarely cause diagnostic difficulty. There is a predictable increase in erythroid precursors associated with a decrease in the M:E ratio. The increased immature erythroids may appear concerning on core biopsy specimens in which they may simulate early myeloid cells, but their erythroid nature is usually easily identifiable in aspirate smears. If difficulty remains, immunostaining with glycophorin or hemoglobin can be used to confirm their erythroid derivation. At high levels, erythropoietin drives megakaryopoiesis, so that megakaryocytes may appear increased as well.

## TOXINS

The effect of toxins on marrow depends on the agent. Benzene is well known as a cause of aplastic anemia and myelofibrosis. Pesticide exposure can also cause transient marrow aplasia. The aplastic marrows are not themselves difficult to diagnose. Rather, the recovery from a toxic insult can be troublesome. In these cases, the marrow tends to recover in waves showing a reactive hyperplasia of synchronized myeloid precursors. This finding can be concerning and raise a differential of AML or acute promyelocytic leukemia (APML). In these cases, clinical correlation may be the key. Often the myeloid cells appeared to be morphologically normal promyelocytes. These cells do not have the morphologic abnormalities associated with APML (folded nuclei, Auer rods). Further investigation of clinical history for toxin or drug exposures, or repeated biopsy after an appropriate interval, typically resolve difficult cases.

**CHEMOTHERAPY EFFECTS—FACT SHEET**

**Definition**
- Marrow changes associated with cytotoxic chemotherapy

**Clinical Features**
- Variable depending on type, extent, and time since chemotherapy

**CHEMOTHERAPY EFFECTS—PATHOLOGIC FEATURES**

**Peripheral Blood**
- Cytopenias not uncommon

**Aspirate Smear**
- Hypocellularity is common
- Erythroid lineage shows dyspoiesis
- Increases in stromal elements are common

**Core Biopsy and Clot Section**
- Hypocellularity is common
- Mild fibrosis may be seen
- Stromal elements may be relatively increased with decreases in hematopoietic elements

**Differential Diagnosis**
- Evaluation for residual and recurrent disease is important

Toxins and drug reactions may also mimic myelodysplastic syndromes (MDSs). For example, arsenic poisoning can result in erythroid hyperplasia and severe dyserythropoiesis resembling MDS. Zinc overexposure, from excessive dietary supplements, results in copper deficiency and a sideroblastic anemia mimicking myelodysplasia. Dyserythropoiesis with vacuolization, ring sideroblasts, and plasma cell iron granules are seen. Valproate therapy or toxicity can induce dysplastic changes that can be mistaken for MDS.

## CHEMOTHERAPY

### Clinical Features

Chemotherapy-related effects are seen in the bone marrow of patients undergoing systemic treatment for a variety of pathologic conditions, most often malignancies.

### Pathologic Features

Antineoplastic chemotherapy can cause marrow suppression and a variety of dyshematopoietic changes. The major changes seen in myeloablative therapy are reviewed in Chapter 14. However, it is important to note that chemotherapy typically consists of antimetabolite drugs. Marrow, being metabolically active, can be easily affected by these medications. In any drug where anemia or myelosuppression can occur as a side effect, dysplasia-like changes can be seen in the marrow. In particular, the various dyserythropoietic changes seen in the myelodysplastic syndromes can be closely replicated by chemotherapy.

## SUMMARY

There are a broad range of disorders affecting the bone marrow that are not due to malignancy, but rather are caused by infections, storage diseases, systemic disorders, or stromal changes. The findings in these disorders are highly variable and may be nonspecific; however, some show distinctive morphologic or immunophenotypic findings that make a diagnosis relatively straightforward. Because of the broad range of hematologic and clinical presentations, many of the conditions may be the primary or diagnostic manifestation of a disease, and their recognition and differentiation from other marrow disorders is important.

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SECTION 2

# Lymphomas

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7

# Small B-Cell Lymphomas

**Paul J. Kurtin, MD**

## INTRODUCTION

This chapter covers the lymphomas of small B lymphocytes: B-cell small lymphocytic lymphoma–chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), splenic marginal zone B-cell lymphoma, nodal marginal zone B-cell lymphoma, and lymphoplasmacytic lymphoma. Though morphologically somewhat similar to one another, these tumors are characterized by wide differences in clinical presentation, phenotype, genetic features, and outcome.

In the past the classification of the small lymphocytic lymphomas was based on the concept that there was a one-to-one correspondence between a lymphoma type and a corresponding normal cell counterpart. This concept is indeed true for follicular lymphoma and extranodal marginal zone B-cell lymphoma of MALT, which are thought to recapitulate the normal germinal center reaction (follicular lymphomas) or arise from normal mucosa associated marginal zone B cells (MALT lymphoma). Small lymphocytic lymphoma–chronic lymphocytic leukemia and mantle cell lymphomas were thought to be the counterparts of naive B lymphocytes that had rearranged their immunoglobulin (Ig) genes, but that had not yet encountered antigen. Thus, one would predict that the cells of these two lymphoma types would have germline sequences of their Ig variable genes as would be characteristic for normal naive B lymphocytes. However, 50% of B-cell small lymphocytic and 25% of mantle cell lymphomas have mutated Ig gene variable regions, indicating that they are neoplasms of post follicular B cells that have encountered antigen. Thus, the World Health Organization (WHO) classification of tumors of the lymphoid tissues does not use the putative normal cell counterpart as the basis for classification of these tumors, but rather attempts to define homogeneous entities based on a combination of clinical, morphologic, immunophenotypic, and genetic attributes. These entities are described in this chapter.

Three tasks confront the pathologist approaching the small B-cell lymphomas: distinguishing the lymphomas from reactive lymphoid hyperplasia, correctly classifying the lymphoma, and providing sufficient phenotypic information to the clinician so that he or she can use any of the immunologic based treatment modalities available. The cornerstone for accomplishing these tasks is careful morphologic observations applied to well-fixed tissue sections on optimally prepared slides. Many of the lymphomas described in this chapter can be diagnosed with confidence solely on the basis of morphologic findings. Notable examples would include most cases of B-cell small lymphocytic lymphoma–chronic lymphocytic leukemia in lymph nodes, follicular lymphomas, and sufficiently sampled prototypic extranodal marginal zone B-cell lymphomas of MALT in the stomach, salivary gland, and lung. In other cases, a limited phenotypic evaluation by either flow cytometry or paraffin section immunohistochemistry suffices to supplement the morphologic evaluation. In the proper morphologic context, Ig light chain restriction in a B-cell population, as demonstrated by immunohistochemistry or flow cytometry, provides strong evidence of a B-cell lymphoma over reactive lymphoid hyperplasia. Once the diagnosis of lymphoma is reached, considering the tumor cell phenotype using a panel of antibodies to CD20, CD10, CD23 BCL-6, BCL-2, cyclin D1, CD3, CD5, and κ and λ Ig light chains (the latter two in paraffin sections to mark plasma cells) is usually sufficient to distinguish among all of the lymphomas discussed in this chapter. Genetic analysis is confined to the limited subset of cases that are not morphologically and phenotypically characteristic. In most cases requiring genetic testing, fluorescence in situ hybridization (FISH) applied to air-dried tumor cell imprints, whole tissue sections, or nuclei extracted from

**TABLE 7-1**
**Differential Diagnosis of Small B-Cell Lymphomas: Morphology**

| Disease | Lymph Node | Spleen | Bone Marrow | Cytology |
|---|---|---|---|---|
| B-cell small lymphocytic/CLL | Diffuse pattern, proliferation centers | Red pulp; cords, sinuses; later white pulp | Intertrabecular nodules, interstitial infiltrates; not paratrabecular | Small lymphocytes, prolymphocytes, and paraimmunoblasts |
| Mantle cell | Diffuse or nodular pattern, atrophic germinal centers | White pulp; atrophic germinal centers, obliterated marginal zones | Intertrabecular nodules, paratrabecular aggregates | Small lymphocytes with nuclear irregularity; no large cells |
| MALT lymphoma | Paracortical infiltrates; surrounding germinal centers | White pulp marginal zones | Intertrabecular nodules, paratrabecular aggregates; intrasinusoidal infiltrates | Centrocyte-like cells, plasma cells occasional large transformed lymphocytes; non-neoplastic germinal centers |
| Splenic marginal zone lymphoma | Nodular perifollicular pattern | White pulp nodules with dimorphic cytologic features | Intertrabecular nodules, paratrabecular aggregates, intrasinusoidal infiltrates | Dimorphic in spleen; mantle cell–like in center of nodules; medium-sized cells; irregular nuclei, abundant pale cytoplasm; occasional large transformed cells in perimeter of nodules |
| Nodal marginal zone | Perisinusoidal or surrounding benign germinal centers and mantle zones | White pulp; small germinal centers, residual non-neoplastic mantle cells | Intertrabecular nodules, paratrabecular aggregates; intrasinusoidal infiltrates | Medium-sized cells; irregular nuclei, abundant pale cytoplasm; occasional large transformed cells |
| Follicular lymphoma | True follicular nodularity | White pulp; germinal centers expanded by benign mantle and marginal zone cells | Intertrabecular nodules, paratrabecular aggregates | Small centrocytes and large centroblasts in varying proportions |
| Lymphoplasmacytic lymphoma | Paracortical and hilar infiltrates, open sinuses | Red pulp and occasionally white pulp | Intertrabecular nodules, paratrabecular aggregates, interstitial infiltrates | Small lymphocyte, plasmacytoid lymphocyte, plasma cell spectrum; varying large cells |

*CLL,* Chronic lymphocytic leukemia; *MALT,* mucosa-associated lymphoid tissue.

formalin-fixed paraffin embedded tissue will resolve the final diagnosis. Tables 7-1 to 7-3 summarize the morphologic, phenotypic, and genetic features that characterize the lymphomas discussed in this chapter.

## B-CELL SMALL LYMPHOCYTIC LYMPHOMA

B-cell small lymphocytic lymphoma (SLL) is an indolent B-cell non-Hodgkin lymphoma that is treated in the WHO classification as a single entity along with B-cell chronic lymphocytic leukemia (CLL). Historically, the term *SLL* was used for lymphomas that were morphologically and immunophenotypically indistinguishable from CLL but lacked lymphocytosis. However, most patients with pure SLL develop bone marrow involvement and lymphocytosis over the course of their disease. SLL in its pure form accounts for less than 10% of CLL/SLL and approximately 5% to 10% of all non-Hodgkin lymphomas. Many early studies on the features of SLL did not exclude blood involvement; therefore there is a lack of accurate information about the presentation, prognosis, and treatment of pure SLL. This chapter will review the pathologic features of lymph node–based SLL, whereas the features of CLL in blood and bone marrow are considered in Chapter 12.

### Clinical Features

The median age at diagnosis is 55 to 65 years, with a male-to-female ratio of 2:1. In the international non-Hodgkin lymphoma classification project, 91% of patients had advanced-stage disease (stage III or IV), and 72% had bone marrow involvement. Almost all patients with SLL have generalized lymphadenopathy at presentation. At diagnosis, 30% have extranodal disease usually resulting from spleen or liver involvement, or both. Approximately 25% of primary splenic lymphomas are SLL.

The most common presentation is generalized lymphadenopathy. B symptoms (fever, weight loss, and

**TABLE 7-2**
**Differential Diagnosis of Small B-Cell Lymphomas: Typical Phenotypes**

| | SIg | CD19 | CD20 | CD23 | CD10/BCL-6 | CD5 | CD3 | Cyclin D1 |
|---|---|---|---|---|---|---|---|---|
| B-cell SLL/CLL | Monoclonal (dim) | + | + (dim) | + | − | + | − | − |
| Mantle cell lymphoma | Monoclonal (bright) | + | + (bright) | − | − | + | − | + |
| Marginal zone lymphoma (MALT, nodal and splenic) | Monoclonal (bright) | + | + (bright) | − | − | ± | − | − |
| Follicular lymphoma | Monoclonal (bright) | + | + (bright) | ± | + | − | − | − |
| Lymphoplasmacytic lymphoma | Monoclonal (also cIg positive monoclonal plasma cells) | + | + | ± | − | − | − | − |

*cIg,* Cytoplasmic immunoglobulin; *CLL,* chronic lymphocytic leukemia; *MALT,* mucosa-associated lymphoid tissue; *SLL,* small lymphocytic lymphoma.

**TABLE 7-3**
**Differential Diagnosis of Small B-Cell Lymphomas: Genetics**

| | Chromosome Abnormality | Genes Involved |
|---|---|---|
| B-cell SLL/CLL | +12; del(13q); del (11q), del (17p), del (6q) | miR-15-16, *DLEU7*, *TP53,* others as yet unknown |
| Mantle cell lymphoma | t(11;14)(q13;q32) | *CCND1/IGH@* |
| MALT lymphoma | t(11;18)(q21;q32)<br>t(14;18)(q32;q21)<br>t(1;14)(p22;q32)<br>t(3;14)(p13;q32)<br>+3, +8, +18<br>del(6q23.3) | *API2/MALT1*<br>*IGH@/MALT1*<br>*BCL10/IGH@*<br>*FOXP1/IGH@*<br>Unknown<br>*TNFAIP3* |
| Nodal marginal zone | +3 | Unknown |
| Splenic marginal zone lymphoma | del(7q) | Unknown |
| Follicular lymphoma | t(14;18)(q32;q21) | *BCL2* |
| Lymphoplasmacytic lymphoma | del(6q21) | Unknown |

*CLL,* Chronic lymphocytic leukemia; *MALT,* mucosa-associated lymphoid tissue; *SLL,* small lymphocytic lymphoma.

night sweats) are uncommon and are seen in less than one third of patients. Anemia and thrombocytopenia are seen in up to one third of patients (hemoglobin <11 g/dL and fewer than 150,000 platelets/mm$^3$). By extension of the international consensus definition of CLL, absolute B lymphocyte count should be less than 5000/μL. A small M component, less than 30 g/L, is seen in 20% of cases. These antibodies are of the IgM type and can have specificity against self antigens present on the red blood cells and platelets, resulting in autoimmune hemolytic anemia and thrombocytopenia. Hypogammaglobulinemia is seen in 40% of patients with SLL, making them vulnerable to infection.

## Pathologic Features

### Morphology

In approximately 80% of cases, SLL completely effaces the node architecture, often with invasion of the capsule and extension into the pericapsular fat (Figure 7-1). In the remainder, neoplastic cells infiltrate the interfollicular areas, sparing small atrophic lymphoid follicles and leaving the sinuses patent. The neoplastic cells in SLL are small (6 to 12 μm) lymphocytes with round nuclear contours, distinctly condensed chromatin, inconspicuous nucleoli, and sparse agranular cytoplasm (Figure 7-2). Although small lymphocytes

## SMALL LYMPHOCYTIC LYMPHOMA—FACT SHEET

### Clinical Features

- Median age, 55 to 65 years
- Male predominance
- Asymptomatic generalized lymphadenopathy
- Eighty percent of patients exhibit Ann Arbor stage IV disease because of bone marrow involvement
- Progresses to leukemic phase, indistinguishable from B-cell chronic lymphocytic leukemia in a high percentage of patients

### Morphology

- Diffuse growth pattern with complete (80%) or partial (20%) involvement
- Small lymphocytes with round nuclei, condensed chromatin, sparse cytoplasm
- Proliferation centers composed of medium-to-large cells with vesicular chromatin and single central nucleoli (prolymphocytes–paraimmunoblasts)
- Transformation to diffuse large B-cell lymphoma (Richter syndrome) or Hodgkin lymphoma in 5%

### Immunophenotype

- $CD19^{+}$, $CD20^{+(dim)}$, $sIg^{+(dim)}$, IgM with or without IgD
- $CD5^{+}$, $CD23^{+}$, $LEF\text{-}1^{+}$
- CD38 and ZAP-70 expression associated with adverse outcome

### Genetics

- +12, del(13q), del(11q), del(17p), del(6q)
- Point mutations in Ig genes present in 50%

### Prognosis and Therapy

- Indolent, incurable disease
- Watch-and-wait approach for asymptomatic stage III and IV
- Low-intensity single- or multiple-agent chemotherapy for symptomatic patients
- 8- to 10-year median survival
- Outcome predicted by International Prognostic Index
- Aggressive disease may be indicated by prominent proliferation centers
- Additional biologic predictors of high risk for adverse outcome: del(17p), del(6q), germline immunoglobulin genes, zap-70, and/or CD38 positive tumor cells
- Transformation to diffuse large B cell lymphoma in 20% heralds aggressive disease

### Differential Diagnosis

- Reactive lymphoid hyperplasia
- Mantle cell lymphoma
- Follicular lymphoma
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue
- Lymphoplasmacytic lymphoma
- Lymphocyte-predominant Hodgkin lymphoma
- Lymphocyte-rich classical Hodgkin lymphoma

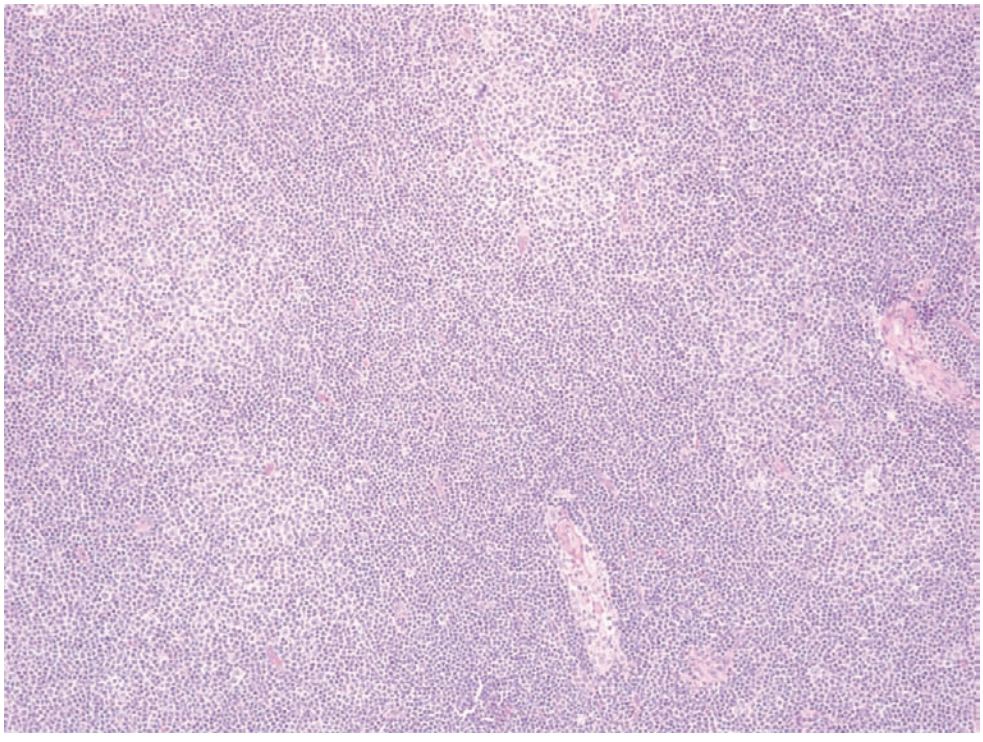

**FIGURE 7-1**

B-cell small lymphocytic lymphoma diffusely effaces the lymph node architecture. The pale areas are proliferation centers.

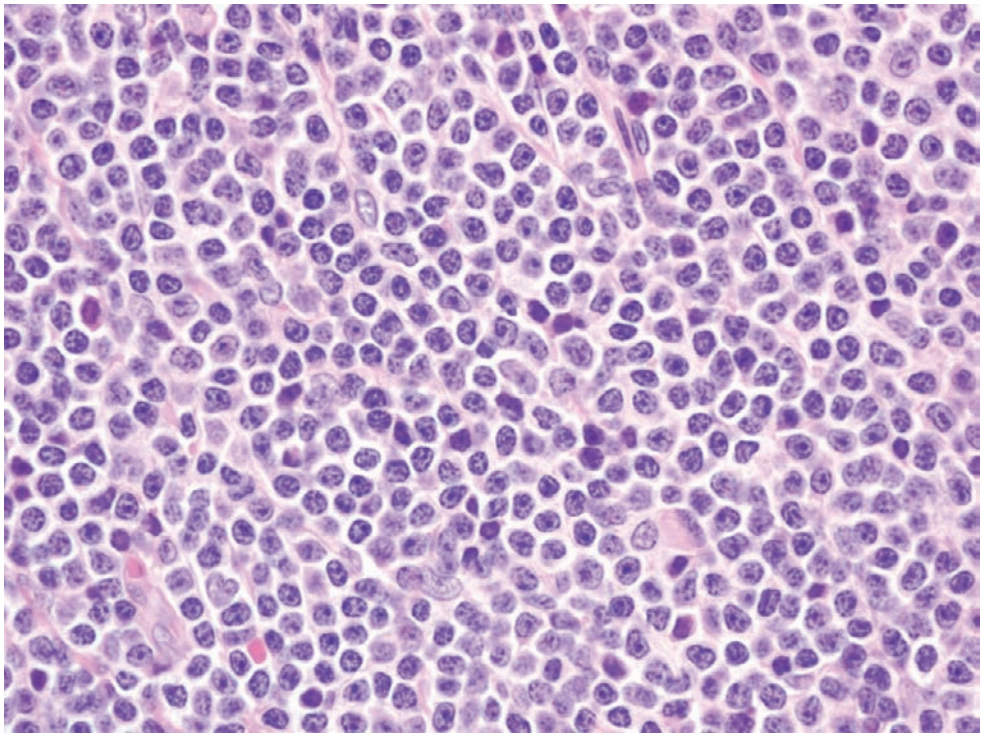

**FIGURE 7-2**

B-cell small lymphocytic lymphoma composed of small lymphocytes with round nuclei, distinctly clumped chromatin, inconspicuous nucleoli, and sparse cytoplasm.

predominate, almost all cases of SLL involving lymph nodes contain admixed intermediate-sized cells termed *paraimmunoblasts;* they are approximately 1.5 times the size of the neoplastic small lymphocytes and have partially condensed chromatin and small, distinct, central nucleoli (Figure 7-3). Paraimmunoblasts can be distributed singly, but more often (90% of cases) they form ill-defined clusters imparting a pale, vaguely nodular pattern at low magnification. The collections of paraimmunoblasts are termed *proliferation centers, growth centers,* or *pseudofollicles*. Proliferation centers are not present in reactive conditions and among the small B-cell lymphomas discussed in this chapter; they occur only in B-cell SLL–CLL. In some instances, pseudofollicles can be prominent and begin to fuse (Figure 7-4); however, this phenomenon should not be mistaken for large cell lymphoma transformation (see later discussion).

When the lymph node architecture is partially preserved, proliferation centers can be located in the interfollicular areas (Figure 7-5), or they can encircle the residual lymphoid follicles. These phenomena are thought to represent early stages of lymph node involvement and are morphologically subtle. The interfollicular pattern can be overlooked easily, and the perifollicular pattern can be mistaken for marginal zone lymphoma.

In the involved spleen, a uniform expansion of the white pulp, creating grossly visible nodules in a miliary fashion, is seen early in the course of the disease. However, at later stages the red pulp becomes involved (Figure 7-6) and the white pulp is obliterated, producing a diffuse pattern of involvement encompassing the entire splenic parenchyma. The microscopic features are similar to the lymph node, with infiltration by small lymphocytes and admixed paraimmunoblasts. When the disease becomes disseminated, essentially any extranodal site can be involved and the same morphologic features are seen, but proliferation centers are not nearly as frequent in extranodal sites as in lymph nodes. Sometimes the infiltrate is sparse and the distinction from lymphoid hyperplasia can be challenging. Special studies to demonstrate monoclonality and to confirm the typical immunophenotype of SLL are prudent in this context.

#### Immunophenotype

The typical immunophenotype of SLL cells is $CD5^+$, $CD10^-$, $CD19^+$, $CD20^+$ (dim), CD22 (dim), $CD23^+$, $CD43^+$, $CD79b^-/dim^+$, $FMC7^-/dim^+$, and surface immunoglobulin (sIg) light chain $dim^+$. Some cases may not have detectable sIg. Cyclin D1 is not expressed. The most reliable of these markers in paraffin section immunohistochemistry are CD5, CD10, CD20, and cyclin D1. CD23 is also helpful but can be difficult to demonstrate on small lymphocytes. However, pseudofollicles are often nicely highlighted by immunostaining for CD23. This immunologic profile of B-SLL is identical to that of B-CLL (see Chapter 12). A reported difference is the expression of lymphocyte function–associated antigen

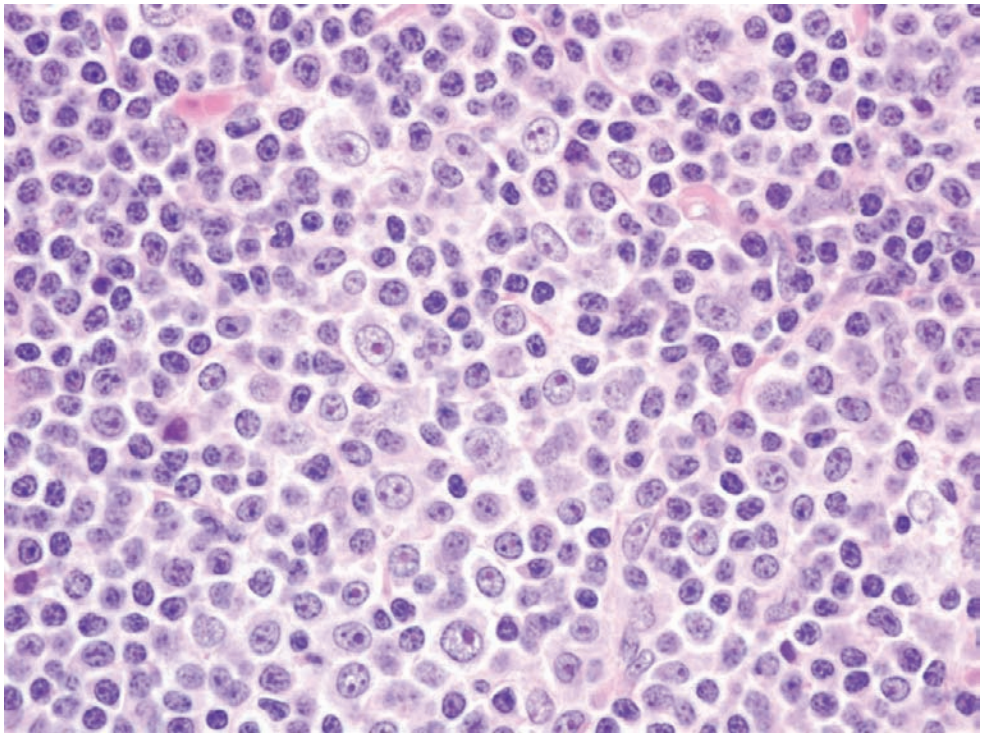

**FIGURE 7-3**
Cellular composition of a proliferation center in B-cell small lymphocytic lymphoma.

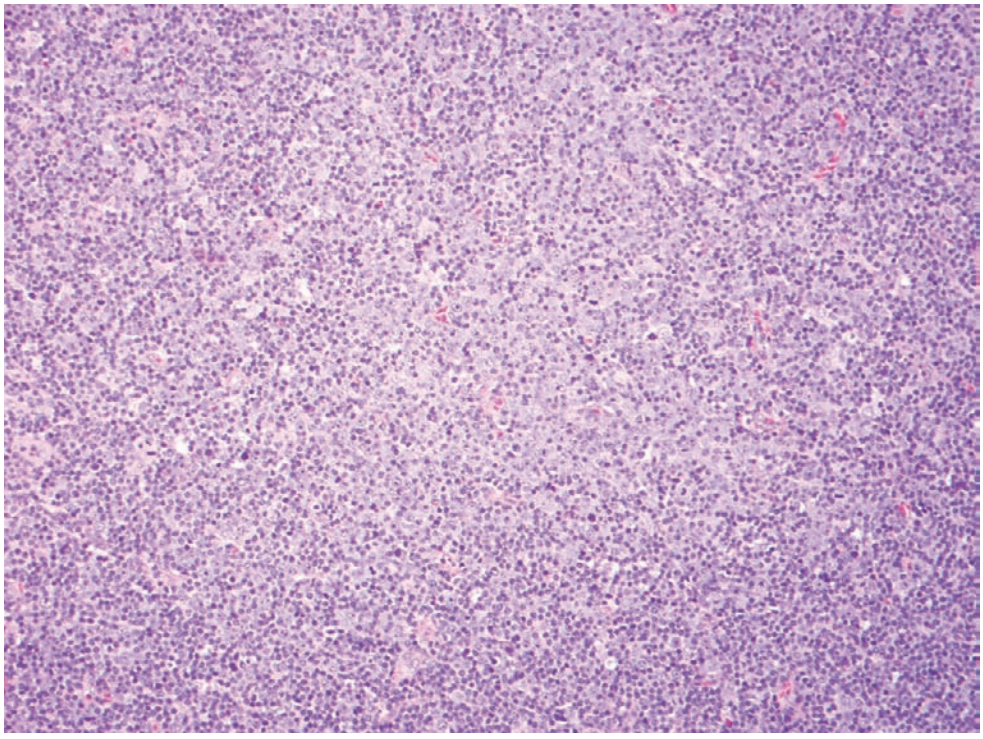

**FIGURE 7-4**
Although prominent, this proliferation center is surrounded by the typical small lymphocytes of B-cell small lymphocytic lymphoma *(right).*

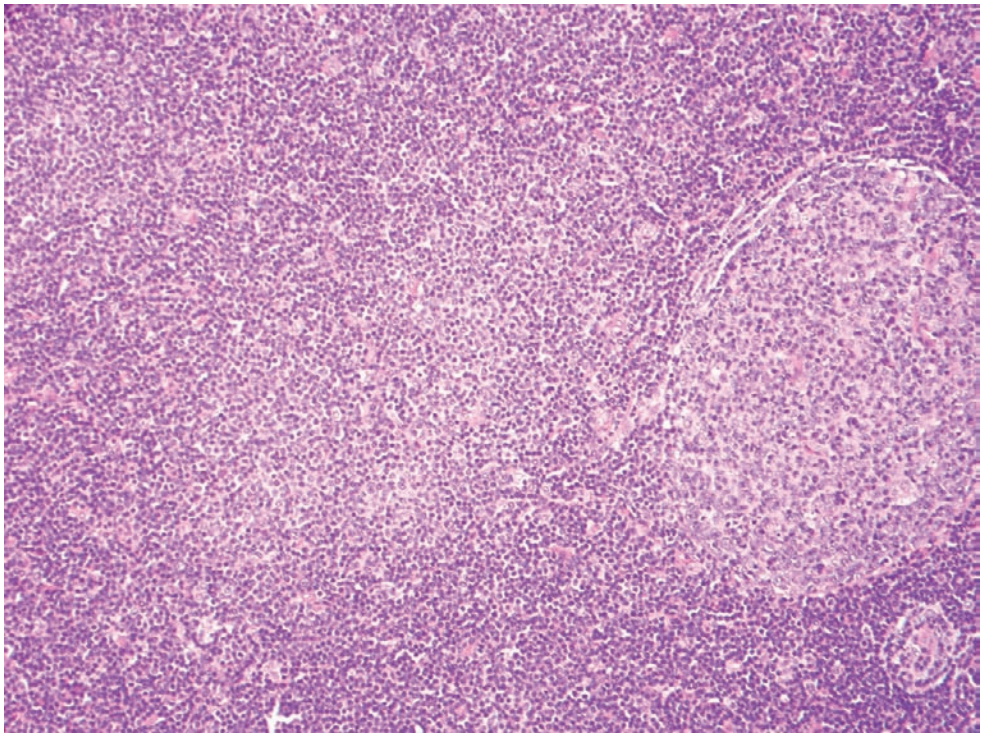

**FIGURE 7-5**

Interfollicular B-cell small lymphocytic lymphoma. A hyperplastic and an atrophic germinal center are present *(right)*. Note the proliferation center *(left),* a low power diagnostic clue to the presence of B-cell SLL.

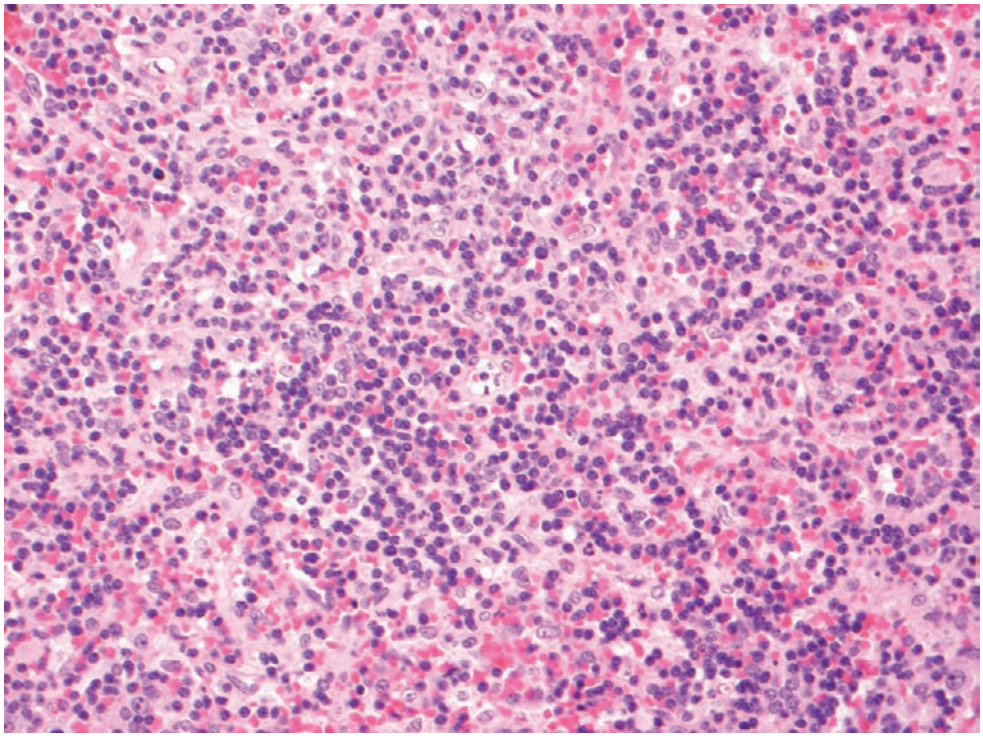

**FIGURE 7-6**

Splenic red pulp infiltrated by B-cell small lymphocytic lymphoma.

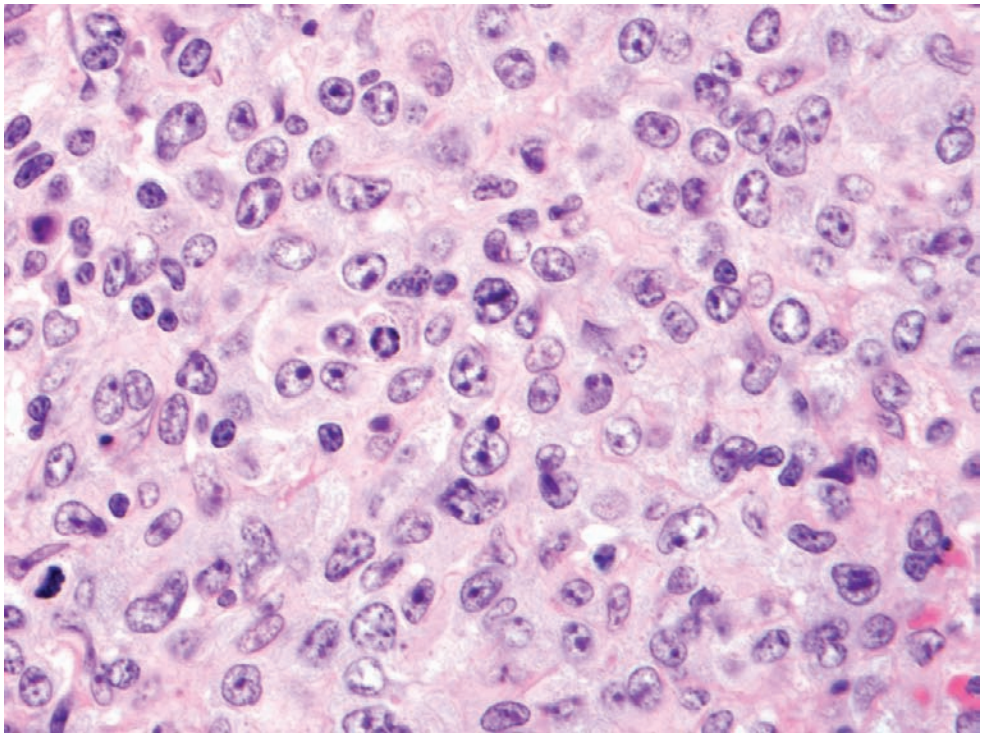

**FIGURE 7-7**

Diffuse large B-cell (Richter's) transformation of B-cell small lymphocytic lymphoma occurred in the same spleen specimen as that illustrated in Figure 7-6.

1, an adhesion molecule, on SLL cells but not CLL. By contrast, CLL cells express the chemokine receptors CCR-1 and CCR-3 that are lacking from SLL cells. LEF-1, a nuclear protein involved in Wnt signaling and CLL cell survival, has recently been shown to be overexpressed in SLL but not in other small B-cell lymphomas. If confirmed, LEF-1 may be another marker useful for diagnosis of CLL/SLL.

CD38 and ZAP-70 expression have been shown to be poor prognostic markers in CLL/SLL and are reviewed in the section on CLL in Chapter 12. It is worth noting that both ZAP-70 and CD38 expression can be assessed by immunostaining and, at least in the case of ZAP-70, there is good correspondence between its detection by flow cytometry and immunohistochemistry.

### Genetics

Clonal immunoglobulin gene rearrangements are detectable in almost all cases. The molecular genetics features are covered in the section on CLL. There are no specific karyotypic abnormalities in B-cell SLL. As with CLL, cases of SLL variably show trisomy 12, del(6q), del(11q), del(13q), and del(17p).

### Transformation

Transformation into an aggressive lymphoma (Richter syndrome) occurs in approximately 5% of SLL cases. Affected patients exhibit rapidly growing masses, elevated serum LDH, and B symptoms. Most commonly the transformation is in the form of diffuse large B-cell lymphoma, which may or may not be clonally related to the B-cell SLL. The morphologic features are similar to those for diffuse large B-cell lymphoma in general. The large B-cell lymphomas are composed of a monomorphous population of large lymphoid cells, resembling centroblasts or immunoblasts that grow in a diffuse pattern, effacing the architecture of the involved tissue (Figure 7-7), including overrunning any residual SLL–CLL in the sample.

Some SLL cases contain prominent, almost coalescing proliferation centers that give the superficial impression of a more aggressive lymphoma. Recognition of the spectrum of small, medium, and larger lymphocytes present in these cases and an appreciation of the manner in which the prominent proliferation centers appear to blend into adjacent small lymphocyte areas helps to distinguish this phenomenon from transformation to diffuse large B-cell lymphoma. Few data exist examining the effect of increased paraimmunoblasts–prolymphocytes on the outcome of SLL. However, two large studies found a correlation between prominent proliferation centers and shorter survival in B-cell CLL. In the most recent study, the authors termed this phenomenon *accelerated CLL;* however, such cases should not be considered transformation to diffuse large B-cell lymphoma.

Finally, rare cases of SLL are complicated by Hodgkin lymphoma, usually of nodular sclerosis or mixed

cellularity types. Single-cell polymerase chain reaction (PCR) studies have shown that the Hodgkin cells in these cases are clonally related to the preexisting CLL clone in some cases but not in others. Epstein-Barr virus (EBV) can be usually be detected in the Reed-Sternberg–like cells. The prognosis for patients with Hodgkin lymphoma complicating B-cell SLL–CLL is worse than the typical SLL, but it is better than that for patients with large B-cell lymphoma transformation.

## Differential Diagnosis

The differential diagnosis of SLL includes reactive lymphoid hyperplasia, lymphocyte predominance Hodgkin lymphoma, lymphocyte-rich classical Hodgkin lymphoma, and most of the small B-cell lymphomas, including mantle cell lymphoma, follicular lymphoma, marginal zone lymphoma, and lymphoplasmacytic lymphoma.

Tissue architectural effacement by monomorphous small lymphocytes is a finding present in B-cell SLL and not lymphoid hyperplasia in which the tissue architecture is intact or the cellular infiltrates contain other cell types, or both. Because proliferation centers do not occur in reactive lymphoid hyperplasia, recognizing these structures is a major factor in distinguishing B-cell SLL from lymphoid hyperplasia. Finally, phenotypic analysis will demonstrate a monotypic B-cell population in B-cell SLL compared with a polytypic B-cell population in hyperplasia. Although normal lymph nodes can contain small populations of non-neoplastic CD5-positive B cells, they are usually confined to the follicular mantle zones and represent only a small subset of the total B-cell population. If there is a substantial population of $CD5^+$ and $CD23^+$ B cells, and if they form aggregates in the interfollicular zones, then B-cell SLL rather than a reactive condition should be considered.

Distinguishing B-cell SLL from the other B-cell malignancies discussed in this chapter is summarized in Tables 7-1 to 7-3. Usually a combination of careful morphologic observations focusing on the features summarized in Table 7-1 together with judicious use of immunophenotyping (see Table 7-2) will allow these tumor types to be distinguished from one another.

One of the much debated areas in the SLL literature is the rare variant of SLL seen in approximately 5% of cases where cells show plasmacytoid features accompanied by an M component. There is little distinction between this variant of SLL and lymphoplasmacytic lymphoma. Cases that are $CD5^+$ and $CD23^+$ and have proliferation centers with limited paraproteinemia should be classified as SLL–CLL. Values of the paraprotein exceeding 30 g/L with hyperviscosity syndrome are highly unlikely in CLL/SLL. There is no consensus as to whether this plasmacytoid variant of SLL carries a poorer prognosis. However, a recent study of 26 such patients showed no difference in the overall survival from an age-, sex-, and stage-matched group of 52 patients with CLL/SLL, albeit with only relatively short follow-up (median of 24 months).

In most cases, lymphocyte-predominant Hodgkin lymphoma grows in a macronodular pattern. The nodules contain small lymphocytes, but also include the typical L and H variants of Reed-Sternberg cells characteristic of this disorder together with variable numbers of epithelioid histiocytes. The nodularity in SLL is due to the proliferation centers that are usually smaller than the nodules of nodular lymphocyte–predominant Hodgkin lymphoma and that lack L and H cells. Phenotypically, the cells in the macronodules of lymphocyte-predominance Hodgkin lymphoma contain T cells immediately surrounding the L and H cells, polyclonal small B cells, and follicular dendritic cells, whereas the small B cells of SLL have a CD5, CD23, and Ig light chain restricted phenotype.

In contrast to B-cell SLL, lymphocyte-rich classical Hodgkin lymphoma contains diagnostic Reed-Sternberg cells expressing CD15 and CD30 with absent CD45 expression. The small lymphocytes represent polyclonal $CD5^-$ B cells and T cells. It should be noted that individual scattered $CD30^+$ Reed-Sternberg–like cells can be seen on occasion in the background of otherwise typical SLL. The background inflammatory infiltrate of classical Hodgkin lymphoma is absent. They are usually EBV positive and represent an EBV-driven phenomenon because of the generally immunosuppressed state of the patient; this should not be considered Hodgkin transformation.

## Prognosis and Therapy

Many studies have investigated the role of different clinical, laboratory, and pathologic parameters in predicting prognosis in SLL. Most concede that there is an uncertain correlation between histology of SLL and disease outcome. In the international Non-Hodgkin's Lymphoma Classification Project, the 5-year overall actuarial survival rates for patients with an international prognostic index of 0/1, 2/3, or 4/5 were 76%, 51%, and 38%, respectively. Similar to CLL, ZAP-70 positivity, trisomy 12, and 17p deletion are predictive of a poor prognosis in patients with SLL.

CLL/SLL is not curable with current conventional therapies. Asymptomatic patients are often observed without treatment, while those with symptomatic early stage disease (stage I and II) may be treated with regional or extended field radiation therapy. Patients with advanced-stage disease (stage III and IV) are usually treated with low-intensity chemotherapy alone or in conjunction with other adjuvant modalities. Purine

analogues, combined with rituxan or with cyclophosphamide and granulocyte colony-stimulating factor, have produced the highest complete remission rates in previously untreated patients.

## MANTLE CELL LYMPHOMA

### Clinical Features

As they are currently defined, mantle cell lymphomas represent 2% to 8% of non-Hodgkin lymphomas in the United States. They occur in older individuals (median age, 63 years) with a male predominance (75% of patients). Most patients exhibit progressive adenopathy involving multiple sites, and there is a relatively high frequency of Waldeyer's tonsillar ring involvement. Splenic involvement in the absence of lymphadenopathy occasionally occurs, and a small subset of patients has multiple intestinal lymphomatous polyps. The staging bone marrows from mantle cell lymphoma patients are frequently positive (70%), and 20% to 30% have morphologically recognizable abnormal circulating lymphocytes. Therefore this disease is almost always widespread (Ann Arbor stage III or IV), with approximately one third of patients having B symptoms at the time of diagnosis.

### Pathologic Features

#### Morphology

The gross pathology of mantle cell lymphoma in lymph nodes is not distinctive. This disorder produces nodal enlargement characterized by homogeneous, tan cut surfaces with or without small nodules. When mantle cell lymphoma involves the spleen, it produces splenic enlargement, usually greater than 1000 g with small, miliary, white nodules (1 to 3 mm) scattered throughout the splenic parenchyma. In a peculiar pattern of involvement in the gastrointestinal tract, mantle cell lymphoma can also produce numerous sessile and pedunculated polyps throughout the entire length of the small and large intestine in a pattern termed *multiple intestinal lymphomatous polyposis*.

Mantle cell lymphomas are characterized by three growth patterns: mantle zone, nodular, and diffuse, in increasing order of frequency. In the mantle zone pattern, prominent mantle zones composed of the neoplastic cells surround reactive germinal centers that can be either normal sized or small and atrophic (Figure 7-8). When the germinal centers are normal size, distinguishing mantle cell lymphoma from follicular hyperplasia can be a challenge unless one recognizes that the architecture of the internodular areas is effaced by the expanded cloud of neoplastic mantle zone cells.

**MANTLE CELL LYMPHOMA—FACT SHEET**

**Clinical Features**
- Two percent to 8% of non-Hodgkin lymphomas
- Median age, 7th decade, male predominance
- Progressive adenopathy
- Splenic enlargement without adenopathy
- Multiple intestinal lymphomatous polyposis
- Leukemic phase
- High stages at clinical presentation

**Morphology**
- Mantle zone, nodular or diffuse architecture
- Monomorphous small lymphocytes with irregular nuclei clumped chromatin, inconspicuous nucleoli and sparse cytoplasm
- Blastoid variants: lymphoblast-like, centroblast-like, pleomorphic

**Immunophenotype**
- $CD19^+$, $CD20^+$, $sIg^+$, IgM with or without IgD
- $CD5^+$
- $CD23^-$ or weakly positive in subset
- Cyclin $D1^+$

**Genetics**
- Clonally rearranged immunoglobulin genes
- t(11;14)(q13;q32)
- Cyclin D1 gene translocated into *IGH@* locus
- Point mutations in Ig genes absent in most, present in some

**Prognosis and Therapy**
- Multiagent chemotherapy with rituximab
- Incurable
- Prognosis suggested by mantle cell lymphoma international prognostic index, pattern of involvement of lymph node, Ki-67 proliferative rate, and blastoid cytology
- 3- to 4-year median survival
- An indolent non-nodal form exists

**Differential Diagnosis**
- Reactive lymphoid hyperplasia
- B-cell small lymphocytic lymphoma
- Follicular lymphoma, grade 1 and grade 2
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
- Lymphoplasmacytic lymphoma
- Lymphocyte-predominant Hodgkin lymphoma
- Lymphocyte-rich classical Hodgkin lymphoma

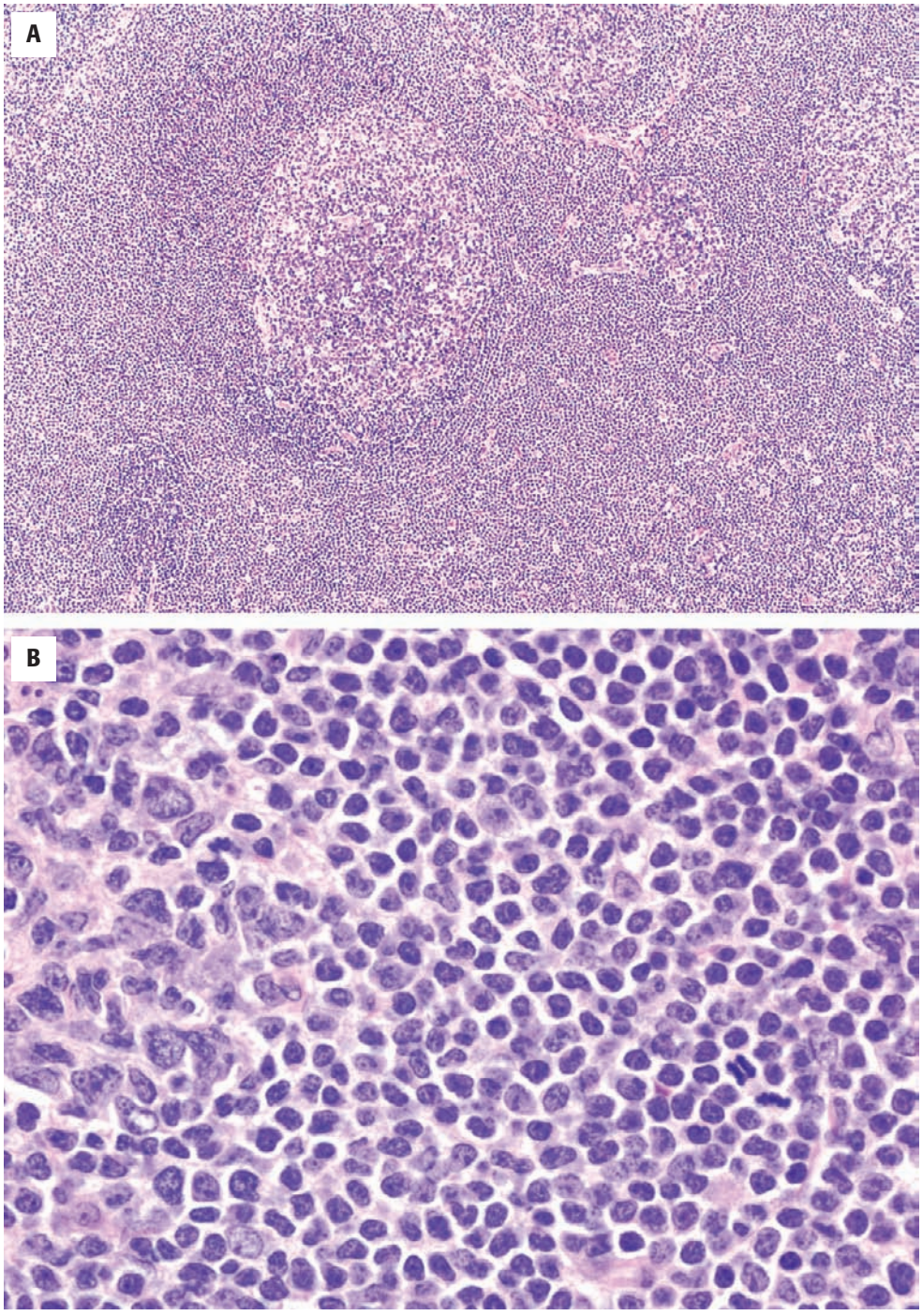


**FIGURE 7-8**

Mantle cell lymphoma, mantle zone pattern. **A,** The neoplastic cells surround hyperplastic germinal centers and infiltrate the interfollicular areas. **B,** Non-neoplastic germinal center cells are illustrated on the left. The mantle cell lymphoma cells occupy the rest of the image.

Numerous coalescing nodules of tumor cells devoid of germinal centers are characteristics of nodular pattern mantle cell lymphoma (Figure 7-9), whereas a diffusely growing lymphoma cell population that effaces the underlying architecture of the involved tissue defines the diffuse pattern (Figure 7-10). Hyalinized small blood vessels course through the lymphoma infiltrates in a substantial fraction of diffuse pattern mantle cell lymphomas and can be a useful low-power morphologic clue to the diagnosis of this tumor type.

In the prototypic case, mantle cell lymphoma is composed of a highly monomorphous population of small lymphocytes with spheroidal nuclei, containing irregularities, cleaves, and grooves. The chromatin is clumped, nucleoli are inconspicuous and the cytoplasm is sparse (Figure 7-11). Proliferation centers and intermixed

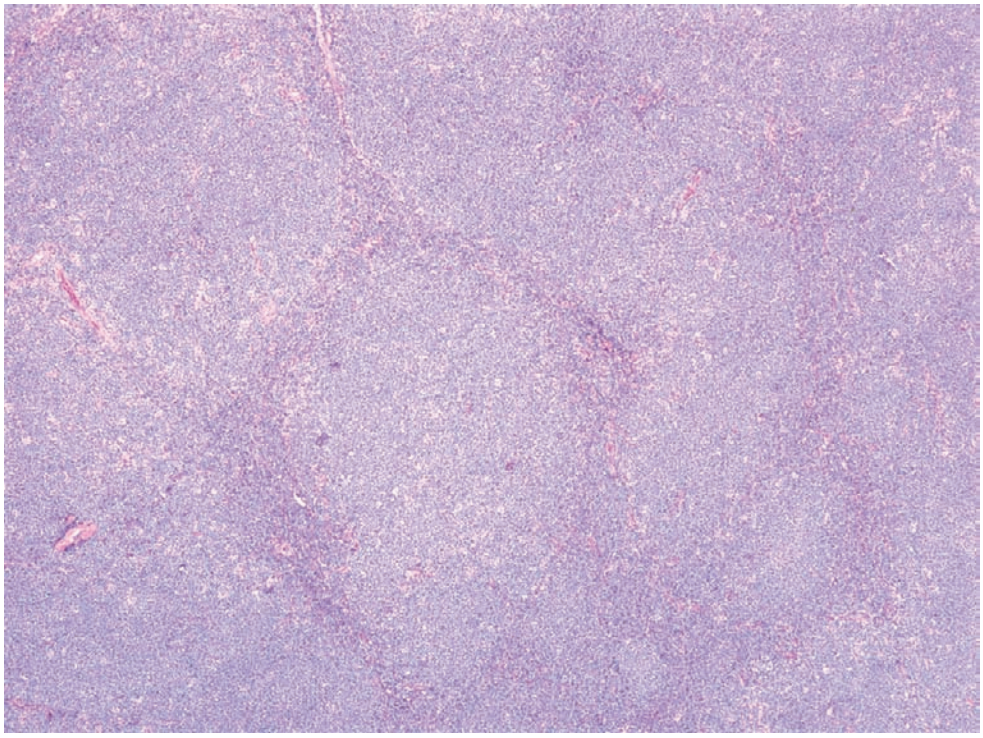

**FIGURE 7-9**

Mantle cell lymphoma, nodular pattern. Near-confluent nodules efface the lymph node architecture.

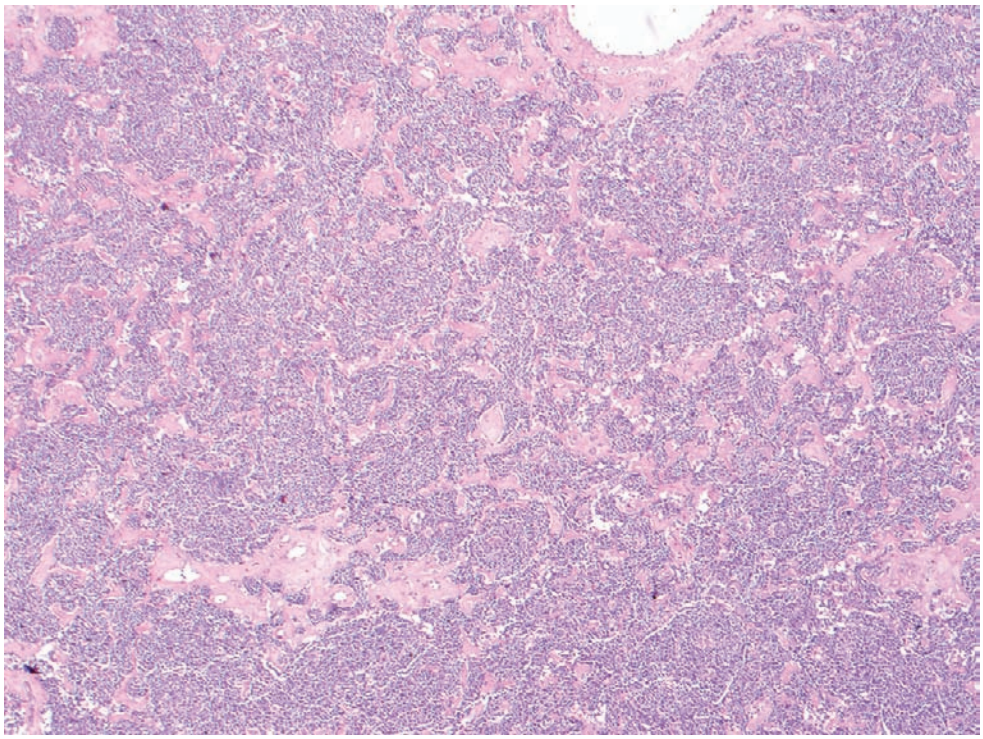

**FIGURE 7-10**

Mantle cell lymphoma, diffuse pattern. Note the blood vessels surrounded by hyalinized collagen.

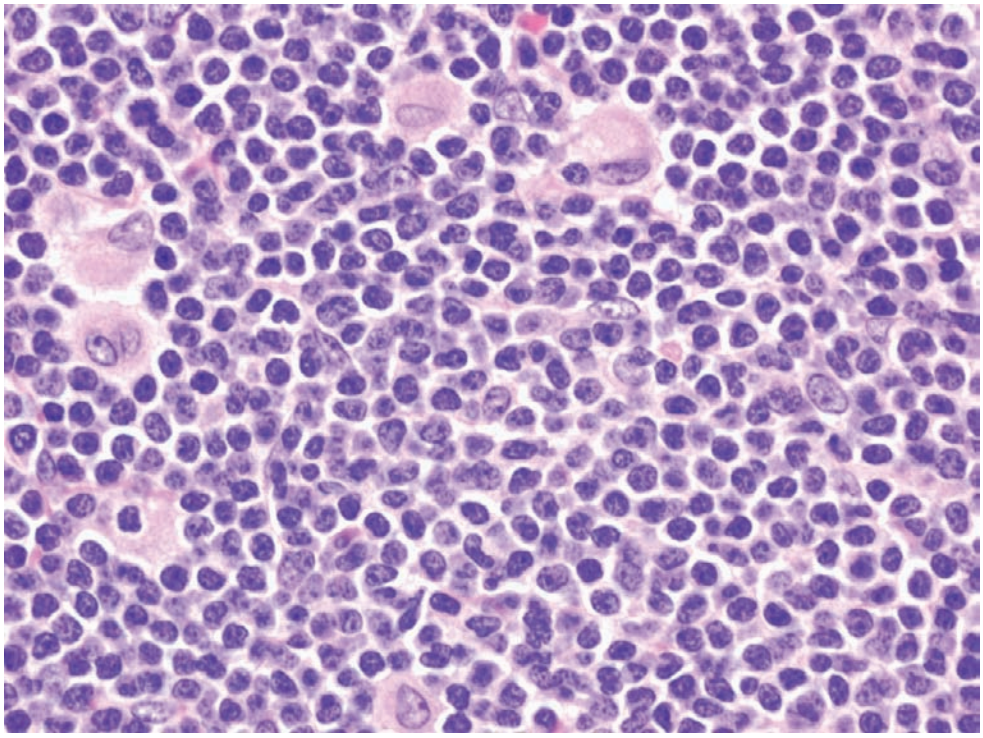

**FIGURE 7-11**

Mantle cell lymphoma, standard cytology. Monomorphous small lymphocytes with irregular nuclei, condensed chromatin, inconspicuous nucleoli, and sparse cytoplasm. Intermixed, singly distributed macrophages are also present.

centroblasts or immunoblasts are absent. In addition, mantle cell lymphomas often contain intermixed, singly distributed, epithelioid macrophages. Because this cell population is unusual in other lymphoma of small B lymphocytes, its presence is a useful clue to the diagnosis of mantle cell lymphoma.

In a small subset of cases, the neoplastic mantle cells have monocytoid characteristics. These characteristics include somewhat larger nuclei and less clumped chromatin than standard mantle cell lymphoma cases, together with voluminous clear or lightly eosinophilic cytoplasm (Figure 7-12). This cytology can mimic exactly the cytology of nodal and splenic marginal zone B-cell lymphomas, highlighting the importance of ancillary studies in supporting the diagnosis of mantle cell lymphoma. It is possible to recognize three other cytologic variants of mantle cell lymphoma. In the first, the neoplastic cells resemble lymphoblasts. They are medium sized with irregular nuclei containing stippled chromatin, small nucleoli, and imperceptible cytoplasm (Figure 7-13, A). They are often associated with numerous mitotic figures. In the second, the neoplastic cells resemble centroblasts with round nuclei, multiple nucleoli often attached to nuclear membranes, and more voluminous amphophilic cytoplasm. In the third, the nuclei of the neoplastic cells are pleomorphic, vary in size and shape, are often hyperchromatic, and have variably prominent nucleoli (see Figure 7-13, B). The WHO lymphoma classification groups these last three cytologic variants into the category of blastoid variant of mantle cell lymphoma. Blastoid mantle cell lymphomas can occur de novo, in which case they are associated with an increased risk for adverse outcomes. Alternatively, standard mantle cell lymphomas with progression can acquire blastoid cytologic characteristics.

### Involvement of Extranodal Sites

Mantle cell lymphomas frequently involve extranodal sites. The bone marrow is involved by lymphoma in 70% of patients at diagnosis. Paratrabecular, nodular, or interstitial infiltrates are found in involved bone marrow biopsy specimens (Figure 7-14). In contrast to the marked monomorphism of the tumor cells of typical mantle cell lymphomas in fixed tissue specimens, the neoplastic cells in air-dried Wright-Giemsa–stained bone marrow aspirate and blood smears are polymorphous (Figure 7-15). Nuclear size and shape vary, the chromatin has a reticulated pattern, and nucleoli can be prominent. Peripheral blood involvement by mantle cell lymphoma can mimic B-cell chronic lymphocytic leukemia, by both absolute lymphocyte count and cytology. Some of the cases previously thought to be B-cell prolymphocytic leukemia that have the t(11;14)(q21;q32) are now considered by most hematopathologists to represent leukemic-phase mantle cell lymphomas. In the spleen, mantle cell lymphoma involves the white pulp and in a subset of cases will demonstrate marginal zone

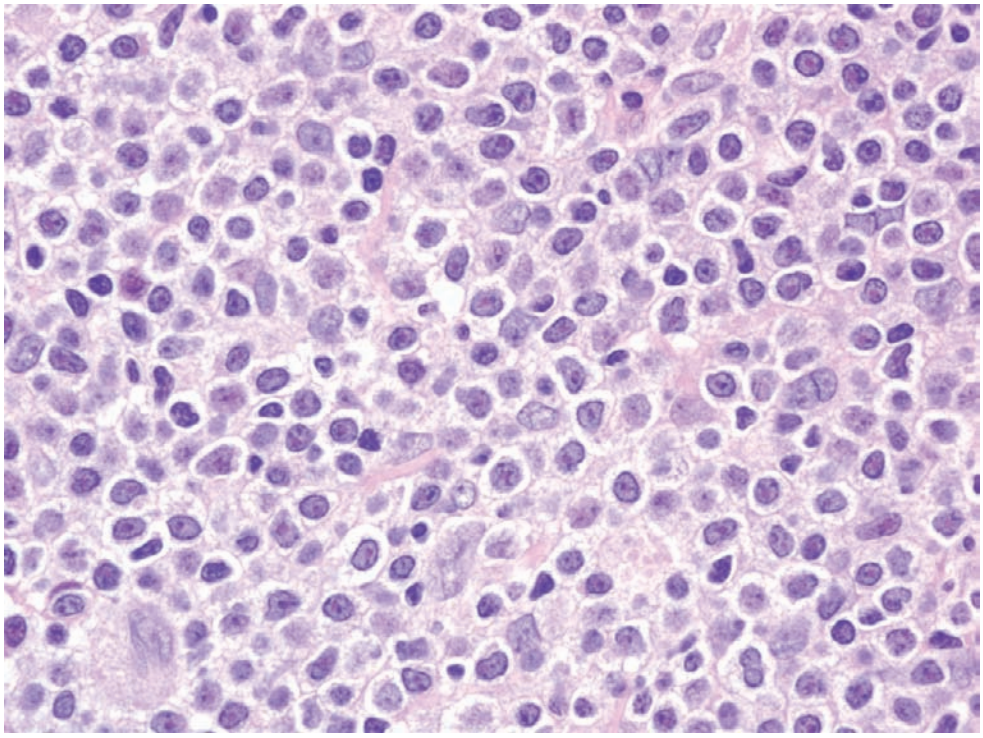

**FIGURE 7-12**
Mantle cell lymphoma, monocytoid cytology.

differentiation toward the periphery of the white pulp nodules. Mantle cell lymphoma is one of the lymphoma types that can cause multiple intestinal lymphomatous polyposis (Figure 7-16).

A clinical variant of mantle cell lymphoma is now recognized, in which patients have splenic involvement without nodal involvement. Isolated leukemic involvement may also be seen with a propensity for kappa light chain restriction. Such patients appear to have mutated $IgV_H$ genes and indolent clinical courses. Bone marrow biopsy in these latter patients demonstrates subtle interstitial involvement by mantle cell lymphoma cells.

### Phenotype

Mantle cell lymphoma cells express pan B lymphocyte antigens such as CD19, CD20 (Figure 7-17, A), CD79a, CD79b, and PAX-5. These cells are positive for IgM or IgM plus IgD and exhibit Ig light chain restriction. More mantle cell lymphoma cases express λ Ig light chain than κ Ig light chain. Greater than 95% are positive for CD5 (see Figure 7-17, B), but negative for other T-cell antigens such as CD3 (see Figure 7-17, C), and most either completely lack CD23 expression or show weak CD23 marking in a subset of the tumor cells (see Figure 7-17, D). Mantle cell lymphoma cells are also usually negative for CD10 and BCL-6. Exceptions to this typical phenotype exist. $CD5^-$ mantle cell lymphoma cases have been described recently; they are recognized on the basis of typical morphologic features and expression of cyclin D1. A small number of mantle cell lymphomas are positive for the follicle center cell marker BCL-6, and mantle cell lymphoma is one of the subtypes of B-cell lymphoma that can coexpress both CD5 and CD10. As a consequence of the t(11;14)(q13;q32) (see later), the nuclei of almost all cases are positive for cyclin D1 (see Figure 7-17, E), a marker demonstrated to best advantage by paraffin section immunohistochemistry.

### Genetics

Almost all cases of mantle cell lymphoma contain a balanced translocation involving the cyclin D1 gene *(CCND1)* on chromosome 11q13. The breakpoints occur in a region of *CCND1* 5′ to the coding region of the gene so that intact cyclin D1 protein can be produced as a result of the translocation. In most cases, the Ig heavy chain gene (*IGH@*) on chromosome 14q32 is the partner in the translocation, but in a small subset of cases the κ Ig light chain gene (2p11) or the λ Ig light chain gene (22q11) are the partner loci. At *IGH@*, the translocation involves the VDJ joining regions of the gene, and *CCND1* comes under the regulatory control of the *IGH@* enhancer sequences; therefore *CCND1* is overexpressed. Because the morphology and phenotype of mantle cell lymphoma overlaps with other small B-cell lymphomas, demonstrating cyclin D1 positivity in the neoplastic cells by paraffin section immunohistochemistry or detecting (*CCND1/IGH@*) fusion by FISH

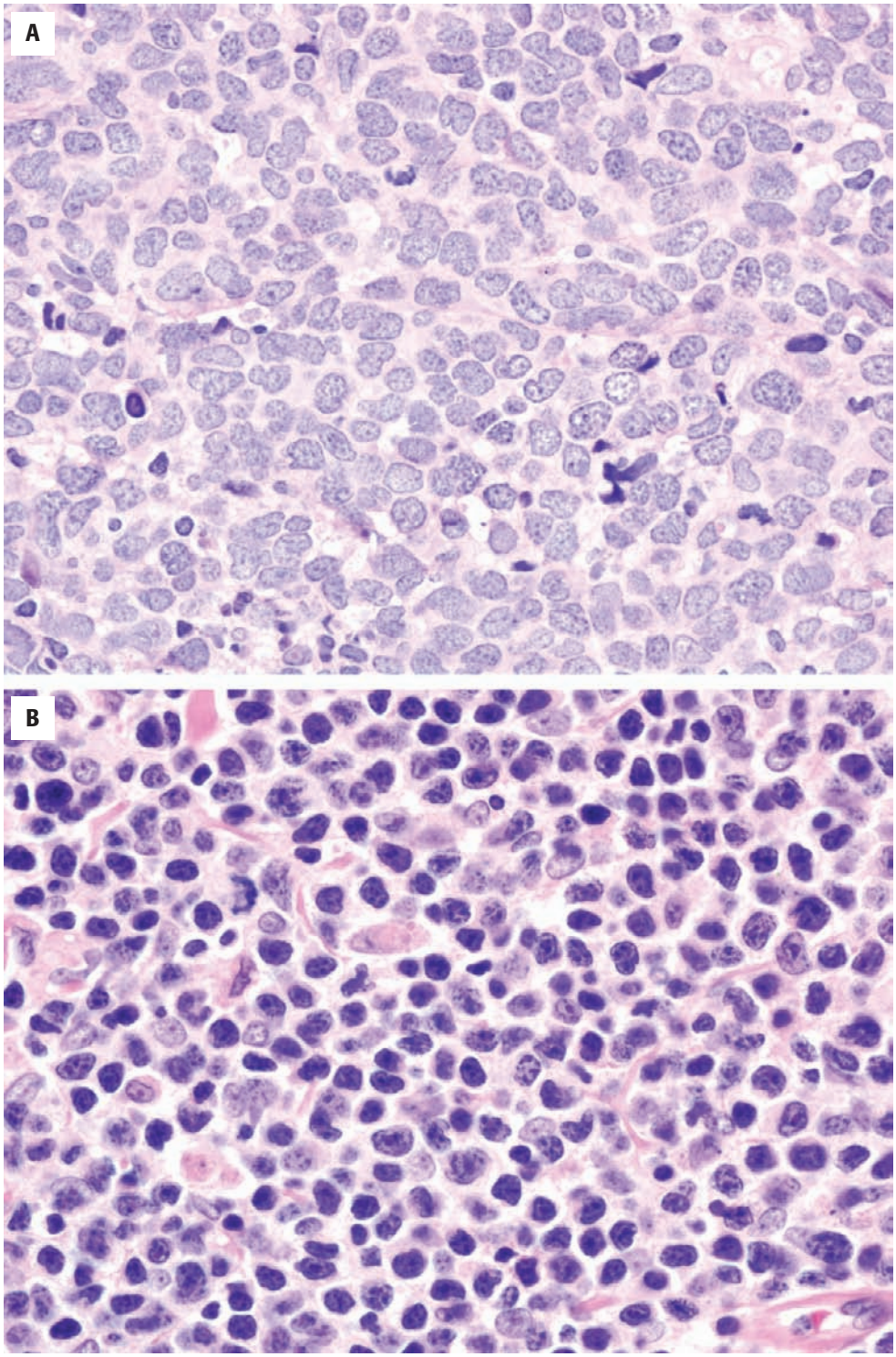


**FIGURE 7-13**

Blastoid variants of mantle cell lymphoma. **A,** Lymphoblastoid cytology. **B,** Pleomorphic cytology.

(Figure 7-18) are useful and are typically considered to be essential confirmatory findings for this diagnosis.

In addition to *CCND1* translocations, mantle cell lymphomas typically contain a high number of other nonrandom chromosome abnormalities; these include gains of 3q26, 7p21, and 8q24 and losses of 1p13-p31, 6q23-q27, 9p21, 11q22-q23, 13q11-q13, 13q14-q34, and 17p13-pter. ATM gene mutations occur in a substantial subset of cases, and trisomy 12 is a relatively frequent abnormality in this disease. Blastoid variants are also characteristically tetraploid, and progression from standard variants to aggressive variants is often associated

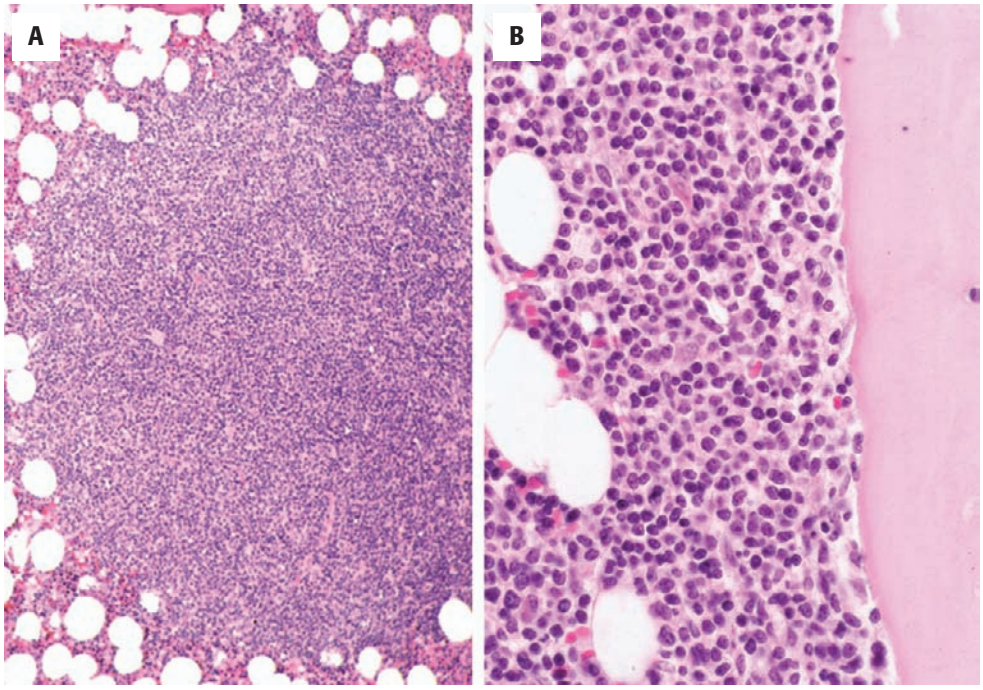


**FIGURE 7-14**

Bone marrow involvement by mantle cell lymphoma. **A,** Intertrabecular nodule. **B,** Paratrabecular aggregate.

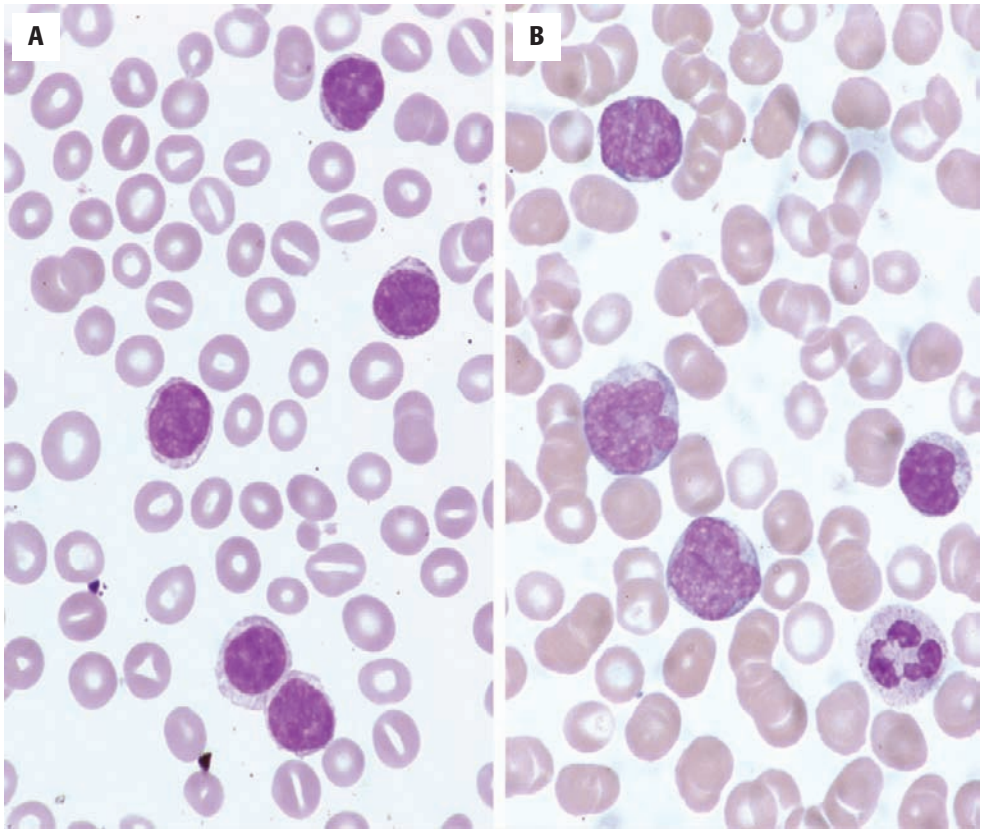


**FIGURE 7-15**

Blood involved by mantle cell lymphoma. **A,** Monomorphous small lymphocytes. **B,** Nuclear size and shape variability and small nucleoli characterize this case.

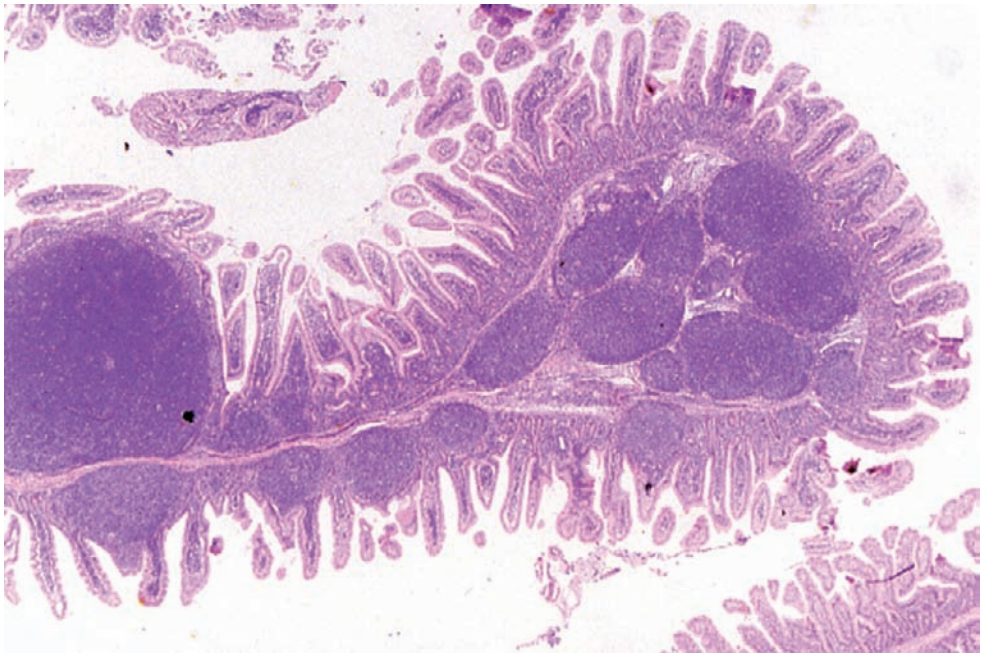

**FIGURE 7-16**
Multiple intestinal lymphomatous polyposis due to mantle cell lymphoma.

with the acquisition of *cMYC* amplification or translocation, p16 deletion or hypermethylation, and/or p53 gene mutation.

The existence of a cyclin D1 negative mantle cell lymphoma has been proposed recently for cases that have typical morphologic and phenotypic attributes ($CD5^+$, $CD23^-$ B-cell lymphoma) of mantle cell lymphoma combined with positivity for cyclin D2 or cyclin D3 and absence of staining of the tumor for p27. These criteria are based on gene expression profiling (GEP) experiments that demonstrated identical GEP signatures of cyclin D1 positive and cyclin D1 negative cases defined in this way. A subset of cyclin D1 negative mantle cell lymphoma cases with translocations involving cyclin D2 or cyclin D3 have also been proposed. These cases are rare and the diagnostic specificity of the cyclin D2 or cyclin D3 positive, p27 negative phenotype has been questioned. This area of research requires additional study and clinical analysis. SOX11 expression by immunohistochemistry is seen in a high proportion (>90%) of mantle cell lymphoma and appears useful in recognition of cyclin D1 negative mantle cell lymphoma. SOX11 is not expressed in other small B-cell lymphomas or DLBCL (including $CD5^+$ DLBCL). It can be expressed in some cases of Burkitt lymphoma and lymphoblastic lymphoma, but these entities are not in the differential diagnosis of mantle cell lymphoma. Routine use of SOX11 has been hampered by lack of a reliable monoclonal antibody.

On a molecular level, clonal Ig heavy and light chain gene rearrangements can be demonstrated in virtually all cases of mantle cell lymphoma. In keeping with the pregerminal center cell theory of origin of mantle cell lymphoma, most cases have no point mutations of *IGH@* or in noncoding sequences of *BCL6*. However, in approximately one third of cases, point mutations in the Ig heavy chain genes can be detected, suggesting a postfollicular center cell genotype and perhaps suggesting a different pathogenesis of this subgroup of cases.

## Differential Diagnosis

The differential diagnosis of mantle cell lymphoma includes all of the lymphoma types addressed in this chapter. Tables 7-1 to 7-3 highlight the features that distinguish among them. In addition, blastoid variant mantle cell lymphoma morphologically resembles B and T precursor lymphoblastic lymphoma–leukemia and acute myeloid leukemia. These diseases are distinguished primarily from mantle cell lymphoma on the basis of phenotype. T precursor lymphoblastic lymphoma–leukemia cells are positive for terminal deoxynucleotidyl transferase (TdT) and have an immature T cell phenotype. B precursor lymphoblastic lymphoma–leukemia is also TdT positive. The blasts express CD34 in a high percentage of the cases, and they have a CD19, CD20 variable, $CD5^-$, sIg, and cyclin D1–negative phenotype. Many examples of myeloid lineage blasts contain azurophilic granules or Auer rods, or both, in Wright-Giemsa–stained smears. By immunohistochemistry applied to paraffin sections, they express myeloperoxidase and lysozyme and lack staining for

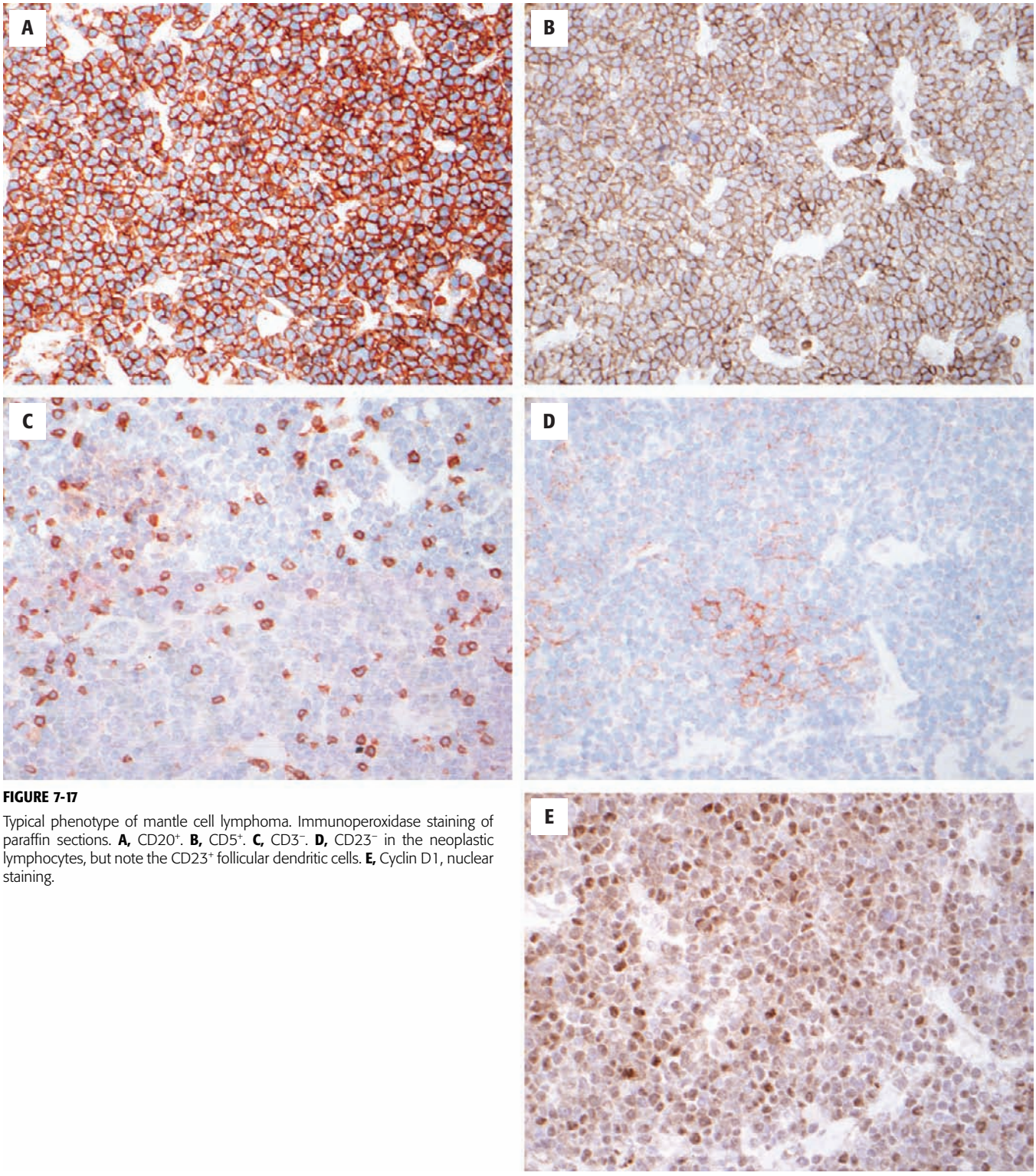


**FIGURE 7-17**

Typical phenotype of mantle cell lymphoma. Immunoperoxidase staining of paraffin sections. **A,** $CD20^+$. **B,** $CD5^+$. **C,** $CD3^-$. **D,** $CD23^-$ in the neoplastic lymphocytes, but note the $CD23^+$ follicular dendritic cells. **E,** Cyclin D1, nuclear staining.

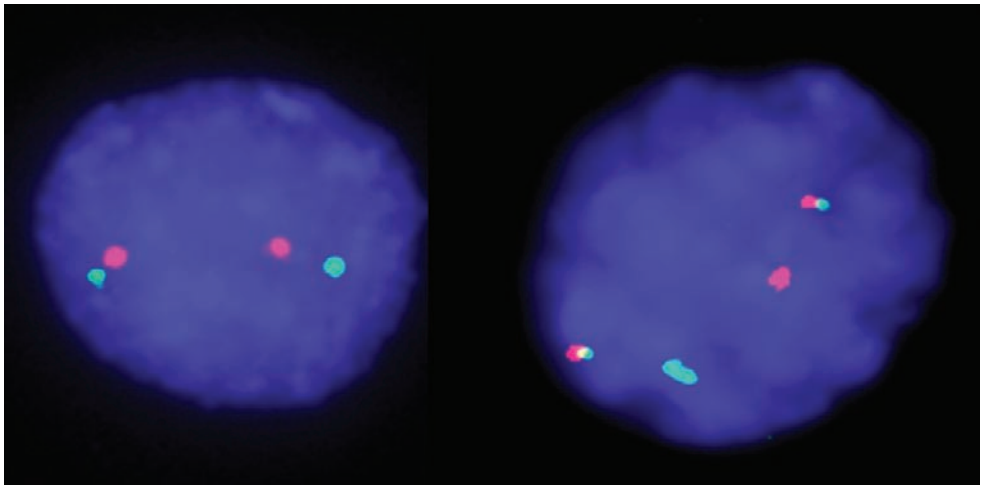

**FIGURE 7-18**

Fluorescence in situ hybridization with probes that recognize *CCND1 (red)* and *IGH@ (green)*. The nucleus on the left is normal; two copies of *CCND1* and two of *IGH* on normal chromosomes 11q13 and 14q32, respectively. On the right is a mantle cell lymphoma nucleus. It contains one normal red *CCND1* and one normal green *IGH@* signal representing the nontranslocated chromosomes 11q13 and 14q32. There are also two yellow fusion signals representing the derivative chromosomes 11 and 14 involved in the reciprocal t(11;14)(q13;q32).

cyclin D1; by flow cytometry, they are variably positive for CD13, CD33, and CD117 (c-kit). Blastoid MCL may also resemble diffuse large B-cell lymphoma (DLBL) but can usually be distinguished from DLBL by expression of CD5 and cyclin D1.

### Prognosis and Therapy

Treatment of mantle cell lymphoma is challenging. There is no standard for initial therapy. Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) with or without Rituximab can produce initial favorable responses, but they are short lived. Purine analogs are not as effective in mantle cell lymphoma as in other small B-cell malignancies. Although initially effective in achieving complete or partial remissions, neither R-CHOP nor high-dose chemotherapy followed by bone marrow transplant is curative in this disorder. The tumor responds to anti-CD20 antibodies. The median survival is only 3 to 4 years, with no plateaus in the survival curves. As noted above, a clinical variant with primarily non-nodal disease (spleen, bone marrow) appears to have an indolent course.

Compared to patients with mantle zone, nodular, and diffuse pattern standard mantle cell lymphoma, those with blastoid variants of mantle cell lymphoma have a worse prognosis. Several groups have attempted to refine prognosis prediction using a proposed mantle cell lymphoma international prognostic index (MIPI). In this scheme, age, performance status, white blood cell count, and lactate dehydrogenase levels are combined into an index that predicts low-, intermediate-, and high-risk groups. A modified MIPI has also been proposed that adds gender and presence or absence of B symptoms to the original MIPI. Improved discrimination into prognostic groups using this approach has been proposed. Finally, proliferative rate as assessed by Ki-67 staining has been suggested as an independent prognostic marker (higher risk associated with proliferation in excess of 30%) in mantle cell lymphoma.

## FOLLICULAR LYMPHOMA

### Clinical Features

Accounting for approximately 20% of all non-Hodgkin lymphomas, follicular lymphomas are the second most common lymphoma type in the United States and Western Europe. The disease occurs with a median age of 60 years and with a slight female predominance. Pediatric cases are rare. Patients most often exhibit gradually progressive or waxing and waning, painless lymph node enlargement involving cervical, supraclavicular, axillary, or inguinal regions. Less common are abdominal presentations, characterized by abdominal or back pain caused by either mesenteric or retroperitoneal adenopathy. Isolated splenomegaly, multiple intestinal lymphomatous polyps, and a peripheral blood leukemic phase superficially resembling chronic lymphocytic leukemia are rare initial manifestations of follicular lymphoma. After staging evaluations, follicular lymphoma patients are usually found to have widespread disease; 40% of patients have spleen involvement, 50% have liver involvement, and 55% to 70% have bone marrow involvement. Most patients present in Ann Arbor stage III or IV. Only 20% have B symptoms (fever, weight loss, night sweats) or elevated lactate dehydrogenase levels.

### FOLLICULAR LYMPHOMA—FACT SHEET

**Clinical**
- Twenty percent of non-Hodgkin lymphomas
- Median age, 7th decade; slight female predominance
- Progressive adenopathy, waxing and waning in some
- Multiple intestinal lymphomatous polyposis
- Primary cutaneous follicular lymphoma
- Pediatric follicular lymphoma with different biology
- Leukemic phase rare
- High stages at clinical presentation in majority of patients

**Morphology**
- Nodular pattern with varying proportions of diffuse growth
- Spectrum of neoplastic centrocytes and centroblasts
- Morphologic variants: floral variant, with plasmacytic differentiation, with marginal zone B cell differentiation
- Grading based on number of centroblasts per 400× field:
  - Grade 1, less than 5
  - Grade 2, between 5 and 15
  - Grade 3, greater than 15
  - Grade 1 to 2 of 3, an acceptable alternative to distinguishing grade 1 from grade 2

**Immunophenotype**
- $CD19^+$, $CD20^+$, $sIg^+$, IgM with or without IgD, occasional cases IgG or $IgA^+$
- $CD10^+$, $BCL\text{-}6^+$
- Associated with CD21 or $CD23^+$ follicular dendritic cells

**Genetics**
- Clonally rearranged immunoglobulin genes
- t(14;18)(q32;q21): *IGH@/BCL2*
- Point mutations in immunoglobulin genes and in 5′ noncoding region of BCL-6 gene

**Prognosis and Therapy**
- Grades 1 and 2 follicular lymphoma:
  - Watch-and-wait approach for asymptomatic stage III and IV
  - Low-intensity, single- or multiple-agent chemotherapy for symptomatic patients
  - 8- to 10-year median survival
  - Outcome predicted by Follicular Lymphoma International Prognostic Index
  - Transformation to diffuse large B cell lymphoma in 20% heralds aggressive disease
- Grade 3 follicular lymphoma:
  - Anthracycline-containing multiagent chemotherapy with rituximab
  - Long term clinical remissions in 40% of patients
  - Outcome predicted by International Prognostic Index

**Differential Diagnosis**
- Follicular lymphoid hyperplasia
- B cell small lymphocytic lymphoma
- Mantle cell lymphoma
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue
- Lymphoplasmacytic lymphoma
- Lymphocyte-predominant Hodgkin lymphoma
- Lymphocyte-rich classical Hodgkin lymphoma

## Pathologic Features

### Morphology

Follicular lymphomas recapitulate the architecture, cytology, and phenotype of normal germinal centers; therefore most grow in a nodular pattern (Figures 7-19 and 7-20). The nodules efface the lymph node architecture, extending from the cortex to the hilum, overrunning the sinuses and invading beyond the capsule of the lymph node into the perinodal soft tissue (Figure 7-21). The neoplastic follicles are crowded together, might coalesce (Figure 7-22), and tend to be smaller and have less prominent mantle zones than normal, reactive follicles. In many cases the neoplastic nodules are accompanied by a diffusely growing component; in rare examples, follicular lymphomas grow in a purely diffuse pattern (Figure 7-23). In the latter instances, they are termed *diffuse follicular lymphoma* by the WHO classification convention (this designation requires phenotypic confirmation including expression of follicular center cell markers CD10 and BCL-6 together with BCL-2; see later). When both nodular and diffuse patterns are present in the same tissue sample, the WHO classification recommends that the percentage of the diffuse component be specified in the report.

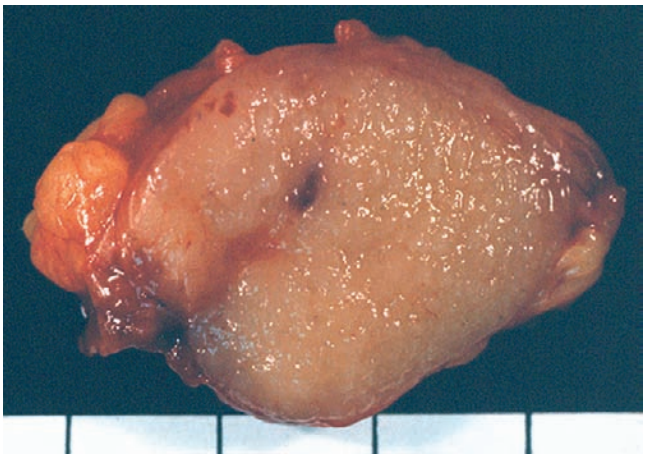

**FIGURE 7-19**
Follicular lymphoma in a lymph node. The multinodular architecture is readily apparent.

Branching, tapering blood vessels associated with varying amounts of collagen fibrosis commonly

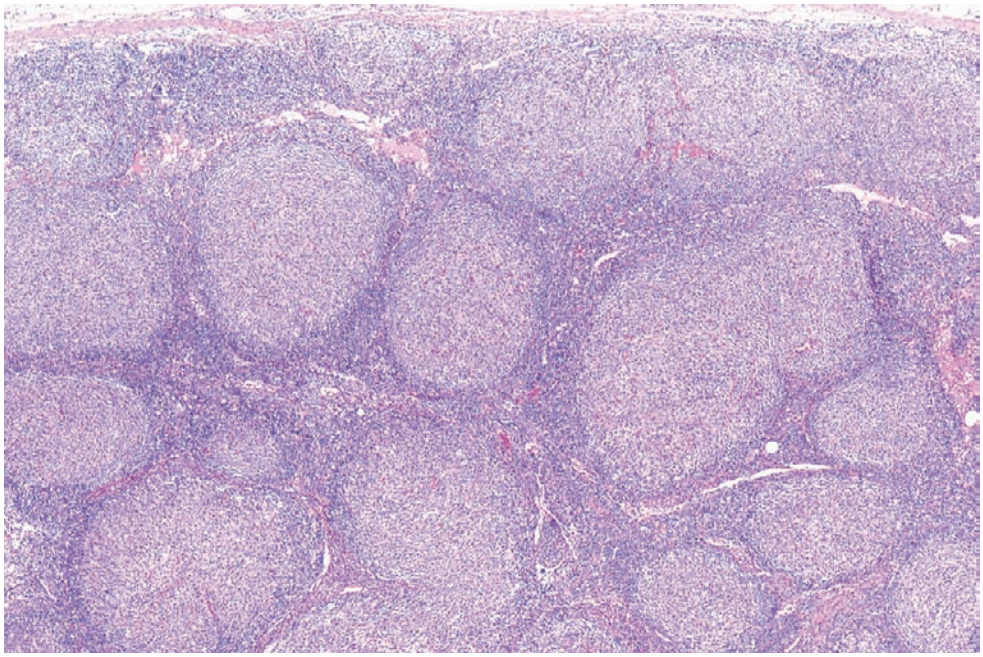

**FIGURE 7-20**

Follicular lymphoma. Multiple neoplastic follicles are crowded into the lymph node.

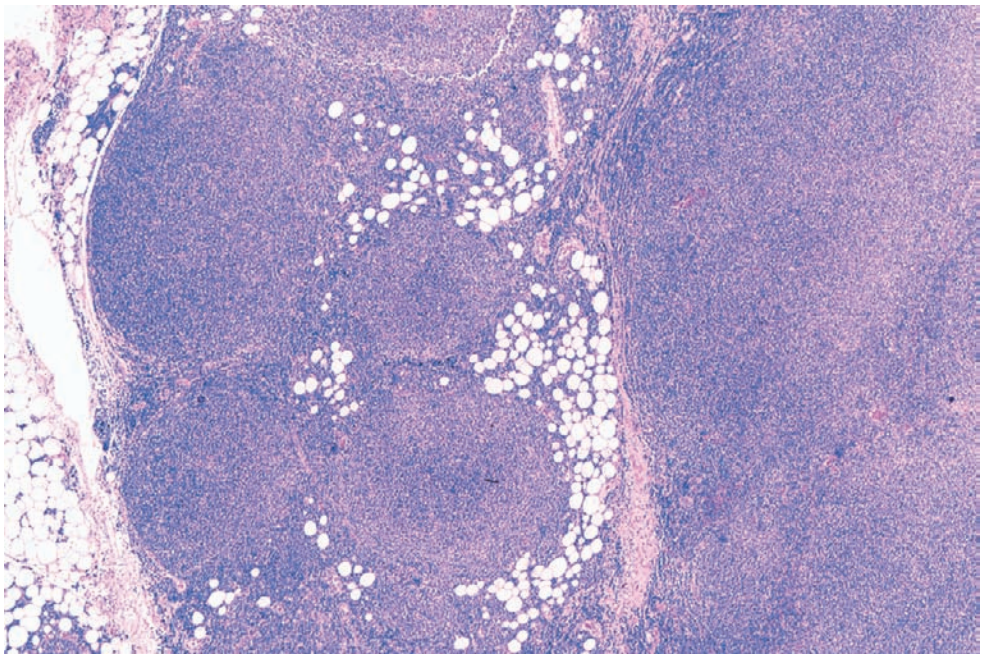

**FIGURE 7-21**

Extensive extracapsular infiltration by follicular lymphoma *(left).* The lymph node architecture is effaced by lymphoma growing in a more vaguely nodular pattern *(right).*

accompany follicular lymphomas (Figure 7-24). The fibrotic areas can be intranodular, perinodular, associated with diffuse areas, or so extensive that they obscure the underlying neoplastic lymphoid infiltrates. In the latter cases, wide sampling of the tissue often uncovers more typical features of follicular lymphoma. The fibrovascular stroma can also surround clusters of neoplastic follicular center cells, packeting them in a manner that suggests an epithelial or a melanocytic malignancy.

Just as there is a spectrum of cell types present in normal germinal centers, follicular lymphomas vary in their cytologic composition from case to case or from area to area in an individual case. Centrocytes predominate in most follicular lymphomas (Figure 7-25, A).

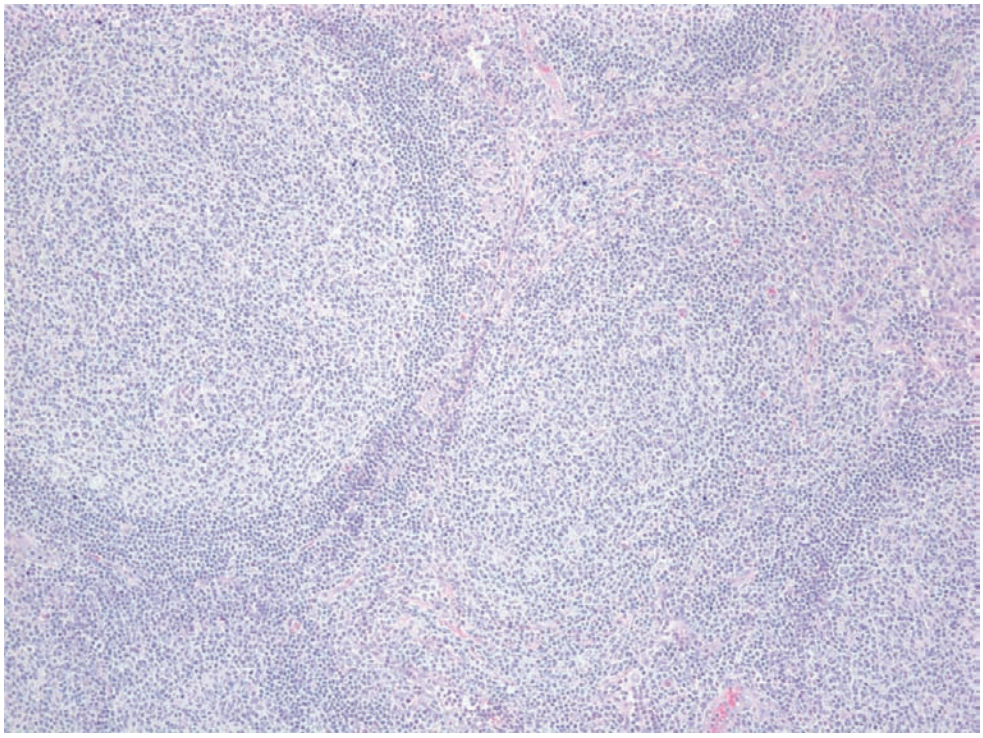

**FIGURE 7-22**
Follicular lymphoma. Nearly confluent neoplasic follicles and interfollicular infiltration by the neoplastic cells.

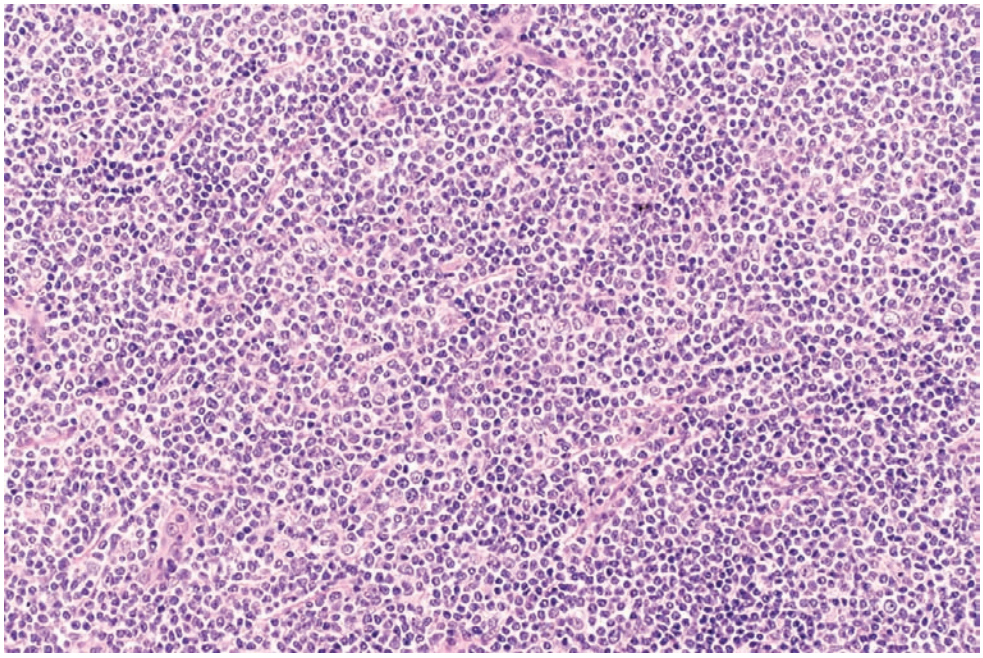

**FIGURE 7-23**
Diffuse follicle center lymphoma.

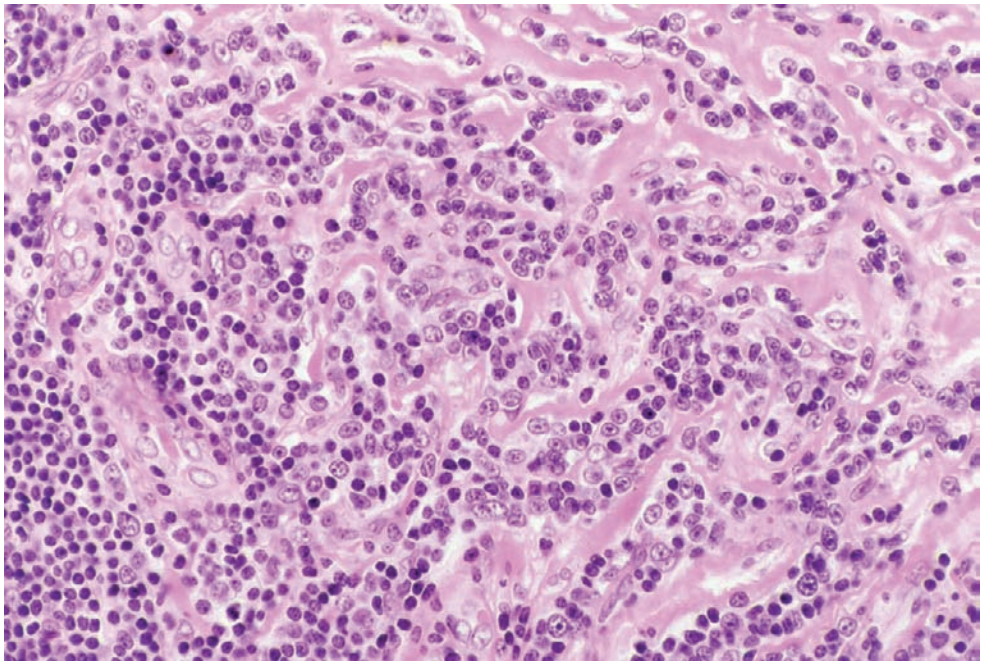

**FIGURE 7-24**
Collagen sclerosis in a follicular lymphoma.

They have nuclei that are basically ovoid shaped with superimposed notches, grooves, and cleaves. Therefore the nuclei often have irregular, angulated contours. In hematoxylin and eosin–stained sections, centrocytes lack nucleoli and the chromatin distribution varies from delicate to coarsely clumped and hyperchromatic. Small and large centrocytes are distinguished from one another based on comparing the nuclei to those of the intermixed macrophages. Small centrocytes have nuclei that are smaller than the nuclei of intermixed histiocytes, whereas large centrocytes have nuclei that are larger than those of the macrophages. The second cell population that can be identified in most examples of follicular lymphoma is the centroblasts (see Figure 7-25, C). They have round nuclei, dispersed chromatin, nucleoli that are usually multiple, and they are adjacent to the nuclear membranes and moderately abundant basophilic cytoplasm. Large and small centroblasts are distinguished from one another based on comparison of the nuclei to those of macrophages similar to the way small and large centrocytes are distinguished from each other.

The relative proportions of centrocytes and centroblasts in a follicular lymphoma case form the basis for grading the neoplasm. Large centroblasts are counted in ten consecutive 400× microscopic fields in ten consecutive follicles. The mean number of centroblasts per 400× field is then determined. Grade 1 is assigned to those cases in which there are less than 5 large centroblasts per 400× field (see Figure 7-25, A). Grade 2 is assigned to those cases with between 5 and 15 centroblasts per 400× field (see Figure 7-25, B) and Grade 3 follicular lymphomas have more than 15 centroblasts per 400× field. Grade 3 is further subdivided into grade 3a and 3b. If virtually all of the cells in the neoplastic follicles are centroblasts, the tumor is assigned grade 3b (see Figure 7-25, C). If there is a mixture of centrocytes together with the centroblasts the lymphoma is graded 3a. Because there is variability between microscopes in the area defined by a 400× field, conversion factors have been published to aid in reproducible grading. Finally, because grade 1 and grade 2 follicular lymphomas comprise a morphologic continuum and because they are both clinically indolent, the WHO classification deems it acceptable (preferable) to use the designation grade 1 to 2 of 3 for cases that meet grading criteria for either grade 1 or grade 2 follicular lymphoma. This approach is particularly useful for small biopsy specimens in which sampling limitations make grading difficult.

There are several unusual morphologic variants of follicular lymphoma that collectively account for no more than 10% of all follicular lymphoma cases. These variants are morphologic reflections of biologic functions of normal follicular center cells: Ig production, plasma cell differentiation, and marginal zone B cell differentiation. When the neoplastic follicular center cells, usually centrocytes, contain intracytoplasmic Ig that distorts the nucleus and forms intracytoplasmic vacuoles, the term *signet ring cell lymphoma* is used. The vacuoles represent dilated rough endoplasmic reticulin containing flocculent material—the Ig. In cases in which the Ig isotype is IgM, the vacuoles are periodic acid–Schiff positive. This unusual morphology probably

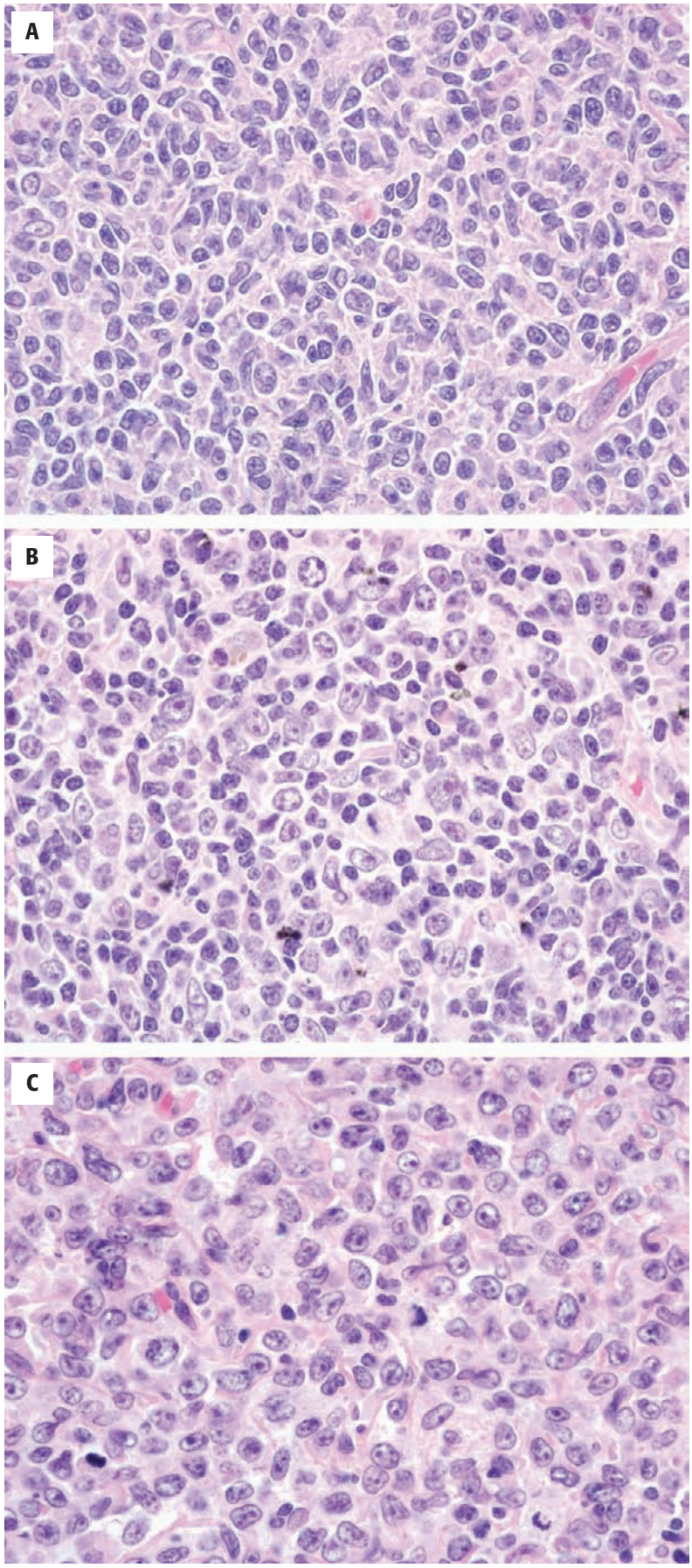


**FIGURE 7-25**

Cytologic composition of follicular lymphoma. **A,** Grade 1. **B,** Grade 2. **C,** Grade 3.

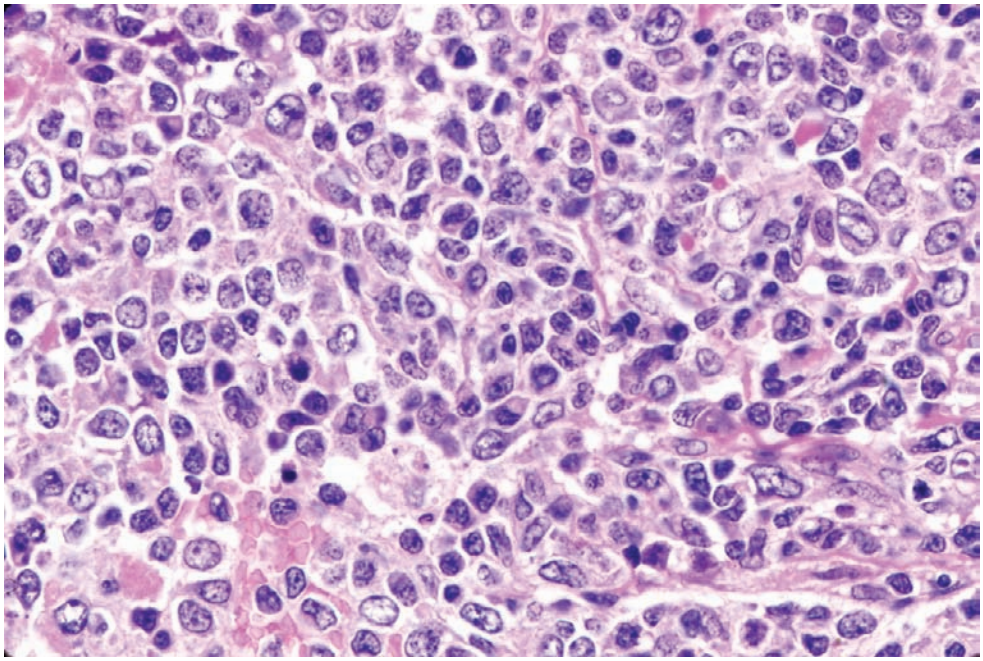

**FIGURE 7-26**

Follicular lymphoma with plasma cell differentiation. The neoplastic follicle is present in the upper right portion of the photograph. The neoplastic plasma cells are clustered in the center.

results from an imbalance between Ig synthesis and the ability of the neoplastic follicular center cells to normally transport the Ig to the surface of the cell. There is no independent prognostic significance to signet ring cell cytology in follicular lymphomas.

Cytologically normal, polyclonal plasma cells, usually distributed between the neoplastic follicles, occur relatively frequently in follicular lymphomas. However, in a small proportion of cases, follicular lymphomas contain intrafollicular or extrafollicular plasma cells that are clonally related to the underlying follicular lymphoma (Figure 7-26). The plasma cells are often cytologically abnormal with nuclear irregularities, and intranuclear (Dutcher bodies) or intracytoplasmic (Russell bodies) Ig inclusions. Using paraffin section immunohistochemistry, the abnormal plasma cells can be shown to express the same light chain type that is expressed by the neoplastic follicular center cells (Figure 7-27). This morphology and immunoarchitecture represents the neoplastic recapitulation of the normal process of plasma cell differentiation of normal germinal center B cells. It is not known whether plasma cell differentiation in follicular lymphomas affects prognosis or is associated with other clinical phenomena, such as monoclonal gammopathy or autoimmune phenomena, at a greater frequency than would be expected for follicular lymphomas without plasma cell differentiation.

A subset of follicular lymphomas exhibits marginal zone B cell differentiation (Figure 7-28). In these cases the neoplastic follicles, composed of centrocytes and centroblasts, are surrounded by coronas of monocytoid-appearing cells with modest nuclear irregularity, partially clumped chromatin, and distinctive, voluminous pale eosinophilic or clear cytoplasm. The immunoarchitecture in these cases is complex. The morphologically more usual follicular lymphoma cells distributed in the centers of the nodules are Ig light chain restricted B cells that express BCL-2 together with CD10 or BCL-6, or both. By contrast, the neoplastic monocytoid-appearing cells, though expressing BCL-2 and the same Ig light chain type as the neoplastic follicular center cells, often lack staining for CD10 and BCL-6. At one time these cases were considered to be composite follicular and monocytoid B-cell lymphomas. However, studies using microdissection and PCR have demonstrated that the marginal zone cells in these cases contain the same Ig gene rearrangement and identical *BCL2/IGH@* gene sequences as the phenotypically and cytologically typical neoplastic follicular center cells. Therefore this process is now thought to represent marginal zone B cell differentiation in follicular lymphoma. It recapitulates the process of marginal zone differentiation that occurs in a subset of normal follicular center cells. In one study there was a suggestion that marginal zone differentiation in follicular lymphomas was a feature indicating increased risk for adverse outcome.

Finally, an architectural variant of follicular lymphoma, termed *floral variant*, has also been described (Figure 7-29). The neoplastic follicles are large and contain clustered neoplastic germinal center cells surrounded by large clouds of non-neoplastic mantle zone lymphocytes.

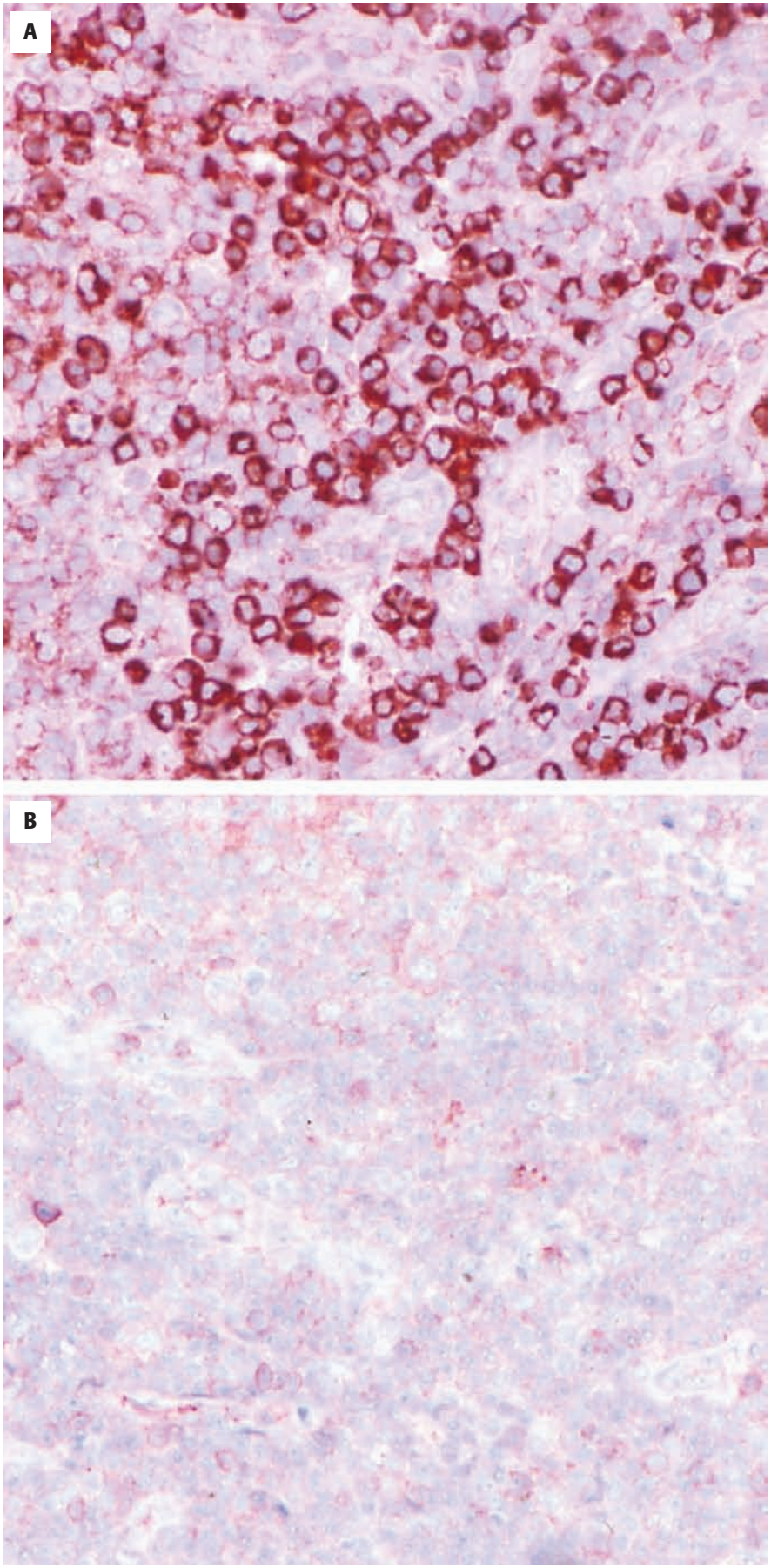


**FIGURE 7-27**

Follicular lymphoma with plasma cell differentiation. Immunoperoxidase stain for κ immunoglobulin light chains **(A)** and λ immunoglobulin light chains **(B).** The plasma cells have a monotypic staining pattern for κ light chains.

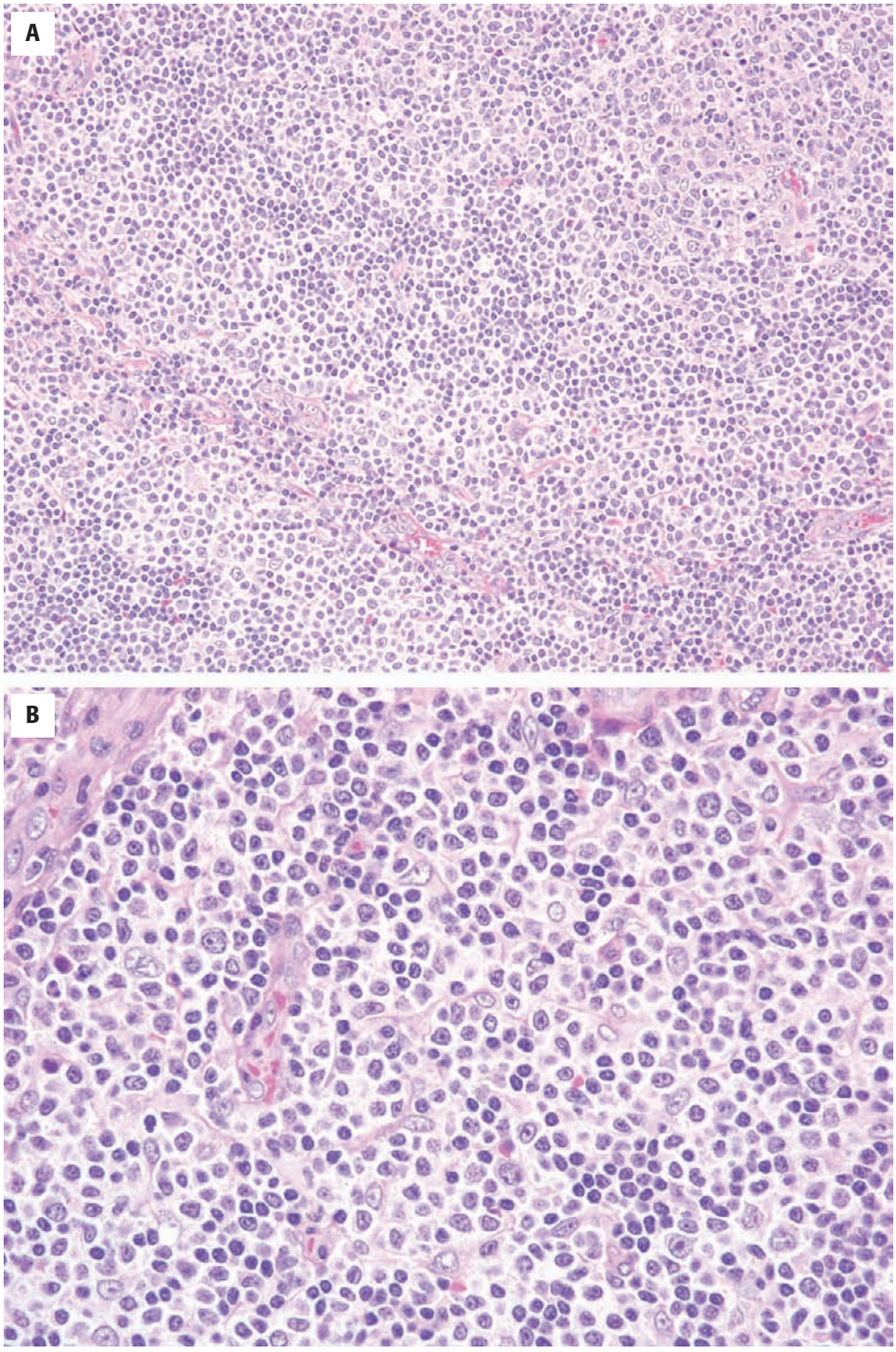


**FIGURE 7-28**

Marginal zone B cell differentiation in a follicular lymphoma. **A,** The neoplastic germinal center *(upper right)*. **B,** Marginal zone differentiation *(lower left)* with the cytology illustrated.

### Phenotype

Follicular lymphoma cells express the pan B lymphocyte antigens CD19, CD20 (Figure 7-30, A), CD22, CD79a, CD79b, and PAX-5. A substantial subset of cases is positive CD23. IgM with or without IgD is the heavy chain type most frequently expressed by follicular lymphomas, but IgG- and IgA-positive cases also occur. In almost all cases, Ig light chain restriction can be demonstrated by flow cytometry or frozen section immunohistochemistry. Rarely the cells of follicular lymphomas do not express Ig. In our experience, surface

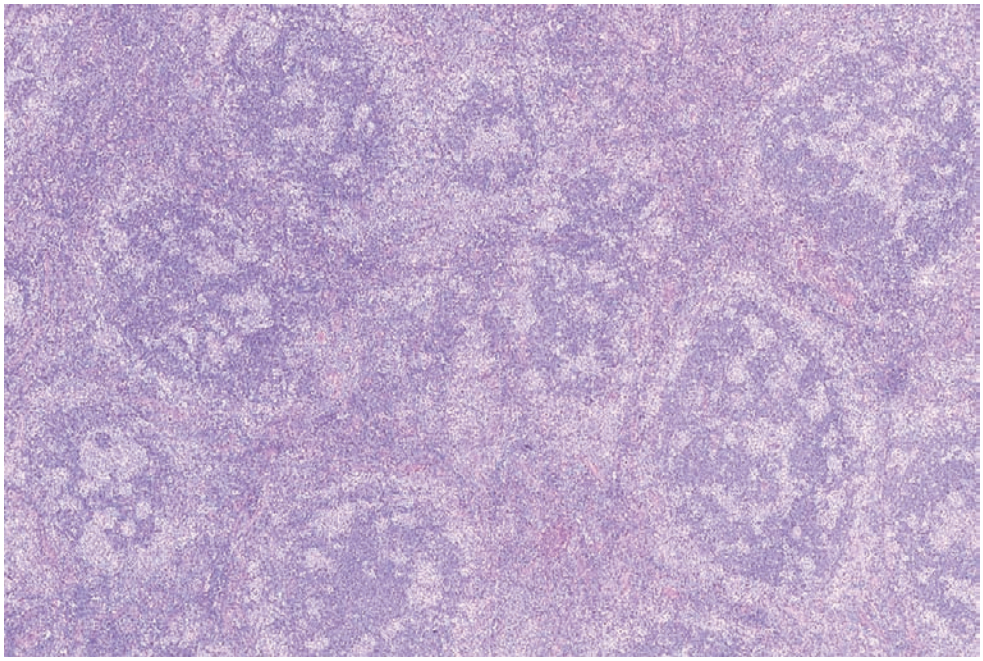

**FIGURE 7-29**
Floral variant follicular lymphoma.

Ig-negative follicular lymphomas are more likely to be grade 3, occur in children and young adults, or be primary in the skin. Similar to normal germinal center B cells, the cells in follicular lymphomas in lymph nodes and anatomic sites other than bone marrow, blood, and skin are positive for CD10 (see Figure 7-30, C) and/or BCL-6 in a high proportion of cases (90%). Compared with flow cytometry, paraffin section immunohistochemistry may be a more sensitive modality by which to detect CD10 expression in these cases. In addition, in approximately half of follicular lymphomas, CD10 is expressed only by the lymphocytes within neoplastic nodules and not by the lymphoma cells present in the interfollicular areas of the lymph node. Furthermore, compared with those involving lymph nodes, a greater fraction of follicular lymphomas in leukemic phase or involving the bone marrow fail to stain for CD10. Thus, particularly in bone marrow and blood, absence of CD10 expression by a $CD5^-$ lymphoma does not exclude follicular lymphoma. With only rare exceptions, follicular lymphomas are negative for CD5 (see Figure 7-30, F). The morphologically recognizable nodular pattern of follicular lymphomas has an immunoarchitecture that recapitulates that of normal germinal centers. Thus, in addition to the lymphomatous B cells, neoplastic follicles contain well-defined meshworks of normal $CD21^+$ or $CD23^+$ (see Figure 7-30, B) follicular dendritic cells, relatively few $CD8^+$ cytotoxic T lymphocytes and varying numbers of $CD3^+$ (see Figure 7-30, E) and $CD4^+$ positive T helper cells and CD57 positive intrafollicular T cells. In most cases the neoplastic follicles are also surrounded by normal, polyclonal, IgD-positive mantle zone lymphocytes.

As a consequence of the t(14;18)(q21;q32) (see later), cytoplasmic BCL-2 protein expression is a hallmark of most follicular lymphoma cases (see Figure 7-30, D). This marker is positive in nearly 100% of grade 1 follicular lymphomas, 75% of grade 3 follicular lymphomas, and a varying percentage of primary cutaneous follicular lymphomas. Because normal follicular center cells lack BCL-2 protein expression, BCL-2 is a useful marker to distinguish follicular lymphoma from follicular lymphoid hyperplasia. In this application, BCL-2 positivity in follicular center cells supports a diagnosis of follicular lymphoma, but the absence of BCL-2 expression by follicular center cells, though characteristic of follicular hyperplasia, does not exclude follicular lymphoma. Because almost all other low-grade B-cell lymphomas physiologically express BCL-2, this marker is not useful to discriminate between follicular lymphoma and other low-grade B-cell lymphoma types.

### Genetics

The genetic hallmark of follicular lymphoma, present in 85% of cases, is the t(14;18)(q32;q21). This abnormality translocates *BCL2* into the VDJ region of the Ig heavy chain gene (*IGH@),* placing *BCL2* under the regulatory control of the *IGH@* promoter and causing overexpression of BCL-2 protein. Because BCL-2 protein is an inhibitor of apoptosis, the translocation rescues the neoplastic cells from apoptotic cell death, causing their

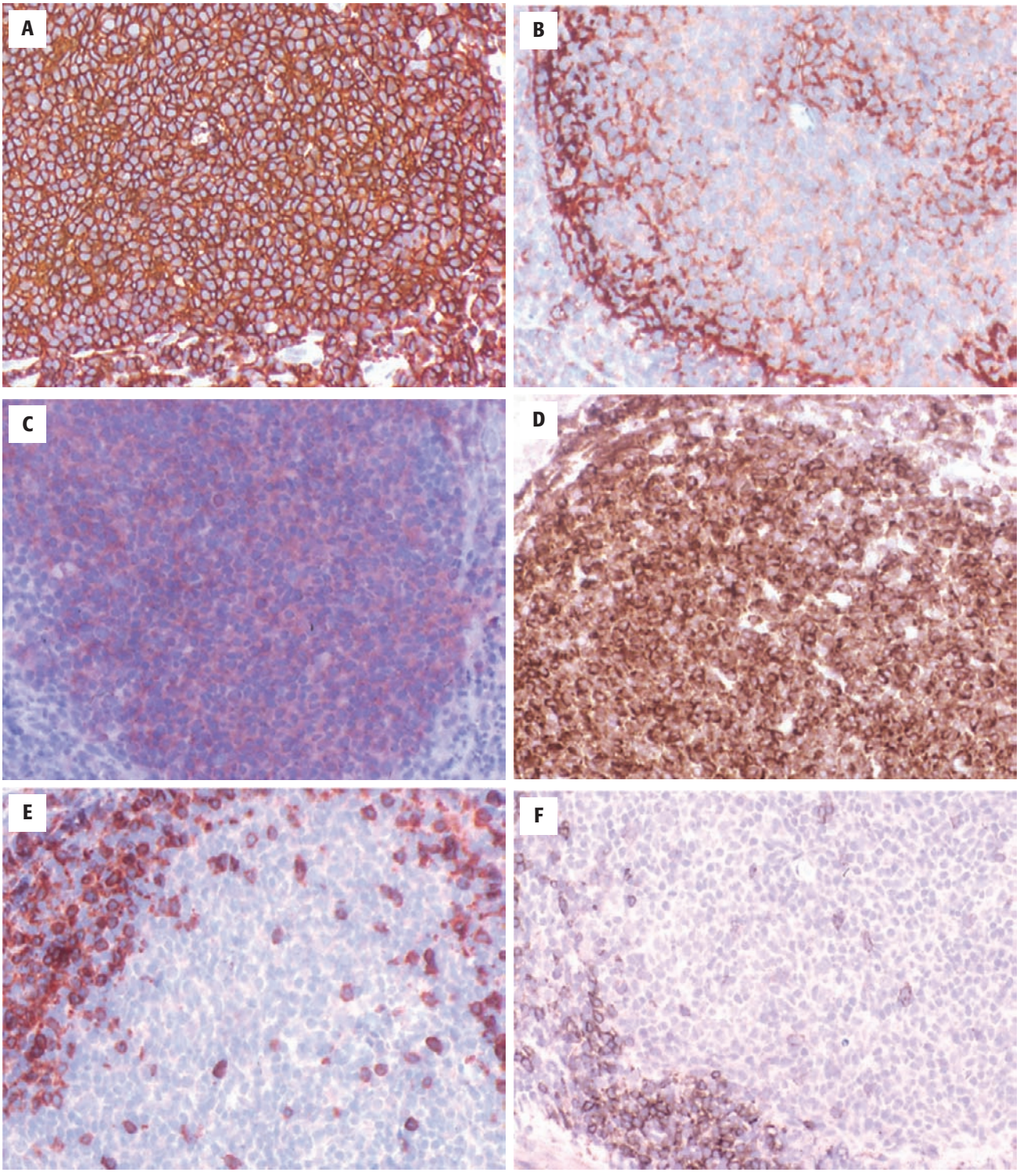


**FIGURE 7-30**

Typical phenotype of follicular lymphoma. Immunoperoxidase staining of paraffin sections of serial sections of a single neoplastic follicle. **A,** $CD20^+$. **B,** $CD23^-$, but note the associated $CD23^+$ follicular dendritic cell meshwork. **C,** $CD10^+$. **D,** BCL-$2^+$. **E,** $CD3^-$. **F,** $CD5^-$.

accumulation in the tissues. The t(14;18) can be demonstrated by routine cytogenetic analysis, PCR techniques with primers that recognize *BCL2* and *IGH@*, and by FISH. The latter can be applied to nuclei extracted from paraffin-embedded tumor tissues, providing a convenient additional diagnostic tool for follicular lymphomas. In most cases, it is unnecessary to demonstrate the t(14;18) in suspected follicular lymphomas, because the morphology and immunoarchitecture are highly characteristic of the entity. However, in those low-grade

B-cell lymphoma cases in which morphology and phenotype are unusual and the differential diagnosis includes follicular lymphoma, demonstrating the t(14;18)(q32;q21) by FISH or PCR helps to confirm the diagnosis of follicular lymphoma. Although the t(14;18)(q32;q21) is the cytogenetic abnormality that is most characteristic of follicular lymphoma, it is usually not the sole karyotypic abnormality in the neoplastic cells. Additional trisomies, monosomies, and other translocations, none of which are specific for follicular lymphoma, usually accompany the t(14;18)(q32;q21).

In a small subset of follicular lymphomas, there are translocations involving *BCL6* at chromosome 3q27. The partner chromosome is usually 14q32 at the *IGH@* locus. The tumor cells in these cases do not contain *BCL2* rearrangements, and these follicular lymphomas are more likely to be examples of grade 3b follicular lymphoma, suggesting that they are pathophysiologically distinct from follicular lymphomas with the t(14;18)(q32;q21).

As in other B-cell lineage lymphomas, follicular lymphomas have clonally rearranged Ig genes. A hallmark of normal follicular center B cells and postfollicular B cells is point mutations in the VDJ regions of the Ig genes and in the 5′ noncoding sequences of *BCL6.* Although the biologic significance of the *BCL6* point mutations is unknown, point mutations in the Ig genes result in Ig molecules that have enhanced affinity for the antigen that has initiated the B-cell immune response. In keeping with their presumed origin from normal follicular center cells, follicular lymphoma cells also contain Ig and *BCL6* gene point mutations.

### Follicular Lymphoma in Extranodal Sites and in Unusual Circumstances

At diagnosis, the bone marrow is involved by follicular lymphoma, grades 1 and 2 in approximately 55% to 70% of cases, and in a lower percentage of follicular lymphoma grade 3 cases. A paratrabecular distribution is characteristic, but intertrabecular nodules and interstitial infiltrates also occur. In the bone marrow, follicular lymphomas are usually composed of a monomorphous population of small centrocytes with few centroblasts, but in a small fraction of cases the whole spectrum of follicle center cell types or monomorphous large centroblasts are present. The cell composition of the lymphoma in the bone marrow might not reflect that of the diagnostic lymph node specimen. Optimally the bone marrow report should include a statement comparing the morphology of the lymphoma in the staging bone marrow sample to that in the lymph node. Follicular lymphomas in the marrow are usually associated with reticulin fibrosis or sparsely and focally involve the bone marrow so that they are not often sampled by bone marrow aspiration. However, when aspirate smears are involved, follicular lymphoma cells are usually small to medium sized with irregular cleaved and grooved nuclei, partially clumped chromatin, inconspicuous nucleoli, and sparse agranular cytoplasm. An overt leukemic phase of follicular lymphoma occurs in no more than 10% of follicular lymphoma patients, but careful examination of the blood will reveal abnormal lymphocytes in the blood smears of many patients with follicular lymphoma. The cytologic features of circulating follicular lymphoma cells are identical to those in aspirate smears of involved bone marrow specimens.

In the spleen, follicular lymphomas preferentially involve the white pulp. Neoplastic centrocytes or centroblasts are present in the centers of white pulp nodules, and from there they can irregularly infiltrate the splenic red pulp. Marginal zone differentiation is common in follicular lymphomas involving the spleen. Halos of medium-sized lymphocytes with abundant, pale cytoplasm and less irregular nuclei than the neoplastic centrocytes surround the neoplastic follicles, making difficult the distinction between follicular lymphoma with marginal zone differentiation and splenic marginal zone lymphoma. Immunoperoxidase stains demonstrating the BCL-2–positive CD10 or BCL-6–positive neoplastic follicular center cells (see later) help to define this lymphoma type in difficult cases. A small subset of splenic follicular lymphomas is BCL-2 negative. These cases typically show less frequent CD10 expression, a higher proportion of grade 3 histology, a higher Ki-67 proliferation rate, and less frequent *BCL2* rearrangements.

Skin involvement can occur in systemic follicular lymphomas, and in this situation they are morphologically, phenotypically, and genetically similar to their nodal counterparts. However, when follicle center lymphomas arise primarily in the skin and only involve this organ, they exhibit important differences from those that arise in lymph nodes. Clinically they produce single or grouped erythematous papules and nodules often in the head and neck, shoulder girdle, upper chest, and back regions. After careful staging excludes extracutaneous disease, patients with primary cutaneous follicle center lymphoma are readily treated by conservative local therapies and rarely disseminate or cause the death of the patient. Histologically, a Grenz zone separates the nodular infiltrates in the dermis from the epidermis. In prototypic cases the centers of the nodules are composed of a mixture of centrocytes and centroblasts without prominent tingible body macrophages or mitotic figures. Distinct mantle cell populations circumscribe the follicular center cells in most cases, and the internodular areas are either sparsely cellular dermal collagen or contain infiltrates of bland T lymphocytes. Phenotypically, cutaneous follicular lymphomas are composed of B cells that express BCL-6 and often CD10. However, the neoplastic lymphoid cells in primary cutaneous follicular lymphomas are more frequently BCL-2 protein and Ig light chain negative than nodal follicular lymphomas;

therefore by both morphology and phenotype they can be difficult to distinguish from cutaneous follicular lymphoid hyperplasia. In difficult cases, establishing that there is an abnormal follicular center cell population in the skin using morphology and immunohistochemistry and showing clonal Ig gene rearrangements by molecular genetics methods are necessary to make the diagnosis of cutaneous follicular lymphoma. The European Organization for Research and Therapy of Cancer and the World Health Organization representatives issued a joint classification for primary cutaneous lymphomas. Those thought to be the cutaneous counterparts of the nodal follicular lymphoma are termed *primary cutaneous follicle center lymphoma* and regardless of the cytologic composition of the neoplastic follicles, they are not graded. Notably, cases showing a completely diffuse pattern, but composed of centrocytic and centroblastic cells (usually with a germinal center phenotype), are also appropriately diagnosed as primary cutaneous follicle center lymphoma.

Follicular lymphoma can manifest in the intestine as multiple sessile and pedunculated polyps—multiple intestinal lymphomatous polyposis. In these cases, numerous nodules of monomorphous follicular center cells extensively infiltrate the lamina propria of the intestine. When small centrocytes predominate, they can be morphologically similar to intestinal lymphomatous polyposis caused by mantle cell lymphoma. Immunophenotyping will readily distinguish between these two lymphoma types. Follicular lymphomatous polyposis in the intestine is almost always associated with mesenteric lymph node involvement; however, duodenal involvement by follicular lymphoma is often confined to that location (approximately half of duodenal follicular lymphomas are confined to the duodenum). In this situation, the lesions almost always involve the second portion of the duodenum, forming periampullary sessile polyps with lamina propria and submucosal nodular lymphoid infiltrates. The lymphomas are grade 1 to 2 and are composed of CD10 or BCL-6 positive, BCL-2 positive tumor cells, or both. As a result, they are pathologically similar to their systemic counterparts; however, there is a high frequency (71%) of association with *Helicobacter pylori*. These tumors show restricted usage of IgVH4 genes, attributes that they share with extranodal marginal zone lymphomas of mucosa associated lymphoid tissue. Therefore primary duodenal follicular lymphomas are hypothesized to be pathophysiologically different than their systemic counterparts.

There are unusual cases in which lymph nodes have normal architecture, but in which the germinal center cells in a subset of the follicles strongly express BCL-2. This phenomenon has been termed *in situ follicular lymphoma*. Before using this designation, there must be certainty that the finding is limited to only a few follicles in the lymph node, that the BCL-2 positive lymphocytes are strongly CD10 and/or BCL-6–positive follicular center cells, and that there is no substantial infiltration of CD10 or BCL-6–positive lymphocytes into the interfollicular areas of the lymph node. When diagnosed in this way, in situ follicular lymphoma can be seen in several clinical settings. In some patients, contemporaneous clinical staging reveals overt disease in other lymph node groups or other extranodal sites. In this situation, it is likely that in situ follicular lymphoma actually represented focal lymph node involvement by disseminated follicular lymphoma; however, in other patients, staging will not uncover any other sites of disease. In this situation, some patients subsequently develop overt follicular lymphoma and others never do; therefore a diagnosis of in situ follicular lymphoma is of uncertain clinical significance independent of rigorous staging and subsequent follow-up.

Pediatric patients can also develop follicular lymphomas, although it is uncommon. Some pediatric cases are clinically and pathologically similar to adult follicular lymphomas, but in children a substantial subset of follicular lymphomas appears to have different clinical, pathologic, and genetic features than follicular lymphomas arising in adults. Typically, children with follicular lymphomas have limited stage disease, often confined to cervical lymph nodes or to Waldeyer's ring. Histologically, the lymph nodes show large neoplastic follicles with serpiginous borders composed of a mixture of centroblasts and centrocytes (grades 2 or 3). The tumor cells show Ig light chain restriction and express CD10 and BCL-6, but lack BCL-2 protein expression and do not contain *BCL2/IGH@* translocations. Pediatric patients with these limited-stage, BCL-2–negative follicular lymphomas have an excellent outcome. By contrast, children with BCL-2–positive grade 1 to 2 follicular lymphomas often have disseminated disease, which is more refractory to therapy.

#### Transformation

Depending on how it is defined, follicular lymphoma transforms to a more aggressive B-cell lymphoma in 20% to 60% of cases. Diffuse large B-cell lymphoma (DLBL) in which the neoplastic cells resemble centroblasts or immunoblasts is the most common tumor to which follicular lymphomas transform (Figure 7-31, A). The large B-cell lymphoma cells express B-cell lineage antigens and usually retain a germinal center B-cell phenotype (CD10 and BCL-6 positive). They typically are clonally related to the follicular lymphomas from which they arise (e.g., contain the same Ig heavy chain rearrangements and identical *BCL2/IGH@* fusion sequences), but contain novel genetic alterations that have been reproducibly observed to be characteristic of transformation. Occasionally the transformed lymphoma has cytologic characteristics of Burkitt lymphoma or B-cell lymphoma, unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma.

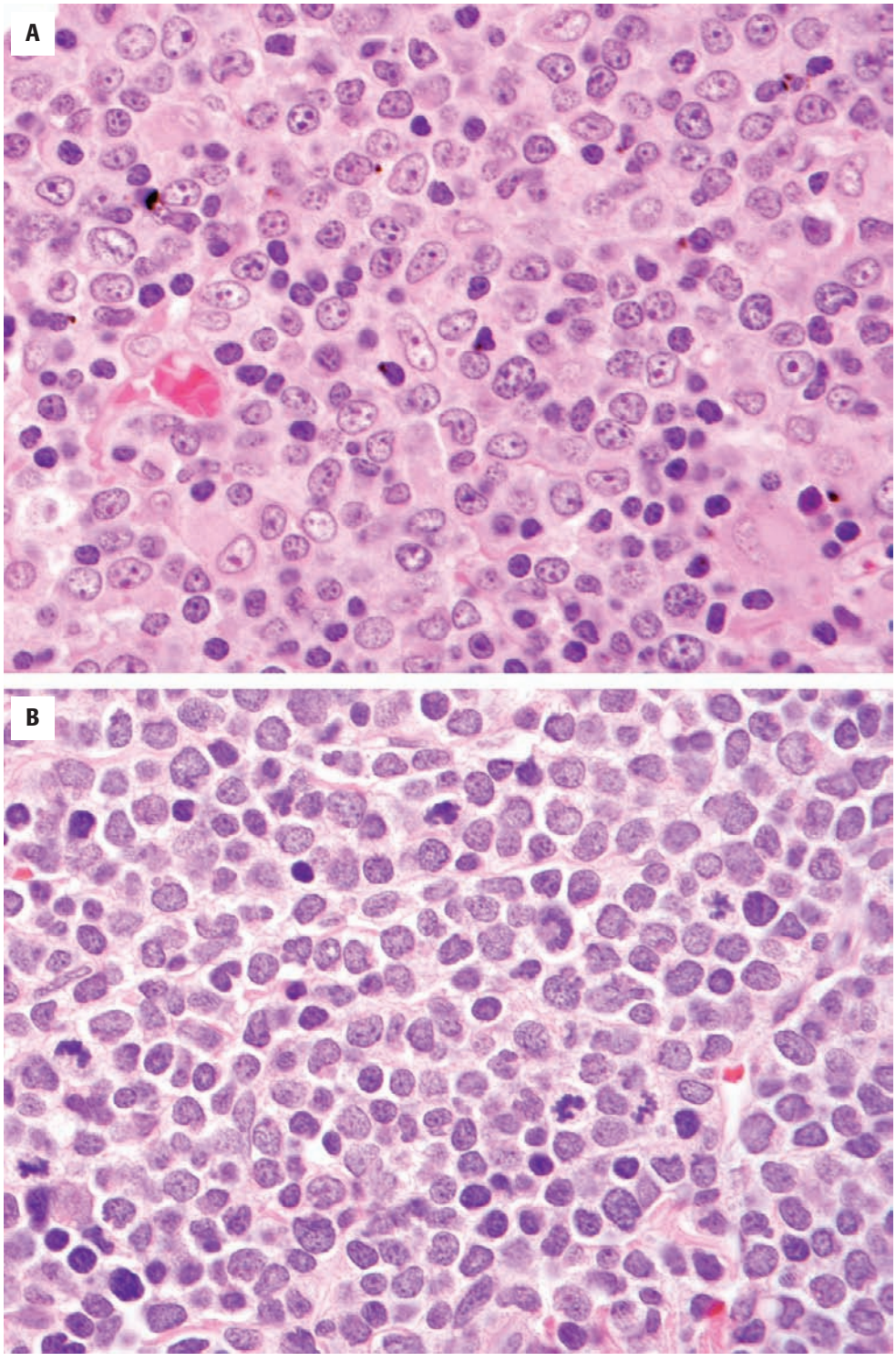


**FIGURE 7-31**

Transformation of follicular lymphoma. **A,** Diffuse large B-cell lymphoma; the cells have cytologic features of centroblasts. Note the large cell size, dispersed chromatin, multiple nucleoli and eosinophilic cytoplasm. **B,** Blastic transformation. The cells are intermediate size with irregular nuclear contours, stippled chromatin, small or inconspicuous nucleoli, and sparse cytoplasm. Note the multiple mitotic figures.

Accompanying this change, the tumor cells can acquire the t(8;14)(q24;q32) involving *C-MYC* (8q24) and *IGH@* (14q32) that is characteristic of these lymphoma types when they arise de novo. However, in the cases in which the follicular lymphomas transform to Burkitt or B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, the preexisting t(14;18)(q32;q21) is present as well, with the transformed lymphocytes representing a t(8;14) positive subclone of the original follicular lymphoma. Finally, rare cases of follicular lymphoma transform to a neoplasm that morphologically is

indistinguishable from lymphoblastic lymphoma (Figure 7-31, B). In these cases the lymphoblastoid neoplastic cells retain the general phenotypic characteristics of follicular lymphomas, including surface Ig expression and absence of TdT. Whatever the form that transformation takes, the disease becomes more clinically aggressive and from that point onward prognosis is guarded. The tumors that acquire abnormalities of *C-MYC* are particularly aggressive.

## Differential Diagnosis

Follicular lymphoma must be distinguished from reactive follicular hyperplasia. Lymph node architectural effacement, uniform size follicles, coalescence of follicles with loss of interfollicular tissue, and monomorphism of the intrafollicular lymphocyte population are all features that favor a diagnosis of follicular lymphoma over follicular hyperplasia. Demonstrating an Ig light chain restricted $CD10^+$ B-cell population by flow cytometry or immunohistochemistry strongly favors a diagnosis of follicular lymphoma over follicular hyperplasia. Demonstrating that nodules of follicular center cells (CD10 or BCL-6–positive, or both, and associated with $CD21^+$ or $CD23^+$ follicular dendritic cell meshworks) express BCL-2 also strongly favors follicular lymphoma over follicular hyperplasia.

Any lymphoma that grows in a nodular pattern is in the differential diagnosis of follicular lymphoma: mantle cell lymphoma, marginal zone B-cell lymphoma, and B-cell chronic lymphocytic leukemia–small lymphocytic lymphoma with proliferation centers. These disorders are morphologically, phenotypically, and genetically distinct from one another and can usually be separated from one another (see Tables 7-1 to 7-3).

Blastic transformation of follicular lymphoma is separated from B and T precursor lymphoblastic lymphoma–leukemia and acute myeloid leukemia in a manner totally analogous to that used for distinguishing these conditions from blastoid mantle cell lymphoma (see previous discussion). Phenotypically, the cells in blastic transformation of follicular lymphoma resemble B precursor lymphoblastic lymphoma–leukemia in that they express CD19 and CD10, but they are usually TdT-negative and $CD34^-$. However, rare case reports have documented clonally related transformations that express TdT.

## Prognosis and Therapy

The treatment approach to and prognosis of follicular lymphomas varies depending on histologic grade and Ann Arbor stage. For grade 1 and 2 follicular lymphomas, choice of therapy is driven by several considerations. It has long been appreciated that follicular lymphoma is an indolent disease and that aggressive therapies do not materially alter the survival. Thus for asymptomatic stage III and IV patients with no vital organ compromise caused by the lymphoma, a watch-and-wait approach is commonly used. When treatment is necessary, rituximab cyclophosphamide, vincristine, and prednisone is often the first line of therapy. In patients receiving this treatment, the lymphoma invariably recurs and the patients can be retreated with the expectation of a subsequent response. Eventually, responsiveness diminishes and the patients succumb to progressive disease or transformation to large B-cell lymphoma. The median survival for these patients is approximately 9 years. Newer treatment approaches involving bone marrow transplant, antibody therapy, lymphoma-specific vaccine therapies, and high-dose chemotherapy have shown promise in early studies and may favorably affect survival in this disease. For grade 3 follicular lymphoma, standard treatment is R-CHOP chemotherapy with rituximab, which has been shown to produce long-term remissions in at least a subset of the treated patients.

Because there is heterogeneity in the clinical outcomes for patients with follicular lymphoma, attempts have been made to devise a prognostic index similar to what has been applied successfully to diffuse large B-cell lymphomas. The Follicular Lymphoma International Prognostic Index has been proposed recently to meet this need. Five adverse prognostic factors were identified: age greater than 60 years, Ann Arbor stage III or IV, elevated serum lactate dehydrogenase level, hemoglobin level less than 12 g/dL, and more than four nodal sites of involvement by lymphoma. Low-risk patients have no or only one adverse factor, intermediate-risk patients have two adverse factors, and high-risk patients have three or more adverse factors. Ten-year survivals of 71%, 51%, and 27% of patients are predicted by the low-, intermediate-, and high-risk groups, respectively.

The primary duodenal follicular lymphomas and primary cutaneous follicle center lymphomas limited to these sites deserve special mention. These lymphomas typically have an indolent, nonprogressive course. The cutaneous cases may require local treatment only, whereas duodenal follicular lymphoma might not require therapy at all. Similarly, in situ follicular lymphoma—which may also be termed *follicular lymphoma–like B cells of undetermined significance*—does not require treatment once staging procedures have ruled out other clinical sites of disease.

Gene expression profiling has identified prognostic gene signatures that reflect immune response by infiltrating T cells and macrophages. Immunophenotypic surrogates such as lymphoma-associated macrophages identified by markers such as CD68 and regulatory T cells identified by FOXP3 are biomarkers that have been

investigated with varying results. Further study is needed before this type of information can be applied in practice.

## EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE

### Clinical Features

Extranodal marginal zone B-cell lymphoma of MALT lymphoma comprises 7% to 8% of all B-cell lymphomas and is a disease of older adults, with a median age of 60 years. There is a slight female predominance. As the name implies, extranodal marginal zone B-cell lymphomas preferentially arise in non–lymph node sites. They are most common in the stomach, salivary glands, lungs, orbit and ocular adnexa, thyroid, skin, and breast. Despite their morphologic resemblance to Peyer patches, MALT lymphomas are actually rare in the intestines, except for a peculiar type of MALT lymphoma—immunoproliferative small intestinal disease. The epidemiology of MALT lymphomas varies by anatomic site. In general, MALT lymphomas arise in organs devoid of or sparsely populated by lymphoid tissue. An inflammatory stimulus, either autoimmune or infectious, recruits lymphocytes and immune accessory cells to these sites, and it is within the inflammatory milieu that MALT lymphomas arise. The inflammatory disorders linked to MALT lymphomas in various anatomic sites are listed in Table 7-4. Because of the association of gastric MALT lymphomas with *Helicobacter pylori* infections (90% of gastric MALT lymphomas are associated with *H. pylori*), there is a higher incidence of this lymphoma type in geographic regions where there is a high incidence of *H. pylori* infection (e.g., the Veneto region of Italy). Despite the ubiquitous distribution of *Campylobacter jejuni,* immunoproliferative small intestinal disease occurs almost exclusively in the Middle East and in the Cape region of South Africa.

The initial manifestations of MALT-type lymphomas vary by the organ primarily involved by the tumor. For the stomach, nonspecific dyspepsia and epigastric pain are common symptoms. For the lung, cough and chest pain herald the symptomatic onset of the disease. For the salivary glands, thyroid, orbit, and skin, tumors involving those sites in association with symptoms of the salient associated autoimmune disease are present. Therefore the presenting manifestations of MALT lymphomas are nonspecific. A subset of MALT lymphoma patients have serum monoclonal gammopathies, usually of IgM isotype. Symptoms of the underlying autoimmune diseases in patients whose lymphomas arise in the salivary gland and the thyroid can also be present concurrently with the onset of MALT lymphoma.

At the time of diagnosis, MALT lymphomas are usually localized to the organ in which they arise. The distribution of MALT lymphomas across the different Ann Arbor stages is likely to vary by anatomic site, but there are few systematically analyzed data that allow for definite conclusions to be made about this point, except for the stomach. For all primary anatomic sites, aggressive staging will uncover bone marrow involvement in 20% of patients, spread to another nonnodal site in 12% of patients, and involvement of lymph nodes in 7.5% of patients. Primary gastric MALT lymphomas have an indolent evolution, remaining localized to the stomach for long periods of time before disseminating to other sites. This behavior is likely to occur in MALT lymphomas originating in other organs as well.

**TABLE 7-4**

**Site-Specific Inflammatory Disorders Related to the Pathogenesis of Malt Lymphomas**

| Anatomic Site | Associated Condition |
|---|---|
| Stomach | *Helicobacter pylori* gastritis |
| Salivary gland | Sjögren syndrome |
| Thyroid | Hashimoto thyroiditis |
| Lacrimal gland | *Chlamydia psittaci* infection (controversial) |
| Skin | *Borrelia burgdorferi* infection |
| Immunoproliferative small intestinal disease | *Campylobacter jejuni* infection |

*MALT,* Mucosa-associated lymphoid tissue.

### Pathologic Features

#### Morphology

Regardless of the extranodal organ involved by the MALT lymphoma, five key elements define the morphology of these tumors: the neoplastic marginal zone B cells, varying numbers of transformed lymphocytes that resemble immunoblasts or centroblasts, plasma cells that can be polyclonal or clonally related to the neoplastic B cells, lymphoepithelial lesions, and reactive germinal centers. These elements are arranged architecturally in a manner that recapitulates the Peyer patch (Figure 7-32). A prototypic gastric MALT type lymphoma is illustrated in Figures 7-33 to 7-35. There is case-to-case variability in the cytologic features of the neoplastic marginal zone B cells. In most cases, the neoplastic marginal zone cells are small to medium sized with irregular nuclear contours, partially clumped

## EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF MUCOSA ASSOCIATED LYMPHOID TISSUE—FACT SHEET

### Clinical Features

- Five percent to 6% of non-Hodgkin lymphomas
- Median age 7th decade, slight female predominance
- Involves extranodal sites: stomach, lung, salivary glands, orbit, skin
- Symptoms depend on presenting anatomic site
- Associated conditions:
  - *Helicobacter pylori* gastritis
  - Sjögren syndrome
  - Hashimoto thyroiditis
- Low Ann Arbor stage at clinical presentation

### Morphology

- Five elements assembled into structures resembling a Peyer patch:
  - Neoplastic small lymphocytes, centrocyte-like cells
  - Transformed lymphocytes resembling centroblasts and immunoblasts
  - Plasma cells, clonally related to the small lymphocytes in 30%
  - Reactive germinal centers
  - Lymphoepithelial lesions
- Follicular colonization in subset

### Immunophenotype

- $CD19^+$, $CD20^+$, $sIg^+$, IgM without IgD, rarely IgG or IgA
- Monotypic plasma cells in 30%
- $CD10^-$ and $CD5^-$
- CD43 variably positive

### Genetics

- Clonally rearranged immunoglobulin genes
- t(11;18)(q21;q21): *BIRC3, MALT1*
- t(14:18)(q32;q21): *IGH@/MALT1*
- t(1:14)(p22:q32): *BCL10/IGH@*
- t(3;14)(p13;q32): *FOXP1/IGH@*
- Trisomies 3, 8, 18 present in cases without translocations
- *TNFAIP3* deletion
- Point mutations in Ig genes

### Prognosis and Therapy

- For gastric MALT lymphoma associated with *H. pylori:*
  - Antibiotic therapy to eradicate *H. pylori*—probability of success decreases with increasing Ann Arbor stage, depth of infiltration of the gastric wall by lymphoma and presence of *API2/MALT1* translocation
- For other anatomic sites:
  - Local radiation
  - Low-intensity, single- or multiple-agent chemotherapy with rituximab
- Excellent prognosis for low-stage gastric MALT lymphoma
- Median survival 5 to 6 years for Ann Arbor stage III and IV patients

### Differential Diagnosis

- Reactive lymphoid hyperplasia
- B-cell small lymphocytic lymphoma
- Mantle cell lymphoma
- Follicular lymphoma, particularly those with plasmacytic or marginal zone B-cell differentiation, or both
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Lymphoplasmacytic lymphoma

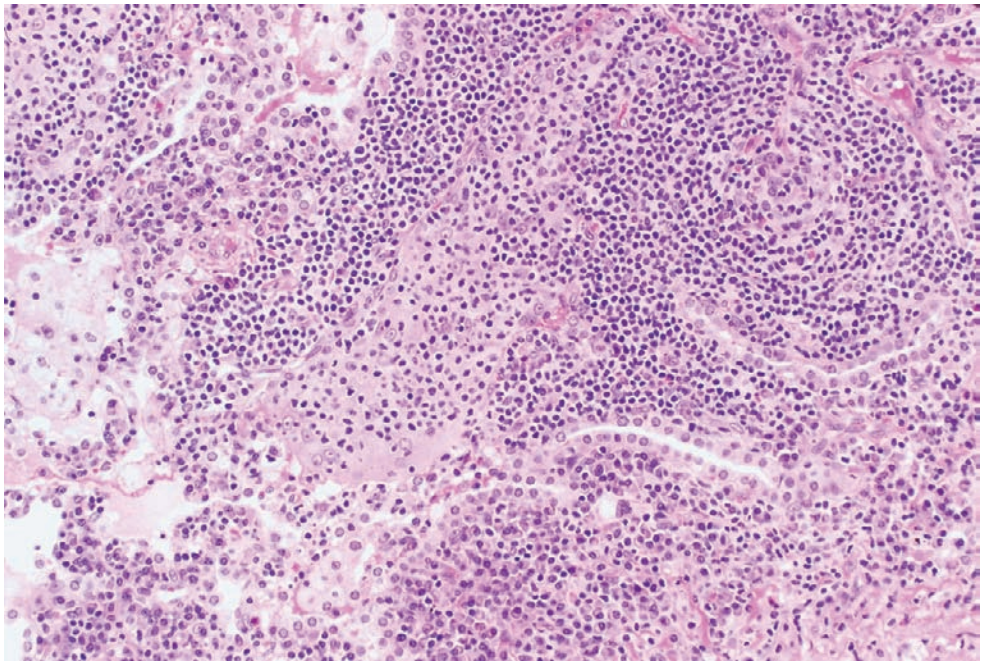

**FIGURE 7-32**

Mucosa-associated lymphoid tissue lymphoma involving lung. The resemblance to a Peyer patch is noted here. There is a germinal center surrounded by neoplastic marginal zone cells that infiltrate into altered pulmonary epithelium forming lymphoepithelial lesions.

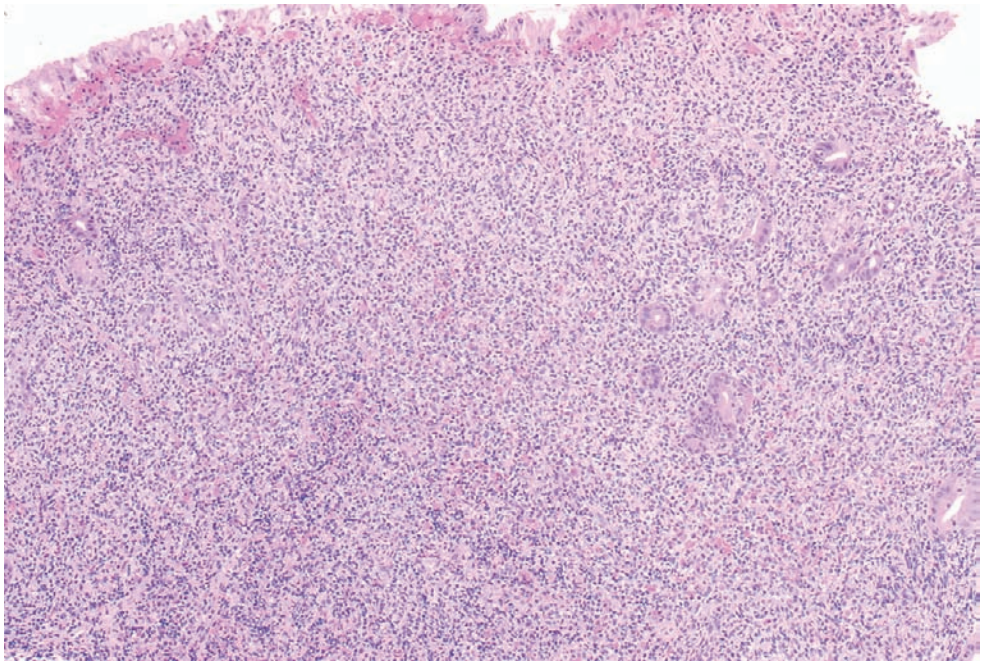

**FIGURE 7-33**

Gastric mucosa-associated lymphoid tissue lymphoma. The tumor cells extensively infiltrate the lamina propria.

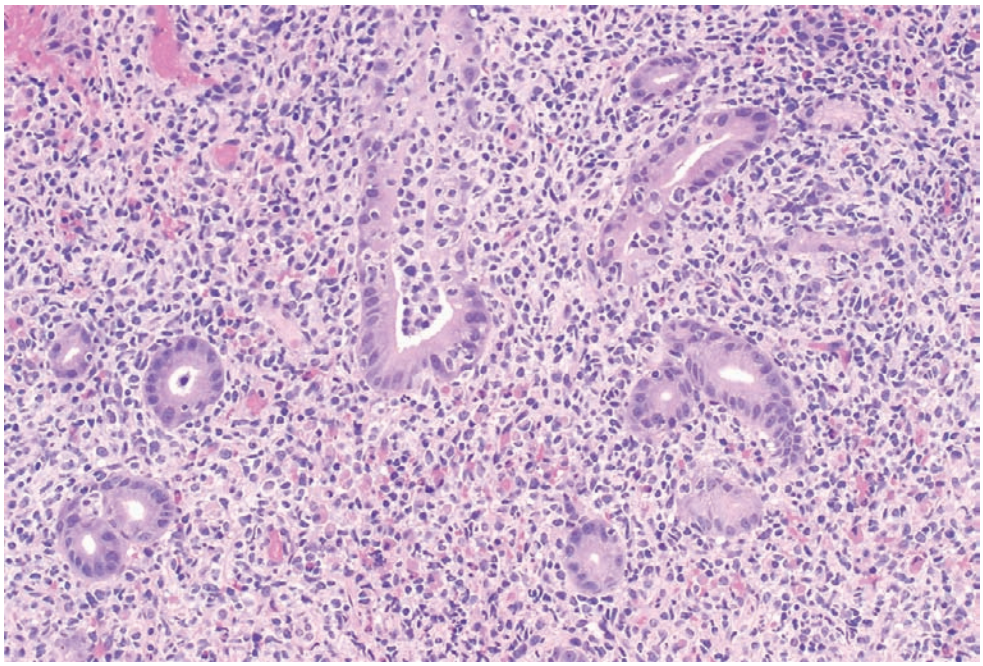

**FIGURE 7-34**

Gastric mucosa-associated lymphoid tissue lymphoma. Residual gastric glands are separated by the neoplastic lymphocytes (centrocyte-like cells). Note the lymphoepithelial lesion in the center of the field.

chromatin, inconspicuous nucleoli, and moderately abundant pale-staining cytoplasm. These cells resemble centrocytes and thus have been termed *centrocyte-like cells* (see Figure 7-35). The tumor cells may have more voluminous clear or pale eosinophilic cytoplasm and resemble monocytoid B cells or have the cytologic characteristics of small lymphocytes with round nuclei, distinctly clumped chromatin, and sparse cytoplasm. In addition to the neoplastic small lymphocytes, most MALT lymphomas also contain variable numbers of singly distributed large transformed lymphocytes with the cytologic features of either centroblasts or

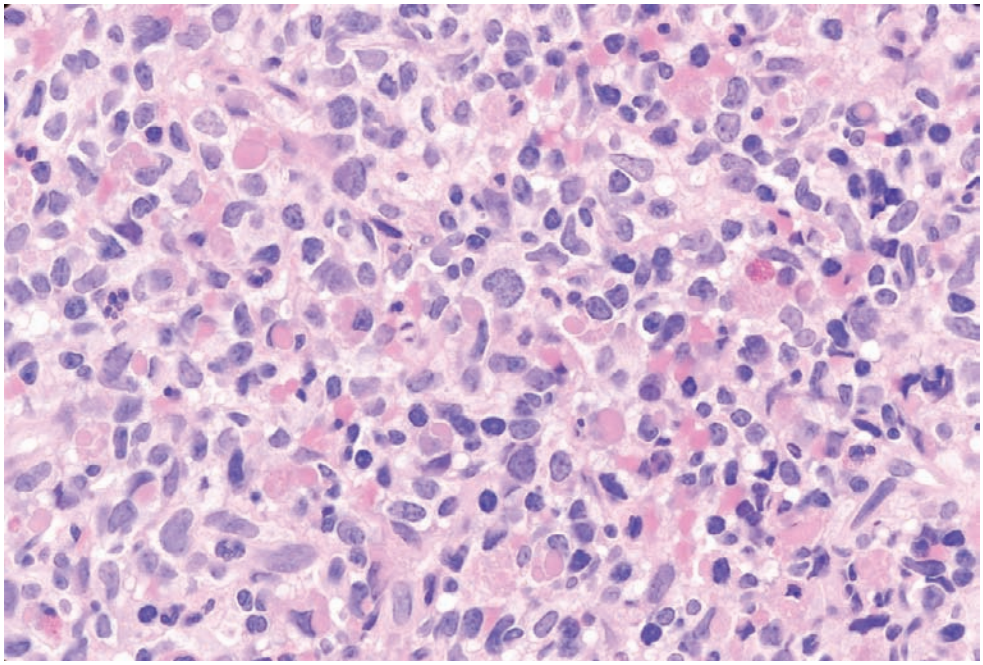

**FIGURE 7-35**

Gastric mucosa-associated lymphoid tissue lymphoma. Varied cytologic composition including centrocyte-like cells, larger transformed lymphocytes, and eosinophilic immunoglobulin inclusions.

immunoblasts. If the large cells begin to form clusters or especially if they aggregate in confluent sheets, then a diagnosis of diffuse large B-cell lymphoma arising in a MALT lymphoma should be entertained. The term *MALT lymphoma* should not be used to refer to an aggressive large B-cell lymphoma arising in an extranodal site; the WHO term *diffuse large B-cell lymphoma* is appropriate in this circumstance. In approximately 30% of cases, Ig light chain restricted plasma cells are part of the tumor (Figure 7-36), and in rare cases they predominate the cellular infiltrates almost to the exclusion of neoplastic marginal zone B lymphocytes. Sometimes the plasma cells contain intranuclear (Dutcher bodies) or intracytoplasmic (Russell bodies) Ig inclusions, or they exhibit nuclear pleomorphism. Characteristically the plasma cells are distributed adjacent to the mucosal surface involved by the lymphoma; if the MALT lymphoma involves the skin, they are most frequently found immediately under the epidermis and at the border between the dermal collagen and the nodular infiltrates of the tumor. Most obvious in gastric, pulmonary, salivary gland, and thyroid gland MALT lymphomas is infiltration of altered epithelium by the neoplastic cells (lymphoepithelial lesions; see Figure 7-34). Lymphoepithelial lesions are unusual in cutaneous, bladder, and conjunctival MALT lymphomas. Lymphoepithelial lesions are composed of three or more neoplastic lymphocytes associated with alteration or destruction of the epithelium. Although lymphoepithelial lesions help to identify a B-cell lymphoma as a MALT-type lymphoma, they can also be seen in a variety of reactive conditions in most mucosa associated anatomic sites. The notable exception is the stomach, in which prominent lymphoepithelial lesions are a diagnostic clue to the presence of lymphoma. Finally, non-neoplastic germinal centers associated with follicular dendritic cells are a common feature in MALT lymphomas. Uncommonly, MALT-type lymphomas are associated with localized amyloid or free Ig light chain deposition that can be so pronounced that the underlying MALT lymphoma is obscured. Wide sampling of the lesion is then necessary to uncover the characteristic cellular infiltrates of the lymphoma.

In some cases of MALT lymphoma, the neoplastic cells infiltrate into the germinal centers (follicular colonization). This phenomenon takes three forms. First, the neoplastic marginal zone cells retain their native cytologic characteristics and replace the follicular center cells. Second, the neoplastic cells enter the germinal center and differentiate into plasma cells. In this situation the germinal center contains a mixture of non-neoplastic follicular center cells and monoclonal plasma cells derived from the MALT lymphoma. Finally, under the influence of the follicular microenvironment, the neoplastic cells can enter the germinal center and transform into large centroblasts. This latter manifestation of follicular colonization is the most difficult to distinguish from florid follicular hyperplasia or grade 3 follicular lymphoma, and it occurs most often in MALT lymphomas arising in the thyroid.

Expansion of the paracortex by a mixed population of small marginal zone B cells with or without plasma

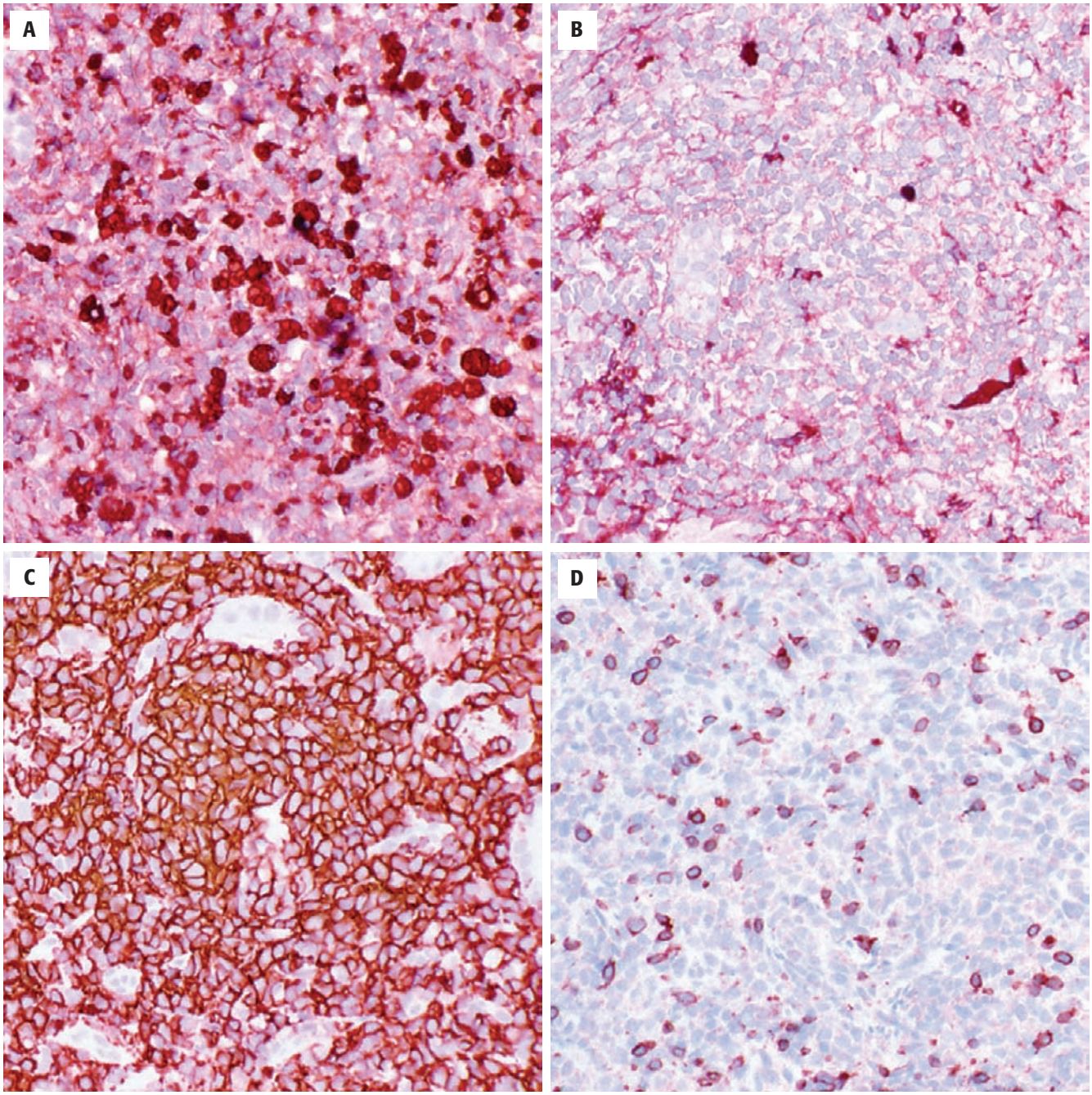


**FIGURE 7-36**

Gastric mucosa-associated lymphoid tissue lymphoma. Immunoperoxidase staining of paraffin sections for κ **(A)** and λ **(B)** immunoglobulin light chains, CD20 **(C),** and CD5 **(D).** The plasma cells show κ light chain restriction. Extracellular immunoglobulin globules also selectively stain for κ light chains. The neoplastic cells express CD20 and are negative for CD5.

cells is the most common pattern of lymph node involvement by MALT lymphoma. In occasional cases, the lymph nodes contain a prominent concentric halo of centrocyte-like cells surrounding germinal centers. This pattern resembles that seen when lymph nodes are involved by splenic marginal zone lymphoma. As a result, it can be difficult to distinguish splenic, nodal, and extranodal marginal zone lymphomas from one another in a lymph node. Considering the anatomic distribution of the lymphoma before classifying it definitively is prudent in this context.

### Immunophenotype

The cells of extranodal marginal zone B-cell lymphoma express the pan B lymphocyte antigens CD19,

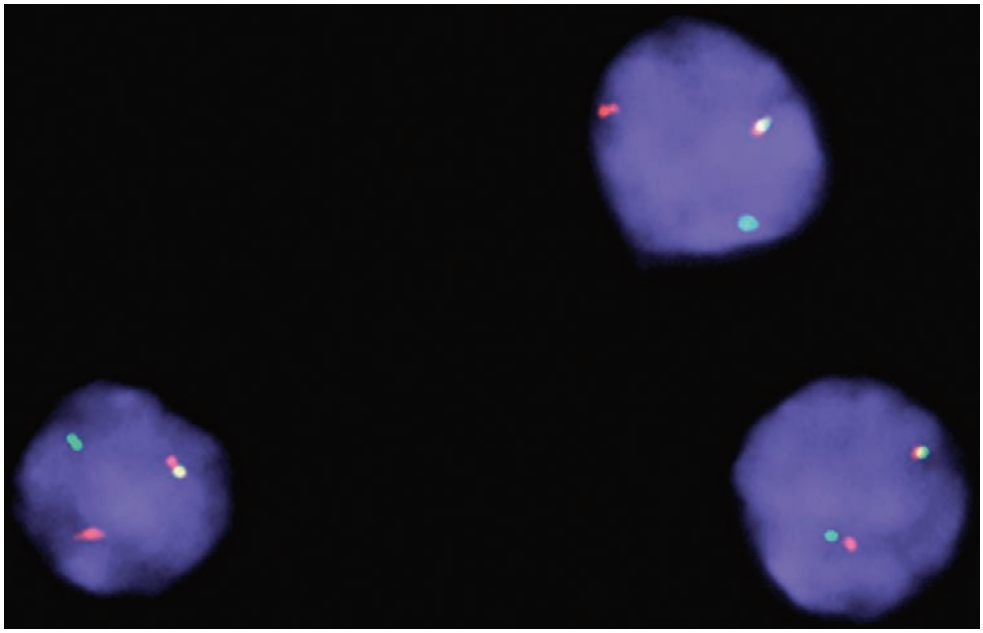

**FIGURE 7-37**

Fluorescence in situ hybridization using probes that recognize the 5′ and 3′ ends of *MALT1* on chromosome 18q21. The cell in the lower right shows the two normal fused 5′ (red fluorochrome tagged) and 3′ (green fluorochrome tagged) signals representing the intact *MALT1* genes on the two normal chromosomes 18q21. The fused signals appear yellow. The upper and left nuclei are MALT lymphoma cells. In each, the 5′ and 3′ *MALT1* signals have split into separate 5′ red and 3′ green signals, indicating that a translocation has occurred with breakpoints in *MALT1*. Each of these nuclei also contain one normal yellow *MALT1* fusion signal representing the nontranslocated chromosome 18q 21.

CD20 (see Figure 7-36), CD22, CD79a, CD79b, and PAX-5 and show either κ or λ light chain restriction. When the intermixed plasma cells are part of the lymphoma, they too express the same Ig light chain as the neoplastic small lymphocytes. MALT lymphoma cells are usually positive for CD21 and CD35. Immunohistochemistry for CD21 and CD35 also highlight the intermixed follicular dendritic cells and helps in the recognition of follicular colonization. Typically the cells of MALT lymphoma are negative for CD5 (see Figure 7-36), CD10, and BCL-6, but these tumors can express CD5 or CD10. CD43 is expressed by a subset of MALT lymphomas. This useful finding helps to distinguish reactive B-cell infiltrates, which exceptionally express CD43 from $CD43^{+}$ MALT lymphomas. Because the pathologist is often in the position of diagnosing an extranodal marginal zone lymphoma of MALT on a small biopsy, a comprehensive phenotypic approach should be applied, first to support a diagnosis of a small B-cell lymphoma (demonstrate light chain restriction in the neoplastic lymphocytes or plasma cells or demonstrate a CD43 positive B-cell population) and second to help to definitively classify the lymphoma (CD10, BCL-6, CD5, CD23, and cyclin D1).

### Genetics

Four translocations are highly specific for extranodal marginal zone B-cell lymphoma of MALT and have different frequencies in MALT lymphomas from different anatomic sites. T(11;18)(q21;q21) is most common and occurs in MALT lymphomas of the stomach, lung, and occasionally orbit (Figure 7-37). As a result of this translocation, two genes (*BIRC3,* also known as *API2* from 11q21, and *MALT1* from 18q21) are fused, producing a chimeric protein coded for by the 5′ end of *BIRC3* and the 3′ end of *MALT1*. In the t(14;18)(q32;q21), a full-length *MALT1* gene is brought under the control of the Ig heavy chain gene enhancer at 14q32, producing overproduction of the MALT1 protein. This translocation is most common in nongastrointestinal MALT lymphomas, particularly in the liver, orbit, skin, and lung. In the t(1;14)(p22;q32), *BCL-10* from 1p22 is brought under the control of the Ig heavy chain enhancer (14q32). Thus BCL-10 protein is overexpressed and localizes to the nucleus of the neoplastic cells for unknown reasons. The t(1;14)(p22;q32) is uncommon and its frequency distribution by involved organ is uncertain. In all these translocations, elements of the signaling pathway from the Ig receptor to activation of nuclear factor κ B (NFκB) are altered, resulting in nonphysiologic activation of NFκB. Finally, rare MALT lymphoma cases have the t(3;14)(p12.1;q32) that involves *FOXP1* and *IGH@*. The pathophysiologic effects of this translocation remain to be determined.

A further genetic abnormality that occurs in MALT lymphomas is deletion or hypermethylation of *TNFAIP3,* a gene found at chromosome 6p23.3 that codes for a protein called A20. A20 is a negative regulator of NFκB signaling, both by directly inhibiting elements of the

NFκB canonical pathway and by effects on ubiquitylation of NFκB pathway members. The net result is that *TNFAIP3* acts as a tumor suppressor, which when deleted or inactivated by hypermethylation results in unrestrained NFκB pathway activation, achieving an effect similar to that caused by three of the other translocations involved in MALT lymphomas.

In addition to the common translocations, trisomies of chromosomes 3, 8, and 18 are also common genetic abnormalities in extranodal marginal zone B-cell lymphomas. Trisomies and the translocations discussed previously are usually mutually exclusive genetic events in MALT lymphomas, suggesting that these tumors arise by multiple pathogenetic pathways, but the pathogenetic and prognostic significance of the trisomies is unknown.

### Differential Diagnosis

Lymphoid hyperplasia in extranodal sites is common as part of a variety of organ-specific inflammatory conditions. MALT lymphomas, with their heterogeneous architecture and cell composition, can closely resemble these reactive processes. In general, monomorphous cell populations, particularly sheets of monocytoid cells, cytologically abnormal plasma cells with nuclear irregularities, and Dutcher bodies and prominent lymphoepithelial lesions in the stomach all indicate a diagnosis of MALT lymphoma. Showing light chain restriction in the B cell or plasma cell population in the right morphological context also supports a MALT lymphoma diagnosis.

Distinguishing MALT lymphomas from other types of indolent B-cell lymphomas can be done using the morphologic, phenotypic, and genetic attributes listed in accompanying Tables 7-1 to 7-3. It should be stressed that all neoplastic lymphoid infiltrates in extranodal sits are not MALT lymphomas. All the lymphoma types discussed in this chapter can involve extranodal sites, with the possible exception of nodal marginal zone B-cell lymphoma because of its operational definition. Therefore, when encountering a neoplastic small lymphocytic infiltrate in an extranodal site, be certain to meet the morphologic and phenotypic criteria for MALT lymphoma (i.e., other indolent lymphoma types, particularly B-cell SLL, follicular lymphoma and mantle cell lymphoma must be excluded) before making a firm diagnosis.

### Prognosis and Therapy

The therapeutic approach to gastric MALT lymphomas is different from the general approach to extranodal marginal zone lymphomas that arise in organs other than the stomach. For gastric MALT lymphomas, clinical stage, presence of *H. pylori,* and presence of the t(11;18)(q21;q21) determine the therapeutic approach and the probability of complete remission of the lymphoma. It has been shown that 73% of gastric MALT lymphoma patients who have Ann Arbor stage IE (lymphoma confined to the stomach), *H. pylori*–positive tumors without the t(11;18)(q21;q21) will achieve a complete remission of the lymphoma treated only with antibiotics to eradicate the *H. pylori* infection. In addition, within the stage IE patient group, increasing depth of invasion of the gastric wall by the lymphoma is associated with increasing risk for treatment failure with antibiotics. Patients with stage IIE (lymphoma in stomach and regional lymph nodes) *H. pylori*– and t(11;18)(q21;q21)–positive tumors have only a 5% chance of complete remission after antibiotic therapy. The lymphomas only gradually respond to antibiotic therapy (up to 18 months), and response can be associated with morphologically occult molecular genetic evidence of persistence of the clonal neoplastic B-cell population. Persistence of the clone as determined by molecular analysis does not preclude long-term clinical remission. Thus, long-term monitoring with endoscopy and biopsies is required to determine whether histologic regression has occurred and to document the need for cytotoxic therapy in unresponsive, symptomatic patients, but the role for molecular monitoring is limited.

Patients with MALT lymphomas confined to single anatomic sites are conventionally treated with either radiation or low-intensity, single- or multiple-agent chemotherapy. Newer approaches using immunotherapy have also produced successful results. For all patients with MALT lymphoma patients, the prognosis is highly favorable.

## SPLENIC MARGINAL ZONE LYMPHOMA

The current definition of the clinical and pathologic features of splenic marginal zone B-cell lymphoma represents the convergence of two separately described disorders: splenic lymphoma with villous lymphocytes (SLVL) and primary splenic marginal zone lymphoma. As initially described, the typical patient with SLVL exhibited absolute lymphocytosis and an enlarged spleen. The lymphocytes were medium sized with voluminous cytoplasm and polar cytoplasmic projections. The spleen contained white pulp expansion by the neoplastic cells. The typical patient with primary splenic marginal zone lymphoma exhibited symptomatic splenomegaly. The spleen contained white pulp–based nodules of neoplastic lymphocytes that resembled the normal splenic marginal zone B cells. Subsequent morphologic review of SLVL spleen specimens, phenotypic studies, and genetic analysis determined that most cases of SLVL and primary splenic marginal zone lymphoma

represented the same entity that is *termed splenic marginal zone lymphoma* in the WHO classification. There are some exceptions. Some cases of follicular lymphoma, lymphoplasmacytic lymphoma, and even mantle cell lymphoma can manifest with symptomatic splenomegaly and a leukemic phase in which the circulating lymphocytes resemble those of SLVL. Conversely, follicular lymphomas and mantle cell lymphomas involving splenic, white pulp can acquire morphologic features nearly indistinguishable from splenic marginal zone lymphoma (marginal zone differentiation). Thus a combined morphologic, phenotypic, and in some cases a genetic approach, must be applied to leukemic cases and spleen specimens to ensure accurate and reproducible diagnosis of splenic marginal zone lymphoma.

## Clinical Features

Splenic marginal zone lymphoma is rare, accounting for no more than 1% to 2% of all non-Hodgkin lymphomas. They occur in older individuals (median age is 68 years) with an equal gender distribution. Most patients exhibit either symptomatic splenomegaly or lymphocytosis associated with an enlarged spleen. Of patients with lymphocytosis (SLVL-like presentation), 77% have splenomegaly. Of patients with symptomatic splenomegaly, 68% have peripheral blood involvement. The bone marrow is positive for lymphoma in at least 80% of patients at diagnosis, and the liver is involved in 30%. Abdominal adenopathy is present in up to 25% of patients, but peripheral and mediastinal adenopathy are rare at diagnosis. Therefore splenic marginal zone B cell lymphoma is characterized by a high frequency of patients (87%) of Ann Arbor stage IV disease at initial presentation. A small number of patients with splenic marginal zone lymphoma have monoclonal gammopathy, usually IgM and far less frequently IgA or IgG isotype.

## Pathologic Features

### Morphology

This disease has its most characteristic morphology in the spleen. Grossly the spleen is enlarged with numerous tan nodules on the cut surface (Figure 7-38). The neoplastic lymphocytes preferentially involve the splenic white pulp, expanding the marginal zones and variably and irregularly infiltrating into the adjacent white pulp (Figures 7-39 and 7-40). In the most characteristic cases, the centers of the white pulp nodules contain a small atrophic germinal center. The neoplastic lymphocytes centrifugally expand out from the margin of the atrophic germinal center and have dimorphic cytologic features

**SPLENIC MARGINAL ZONE LYMPHOMA—FACT SHEET**

**Clinical Features**
- One percent of non-Hodgkin lymphomas
- Median age, 7th decade; no gender predominance
- Symptomatic splenomegaly
- Frequent peripheral blood leukemic phase (splenic lymphoma with villous lymphocytes)
- Paraprotein in 25%
- High Ann Arbor stage at diagnosis

**Morphology**
- Splenic white pulp nodules with infiltration of the red pulp
- Dimorphic cytology: small lymphocytes resembling mantle zone lymphocytes in center of nodules, larger cells resembling normal splenic marginal zone lymphocytes at periphery of nodules
- Provisional entity: splenic diffuse red pulp small B-cell lymphoma possibly related to SMZL
- Bone marrow with intertrabecular nodular, paratrabecular and intravascular infiltrates

**Immunophenotype**
- $CD19^+$, $CD20^+$, $sIg^+$, IgM with or without IgD, occasional cases IgG or $IgA^+$
- $CD5^{\pm}$, $CD10^-$, BCL-6 negative
- May express CD11c, CD22, and CD103 (dim)

**Genetics**
- Clonally rearranged immunoglobulin genes
- del 7q31-32 in subset
- +3 in subset
- Point mutations in immunoglobulin genes and in 5′ noncoding region of *BCL6* in subset, germline immunoglobulin and *BCL6* genes in subset

**Prognosis and Therapy**
- Watch and wait approach for asymptomatic patients
- Splenectomy or splenic radiation for patients with symptomatic splenomegaly or cytopenia
- Low-intensity single- or multiple-agent chemotherapy with rituximab for symptomatic patients
- Eight- to 10-year median survival
- Transformation to diffuse large B-cell lymphoma in 13% heralds aggressive disease

**Differential Diagnosis**
- Marginal zone B-cell hyperplasia
- Mantle cell lymphoma with marginal zone B-cell differentiation in the spleen
- Follicular lymphoma with marginal zone B-cell differentiation in the spleen
- Hairy cell leukemia

(Figure 7-41). The neoplastic cells immediately adjacent to the germinal center have cytologic characteristics similar to mantle zone lymphocytes with round nuclei, clumped chromatin, and sparse cytoplasm. These in turn are surrounded by coronas of neoplastic cells that resemble normal splenic marginal zone lymphocytes (small to medium sized with partially dispersed chromatin small or inconspicuous nucleoli and abundant pale staining cytoplasm). In most cases, the neoplasm includes large transformed lymphocytes with features similar to immunoblasts near the border between the expanded white pulp and the adjacent red pulp. Cases with the cytologic and phenotypic features of splenic marginal zone lymphomas, but with a diffuse red pulp infiltrative pattern, were previously considered to be diffuse variants of splenic marginal zone lymphomas. They now are considered a provisional entity termed *splenic diffuse red pulp small B-cell lymphoma*. The attributes of this provisional entity are discussed in the section on differential diagnosis.

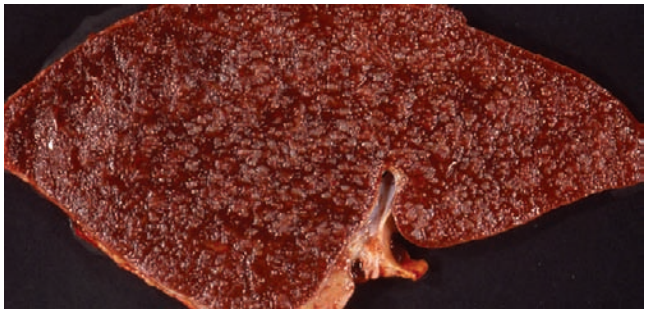

**FIGURE 7-38**

Splenic marginal zone lymphoma. Note the prominence of the white pulp nodules.

In lymph nodes, splenic marginal zone lymphomas recapitulate the splenic architecture (Figure 7-42). The neoplastic cells surround non-neoplastic germinal centers or produce multifocal cortical nodules without germinal centers. Often the lymph node sinuses are dilated. In the liver, splenic marginal zone lymphomas produce multifocal portal nodules and irregularly infiltrate into the hepatic sinusoids. Bone marrow involvement by splenic marginal zone lymphoma is highly characteristic (Figure 7-43). Although intertrabecular nodules, paratrabecular aggregates, and interstitial infiltrates can occur, the neoplastic lymphocytes typically involve the bone marrow vasculature. Immunohistochemistry for CD20 is particularly useful to highlight the linear arrays of intravascular neoplastic cells. In a patient who presents with splenomegaly and lymphocytosis, demonstrating by flow cytometry that the circulating abnormal lymphocytes have the proper phenotype for marginal zone lymphoma (see below) and that the bone marrow has intravascular involvement, one can usually make a confident diagnosis of splenic marginal zone lymphoma without splenectomy or lymph node biopsy. The typical cytology of splenic marginal zone lymphoma in blood is represented in Figure 7-44.

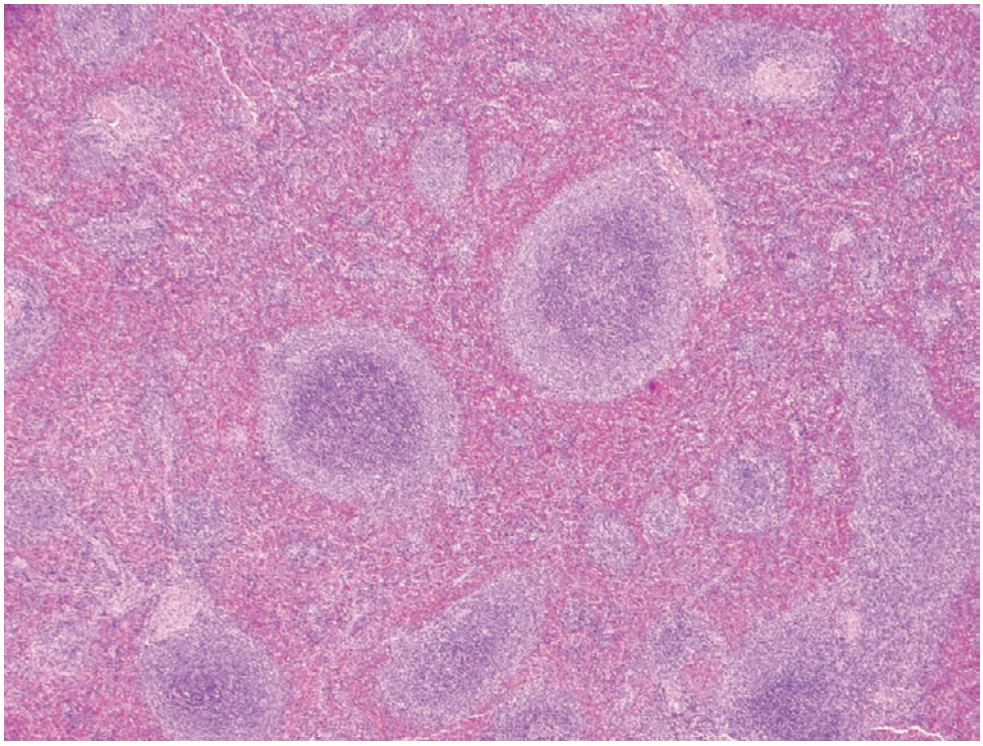

**FIGURE 7-39**

Splenic marginal zone lymphoma. There is expansion of the white pulp by the neoplasm.

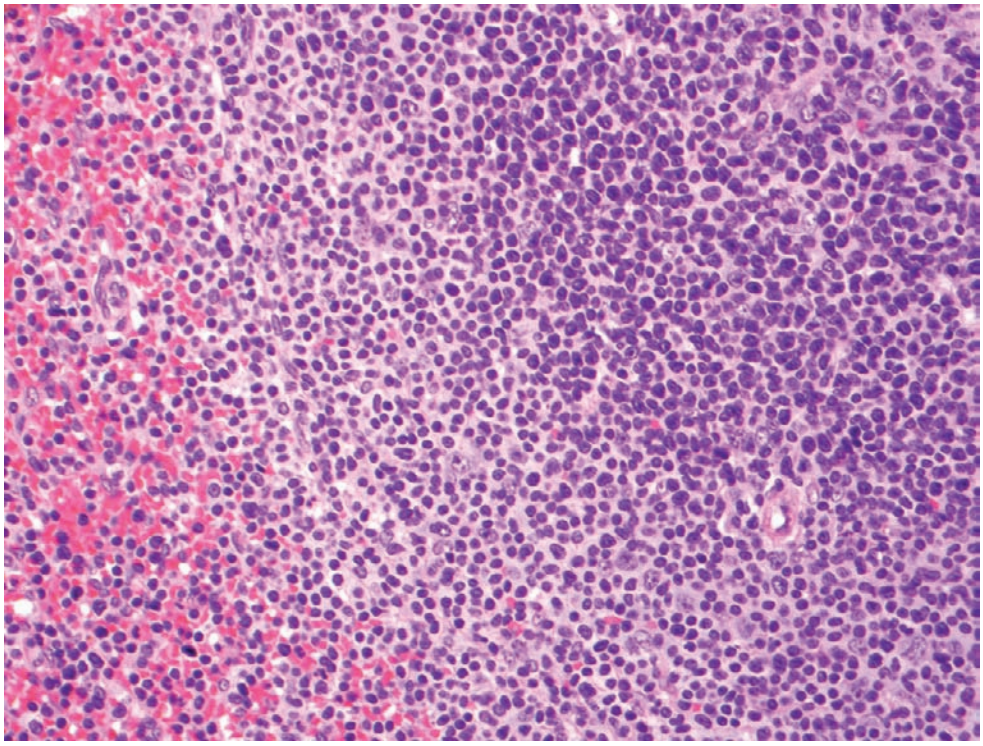

**FIGURE 7-40**

Splenic marginal zone lymphoma. Note the dimorphic cytologic features, neoplastic small mantle zone–like cells in the center of the nodule and marginal zone–like cytologic features of the neoplastic cells at the periphery of the nodule. Note also the infiltration of the red pulp by the neoplastic cells at the periphery of the white pulp nodule.

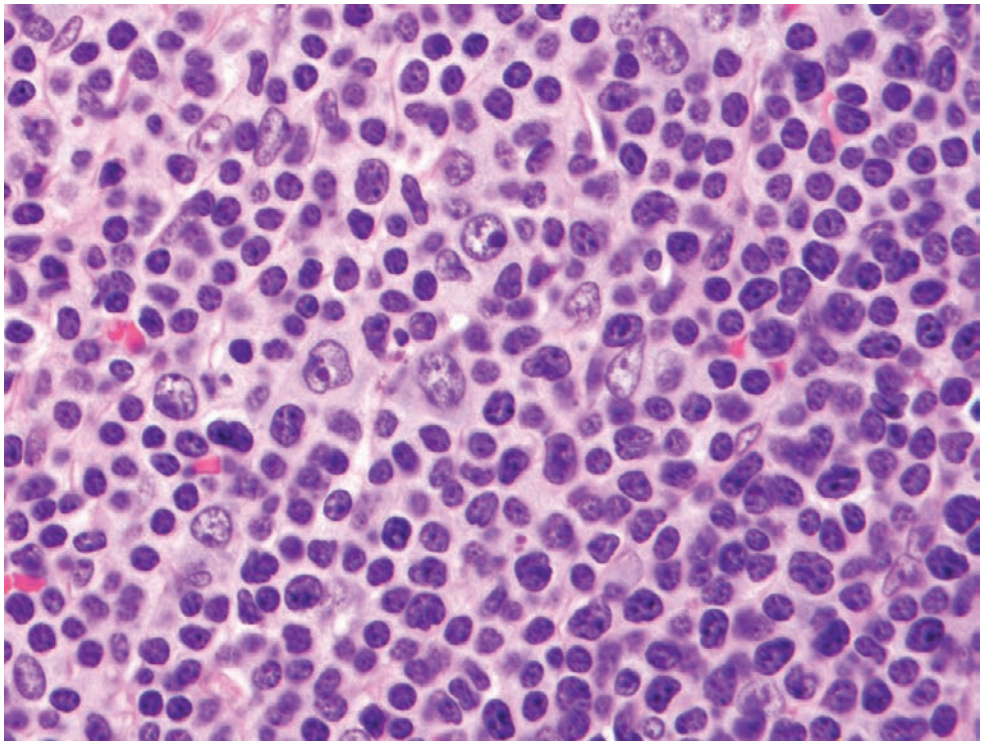

**FIGURE 7-41**

Splenic marginal zone lymphoma. Cytologic features, including scattered large transformed lymphocytes.

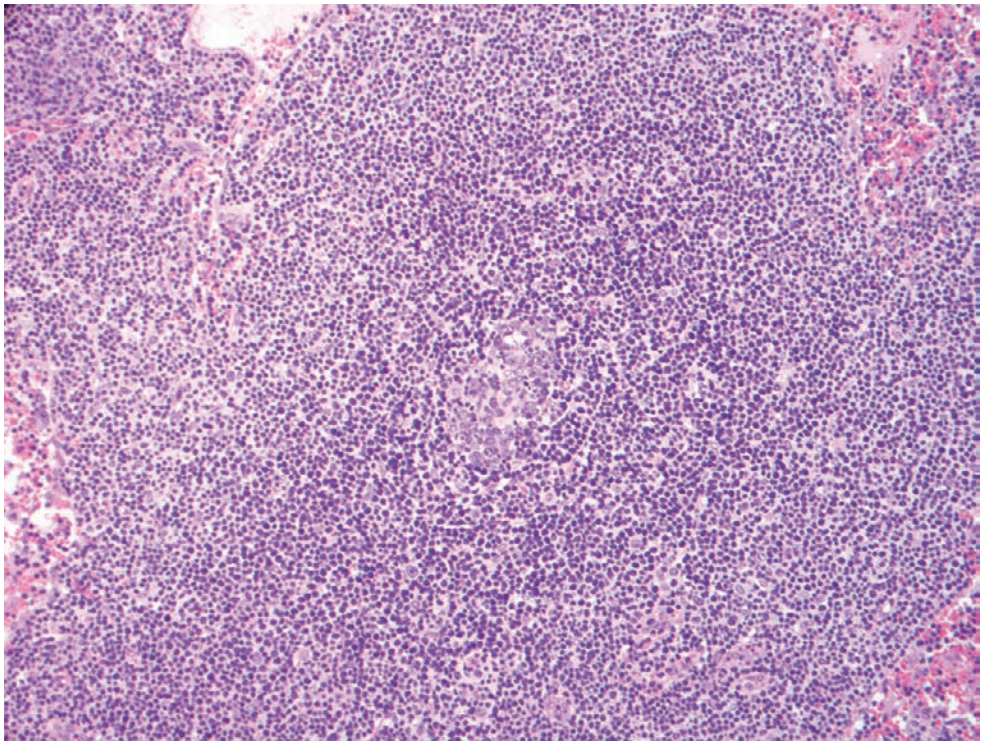

**FIGURE 7-42**

Splenic marginal zone lymphoma involving a lymph node. In the center of the nodule is an atrophic non-neoplastic germinal center surrounded by the same dimorphic cell population as is present in the spleen.

### Phenotype

The cells of splenic marginal zone B-cell lymphomas express the pan B lymphocyte antigens CD19, CD20, CD22, and PAX-5 and show either κ or λ Ig light chain restriction. Most frequently, they express IgM and are weakly positive for IgD; they are rarely IgG or IgA positive. Occasionally it is possible to demonstrate that plasma cells within the non-neoplastic germinal centers or at the margins of the neoplastic nodules are part of the lymphoma, because they too express the same Ig light chain as the neoplastic small lymphocytes. Splenic marginal zone lymphoma cells are occasionally positive for CD5 (20% of cases), DBA.44, CD11c, and CD103. Care must be taken in these instances to exclude mantle cell lymphoma and hairy cell leukemia; however, typically the cells of this lymphoma type are negative for CD10, CD21, CD35, BCL-6, CD103, and CD5.

### Genetics

There is no single cytogenetic abnormality that is exclusively observed in splenic marginal zone lymphomas. The two most common anomalies are del7q31-32 and trisomy 3. The former is described in up to 40% of splenic marginal zone lymphoma cases and the latter in 15%. In one study, translocations involving 7q31-32 were associated with cyclin dependent kinase 6 (CDK6) overexpression. But most cases of splenic marginal zone lymphoma with chromosome 7q abnormalities have only deletions of the 7q31-32 region, not translocations. Thus the pathogenetic role of *CDK6* in this lymphoma type is uncertain. Other translocations that have been described in rare cases of splenic marginal zone lymphomas involve the Ig heavy chain locus and the following genes: *PAX-5, BCL-6, BCL-3, IRF-4,* and *CCND3.* The specificity of these translocations for splenic marginal zone lymphoma is uncertain.

On a molecular level, clonal Ig heavy and light chain gene rearrangements can be demonstrated in virtually all cases of splenic marginal zone lymphoma. There is variability in the reported proportions of cases that have point mutations of *IGH@* or in noncoding sequences of *BCL-6*. Some authors have detected frequent point mutations in the *IGH@* loci, whereas others have shown that only a small proportion of cases have point mutations in the 5′ noncoding regions of *BCL-6*. Most normal splenic marginal zone B cells have Ig point mutations and are thought to represent postgerminal center memory B cells. The data seem to suggest that splenic marginal zone lymphoma, like other low-grade B-cell lymphomas, is also heterogeneous with respect to Ig and BCL-6 gene mutations and represents a mixture of cases analogous to either prefollicular or postfollicular B cells.

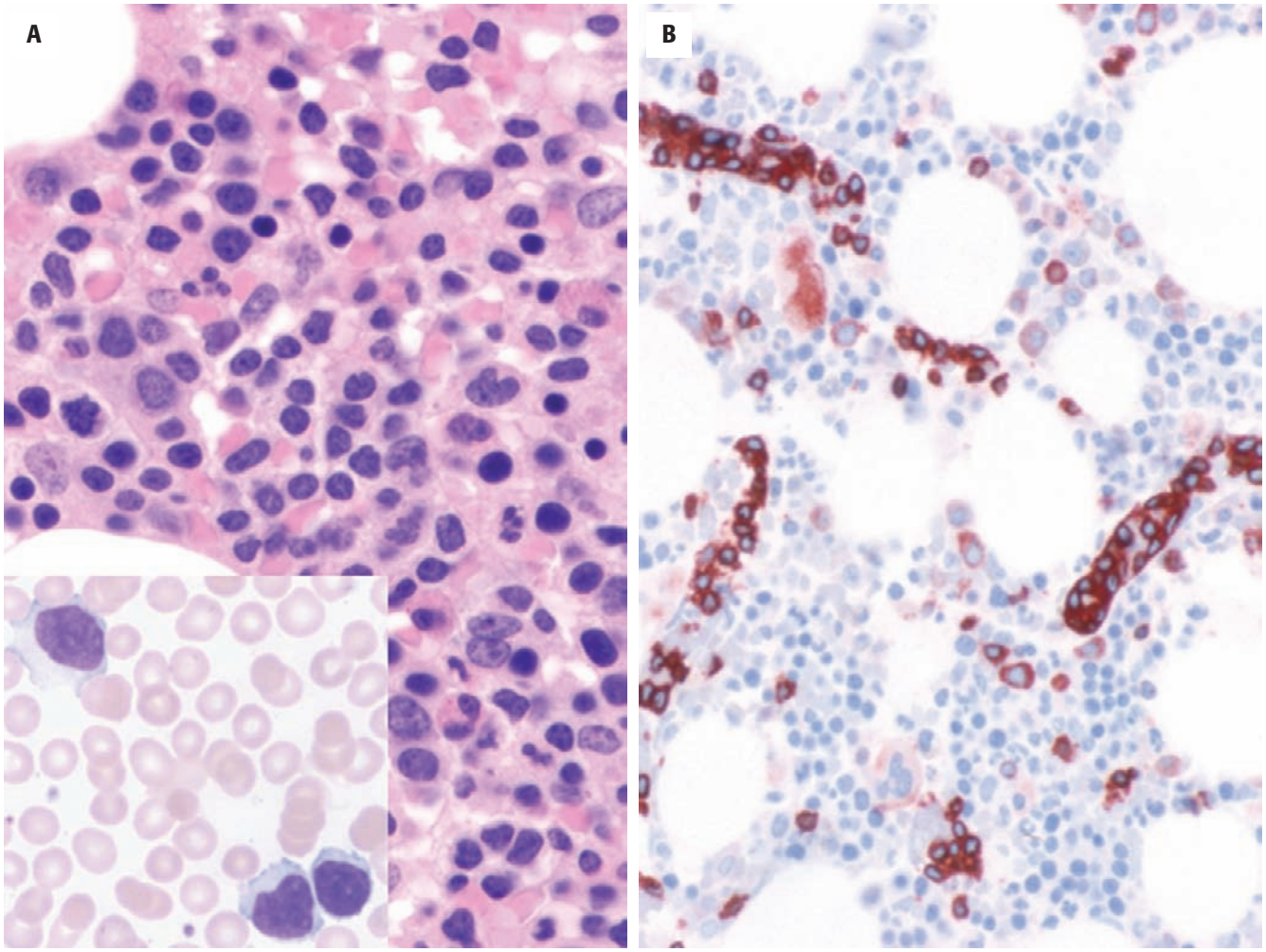


**FIGURE 7-43**

Marginal zone B-cell lymphoma involving bone marrow. **A,** Interstitial involvement of the bone marrow biopsy specimen. Note the cytology of the neoplastic cells with voluminous cytoplasm in the peripheral blood *(inset)*. **B,** An immunoperoxidase stain for CD20 highlighting the linearly arranged intravascular neoplastic cells.

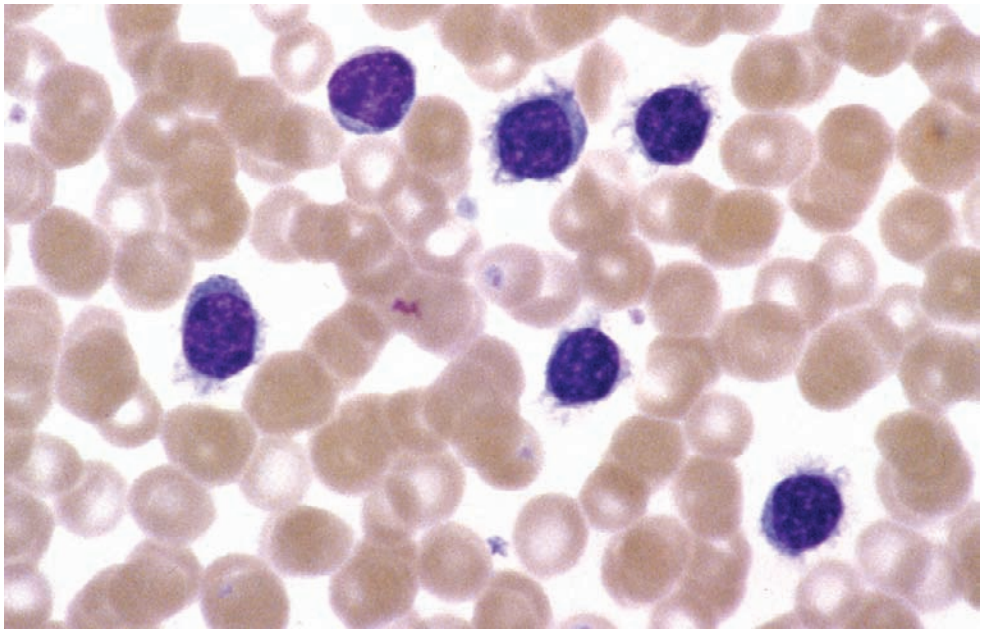

**FIGURE 7-44**

Peripheral blood involvement by splenic marginal zone lymphoma. Note the villous cytoplasmic projections.

### Differential Diagnosis

Reactive follicular hyperplasia in the spleen can be associated with prominent marginal zone B-cell populations. It is possible to observe up to 10 layers of marginal zone cells surrounding the follicular mantle in reactive conditions. The borders of the reactive marginal zone cells with the adjacent red pulp are sharply defined, with little or no marginal zone cells infiltrating the adjacent red pulp. Non-neoplastic marginal zone B cells are polytypic with respect to κ and λ light chain staining.

In the spleen, the differential diagnosis includes grades 1 and 2 follicular lymphoma and mantle cell lymphoma. These lymphomas selectively involve the splenic white pulp and can exhibit marginal zone B-cell differentiation. Thus a careful morphologic and phenotypic approach is needed to separate them from one another. In follicular lymphoma, neoplastic centrocytes with a variable mix of centroblasts are present in the centers of the white pulp nodules. They are monotypic for either κ or λ light chains and they express CD10, BCL-6, and BCL-2. The cells in the regions of marginal zone differentiation can lose CD10 and BCL-6 positivity; therefore it is important to focus on the centers of the nodules. In splenic marginal zone lymphoma with atrophic germinal centers, the non-neoplastic germinal center cells compose a smaller proportion of the total white pulp cell population, they are polytypic in κ and λ light chain stains and they are BCL-2 negative. In difficult cases, it is possible to demonstrate the t(14;18)(q32;q21) by either cytogenetics of FISH in a high proportion of splenic follicular lymphomas. Mantle cell lymphomas also selectively involve splenic white pulp with central atrophic germinal centers and often a dimorphic cytologic appearance because of marginal zone differentiation at the periphery of the nodules. They are CD5 positive, CD23 negative and very importantly positive for cyclinD1, whereas splenic marginal zone lymphomas lack cyclinD1 expression. Again, cytogenetics of FISH showing t(11;14)(q21;q32) supports a diagnosis of mantle cell lymphoma.

A provisional entity in the WHO lymphoma classification scheme is termed *splenic diffuse red pulp small B-cell lymphoma*. It has many characteristics in common with splenic marginal zone lymphomas. It occurs in older adults with a male predominance and produces splenomegaly with frequent involvement of blood and bone marrow. Circulating lymphocytes in some cases have been described as *villous,* similar to splenic marginal zone lymphoma. In the spleen this lymphoma produces diffuse red pulp infiltrates with obliteration of the white pulp. The cells are medium sized with round to irregular nuclei, partially clumped chromatin, inconspicuous nucleoli, and abundant pale cytoplasm (Figure 7-45). In immunohistochemical or flow cytometry analyses, the tumor cells are $CD19^+$ and $CD20^+$, they often express CD11c, CD22, and DBA.44, but they are negative for CD103, cyclin D1 and annexin A1, features that separate them from hairy cell leukemia (Figure 7-46). This lesion seems related to splenic marginal zone lymphoma, but is histopathologically distinct. It is clearly separable from hairy cell leukemia, both by pathologic features and clinical features, including failure to respond to purine analog therapy; however, its relationship to hairy cell leukemia variant remains uncertain, provided that it is considered a distinct entity.

In blood, hairy cell leukemia can morphologically and phenotypically resemble splenic marginal zone lymphoma. In the bone marrow, hairy cell leukemia infiltrates occur almost exclusively in the interstitium, with hemorrhage and disproportionate suppression of hematopoiesis relative to the degree of involvement by the leukemia. Nodular and intravascular infiltrates are uncommon in hairy cell leukemia and should suggest marginal zone lymphoma. In the spleen, hairy cell leukemia involves red pulp with blood lakes. The white pulp is atrophic in contrast to the prominent white pulp involvement by splenic marginal zone lymphoma.

### Prognosis and Therapy

Splenic marginal zone lymphoma has an indolent clinical course with an overall 5-year survival rate of 65% to 78%, but cures are rare. Therefore asymptomatic patients can be followed without treatment. Symptomatic splenomegaly associated with varying degrees of anemia, thrombocytopenia, and neutropenia is often treated by splenectomy or splenic radiation alone. These therapies will usually produce relatively long standing symptomatic improvement and rapid resolution of the cytopenia. Low-intensity chemotherapy with alkylating agents or purine nucleoside analogs can produce partial and complete remissions, and newer therapies using anti-CD20 antibodies have been associated with at least partial responses. A subset of patients with splenic marginal zone lymphoma has a more aggressive clinical course. Risk factors for more guarded prognosis include failure to achieve a complete remission with first-line therapy, involvement of nonhematopoietic sites at diagnosis, high performance status scores, and p53 expression by the neoplastic cells.

## Nodal Marginal Zone B-Cell Lymphoma

### Clinical Features

Nodal marginal zone B-cell lymphomas are uncommon. They occur at a median age of 60 to 65 years, but pediatric patients with this lymphoma type have been

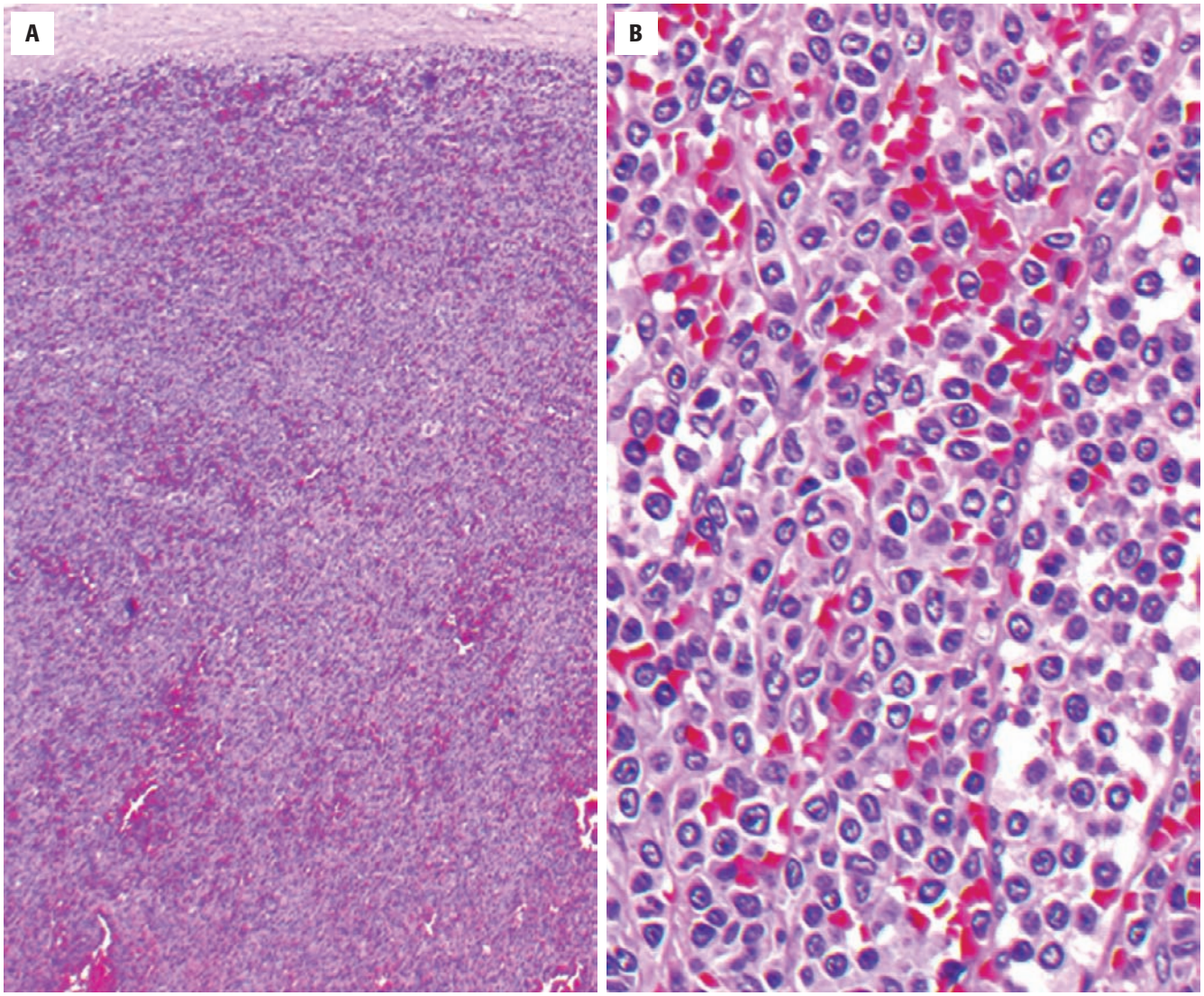


**FIGURE 7-45**

Splenic diffuse red pulp small B-cell lymphoma. **A,** Diffuse red pulp infiltration of the spleen. **B,** The typical cytology is illustrated. Neoplastic cells expand both splenic cords and sinusoids.

## NODAL MARGINAL ZONE B-CELL LYMPHOMA—FACT SHEET

### Clinical Features

- 1.8% of lymphomas
- Median age, 8th decade
- Female predominance
- Slowly progressive or waxing and waning lymphadenopathy

### Morphology

- Perisinusoidal and interfollicular infiltrates
- Medium size lymphocytes with round to irregular nuclei and voluminous clear cytoplasm mimicking nodal monocytoid B cells

### Immunophenotype

- $CD19^+$, $CD20^+$, $sIg^+$, IgM without IgD, rarely IgG or IgA
- Monotypic plasma cells in 30%
- $CD10^-$ and $CD5^-$
- CD43 variably positive
- BCL-2 frequently positive

### Genetics

- Clonally rearranged immunoglobulin genes
- Trisomies 3, 8, 18 present in some cases

### Prognosis and Therapy

- Low-intensity, single- or multiple-agent chemotherapy with rituximab
- Excellent prognosis for Ann Arbor stage I and II
- Median survival, 5 to 6 years for Ann Arbor stage III and IV

### Differential Diagnosis

- Reactive lymphoid hyperplasia, particularly toxoplasmic lymphadenitis, cytomegalovirus lymphadenitis human immunodeficiency virus associated lymphadenitis, and cat scratch disease
- B-cell small lymphocytic lymphoma
- Mantle cell lymphoma
- Follicular lymphoma, particularly those with marginal zone B-cell differentiation
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue
- Lymphoplasmacytic lymphoma
- Hairy cell leukemia

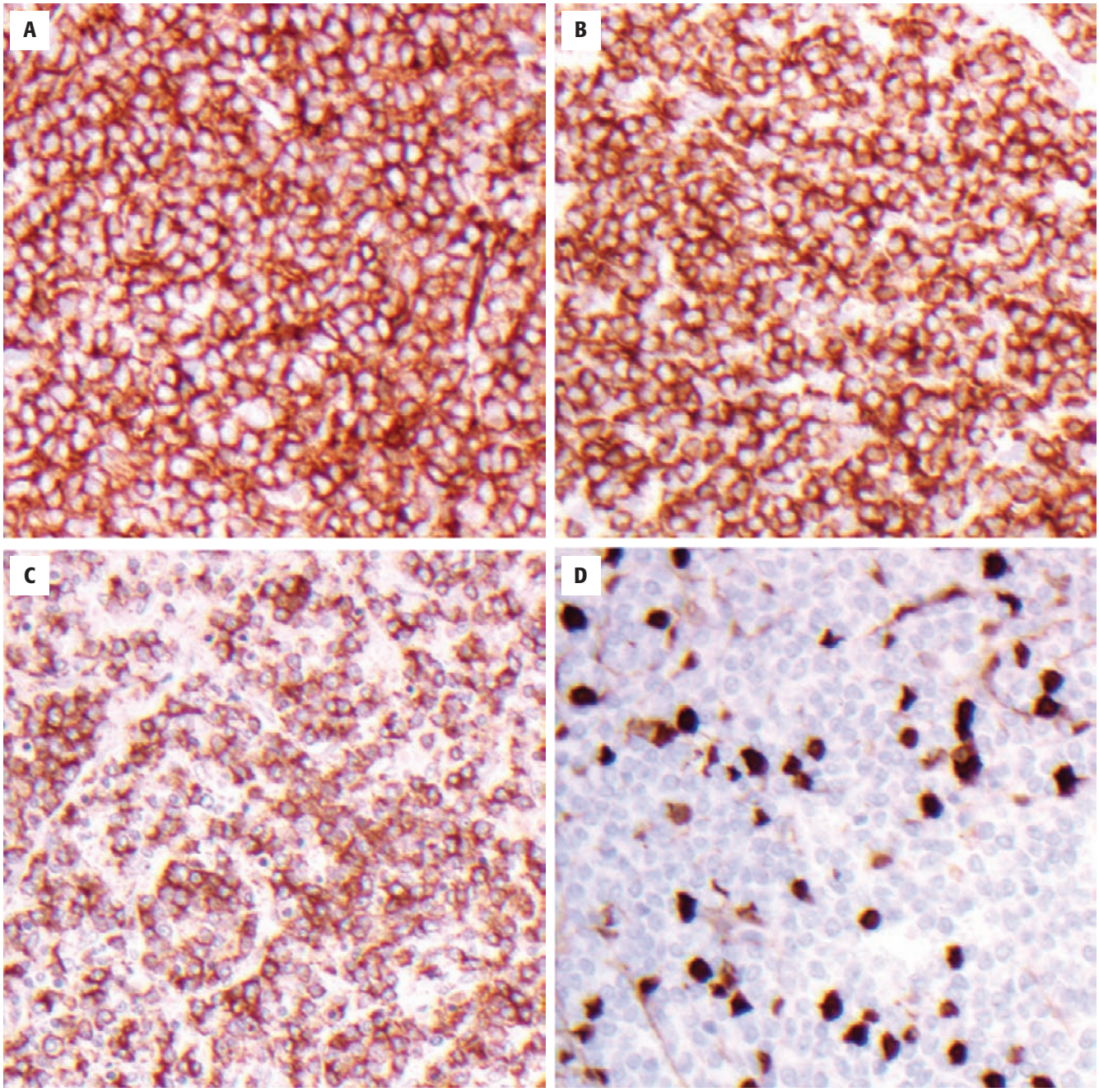


**FIGURE 7-46**

Phenotype of splenic diffuse red pulp small B-cell lymphoma. The neoplastic cells express CD22 **(A),** CD11c **(B),** and DBA.44 **(C)** and are negative for annexin A1 **(D).** The annexin A1–positive cells are granulocytes.

reported. Most studies note a striking female predominance (male-to-female ratio, 1:1.5 to 1:5). The disease appears with painless, slowly progressive or waxing and waning lymph node enlargement. Cervical region lymph nodes are most commonly affected, followed by inguinal and retroperitoneal lymphadenopathy. Although most reports suggest that 50% to 65% of patients with this lymphoma type have Ann Arbor stage I or II disease, some reports indicate advanced stage in up to 71% of patients, with bone marrow involvement in 32%. B symptoms are unusual. Because there is considerable morphologic and phenotypic overlap among nodal, splenic, and extranodal MALT-type marginal zone B-cell lymphomas, involvement of the spleen and of anatomic sites commonly affected by MALT lymphoma should be excluded by staging before a case is accepted as a nodal marginal zone B-cell lymphoma.

## Pathologic Features

### Morphology

In prototypic cases, abnormal lymphocytes distributed immediately adjacent to the lymph node sinusoids and surrounding the residual germinal centers alter but do not completely efface the lymph node architecture (Figure 7-47). From there the tumor spreads in a diffuse pattern to totally replace the underlying lymph node structure and is associated with variable degrees of capsular and trabecular fibrosis. The neoplastic lymphocytes usually are monomorphous and medium sized with round to irregular nuclear contours, partially condensed chromatin, inconspicuous nucleoli, and voluminous clear cytoplasm with distinct cell membranes (Figure 7-48). They are mixed with variable numbers of neutrophils and large transformed lymphocytes resembling immunoblasts or centroblasts and few T cells.

Cytologic variation of nodal marginal zone lymphoma has been described. In some cases the neoplastic lymphocytes have sparse cytoplasm together with greater nuclear irregularity, and the neoplastic cells resemble the small centrocytes of follicular lymphomas. In other cases, admixed monotypic plasma cells, clonally related to the neoplastic lymphocytes, are present. In the purest examples of this disorder, plasma cell differentiation is not present.

In the bone marrow, marginal zone lymphoma of nodal, splenic, and MALT types are similar to one another. They can produce intertrabecular nodules, paratrabecular aggregates, or interstitial infiltrates; however, a characteristic feature of marginal zone lymphoma in bone marrow is intrasinusoidal involvement (see Figure 7-43).

### Phenotype

The cells of nodal marginal zone B-cell lymphomas express the pan B lymphocyte antigens CD19, CD20, CD22, and PAX-5 and show either κ or λ Ig light chain restriction. They express IgM and rarely IgG or IgA, but in contrast to splenic marginal zone B-cell lymphomas they are usually negative for IgD. When the intermixed plasma cells are part of the lymphoma, they too express the same Ig light chain as the neoplastic small lymphocytes. In contrast to normal monocytoid B cells that do not express BCL-2 or CD43, nodal marginal zone lymphoma cells are BCL-2 or CD43 positive in many cases. Typically the cells of this lymphoma type are negative for CD10, CD21, CD35, BCL-6, and CD5.

### Genetics

No nodal marginal zone B-cell lymphoma-specific chromosomal abnormalities have been detected. Trisomy 3 is the most common abnormality associated with

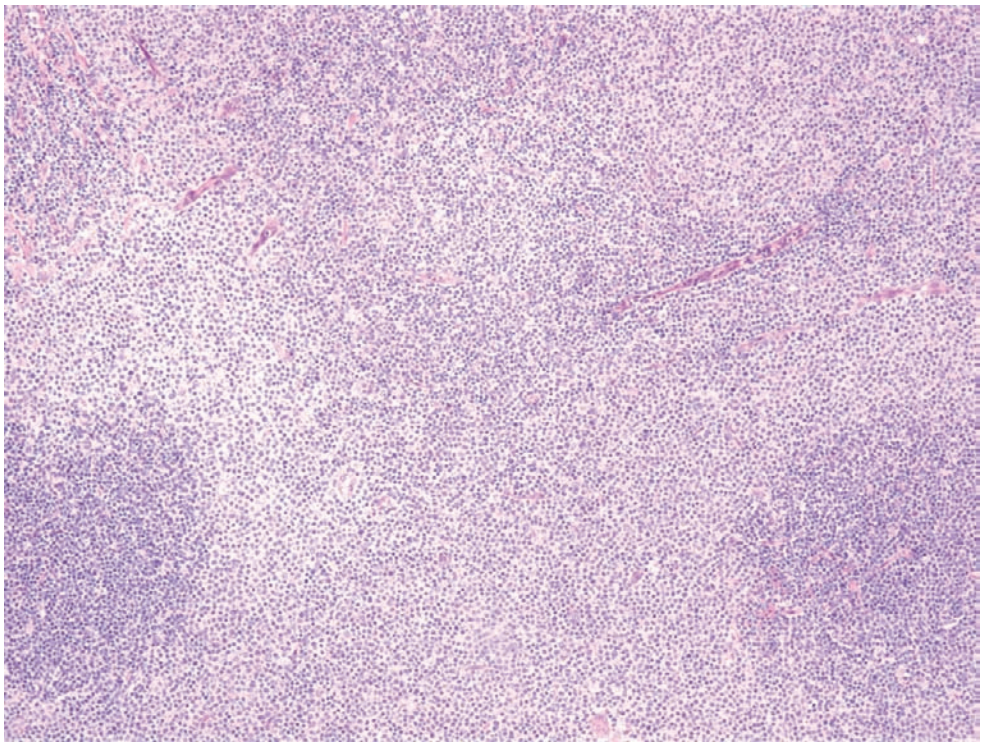

**FIGURE 7-47**

Nodal marginal zone B-cell lymphoma. The neoplastic cells *(pale zones)* are situated adjacent to the lymph node sinusoids and completely surround non-neoplastic primary follicles *(dark zones)*.

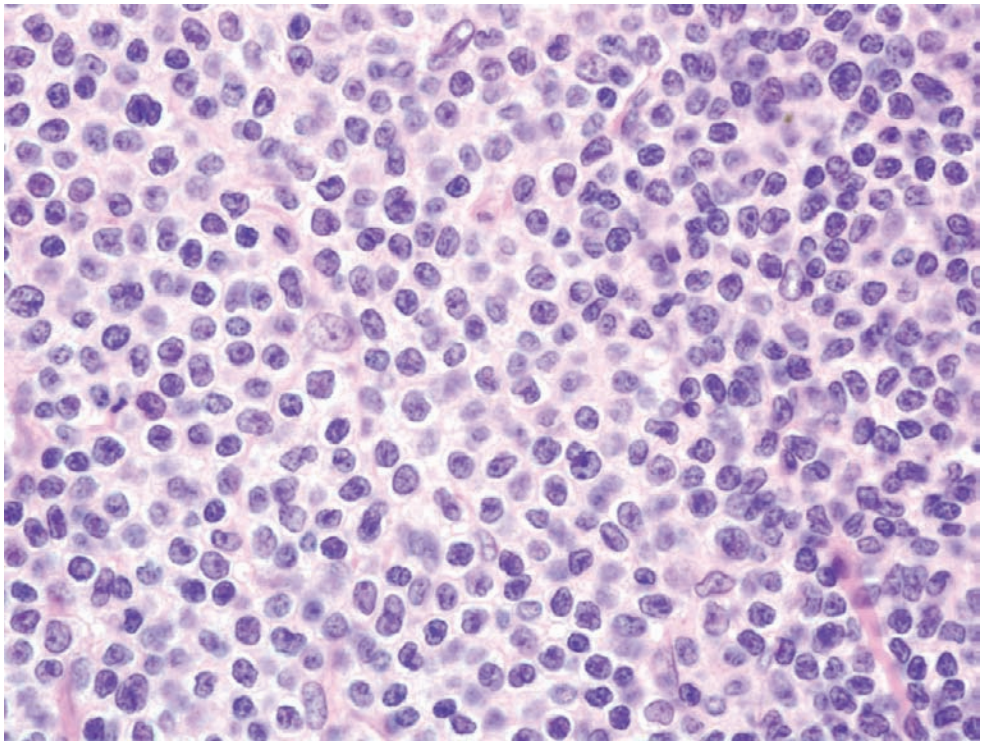

**FIGURE 7-48**
Nodal marginal zone B-cell lymphoma. Typical cytology.

nodal marginal zone B-cell lymphoma, but this finding is also present in a variety of other lymphoma types, including splenic and MALT type marginal zone B-cell lymphoma. The chromosome translocations found in extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue are not detected in rigidly defined nodal marginal zone B-cell lymphomas using the criteria described previously for prototypic cases without plasma cell differentiation.

Clonal Ig gene rearrangements occur in nodal marginal zone lymphomas. Point mutations in the Ig genes have been described in some cases, but others have non-mutated Ig genes. Thus, some nodal marginal zone B-cell lymphomas appear to be more similar to naive B cells (no point mutations) and others to postfollicular memory B cells (mutated Ig genes).

## Differential Diagnosis

Nodal marginal zone B-cell lymphomas must be distinguished from monocytoid B-cell hyperplasia. Monocytoid B-cell hyperplasia rarely occurs as the sole abnormality in the lymph node. It is usually accompanied by follicular hyperplasia with or without epithelioid macrophage clusters that impinge on follicles. Hyperplastic monocytoid B cells do not completely surround reactive follicles and bridge between adjacent completely surrounded germinal centers. Reactive marginal zone B cells are polytypic with respect to κ and λ light chain staining, and they are BCL-2 negative. Nodal marginal zone B-cell lymphoma cells show Ig light chain restriction, and they are BCL-2 positive.

Clues to the diagnosis of other low-grade lymphoma types easily confused with nodal marginal zone B-cell lymphoma are presented in Tables 7-1 to 7-3. It should be noted that nodal marginal zone B-cell lymphoma is partly a diagnosis of exclusion. It is necessary to exclude marginal zone B-cell differentiation follicular and mantle cell lymphoma (done by recognizing the specific features of the other lymphoma types in addition to the marginal zone B cells) and exclude MALT type lymphoma by staging studies that show no involvement of extranodal sites.

In the bone marrow, nodal marginal zone B-cell lymphoma must be distinguished from other disorders that produce intrasinusoidal B-cell infiltrates: polyclonal B cell lymphocytosis and intravascular lymphomatosis. Polyclonal B-cell lymphocytosis produces peripheral blood lymphocytosis, but the abnormal B cells show polytypic expression of κ and λ Ig light chains. *Intravascular lymphomatosis* is a term that is usually reserved for large B-cell lymphoma selectively involving vascular spaces without infiltration into the adjacent parenchyma of the involved organ. The large size and prominent nucleoli of the neoplastic cells in intravascular lymphomatosis should be obvious clues to this diagnosis

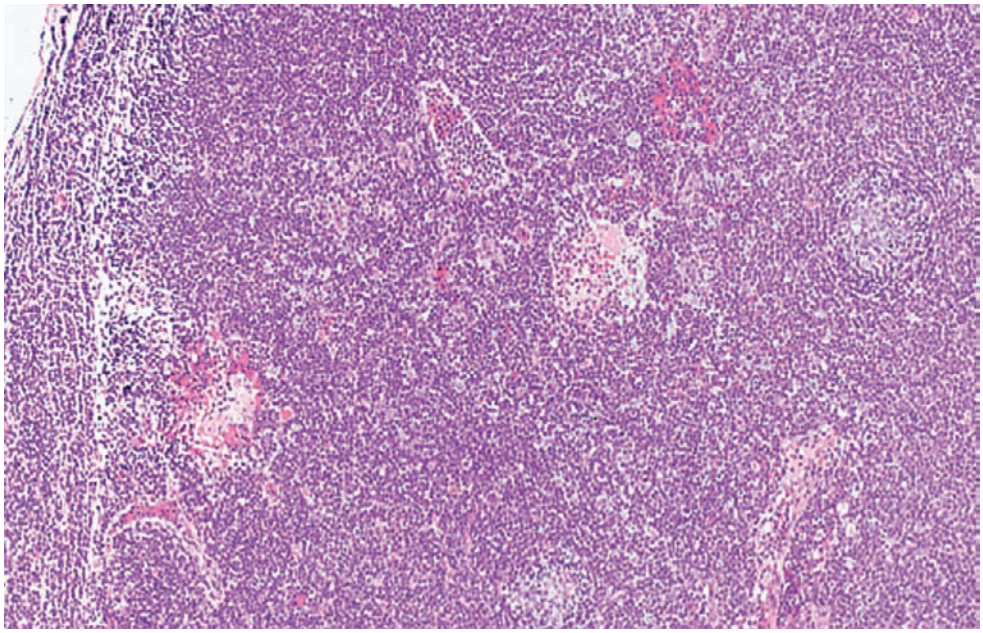

**FIGURE 7-49**

Lymphoplasmacytic lymphoma in a lymph node, infiltrating the capsule and present in the interfollicular zones, but sparing the patent lymph node sinusoids.

once the intravascular infiltrates are recognized by morphology and staining for CD20. T-cell and natural killer cell granular lymphocytic leukemia and hepatosplenic T-cell lymphoma also produce intrasinusoidal bone marrow infiltrates, but the cytolytic T-cell or natural killer cell phenotype of these disorders allows them to be distinguished from bone marrow involvement by marginal zone B-cell lymphoma.

### Prognosis and Therapy

Nodal marginal zone B-cell lymphomas are approached in a manner similar to extragastric MALT lymphomas. They are treated if necessary with single- or multiple-agent, low-intensity chemotherapy with rituximab. For patients with stage I or II disease, there is an excellent prognosis. For those with more advanced stage disease, the literature on outcome is inconsistent. Some reports document a very indolent clinical course with long survivals, others a clinical course that is more aggressive with more limited median survivals.

## LYMPHOPLASMACYTIC LYMPHOMA

### Clinical Features

Lymphoplasmacytic lymphoma is a neoplasm of older adults with a slight male predominance; it is an uncommon neoplasm. Usually, lymphoplasmacytic lymphoma affects the bone marrow with less common involvement of spleen and lymph nodes and is associated with IgM paraproteinemia and manifestations of hyperviscosity or cryoglobulinemia (Waldenström macroglobulinemia). Whereas most patients with Waldenstrom macroglobulinemia have lymphoplasmacytic lymphoma, patients with IgM paraproteins in general can have a variety of B-cell lymphoma types, and not all cases of lymphoplasmacytic lymphoma are associated with an IgM paraprotein. Another clinically distinctive manifestation of lymphoplasmacytic lymphoma is the cases that arise in association with hepatitis C. The liver is frequently involved and there is cryoglobulinemia because of the production of monoclonal IgM cryoglobulins by the neoplastic cells. Once cases of Waldenström macroglobulinemia and hepatitis C–related lymphoplasmacytic lymphomas are excluded, lymphoplasmacytic lymphoma as a disease predominantly involving lymph nodes is rare.

### Pathologic Features

#### Morphology

The neoplastic cells infiltrate in the paracortex and hilum of the lymph node, often with sparing or dilatation of the subcapsular and marginal sinuses (Figure 7-49). Because of this distribution, lymphoplasmacytic lymphoma involvement of lymph nodes can be subtle. In more advanced cases there is diffuse effacement of the lymph node architecture (Figure 7-50). The

## LYMPHOPLASMACYTIC LYMPHOMA—FACT SHEET

### Clinical Features

- 1.5% of nodal lymphomas
- Median age, 7th decade
- Male predominance
- IgM paraproteinemia with or without symptoms of hyperviscosity (Waldenström macroglobulinemia) and bone marrow involvement in most
- Slowly progressive or waxing and waning lymphadenopathy in uncommon subset of patients with primarily lymph node based disease

### Morphology

- Paracortical infiltrates with open lymph node sinuses
- Diffuse architectural effacement of lymph node
- Spectrum of small lymphocytes, plasmacytoid lymphocytes plasma cells and immunoblasts in varying proportions in each case
- Abnormal Ig production: Dutcher bodies, Russell bodies, amyloid or free Ig light chain deposits
- Some cases with epithelioid macrophage clusters that obscure presence of lymphoma

### Immunophenotype

- $CD19^+$, $CD20^+$, $sIg^+$, IgM without IgD, rarely IgG or IgA
- Monotypic plasma cells in all (defining feature)
- $CD10^-$ and $CD5^-$
- CD43 variably positive
- Genetics
- For lymphoplasmacytic lymphoma associated with Waldenstrom macroglobulinemia: del(6)(q21)

### Prognosis and Therapy

- Low-intensity single- or multiple-agent chemotherapy
- Rituximab
- Plasma exchange for transient relief of symptoms of hyperviscosity
- Survival
  - Asymptomatic Waldenström macroglobulinemia: similar to age matched controls
  - Symptomatic Waldenström macroglobulinemia: median survival, 5 to 7 years
  - Lymph node–based disease without Waldenström macroglobulinemia: median survival, 4 years

### Differential Diagnosis

- Reactive lymphoid hyperplasia, particularly autoimmune disease associated lymphadenitis, IgG4 related sclerosing–autoimmune disorder, and plasma cell variant Castleman disease
- B-cell small lymphocytic lymphoma
- Mantle cell lymphoma
- Follicular lymphoma, particularly those with plasma cell differentiation
- Nodal marginal zone B-cell lymphoma
- Splenic marginal zone B-cell lymphoma
- Extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue
- Plasma cell neoplasms

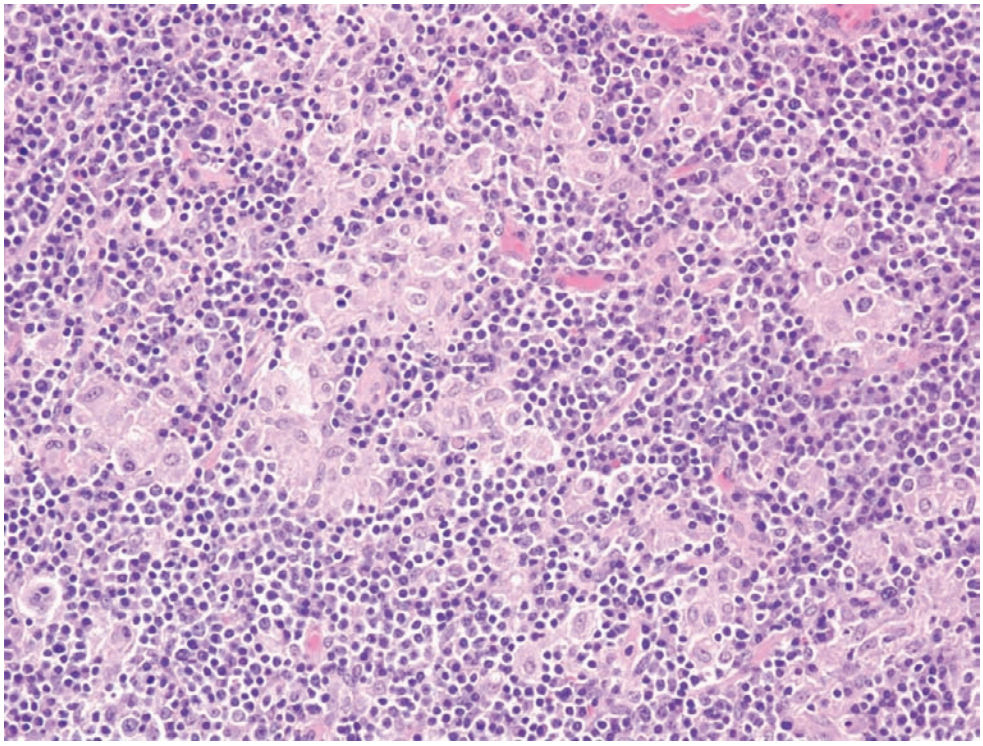

**FIGURE 7-50**

Lymphoplasmacytic lymphoma diffusely effaces lymph node architecture and is associated with clustered epithelioid macrophages.

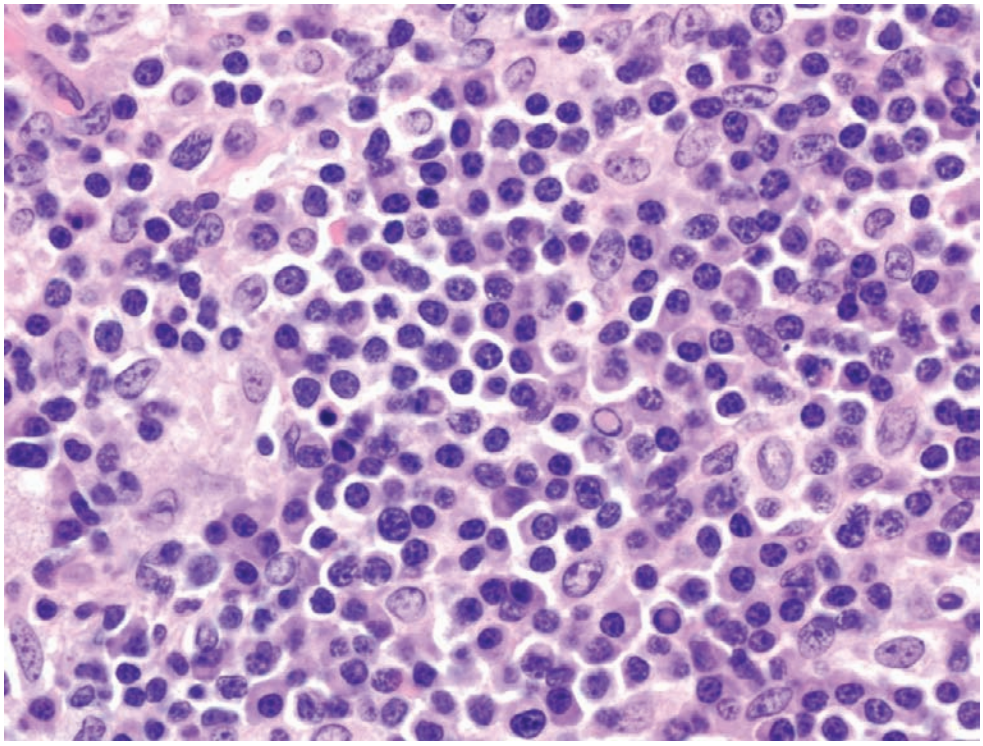

**FIGURE 7-51**

Lymphoplasmacytic lymphoma. Note the cytologic spectrum: small lymphocytes, plasmacytoid lymphocytes, and plasma cells and the Dutcher body in the center of the field.

neoplastic cells span a spectrum from small lymphocytes with clumped chromatin, inconspicuous nucleoli, and sparse cytoplasm through cells with features intermediate between the plasma cells and small lymphocytes (i.e., plasmacytoid lymphocytes) to plasma cells (Figure 7-51). Whereas they may be evenly mixed together, the more common finding is concentration of the plasma cells near blood vessels and lymph node sinuses. In some cases, large transformed lymphocytes, which are cytologically similar to immunoblasts or centroblasts, are mixed evenly with the smaller lymphocytes and plasma cells. The proportions of each of the cell types vary greatly from case to case, forming the traditional basis for the subclassification of lymphoplasmacytic lymphomas into lymphoplasmacytoid (small lymphocytes and plasmacytoid lymphocytes, few to no large cells), lymphoplasmacytic (small lymphocytes and mature plasma cells, few large cells) and polymorphous (small lymphocyte to plasma cell spectrum plus large cells increased over 5% of the total cell population, but not forming large aggregates of sheets). These three subtypes are not recognized by the WHO classification of lymphoplasmacytic lymphomas, but maintaining the subclassification as an intellectual exercise is useful so that the pathologist is cognizant of the morphologic spectrum of this lymphoma type. In addition, the polymorphous type has been associated with a less favorable survival than the other types. As with any neoplastic plasmacytic disorder, Dutcher bodies and Russell bodies are found in the tumor cells in almost all cases, but finding them may require a persistent search. Other features of abnormal Ig production, such as crystalline Ig deposits in the tumor cells (Figure 7-52, A) or in macrophages (see Figure 7-52, B) and focal or extensive deposition of free Ig light chains or amyloid, occur in a small fraction of the cases. An increase in reactive mast cells, clusters of epithelioid macrophages that can obscure the presence of lymphoma, and focal deposits of hemosiderin often accompany the lymphoma cells. By convention, proliferation centers as seen in B cell small lymphocytic lymphoma are absent. In the spleen lymphoplasmacytic lymphomas preferentially involve the red pulp, but occasionally are more white pulp based. In lymphoplasmacytic lymphoma involved bone marrows, the abnormal lymphoplasmacytic infiltrates can form any combination of paratrabecular or intertrabecular lymphoid aggregates or involve the bone marrow interstitium (Figure 7-53). The cytologic spectrum of lymphocytes, plasmacytoid lymphocytes, and plasma cells is probably best appreciated in this disease in Wright-Giemsa–stained bone marrow aspirates (Figure 7-54).

### Phenotype

The neoplastic lymphocytes and plasmacytoid lymphocytes express the pan B lymphocyte antigens CD19, CD20, and PAX-5 and show either κ or λ Ig light chain restriction. The cells in up to 61% of cases express

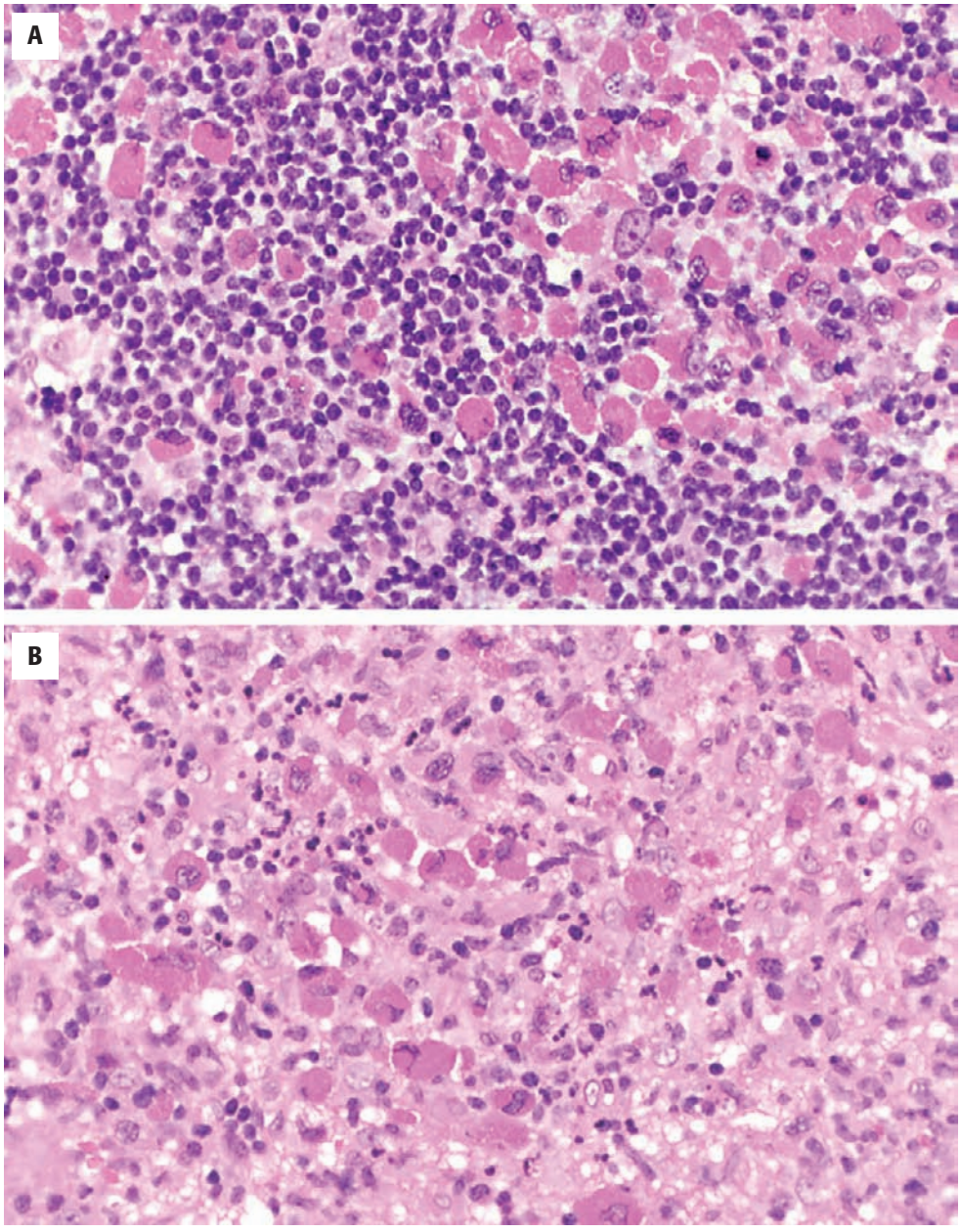


**FIGURE 7-52**

Immunoglobulin inclusions in lymphoplasmacytic lymphoma. **A,** Abnormal globules and crystals of immunoglobulin in neoplastic plasma cells. **B,** The cells containing the abnormal immunoglobulin inclusions are associated with a prominent infiltrate of non-neoplastic macrophages.

CD23. They are typically negative for CD5 and CD10, but cases with expression of these markers have been well described. The neoplastic plasma cells express CD138 and express the same Ig light chain type as the neoplastic lymphocytes. Typically lymphoplasmacytic lymphoma cells are positive for IgM, lack staining for IgD, and rarely are positive for IgG or IgA.

### Genetics

Although early studies suggest that t(9;14)(p13;q32) is a common recurring chromosome abnormality in lymphoplasmacytic lymphomas, current data have challenged this conclusion. None of the recently karyotyped or FISH analyzed cases of Waldenström macroglobulinemia or extramedullary lymphoplasmacytic lymphomas had this abnormality. Whereas a small number of lymphoplasmacytic lymphomas probably harbor the t(9;14)(p13;q32), this translocation is neither frequent nor specific for lymphoplasmacytic lymphoma. However, karyotypic and FISH studies have repeatedly demonstrated deletions in the long arm of chromosome 6 in many samples from Waldenström macroglobulinemia patients. Thus, though not specific for lymphoplasmacytic lymphoma, del(6)(q21) is the most common abnormality in lymphoplasmacytic lymphoma.

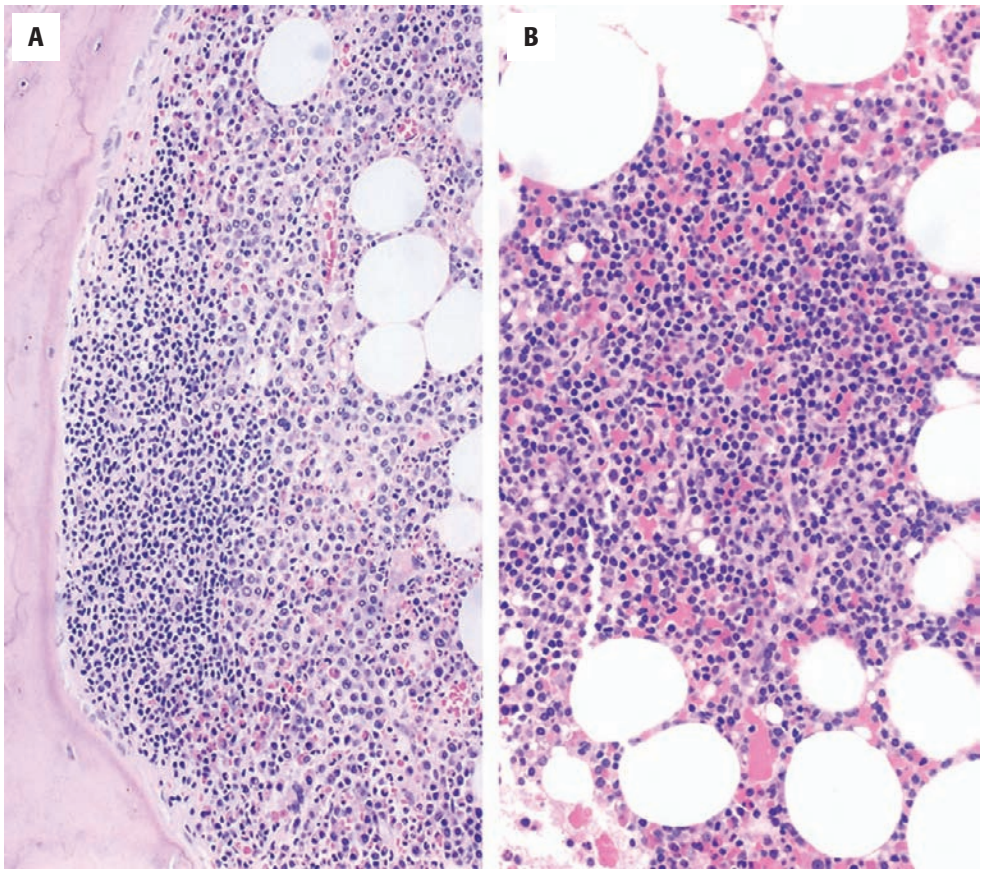


**FIGURE 7-53**

Bone marrow involvement by lymphoplasmacytic lymphoma. **A,** Paratrabecular pattern. **B,** Intertrabecular aggregate.

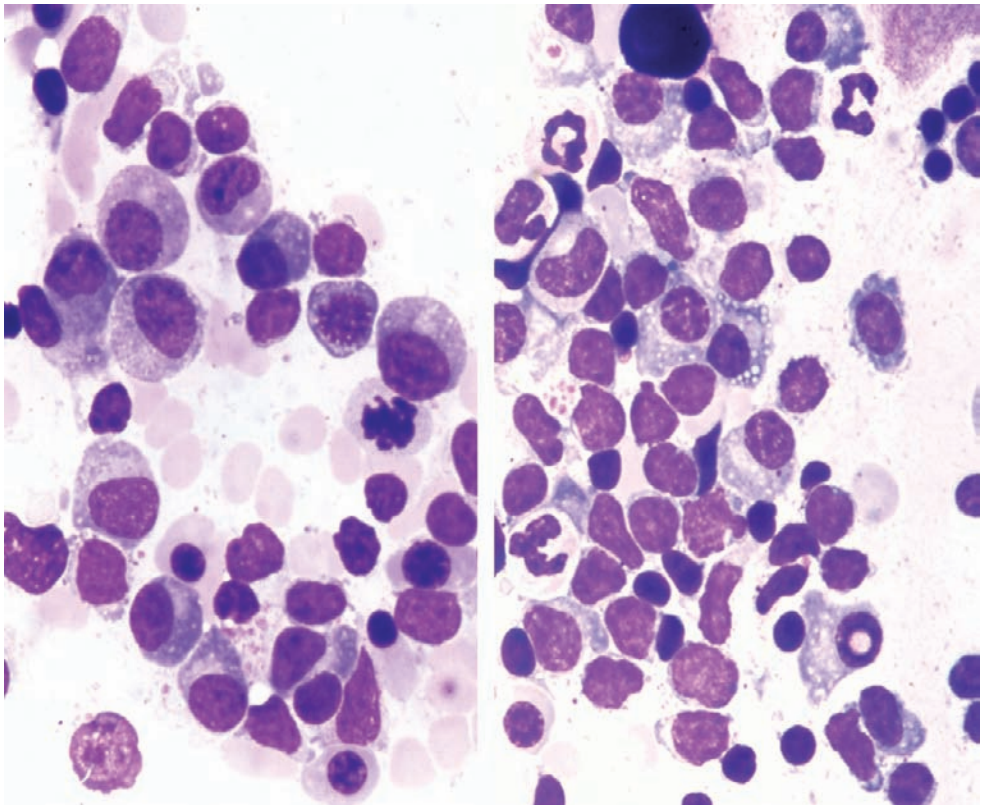

**FIGURE 7-54**

Bone marrow involvement by lymphoplasmacytic lymphoma—aspirate smears stained with Wright-Giemsa. In both of these cases, the cell spectrum, small lymphocytes, plasmacytoid lymphocytes, and plasma cells are readily apparent.

Compared to other B-cell lineage neoplasms, including multiple myeloma, lymphoplasmacytic lymphoma is unusual for its lack of chromosomal translocations involving the Ig heavy chain gene locus (14q32).

Clonal Ig gene rearrangements can be demonstrated by PCR or Southern blot techniques in almost all cases of lymphoplasmacytic lymphomas. The Ig genes in lymphoplasmacytic lymphoma cells contain point mutations, without intraclonal heterogeneity; however, there is no molecular evidence of switch recombination. Thus the neoplastic cells are postgerminal center B cells in which Ig isotype switch events have not occurred.

## Differential Diagnosis

Nonspecific reactive conditions associated with marked plasma cell infiltrates are in the differential diagnosis of lymphoplasmacytic lymphoma. These cases can be readily demonstrated to be reactive using paraffin section immunohistochemistry and showing that the plasma cells have a polytypic staining pattern for κ and λ Ig light chains. Plasma cell variant Castleman disease should be considered any time there is a striking interfollicular plasmacytosis in a specimen resembling lymph node. Clinically, plasma cell variant Castleman disease produces a single dominant mass, sometimes with enlarged adjacent lymph nodes. Although serum gamma globulin levels in plasma cell variant Castleman disease may be elevated, there is polyclonal hypergammaglobulinemia, not a paraprotein. Histologically, plasma cell variant Castleman disease is characterized by regressive transformation of germinal centers, and sheets of interfollicular plasma cells that at least in areas overrun the lymph node sinuses. The plasma cells are polyclonal in regard to light chain staining. Lymphadenopathy, characterized by expanded paracortical regions rich in IgG4-positive plasma cells have been described as part of the IgG4-related sclerosing–autoimmune disease. In these cases, the patients have other autoimmune phenomena, such as pancreatitis, sialadenitis, and pulmonary infiltrates and high serum levels of IgG4. In the lymph nodes and other sites involved by this disorder, the plasma cells are polytypic for κ and λ light chains, and it can be distinguished from lymphoplasmacytic lymphoma.

The other low-grade lymphomas that can be confused with lymphoplasmacytic lymphoma can be distinguished from one another by using the criteria in Tables 7-1 to 7-3. In particular, prominent monocytoid B-cell populations are rare in lymphoplasmacytic lymphoma and if present should suggest marginal zone B-cell lymphoma, particularly MALT-type lymphoma.

Plasmacytoma and multiple myeloma involving lymph nodes produce sheets of plasma cells that efface the architecture without intermixed small lymphocytes or plasmacytoid lymphocytes. The neoplastic plasma cells in less than 1% of plasmacytomas and cases of multiple myeloma express IgM, so assessing the Ig isotype by immunohistochemistry or by serum or urine immunoelectrophoresis in patients with paraproteins can help to distinguish lymphoplasmacytic lymphoma from these other plasma cell dyscrasias. Multiple myeloma with t(11;14)(q13;q32) can have a lymphoplasmacytoid morphology, express CD20, and have an IgM paraprotein. Thus evaluation of suspected lymphoplasmacytic lymphomas for cyclin D1 by immunostaining can be useful to avoid misdiagnosis since t(11;14)(q13;q32)-positive myelomas show strong nuclear cyclin D1 expression. Finally, in difficult cases, deferring to the clinical distribution of disease can help to define the underlying disorder: lymph node and spleen only favors lymphoplasmacytic lymphoma; lytic bone lesions, hypercalcemia, and renal failure, favor plasma cell myeloma.

## Prognosis and Therapy

Lymphoplasmacytic lymphomas whether present as part of the clinical syndrome, Waldenström macroglobulinemia, or arising de novo in lymph nodes are approached in a manner similar to other low-grade B-cell lymphomas. The lymphomas are treated if necessary with single- or multiple-agent, low-intensity chemotherapy regimens that can include alkylating agents, purine nucleoside analogs, and monoclonal antibodies. Symptoms of severe hyperviscosity syndrome resulting from high IgM levels can be effectively but transiently treated with plasma exchange. For Waldenström macroglobulinemia, the prognosis primarily depends on whether the patient is symptomatic. Survival in asymptomatic patients is similar to that for age- and gender-matched control patients. There is a median survival of 7 years and a fivefold risk of death compared with age- and gender-matched control patients for symptomatic patients. Because lymph-node based presentations of lymphoplasmacytic lymphoma are unusual, and the diagnostic criteria for this entity have been refined relatively recently, data about the prognosis of lymphoplasmacytic lymphoma are limited. However, patients with this disease are thought to have a survival that falls between that for grades 1 and 2 follicular lymphoma on the favorable side and mantle cell lymphoma on the unfavorable side.

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The complete reference list is available online at www.expertconsult.com.

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8

# Diffuse Aggressive B-Cell Lymphomas

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## ■ INTRODUCTION

This chapter covers diffuse aggressive B-cell lymphomas in immunocompetent patients. In the 2008 World Health Organization classification concepts regarding diffuse and aggressive B-cell lymphomas were revised to reflect current thinking and several new entities were added. Table 8-1 shows the entities considered under this topic. Most of the entries in Table 8-1 will be covered in this chapter. Immunodeficiency-related diffuse aggressive lymphomas will be discussed in Chapter 10. De novo diffuse large B-cell lymphoma (DLBCL) represents the most common type of non-Hodgkin lymphoma. It is a clinically and biologically heterogeneous disease. For the purposes of discussion, DLBCL can be divided into morphologic variants as well as clinically and biologically distinct subtypes (see Table 8-1). It is worth mentioning that DLBCL can also result from transformation of a preexisting low-grade lymphoma such as chronic lymphocytic leukemia–small lymphocytic lymphoma (i.e., CLL/SLL), follicular lymphoma, or marginal zone lymphoma. Unless the history is known or a low-grade component is represented in the biopsy (so-called composite histology), it might not be possible to distinguish these transformed lymphomas from de novo DLBCL.

Other types of large B-cell lymphomas (LBCLs) are recognized (Table 8-2) and include DLBCL associated with chronic inflammation, primary mediastinal LBCL, intravascular LBCL, lymphomatoid granulomatosis, *ALK*-positive LBCL, plasmablastic lymphoma, primary effusion lymphoma, and LBCL arising in human herpes virus 8 (HHV8)-associated multicentric Castleman disease. The latter two entities are discussed with the immunodeficiency-related lymphomas (see Chapter 10) and will not be reviewed here. This chapter also discusses Burkitt lymphoma (BL) and the newly created diagnostic entity B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL.

## ■ DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

### Clinical Features

De novo DLBCL is the most common type of non-Hodgkin lymphoma, representing 30% to 40% of all non-Hodgkin lymphomas. Although it can occur in children, this disease occurs primarily in adults, with a median age of onset in the seventh decade. Like most lymphomas, there is a male predominance. Patients may have a rapidly enlarging mass. This usually represents a lymph node; however, occurrence at an extranodal site is common (40%). The most common extranodal site is the gastrointestinal tract, followed by the skin, although essentially any extranodal site can be involved. B symptoms (weight loss, fever, night sweats) are seen in approximately 30% of patients.

### Pathologic Features

#### Histopathology

By definition, the malignant cells proliferate and infiltrate in a diffuse pattern. Usually there is complete effacement of the lymph node architecture, although occasionally there is partial involvement. In the lymph node, partial involvement can manifest as interfollicular or sinusoidal infiltration. Sclerosis can be seen in some cases and may even impart a pseudonodular pattern. Lack of true follicle formation can be confirmed with immunostaining showing lack of a follicular dendritic cell meshwork that is typical of follicular lymphomas. In extranodal sites, obliteration of the normal epithelial components is seen. In a mucosal site such as the stomach, epithelial invasion with formation of lymphoepithelial lesions is sometimes seen. Although

**DIFFUSE LARGE B-CELL LYMPHOMA—FACT SHEET**

**Clinical Features**
- Most common type of non-Hodgkin lymphoma
- Median age of onset, 7th decade; males affected more than females
- Rapidly enlarging mass

**Morphology**
- Diffuse infiltrate of large cells
- Morphologic variants
  - Centroblasts
  - Immunoblasts
  - Anaplastic

**Immunophenotype**
- $CD19^+$, $CD20^+$, $sIg^+$
- $CD10^+$ (subset), $BCL2^+$ (subset), $BCL6^+$ (subset), $MUM1^+$ (subset)
- Germinal center B-cell type:
  - Hans: $CD10^+$ or $CD10^-/BCL6^+/MUM1^-$
  - Choi: $GCET1^+/MUM1^-$, $GCET1^-/CD10^-$
  - $GCET1^-/CD10^-/BCL6^+/FOXP1^-$
- Non-germinal center B-cell:
  - Hans: $CD10^-/BCL6^-$ or $CD10^-/BCL6^+/MUM1^+$
  - Choi: $GCET1^+/MUM1^+$, $GCET1^-/CD10^-/BCL6^-$
  - $GCET1^-/CD10^-/BCL6^+/FOXP1^+$

**Molecular Genetics**
- Rearranged immunoglobulin genes
- 20% *IGH@/BCL2*
- 30% 3q27 abnormalities
- Gene expression profiling suggests germinal center, activated B-cell, and type 3

**Prognosis and Therapy**
- Anthracycline-containing multiple-agent chemotherapy plus rituximab
- 55%, 5-year survival, outcome can be stratified based in the International Prognostic Index

**Differential Diagnosis**
- Peripheral T-cell lymphoma, Burkitt lymphoma, lymphoblastic lymphoma, blastoid or pleomorphic mantle cell lymphoma, myeloid sarcoma, carcinoma, melanoma

**TABLE 8-1**

**Variants and Subtypes of DLBCL**

| |
|---|
| ***DLBCL, Morphologic Variants*** |
| Centroblastic |
| Immunoblastic |
| Anaplastic |
| ***DLBCL, Subtypes*** |
| T-cell–histiocyte rich large B-cell lymphoma |
| Primary DLBCL of the central nervous system |
| Primary cutaneous DLBCL, leg type |
| EBV-positive DLBCL of the elderly |

*DLBCL,* Diffuse large B-cell lymphoma; *EBV,* Epstein-Barr virus.

**TABLE 8-2**

**Other Large B-Cell Lymphomas Seen in Immunocompetent and Immunocompromised Populations**

| |
|---|
| ***LBCL, Immunocompetent*** |
| DLBCL associated with chronic inflammation |
| Primary mediastinal large B-cell lymphoma |
| Intravascular large B-cell lymphoma |
| Lymphomatoid granulomatosis |
| ALK-positive large B-cell lymphoma |
| ***LBCL, Immunocompromised*** |
| Plasmablastic lymphoma |
| Primary effusion lymphoma |
| Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease |

*ALK,* Anaplastic lymphoma kinase; *DLBCL,* diffuse large B-cell lymphoma; *HHV,* human herpesvirus; *LBCL,* large B-cell lymphoma.

this is a feature of mucosa-associated lymphoid tissue (MALT)-type lymphomas, such LBCLs are currently considered and classified as extranodal DLBCL.

Cytologically heterogeneity and morphologic variants are recognized. Common to all is a nuclear chromatin pattern that is open and vesicular compared to the condensed chromatin pattern of small B-cell lymphomas. Nucleoli may be multiple or single and variably sized. Cells are usually large (compared to the nuclear size of a benign histiocyte) but can occasionally be intermediate in size (approximately the same size as a benign histiocyte). The recognized morphologic variants are centroblastic, immunoblastic, and anaplastic (Figures 8-1 and 8-2). Centroblastic cells are characterized by round to oval large nuclei with vesicular chromatin and multiple (two to four) small nucleoli that are closely applied to the nuclear membrane. Cytoplasm is scant but visible, particularly on Giemsa staining, in which it has a basophilic quality. In some cases, polylobated nuclei predominate as opposed to the typical round contours. The occurrence of polylobated nuclei typically imparts a smaller cell size and can make distinction from small B cell lymphomas difficult. Multilobated cells are common in some extranodal lymphomas, such as primary

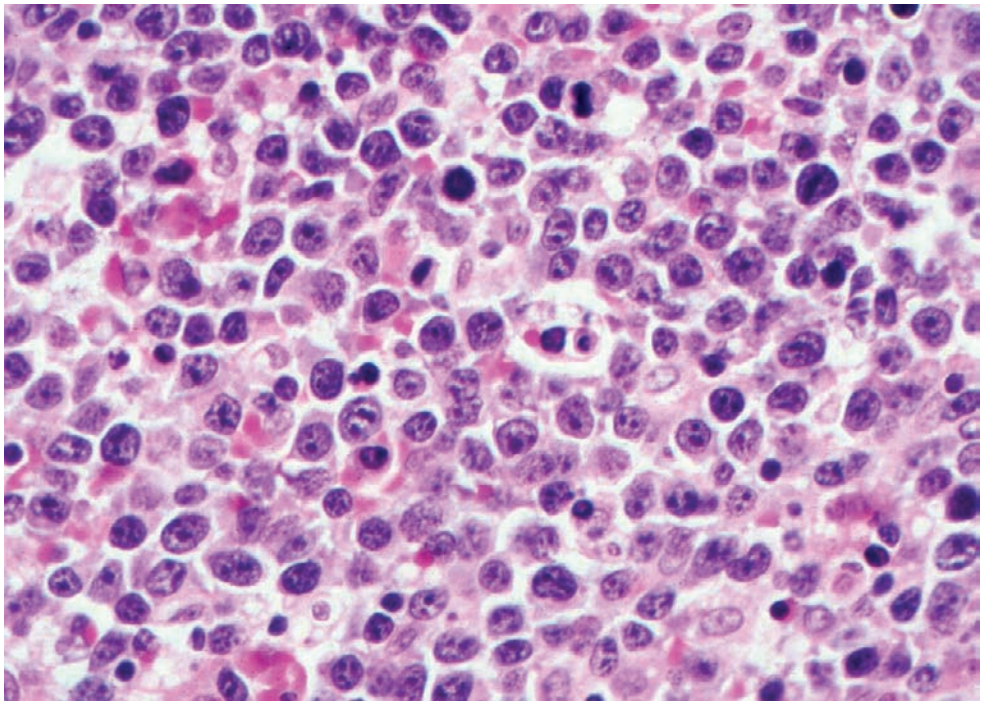

**FIGURE 8-1**

Diffuse large B-cell lymphoma, centroblastic type. The cells are round with multiple small nucleoli.

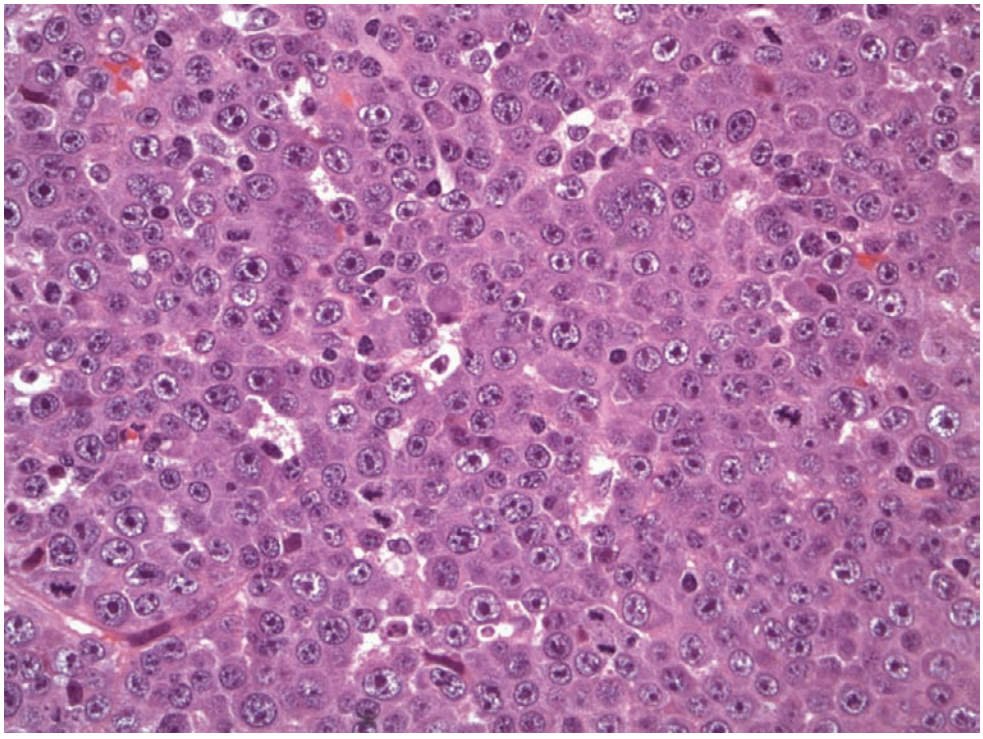

**FIGURE 8-2**

Diffuse large B-cell lymphoma, immunoblastic type. Many cells have large prominent central nucleoli.

lymphoma of bone. Immunoblasts are large cells with a single, prominent central nucleolus and more abundant basophilic cytoplasm. Plasmacytoid differentiation can be seen. Diagnosis of the immunoblastic variant is appropriate when more than 90% of cells are of this type. Some studies, including a recent study with patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in a phase III clinical trial, have suggested a worse outcome for this variant. The anaplastic variant is composed of sheets of anaplastic, atypical cells with bizarre multilobated–multinucleated cells that may resemble Reed-Sternberg cells. This term reflects both cytologic features and pattern of growth. For example, anaplastic DLBCL may grow within lymph node sinuses, reminiscent of an epithelial tumor. *Pleomorphic* may be a better term than *anaplastic,* because the latter can be confused with anaplastic large cell lymphoma of T-cell lineage.

Other unusual morphologic variants have been described, and it is useful to be aware of them primarily because they can initially be mistaken for other non-hematolymphoid tumors until immunophenotyping is performed. These variants include signet ring cell, microvillus, myxoid, and spindle cell variants (Figure 8-3).

Approximately 5% to 15% of patients with DLBCL have bone marrow involvement, and this is associated with an adverse outcome. The histology, however, is highly variable. The pattern can be paratrabecular, interstitial, or diffuse. The diffuse pattern is uncommon and is usually seen when the lymphomatous infiltrate is composed of large cells. The cytology is often discordant (approximately half of the cases), meaning the marrow infiltrate is composed of small lymphocytes or mixed small and large lymphocytes. Studies have shown that patients with concordant large cells in the bone marrow have a poor response to therapy and poor overall survival. This finding is independent of the International Prognostic Index (IPI) score. The presence of discordant marrow involvement at the time of staging (small B-cells in the marrow) has recently been shown to be a predictor of inferior progression-free survival, but it is not independent of the clinical IPI variables and discordant disease travels with older age, elevated serum lactate dehydrogenase (LDH), advanced stage, and increased number of extranodal sites.

#### Immunophenotype

The immunophenotype of DLBCL is heterogeneous. These lymphomas express pan B-cell markers CD19, CD20, and CD22. CD10 is expressed in 25% to 50% of cases. CD5 is uncommonly expressed (<10% of cases). Surface immunoglobulin (Ig) is expressed in most cases, but in approximately 10% of cases it is undetectable by flow cytometry. BCL6 is expressed in 50% to 90% of cases and BCL2 (depending on threshold cut-point) in approximately 60%. Expression of Ki-67 is highly variable and can range from 30% to more than 95%. Immunophenotyping has been used to help predict outcome.

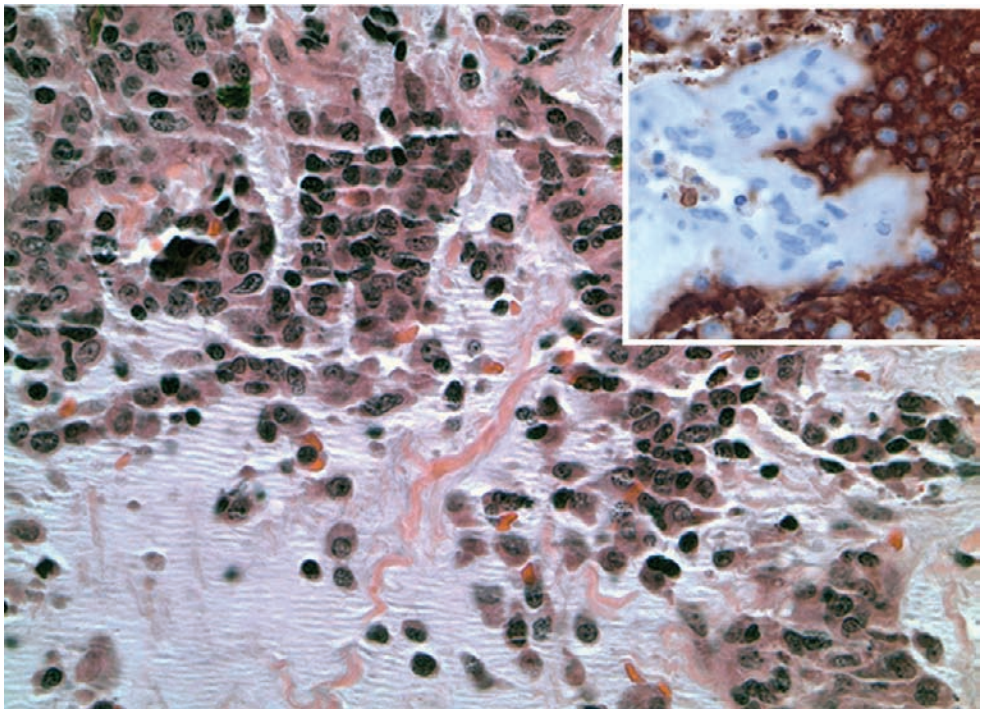

**FIGURE 8-3**

Myxoid variant of diffuse large B-cell lymphoma. The lymphoma cells have vesicular chromatin and moderate amounts of eosinophilic cytoplasm. The *inset* shows the CD20 immunostain, confirming a B-cell lineage.

BCL2 expression has been shown in many studies to be a predictor of poor outcome in DLBCL in the era before the introduction of rituximab. Gene expression profiling (GEP) has shown that DLBCL can be classified into prognostically significant subgroups based on the cell of origin, namely germinal center B cell (GCB) versus the activated B cell (ABC) type. This technique also includes an unclassifiable category, accounting for approximately 15% to 20% of cases. Algorithms based on immunohistochemical (IHC) staining have been devised that differentiate DLBCL with close concordance to the GEP classification scheme. The Hans algorithm uses the expression pattern of CD10, BCL6, and MUM1 and has approximately 80% concordance with the GEP classification. The Choi algorithm uses the expression pattern of CD10, BCL6, MUM1, GCET1, and FOXP1 and has approximately 93% concordance with the GEP classification (Figure 8-4). A new approach

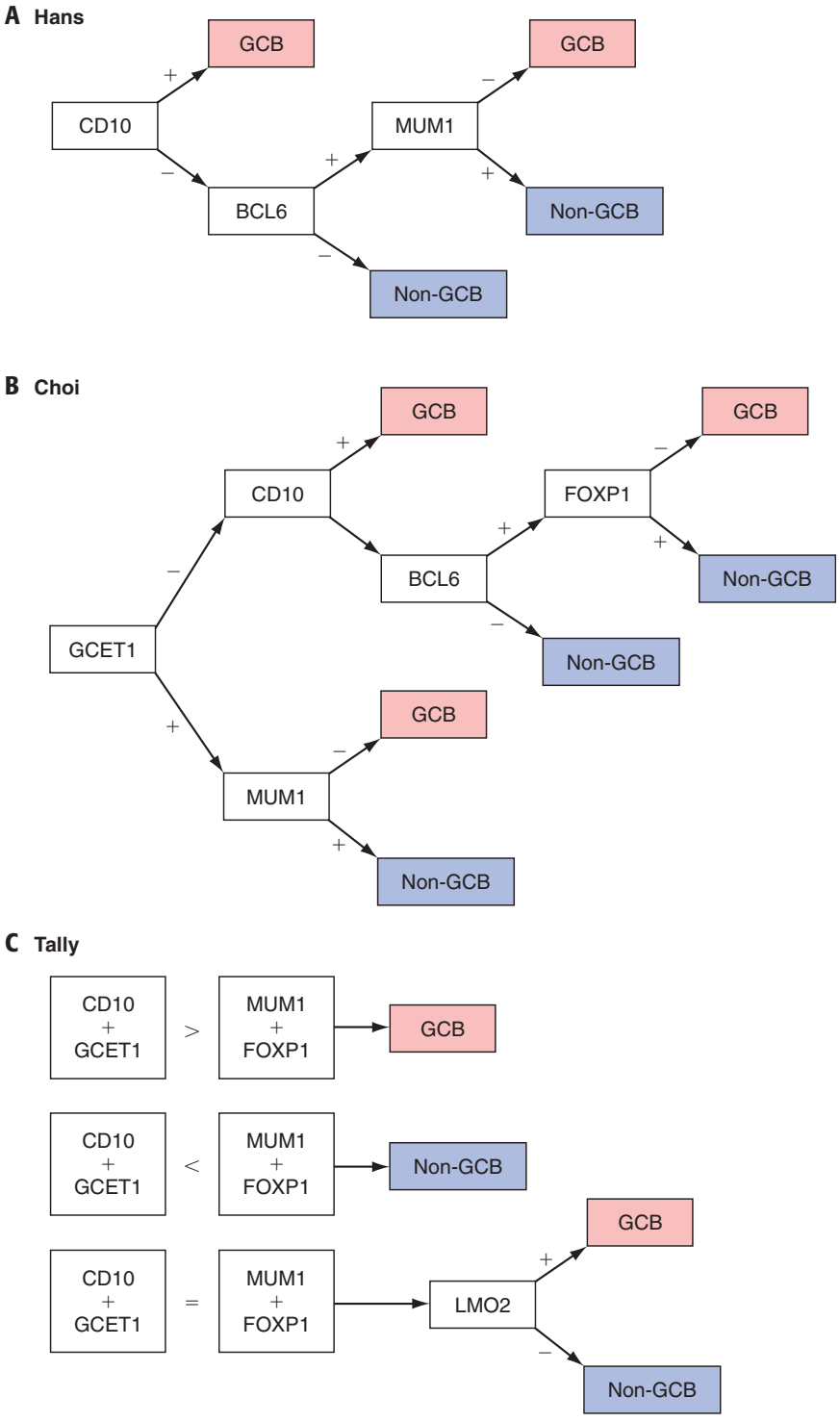


**FIGURE 8-4**

Algorithms for immunohistochemical discrimination between germinal center B-cell type and nongerminal center B-cell type in diffuse large B-cell lymphoma. **A,** Hans algorithm. Positivity is defined as ≥30% positive cells. **B,** Choi algorithm. Positivity for CD10 and BCL6 is defined as ≥30% positive cells. Positivity for MUM1, GCET1, and FOXP1 is defined as ≥80% positive cells. **C,** Tally algorithm. Positivity for CD10, GCET1, MUM1, FOXP1, and LMO2 is defined as ≥30% positive cells. Positive stains are assigned a score of 1, negative stains a score of 0. (***A*** *from Hans CP, Weisenburger DD, Greiner TC, et al: Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using tissue microarray,* Blood *103:275–282, 2004;* ***B*** *from Choi WWL, Weisenburger DD, Greiner TC, et al: A new algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy,* Clin Cancer Res *15:5494–5502, 2009;* ***C*** *from Meyer PN, Fu K, Greiner TC, et al: Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab,* J Clin Oncol *29:200–207, 2011.)*

referred to as the *tally algorithm* uses a combination of IHC antibodies but without regard to order, and shows the highest concordance with GEP. Germinal center markers (CD10 and GCET1) and activated B-cell markers (MUM1 and FOXP1) are scored (≥30% of cells), and cases are classified based on whether more germinal center or ABC markers are present. In case of a tie, LMO2 is used to determine subtype (≥30% + Cells = GCB; <30% + Cells = non-GCB).

There are conflicting data regarding the prognostic significance of cell-of-origin distinctions in both treatment eras (i.e., before and after the introduction of rituximab), in part because of the vagaries of IHC and the fact that, in contrast to GEP, the IHC algorithms do not include an unclassifiable category but instead default cases to either GCB or non-GCB categories. Indeed a large multicenter series evaluating these immunohistochemical markers suggested that only BCL2 and Ki-67 were of prognostic significance in R-CHOP treated patients. While of interest in the research setting, the routine use of IHC for cell of origin classification or prognostication must await further standardization and validation before clinical decisions are based on the results.

### Molecular Genetics

Since DLBCLs are monoclonal B-cell neoplasms, Ig genes are usually rearranged while T-cell receptor genes are typically in the germline configuration. *BCL2, BCL6,* and *MYC* rearrangements are found in approximately 20% , 30% , and 5% of DLBCLs, respectively. Presence of *MYC* translocation has been shown to be a poor prognostic indicator in DLBCL patients treated with R-CHOP.

Our understanding of the molecular heterogeneity of DLBCLs has been advanced by gene expression profiling. This powerful tool makes use of cDNA or oligonucleotide microarrays and is capable of analyzing global gene expression patterns in lymphoma. As reviewed in Chapter 24, DLBCL can be segregated into at least three subgroups based on similarities to normal cell types: GCB, ABC, and the unclassifiable group, which shows a probability of less than 90% based on gene expression of belonging to either GCB or ABC subtypes (Figure 8-5). The IHC classifiers use GCB versus non-GCB categories only and thus the unclassifiable cases may be variably distributed. Large studies using GEP have confirmed the prognostic importance of GCB versus ABC in the R-CHOP era, whereas IHC-based studies have been reported variably. Mini-gene predictors, using two to six genes, have also been described that appear to be independent of the clinical IPI variables. The cell-of-origin distinctions based on GEP also have underpinnings in biology. Translocation (14;18) is almost exclusively found within the GCB subtype, whereas 3q27 translocations targeting *BCL6* are enriched within the ABC subtype.

In addition to large-scale genetic events, recurrent mutations are also differentially found, such as activating mutations of *EZH2* exclusively in GCB type and *BLIMP1* mutations found only in the ABC group. Next generation sequencing (NGS) of DLBCL has identified recurring mutations in many genes, with those involved in epigenetic regulation appearing as frequent targets of somatic mutation. Inactivating mutations of *MLL2* (32% of DLBCL) and mutations of *MEF2B* (11% of DLBCL) were identified and, together with the previously identified alterations in *EZH2, CREBBP,* and *EP300,* point to dysregulated histone acetylation and methylation as driver events in the pathogenesis of DLBCL.

In aggregate, these survival data and molecular findings further underscore the rationale behind distinguishing DLBCL cases based on cell of origin. Constitutive activation of specific pathways in these subtypes, such as NFκB and JAK signaling in ABC type lymphomas, supports the potential effects of specific biological therapies for patients with GCB versus ABC type DLBCLs. NGS studies are increasing our understanding of the genetics of DLBCL and will undoubtedly also affect therapeutic strategies.

## Differential Diagnosis

The differential diagnosis of DLBCL includes other hematopoietic neoplasms of intermediate to large cells, including T-cell lymphomas, BL, blastoid and pleomorphic variants of mantle cell lymphoma, lymphoblastic lymphoma, and myeloid sarcoma (chloroma). These neoplasms can usually be easily distinguished from DLBCL by routine immunophenotyping. T-cell lymphomas can be identified because of a lack of B-cell markers and the expression of T-cell associated antigens such as CD2, CD3, CD5, and CD7 by the malignant cells. BL generally has a high mitotic index with a starry-sky pattern and is composed of medium-sized cells with multiple small nucleoli. *MYC* translocations are virtually always present in BL; however, this finding is not specific because *MYC* translocation can be seen as primary or secondary events in DLBCL. Importantly, BL cells have a characteristic immunophenotype with expression of CD20, CD10, and BCL6, an absence of BCL2 expression, and a high proliferative rate in nearly all cases. Therefore consideration of morphologic, immunophenotypic, and molecular genetic features is required for accurate diagnosis. Blastoid and pleomorphic mantle cell lymphoma can resemble DLBCL but will typically express CD5 and cyclin D1, with the

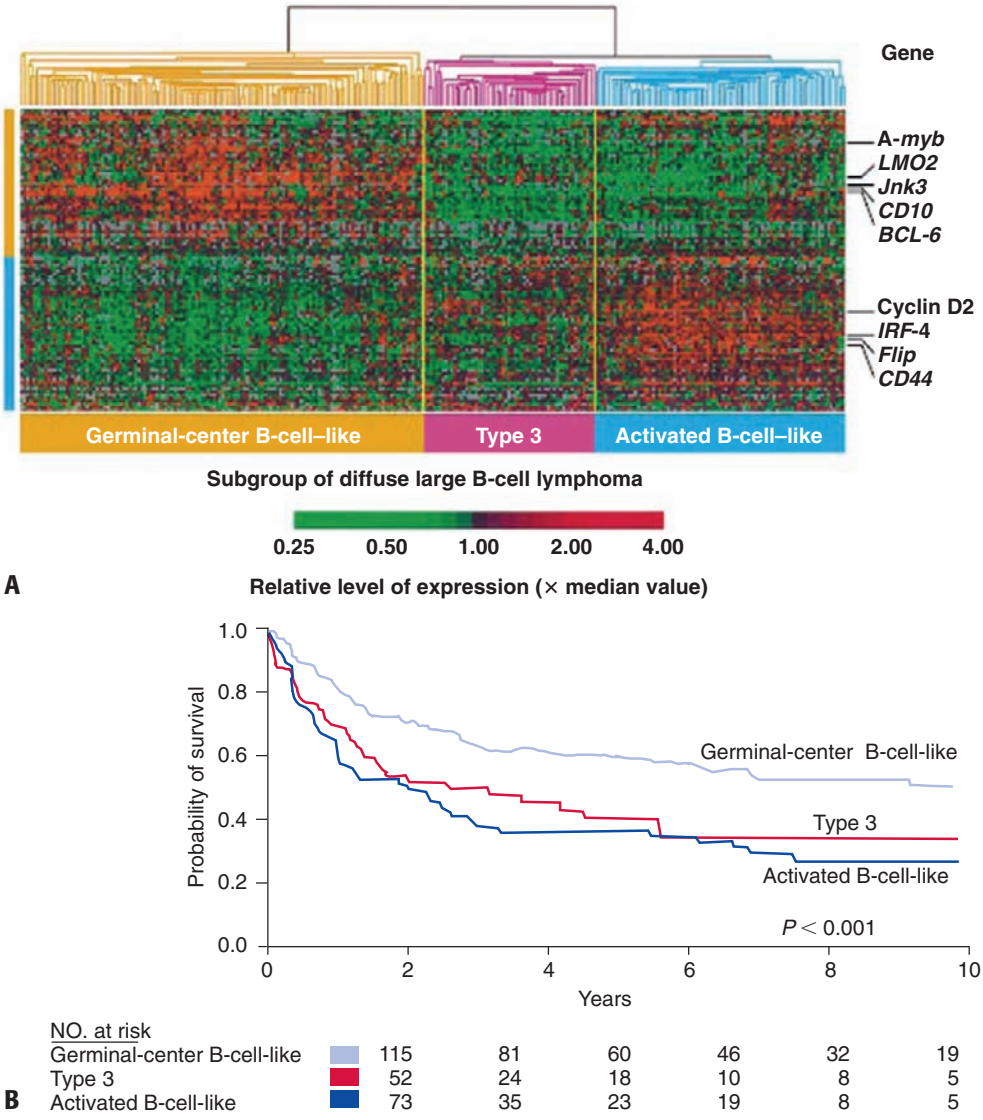


**FIGURE 8-5**

Classification of diffuse large B-cell lymphoma. **A,** Diffuse large B-cell lymphoma (DLBCL) expression profiling showed that cases can be subclassified based on their differential expression of a specific set of genes. **B,** This type of subclassification has prognostic significance with germinal center types of DLBCL having a favorable prognosis independent of the International Prognostic Index.

latter in a strong and uniform pattern not seen in DLBCL. Lymphoblastic lymphoma cells have a fine, blastic chromatin pattern unlike the more vesicular chromatin of DLBCL, and the neoplastic cells will usually be positive for terminal deoxynucleotidyl transferase (TdT) or CD34, or both. Myeloid sarcoma will lack B-cell markers and express myeloid markers such as CD13, CD33, lysozyme, myeloperoxidase, or CD68. Pleomorphic examples of DLBCL may mimic other anaplastic neoplasms (carcinomas including small cell carcinoma, melanomas, or sarcomas). Expression of B-cell markers and lack of markers expected in these other nonlymphoid malignancies, such as keratins or melanoma-associated antigens, will aid in correct diagnosis.

## Prognosis and Therapy

The current standard of care treatment for DLBCL combines the multiple-agent chemotherapy regimen CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) with humanized anti-CD20 monoclonal antibody, rituximab (R-CHOP). With this type of therapy, patients can be cured in approximately 60% to 70% of cases, representing a significant improvement over CHOP alone. Prognosis of patients can also be stratified by the five clinical factors of the IPI score (Table 8-3). Segregation of DLBCL into GCB and non-GCB type provides further prognostic information in some studies, independent of the IPI.

**TABLE 8-3**
**International Prognostic Index**

| Prognostic Factors | Risk Score* | | 5 Year OS (Months) |
|---|---|---|---|
| Age (>60 years) | Low | 0, 1 | 73 |
| LDH (nL) | Low Int | 2 | 51 |
| Performance status (>1) | High Int | 3 | 43 |
| Stage (III, IV) | High | 4, 5 | 26 |
| Extranodal (>1 site) | | | |

*The risk score is the sum of the number of prognostic factors (0-5).
*Int,* Intermediate; *LDH,* lactate dehydrogenase; *OS,* overall survival.

**T-CELL–HISTIOCYTE RICH LARGE B-CELL LYMPHOMA—FACT SHEET**

**Clinical Features**
- Middle-age adults, males affected more than females
- Lymph node, spleen, liver, and bone marrow involvement common

**Morphology**
- Large atypical lymphocytes rare (<10% of cells)
- Numerous small lymphocytes and histiocytes (>90% or cells)
- Diffuse pattern of infiltration

**Immunophenotype**
- Large atypical lymphocytes: $CD45^+$, $CD20^+$ B cells
- Background small lymphocytes: $CD3^+$ T cells

**Molecular Genetics**
- Gains of Xq, 4q13, Xp21, and 18q21
- Loss of 17p

**Prognosis and Therapy**
- Anthracycline based multiple-agent chemotherapy
- Forty-five percent to 58%, 5-year survival

**Differential Diagnosis**
- Classical Hodgkin lymphoma, lymphocyte predominant Hodgkin lymphoma, peripheral T-cell lymphoma

## T-CELL/HISTIOCYTE-RICH LARGE B-CELL LYMPHOMA

### Clinical Features

T-cell/histiocyte-rich LBCL (TC/HRBCL) is primarily a disease of young to middle-aged adults, with a median age of onset of 30 years, and affects men more often than women. Patients usually present with B symptoms, advanced stage disease (III and IV), and a high IPI score. Extranodal disease is common with the spleen, liver, and bone marrow being the most commonly involved extranodal sites.

### Pathologic Features

#### Histopathology

Lymph nodes involved by TC/HRBCL show architectural effacement by a diffuse cellular infiltrate consisting predominantly (>90%) of small T-cells and nonepithelioid histiocytes. Malignant cells are scattered singly amongst the cellular background, although in some cases they may cluster. The cells are large, highly atypical cells that are often multilobated with variably prominent nucleoli (Figure 8-6). In some cases the cells can resemble the LP cells characteristic of nodular lymphocyte predominance Hodgkin lymphoma (NLPHL) or Hodgkin and Reed-Sternberg cells characteristic of classical Hodgkin lymphoma (HL). Some cases may have cells that have more conventional immunoblastic or centroblastic cells.

Splenic involvement occurs in a multifocal and micronodular pattern with expansion of the white pulp by an infiltrate of cells similar in composition to that described in the lymph node. The red pulp is not usually involved. Within the liver the lymphomatous infiltrate is confined principally to the portal tracts. Bone marrow involvement in TC/HRBCL occurs at a significantly higher rate than de novo DLBCL (60%). Involvement is often paratrabecular with a polymorphous infiltrate of small T-cells, histiocytes, and scattered large atypical $CD20^+$ B-cells. Small B cells are typically scarce.

#### Immunophenotype

As noted, most cells in the infiltrate are reactive $CD3^+$ T-cells or $CD68^+$ histiocytes. Only occasional $CD20^+$ large B-cells should be seen, and small $CD20^+$ B-cells should be absent or only rarely seen (Figure 8-7). The immunophenotype of the malignant cells is somewhat heterogeneous. The malignant cells are positive for CD45 and the pan B-cell marker CD20; however, expression of CD10, BCL6, and BCL2 is variable. Some have suggested, based on BCL6 expression, that a germinal center origin can be entertained, but others could not replicate these findings. It is likely that TC/HRBCL, as currently described, is heterogeneous, in part because of a lack of precise diagnostic criteria. Many believe that a subset of cases within the category of TC/HRBCL might define a specific entity, closely related to NLPHL. Evidence in support of this theory includes the occurrence of the two lymphomas in the same patient and an identical immunophenotype of large B-cells in both ($CD20^+$,

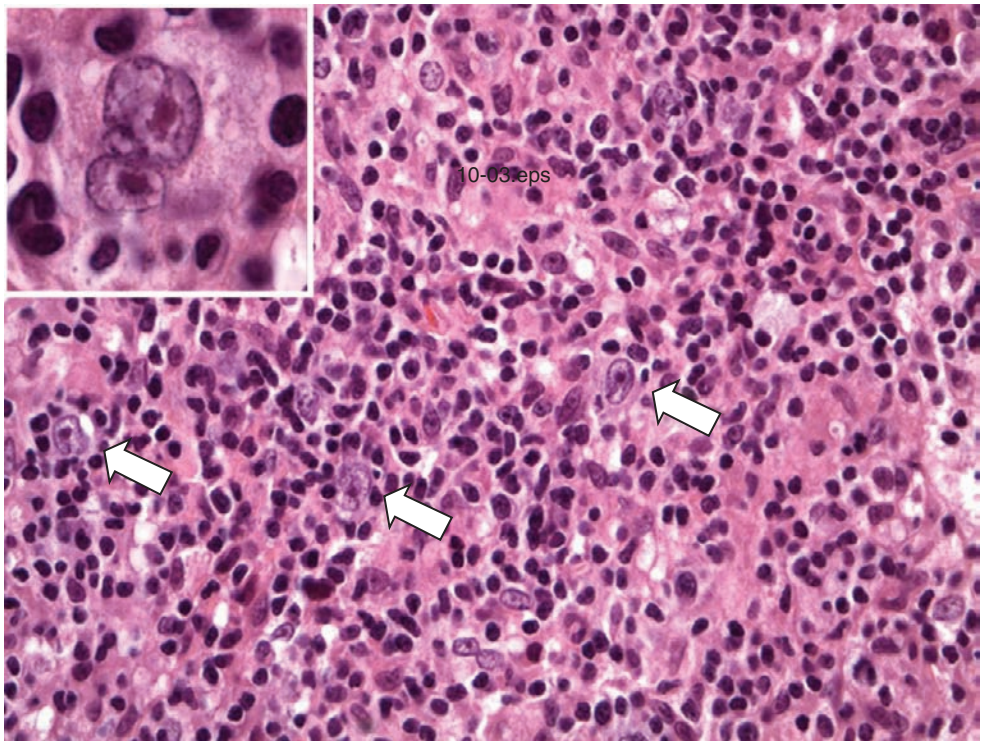


**FIGURE 8-6**

T-cell–histiocyte-rich large B-cell lymphoma. Low magnification shows predominance of small lymphocytes, scattered histiocytes, and occasional atypical large cells that represent the malignant cells *(arrows).* The *inset* shows one of these atypical lobulated cells.

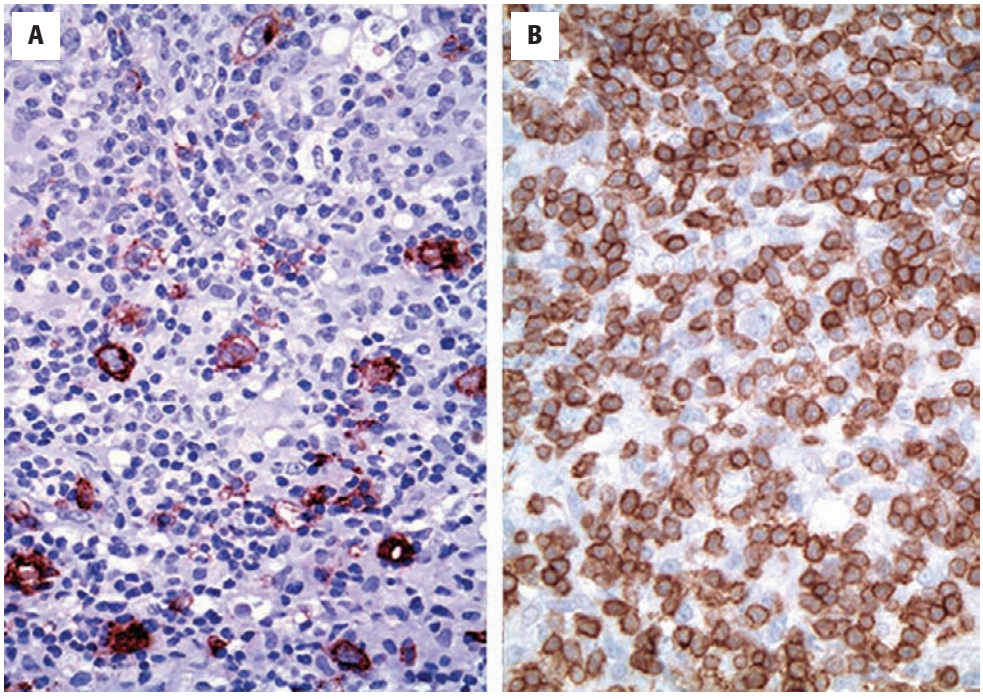


**FIGURE 8-7**

T-cell–histiocyte-rich large B-cell lymphoma. Immunostains for CD20 **(A)** and CD3 **(B)** show that these large cells are $CD20^+$ B cells. The small lymphocytes are reactive T cells.

CD79a$^+$, BCL6$^+$, EMA$^+$, and CD75$^+$). EBV is absent. The distinguishing features between TC/HRBCL and NLPHL rely primarily on the surrounding non-neoplastic microenvironment.

#### Molecular Genetics

There are few studies investigating the molecular genetics of TC/HRBCL. One study, using comparative genomic hybridization, showed that genetic imbalances in TC/HRBCL are common, with approximately 4.7 abnormalities per tumor. The most common abnormalities include gains of Xq, 4q13, Xp21, and 18q21 and loss of 17p.

### Differential Diagnosis

TC/HRBCL must be distinguished from NLPHL, classical HL, and T-cell lymphomas. In differentiating NLPHL from TCRBCL, a diffuse pattern and lack of a follicular dendritic cell meshwork by CD21 or CD35 staining supports a diagnosis of TC/HRBCL over NLPHL. Furthermore, NLPHL contains large numbers of small non-neoplastic IgD$^+$ B-cells and increased numbers of CD57$^+$, PD1$^+$ T-cells within the nodules; these are not features of TC/HRBCL. Strong expression of CD20 and CD45 in the atypical large cells with lack of CD15 helps to exclude classical HL and T-cell lymphoma.

### Prognosis and Therapy

TC/HRBCL is an aggressive lymphoma. Treatment is usually with a multiple-agent anthracycline-containing regimen (CHOP) plus rituximab. Five-year overall survival rates with CHOP alone are reported to be in the range of 45% to 58%, similar to DLBCL. The effect of adding rituximab is not currently known. Distinguishing TC/HRBCL from NLPHL and classical HL is important because therapies for the HLs do not adequately treat TC/HRBCL.

## Primary Diffuse Large B-Cell Lymphoma of the Central Nervous System

### Clinical Features

Primary DLBCL of the central nervous system (PCNSL) is a rare tumor accounting for 1% of non-Hodgkin lymphomas and 5% of brain tumors. It is the most common type of primary central nervous system (CNS) lymphoma and encompasses all cases of DLBCL that are localized to the brain or eye parenchyma. DLBCL occurring in the CNS in the setting of immunodeficiency is no longer included in this diagnostic category. The median age of onset is 55 years, with a slight male predominance. Patients may have headache, mental status changes, or focal neurologic symptoms related to mass effect at the site of involvement. In one series, 81% of cases occurred in a supratentorial location, 7% infratentorially, and 12% in both locations. Because this neoplasm grows rapidly, the duration of signs and symptoms is on the order of weeks.

**PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE CENTRAL NERVOUS SYSTEM—FACT SHEET**

**Clinical Features**
- Rare tumor, but most common type of primary CNS lymphoma
- Supratentorial location most common
- Median age at diagnosis, 55 years
- Presenting symptoms include mental status changes, headache, focal neurologic deficit

**Morphology**
- Sheets of large centroblastic or immunoblastic cells
- Perivascular distribution

**Immunophenotype**
- CD20$^+$, BCL2$^{-/+}$, BCL6$^{-/+}$, MUM1$^+$, EBV$^-$

**Molecular Genetics**
- Rearranged immunoglobulin genes
- del6q21-22 and *BCL6* rearrangement

**Prognosis and Therapy**
- Multiple-agent therapy with high-dose methotrexate and radiation
- Aggressive lymphoma, median survival approximately 3 years (age >60 years is poor prognostic factor)

**Differential Diagnosis**
- Burkitt lymphoma, metastatic carcinoma, melanoma, inflammatory reaction (encephalitis)

### Pathologic Features

#### Histopathology

The tumor resembles other DLBCLs cytologically. In a series of 62 PCNSL cases in immunocompetent patients, 87% were centroblastic and 13% were immunoblastic. The pattern of involvement is diffuse with a propensity for perivascular involvement (Figure 8-8). The diffuse pattern is more readily seen when a large mass is biopsied; perivascular involvement may be the

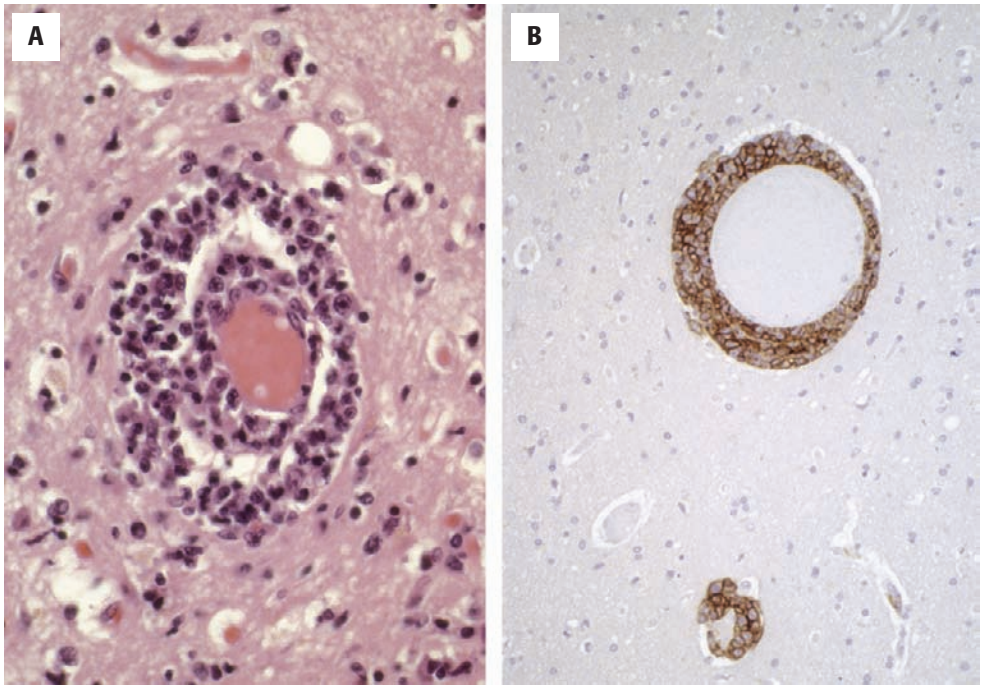


**FIGURE 8-8**

Primary diffuse large B-cell lymphoma of the central nervous system. **A,** Lymphoma cells often show a perivascular distribution. **B,** CD20 stain confirms B-cell lineage.

only pattern seen in smaller stereotactic biopsy specimens. Necrosis and surrounding gliosis are common associated features.

#### Immunophenotype

The lymphoma cells express pan B-cell markers CD19 and CD20. CD10 is expressed in less than 20% of cases, BCL6 in 60% to 80% of cases, and MUM1 in more than 90% of cases. The reported expression of BCL2 is variable, but is seen in at least 50% of cases. Using the Hans algorithm, approximately 80% to 95% of cases are classified as non-GCB type; this is due to the frequent expression of MUM1. Because many cases coexpress BCL6, it has been suggested that this tumor is derived from a B-cell in the late germinal center to early post-germinal center stage of development. EBV is usually absent.

#### Molecular Genetics

Monoclonal immunoglobulin gene rearrangement is present. There are no specific diagnostic molecular genetic abnormalities identified in PCNSL, but abnormalities of 1q21, 6q, 7q, and 14q are reported. Comparative genomic hybridization studies have shown that gains outnumber losses. Chromosome 12q shows the most frequent gains (63%). The most commonly deleted locus is located at chromosome 6q (47%), with 6q21-22 being the commonly deleted region. Rearrangement of the *BCL6* gene is also seen in a significant minority of cases (23% to 37%). Gene expression profiling shows that PCNSL is a tumor of late germinal center cells and its transcriptional signature closely resembles that of systemic DLBCL. Using an integrative analysis combining GEP and high-resolution array comparative genomic hybridization, it has recently been shown that 65% of PCNSLs reveal deletions of 9p21.3 involving *CDKN2A* as well as a number of previously unrecognized deletions associated with diminished expression of the candidate genes.

## Differential Diagnosis

The differential diagnosis is similar to that of DLBCL at any site. Given the perivascular arrangement of lymphoid cells, inflammatory conditions such as encephalitis can be considered. However, the cytologic atypia and predominance of B-cells help differentiate PCNSL from inflammatory conditions. EBV studies are helpful for distinguishing PCNSL from lymphomatoid granulomatosis.

## Prognosis and Therapy

Therapy is generally multiple-agent therapy with high-dose methotrexate combined with radiotherapy. The latter is generally withheld in older patients because of

unacceptable toxicity. This lymphoma is aggressive, with a median overall survival of 37 months. An important prognostic factor is age. Patients older than 60 years fare substantially worse than those younger than 60 years. Deletion of chromosome 6q21-22 and rearrangement of *BCL6* have also been shown to correlate with a decreased overall survival. Low expression of C4orf7 has been recently linked to inferior overall survival.

## PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE

### Clinical Features

Primary cutaneous DLBCL, leg type, is primarily a disease of elderly adults (median age, 77 years) with a female predominance. It usually manifests with localized disease of the skin with a single or multiple, localized, rapidly growing reddish-brown plaques. The lower leg is the most frequent site of involvement, but other sites can be involved as well. Dissemination to nodal or extranodal sites is common with disease progression.

**PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE—FACT SHEET**

**Clinical Features**
- Most common in older patients, females affected more than women
- Rapidly enlarging mass on the lower extremities

**Morphology**
- Diffuse sheets of large centroblasts and immunoblasts
- Absent or minimal stromal response

**Immunophenotype**
- $CD20^+$, $CD10^-$, $BCL6^+$, $MUM1^+$, $FOXP1^+$, $BCL2^+$, cIgM

**Molecular Genetics**
- Rearranged immunoglobulin genes
- Amplification of 18q21 (*BCL2* and *MALT1*)
- Deletion of 9p21 *(CDKN2A)*
- Translocation of *BCL6, MYC,* and/or *IGH@* present in many cases

**Prognosis and Therapy**
- Aggressive lymphoma with 50%, 5-year survival
- Treatment with anthracycline-containing, multiple-agent chemotherapy

**Differential Diagnosis**
- Secondary involvement in skin of systemic DLBCL
- Cutaneous lymphoblastic lymphoma

### Pathologic Features

#### Histopathology

The tumor is composed of diffuse sheets of medium-to-large centroblasts and immunoblasts without admixed small centrocytes (Figure 8-9). The cells have a characteristic round nuclear shape. There is little stromal reaction, and few infiltrating small non-neoplastic lymphocytes are present. Mitoses are easily seen. The epidermis is usually uninvolved.

#### Immunophenotype

The tumor is positive for the pan B-cell marker CD20. The vast majority of cases (>85%) express BCL2, MUM1, and FOXP1 and lack expression of CD10. BCL6 expression is seen in a variable number of cases. Cytoplasmic IgM with or without coexpression of IgD is reported in virtually all cases.

#### Molecular Genetics

Monoclonal *IGH@* rearrangement is present. The tumor lacks t(14;18); however, translocations involving *BCL6*, *MYC,* and *IGH@* are common. The two most common recurring genetic abnormalities are amplification of chromosome 18q21, where the *BCL2* and *MALT1* genes are located, and deletion of chromosome 9p21.3, where the *CDKN2A* gene is located. Amplification of *BCL2* is believed to be the mechanism responsible for the frequent expression of BCL2 protein in seen in this tumor. Gene expression profiling shows that the tumor is similar to activated B-cell–like DLBCL.

#### Differential Diagnosis

The main differential diagnosis of DLBCL, leg type, is cutaneous involvement by systemic DLBCL. This can be excluded only by careful staging procedures and knowledge of the clinical history. Another B-cell lymphoma that must be excluded is B lineage lymphoblastic lymphoma. Cutaneous lymphoblastic lymphoma is rare. Unlike primary cutaneous DLBCL, leg type, lymphoblastic lymphomas usually express CD10 and TdT. Primary cutaneous follicle center lymphoma may be composed of large cells but often lack BCL2, express germinal center markers such as CD10 and uncommonly express cytoplasmic IgM.

### Prognosis and Therapy

Treatment with systemic anthracycline-containing multiple-agent immunochemotherapy is the preferred type of therapy for this lymphoma. Primary cutaneous

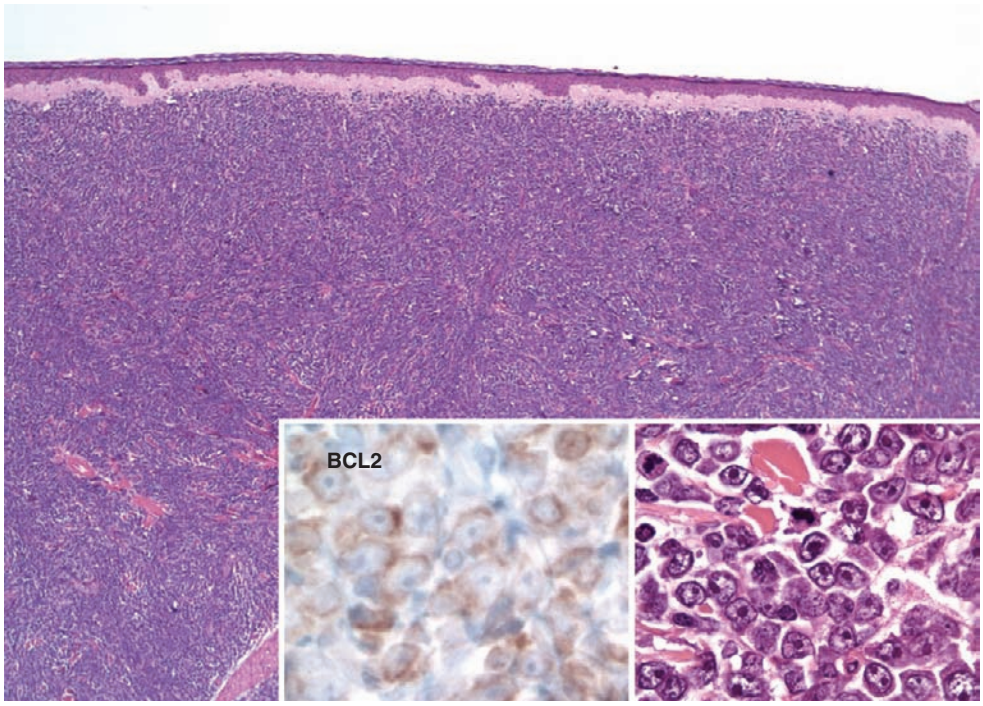


**FIGURE 8-9**

Cutaneous large B-cell lymphoma, leg type. Low magnification shows a diffuse infiltrate of atypical large cells that express CD20. The *inset* in the lower right corner shows a predominance of large round immunoblastic cells. Expression of BCL2 is characteristic.

DLBCL, leg type, has an intermediate clinical behavior with a 50%, 5-year survival. Adverse prognostic factors include advanced age, multiple skin lesions, and disease localized to the leg. Some authors have suggested that it is the expression of BCL2 protein, a known poor prognostic indicator in other lymphomas, along with other clinical factors that contribute to the poor outcome of this lymphoma, rather than location on the leg. Recent studies have shown that deletion of 9p21.3 or methylation of the promoter region of *CDKN2A* are associated with an unfavorable prognosis.

## EPSTEIN-BARR VIRUS–POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA OF THE ELDERLY

### Clinical Features

Epstein-Barr virus (EBV)–positive DLBCL of the elderly is the monoclonal counterpart of a related group of diseases collectively known as *EBV-positive B-cell lymphoproliferative disorders*. It is seen most commonly in Southeast Asia, particularly Japan, although less commonly it is seen in Western countries. The disease is a result of age-associated immune senescence and typically affects patients older than 50 years without known immunodeficiency or previous history of an EBV-associated lymphoproliferative disorder (i.e., DLBCL associated with chronic inflammation, lymphomatoid granulomatosis, primary effusion lymphoma or plasmablastic lymphoma). EBV positivity in DLBCL is proportionately more common with increasing age, reaching a peak after the age of 90 years, although peak incidence occurs in the eighth decade. Rare cases have been reported in patients younger than 50 years of age. It is important to exclude other causes of underlying immunodeficiency before making the diagnosis in this age group.

EBV-positive DLBCL affects men slightly more frequently than women and appears in lymph nodes and at extranodal sites, including the skin, gastrointestinal tract, lung, and tonsil. Greater than 50% of patients exhibit B symptoms, elevated LDH, and advanced-stage disease, although solid organ and bone marrow involvement are relatively infrequent.

### Pathologic Features

#### Histopathology

EBV-positive DLBCL is morphologically heterogeneous. It can be divided into polymorphous and large-cell lymphoma subtypes; however, these are of no clinical significance and usually both morphologies can be seen in the same specimen when examined thoroughly. In all cases, the tumor consists of a polymorphic or monomorphic infiltrate of immunoblast-like

**EBV-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA OF THE ELDERLY—FACT SHEET**

**Clinical Features**
- Affects patients older than 50 years with no history of immunosuppression
- Usually appears with B symptoms and advanced stage
- Nodal and extranodal disease common; solid organs and bone marrow not usually involved
- Rare in Western countries; more common in Japan

**Morphology**
- Polymorphic or monomorphic infiltrate of immunoblast-like transformed cells or Hodgkin and Reed-Sternberg–like cells.
- Mixed inflammatory cells in polymorphic infiltrate

**Immunophenotype**
- $CD20^+$, $CD79a^+$, $CD10^-$, $BCL6^-$, $MUM1^+$, $CD30^{+/-}$, $CD15^-$, $EBV^+$

**Molecular Genetics**
- Clonally rearranged *IGH@* gene
- Clonal EBV genome

**Prognosis and Therapy**
- Combination immunochemotherapy (CHOP-R)
- Median survival, 2 years

**Differential Diagnosis**
- Infectious mononucleosis
- Classical Hodgkin lymphoma

transformed cells and Hodgkin and Reed-Sternberg (HRS)-like cells that efface normal architecture. Inflammatory cells, including small reactive lymphocytes, plasma cells, histiocytes and epithelioid cells, are admixed in polymorphic areas. Foci of geographic necrosis are also common and reminiscent of PTLD.

#### Immunophenotype

The malignant cells in EBV-positive DLBCL of the elderly express the pan B-cell antigens CD20 and CD79a and exhibit a nongerminal center immunophenotype, being negative for CD10 and BCL6 and positive for MUM1. Approximately three fourths of cases express CD30, whereas CD15 is universally absent. EBV is present in all cases and is best detected by in situ hybridization for EBV-encoded RNA (EBER).

#### Molecular Genetics

EBV-positive DLBCL of the elderly is a monoclonal disorder, and rearrangement of the *IGH@* gene is usually detected. A clonal EBV genome is also usually identified.

### Differential Diagnosis

The main differential diagnosis includes infectious mononucleosis and EBV-positive classical Hodgkin lymphoma (classical HL). Unlike EBV-positive DLBCL of the elderly, infectious mononucleosis shows preservation of lymphoid architecture with preferential expansion of the paracortex by a polymorphous cellular infiltrate. Zonal necrosis is uncommon and a monoclonal B-cell population is not detected. The HRS cells of classical HL usually differ from the HRS-like cells of EBV-positive DLBCL of the elderly, in that they do not express CD79a, usually lack CD20, and are positive for CD15 in most cases. In addition, reactive T-cells with an activated immunophenotype ($TIA1^+$/$perforin^+$) are common in EBV-positive DLBCL of the elderly but infrequent in classical HL.

### Prognosis and Therapy

Standard treatment of EBV-positive DLBCL of the elderly consists of combination immunochemotherapy, usually rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Despite therapeutic intervention, it remains an aggressive disease with a median survival of approximately 2 years. Indicators of poor prognosis include advanced age, the presence of B symptoms, advanced stage, multiple extranodal sites of involvement, and an elevated LDH.

## DIFFUSE LARGE B-CELL LYMPHOMA ASSOCIATED WITH CHRONIC INFLAMMATION

### Clinical Features

DLBCL associated with chronic inflammation is a rare lymphoma occurring at sites of long-standing chronic inflammation within body cavities and enclosed spaces. It is associated with latent EBV-infection (type III latency) and is believed to arise as a result of acquired local immunosuppression within the inflammatory milieu.

The most commonly affected site is the pleural cavity (called *pyothorax-associated lymphoma* [PAL]). PAL usually occurs in the setting of an artificial pneumothorax created to treat pulmonary or pleural tuberculosis. It is seen most frequently in Japan, where artificial pneumothorax was common; it is seen only occasionally in Western countries. The delay in lymphoma development after the onset of inflammation is greater than 20 years. As such, PAL tends to be a disease of older adults, with the median age occurring in the seventh decade (61 to 70 years). Men are affected more frequently than

**DIFFUSE LARGE B-CELL LYMPHOMA ASSOCIATED WITH CHRONIC INFLAMMATION—FACT SHEET**

**Clinical Features**
- Occurs in the setting of long-standing chronic inflammation in body cavities or enclosed spaces
- Pleural cavity most commonly affected (pyothorax-associated lymphoma)
- Predominantly older men (male-to-female ratio, >5:1)
- Fever, chest or back pain, and respiratory symptoms
- Low-stage disease
- Other affected sites include bone, joints, and skin

**Morphology**
- Diffuse infiltrate of large immunoblastic or plasmacytic cells

**Immunophenotype**
- $CD20^{+}$, $CD79a^{+}$, $PAX5^{+}$, $CD10^{-}$, $BCL6^{-}$, $MUM1^{+}$, $CD138^{-/+}$, $CD30^{-/+}$, $EBV^{+}$, $HHV8^{-}$

**Molecular Genetics**
- Clonally rearranged immunoglobulin genes
- Approximately 67% have mutated p53
- Unique gene expression profile with overexpression of *IFI27*

**Prognosis and Therapy**
- Chemotherapy, radiation therapy, or surgery
- Five-year overall survival, 22% to 35%

**Differential Diagnosis**
- Primary effusion lymphoma
- DLBCL, not otherwise specified
- Nonhematopoietic malignancy

women (>5:1 male-to-female ratio). Patients usually have a large pleural or lung-based mass, or both, with associated chest pain, respiratory symptoms, or hemoptysis. Fever, an elevated LDH, and low-stage disease are also common.

Other affected sites include bone with osteomyelitis, periarticular soft tissue and joints with metallic implants, chronic skin ulcers, and sites of surgical mesh implants. Rare cases occurring near hydroceles and within an atrial myxoma with similar features have also been reported and may also be considered in this category. Lymphoma usually develops at these sites after at least 10 years of chronic inflammation. Patients complain of a mass or localized pain, or both.

## Pathologic Features

### Histopathology

DLBCL of chronic inflammation is morphologically indistinguishable from DLBCL–not otherwise specified. It usually consists of large, atypical lymphocytes with immunoblastic features. Plasmacytic differentiation or large anaplastic cells are encountered occasionally.

### Immunophenotype

The malignant lymphocytes express the pan B-cell antigens CD20, CD79a, and PAX5. They exhibit a non-germinal center immunophenotype being negative for CD10 and BCL6 and positive for MUM1. CD138 and CD30 are variably expressed. Occasionally tumor cells are positive for T-cell–associated antigens CD2, CD3, or CD4. EBV is positive and is best detected by in situ hybridization for EBER. HHV8 is absent.

### Molecular Genetics

DLBCL associated with chronic inflammation is a monoclonal B-cell lymphoma and clonal rearrangement of Ig genes is present. Approximately 67% of cases are associated with mutation of *TP53*. Compared to DLBCL–not otherwise specified, DLBCL of chronic inflammation exhibits a unique gene expression profile with overexpression of genes involved in apoptosis, signal transduction, and interferon response with the *IFI27* (interferon-α-inducible protein 27) gene being one of the most overexpressed. Clonal genomic EBV DNA is also detected.

## Differential Diagnosis

The differential diagnosis includes primary effusion lymphoma (PEL), DLBCL–not otherwise specified, and nonhematopoietic malignancies. DLBCL associated with chronic inflammation differs and can be distinguished from PEL because it forms a solid mass, expresses pan B-cell antigens (CD20 and CD79a), and is negative for HHV8. The distinction between DLBCL associated with chronic inflammation and DLBCL–not otherwise specified requires an adequate history and knowledge of whether there is a history of long-standing chronic inflammation at the biopsied site. Anaplastic-appearing nonhematopoietic malignancies such as carcinoma, mesothelioma, or sarcoma may exhibit some similarities with DLBCL associated with chronic inflammation; however, the nonhematopoietic tumors lack CD45 and CD20 and express other tumor specific antigens (e.g., keratin, calretinin) that are not expressed by DLBCL.

## Prognosis and Therapy

Treatment of DLBCL associated with chronic inflammation may consist of any combination of chemotherapy (R-CHOP), radiotherapy, or surgery. Despite

treatment, the disease is clinically aggressive with overall 5-year survival ranging from 22% to 35%. Indicators of poor prognosis include male sex, poor performance status, advanced-stage disease, and an elevated LDH.

## PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

### Clinical Features

Primary mediastinal large B-cell lymphoma (PMBCL) appears most commonly in young to middle-aged women (median age 39 years; male-to-female ratio, 1:2) as a bulky mediastinal mass arising from the thymus with or without regional adenopathy. The majority of patients have stage I or II disease. Bone marrow involvement is extremely uncommon. Symptoms are usually related to mass effect and include superior vena cava syndrome.

**PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA—FACT SHEET**

**Clinical Features**
- Young to middle-aged adults; females affected more than males
- Mediastinal mass can result in compression (superior vena cava syndrome) of local structures

**Morphology**
- Large centroblastic or polylobated cells with clear cytoplasm
- Fine compartmentalizing alveolar fibrosis
- Some cells may resemble Hodgkin and Reed-Sternberg cells

**Immunophenotype**
- $CD19^{+}$, $CD20^{+}$, $CD10^{-/+}$, $BCL2^{+/-}$, $BCL6^{+/-}$, $CD30^{+/-}$ (weak, focal), $CD45RB^{+}$, $MAL^{+/-}$, nuclear $cREL^{+}$, $Grb2^{+}$

**Molecular Genetics**
- Rearranged immunoglobulin genes
- Gains of 9p, amplification of 2p involving *cREL*
- Translocations in CIITA

**Prognosis and Therapy**
- Anthracycline-based, multiple-agent chemotherapy
- Seventy-five percent event free survival can be achieved and maintained without late relapses

**Differential Diagnosis**
- Classical Hodgkin lymphoma
- Other large cell neoplasms (carcinoma, germ cell tumor)

### Pathologic Features

#### Histopathology

This lymphoma is characterized by sheets of large, centroblastic cells, often with abundant clear cytoplasm. Heterogeneity can be seen with some cases containing medium-sized cells and others containing large atypical polylobated cells, sometimes resembling Hodgkin and Reed-Sternberg cells. There is usually a fine sclerotic stroma present that can result in compartmentalization of the tumor cells (Figure 8-10). Occasionally, interpretation of biopsy specimens is difficult because of the crush artifact inherent in sclerotic tissue that distorts the appearance of cells. Moreover, the small biopsy size and crush artifact associated with mediastinoscopy techniques further contribute to diagnostic difficulty. Close comparison of atypical cells to the small reactive lymphocytes in the same area will aid in the recognition of the malignant cells.

#### Immunophenotype

The lymphoma cells express pan B-cell markers such as CD19, CD20, and CD79a. CD5 is not expressed, but CD10 can be seen in a minority (20%) of cases. BCL6 is expressed in approximately 50% of cases. BCL2 and MUM1 have been reported in approximately 75% of cases. Many of these lymphomas lack detectable surface Ig, although B-cell transcription factors Oct2 and Bob.1 are present. CD30 is commonly expressed (up to 85% of cases), but usually in a weak or focal pattern. CD30 expression should not be considered definitive evidence of classical HL. Unlike classical HL, CD45RB is expressed in PMBCL. The neoplastic cells in PMBCL typically lack expression of CD15, but CD23 is expressed in approximately 70% of cases. EBV is absent.

Expression of MAL, a lipid-raft protein involved in intracellular transport and T-cell development, has been shown to be specific for this type of lymphoma and can be seen in more than 50% of cases (Figure 8-11). c-REL is also characteristically expressed in its active form (nuclear), similar to classical HL. TRAF1 is also frequently coexpressed.

#### Molecular Genetics

These lymphomas have rearranged Ig genes and germline T-cell receptor genes. Rearrangements of *BCL2, BCL6,* and *MYC* are not seen. Translocations involving the *CIITA* gene, the master regulator of MHC class II expression (HLA-DR), are seen in 38% of cases. The fusion partners are promiscuous, but involve PD-1 ligands in 50% of cases. PMBCL does have characteristic molecular genetic features with gains of chromosome 9p24 encompassing *JAK2, PD-L1, PD-L2,* and

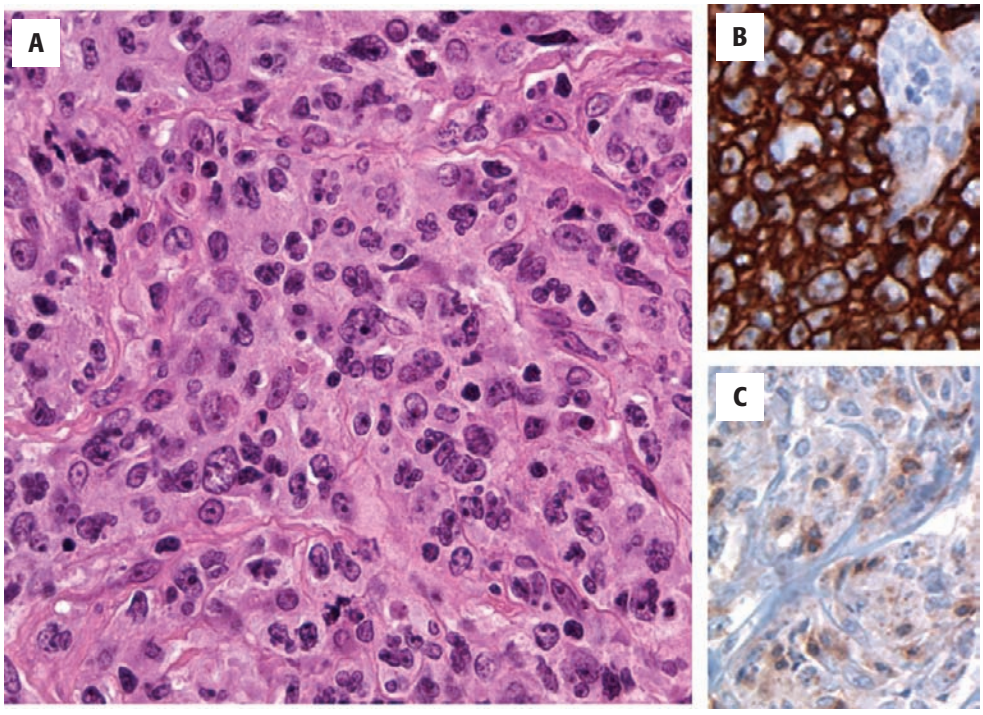


**FIGURE 8-10**

Primary mediastinal large B-cell lymphoma. **A,** The lymphoma cells are large with lobulated nuclei set in a background of fine sclerotic stroma. The cells are positive for CD20 **(B)** and negative for CD3 **(C)**.

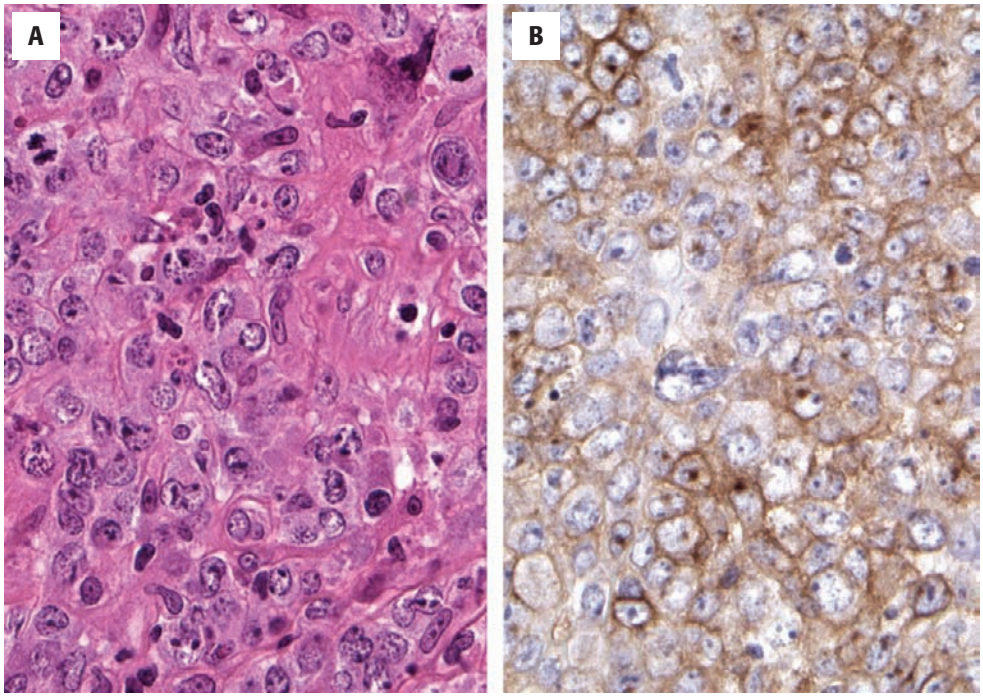


**FIGURE 8-11**

Another case of primary mediastinal large B-cell lymphoma. **A,** Characteristic morphologic features. **B,** MAL is also characteristic.

amplification of chromosome 2p involving *c-REL*. As with other lymphomas, gene expression profiling has revealed interesting features. The overall expression profile shows that PMBCL has a unique signature, but suggests it is closely related to classical HL. In particular, as noted previously, MAL is noted to be overexpressed, a feature peculiar to PMBCL and not typically seen in DLBCL. As might be expected from *c-REL* amplification, NFκB transcriptional targets have also been shown to be activated in this lymphoma, resulting in an antiapoptotic effect. This pathway may be a target of future therapeutic agents. Similarly, amplifications involving 9p24 appear to preferentially lead to overexpression of PD-L2>PD-L1. Overexpression of *JAK2* can also lead to increased expression of the PD-1 ligands and strongly suggest that a dominant pathogenetic mechanism in PMBCL is escape from immunosurveillance. Specific inhibitors to both JAK2 and the PD-1 ligands are currently being tested in phase I trials of unrelated tumors, but could conceivably become targeted treatments for clinically aggressive variants of PMBCL.

### Differential Diagnosis

The main differential diagnostic considerations for PMBCL are DLBCL arising in mediastinal lymph nodes and classical HL. Regarding DLBCL, the histologic features of centroblastic cells with clear cytoplasm in a sclerotic stroma all support the diagnosis of PMBCL. Currently, however, PMBCL is a clinicopathologic diagnosis and awareness of the clinical scenario is required—specifically, the presence of a mediastinal mass without significant systemic disease or a prior history of lymphoma. Expression of nuclear c-REL and MAL will strongly support the diagnosis of PMBCL. Similarly, expression of STAT1 and TRAF1, particularly coexpressed with nuclear c-REL, can be useful for distinguishing PMBCL. Because expression of CD30 is seen in PMBCL, classical HL can be a diagnostic consideration in a mediastinal biopsy. Unlike classical HL, PMBCL expresses CD45 and lacks strong expression of CD15. MAL expression supports PMBCL; however, a small minority of cases of classical HL (10% to 20%) express MAL. Recently, expression of growth factor receptor-bound protein 2 (Grb2) has been shown to be useful in distinguishing PMBCL from classical HL, because it is universally expressed by PMBCL, but only expressed by the neoplastic cells in classical HL in 10% of cases.

Rare cases may have morphologic and phenotypic features that overlap between a diagnosis of PMBCL and classical HL and are now recognized as mediastinal gray-zone lymphomas. These cases warrant a diagnosis of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical HL. Translocations involving *CIITA* occur in 24% of these cases, further testament to the relatedness of these borderline lesions.

### Prognosis and Therapy

Therapy for PMBCL consists of multiple-agent anthracycline-containing regimens, such as CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, predinsone), with rituximab followed by involved field radiation. Using this approach, an 80% complete remission rate and 75% event-free survival can be achieved. Recently, the infusion therapy EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) has shown promising results in treating PMBCL. The disease course following conventional chemotherapy appears distinct from DLBCL. PMBCL does not appear to result in late relapses, a feature that is sometimes seen in DLBCL.

## INTRAVASCULAR LARGE B-CELL LYMPHOMA

### Clinical Features

Intravascular large B-cell lymphoma (IVLBCL) is an uncommon type of extranodal DLBCL characterized by the selective localization of lymphoma cells to the intravascular space, most commonly involving the small and medium sized blood vessels. Patients are typically older adults (median age, 71 years), with men and women affected equally. Distinct clinical entities are currently recognized and are distinguished by the geographic origin of the patient. Patients originating from Southeast Asian countries typically exhibit B symptoms, hepatosplenomegaly, bone marrow involvement, thrombocytopenia, and hemophagocytic syndrome. Patients from Western countries exhibit B symptoms and disease localized to the central nervous system and skin. A cutaneous variant is also recognized. The cutaneous variant is seen predominantly in young women from Western countries. Patients with cutaneous variant IVLBCL generally have a good performance status and have disease limited to the skin. Because of the nonspecific nature of the presenting symptoms and the lack of an identifiable mass, the diagnosis of IVLBCL is often delayed, sometimes being made at autopsy. In suspected cases with no localizing findings, random skin biopsies may prove helpful in establishing a diagnosis.

**INTRAVASCULAR LARGE B-CELL LYMPHOMA—FACT SHEET**

**Clinical Features**
- Median age at diagnosis, 71 years
- Affects men and women equally
- Nonspecific dermatologic, neurologic, or constitutional symptoms
- Asian variant: patient from Southeast Asian countries with B symptoms, hepatosplenomegaly, bone marrow involvement, thrombocytopenia, hemophagocytic syndrome
- Western variant: patients from Western countries with B symptoms, CNS and skin involvement
- Cutaneous variant: young women from Western countries with disease localized to the skin

**Morphology**
- Intravascular localization of large lymphoma cells
- Intrasinusoidal involvement in bone marrow, liver, and spleen

**Immunophenotype**
- $CD20^{+}$, $CD5^{-/+}$, $CD10^{-/+}$, $BCL6^{-/+}$, $MUM1^{+}$, $BCL2^{+}$

**Molecular Genetics**
- Clonally rearranged immunoglobulin genes
- No recurrent abnormalities identified

**Prognosis and Therapy**
- *Asian and Western variants:* aggressive course with poor outcome unless early diagnosis and institution of anthracycline-containing multiple-agent chemotherapy
- Cutaneous variant: significantly longer survival than other variants

**Differential Diagnosis**
- Nonintravascular non-Hodgkin B-cell lymphoma with minor intravascular component
- Intravascular large cell lymphoma of T cell or NK cell origin
- Intravascular undifferentiated carcinoma

## Pathologic Features

### Histopathology

Although almost any tissue can be involved, the most commonly biopsied site is skin. The unifying feature is the localization of the lymphoma cells to the lumina of small blood vessels and capillaries (Figure 8-12). Minimal extravasation of tumor cells may be seen. The lymphoma cells are large with vesicular chromatin and prominent nucleoli. Cases composed of anaplastic cells and small atypical cells have also been reported. Mitotic figures are often present. Bone marrow, liver, and spleen show sinusoidal involvement. Hemophagocytosis is seen in some cases, typically in patients of Asian origin. By nature the infiltrate can be subtle, and close attention to contents of vascular spaces is required to avoid overlooking this lymphoma. Particularly in the bone marrow, recognition of subtle involvement may be greatly aided by routine immunohistochemistry (see later discussion).

### Immunophenotype

By definition the tumor cells are B-cells and express pan B-cell antigens, such as CD20 and CD79a. CD5, CD10, BCL6, and MUM1 are expressed in approximately 38%, 13%, 26%, and 95% of cases, respectively. BCL2 is expressed in approximately 90% of cases. EBV is usually not present in these lymphomas; rare EBV-positive cases have been reported in the setting of immunodeficiency. One study has reported a lack of expression of the adhesion molecules CD29 (β1 integrin) and CD54 (ICAM-1), which might explain the unusual localization of this lymphoma.

### Molecular Genetics

These lymphomas have clonally rearranged immunoglobulin genes. The *IGH@/BCL2* translocation is not identified. Given the few cases that have been studied, no recurrent genetic abnormalities are known. False-negative clonality studies can occur because of sensitivity problems related to small amounts of tumor cells in most biopsies.

## Differential Diagnosis

The differential diagnosis includes other nonintravascular lymphoma cases of DLBCL with a minor intravascular component. Other lineage lymphomas (T and natural killer [NK] cell) should be considered, but can be excluded by immunophenotyping. Undifferentiated carcinomas can be seen within blood vessels and may resemble IVLBCL morphologically; however, they will express epithelial antigens and lack of expression of CD45 and B-cell markers, thus excluding a diagnosis of IVLBCL. Vascular tumors can also be considered because of the location of the malignant cells. In fact, before widespread immunophenotyping in paraffin sections, an endothelial origin had been postulated. Differentiation from endothelial tumors is easily accomplished with immunostaining for B-cell markers and endothelial markers such as CD31 and CD34.

## Prognosis and Therapy

Given the rarity of this lymphoma, studies with large numbers of patients have not been performed; however,

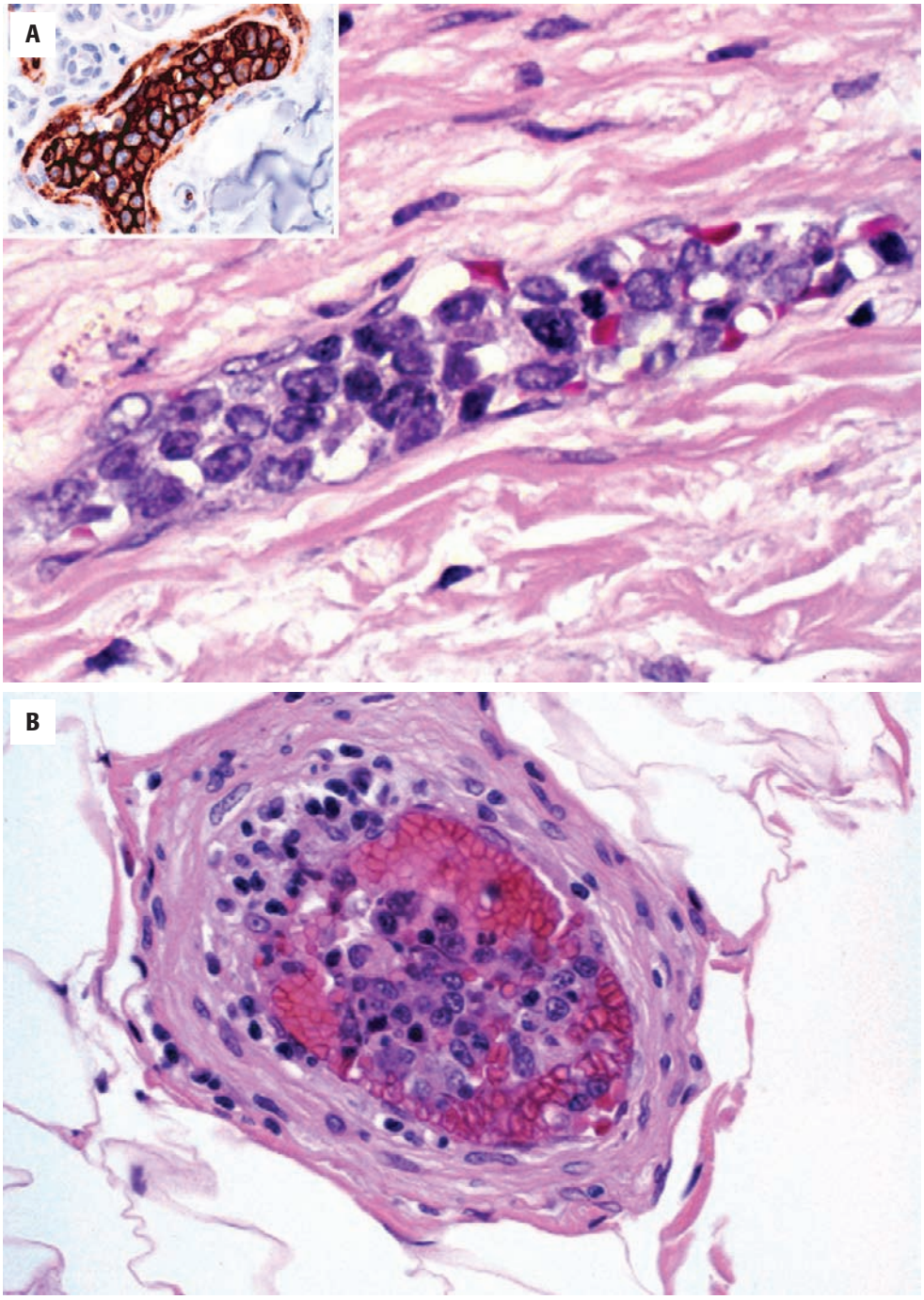


**FIGURE 8-12**

Intravascular lymphoma. **A,** The lymphoma cells are confined to the lumina of vessels. The *inset* shows expression of CD20. **B,** This case shows the lymphoma cells admixed with the erythrocytes within the blood vessel. It was the only vessel involved in this biopsy of a peripheral nerve performed for neurologic symptoms related to this undiagnosed lymphoma.

this lymphoma is aggressive with a generally poor outcome. Survival is often measured in weeks to months. When the diagnosis is made early, anthracycline-containing multiple-agent chemotherapy has resulted in complete remission and long-term survival. One study reported a 33%, 3-year survival. A more favorable outcome is reported in patients with the cutaneous variant of the disease.

## ALK-POSITIVE LARGE B-CELL LYMPHOMA

### Clinical Features

ALK-positive LBCL is a rare lymphoma. It can appear at any age but occurs most frequently in adult males (male-to-female ratio, 5:1) at a median age of 43 years.

**ALK-POSITIVE LARGE B-CELL LYMPHOMA—FACT SHEET**

**Clinical Features**
- Rare disorder affecting men more often than women
- Median age, 43 years
- Rapidly enlarging mass

**Morphology**
- Sheets of large immunoblast or plasmablast-like cells
- Sinusoidal distribution in lymph nodes

**Immunophenotype**
- $CD45^{-/+}$, $CD3^{-}$, $CD20^{-}$, $CD30^{-}$, $CD138^{+}$, $EMA^{+}$, cytoplasmic $IgA^{+}$, $ALK^{+}$
- $EBV^{-}$, $HHV8^{-}$

**Molecular Genetics**
- Clonally rearranged immunoglobulin genes
- t(2;17)(p23;q23) in majority of cases

**Prognosis and Therapy**
- Anthracycline-containing combination chemotherapy
- Median survival, 12 months

**Differential Diagnosis**
- Other non-Hodgkin lymphomas with plasmablastic morphology
- $ALK^{+}$ anaplastic large cell lymphoma
- Epithelial malignancies

It can be included under the rubric of plasmablastic lymphomas, because this morphology and immunophenotype are characteristic. It is primarily a disease of lymph nodes and appears at an advanced stage (III and IV) in the majority of patients. Extranodal disease is less common, but has been reported in the nasopharynx, liver, spleen, gastrointestinal tract, soft tissue, and bone.

## Pathologic Features

### Histopathology

The tumor typically infiltrates lymph node sinuses. The neoplastic cells are large and immunoblast-like with round nucleoli, prominent central nucleoli, and abundant basophilic cytoplasm. Plasmablastic differentiation is common, and Reed-Sternberg–like cells or multinucleated anaplastic large cells may be seen (Figure 8-13). Foci of necrosis are often present. Extranodal sites show a similar cellular infiltrate, but lack the sinusoidal pattern of involvement seen in lymph nodes.

### Immunophenotype

This lymphoma has a distinctive immunophenotype. Tumor cells are negative for pan B-cell markers CD19, CD20, CD22, and CD79a, as well as T-cell–associated antigens CD2, CD3, CD5, CD7, and CD8, although CD4 can be weakly positive in a subset of cases. CD45 is only weakly and focally positive or completely absent. The neoplastic cells express the plasma cell–associated antigens CD138 and EMA and are positive for monoclonal cytoplasmic Ig, usually IgA. CD30 is negative or only focally and weakly positive. ALK is expressed in all cases. Similar to ALK-positive anaplastic large cell lymphoma (ALK-positive ALCL), the pattern of ALK expression correlates with the underlying *ALK*-associated genetic abnormality (see later discussion). Most cases express ALK in a granular cytoplasmic pattern with the cytoplasmic, nuclear, and nucleolar pattern much less frequently described. EBV and HHV8 are not detected.

### Molecular Genetics

The lymphoma has monoclonal rearrangement of the *IGH@* gene. Genetic studies show that most cases harbor the t(2;17)(p23;q23), which fuses the clathrin heavy chain gene *(CLTC)* at chromosome 17q23 with the *ALK* gene at chromosome 2p23. This genetic abnormality is associated with the granular cytoplasmic pattern of ALK protein expression seen by immunohistochemical staining. Fewer cases harbor the t(2;5)(p23;q35) commonly seen in ALK-positive ALCL. This abnormality is associated with the cytoplasmic, nuclear, and nucleolar pattern of ALK protein expression seen by immunohistochemical staining. Other less common translocations have been described involving *SEC31A* on chromosome 3q27 and *SQSTM1* on chromosome 5q35.1.

## Differential Diagnosis

ALK-positive LBCL shares many morphologic and immunohistochemical features with other tumors including plasmablastic lymphoma, primary effusion lymphoma, ALK-positive ALCL and epithelial tumors. Careful application and interpretation of immunohistochemical stains (e.g., CD138, ALK, EBV, HHV8, CD30, Igs) will help to reveal the true nature of the neoplastic B-cells. Laboratories that use screening IHC approaches should be cautious, because this lymphoma is easily confused with epithelial tumors showing sinusoidal lymph node infiltration.

## Prognosis and Therapy

Patients with ALK-positive LBCL typically follow an aggressive clinical course. Treatment usually consists of anthracycline-containing multiple-agent chemotherapy such as CHOP. Rituximab is not usually included

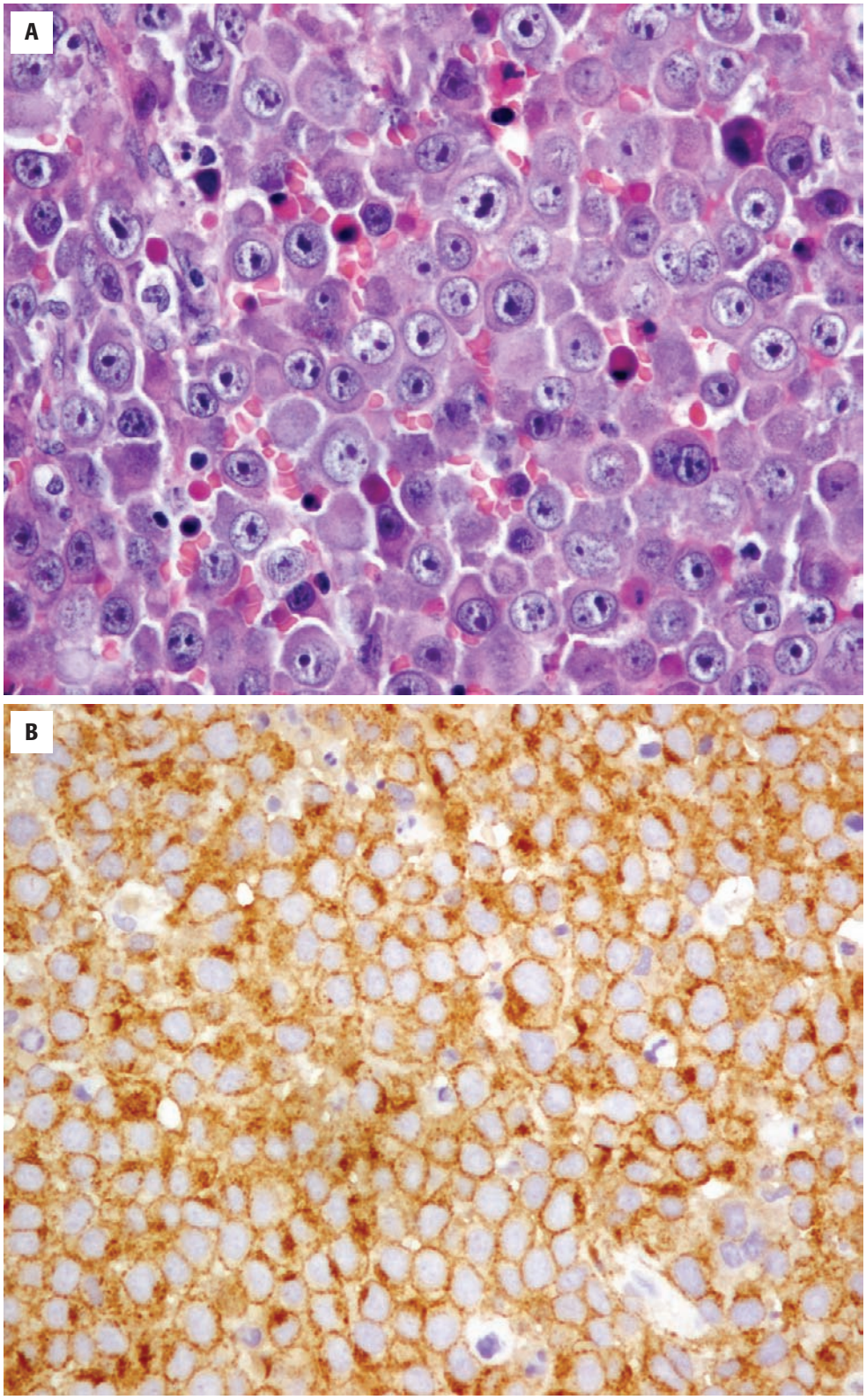


**FIGURE 8-13**

ALK-positive large B-cell lymphoma. **A,** The cells have immunoblastic morphology with plasmacytoid features. The cells usually lack expression of B-cell markers such as CD20. **B,** ALK staining shows the characteristic cytoplasmic granular staining pattern. This is in contrast to the nuclear and cytoplasmic staining seen in the majority of anaplastic large cell lymphomas.

because of the lack of CD20 expression by the tumor. Despite therapeutic intervention, the prognosis remains poor and nearly half of the patients die within the first year, with a median survival of 12.2 months.

## LYMPHOMATOID GRANULOMATOSIS

Inclusion of lymphomatoid granulomatosis (LyG) into this chapter is somewhat arbitrary given the heterogeneity of histopathology and behavior; however, because higher-grade lesions can be considered aggressive B-cell neoplasms it is covered here. Our concept of this disease has changed over time. Originally it was described in the context of studies of Wegener's granulomatosis. In 1972, Liebow used the term *LyG* to emphasize the similarity of LyG to Wegener's granulomatosis; however, LyG was not believed to be caused by granulomatous inflammation. Instead it was thought to represent a destructive lymphoid proliferation. Because T-cells predominate in the infiltrate, it has also been considered a T-cell disorder, often along the spectrum of what is known as extranodal NK/T-cell lymphoma, nasal type (so called angiocentric immunoproliferative lesions). It is currently known that most cases are EBV-driven B-cell lymphoproliferative disorders with variable histology and outcomes.

### LYMPHOMATOID GRANULOMATOSIS—FACT SHEET

**Clinical Features**
- Rare disorder
- Most commonly presents in adults (40 to 69 years old) with fever, cough, dyspnea, and weight loss
- Manifests as lung mass (bilaterally) on chest radiograph

**Morphology**
- Angiocentric and angiodestructive lesions composed of a polymorphous infiltrate of small lymphocytes, histiocytes, plasma cells, and variable numbers of immunoblasts
- Hodgkin-like cells can be seen
- Cytologic grading is based on the presence of large cells ($EBV^+$)
  - Grade I: <5 $EBV^+$ cells per hpf
  - Grade II: 5-50 $EBV^+$ cells per hpf
  - Grade III: >50 $EBV^+$ cells per hpf
- Necrosis is seen in higher-grade lesions

**Immunophenotype**
- Numerous small T cells
- Large atypical immunoblast and Hodgkin-like cells are $CD20^+$ B cells and $EBV^+$

**Molecular Genetics**
- $EBV^+$ B cells are monoclonal, best detected in higher grade lesions
- Immunoglobulin gene rearrangement studies may be negative in lower grade lesions
- T-cell receptor gene rearrangement usually negative

**Prognosis and Therapy**
- Single or multiagent chemotherapy, immunomodulatory therapy
- Variable clinical course; higher-grade lesions have an aggressive clinical course with median survival of 18 months

### Clinical Features

LyG is a rare disorder with few published case series and case reports, making generalization difficult. LyG can be seen at almost any age, but most cases occur in the fourth to sixth decades, with a male predominance (male-to-female ratio, 2:1). Patients are almost always symptomatic and appear most commonly with fever, cough, dyspnea, weakness, and weight loss. The chest radiograph usually shows multiple bilateral rounded masses. Besides the lung, other commonly involved sites include skin, central nervous system, kidney, spleen, liver, and lymph node. The disorder generally produces nodular masses in these tissues. Although not associated with autoimmune diseases, some studies have suggested T-cell defects and a type of immunodeficiency. For this reason some have considered this disorder in a similar light as posttransplantation lymphoproliferative disorders.

### Pathologic Features

#### Histopathology

LyG most commonly manifests in the lung as nodules composed of a polymorphous cellular population of small lymphocytes, plasma cells, immunoblasts, and histiocytes. Well-formed granulomas are not part of the infiltrate. Large, atypical immunoblast-like cells are variably present—ranging from absent or rare to frequent. Some cells may resemble Hodgkin–Reed-Sternberg cells. At the periphery of the nodules, an angiocentric or angiodestructive lymphoid infiltrate can be seen and may contribute to the necrosis that may be present. Lesions of LyG have been graded cytologically based on the presence of large atypical cells. These grading criteria were developed at a time when LyG and angiocentric NK/T-cell lymphomas were often grouped together because of imprecise definitions. Currently, grading based on the number of large EBV-positive cells is recommended. Lesions that consist of sheets of large atypical B-cells and lack an inflammatory background should be classified as DLBCL.

- *Grade I:* lesions show a polymorphous lymphoid infiltrate without cytologic atypia, little or no necrosis, and rare (fewer than five cells per high-power field [hpf]) EBV-positive B-cells (by EBER in situ hybridization).

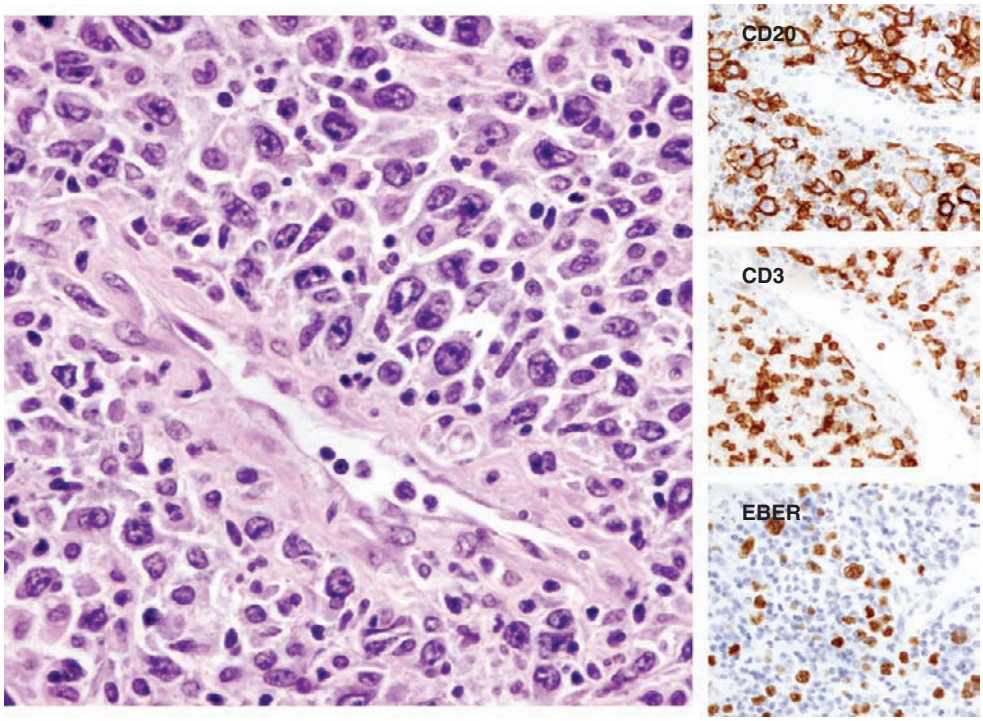


**FIGURE 8-14**

Lymphomatoid granulomatosis, grade III. This open lung biopsy specimen shows an atypical infiltrate composed of large lymphoid cells and admixed small lymphocytes. The large cells are $CD20^+$ B cells, and the small lymphocytes are T cells. Epstein-Barr virus–encoded RNA (EBER) is present in the large cells.

- *Grade II:* lesions show occasional large atypical lymphoid cells with or without necrosis and between 5 and 50 EBV-positive B-cells per hpf.
- *Grade III:* lesions maintain an inflammatory background but contain many large atypical cells (including Hodgkin-like cells) that may form clusters. Necrosis and numerous (>50 cells per hpf) EBV-positive B-cells are present (Figure 8-14). The EBV-positive B-cells can be shown to be monoclonal.

In the skin, a commonly involved site, papules or nodules with an angiocentric–angiodestructive dermal lymphoid infiltrate composed predominantly of small T-cells are seen. Ulceration is common and EBV-positive cells are present, but not in large numbers. Indurated plaques can also develop but are usually EBV negative.

Bone marrow involvement is uncommon in LyG. When involvement is present, nonparatrabecular atypical lymphoid infiltrates can be seen. Hemophagocytic syndrome has been reported and manifests similarly in other settings.

#### Immunophenotype

The background small lymphocytes of LYG are $CD3^+$ T-cells. $CD4^+$ and $CD8^+$ cells are present, with $CD4^+$ cells predominating. The large atypical cells are $CD20^+$ B-cells that express EBV by EBER in situ hybridization and EBV LMP1 by immunohistochemistry. CD30 may be expressed, but CD15 is absent.

#### Molecular Genetics

Immunoglobulin gene rearrangement can be demonstrated by PCR in most cases of grade II and III LyG. Less consistent documentation of a B-cell clone is seen in grade I lesions. When multiple lesions are tested for clonality, different clones may be found, analogous to what may occur in posttransplantation lymphoproliferative disorders. T-cell receptor gene rearrangement is usually negative.

## Differential Diagnosis

Wegener's granulomatosis is in the differential diagnosis of LyG; it has a clinical presentation similar to LyG with constitutional symptoms and involvement of the skin and kidney. The former often involves the upper respiratory tract, unlike LyG, which involves the lower respiratory tract. Histologically, both can have an inflammatory infiltrate with necrosis. Well-formed necrotizing granulomata are not usually seen in LyG, and the presence of large atypical immunoblast or Hodgkin-like $CD20^+$

B-cells favors LyG. Likewise, EBV-positive atypical cells are seen in LyG. Finally, demonstration of monoclonal *IGH@* rearrangement is characteristic of higher-grade LyG.

Many older series of LyG likely included cases of extranodal, NK/T-cell lymphoma, nasal type. This lymphoma manifests as an EBV-positive angiocentric and angiodestructive lymphoproliferative disorder and it has similar features to LyG; however, it usually involves the nasal cavity as opposed to the lung. Extranodal NK/T-cell lymphoma, nasal type cells are cytologically atypical, as opposed to the T-cells in cases of LyG. Large atypical EBV-positive B-cells are not a feature of extranodal NK/T-cell lymphoma, nasal type.

### Therapy and Prognosis

The clinical course of LyG is variable. A waxing and waning course can be seen with spontaneous recovery in some cases. Patients with higher-grade lesions follow an aggressive clinical course, with median survival of less than 18 months. Some patients progress to overt DLBCL. Single-agent (cyclophosphamide) or combination immunotherapy and anthracycline-containing multiple-agent chemotherapy (R-CHOP) have been used to treat LyG. Because of the paucity of studies, clear guidelines are lacking. Given the presumed immunodeficiency state, immunomodulatory therapies (interferon-α 2b) have been attempted with some success.

## Plasmablastic Lymphoma

### Clinical Features

Plasmablastic lymphoma (PBL) is a rare lymphoma associated with immunosuppression. It occurs most frequently in HIV-infected patients, but is also seen in patients receiving immunosuppressive therapy, the elderly with age-associated immunosuppression, and rarely immunocompetent patients. PBL is primarily a disease of adults, affecting men more often than women. In the setting of HIV infection, the most frequent site of involvement is the oral cavity. It also affects other extranodal sites with a predilection for mucosal tissues, including the sinonasal cavities, orbit, respiratory and gastrointestinal tracts, skin, and bone. Lymph node involvement is uncommon and is more frequent in immunocompetent patients. In the majority of patients, PBL appears at an advanced stage with an intermediate- to high-risk IPI.

**PLASMABLASTIC LYMPHOMA—FACT SHEET**

**Clinical Features**
- Rare disorder associated with immunosuppression (HIV, iatrogenic, age-associated)
- Affects men more than women
- Appears at high stage

**Morphology**
- Sheets of large immunoblast or plasmablast-like cells
- Mature plasma cells may be admixed

**Immunophenotype**
- $CD45^-$, $CD3^-$, $CD20^-$, $CD38^+$, $CD79a^{+/-}$, $CD138^+$, $BCL6^{-/+}$, $PAX5^-$, $MUM1^+$, $EMA^{+/-}$, $clg^{+/-}$, $CD30^{-/+}$, $EBV^+$, $HHV8^-$, $ALK^-$

**Molecular Genetics**
- Clonally rearranged immunoglobulin genes
- Approximately 50% of cases associated with *MYC* rearrangement, usually t(8;14)

**Prognosis and Therapy**
- Anthracycline-containing combination chemotherapy
- Highly active anti-retroviral therapy for HIV
- Median survival, 8 to 14 months

**Differential Diagnosis**
- Plasmacytoma
- Primary effusion lymphoma
- $ALK^+$ diffuse LBCL

### Pathologic Features

#### Histopathology

Plasmablastic lymphoma infiltrates in a diffuse pattern. The neoplastic cells are large and atypical with immunoblastic, plasmablastic, or plasmacytic features, including eccentric nuclei, prominent central or peripheral nucleoli, basophilic cytoplasm, and a perinuclear hof (Figure 8-15). Mature-appearing plasma cells may also be present. Mitotic figures, apoptotic bodies, and tingible body macrophages are also featured.

#### Immunophenotype

The tumor cells of PBL exhibit features of terminally differentiated B-cells and express antigens in a pattern similar to plasma cells. They are invariably positive for CD38, CD138, and MUM1; variably positive for CD79a, cytoplasmic Ig, EMA, and CD30; and usually negative for CD45, CD20, and PAX5, although these antigens are occasionally weakly expressed. BLIMP1 and XBP-1, markers of terminal B-cell differentiation, are also usually expressed. These two markers are not often

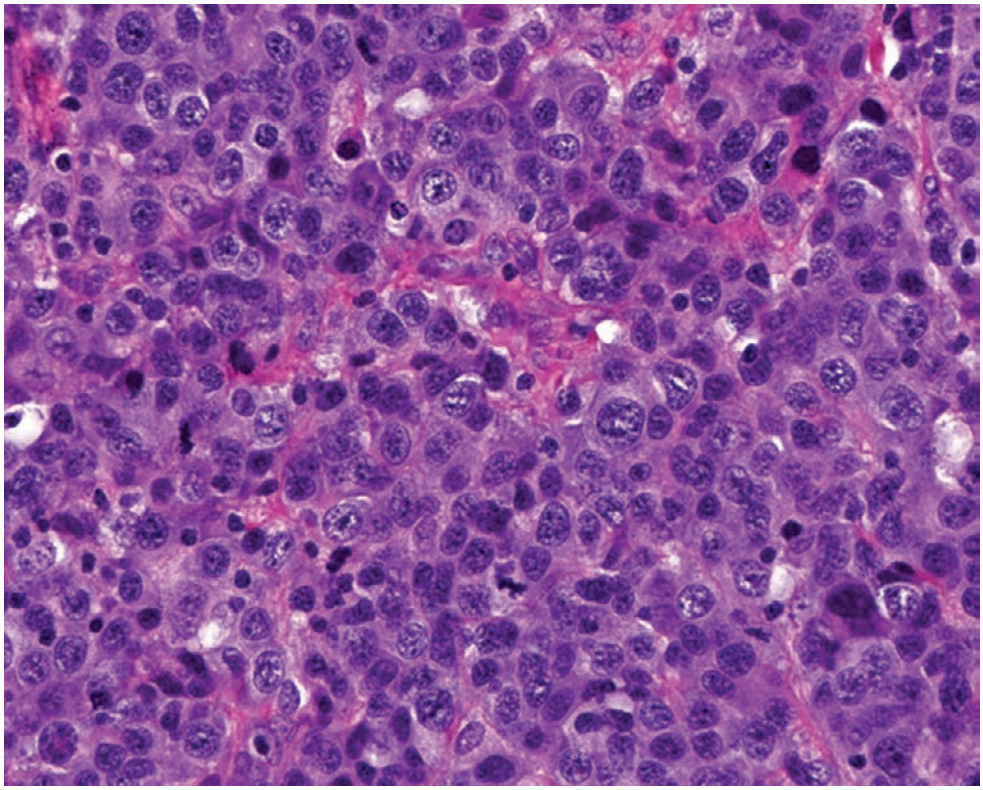

**FIGURE 8-15**

Plasmablastic lymphoma. The malignant lymphocytes are large and atypical with plasmacytic features.

routinely available but further support the plasmacytic differentiation of these lymphomas. PBL usually does not express CD56, distinguishing it from true plasma cell neoplasms. The proliferation index is in excess of 90%, and approximately 70% to 75% of cases are associated with EBV infection in a type I latency pattern (best detected by in situ hybridization for EBER). HHV8 infection is not present.

### Molecular Genetics

Plasmablastic lymphoma exhibits monoclonal rearrangement of the *IGH@* gene. *MYC* gene rearrangement is a recurring secondary genetic abnormality in PBL, occurring in approximately 50% of cases. The translocation partner is usually *IGH@*. It is seen in the setting of a complex karyotype; however, rearrangements of *BCL2* and *BCL6* are not usually observed and PBL is not considered part of the spectrum of "double-hit" lymphomas.

## Differential Diagnosis

The differential diagnosis includes other lymphoid neoplasms with plasmacytic features such as ALK-positive DLBCL, PEL, and plasmacytoma. PBL is distinguished from ALK-positive DLBCL by its lack of expression of the ALK protein, and absence of HHV8 co-infection distinguishes PBL from primary effusion lymphoma. Differentiation from plasmacytoma can be challenging. Features that help distinguish PBL from plasmacytoma include its association with an immunodeficient state and the presence of EBV infection. In addition, the identification of a *MYC* gene rearrangement can help to distinguish PBL from plasmacytoma, as *MYC* rearrangement is rare in the latter disorder.

## Prognosis and Therapy

PBL is an aggressive lymphoma and a standard therapy has not yet been established. Treatment usually consists of chemotherapy with various regimens including CHOP, R-CHOP, and cyclophosphamide, vincristine, doxorubicin, high dose methotrexate/ifosfamide, etoposide, high-dose cytarabine (CODOX-M/IVAC). The use of a rituximab-containing regimen is of uncertain utility if the neoplastic cells do not express CD20. In the HIV-infected population, the addition of highly active antiretroviral therapy also improves prognosis; however, overall survival is short despite therapeutic intervention, with a median survival of less than 8 to 14 months.

## BURKITT LYMPHOMA

### Clinical Features

Burkitt lymphoma (BL) is a highly aggressive, mature B-cell neoplasm occurring primarily in children and young adults. It can manifest as a lymphomatous mass or occasionally as a leukemic process. In former classifications it was considered a form of acute leukemia (e.g., French-American-British Classification L3 ALL); however, it is no longer considered in the spectrum of acute leukemias. Three clinical variants are recognized: endemic, sporadic, and immunodeficiency-associated BL. The latter is treated in detail in Chapter 10.

Endemic BL occurs principally in equatorial Africa and Papua, New Guinea. In Africa it is the most common childhood malignancy and was first described in detail by Dennis Burkitt in 1958. It is seen in early childhood (4 to 7 years old) with a male predominance and has a predilection for extranodal involvement of the jaw, abdomen, orbit, and paraspinal region. The association with EBV infection is strong and is seen in essentially all endemic cases. The sporadic type of BL is seen throughout the world, including Europe and North America. It occurs mainly in children and young adults and decreases in frequency with increasing age. It is a relatively rare lymphoma, accounting for 1% of all lymphoma in adults. In children, however, it is among the most common types. Sites of involvement include the abdomen, nasopharynx, and bone marrow. Lymph nodes are not frequently involved in younger patients, but are more frequently involved in older patients.

**BURKITT LYMPHOMA—FACT SHEET**

**Clinical Features**
- Appears most commonly in children and young adults
- Rapidly enlarging mass, extranodal sites common
- Endemic (African), sporadic, and immunodeficiency-related types

**Morphology**
- Starry-sky pattern
- Intermediately sized cells (small noncleaved cells)
- Numerous mitoses
- Characteristic imprint morphology with abundant basophilic cytoplasm and vacuoles (FAB-L3 morphology)

**Immunophenotype**
- $CD19^+$, $CD20^+$, $CD10^+$, $BCL6^+$, $CD5^-$, $BCL2^-$, surface immunoglobulin positive
- Ki-67, approximately 100%

**Molecular Genetics**
- t(8;14)(q24;q32), t(8;22)(q24;q11), or t(2;8)(p12;q24)
- No rearrangement of *CCND1, BCL6,* or *BCL2*
- Noncomplex karyotype

**Prognosis and Therapy**
- Aggressive, rapidly growing lymphoma
- High-intensity, short-duration therapy such as hyper-CVAD used
- Complete remission possible in 75% to 90%

### Pathologic Features

#### Histopathology

The typical morphologic feature of BL is a diffuse infiltrate of monomorphous, medium-sized cells. At low magnification there is a starry-sky pattern, which refers to the mottled or moth-eaten appearance produced by the pale tingible body macrophages scattered throughout the lymphomatous infiltrate. These macrophages contain cellular debris produced from phagocytosis of apoptotic lymphoma cells. Rarely, a follicular pattern may be seen, possibly representing colonization of preexisting reactive germinal centers. The cells are monotonous, with nuclei that are approximately the size of benign histiocyte nuclei. The chromatin is vesicular and there are multiple small basophilic nucleoli (Figure 8-16). Cytoplasm is basophilic and moderate in amount. The cells often sit slightly apart from one another, imparting a squared-off or cobblestone pattern. The imprint morphology is characteristic and consists of blastic cells with moderate amounts of deeply basophilic cytoplasm with punched-out vacuoles that contain lipid, which can be demonstrated with oil-red O staining. Vacuoles are not specific and can be seen in non-Burkitt large-cell lymphomas and are particularly frequent in anaplastic large-cell lymphoma. Variant morphologies have been described. Patients with HIV may have more variation in cell size and plasmacytic features. Cases with slightly more morphologic heterogeneity but otherwise exhibiting the immunophenotypic and molecular genetic features of BL should still be diagnosed as BL.

#### Immunophenotype

BL cells express the pan B-cell markers CD19, CD20, and CD79a. They are positive for cytoplasmic IgM and surface Igs, and they universally express CD10 and BCL6 and lack BCL2 in the vast majority of cases. Some cases may show weak and focal expression of BCL2. BL cells do not express MUM1 or cyclin-D1. Cases that are associated with EBV (endemic or sporadic) are positive for ENBA-1 and EBER but lack LMP-1 and EBNA-2, which is consistent with the type I latency pattern of EBV infection. Ki-67 staining shows that almost 100% of tumor cells are positive, reflecting the underlying biology of MYC overexpression driving tumor cells into

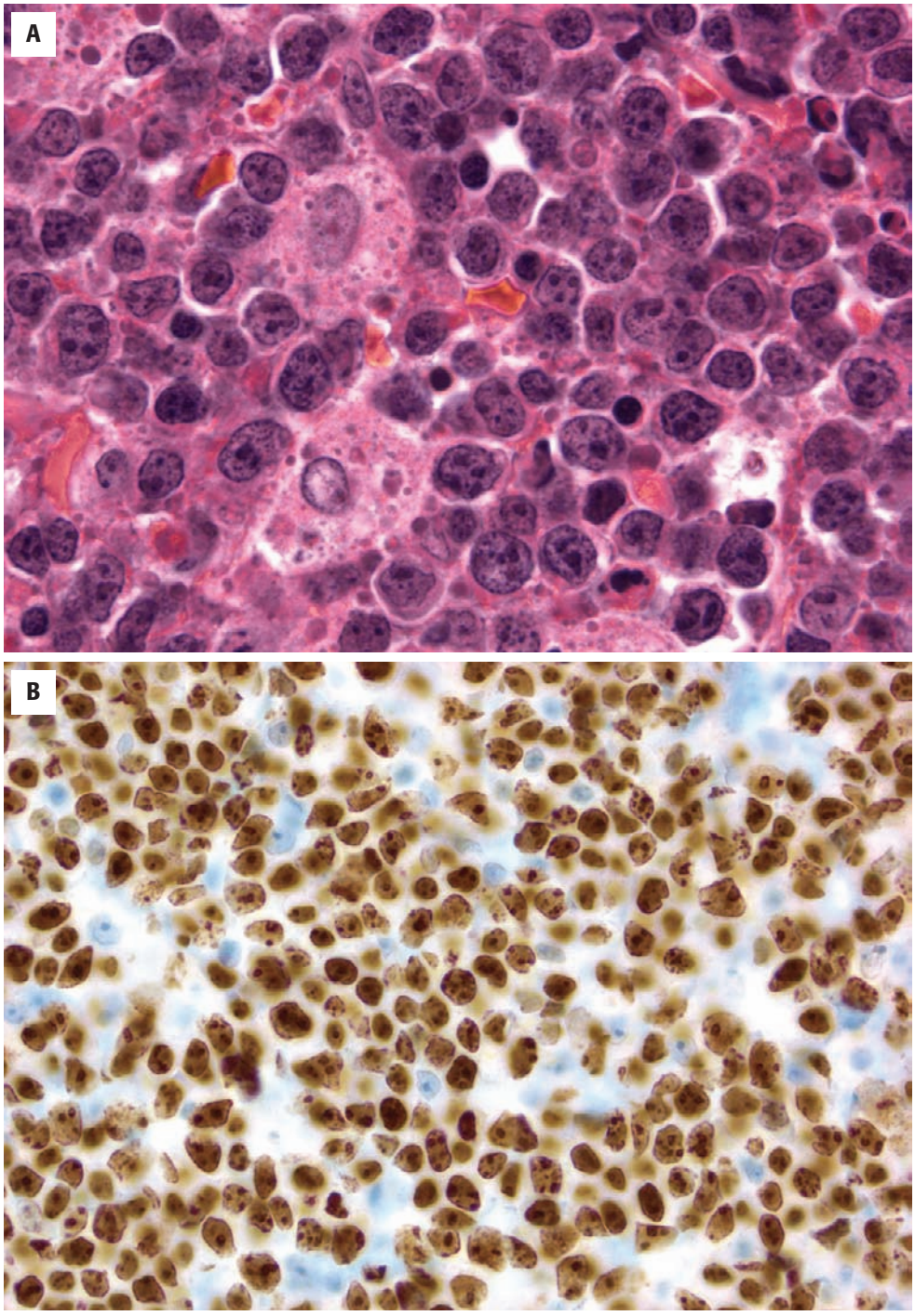


**FIGURE 8-16**

Burkitt lymphoma. **A,** The cells are monotonous intermediate-sized cells (similar in size to a benign histiocyte) with multiple inconspicuous nucleoli. The characteristic immunophenotype is $CD10^{-}$, BCL2-negative. **B,** The Ki-67 labeling index approaches 100% because of the presence of *MYC* deregulation.

cell cycle. Predominantly nuclear localization of MYC protein by immunohistochemistry and expression of the pre–B-cell receptor protein VpreB3 is seen in the vast majority of BL and may also be useful for detecting *MYC* translocations in DLBCL and B-cell lymphoma unclassifiable, with features intermediate between DLBCL and BL that harbor *MYC* translocation.

### Molecular Genetics

Regardless of the clinical variant, translocation of *MYC* (chromosome 8q24) is present and is considered the primary transforming event. One of three partner genes is involved: Ig heavy chain (*IGH@*) at chromosome 14q32, κ light chain (*IGK@*) at chromosome 2p11,

or λ light chain (*IGL@*) at chromosome 22q11. In most cases, fluorescence in situ hybridization can be used to detect these translocations. Cryptic and variant *MYC/IGH@* translocations are described that cannot be detected using standard *MYC* break-apart probes alone. To prevent misdiagnosis, fusion probes that target the specific translocations should be used to confirm the presence of a *MYC* translocation. Rarely, *MYC* rearrangement cannot be demonstrated. Some cases may represent false-negative fluorescence in situ hybridization studies. Most cases are true-negatives. and there is evidence that BL cases without *MYC* rearrangement harbor alternate pathways for MYC deregulation with some studies pointing towards altered expression of micro RNAs involved in MYC protein expression Further research is required to fully characterize these cases at the molecular level. Cases without demonstrable *MYC* rearrangement that otherwise meet the diagnosis criteria for BL should still be diagnosed as BL.

There is considerable heterogeneity in the *MYC* breakpoint in BL. In the t(8;14), breakpoints are 5′ to *MYC*, whereas in the t(2;8) and the t(8;22) the breakpoints are 3′ to *MYC*. In the 5′ breakpoint of the t(8;14) there is a propensity for the breakpoint to be far upstream (>100 kB) from *MYC* and also to involve the J (joining) region of *IGH@*; this is seen in endemic BL. In sporadic cases, the breakpoint is immediately 5′ to *MYC* and involves the switch regions of *IGH@*. Translocations involving the first exon or intron of *MYC* are designated class I; those involving the immediately upstream region as class II; and those far upstream as class III (Table 8-4).

While *MYC* rearrangement is a diagnostic feature of BL it is not specific for the disease as other B-cell non-Hodgkin lymphomas, including mantle cell lymphoma, follicular lymphoma, and DLBCL (DLBCL) can harbor *MYC* rearrangements. In the latter group of lymphomas, *MYC* rearrangement is often a secondary genetic event related to disease transformation. Detection of other recurring genetic abnormalities, including rearrangement of the *CCDN1*, *BCL2,* and *BCL6* genes precludes a diagnosis of BL.

Secondary genetic changes are common BL, but are usually fewer than two in number and complex karyotypes are unusual. The most frequently observed recurring abnormalities include gains at chromosomes 1q, 7, and 12 and losses of 6q, 13q32-34, and 17p.

Gene expression profiling has identified a unique molecular profile in BL that predicts for good response to therapy and distinguishes BL from DLBCL. Interestingly, some cases with the BL molecular profile meet the diagnostic criteria for DLBCL by morphology, immunophenotype, and genetic features, whereas some cases that satisfy the criteria for a diagnosis of BL lack the BL molecular profile and have a worse clinical outcome. This finding serves to highlight the imprecise nature of the current diagnostic criteria in BL.

## Differential Diagnosis

The differential diagnosis includes other diffuse aggressive B-cell lymphomas, such as B-cell precursor lymphoblastic lymphoma, blastoid variant of mantle cell lymphoma, and de novo or transformed DLBCL. The diagnostic features of these various lymphomas are listed in Table 8-5. The first two entities are easily distinguished from BL by morphologic and immunophenotypic features. Lymphoblastic lymphoma, like BL, is often composed of medium-sized cells, but it exhibits more evenly dispersed, fine, blastic chromatin. Unlike BL, lymphoblastic lymphoma expresses TdT or CD34, or both, in almost all cases and lacks surface Ig. Blastoid mantle cell lymphoma can mimic BL morphologically, but differs from BL by its expression of CD5 and cyclin-D1.

The main differential diagnostic consideration in BL is DLBCL. DLBCL is often composed of large atypical cells with centroblastic or immunoblastic morphology, but is occasionally composed of medium-sized, centroblast-like cells resembling BL. Failure of the lymphoma to express CD10, expression of BCL2, or a Ki-67 proliferation index less than 95% favors DLBCL and helps to exclude BL. Absence of a *MYC* rearrangement, or a *MYC* rearrangement in the setting of a complex karyotype, also helps to exclude BL. Rarely, it may not be possible to differentiate between BL and DLBCL.

**TABLE 8-4**
**Genetic Features of Burkitt Lymphoma**

| | Endemic (African) | Sporadic | HIV-Associated |
|---|---|---|---|
| ***Class*** | III | I and II | I |
| MYC breakpoint | >100 kB 5′ of *MYC* | Located in first exon/intron or immediately 5′ of *MYC* | Within first exon/intron of *MYC* |
| *IGH@* breakpoint | Predominantly J region | Predominantly Switch region | Predominantly Switch region |

**TABLE 8-5**
**Differential Diagnosis of Burkitt Lymphoma**

| Lymphoma Type | Histology | Immunophenotype | Proliferation Index | Molecular Genetics |
|---|---|---|---|---|
| Burkitt lymphoma | Intermediately sized monotonous cells with multiple small nucleoli, starry-sky pattern | $CD19^+$, $CD20^+$, $CD5^-$, $CD10^+$, $BCL2^-$, cyclin $D1^-$, $TdT^-$ | ≈100% | *MYC* rearrangement in all cases |
| DLBCL (de novo or transformed B-cell non-Hodgkin lymphoma) | Large centroblastic, immunoblastic, or intermediately sized, small centroblastic cells | $CD19^+$, $CD20^+$, $CD5^{-/+}$, $CD10^{+/-}$, $BCL2^+$, cyclin $D1^-$, $TdT^-$ | <90% | *MYC* rearrangement (10%), t(14;18) (20%), *BCL6* abnormality (20%) |
| Blastoid MCL | Intermediately sized cells resembling lymphoblasts | $CD19^+$, $CD20^+$, $CD5^+$, $CD10^-$, $BCL2^+$, cyclin $D1^+$ | <90% | t(11;14) in virtually 100% of cases |
| Lymphoblastic lymphoma | Lymphoblasts | $CD19^+$, $CD20^{-/+}$, $CD5^-$, $CD10^+$, $BCL2^+$, cyclin $D1^-$, $TdT^+$ | <90% | Lacks *MYC* rearrangement |

*DLBCL,* Diffuse large B-cell lymphoma; *MCL,* mantle cell lymphoma.

These cases warrant a diagnosis of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (see later discussion).

### Therapy and Prognosis

Therapy consists of aggressive, short-duration chemotherapeutic regimens with CNS prophylaxis. The high-intensity, short-duration strategy is needed because of the high growth fraction and the need to avoid the potential for rapid growth and resistance development between chemotherapy cycles. It is beyond the scope of this chapter to review therapeutic protocols. However, common regimens include CODOX-M/IVAC and hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, and cytarabine). Complete remissions have been reported in 75% to 90% of patients, with overall survival at 2 years of greater than 70% in some studies. Relapses typically occur within 1 year of treatment.

## B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND BURKITT LYMPHOMA

B-cell lymphoma, unclassifiable (BCLU), with features intermediate between DLBCL and Burkitt lymphoma is a new diagnostic category included in the most recent (2008) World Health Organization classification of lymphoid tumors. This category has its basis in gene array studies recognizing cases with genetic and histopathologic features that were intermediate between DLBCL and BL. In the past, diseases that meet the criteria for inclusion in this category have been called *atypical Burkitt lymphoma, Burkitt-like lymphoma,* or *gray-zone lymphoma*. This diagnostic category does not represent a well-defined clinicopathologic entity, but rather a heterogeneous group of de novo or transformed high-grade B-cell lymphomas that cannot be classified more accurately, because they do not meet the diagnostic criteria required for BL or DLBCL.

### Clinical Features

BCLU is a rare disease. It is usually diagnosed in older adult patients, with men and women affected equally. Patients exhibit a rapidly enlarging mass involving lymph nodes or extranodal sites. There is no predilection for any particular body site. Advanced-stage disease is more common than in BL, and bone marrow involvement is often seen.

### Pathologic Features

#### Histopathology

BCLU is a morphologically heterogeneous disease. Some cases closely resemble BL and are composed of a monotonous population of medium-sized cells with vesicular chromatin, small inconspicuous nucleoli, and moderate basophilic cytoplasm. These cases usually

**B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND BURKITT LYMPHOMA—FACT SHEET**

**Clinical Features**
- Appears in older patients with advanced stage
- Rapidly enlarging mass
- Highly aggressive disease

**Morphology**
- Burkitt-like morphology
- DLBCL-like morphology

**Immunophenotype**
- Burkitt lymphoma-like: $CD20^+$, $CD10^+$, $BCL6^{+/-}$, $BCL2^+$, MIB1 < 95%
- DLBCL-like: $CD20^+$, $CD10^+$, $BCL6^+$, $BCL2^-$, MIB1 approximately 100%

**Molecular Genetics**
- Clonally rearranged immunoglobulin genes
- *MYC* rearranged in most cases
- Complex karyotype common
- Concurrent *BCL6* and/or *BCL2* rearrangement (double-hit lymphoma)

**Prognosis and Therapy**
- Resistant to multiple-agent chemotherapy
- Poor prognosis
- Double-hit lymphoma survival measured in months

have one or more immunophenotypic or karyotypic abnormalities that are not consistent with BL and preclude its diagnosis (see later). Conversely, other cases are reminiscent of DLBCL with greater nuclear pleomorphism and increased numbers of large atypical cells within the infiltrate; however, these cases usually have an immunophenotype or karyotype that is consistent with BL.

#### Immunophenotype

All cases of BCLU are composed of B-cells that express the pan B-cell antigens CD20 and CD79a and lack TdT. Cases of BCLU that morphologically resemble BL usually have an immunophenotype that is inconsistent with BL and precludes its diagnosis. Typical inconsistent features include strong expression of BCL2, absence of BCL6 expression, and a proliferation index less than 95% . Cases of BCLU that are morphologically in keeping with DLBCL must express CD10 and BCL6, lack expression of BCL2, and have a proliferation index of approximately 100% . If the latter group does not express this immunophenotype, a diagnosis of DLBCL is appropriate.

#### Molecular Genetics

Lymphomas in this category show monoclonal rearrangement of the *IGH@* gene. As discussed in the previous section, rearrangement of the *MYC* gene is the genetic hallmark of BL. Cases that lack an *MYC* rearrangement, but are morphologically and phenotypically consistent with BL, should still be diagnosed as BL if other genetic abnormalities are not present (see later discussion). Cases with a *MYC* rearrangement that are otherwise consistent with DLBCL should be diagnosed as DLBCL and not BCLU, because *MYC* rearrangement can be seen in a subset of DLBCL. Indeed, such cases are more common in routine practice than de novo BL cases. In BCLU, *MYC* rearrangement is common but shows features not typical for BL. These features include *MYC* rearrangement with non-Ig partner genes, complex karyotype, and concurrent rearrangement of the *BCL2* or *BCL6* genes, or both. Cases with the latter abnormality are often referred to as *double-hit* or *triple-hit lymphomas*.

The best characterized double-hit lymphomas are those with *MYC* and *BCL2* rearrangement (Figure 8-17). Although common among the BCLU category, the combination of these genetic abnormalities has been described in cases of DLBCL and B-cell lymphoblastic lymphoma. These lymphomas express BCL2, CD10, and BCL6, prompting some to suggest that neoplasms harboring this abnormality represent an aggressive variant of transformed follicular lymphoma. Regardless, these tumors are associated with aggressive disease, and patients exhibit advanced-stage including involvement of extranodal sites, bone marrow, and the CNS.

## Differential Diagnosis

The main differential diagnostic considerations are BL and DLBCL. Close attention to morphologic, immunophenotypic, and genetic features and application of the diagnostic criteria previously described in this chapter will help in making an accurate diagnosis. Cases with double or triple hits as described previously are, by definition, included in this BCLU category. In general, cases that do not fulfill the diagnostic criteria for either DLBLC or BL but have a combination of morphologic, phenotypic, and genetic features of between the two of these two disease entities are appropriate for inclusion

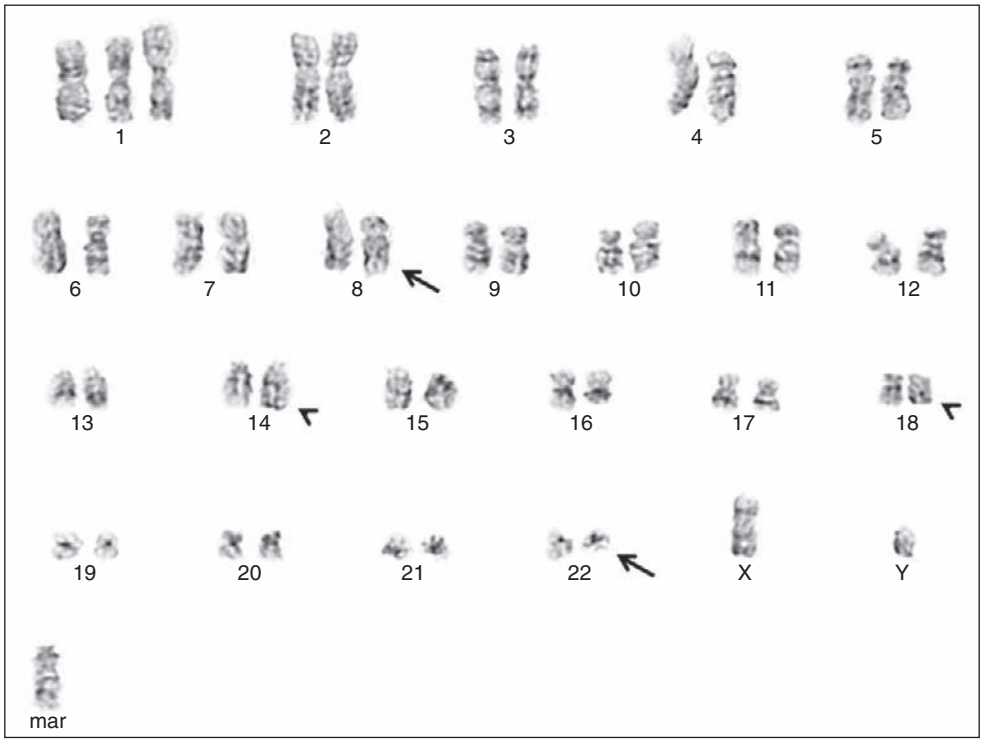


**FIGURE 8-17**

Double-hit genetic abnormality. Complex karyotype with a double-hit abnormality including a t(8;22) *MYC* rearrangement *(arrows)* and t(14;18) *BCL2* rearrangement *(arrowheads)*.

in the BCLU category. In the future, better definitions based on gene expression or other genetic data may be forthcoming and refine or replace this category.

## Therapy and Prognosis

These lymphomas are aggressive, and a standard approach to therapy has not yet been established. Double-hit cases are usually refractory to multiple-agent chemotherapy, and patient survival is measured in the order of months.

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The complete reference list is available online at www.expertconsult.com.

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9

# Peripheral T-Cell Lymphomas

**William R. Macon, MD**

## INTRODUCTION

Peripheral T-cell lymphomas (PTCLs) are much less common than B-cell lymphomas, and they constitute approximately 10% of non-Hodgkin lymphomas (NHLs) in the United States and Europe. PTCLs are derived from post-thymic T cells and generally arise in lymphoid tissues "peripheral" to the thymus, such as the lymph nodes, spleen, gastrointestinal tract, and skin. PTCLs have a mature T-cell phenotype and occur most frequently in adults. Because natural killer (NK) cells and T cells arise from a common progenitor cell and have some overlapping properties, NK cell lymphomas are considered together with PTCLs in the World Health Organization (WHO) classification. The mature T- and NK-cell neoplasms can be grouped according to clinical presentation: leukemic, nodal, and extranodal. This chapter addresses the nodal and extranodal PTCLs and NK cell lymphomas recognized in the WHO classification.

## ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

Angioimmunoblastic T-cell lymphoma (AILTCL) is primarily a nodal type of PTCL. It was originally described in the early 1970s as a non-neoplastic abnormal immune reaction, variously termed *angioimmunoblastic lymphadenopathy with dysproteinemia, immunoblastic lymphadenopathy, lymphogranulomatous X,* and *immunodysplastic disease*. The disease was characterized by generalized lymphadenopathy, fever, hypergammaglobulinemia, various autoimmune phenomena, and often fatal outcome. Later, an immunoblastic lymphadenopathy–like malignant lymphoma was described and suggested angioimmunoblastic lymphadenopathy with dysproteinemia was actually a type of PTCL. Subsequently, it has been shown that most cases of angioimmunoblastic lymphadenopathy with dysproteinemia had clonal rearrangements of T-cell receptor (TCR) genes and also had nonrandom chromosomal abnormalities. Therefore lymphoproliferation with features of angioimmunoblastic lymphadenopathy with dysproteinemia are generally accepted as being de novo AILTCL in the WHO classification. The subject of whether a benign counterpart of AILTCL truly exists remains controversial.

### Clinical Features

Patients with AILTCL typically are middle-aged to elderly adults with a systemic illness characterized by generalized lymphadenopathy, fever, weight loss, hepatosplenomegaly, skin rash, and polyclonal hypergammaglobulinemia. There is a nearly equal incidence between genders. Most patients have stage III or IV disease at presentation. Autoimmune hemolytic anemia is seen in up to 45% of cases. AILTCL is moderately aggressive with median survival of 1 to 2 years.

Progression to a more aggressive tumor with immunoblastic morphology occasionally occurs. Such transformations are usually of the T-cell phenotype, but occasionally may be of B-cell lineage.

### Pathologic Features

#### Microscopic Findings

The nodal architecture is usually effaced by a diffuse lymphoproliferation that may extend beyond the capsule into the pericapsular soft tissue (Figure 9-1). Follicular centers are generally absent and contain an abnormal proliferation of follicular dendritic cells (FDCs). One of the most striking features of AILTCL is the proliferation of branching high endothelial venules that are accentuated by periodic acid–Schiff with hematoxylin staining. The lymphoid infiltrate may appear hypocellular and consists of a mixture of small lymphocytes and immunoblasts, the latter having clear cytoplasm and clustering around vessels. Plasma cells, eosinophils, and

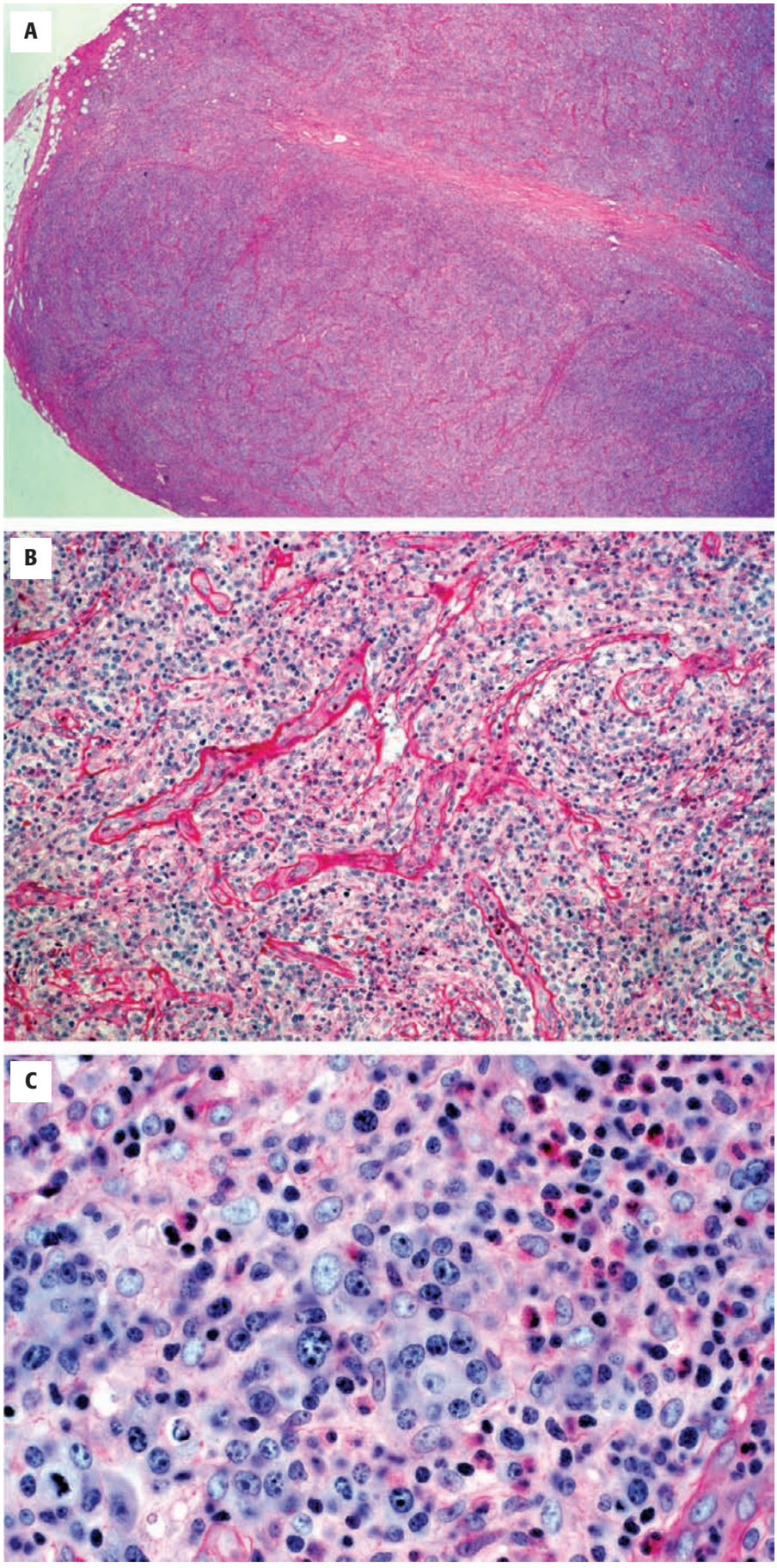


**FIGURE 9-1**

Angioimmunoblastic T-cell lymphoma: microscopic features. **A,** At low magnification, the lymph node architecture is completely effaced by a diffuse lymphoproliferation that appears hypervascular in this periodic acid-Schiff stain. **B,** At intermediate magnification, the branching pattern of the numerous high endothelial venules can be appreciated in this periodic acid-Schiff stain. **C,** At high magnification, clusters of immunoblasts with clear cytoplasm are present within a polymorphic reactive cell background that includes frequent eosinophils.

**ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA—FACT SHEET**

**Definition**
- Nodal T-cell lymphoma characterized by clusters of T-cell immunoblasts, hypervascularity, polymorphic inflammatory cell infiltrate, and proliferated FDC networks

**Incidence and Location**
- Uncommon (1% to 2% of all NHLs but 15% to 20% of peripheral T-cell lymphomas)
- Approximately 1000 new cases per year in the United States

**Morbidity and Mortality**
- Median survival: 1 to 2 years

**Gender and Age Distribution**
- Middle-aged to elderly adults
- No gender predilection

**Clinical Features**
- Generalized lymphadenopathy (stage III or IV disease), hepatosplenomegaly, and skin rash
- B symptoms
- Polyclonal hypergammaglobulinemia and autoimmune hemolytic anemia

**Prognosis and Therapy**
- Moderately aggressive disease with fair prognosis
- Chemotherapy

**ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Partially to completely effaced nodal architecture
- Diffuse lymphoproliferation with clusters of neoplastic T-cell immunoblasts, often in a perivascular distribution
- Proliferation of high endothelial venules
- Disrupted and proliferated FDC networks
- Polymorphic reactive cell background

**Immunophenotypic Findings**
- $CD4^+$ T-cell immunoblasts that often have aberrant phenotype
- CD10, CD279, and CXCL13 frequently expressed
- CD21, CD35, and clusterin staining of disrupted and proliferated FDC networks

**Molecular and Cytogenetic Findings**
- Clonal TCR gene rearrangements in 75% of cases
- EBV genome–positive B-cell immunoblasts in background
- No recurring chromosomal translocations, but trisomies 3 and 5, an additional X chromosome, and 1p alterations common
- Derivation from follicular T-helper cells

**Differential Diagnosis**
- T cell–rich large B-cell lymphoma variant of diffuse large B-cell lymphoma
- Mixed cellularity Hodgkin lymphoma
- Florid reactive processes

histiocytes are often present in the background. Occasionally, clusters of epithelioid histiocytes impart a granulomatous appearance. This combination of morphologic features is most commonly seen in AILTCL and is regarded as pattern III. Pattern I has partially preserved architecture, hyperplastic follicles with poorly preserved mantles, and inconspicuous neoplastic cells distributed around the follicles. Pattern II has depleted or "burnt out" lymphoid follicles. FDCs are normal to only slightly increased in patterns I and II.

#### Immunophenotype

The neoplastic clear cells of AILTCL express the pan T-cell antigens CD2, CD3, CD5, and CD7 (Figure 9-2); however, aberrant loss of one or more of these antigens is common. The tumor cells demonstrate a $CD4^+$ (T-helper cell) phenotype and often express CD10. Recently, the chemokine CXCL13 has been shown to be expressed in 90% of AILTCL, but only in 10% of peripheral T-cell lymphoma, NOS. Disrupted and expanded FDC networks can be recognized using antibodies to CD21, CD35, and clusterin, a feature that helps to distinguish AILTCL from other PTCLs. Most AILTCLs are derived from noncytotoxic αβ T cells. Their coexpression of CXCL13, CD10, PD-1 (CD279), and inducible costimulator (ICOS), as well as their association with expanded FDC networks suggest an origin from follicular T-helper (TFH) cells.

#### Molecular and Cytogenetic Findings

Approximately 75% of cases of AILTCL will demonstrate clonal rearrangement of TCR genes. Many cases will also show an oligoclonal or small clonal population of B cells. Indeed, dominant B-cell clones in the absence of obvious DLBL (see later discussion) have been reported in AILTCL, reflecting the immune dysregulation present in these patients. Trisomies 3 and 5, an additional X chromosome, and 1p alterations are the karyotypic abnormalities found most frequently; however, no single translocation is associated with the majority of cases. Gene expression profiling has shown a strong contribution by non-neoplastic cell constituents with overexpression of B-cell– and FDC-related genes and genes related to extracellular matrix and vascular biology. The tumor cell signature appears enriched in genes normally expressed by TFH cells, which has further established a TFH-cell derivation for AITCL.

Epstein-Barr virus (EBV) genomes are detected in many cases, and they appear to be in B-cell

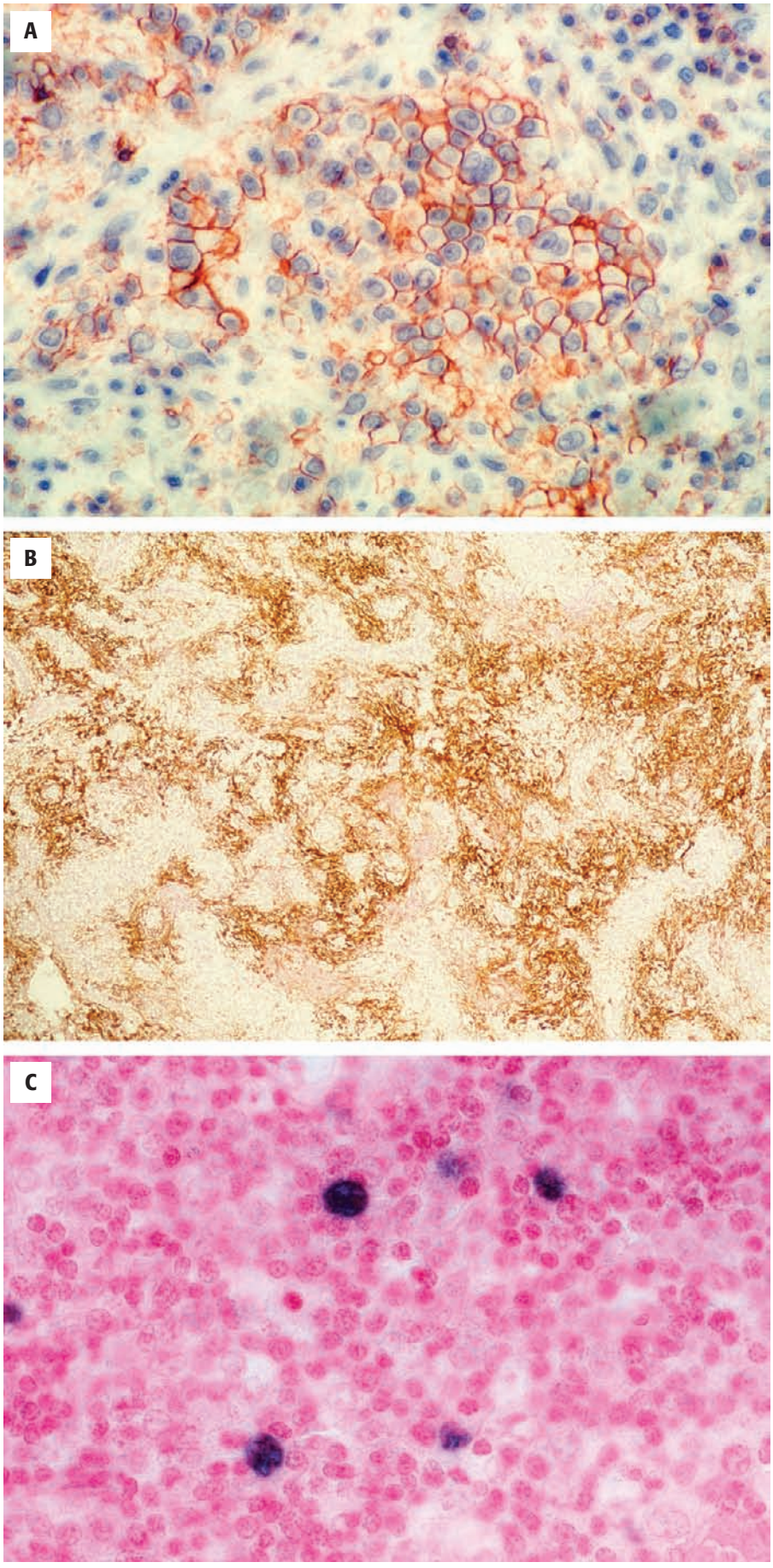


**FIGURE 9-2**

Angioimmunoblastic T-cell lymphoma: immunophenotypic features and in situ hybridization. **A,** $CD3^+$ T-cell immunoblasts are easily identified in this paraffin immunoperoxidase stain. **B,** Disrupted and proliferated CD21-staining follicular dendritic cell networks stand out in this paraffin immunoperoxidase stain. **C,** A background of Epstein-Barr virus (EBV) genome–positive immunoblasts is detected by in situ hybridization using probes for EBV-encoded RNA.

immunoblasts (see Figure 9-2). A specific role for EBV in the pathogenesis of AILTCL remains unproven.

### Differential Diagnosis

AILTCL must be distinguished from the T-cell–rich large B-cell lymphoma variant of diffuse large B-cell lymphoma, mixed cellularity classical Hodgkin lymphoma, and some florid reactive lymph nodes. T-cell–rich large B-cell lymphomas do not have clusters of clear T-cell immunoblasts and may be immunoglobulin light chain restricted. A diagnosis of T-cell–rich large B-cell lymphoma may require confirmation by demonstrating clonal immunoglobulin gene rearrangements in the absence of T-cell clonality. Mixed cellularity classical Hodgkin lymphoma will generally show classic Reed-Sternberg cells or Reed-Sternberg cell variants that are $CD45^-$, $CD15^+$, $CD30^+$, and fascin positive; the lack of proliferated FDC networks may also be a helpful distinguishing feature. Both florid reactive processes and AILTCL can show an expanded T zone, paracortical area, or vascular proliferation. Reactive processes generally show preserved germinal centers and no evidence of abnormal FDC networks. In contrast, AILTCL will usually show loss of or burnt-out germinal centers plus abnormal FDC networks. Reactive processes generally lack clonal TCR gene rearrangements.

$EBV^+$ B-cell lymphomas, principally DLBL but also rarely classical Hodgkin lymphoma, can complicate AILTCL; this can occur in up to 23% of cases. These cases are thought to arise as part of the overall immunosuppressed state of the patient; therefore when making a diagnosis of AITL, always search for evidence of a second lymphoma such as $EBV^+$ DLBL. Diagnosis of DLBL or Hodgkin lymphoma should be made only when clear histopathologic evidence, such as large sheets of large B-cells in the former, is present.

## ANAPLASTIC LARGE-CELL LYMPHOMA

Anaplastic large-cell lymphoma (ALCL) was first described in 1985 and is one of the two most common nodal PTCLs. It accounts for 3% of all adult NHLs and 10% to 30% of pediatric lymphomas. Prototypic features include a pleomorphic large-cell infiltrate with involvement of nodal sinuses, immunoreactivity with anti-CD30 antibodies, and the presence of a nonrandom chromosomal translocation, t(2;5)(p23;q35), in approximately 60% of cases. Primary ALCL can be subdivided into systemic (nodal) and cutaneous forms, with the latter being discussed in the section on primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders. Secondary ALCL is a morphologic transformation of another PTCL type that acquires expression of CD30. The WHO classification separates primary systemic ALCL into anaplastic lymphoma kinase (ALK)-positive and ALK-negative disease entities.

### Clinical Features

There is a bimodal age distribution for systemic ALCL, similar to Hodgkin lymphoma, with a large peak in childhood or young adults (mostly ALK positive) and a small peak in older adults (mostly ALK negative). Patients often have lymphadenopathy extranodal masses, or both, B symptoms (e.g., fever, night sweats, and weight loss), and stage III or IV disease. There is a male predominance in younger patients and a slight female predominance in older patients. Systemic ALCL is moderately aggressive, and prognosis is closely related to ALK expression. Patients with ALK-positive tumors have the most favorable response to treatment with up to 80% 5-year overall survival rate, whereas those with ALK-negative tumors have an approximately 40% 5-year overall survival rate. ALK-negative ALCLs that have an indolent course include those restricted to the oral

**ANAPLASTIC LARGE CELL LYMPHOMA—FACT SHEET**

**Definition**
- Systemic (nodal) T-cell lymphoma characterized by pleomorphic $CD30^+$ large T cells that often involve nodal sinuses

**Incidence and Location**
- Uncommon (3% of all adult NHLs but 10% to 30% of pediatric lymphomas)

**Morbidity and Mortality**
- ALK-positive ALCL: 80% 5-year overall survival rate
- ALK-negative ALCL: 40% 5-year overall survival rate

**Gender and Age Distribution**
- Bimodal age distribution with large peak in childhood or young adults (mostly ALK-positive) and small peak in older adults (mostly ALK-negative)
- Male predominance in younger patients and slight female predominance in older patients

**Clinical Features**
- Lymphadenopathy or extranodal masses (stage III or IV disease), or both
- B symptoms

**Prognosis and Therapy**
- Moderately aggressive disease with prognosis related to ALK expression
- Chemotherapy
- Anti-CD30 monoclonal antibody conjugated with monomethyl auristatin E (brentuximab vedotin)

**ANAPLASTIC LARGE CELL LYMPHOMA—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Partially to completely effaced tissue architecture or confined to nodal sinuses
- Pleomorphic large tumor cells with circular or horseshoe-shaped nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm (hallmark cells)
- Subtypes among ALK-positive tumors: common (70%), lymphohistiocytic (10%), small cell (5% to 10%), and Hodgkin-like (3%)

**Immunophenotypic Findings**
- $CD30^+$ tumor cells
- T-cell phenotype (80% to 90%); null cell phenotype (10% to 20%)
- ALK positive (70% to 80%); ALK negative (20% to 30%)

**Molecular and Cytogenetic Findings**
- Clonal TCR gene rearrangements in 90% of cases
- EBV genome negative
- t(2;5)(p23;q35) in 60% of cases (70% to 80% of ALK-positive cases)
- t(6;7)(p25.3;q32.3) in some ALK-negative cases

**Differential Diagnosis**
- Metastatic carcinomas and melanomas
- Histiocytic sarcomas
- Classical Hodgkin lymphomas
- PTCL, NOS
- Reactive processes (lymphohistiocytic and small cell variants of ALCL)

cavity and those associated with recurrent seromas around breast implants. Extranodal sites most commonly involved, in order of frequency, are skin, bone, soft tissues, lung, and liver.

## Pathologic Features

### Microscopic Findings

Histopathologic features are distinctive. The large cells, which may appear cohesive, extend from the subcapsular sinuses into the paracortical region, often sparing germinal centers (Figure 9-3). The neoplastic cells may sometimes be largely confined to the nodal sinuses, although, more often a complete architectural effacement is noted. The hallmark tumor cell is a large transformed cell with irregular nuclear contours, a prominent nucleolus, and abundant eosinophilic cytoplasm. Multinucleated tumor cells may have nuclei arranged in a circular pattern (i.e., wreath cells) or in a horseshoe shape. Several morphologic subtypes have been described, subdivided according to tumor cell size, nuclear pleomorphism, types of reactive cells, and degree of fibrosis. Histologic types that have been described for ALK-positive ALCL include the common type with pleomorphic or monomorphic large tumor cells (approximately 70% of ALCLs), lymphohistiocytic variant (10%), small cell variant (5% to 10%), and Hodgkin-like variant (3%). In the lymphohistiocytic and small cell variants, large tumor cells are a relatively minor population in a background of small, irregular lymphocytes. Occasional cases have abundant histiocytes, fibrosis, and increased neutrophils. In the small cell variant, the large tumor cells can often be found around vessels. The Hodgkin-like variant has morphologic features resembling nodular sclerosis classical Hodgkin lymphoma.

Unlike other large cell lymphomas, bone marrow involvement might not be obvious on routine hematoxylin and eosin–stained biopsy sections because of the propensity for single tumor–cell infiltration in ALCL. Studies have shown that with routine hematoxylin and eosin sections alone, approximately 10% of cases will show detectable involvement. However, if bone marrow evaluation is done in combination with staining for CD30 or ALK, or both, the percentage of cases with detectable involvement is closer to 30%.

### Immunophenotype

The tumor cells of ALCL are always $CD30^+$, usually with both cytoplasmic membrane and Golgi region staining by immunohistochemistry (Figure 9-4). The majority of ALCLs have a $CD4^+$ T-cell phenotype, but CD3 is absent in approximately 75% of cases. Approximately 10% to 20% of cases do not express T-cell antigens and are regarded as null cell type, but they usually can be shown to be of T-cell origin if T-cell gene rearrangement studies are performed. Although B-cell cases with an anaplastic morphology and CD30 positivity were accepted in the original definition of ALCL, they are now included among the diffuse large B-cell lymphoma category in the WHO classification. Most ALCLs are $CD45^+$; however, 20% to 40% of cases may lack or have only weak staining. Approximately 60% of cases are epithelial membrane antigen positive. Approximately 70% to 80% of ALCLs are ALK positive, with the majority of these demonstrating both diffuse cytoplasmic and nuclear staining. Other ALK-positive ALCLs have cytoplasmic or more rarely membranous staining only. When this occurs, a variant *ALK* translocation is present other than *NPM1-ALK*. ALCLs are derived from cytotoxic T cells, as the tumor cells routinely stain for TIA-1, granzyme B, or perforin. A small subset (15% to 25%) of ALCL cases is also $CD15^+$.

### Molecular and Cytogenetic Findings

Most ALCLs (90%) have clonal TCR gene rearrangements. Tumor cells are negative for the EBV genome. A t(2;5)(p23;q35) chromosomal abnormality is present in

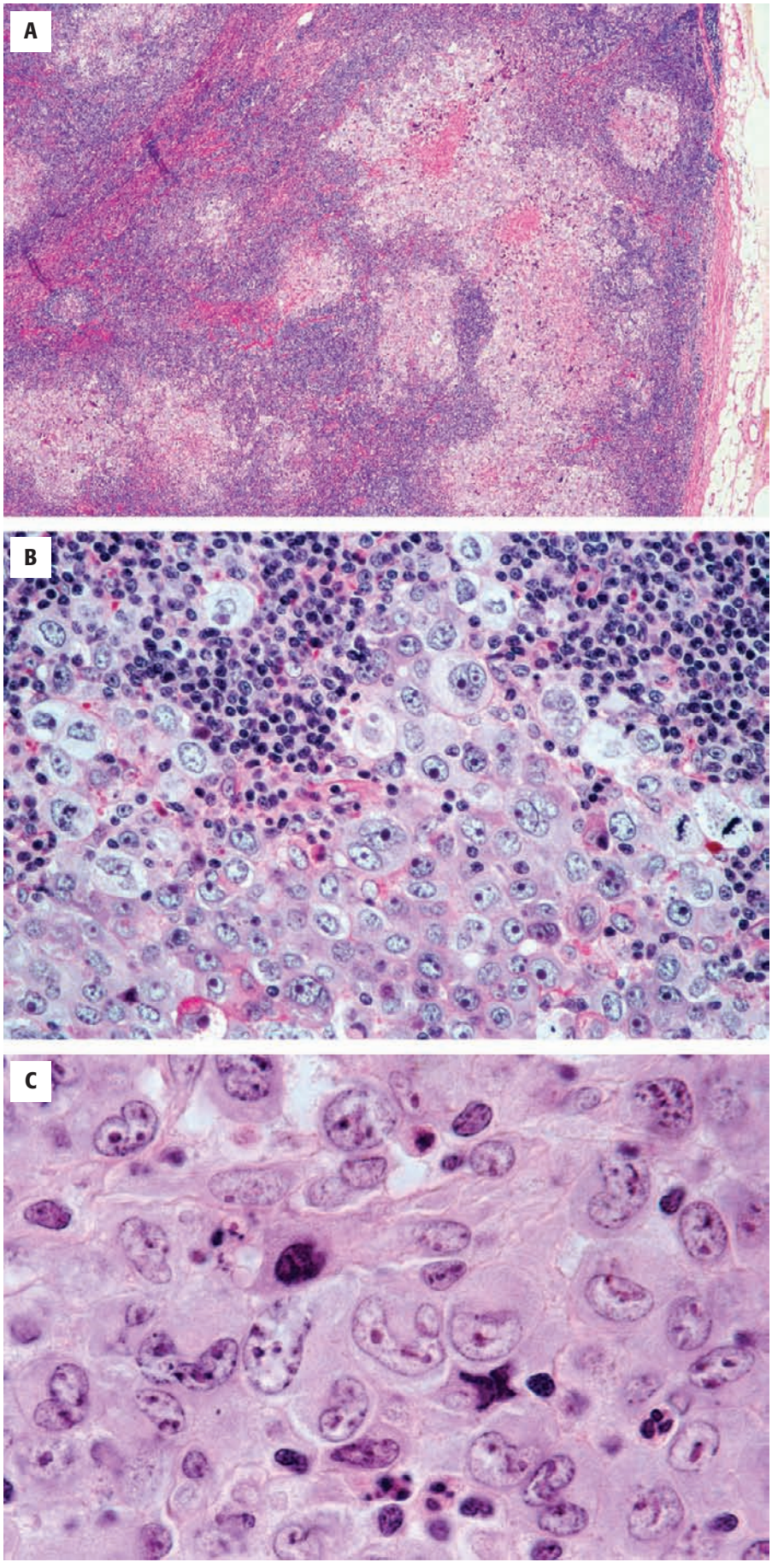


**FIGURE 9-3**

Anaplastic large cell lymphoma: microscopic features. **A,** At low magnification, neoplastic cells infiltrate and expand the lymph node sinuses. **B,** At intermediate magnification, large tumor cells appear cohesive. **C,** At high magnification, the large tumor cells show pleomorphic nuclei and abundant eosinophilic cytoplasm. A hallmark cell with horseshoe-shaped nucleus is located centrally.

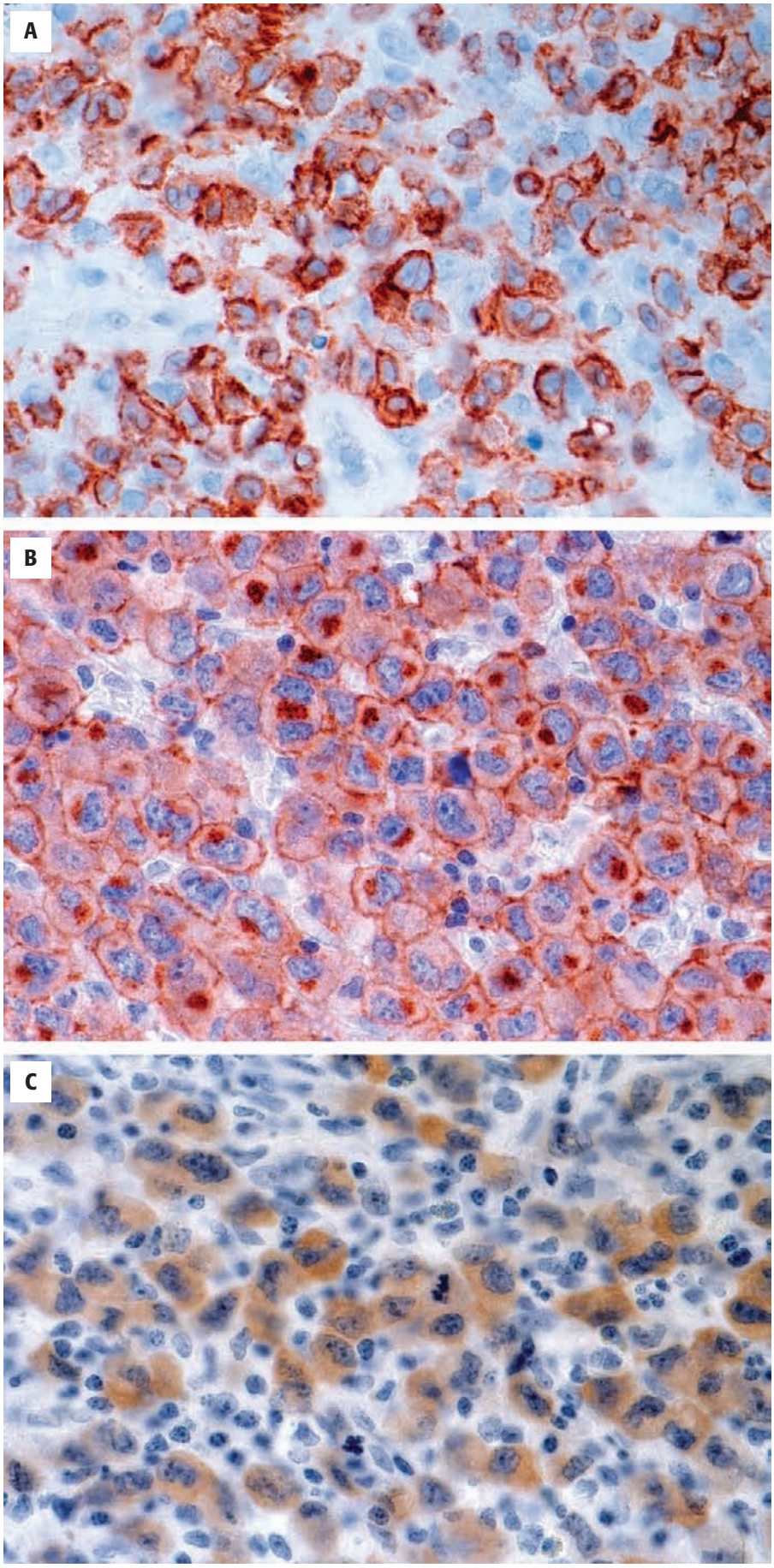


**FIGURE 9-4**

Anaplastic large cell lymphoma (ALCL): immunophenotypic features. **A,** The tumor cells are $CD3^+$ in this paraffin immunoperoxidase stain. **B,** Uniform strong cytoplasmic membrane and Golgi region CD30 positivity are seen in the tumor cells of most ALCLs. **C,** The tumor cells are ALK-1 positive in this paraffin immunoperoxidase stain.

most ALCLs, which translocates a novel *ALK* gene on chromosome 2p23 next to the nucleolar organizing gene, nucleophosmin (*NPM*), on chromosome 5q35. This translocation results in a fusion protein that can be detected using antibodies that recognize ALK, thus producing a cytoplasmic, nuclear, and nucleolar staining pattern. A number of variant translocations have also been described that result in translocation of ALK to other gene partners, including some on chromosomes 1q21, 1q25, 3q21, 4q33 Xq11-12, 17q23, 17q25, 19p13.1, and 22q11.2 and in inversions of 2p23-2q35. These variant translocations can be seen in 20% or more of ALK-positive cases and are often associated with a cytoplasmic-only staining pattern with antibodies to ALK. These *ALK* translocations are seen mostly in young patients and in primary nodal cases. The only recurrent translocation identified among systemic ALK-negative ALCLs is t(6;7)(p25;q32.3) involving *DUSP22* and *FRA7*. Finally, gene expression profiling can show molecular separation of ALK-positive ALCL from ALK-negative ALCL, PTCL, not otherwise specified (NOS), and adult T-cell leukemia–lymphoma. This finding supports the separate classification of these lymphomas.

### Differential Diagnosis

Nodal ALCLs must be distinguished from metastatic carcinomas, and melanomas and from histiocytic sarcomas, because of their sinusoidal infiltrates. Other common-type ALCLs and the Hodgkin-like variant must be distinguished from classic Hodgkin lymphomas. The lymphohistiocytic and small cell variants of ALCL need to be distinguished from reactive processes. Distinguishing among these different entities can generally be accomplished using appropriate immunohistochemical markers because Reed-Sternberg cells of Hodgkin lymphoma, in addition to CD30 expression, are almost always positive for PAX5, often express CD15, and lack T-cell markers

ALK-negative ALCLs may be difficult to differentiate from $CD30^+$ PTCL, NOS. Presence of hallmark cells, growth patterns such as sinusoidal involvement and t(6;7)(p25;q32.3) favor a diagnosis of ALK-negative ALCL. The separation is not entirely academic because some reports demonstrate an intermediate outcome of ALK-negative ALCL, between the favorable outcome of ALK-positive ALCL and the poor outcome of PTCL, NOS. However, current first-line therapies for ALCL and PTCL, NOS are not yet based on histologic type.

The seroma-associated ALCLs seen in the setting of breast implantation should be specifically recognized given the favorable and indolent nature of this lymphoproliferation. Although pathologically the cells have the cytologic and immunophenotypic features typical of ALK-negative ALCL, they occur in a fibrinous and serous background without tissue infiltration and can be identified readily based on the clinical context.

## Peripheral T-Cell Lymphoma, NOS

PTCL, NOS, is one of the two most common nodal PTCLs, but it can occur in extranodal sites as well. This category is a catch-all for PTCLs that are yet to be well defined in terms of their classification into distinct entities.

### Clinical Features

Most patients with PTCL, NOS, are adults with generalized lymphadenopathy and B symptoms. There is no gender predilection. These are aggressive lymphomas, and patients have a 25% 5-year overall survival rate.

### Pathologic Features

#### Microscopic Findings

Tissue architecture is generally effaced by a diffuse lymphoproliferation that consists of tumor cells of varying size, often within a polymorphic reactive cell

**PERIPHERAL T-CELL LYMPHOMA, NOS—FACT SHEET**

**Definition**
- Nodal or extranodal T-cell lymphoma, the features of which do not fit any of the defined T-cell lymphoma entities

**Incidence and Location**
- Uncommon, but one of the two most frequently occurring nodal T-cell lymphomas

**Morbidity and Mortality**
- 25% 5-year overall survival rate

**Gender and Age Distribution**
- Adults
- No gender predilection

**Clinical Features**
- Generalized lymphadenopathy (stage III or IV disease)
- B symptoms

**Prognosis and Therapy**
- Aggressive disease with poor prognosis
- Chemotherapy

**PERIPHERAL T-CELL LYMPHOMA, NOS—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Effaced architecture
- Diffuse lymphoproliferation with tumor cells of varying size, often with polymorphic reactive cell background
- T-zone, lymphoepithelioid cell, and follicular variants

**Immunophenotypic Findings**
- Variable T-cell antigen expression that is often aberrant

**Molecular and Cytogenetic Findings**
- Clonal TCR gene rearrangements in most cases
- Tumor cells contain EBV genome in few cases
- t(5;9)(q33;q22) in some PTCLs with follicular growth pattern
- t(6;14)(p25;q11.2) in rare cytotoxic PTCLS involving skin and bone marrow

**Differential Diagnosis**
- B-cell lymphomas, including follicular lymphoma
- Classical Hodgkin lymphomas
- Adult T-cell leukemia–lymphoma
- Reactive processes (T-zone and lymphoepithelioid cell variants)

background. Large tumor cells may have hyperlobate nuclei or resemble Reed-Sternberg cells. Two morphologic variants that do not appear to be clinicopathologic entities have been recognized: T-zone lymphoma and lymphoepithelioid cell (Lennert) lymphoma (Figure 9-5). T-zone lymphoma has an interfollicular growth pattern and is composed of cytologically atypical small lymphocytes that often have clear cytoplasm. Other inflammatory cells such as eosinophils and plasma cells are often admixed, lending a polymorphous appearance that can cause further confusion in diagnosis. The secondary lymphoid follicles may be hyperplastic and infiltrated by the tumor cells. Lymphoepithelioid cell lymphoma has a proclivity to involve Waldeyer's ring and cervical lymph nodes. Tissues are effaced, and there are numerous evenly dispersed clusters of epithelioid histiocytes between which are infiltrates of mostly small tumor cells. Another recognized variant has tumor cells that selectively infiltrate lymphoid follicles producing a follicular growth pattern, but this tumor may be related to AILTCL because of its apparent derivation from TFH.

#### Immunophenotype

Given that PTCL, NOS, is likely a heterogeneous category, there is no specific diagnostic immunophenotype. Most PTCLs, NOS, have an aberrant T-cell phenotype, with loss of CD3 or CD7 most frequently observed. Most PTCLs, NOS, are $CD4^+$ $\alpha\beta$ T-cell lymphomas, although some are $CD8^+$ and a few have $\gamma\delta$ TCRs. Some of these lymphomas have a cytotoxic T-cell phenotype or express NK cell–associated antigens such as CD56. Follicular variants are $CD4^+$ T cells that express TFH markers such as CD10, BCL6, and PD1 (CD279).

#### Molecular and Cytogenetic Findings

Most of these lymphomas have clonal TCR gene rearrangements, and a few may have EBV genomes in the tumor cells or in non-neoplastic B-cell immunoblasts. No consistent cytogenetic abnormality has been identified for most PTCL, NOS, but a t(5;9)(q33;q22) has been detected in some PTCLs with a follicular growth pattern. This translocation results in a novel *ITK-SYK* fusion tyrosine kinase that mimics activated TCRs and has been shown to be capable of producing T-cell lymphoma in animal models. Two cytotoxic PTCLs, NOS, with skin and bone marrow involvement and a t(6;14)(p25;q11.2) involving *IRF4* and *TCRA* have also been described and might represent a distinct clinicopathologic entity.

## Differential Diagnosis

PTCL, NOS, must be distinguished from B-cell lymphomas and occasionally from classical Hodgkin lymphomas. This can generally be accomplished by immunophenotyping, but gene rearrangement studies may also be helpful. It should be remembered that rare cases of PTCL, NOS, have been reported to express CD30 and CD15, mimicking Hodgkin lymphoma. However, there is often a monomorphic appearance of lymphoma cells or expression of multiple T-cell markers, or both, often including CD4 in such cases of PTCL. Some PTCLs, NOS, cannot be distinguished from adult T-cell leukemia–lymphoma without knowledge of the patient's human T-lymphotropic virus (HTLV)-1 status. Similarly, lymph node involvement by other T-cell leukemia or lymphomas are difficult to distinguish from PTCL, NOS, without clinical history. T-PLL often involves lymph nodes and without clinical history it might not be possible to make the appropriate diagnosis unless TCL-1 staining is performed and shown to be positive. Lymph node involvement by mycosis fungoides (MF) also requires knowledge of the history of MF to most appropriately make the diagnosis. The T-zone and lymphoepithelioid cell variants must not be confused with reactive processes. The latter can be mistaken for a granulomatous process because of the prominent epithelioid histiocytic reaction, but attention should be focused on the accompanying lymphoproliferation that effaces the tissue architecture. PTCL with a follicular growth pattern may be misinterpreted as a follicular lymphoma.

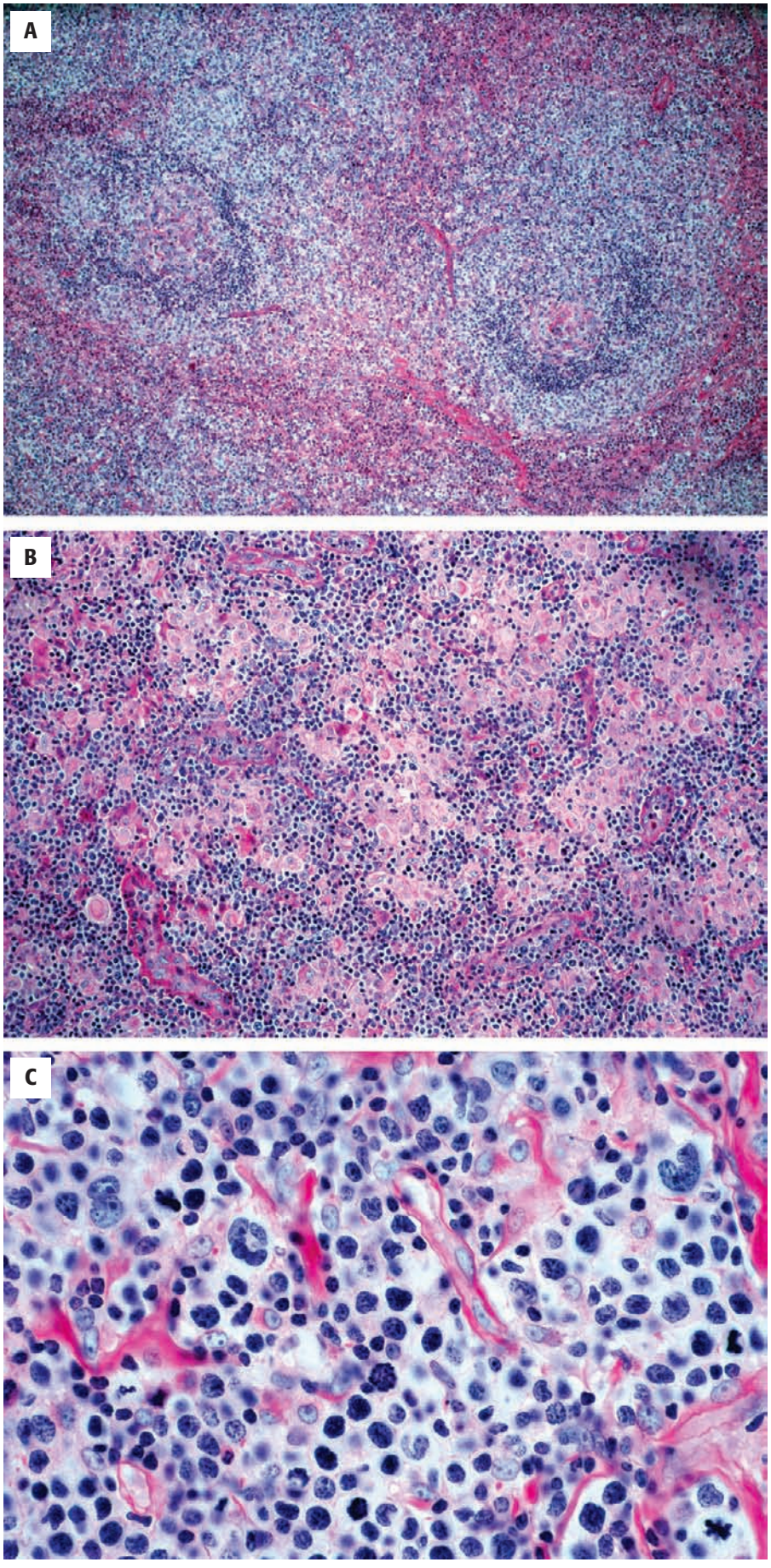


**FIGURE 9-5**

Peripheral T-cell lymphoma (PTCL), NOS: microscopic features. **A,** At low magnification, the lymph node architecture is markedly distorted by a paracortical infiltrate of neoplastic T cells that surround two residual non-neoplastic secondary lymphoid follicles in this T-zone lymphoma variant of PTCL, NOS. **B,** At low magnification, the lymph node architecture is effaced by a diffuse infiltrate of neoplastic T cells that lie between evenly dispersed clusters of epithelioid histiocytes in this lymphoepithelioid cell (Lennert) lymphoma variant of PTCL, NOS. **C,** At high magnification, pleomorphic tumor cells with clear cytoplasm, some resembling Reed-Sternberg cells, are present in another PTCL, NOS.

## EXTRANODAL NATURAL KILLER/T-CELL LYMPHOMA, NASAL TYPE

As its name implies, extranodal NK/T-cell lymphoma, nasal type, is virtually always extranodal in presentation and most commonly occurs in the nasal cavity, nasopharynx, or palate. This tumor has also been called *polymorphic reticulosis* and *lethal midline granuloma*. It is most prevalent in eastern Asia, Mexico, and Central and South America and is generally associated with EBV. Most cases are derived from NK cells, but some arise from cytotoxic T cells.

### Clinical Features

Patients often have nasal obstruction, epistaxis, or midfacial destructive lesions with extension of the tumor into the paranasal sinuses and orbit. B symptoms may occur. The disease frequently disseminates to the skin or gastrointestinal tract. Other patients may exhibit cutaneous, gastrointestinal, or testicular tumors without apparent nasal involvement (i.e., extranasal NK/T-cell lymphomas). Lymph nodes are usually not involved. The clinical course is typically aggressive, but some of the lymphomas respond well to systemic chemotherapy. Patients with extranasal presentation tend to have a poorer overall survival compared to those with nasal disease.

**EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE—FACT SHEET**

**Definition**
- Extranodal lymphoma of NK or cytotoxic T cells, most frequently occurring in nasal cavity or nasopharynx

**Incidence and Location**
- Rare
- Most prevalent in east Asia, Mexico, and Central and South America

**Morbidity and Mortality**
- Variable

**Gender and Age Distribution**
- Adults
- Male predominance

**Clinical Features**
- Nasal obstruction, epistaxis, or midfacial destructive lesions
- B symptoms

**Prognosis and Therapy**
- Aggressive disease with variable prognosis
- Chemotherapy

### Pathologic Features

#### Microscopic Findings

Regardless of site, the lymphoma will often have an angiocentric, angioinvasive, and angiodestructive infiltrate of cytologically atypical lymphocytes of varying size (Figure 9-6). Many cases will have a diffuse growth of tumor cells. Necrosis is often present and may be extensive. Occasionally, there is a polymorphic inflammatory cell background.

#### Immunophenotype

Tumors derived from NK cells typically have a $CD2^+$ and $CD56^+$ phenotype with expression of cytotoxic granule–associated proteins, TIA-1, granzyme B, and perforin (Figure 9-7). The NK cells are surface $CD3^-$ and may have cytoplasmic CD3ε staining by immunohistochemistry, but do not express TCR-αβ or TCR-γδ proteins. They may be $CD8^+$ but generally lack CD4 and CD5. CD7 is variably present. The few tumors that are T cell derived will have a similar phenotype except for expression of surface CD3 and TCR proteins.

**EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Angiocentric, angioinvasive, and angiodestructive infiltrate of cytologically atypical lymphocytes of varying size

**Immunophenotypic Findings**
- NK cell tumors: $CD2^+$, surface $CD3^-$, $CD56^+$, TCR-αβ negative, and TCR-γδ negative, and expression of cytotoxic granule–associated proteins
- T-cell tumors: $CD2^+$, surface $CD3^{+/-}$, $CD56^{+/-}$, TCR-αβ positive, or TCR-γδ positive, and expression of cytotoxic granule–associated proteins

**Molecular and Cytogenetic Findings**
- NK cell tumors: no clonal TCR gene rearrangements
- T-cell tumors: clonal TCR gene rearrangements
- EBV genome in neoplastic NK cells or in tumor cells of many T-cell cases
- No recurring chromosomal translocations, but del(6)(q21q25) or i(6)(p10) may be present

**Differential Diagnosis**
- Lymphomatoid granulomatosis
- Wegener granulomatosis
- Diffuse, large B-cell lymphomas
- Subcutaneous panniculitis–like T-cell lymphomas (skin cases)
- NK-cell enteropathy (gastrointestinal cases)

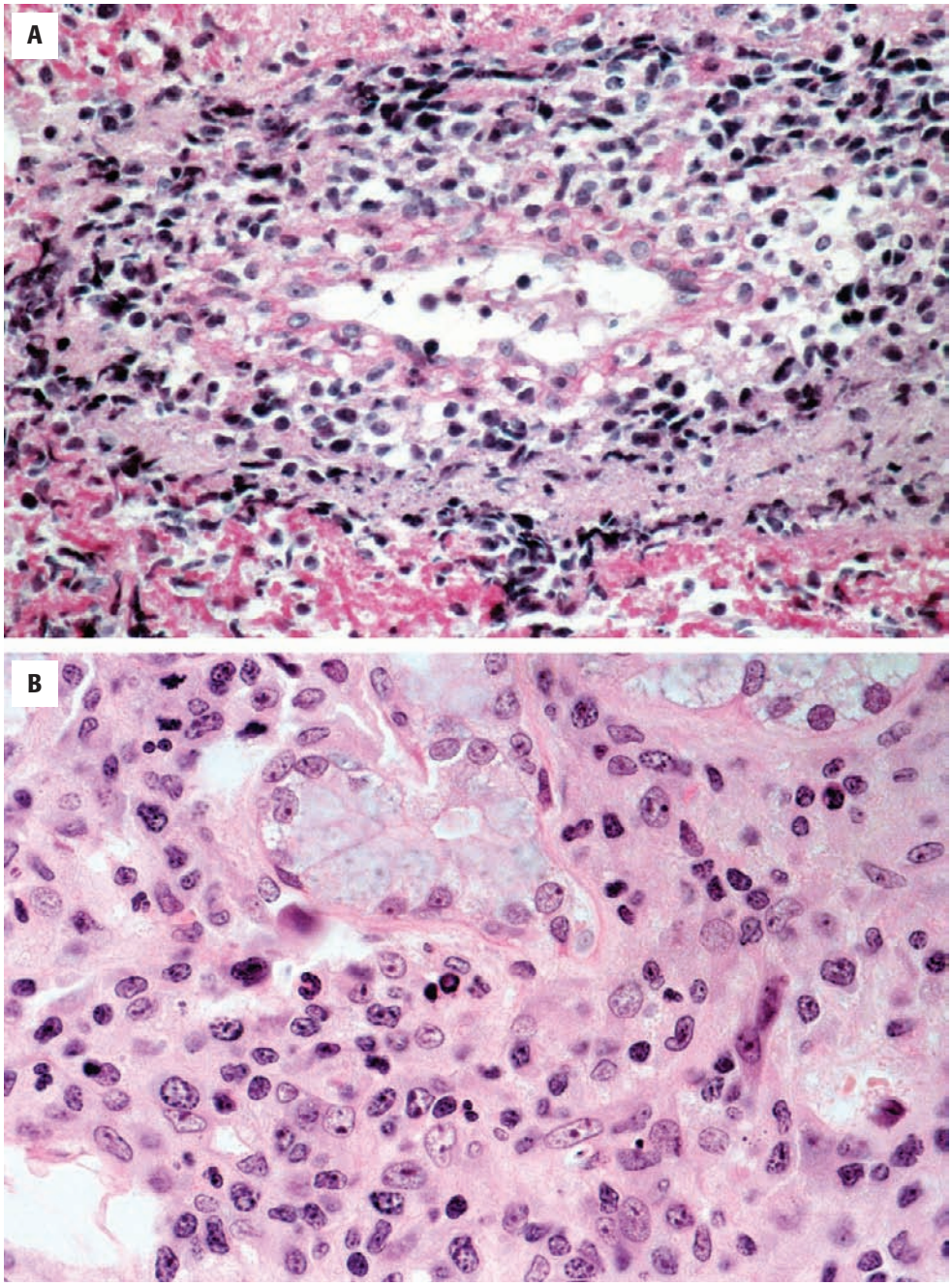


**FIGURE 9-6**

Extranodal natural killer/T-cell lymphoma, nasal type: microscopic features. **A,** At intermediate magnification, an angiocentric infiltrate of pleomorphic lymphocytes in the nasal cavity is associated with necrosis at the upper and lower margins of the field of view. **B,** At high magnification, pleomorphic lymphocytes are present between intact glands in the nasal mucosa.

### Molecular and Cytogenetic Findings

TCR genes have a germline configuration in all cases derived from NK cells, whereas those that arise from T cells often have clonal TCR gene rearrangements. The tumor cells are generally EBV positive (see Figure 9-7). The most common cytogenetic abnormality is del(6)(q21q25) or i(6)(p10). Gene expression profiling shows distinct clustering of extranodal NK/T-cell lymphoma, nasal type, from cytotoxic PTCL, NOS.

## Differential Diagnosis

Extranodal NK/T-cell lymphoma, nasal type, has histologic features similar to those of lymphomatoid granulomatosis. However, lymphomatoid granulomatosis is recognized as a lymphoma of EBV-positive large B cells that are angiocentric, angioinvasive, and angiodestructive and have an accompanying reaction rich in T cells. Immunophenotyping should differentiate the two

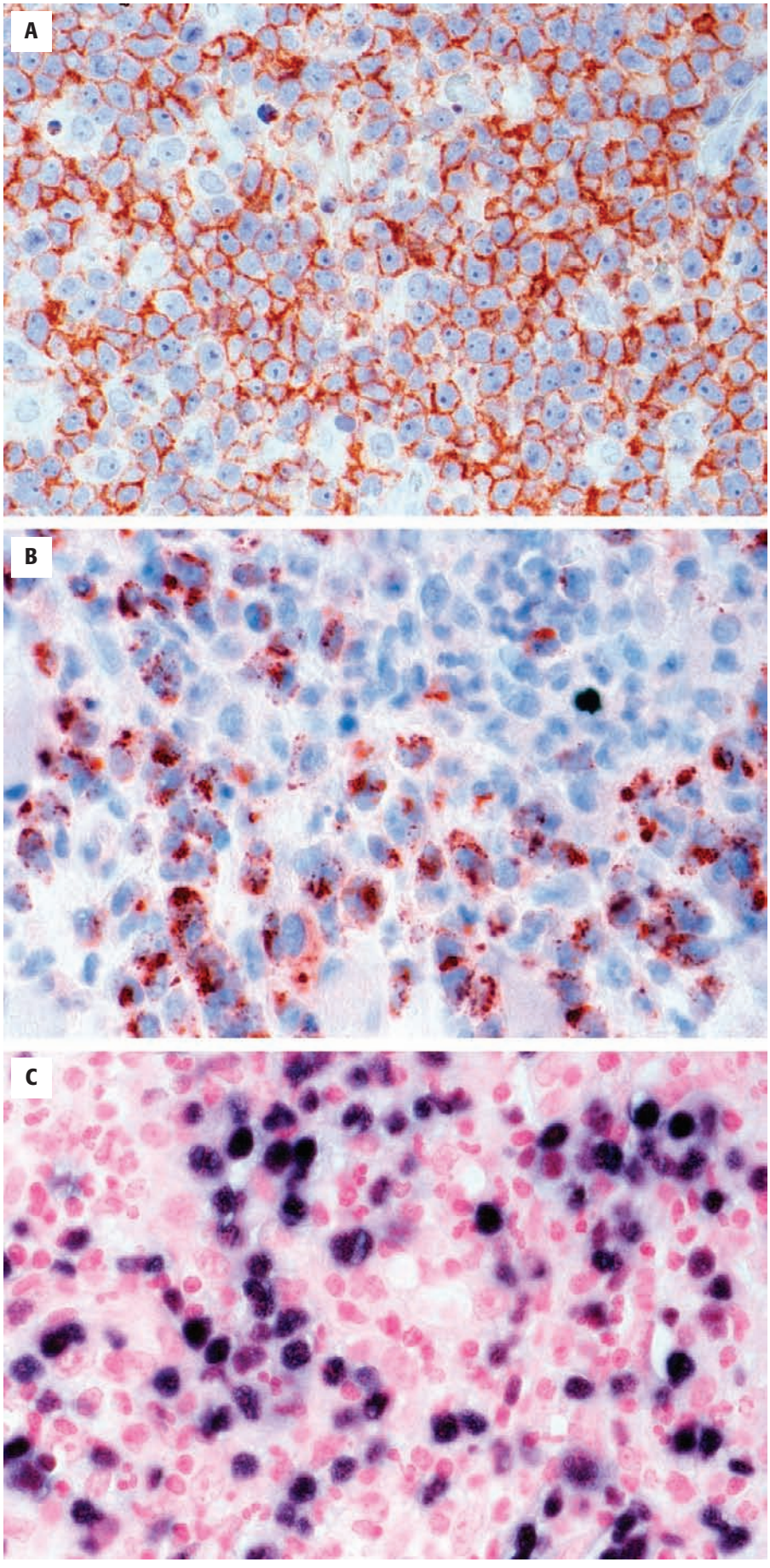


**FIGURE 9-7**

Extranodal natural killer (NK)/T-cell lymphoma, nasal type: immunophenotypic features and in situ hybridization. **A,** Neoplastic NK cells, or sometimes T cells, generally express CD56 as in this paraffin immunoperoxidase stain. **B,** The tumor cells have a cytotoxic lymphocyte phenotype, as shown by the granzyme B positivity in this paraffin immunoperoxidase stain. **C,** Epstein-Barr virus (EBV) genome is generally detected in the tumor cell nuclei as in this in situ hybridization stain using probes for EBV-encoded RNA.

lymphomas, and lymphomatoid granulomatosis will likely have clonal immunoglobulin gene rearrangements not seen in extranodal NK/T-cell lymphoma, nasal type. Nasal lesions occasionally will need to be distinguished from Wegener's granulomatosis, which can be accomplished with a complete immunophenotypic work-up. Extranodal NK/T-cell lymphomas, nasal type, that have a diffuse growth pattern may resemble diffuse large B-cell lymphomas, but immunophenotyping will separate them. Cutaneous extranodal NK/T-cell lymphomas, nasal type, may infiltrate subcutaneous tissue in a panniculitic pattern requiring distinction from subcutaneous panniculitis–like T-cell lymphoma (SPTCL). This can be accomplished by immunophenotyping and EBV studies, with SPTCL showing no EBV-positive cells.

NK/T-cell lymphoma in the gastrointestinal tract should also be distinguished from the recently described syndrome of *NK-cell enteropathy*. At present, patients with NK-cell enteropathy appear to complain of vague abdominal symptoms and demonstrate erythematous and ulcerative mucosal lesions composed of medium to large atypical cells with irregular nuclei, mature chromatin, and small nucleoli. The cells infiltrate the mucosa and displace glandular elements, but do not show epitheliotropism; they rarely involve the submucosa. However, extensive necrosis, angiocentricity, and angiodestruction are not seen as in nasal type extranodal NK/T-cell lymphoma. The immunophenotype of NK-cell enteropathy (cytoplasmic $CD3^+$, $CD7^+$, $CD56^+$, $CD5^-$, $TIA1/GzB^+$) may be similar to extranodal NK/T-cell lymphoma, but EBV is absent. Furthermore, the Ki67 proliferative index is usually low (approximately 25%). T-cell clonality studies are polyclonal, and patients appear to have an indolent, nonprogressive course.

## SUBCUTANEOUS PANNICULITIS–LIKE T-CELL LYMPHOMA

SPTCL is a rare form of PTCL that produces subcutaneous nodular lesions and is composed of cytotoxic αβ T cells. In prior classifications, similarly appearing lymphomas derived from γδ T-cells were included as SPTCL, but these have since been separated and classified as primary cutaneous γδ T-cell lymphoma because of their different clinical behavior.

### Clinical Features

SPTCLs usually manifest with multiple, nontender, small to large (0.5 to 12 cm), erythematous subcutaneous nodules or plaques on the extremities, trunk, or both. The face and head are occasionally involved. Most patients are younger adults (median age, 43 years; range, 5 months to 84 years), with a slight female predominance. B symptoms are common. Most patients have stage I disease localized to the skin without dissemination to lymph nodes, liver, spleen, or bone marrow.

Exceptional cases with terminal bone marrow involvement and overt leukemia have been reported. Patients generally follow one of two clinical courses: most have a prolonged indolent period with regressing and recurring lesions that eventually progress to aggressive disease, whereas others have aggressive disease from the outset. Clinically aggressive disease appears to correlate with the presence and severity of hemophagocytosis rather than systemic dissemination of lymphoma. The hemophagocytic syndrome (hepatosplenomegaly, peripheral blood cytopenia, and hypertriglyceridemia) develops in most patients at some time during their course, but may not occur for up to 5 years after the initial diagnosis. Patients with clinically aggressive disease and hemophagocytic syndrome usually die of their disease despite aggressive chemotherapy. Median survival in these patients is approximately 2 years, but overall survival ranges from 6 months to 11 years. Death is usually due to hemorrhagic and infectious complications of pancytopenia and coagulation abnormalities.

**SUBCUTANEOUS PANNICULITIS–LIKE T-CELL LYMPHOMA—FACT SHEET**

**Definition**
- Lymphoma of cytotoxic T cells that produces subcutaneous nodular lesions

**Incidence and Location**
- Rare

**Morbidity and Mortality**
- Median survival: 2 years
- Overall survival: 6 months to 11 years

**Gender, Race, or Age Distribution**
- Younger adults
- Slight female predominance

**Clinical Features**
- Nontender, erythematous subcutaneous nodules usually on extremities or trunk, or both (stage I disease)
- B symptoms
- Hemophagocytic syndrome

**Prognosis and Therapy**
- Variably indolent to aggressive disease
- Chemotherapy

## Pathologic Features

### Microscopic Findings

SPTCLs have a lobular panniculitic infiltrate of pleomorphic small, medium, and large lymphocytes with highly irregular hyperchromatic nuclei (Figure 9-8). The tumor is predominantly localized to the subcutis, where there is infiltration between fat cells, often with rimming of the neoplastic cells around fat spaces. Infiltration of interlobular septae may occur. The deep reticular dermis may also be involved in some cases, but the upper reticular dermis, papillary dermis, and epidermis are spared. Vascular invasion is common, but angiodestructive growth with fibrinoid necrosis is not seen. Karyorrhexis and fat necrosis are always present to various degrees. Benign histiocytes with voluminous foamy cytoplasm and bland, eccentric nuclei are usually present and often exhibit erythrophagocytosis or phagocytosis of nuclear fragments (cytophagocytosis). Occasional cases show striking sarcoid-like granulomatous reactions with giant cells mimicking granulomatous panniculitis. Scattered plasma cells are often seen, but eosinophils and neutrophils are absent or rare.

### Immunophenotype

The tumor cells of SPTCLs always express a cytotoxic T-cell phenotype. The typical profile is $CD2^+$, $CD3^+$, $CD5^+$, $CD7^+$, $CD8^+$, TIA-1 positive, and granzyme B positive. Similar to other PTCLs, loss or diminished expression of pan–T-cell antigens may be seen, particularly lack of CD5 or CD7. SPTCLs are derived from αβ T cells ($\beta F1^+$). PTCLs resembling SPTCL but composed of γδ T cells are segregated from SPTCLs in the category of primary cutaneous PTCLs.

**SUBCUTANEOUS PANNICULITIS–LIKE T-CELL LYMPHOMA—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Lobular panniculitic infiltrate of pleomorphic lymphocytes of varying size
- Neoplastic cells often rim fat spaces
- Karyorrhexis and fat necrosis always present

**Immunophenotypic Findings**
- $CD8^+$, TCR-αβ positive, and expression of cytotoxic granule–associated proteins

**Molecular and Cytogenetic Findings**
- Clonal TCR gene rearrangements in most cases
- EBV genome negative
- No recurring chromosomal translocations

**Differential Diagnosis**
- Reactive panniculitis
- Cutaneous γδ T-cell lymphomas
- Extranodal NK/T-cell lymphoma, nasal type
- Cutaneous dissemination of enteropathy-associated T-cell lymphomas
- Diffuse large B-cell lymphomas

### Molecular and Cytogenetic Findings

Clonal rearrangement of TCRs has been demonstrated in most cases. SPTCLs are EBV negative. No recurring cytogenetic abnormalities have been reported.

## Differential Diagnosis

The differential diagnosis for SPTCLs includes benign panniculitis such as lupus profundus and other cutaneous and subcutaneous lymphomas, particularly cutaneous γδ T-cell lymphoma and extranodal NK/T-cell lymphoma, nasal type, as discussed previously. Features that favor lupus profundus include involvement of the epidermis, presence of numerous plasma cells, reactive germinal centers, and B cells. Most reports show polyclonal gene rearrangement. Concomitant clinical features or existing diagnosis of lupus erythematosus also favor lupus profundus over lymphoma. In difficult cases, repeated biopsies may be required. Subcutaneous dissemination by other PTCLs, such as enteropathy-associated T-cell lymphoma, and by some diffuse large B-cell lymphomas must be considered and can usually be differentiated by immunohistochemistry or with detailed clinical information.

# HEPATOSPLENIC T-CELL LYMPHOMA

T cells can be subdivided based on the nature of their surface TCRs. Normally the majority (95%) of T cells are of the αβ type, and a minority (5% or less) are of the γδ type. γδ T cells are preferentially found in the small bowel epithelium, skin, and splenic red pulp. These normal γδ T-cell subpopulations are thought to be the normal counterparts for several subtypes of γδ T-cell lymphomas, including some enteropathy-associated T-cell lymphomas, primary cutaneous γδ T-cell lymphomas, and most hepatosplenic T-cell lymphomas (HSTCLs). Subsequent to the initial descriptions identifying hepatosplenic γδ T-cell lymphomas, clinically identical cases of hepatosplenic αβ T-cell lymphoma have been described. Therefore the WHO classification uses the term *HSTCL* rather than the originally proposed term *hepatosplenic γδ T-cell lymphoma*. HSTCL also appears to be the best current designation for the previously described unusual PTCLs, erythrophagocytic

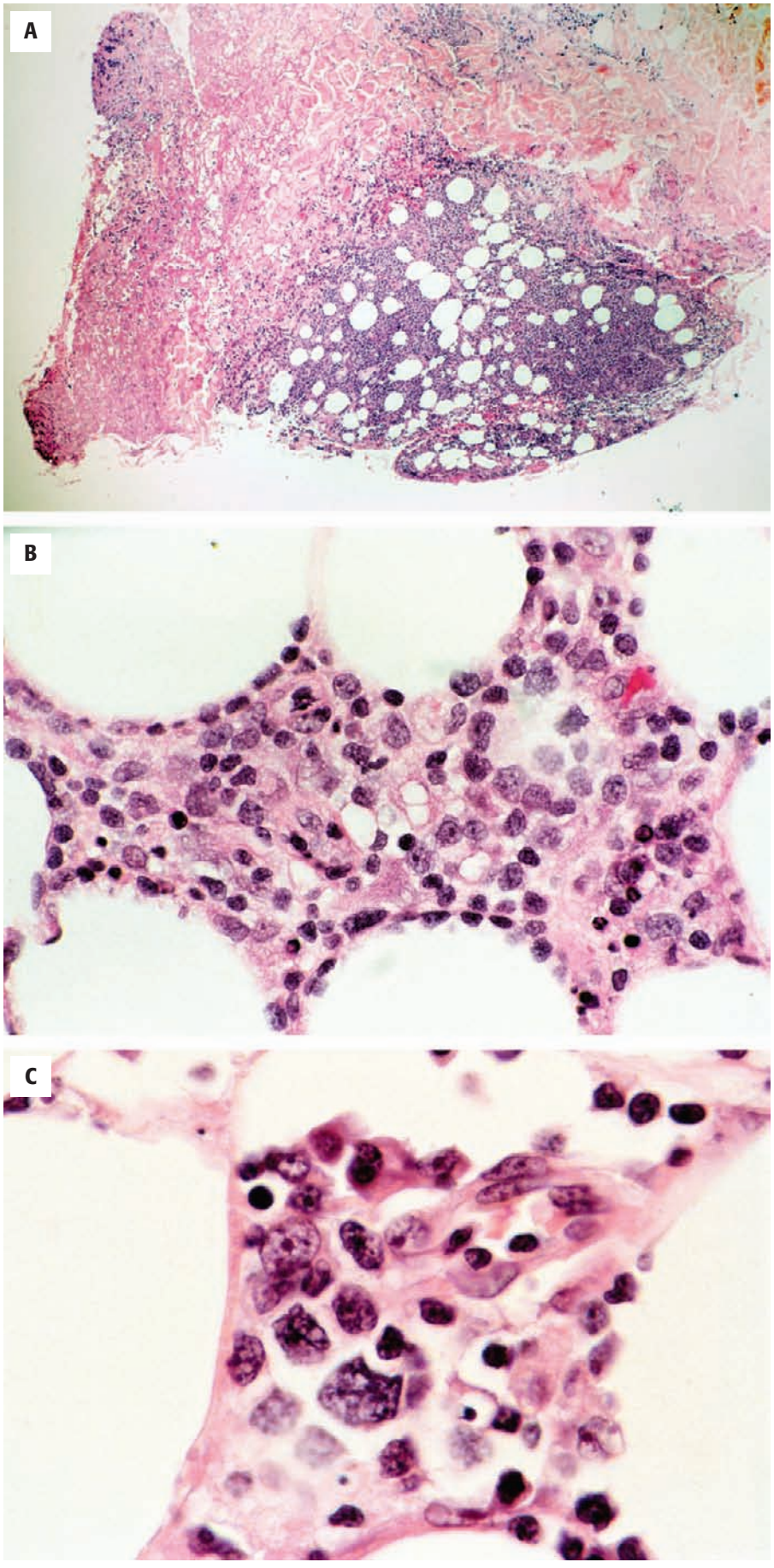


**FIGURE 9-8**

Subcutaneous panniculitis–like T-cell lymphoma: microscopic features. **A,** At low magnification, there is a lobular panniculitis-like lymphoid infiltrate in this skin excision. **B,** At intermediate magnification, tumor cells are seen in the inter–fatty spaces, and some show tight rimming around the adipocytes. **C,** At high magnification, the cytologic pleomorphism of the tumor cells is appreciated.

Tγ lymphoma, and S-100–positive T-cell lymphoproliferative disorder. Both of these have clinicopathologic features nearly identical to those of HSTCLs.

## Clinical Features

Most patients with HSTCLs exhibit B symptoms, hepatosplenomegaly (often massive), no lymphadenopathy, moderate anemia, and marked thrombocytopenia. Leukocytosis may be present in those with peripheral blood involvement. The γδ type is most commonly seen in young adult males, whereas the αβ type tends to occur more frequently in females. Approximately 20% of HSTCLs occur in the setting of chronic immunosuppressive therapy with thiopurine following solid organ transplantation or in patients with inflammatory bowel disease. HSTCL is aggressive, and most patients die within 2 years of diagnosis, even if an initial remission is achieved with therapy.

**HEPATOSPLENIC T-CELL LYMPHOMA—FACT SHEET**

**Definition**
- Extranodal lymphoma of inactivated cytotoxic T cells that produces massive hepatosplenomegaly and no lymphadenopathy

**Incidence and Location**
- Rare

**Morbidity and Mortality**
- Median survival: less than 2 years
- May be associated with chronic immunosuppressive therapy using thiopurine

**Gender and Age Distribution**
- Young adult males predominate in γδ type
- Females predominate in αβ type

**Clinical Features**
- Massive hepatosplenomegaly and no lymphadenopathy
- B symptoms
- Moderate anemia and marked thrombocytopenia common

**Prognosis and Therapy**
- Aggressive disease with poor prognosis

**HEPATOSPLENIC T-CELL LYMPHOMA—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Infiltration of splenic red pulp cords and sinuses, hepatic sinusoids, and bone marrow sinuses by small- to intermediate-sized lymphocytes

**Immunophenotypic Findings**
- $CD4^-$, $CD5^-$, and $CD8^-$ γδ (majority) or αβ (minority) T cells with an inactivated (TIA-1–positive, granzyme B–negative, and perforin-negative) cytotoxic T-cell phenotype
- May coexpress multiple killer immunoglobulin-like receptor isoforms

**Molecular and Cytogenetic Findings**
- Clonal TCR gene rearrangements in most cases
- EBV genome negative
- Isochromosome 7q often present along with trisomy 8 and loss of a sex chromosome

**Differential Diagnosis**
- Histiocytic sarcoma

## Pathologic Features

### Microscopic Findings

This lymphoma preferentially infiltrates the cords and sinuses of the splenic red pulp, hepatic sinusoids, and marrow sinuses (Figure 9-9). Tumor cells are generally small to intermediate in size, but some cases may have a predominance of large cells. There may be an associated hemophagocytosis by benign histiocytes. A leukemic phase may develop as the disease progresses. Circulating tumor cells are generally agranular, but cytoplasmic granules have been detected by electron microscopy in some cases.

### Immunophenotype

The characteristic phenotype is $CD2^+$, $CD3^+$, $CD4^-$, $CD5^-$, $CD7^+$, and $CD8^-$ (see Figure 9-9). The majority of HSTCLs are TCR-γδ positive, but a smaller number of cases are TCR-αβ positive. NK cell–associated antigens, such as CD16 and CD56, are often expressed. Furthermore, these lymphomas have an inactivated cytotoxic T-cell phenotype, with expression of the cytolytic granule–associated proteins TIA-1 and granzyme M, but not granzyme B or perforin. HSTCLs often have aberrant coexpression of multiple killer immunoglobulin–like receptor isoforms along with dim or absent CD94. Most γδ HSTCLs express the Vδ1 variable region, indicating that they are likely derived from the subset of γδ T cells that normally is present in the splenic red pulp.

### Molecular and Cytogenetic Findings

Rearrangements of TCR-γ and -δ genes can be seen in most cases; a few may have TCR-β–chain gene rearrangements, particularly those of the αβ T-cell type.

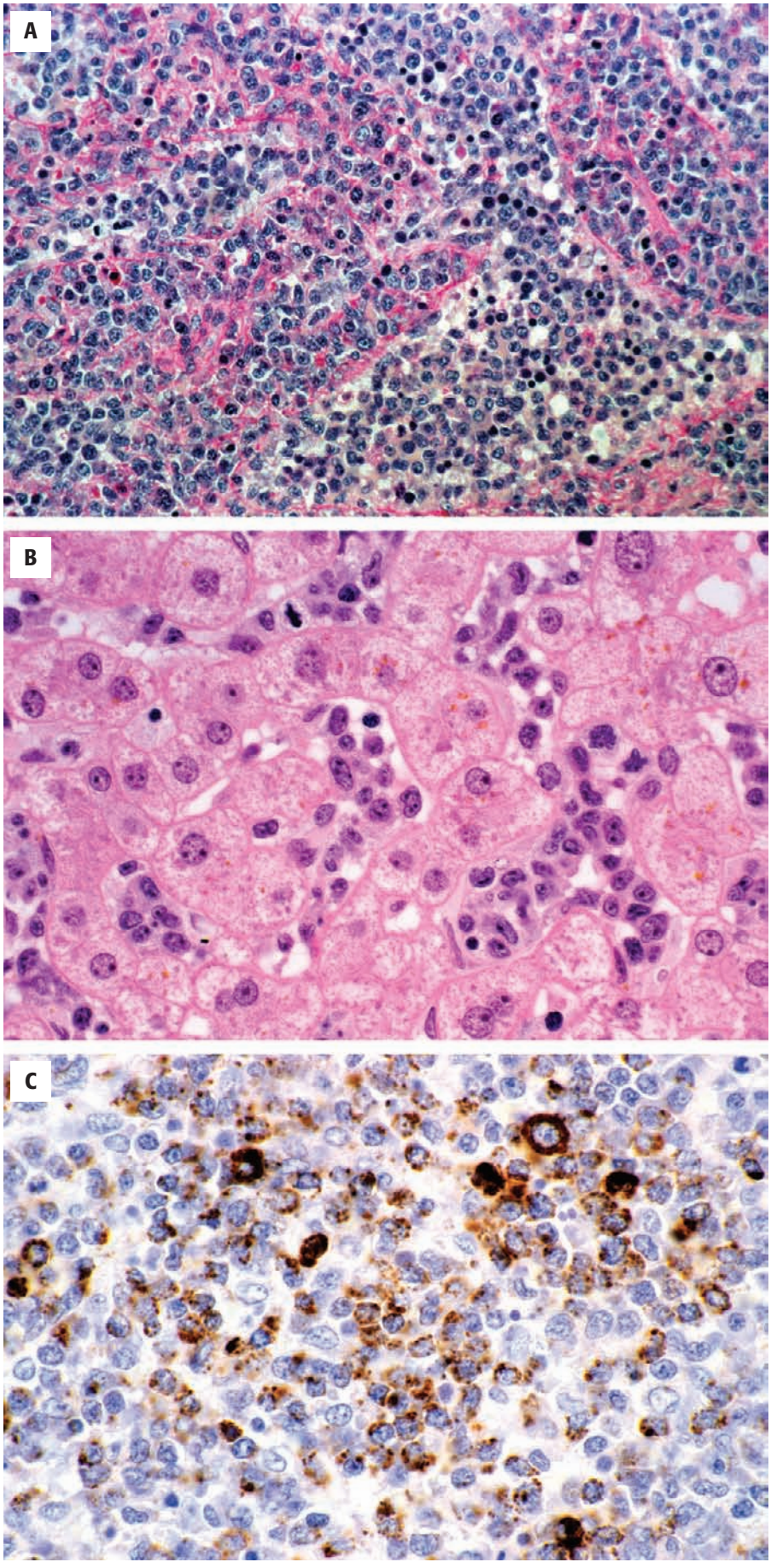


**FIGURE 9-9**

Hepatosplenic T-cell lymphoma: microscopic and immunophenotypic features. **A,** At intermediate magnification, the neoplastic T cells are distributed in the splenic red pulp, where they expand and fill sinuses. **B,** At high magnification, intermediate-sized tumor cells are prominently displayed in hepatic sinusoids. **C,** The neoplastic T cells are TIA-1 positive, indicating their cytotoxic lymphocyte phenotype in this paraffin immunoperoxidase stain.

Karyotypic studies often show isochromosome 7q, which may be accompanied by trisomy 8 and loss of a sex chromosome.

### Differential Diagnosis

Few disorders exhibit with the clinicopathologic features of HSTCL. Some cases of histiocytic sarcoma may have a similar clinical course, disease distribution, and presence of hemophagocytosis. However, histiocytic sarcoma can usually be ruled out by immunophenotypic studies.

## ENTEROPATHY-ASSOCIATED T-CELL LYMPHOMA

The gastrointestinal tract is the most common site of primary extranodal NHLs. The vast majority of these are B-cell lymphomas that presumably arise from mucosa-associated lymphoid tissue (MALT). Primary intestinal T-cell lymphomas are rare, but their clinicopathologic features are so distinct that they are recognized as a separate entity in the WHO classification. These lymphomas arise from intestinal mucosal T cells that have cytotoxic function. These lymphomas are regarded as being enteropathy associated if there is clinical evidence of malabsorption or if there is villous atrophy of the mucosa. Enteropathy associated T-cell lymphoma (EATL) has been recognized with increased frequency in populations prone to celiac disease. A genetic predisposition may be present because some patients with EATL appear to have the celiac disease–associated DQA1*0501,DQB1*0201 HLA genotype.

### Clinical Features

Most patients with EATLs are middle aged to elderly. It is unusual for these lymphomas to appear before 40 years of age. There is a slight male preponderance. The most common presenting symptoms are abdominal pain and weight loss. Diarrhea may be present but is not frequent. There may be signs of acute obstruction or spontaneous perforation. The tumor occurs most commonly in the jejunum or ileum, although rare reported cases have been documented in the duodenum, stomach, and colon. Hematologic findings can include anemia and leukocytosis without lymphocytosis. Patients may have a history of celiac disease or other malabsorptive problem. In particular, patients may have a history of refractory celiac disease or celiac disease with development of intestinal ulceration (ulcerative jejunitis). These T-cell lymphomas are aggressive, and most patients die of their disease within 2 years of diagnosis.

**ENTEROPATHY-ASSOCIATED T-CELL LYMPHOMA—FACT SHEET**

**Definition**
- Extranodal lymphoma of cytotoxic T cells that produces small bowel lesions and masses

**Incidence and Location**
- Rare

**Morbidity and Mortality**
- Median survival: less than 2 years
- Type I EATL often associated with celiac disease unlike type II EATL

**Gender and Age Distribution**
- Adults
- Slight male predominance

**Prognosis and Therapy**
- Aggressive disease with poor prognosis

### Pathologic Features

#### Microscopic Findings

The small intestine may have single or multifocal lesions, and the neoplastic cells always involve the mucosa (Figure 9-10). There is often ulceration, particularly in the jejunum (ulcerative jejunitis), and there may be villous atrophy. Lymphoma cells also may infiltrate residual glandular structures producing lymphoepithelial lesions, such as in extranodal marginal zone B-cell lymphomas of the MALT type. This epitheliotropism is also reminiscent of that seen in the skin in patients with mycosis fungoides. The lymphoma may extend through the bowel wall, resulting in perforation. Regional lymph nodes are often involved. Tumor cells are usually medium to large in size with oval to pleomorphic nuclei, are admixed with inflammatory cells, and may be associated with necrosis (type I EATL). Some cases have monomorphic small- to medium-sized tumor cells with round nuclei, and there is no significant associated inflammatory cell background and little, if any, necrosis (type II EATL). There is generally abundant clear to pale eosinophilic cytoplasm, and mitotic activity is usually brisk.

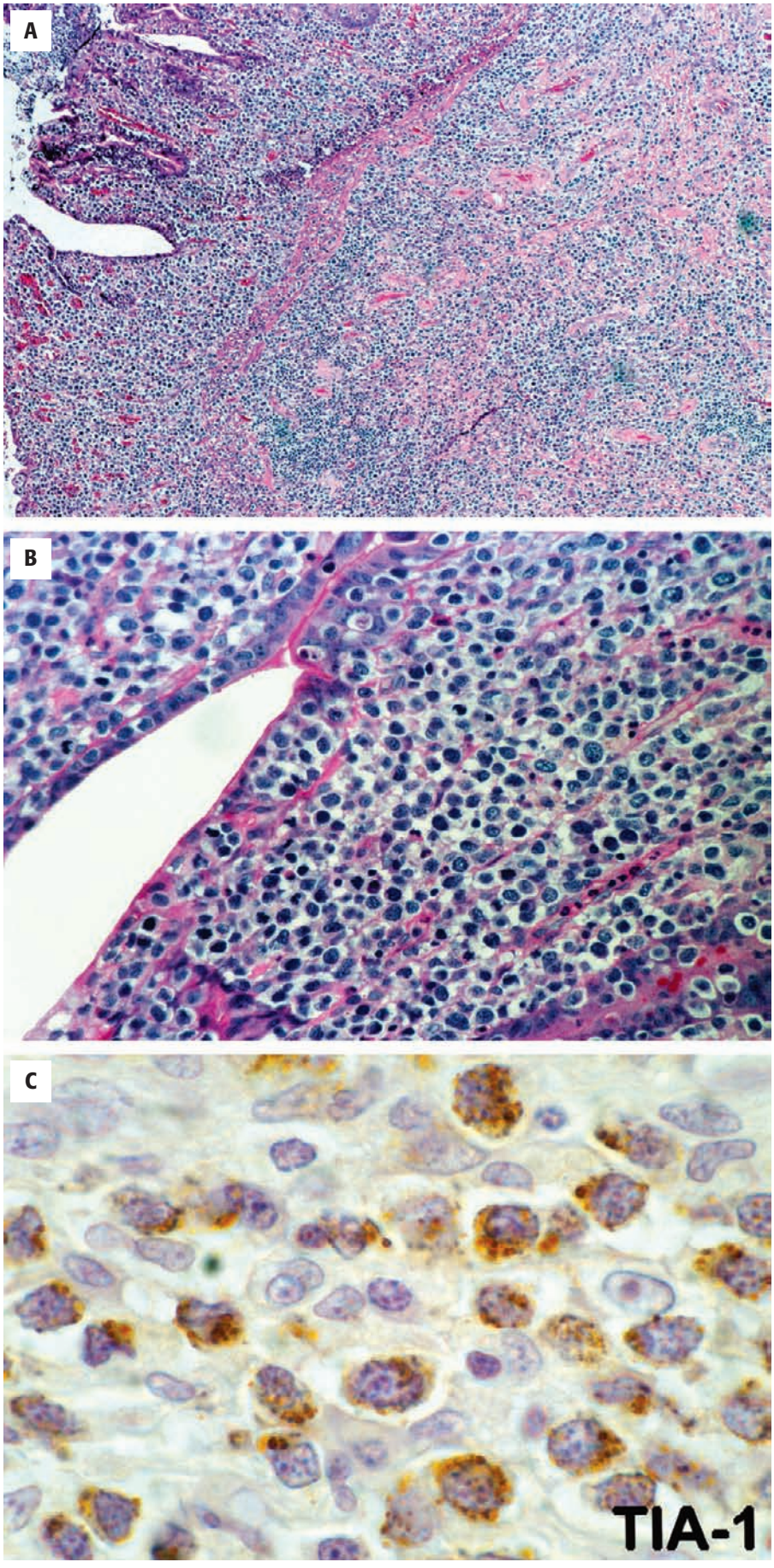


**FIGURE 9-10**

Enteropathy-type T-cell lymphoma: microscopic features and immunophenotypic features. **A,** At low magnification, the small bowel surface shows villous blunting, and there is a dense, diffuse lymphoproliferation throughout the mucosa and submucosa. **B,** At intermediate magnification, neoplastic T cells with clear cytoplasm destroy most of the glandular pattern in the mucosa, but a residual gland at the lower right is infiltrated by occasional tumor cells. **C,** The pleomorphic T cells are TIA-1 positive, indicating their cytotoxic lymphocyte phenotype in this paraffin immunoperoxidase stain.

**ENTEROPATHY-ASSOCIATED T-CELL LYMPHOMA—PATHOLOGIC FEATURES**

**Microscopic Findings**

- Type I EATL: Infiltrate of medium to large tumor cells with oval to pleomorphic nuclei that are frequently associated with inflammatory cells and necrosis
- Type II EATL: Infiltrate of small to medium tumor cells with round nuclei and little to no association with inflammatory cells and necrosis
- Villous atrophy consistent with malabsorptive disease may be present in mucosa adjacent to the tumor

**Immunophenotypic Findings**

- Type I EATL: $CD3^+$, $CD4^-$, $CD5^-$, $CD8^{-/+}$, $CD30^{-/+}$, $CD56^-$, TCR-αβ positive, and expression of cytotoxic granule–associated proteins
- Type II EATL: $CD3^+$, $CD4^-$, $CD5^-$, $CD8^+$, $CD30^{-/+}$, $CD56^+$, TCR-αβ positive, and expression of cytotoxic granule-associated proteins

**Molecular and Cytogenetic Findings**

- Clonal TCR gene rearrangements in most cases
- EBV genome in tumor cells in a few cases
- No recurring chromosomal translocations; gains at chromosomes 1q and 5q for type I EATL and *MYC* amplification for type II EATL

**Differential Diagnosis**

- Celiac disease
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Diffuse large B-cell lymphomas
- PTCL, NOS
- ALCLs (for $CD30^+$ cases)
- Histiocytic sarcoma

#### Immunophenotype

The phenotype is variable, and type I EATL is often $CD2^+$, $CD3^+$, $CD4^-$, $CD5^-$, $CD7^+$, $CD8^{-/+}$, $CD56^-$, and TCR-αβ positive (see Figure 9-10). Type II EATL is often $CD8^+$, $CD56^+$, and TCR-αβ positive. A few cases are TCR-γδ positive. The presence of TIA-1 and variable marking for granzyme B in the tumor cells indicate these lymphomas arise from cytotoxic T cells. Some cases may be $CD30^+$ and must be distinguished from ALCLs.

#### Molecular and Cytogenetic Findings

Many cases demonstrate clonal TCR-β–chain gene rearrangements. The EBV genome can be detected in the tumor cells in a few cases. Karyotypic abnormalities may be present, but a specific pattern has not been identified. Recent studies using comparative genomic hybridization, however, have shown gains involving chromosomes 9q (58% of cases), 7q (24%), 5q (18%), and 1q (16%), with losses at chromosomes 8p (24%), 13q (24%), and 9p (18%). Type I EATL, which is pathogenetically linked to celiac disease, has a higher frequency of chromosomal gains of 1q and 5q. Type II EATL, which is not closely linked to celiac disease, has *MYC* oncogene locus gain.

### Differential Diagnosis

Celiac disease is a consideration because 5% to 10% of these patients will develop an EATL, and there may be similar histologic features between celiac disease and EATL, such as villous atrophy and crypt hyperplasia. However, the lymphoma will have a much more extensive lymphoid infiltrate that also frequently exhibits too much nuclear pleomorphism to be a reactive process. Biopsy specimens from patients with ulcerative jejunitis or refractory celiac disease should be searched carefully for atypical T cells within the ulcer bed and evidence of abnormal T-cell phenotype or T-cell clonality in order to exclude EATL.

Extranodal marginal zone B-cell lymphomas of the MALT type are well known to have lymphoepithelial lesions that also may be seen in EATLs. However, the latter generally have more pleomorphism, greater mitotic activity, and no association with lymphoid follicles. Furthermore, immunophenotyping readily distinguishes the B-cell lymphoma from the T-cell process. Immunoproliferative small intestinal disease, or Mediterranean lymphoma, is a special type of MALT lymphoma that predominantly involves the jejunum and is associated with malabsorption and α heavy chain disease.

Lymphomas composed of neoplastic large cells must be distinguished from diffuse large B-cell lymphomas, PTCL, NOS, and ALCLs for $CD30^+$ cases; this can be done largely with complete immunohistochemical staining. NK cell enteropathy can mimic EATL, particularly when ulceration is present; however, there is no epitheliotropism and cells are NK type and lack surface CD3. Histiocytic sarcoma enters the differential diagnosis because of the cytologic appearance of the tumor cells. In fact, most cases of EATL were incorrectly classified as malignant histiocytosis until adequate phenotyping studies were performed.

## MYCOSIS FUNGOIDES AND SÉZARY SYNDROME

Cutaneous T-cell lymphomas (CTCLs) are a group of T-cell lymphomas that are primarily localized to the skin and presumably derived from T cells of the skin-associated lymphoid tissue. MF, although rare, is the most common form of CTCL. MF and its leukemic

counterpart, Sézary syndrome (SS), are specific clinicopathologic subtypes of CTCL and represent the majority of CTCLs in Western countries.

## Clinical Features

MF occurs most frequently in middle-aged to elderly adults, and approximately two thirds of cases are in males. The disease is generally protracted and is typically preceded by years of nonspecific scaly rash that is often localized and on the trunk. MF has four clinical appearances in the skin: patches, plaques, tumors, and generalized erythroderma. The erythematous patches and thickened plaques can persist for years, become more generalized in distribution, and eventually progress to nodular tumors. Extracutaneous dissemination to lymph nodes and visceral organs occurs late in the disease course. Bone marrow involvement is uncommon. Patients with SS have generalized erythroderma, lymphadenopathy, and peripheral blood involvement.

Survival is dependent on stage. Patients with limited patch- or plaque-stage MF have survival rates equal to that of age-matched controls. Ten-year disease-specific survival rate declines to 42% for tumor-stage MF and 20% for those with histologically proven lymph node involvement. Modern staging systems include skin, lymph node, visceral, and blood assessment. Enumeration of percent (≤5%, >5%, and absolute count >1000/μL) Sézary cells separate patients with absence of significant, low, and high tumor burden, respectively. High CD4:CD8 ratio (≥10), or abnormal immunophenotype by flow cytometry and detection monoclonal TCR gene rearrangement in the blood also influence staging; therefore pathologists may be asked to perform this type of analysis in MF patients. Treatment is usually conservative (i.e., skin targeted) with systemic chemotherapy reserved for advanced disease. Transformation to large-cell lymphoma portends an aggressive clinical course.

Clinical variants of MF include pagetoid reticulosis, granulomatous slack skin, and follicular mucinosis. Pagetoid reticulosis presents as localized patches or plaques. Granulomatous slack skin appears with pendulous folds of skin, usually in the axilla or groin. Folliculotropic MF is a variant that preferentially involves hair follicles and manifests as grouped acneiform papules or plaques on the head and neck. Alopecia is common and mucinorrhea may be present.

### MYCOSIS FUNGOIDES AND SÉZARY SYNDROME—FACT SHEET

**Definition**
- Mycosis fungoides: a specific type of CTCL derived from $CD4^+$ T cells of skin-associated lymphoid tissue
- SS: leukemic counterpart of MF accompanied by generalized erythroderma and lymphadenopathy

**Incidence and Location**
- Rare, but the most common form of CTCL

**Morbidity and Mortality**
- Survival equal to age-matched controls for limited patch or plaque stage
- 42% 10-year disease-specific survival for tumor stage
- 20% 10-year disease-specific survival with proven lymph node involvement

**Gender and Age Distribution**
- Middle-aged to elderly adults
- Males predominate 2:1

**Clinical Features**
- Persistent skin rash (patches, plaques, tumors, and generalized erythroderma) often localized and on trunk
- Clinical variants: pagetoid reticulosis, granulomatous slack skin, and follicular mucinosis

**Prognosis and Therapy**
- Generally indolent disease with survival dependent on stage
- Skin-targeted therapy for limited-stage disease
- Chemotherapy for advanced-stage disease

## Pathologic Features

### Microscopic Findings

The histologic appearance of MF varies with the type of clinical lesion. The classic histologic pattern of MF is associated with plaque-stage lesions and is characterized by a dense bandlike lymphocytic infiltrate in the superficial papillary dermis, epidermal infiltration (epidermotropism) with formation of Pautrier microabscesses, and cytologically atypical, hyperchromatic cerebriform lymphocytes (Figure 9-11). In patch- or early plaque-stage lesions, the infiltrate is less dense and less cytologically atypical and may not have Pautrier microabscesses. In these early lesions, the most reliable histologic features are the presence of medium to large (7 to 9 μm nuclear diameter [approximately the size of basal keratinocyte nuclear diameter]) cerebriform cells in the epidermis or in clusters in the dermis. Epidermotropism as single cells align along basal keratinocytes of the epidermal-dermal junction, absence of significant papillary dermal fibrosis, and absence of significant numbers of blast-like cells are other useful histologic features of early MF. However, the efficacy of single histologic features in the diagnosis of early MF is generally poor. Pautrier microabscesses strongly suggest MF when present, but they are often absent in early lesions and are not entirely specific. The level of certainty in the

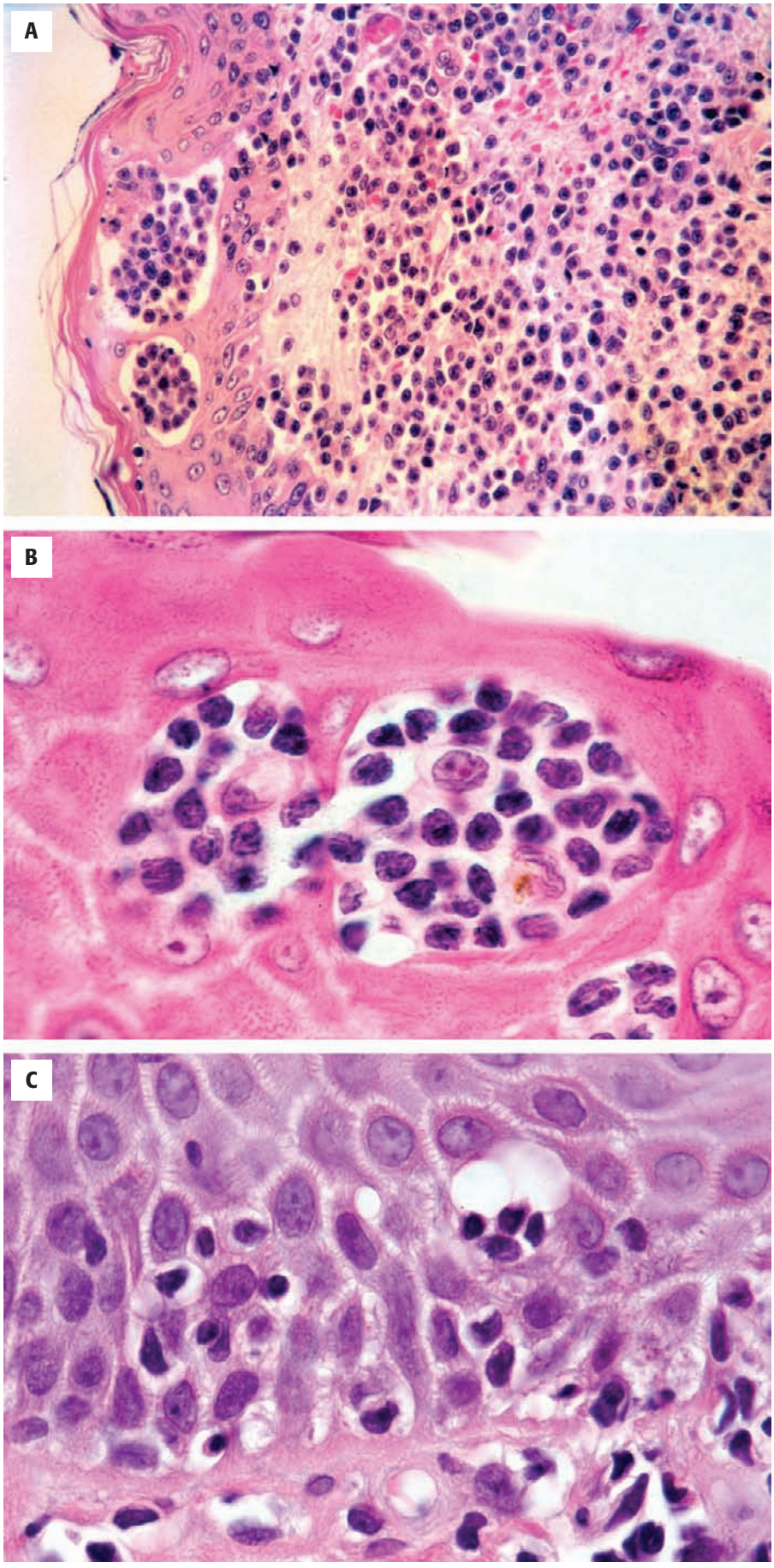


**FIGURE 9-11**

Mycosis fungoides (MF): microscopic features. **A,** At low magnification, this skin biopsy specimen contains a dense lichenoid lymphocytic infiltrate in the superficial dermis that is epidermotropic, including formation of Pautrier microabscesses. **B,** At high magnification, the Pautrier microabscess contains numerous hyperchromatic cerebriform lymphocytes. **C,** At high magnification, hyperchromatic, medium to large cerebriform lymphocytes are singly distributed or in a focal small cluster in the epidermis in this early lesion of MF.

**MYCOSIS FUNGOIDES AND SÉZARY SYNDROME—PATHOLOGIC FEATURES**

**Microscopic Findings**

- Patch stage: slight superficial dermal lymphocytic infiltrate with medium to large cerebriform cells in the epidermis, often as single cells aligned along the basement membrane or in dermal clusters
- Plaque stage: dense bandlike superficial dermal infiltrate of cytologically atypical, hyperchromatic, cerebriform lymphocytes exhibiting epidermotropism and possibly forming Pautrier microabscesses
- Tumor stage: top-heavy dermal infiltrate of medium to large pleomorphic cerebriform lymphocytes that often lack epidermotropism
- Large cell transformation defined by presence of greater than 25% large lymphoid cells in the dermis
- Erythroderma: mild superficial dermal infiltrate of cerebriform lymphocytes that have limited epidermotropism but are frequently in the peripheral blood

**Immunophenotypic Findings**

- $CD4^+$ T cells that are often $CD7^-$
- $CD30^+$ tumor cells in large cell transformation

**Molecular and Cytogenetic Findings**

- Clonal TCR gene rearrangements in most cases
- No recurring chromosomal translocations

**Differential Diagnosis**

- Reactive T-cell infiltrates (parapsoriasis, drug eruptions, contact dermatitis, benign erythroderma, and follicular mucinosis)
- Skin involvement by other peripheral T-cell lymphomas, including adult T-cell leukemia or lymphoma

diagnosis of MF increases as the percentage, size, and degree of nuclear contour irregularities increase. In equivocal cases, it is best to call the biopsy specimen suspicious but not diagnostic and to recommend a repeated biopsy and clinical follow-up.

In tumor-stage MF, the diagnosis of lymphoma is usually not an issue. Instead, the problem is differentiating MF from cutaneous involvement by other PTCLs. Tumor-stage MF often becomes nonepidermotropic but still maintains a top-heavy pattern with the broadest part of the infiltrate in the superficial dermis. Tumor-stage MF usually extends deep into the reticular dermis and often involves the superficial subcutaneous tissue. Cytologic atypia is usually prominent in tumor-stage MF with an increased percentage of medium to large, pleomorphic cerebriform cells. Large-cell transformation, defined by the presence of more than 25% large lymphoid cells in the dermis, is seen in up to 50% of tumor-stage MF cases.

Erythrodermic MF in patients with SS can be difficult to diagnose. The cutaneous infiltrates in SS tend to show less epidermotropism and are more frequently nondiagnostic than patch or plaque stage MF. Pautrier microabscesses are present in approximately 50% of erythrodermic SS skin biopsy specimens. Multiple or repeated biopsies may be necessary for a definitive diagnosis of MF in the skin in patients with SS. However, the diagnosis can often be confirmed by morphologic evaluation of the peripheral blood for circulating Sézary cells in combination with flow cytometry to detect restricted TCR Vβ chain use and TCR gene rearrangement studies. Sézary cells are enlarged lymphocytes with complex nuclear folds and scant cytoplasm. Nuclear chromatin in large Sézary cells is more finely dispersed than in normal peripheral blood lymphocytes. Small Sézary cells can be difficult to recognize in peripheral blood smears. There is currently no consensus on the number of Sézary cells required to diagnosis SS. The minimum criterion is more than five Sézary cells per 100 lymphocytes. More stringent criteria require that Sézary cells constitute at least 10% to 20% of peripheral blood lymphocytes or that there is an absolute Sézary cell count of more than 1000/μL.

Regarding the variants, pagetoid reticulosis manifests as an epidermotropic atypical lymphoid infiltrate limited to the epidermis. No dermal component should be present. Pagetoid reticulosis should not be considered when lesions are generalized. Granulomatous slack skin shows a dense and destructive dermal atypical lymphoid infiltrate with cerebriform nuclei. Macrophages are present and produce a prominent granulomatous component with multinucleated giant cells. Folliculotropic MF has an atypical perivascular and periadnexal lymphoid infiltrate that demonstrates folliculotropism. Mucinous degeneration is often present within the involved follicles. Uncommonly, there may be involvement of the eccrine glands (so-called syringotropic MF).

Extracutaneous dissemination of MF most commonly involves peripheral lymph nodes draining involved skin, but it can also involve the liver, spleen, lungs, and other sites. Extracutaneous MF can be recognized histologically by infiltrates of pleomorphic cerebriform cells. Large-cell transformation of MF can occur initially in extracutaneous sites, giving the appearance of diffuse large-cell lymphoma or ALCL. Bone marrow involvement by MF is often subtle and rarely extensive or hematologically significant. MF in the bone marrow forms lymphoid aggregates with atypical cerebriform cells, but in SS it often shows subtle interstitial infiltrates with small clusters of Sézary cells.

#### Immunophenotype

MF is a neoplasm of $CD4^+$ memory, helper/inducer T cells, but rare $CD8^+$ suppressor/cytotoxic cases are reported. In addition to CD4, these lymphomas express most T-cell antigens expressed by normal peripheral blood T cells (CD2, CD3, CD5, and TCR-αβ), but they

are often negative for CD7. Absent or diminished expression of T-cell antigens other than CD7 supports a diagnosis of MF over a reactive T-cell proliferation; however, these aberrant T-cell phenotypes generally do not occur in early MF, but rather in advanced plaque or tumor stage lesions. Loss of CD26 on neoplastic $CD4^+$ T cells is characteristically seen in Sézary syndrome. CD30 can be expressed in transformed cases and does not have the association of a good prognosis that is seen in de novo $CD30^+$ cutaneous lymphoproliferative disorders.

#### Molecular and Cytogenetic Findings

Clonal rearrangement of TCR genes (TCR-β and TCR-γ) can usually be demonstrated. Characteristic cytogenetic abnormalities for MF have not been identified, although there may be an association with lp22-1p36 abnormalities.

### Differential Diagnosis

The differential diagnosis for MF-SS includes reactive conditions such as parapsoriasis, drug eruptions, contact dermatitis, benign erythroderma, and follicular mucinosis; cutaneous involvement by other PTCLs are also in the differential diagnosis. Immunophenotyping may be of limited usefulness in distinguishing early MF from benign dermatitis because a lack of CD7 can be seen in both, and aberrant T-cell antigen expression is rarely seen in early MF. A CD4:CD8 ratio of more than 10:1 favors MF, but this can also occur in benign dermatitis. Furthermore, clonal TCR gene rearrangements can be detected in some dermatitides. Therefore multiple skin biopsies and clinical correlation are often necessary to make the correct diagnosis. The demonstration of the same TCR rearrangement amplicon in two separate biopsy specimens strongly supports a diagnosis of lymphoma. The differential diagnosis with adult T-cell leukemia–lymphoma has already been discussed.

## PRIMARY CUTANEOUS $CD30^+$ T-CELL LYMPHOPROLIFERATIVE DISORDERS

The primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders are the second most common group of CTCLs and consist of a spectrum of skin lesions composed of atypical large $CD30^+$ T cells. Primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders include primary cutaneous ALCL (C-ALCL), lymphomatoid papulosis (LyP), and borderline lesions. The latter is a term used when the clinicopathologic features of the lesion are not distinguishable for C-ALCL or LyP.

### Clinical Features

Primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders occur mainly in older adults and males more frequently than in females. Primary C-ALCL typically manifests as a primary disease of the skin with solitary or localized nodules. The lesions may spontaneously regress but generally recur. The disease often responds to local excision and radiation therapy, if isolated in location. The overall 5-year survival in primary C-ALCL approaches 90% with current therapy. Dissemination to regional lymph nodes may occur, but visceral involvement is rare. LyP is a chronic, relapsing condition with a benign clinical course. It appears as spontaneously regressing papules typically isolated to the extremities. Localized therapy may reduce the number of lesions and relapses; however, the disease will continue its natural course upon discontinuation of therapy. Approximately 10% to 20% of cases will progress to lymphoma. Extracutaneous dissemination occurs only in those cases with lymphomatous progression.

**PRIMARY CUTANEOUS $CD30^+$ T-CELL LYMPHOPROLIFERATIVE DISORDERS—FACT SHEET**

**Definition**
- A spectrum of skin disorders containing atypical large $CD30^+$ T cells

**Incidence and Location**
- Rare, but second most common form of CTCL

**Morbidity and Mortality**
- Primary cutaneous anaplastic large cell lymphoma: 90% 5-year overall survival
- Lymphomatoid papulosis: benign clinical course of long duration

**Gender and Age Distribution**
- Older adults
- Males predominate

**Clinical Features**
- Primary cutaneous anaplastic large cell lymphoma: solitary or localized skin nodules that may spontaneously regress but generally recur
- Lymphomatoid papulosis: spontaneously regressing papules typically isolated on extremities

**Prognosis and Therapy**
- Primary cutaneous anaplastic large cell lymphoma: skin-targeted therapy for limited stage disease and chemotherapy for extracutaneous disease
- Lymphomatoid papulosis: low-dose methotrexate and psoralen with ultraviolet A

## Pathologic Features

### Microscopic Findings

The infiltrates of primary C-ALCL are diffuse and extend from the papillary dermis to the subcutaneous tissue (Figure 9-12). There is often ulceration but little epidermotropism. The cytologic features of the tumor cells are similar to those in systemic ALCL. An inflammatory cell background may be present but is not prominent.

LyP has several histologic appearances, but the lesions are generally wedge-shaped dermal infiltrates of cytologically atypical lymphocytes admixed with a polymorphic inflammatory cell reaction. LyP, type A, contains Reed-Sternberg–like cells with numerous inflammatory cells, producing a resemblance to classical Hodgkin lymphoma. LyP, type B, is uncommon and has a predominance of cerebriform lymphocytes and a less pronounced inflammatory cell infiltrate. LyP, type C has confluent sheets of large tumor cells characteristic of primary C-ALCL, but regressing papules clinically.

**PRIMARY CUTANEOUS CD30⁺ T-CELL LYMPHOPROLIFERATIVE DISORDERS—PATHOLOGIC FEATURES**

**Microscopic Findings**

- Primary cutaneous ALCL: diffuse dermal infiltrate of numerous atypical large cells that extends to subcutaneous tissue but has little epidermotropism
- Lymphomatoid papulosis: wedge-shaped dermal infiltrate of variable numbers of atypical large cells admixed with polymorphic reactive cell background
  - Type A: Reed-Sternberg–like cells with numerous inflammatory cells
  - Type B: Cerebriform lymphocytes and modest inflammatory cell infiltrate
  - Type C: Confluent sheets of atypical large cells morphologically but regressing papules clinically

**Immunophenotypic Findings**

- CD30⁺ atypical large cells that usually express CD4, have a cytotoxic lymphocyte phenotype, and are ALK negative

**Molecular and Cytogenetic Findings**

- Clonal TCR gene rearrangements in most primary C-ALCLs and LyP
- *IRF4* (6p25.3) translocation present in approximately 30% of primary C-ALCLs, but rare in LyP and transformed MF

**Differential Diagnosis**

- Secondary cutaneous involvement by systemic ALCL
- Large cell transformation of MF

### Immunophenotype

Greater than 75% of the atypical large cells are CD30⁺ in primary C-ALCL and LyP, types A and C (see Figure 9-12, C). The cerebriform lymphocytes in LyP, type B, are typically negative for CD30. The atypical T cells in all these lesions are usually CD4⁺ and often have a cytotoxic lymphocyte phenotype with expression of TIA-1, granzyme B, and perforin. An aberrant T-cell phenotype is common, and the atypical cells are consistently ALK-negative.

### Molecular and Cytogenetic Findings

Clonal TCR gene rearrangements are found in most primary C-ALCLs and in approximately 60% of LyP. Translocations involving *ALK* are not seen. However, 6p25.3 translocations involving in the region of *IRF4* and *DUSP22* can be seen in nearly 30% of primary C-ALCL and are rarely observed in LyP and transformed MF. This translocation is not present in systemic ALCL or in other PTCLs studied. Gene expression profiling shows marked differences in expression patterns of chemokine receptors and apoptosis regulators between primary C-ALCL and primary cutaneous PTCL, NOS.

## Differential Diagnosis

Primary cutaneous ALCL must be distinguished from secondary cutaneous involvement by systemic ALCL, from large cell transformation of MF, and from LyP. It is therefore often critical in skin lesions to correlate the histopathology and immunostain findings with clinical or clonality studies, or both, to classify these lesions accurately in a specific patient.

# PRIMARY CUTANEOUS PERIPHERAL T-CELL LYMPHOMAS: RARE SUBTYPES

CTCLs also include three rare types of primary cutaneous PTCLs: primary cutaneous γδ T-cell lymphoma, primary cutaneous CD8⁺ aggressive epidermotropic cytotoxic T-cell lymphoma, and primary cutaneous CD4-positive small/medium T-cell lymphoma. All these types must be distinguished from MF or one of its variants by their clinicopathologic features.

## Clinical Features

Primary cutaneous γδ T-cell lymphomas occur mainly in adults who have B symptoms. There is no gender predilection. Skin lesions are often generalized, mostly

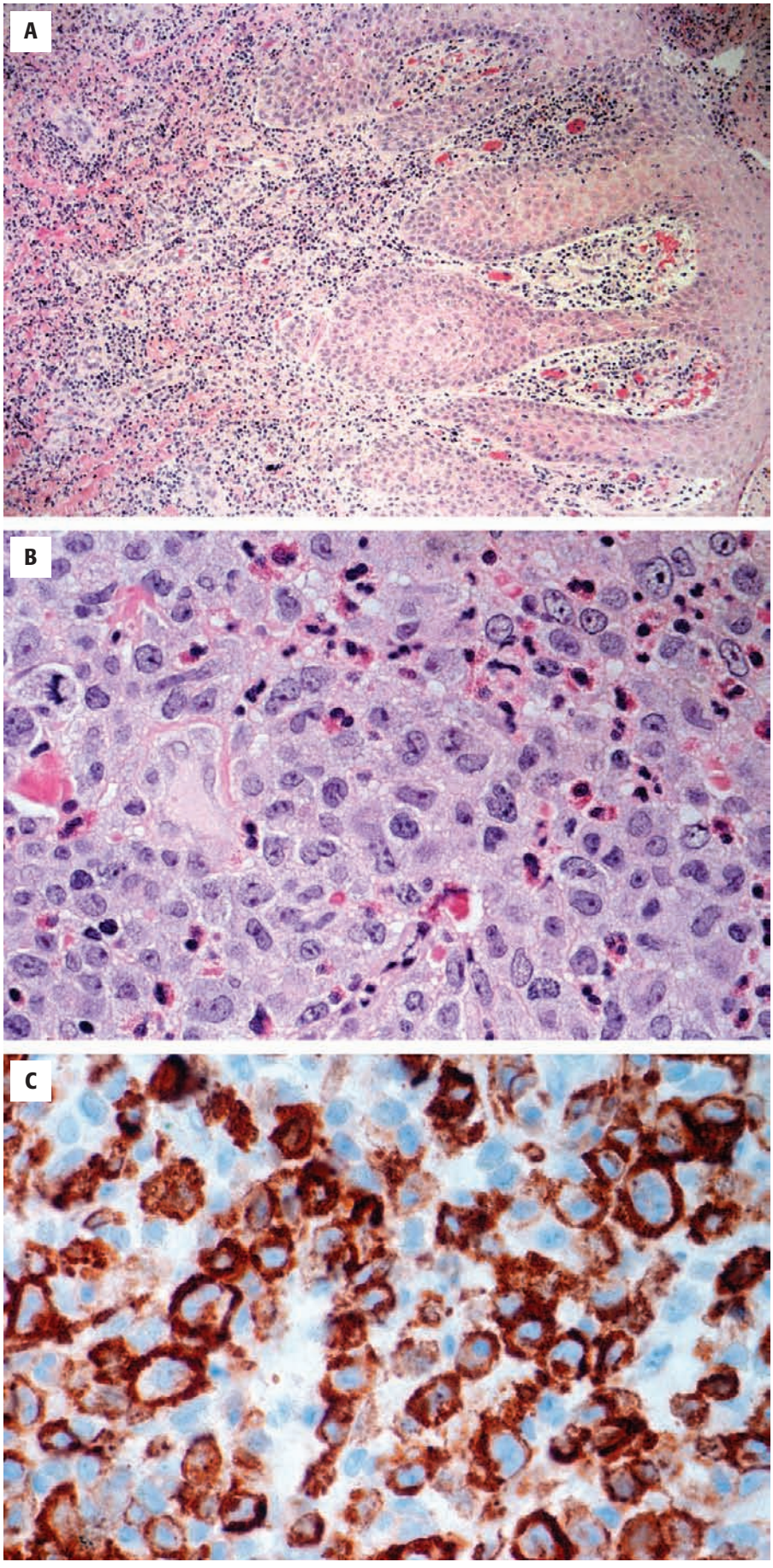


**FIGURE 9-12**

Primary cutaneous CD30⁺ T-cell lymphoproliferative disorders: microscopic features and immunophenotypic features. **A,** At low magnification, this skin excision exhibits pseudoepitheliomatous hyperplasia associated with a dermal lymphocytic infiltrate. **B,** At high magnification, there is a mixed lymphoeosinophilic infiltrate in which most of the lymphocytes are large. **C,** The large lymphocytes are CD30⁺ in this paraffin immunoperoxidase stain.

on the extremities. The lesions vary from papules, patches, and plaques to deep dermal or subcutaneous nodules that may be ulcerated. The tumor frequently disseminates to mucosal and other extranodal sites, but lymph nodes, spleen, and bone marrow are usually spared. A hemophagocytic syndrome may develop. Median survival is less than 2 years.

Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma occurs mainly in adults with generalized skin lesions that vary from superficial hyperkeratotic patches and plaques to eruptive papules and centrally ulcerated nodules. The disease may disseminate to visceral sites, but lymph nodes are usually spared. Median survival is less than 3 years.

**PRIMARY CUTANEOUS PERIPHERAL T-CELL LYMPHOMA, RARE SUBTYPES—FACT SHEET**

**Definition**

- Three subtypes of primary cutaneous PTCLs that are distinguishable from the much more common MF and primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders: primary cutaneous γδ T-cell lymphoma, primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma, and primary cutaneous $CD4^+$ small/medium T-cell lymphoma

**Incidence and Location**

- Extremely rare

**Morbidity and Mortality**

- Primary cutaneous γδ T-cell lymphoma and primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: both subtypes aggressive with median survival less than 3 years
- Primary cutaneous $CD4^+$ small/medium T-cell lymphoma: indolent with 5-year survival approximately 80%

**Gender and Age Distribution**

- Adults
- No gender predilection

**Clinical Features**

- Primary cutaneous γδ T-cell lymphoma: generalized papules, patches, and plaques to deep dermal or subcutaneous nodules that may be ulcerated, followed by dissemination to mucosal and other extranodal sites
- Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma: generalized superficial hyperkeratotic patches and plaques to eruptive papules and centrally ulcerated nodules followed by extranodal dissemination
- Primary cutaneous $CD4^+$ small/medium T-cell lymphoma: solitary plaques or nodules preferentially in head and neck region, but sometimes in upper extremities or trunk

**Prognosis and Therapy**

- Primary cutaneous γδ T-cell lymphoma and primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: chemotherapy but poor prognosis for both
- Primary cutaneous $CD4^+$ small/medium T-cell lymphoma: good prognosis with surgical excision or local irradiation

Primary cutaneous $CD4^+$ small/medium T-cell lymphoma occurs mainly in asymptomatic adults as solitary plaques or nodules preferentially in the head and neck region or sometimes in the upper extremities or trunk. The disease is indolent, and surgical excision or local irradiation is usually the treatment of choice. There is a 5-year survival rate of approximately 80%.

## Pathologic Features

### Microscopic Findings

The infiltrates of primary cutaneous γδ T-cell lymphomas have several histologic appearances that may be present within the same lesion or between different lesions. Epidermotropism varies from focally mild to overtly pagetoid. Subcutaneous lesions resemble SPTCL but are often accompanied by a dermal component not seen in SPTCL. Tumor cells are typically medium to large in size with clumped chromatin. Karyorrhexis and necrosis are common, and angioinvasion may be seen.

Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphomas have marked pagetoid epidermotropism, possibly associated with a lichenoid pattern (Figure 9-13). Invasion and destruction of adnexal structures is common, and angioinvasion may be present. Tumor cells vary in size from small-medium to medium-large and are pleomorphic.

Primary cutaneous $CD4^+$ small/medium T-cell lymphomas show dense nodular dermal infiltrates that may extend into the subcutis (Figure 9-14). Epidermotropism, if present, is not conspicuous. Tumor cells are typically small-medium in size and are pleomorphic. Pleomorphic large cells, if present, compose less than 30% of the infiltrate. Reactive small lymphocytes and histiocytes may be frequent.

### Immunophenotype

The tumor cells of primary cutaneous γδ T-cell lymphoma are typically $CD3^+$, $CD4^-$, $CD5^-$, $CD7^{+/-}$, $CD8^-$, $CD56^+$, TCR-$\alpha\beta^-$ and TCR-$\gamma\delta^+$. Tumors resembling SPTCL preferentially express the TCRVδ2 epitope, which is the form found in normal cutaneous γδ T-cells. All cases have strong expression of cytotoxic granule-associated proteins such as TIA-1 and granzyme B.

The tumor cells of primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma are typically $CD3^+$, $CD4^-$, $CD5^-$, $CD7^{+/-}$, $CD8^+$, $CD56^-$, TCR-$\alpha\beta^+$, and TCR-$\gamma\delta^-$ (see Figure 9-13). These cells express cytotoxic granule-associated proteins such as TIA-1 and granzyme B.

The tumor cells of primary cutaneous $CD4^+$ small/medium T-cell lymphoma are typically $CD3^+$, $CD4^+$,

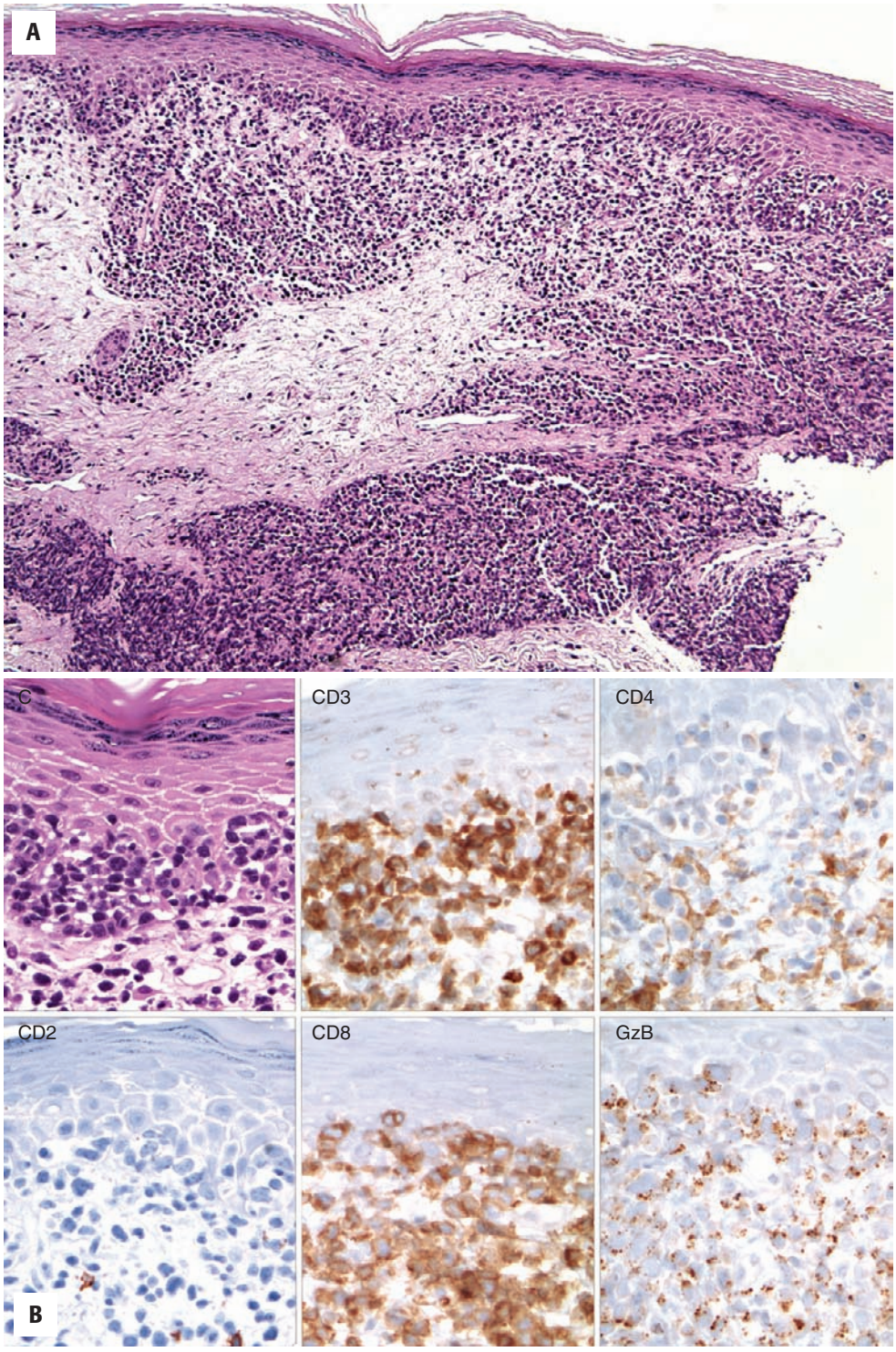


**FIGURE 9-13**

Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: microscopic features and immunophenotypic features. **A,** At low magnification, this skin biopsy shows an epidermotropic and lichenoid lymphoid infiltrate that extends into the deep dermis. **B,** At high magnification in the *upper left panel,* small to medium-sized tumor cells show striking epidermotropism. The accompanying paraffin immunoperoxidase stains show a $CD3^+$, $CD8^+$, and granzyme $B^+$ phenotype for the neoplastic cells that also lack CD4 and have aberrant loss of CD2.

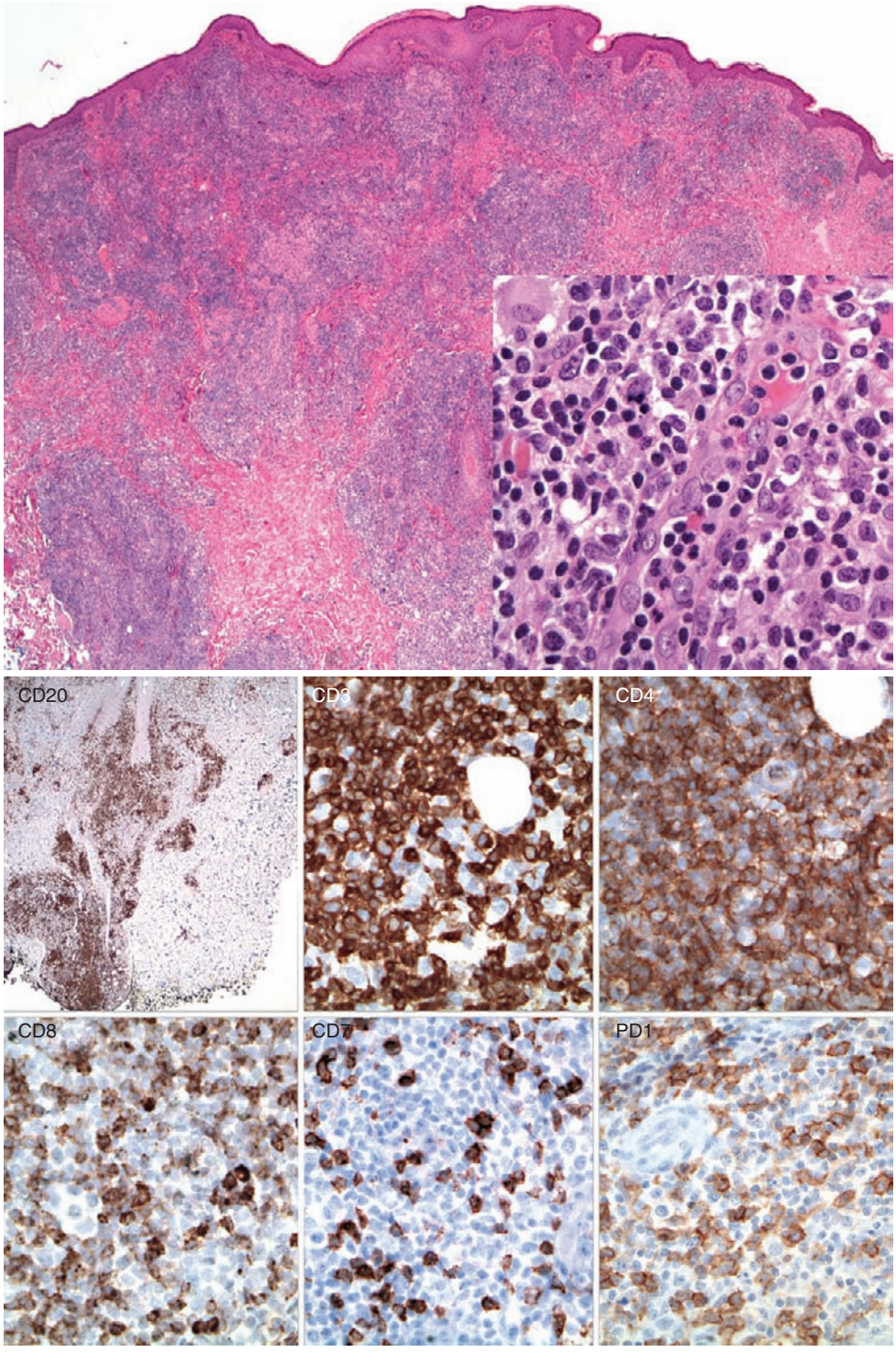


**FIGURE 9-14**

Primary cutaneous $CD4^+$ small/medium T-cell lymphoma: microscopic features and immunophenotypic features. At low magnification, this skin excision exhibits a patchy dense dermal lymphoid infiltrate with no significant epidermotropism. High magnification *(inset)* shows mostly pleomorphic small lymphocytes and some medium-sized lymphocytes admixed with histiocytes. At high magnification, the paraffin immunoperoxidase stains show a $CD3^+$, $CD4^+$, and $CD7^-$ phenotype for the tumor cells, some of which coexpress PD1 (CD279). There are intermixed non-neoplastic $CD20^+$ B cells and reactive $CD7^+$ and $CD8^+$ T cells.

**PRIMARY CUTANEOUS PERIPHERAL T-CELL LYMPHOMA, RARE SUBTYPES—PATHOLOGIC FEATURES**

**Microscopic Findings**

- Primary cutaneous γδ T-cell lymphoma: variable with focal mild to overtly pagetoid epidermotropism, dermal, and subcutaneous involvement by medium to large tumor cells associated with karyorrhexis and necrosis
- Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: marked pagetoid epidermotropism by pleomorphic small-medium to medium-large tumor cells, possibly associated with lichenoid pattern and destruction of adnexal structures
- Primary cutaneous $CD4^+$ small/medium T-cell lymphoma: dense nodular dermal infiltrates of pleomorphic small-medium tumor cells that may extend into subcutis, but have no conspicuous epidermotropism or significant large cell component

**Immunophenotypic Findings**

- Primary cutaneous γδ T-cell lymphoma: $CD3^+$, $CD4^-$, $CD5^-$, $CD7^{+/-}$, $CD8^-$, $CD56^+$, TCR-$\alpha\beta^-$, TCR-$\gamma\delta^+$, TIA-$1^+$, and granzyme $B^+$
- Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma: $CD3^+$, $CD4^-$, $CD5^-$, $CD7^{+/-}$, $CD8^+$, $CD56^-$, TCR-$\alpha\beta^+$, TCR-$\gamma\delta^-$, TIA-$1^+$, and granzyme $B^+$
- Primary cutaneous CD4-positive small/medium T-cell lymphoma: $CD3^+$, $CD4^+$, $CD8^-$, $CD30^-$, $PD1^+$, TCR-$\alpha\beta^+$, TCR-$\gamma\delta^-$, TIA-$1^-$, and granzyme $B^-$

**Molecular and Cytogenetic Findings**

- Clonal TCR gene rearrangements in all three subtypes
- No association with EBV
- No specific genetic abnormalities

**Differential Diagnosis**

- MF or its variants for all three subtypes
- SPTCL for primary cutaneous γδ T-cell lymphomas having subcutaneous predominance
- Cutaneous lymphoid hyperplasia for primary cutaneous $CD4^+$ small/medium T-cell lymphoma
- Cutaneous B-cell lymphomas for primary cutaneous $CD4^+$ small/medium T-cell lymphomas with numerous non-neoplastic B-cells

$CD8^-$, $CD30^-$, TCR-$\alpha\beta^+$, and TCR-$\gamma\delta^-$ (see Figure 9-14); they do not express cytotoxic granule-associated proteins. Frequent small B cells are often intermixed. These lymphomas may be related to TFH cells because they have also been shown to express TFH-associated markers such as PD-1, CXCL-13, and BCL6.

#### Molecular and Cytogenetic Findings

Clonal TCR gene rearrangements are generally found in all three of these rare primary cutaneous PTCL subtypes. If clonal TCR-β chain gene rearrangements are detected in primary cutaneous γδ T-cell lymphoma, then they are nonproductive. None of these rare subtypes is EBV-associated. Specific genetic abnormalities have not been described for any of the three subtypes.

### Differential Diagnosis

All three rare subtypes must be distinguished from MF or one of its variants. Presence of typical patches or plaques in the appropriate distribution along with epidermotropism of $CD4^+$ T-cells supports a diagnosis of MF. Primary cutaneous γδ T-cell lymphomas with predominant subcutaneous involvement require a distinction from SPTCL. Immunophenotyping demonstrating that the tumor cells are indeed γδ type strongly supports primary cutaneous γδ T-cell lymphoma as opposed to these other rare types and SCPTCL. Primary cutaneous $CD4^+$ small/medium T-cell lymphomas can be misidentified as cutaneous lymphoid hyperplasia or as a cutaneous B-cell lymphoma when there are numerous intermixed non-neoplastic B cells. Demonstration of monoclonality and expression of TFH markers on the atypical $CD4^+$ T cells will support the diagnosis. Drug reactions associated with lymphoid hyperplasia, such as anticonvulsants, should also be considered, since these lymphoproliferative lesions can be monoclonal T-cell dominant reactions. Clinical history is important for arriving at the most appropriate diagnosis.

## EPSTEIN-BARR VIRUS–POSITIVE T-CELL LYMPHOPROLIFERATIVE DISORDERS OF CHILDHOOD

There are two main EBV-associated T-cell lymphoproliferative disorders that manifest in the pediatric age group and occasionally in young adults: systemic EBV-positive T-cell lymphoproliferative disease of childhood and hydroa vacciniforme–like lymphoma. Both have increased frequency in Asians and in Native Americans from Central and South America and Mexico.

### Clinical Features

Systemic EBV-positive T-cell lymphoproliferative disease of childhood occurs shortly after primary acute EBV infection or in the setting of chronic active EBV infection. The clinical course is fulminant with fever, hepatosplenomegaly, cytopenia, rapid progression to multiorgan failure, sepsis, and death within days to weeks. A hemophagocytic syndrome usually develops. Cases of systemic EBV-positive T-cell lymphoproliferative disorder of

childhood have previously been reported under such terms as *fulminant hemophagocytic syndrome, fatal EBV-associated hemophagocytic syndrome,* and *severe chronic active EBV infection*. It is associated with abnormal EBV serology with low or absent anti-VCA IgM antibodies and high viral loads.

Hydroa vacciniforme–like lymphoma manifests as papulovesicular eruptions on sun-exposed skin that generally ulcerate and scar. Patients exhibit hypersensitivity to sunlight and insect bites, particularly mosquito bites. The clinical course is often protracted for a decade or more before progression to systemisc disease, which behaves more aggressively with fatal outcome. B symptoms accompanied by lymphadenopathy and hepatosplenomegaly occur with systemic involvement.

**EPSTEIN-BARR VIRUS–POSITIVE T-CELL LYMPHOPROLIFERATIVE DISORDERS OF CHILDHOOD—FACT SHEET**

**Definition**
- Two EBV-associated T-cell lymphoproliferative disorders in the pediatric age group: systemic EBV-positive T-cell lymphoproliferative disease of childhood and hydroa vacciniforme–like lymphoma

**Incidence and Location**
- Rare

**Morbidity and Mortality**
- Systemic EBV-positive T-cell lymphoproliferative disease of childhood: most cases fulminant with death in days to weeks after onset
- Hydroa vacciniforme–like lymphoma: protracted course often lasting more than a decade, but fatal outcome

**Race and Age Distribution**
- Children and occasionally young adults
- Increased frequency in Asians, and in Native Americans from Central and South America and Mexico for both entities

**Clinical Features**
- Systemic, EBV-positive T-cell lymphoproliferative disease of childhood: occurs shortly after primary acute EBV infection or in the setting of chronic active EBV infection with rapid progression to hemophagocytic syndrome, multiorgan failure, sepsis, and death
- Hydroa vacciniforme–like lymphoma: papulovesicular eruptions on sun-exposed skin that generally ulcerate and scar in patients who exhibit hypersensitivity to sunlight and insect bites, particularly mosquito bites

**Prognosis and Therapy**
- Systemic EBV-positive T-cell lymphoproliferative disease of childhood: poor response to any therapy
- Hydroa vacciniforme–like lymphoma: refractory to chemotherapy, but cures possible with allogeneic hematopoietic stem cell transplantation

## Pathologic Features

### Microscopic Findings

Systemic EBV-positive T-cell lymphoproliferative disease of childhood preferentially infiltrates the liver and spleen, followed by lymph nodes, bone marrow, skin, and lung. Splenic infiltrates are mainly in the sinuses, and the white pulp is diminished. Hepatic infiltrates are portal and sinusoidal. The tumor cells are often small without significant cytologic atypia, but cases with pleomorphic medium- to large-sized cells have been described. Hemophagocytosis is often pronounced.

Hydroa vacciniforme–like lymphoma extends from the epidermis to the subcutis, and there is frequent ulceration. Tumor cells are small to medium in size without significant cytologic atypia. Angiocentric and angioinvasive foci may be present.

### Immunophenotype

Tumor cells of systemic EBV-positive T-cell lymphoproliferative disease of childhood are typically $CD3^+$, $CD56^+$, and TIA-$1^+$. The cells are often $CD8^+$ after acute

**EPSTEIN-BARR VIRUS–POSITIVE T-CELL LYMPHOPROLIFERATIVE DISORDERS OF CHILDHOOD—PATHOLOGIC FEATURES**

**Findings**
- Systemic EBV-positive T-cell lymphoproliferative disease of childhood: splenic sinus and hepatic portal and sinusoidal infiltrates of usually small tumor cells, often accompanied by striking hemophagocytosis
- Hydroa vacciniforme–like lymphoma: epidermal to subcutaneous infiltrates of small to medium-sized tumor cells, often associated with ulceration

**Immunophenotypic Findings**
- Systemic, EBV-positive T-cell lymphoproliferative disease of childhood: $CD3^+$, $CD56^+$, and TIA-$1^+$; $CD8^+$ after acute primary EBV infection, but $CD4^+$ in setting of severe chronic active EBV infection
- Hydroa vacciniforme–like lymphoma: $CD3^+$, $CD56^{+/-}$, and TIA-$1^+$

**Molecular and Cytogenetic Findings**
- Clonal TCR gene rearrangements for both entities
- EBV-positive tumor cell by in situ hybridization for both
- Clonal EBV by terminal repeat analysis

**Differential Diagnosis**
- Systemic EBV-positive T-cell lymphoproliferative disease of childhood: aggressive NK-cell leukemia and HSTCL
- Hydroa vacciniforme–like lymphoma: extranodal NK/T-cell lymphoma, nasal type

primary EBV infection, but $CD4^+$ in the setting of severe chronic active EBV infection. Immunophenotyping may be needed to demonstrate the true extent of infiltrate in cases with minimal cytologic atypia.

Tumor cells of hydroa vacciniforme–like lymphoma have a cytotoxic T-cell phenotype and may express CD56. A few cases have NK-cell derivation.

#### Molecular and Cytogenetic Findings

Clonal rearrangement of TCR genes can usually be demonstrated in both systemic EBV-positive T-cell lymphoproliferative disease of childhood and hydroa vacciniforme–like lymphoma. Tumor cells are EBV positive by in situ hybridization, and EBV is monoclonal by terminal repeat analysis.

## Differential Diagnosis

Systemic EBV-positive T-cell lymphoproliferative disease of childhood has some overlapping features with aggressive NK-cell leukemia; however, the latter tends to occur more frequently in middle-aged adults rather than in the pediatric age group. HSTCL should be included in the initial differential diagnosis, but can be excluded by the phenotypic attributes of the tumor cells and their lack of EBV. Hydroa vacciniforme–like lymphoma must be distinguished from cutaneous presentation of extranodal NK/T-cell lymphoma, nasal type. X-linked lymphoproliferative disease caused by *SAP* mutation is characterized by an initial fulminant acute EBV infection with organomegaly and hemophagocytic syndrome. EBV-infected B cells and activated T cells are seen as opposed to the EBV-infected T/NK cells of these systemic EBV-positive T-cell disorders.

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10

# Immunodeficiency-Related Lymphoproliferative Disorders

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## INTRODUCTION

Lymphoproliferative disorders are innately disorders of the immune system, and the immunodeficiency-related lymphoproliferative disorders are of particular interest because they offer a paradigm for studying the relationships between the immune system and the evolution of neoplastic disease. Although the various entities offer a complicated spectrum of lymphoid proliferations with heterogeneous morphologies, they have a number of features in common, including the tendency to involve extranodal sites and to be infected with the Epstein-Barr virus (EBV).

## POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS

Posttransplantation lymphoproliferative disorders (PTLDs) occur in patients who have undergone bone marrow or solid organ transplantation and associated immunosuppressive therapy to prevent graft rejection.

### Clinical Features

According to the United Network for Organ Sharing (UNOS), the 5-year cumulative incidence of PTLD is 3.26% for children and 0.91% for adults (Organ Procurement and Transplantation Network [OPTN]/UNOS Ad Hoc Disease Transmission Advisory Committee Report to the Board of Directors 2009). PTLD is most common in children younger than 10 years and adults older than over 60 years. It is the second most common posttransplant malignancy in children, and the second most common malignancy after skin cancer in adults. The risk of PTLD is greatest in the first year to 18 months, but can occur any time after transplant. Treatment decisions are made based on the histologic type, grade, stage, and site of tumor, assessment of clinical state including transplant organ function, and capacity to tolerate therapy.

Patients who are EBV negative before transplantation and then seroconvert are at the greatest risk for PTLD. With early preventive strategies late PTLD is becoming relatively more common. Greater risk occurs with more complex and multiple organ transplants, which require greater immunosuppression, including the use of cyclosporine A and monoclonal antibody OKT3. In bone marrow transplant recipients, PTLD is of donor rather than recipient origin, and there is increased risk with T-cell depletion, severe graft-versus-host disease, anti-CD3 immunotherapy, and degree of HLA mismatch.

Plasma cell hyperplasia–infectious mononucleosis (IM)–like PTLDs tend to occur in younger age groups, often children and involve the head and neck, particularly tonsil and Waldeyer's ring. These lesions usually regress with surgical excision or reduction in immunotherapy. Cases of polymorphic PTLD may regress in response to decreased immunosuppression, but more aggressive lesions will either require chemotherapy (including the use of anti-CD20 rituximab), and radiation. In the more advanced forms of PTLD there may be no response or progression despite therapy. Monomorphic PTLDs are often extranodal, but may present in bone marrow or lymph nodes. These PTLDs tend to involve older age groups (median age, 56 years) and are often aggressive despite chemotherapy or radiation therapy. T-cell PTLD is uncommon, usually EBV negative, and tends to be aggressive and refractory to therapy.

**POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER—FACT SHEET**

**Definition**
- Heterogeneous group of lymphoid proliferations occurring in the posttransplantation setting, frequently extranodal and usually positive for EBV

**Clinical Features**
- Plasma cell hyperplasia–IM-like PTLDs usually in younger age group and often involve tonsils or Waldeyer's ring
- More advanced lesions often extranodal, including the transplanted organ

**Pathologic Features**
- Early lesions: Plasma cell hyperplasia–IM-like PTLD
- Polymorphic PTLD
- Monomorphic PTLD (classified like the corresponding lymphoma)

**B-Cell Neoplasms**
- Diffuse large B-cell lymphoma
- Burkitt lymphoma
- Myeloma
- Other

**T/NK-Cell Neoplasms**
- Peripheral T-cell lymphoma, NOS
- Hepatosplenic T-cell lymphoma
- NK cell leukemia–lymphoma
- Other

**Immunohistochemistry**
- Early lesions (plasma cell hyperplasia, IM) are polyclonal; the remainder are monoclonal for immunoglobulin expression
- EBV is demonstrated by in situ hybridization for EBER or expression of LMP-1; EBER is more sensitive and should be used as a screening test

**Molecular Genetics**
- Plasma cell hyperplasia is germline for immunoglobulin genes, and EBV polyclonal or oligoclonal
- Both polymorphic and monomorphic PTLDs are monoclonal for immunoglobulin genes and EBV
- Polymorphic PTLD lacks oncogene abnormalities except for *BCL6*
- Advanced lesions frequently have oncogene or suppressor gene abnormalities

**Prognosis and Therapy**
- Early lesions may respond to excision or reduction of immunosuppression
- More advanced lesions may progress despite chemotherapy or radiation therapy; treatment with anti-CD20 (Rituximab) has been effective in some cases

## Pathologic Features

There is a spectrum of histologic appearances with PTLDs ranging from the early lesions (plasmacytic hyperplasia–IM-like), polymorphic PTLD, and monomorphic PTLD, which includes malignant lymphoma and myeloma (Table 10-1).

In plasmacytic hyperplasia–infectious mononucleosis–like PTLD, which often involves lymph nodes and tonsils, there is retention of the overall architecture with expansion of interfollicular areas by a proliferation of plasma cells, admixed with small lymphocytes and occasional immunoblasts (Figure 10-1). These immunoblasts are usually single and there is minimal cytologic atypia. Some cases may have histologic features of follicular or other lymphoid hyperplasia without typical features of IM.

In polymorphic PTLD there is destruction of architecture and a lymphoid infiltrate showing variable plasmacytic differentiation including immunoblasts, which may be found in small clusters. Atypical lymphoid cells with irregular nuclear outlines may be present, and there may be foci of necrosis (Figure 10-2).

Monomorphic PTLD resembles lymphoma in the immunocompetent population and includes diffuse large B-cell lymphomas, which may have an immunoblastic appearance, Burkitt lymphoma (BL), myeloma, plasmacytoma, Hodgkin lymphoma (HL), and T-cell lymphomas. Indolent small B-cell lymphomas after transplant are generally not considered part of the spectrum of PTLD.

Burkitt lymphoma after transplant (Figure 10-3) often manifests with high-stage disease and bone marrow involvement. It usually occurs late after transplant (average, 4.5 years). Plasmacytoma-like PTLD is relatively rare (Figure 10-4) and is similar to extramedullary plasmacytoma in other settings, involving extranodal sites such as the gastrointestinal tract or lymph nodes.

Classical HL is a late-occurring PTLD (mean, 4 years after transplantation, but possibly 7 years or longer). Mixed cellularity type is most common, and the Reed-Sternberg cells are EBV positive (Figure 10-5). The differential includes polymorphous Hodgkin-like PTLD, and immunostaining may be helpful in the differential (Reed-Sternberg cells should have the classical phenotype $CD15^+$, $CD30^+$, usually negative for OCT-2 or BOB.1).

T-cell PTLDs range in incidence from 4% to 14% of PTLD. They should be classified according to the World Health Organization classification of T-cell lymphomas, and include the entire spectrum of T/natural killer (NK) cell neoplasms. T-cell PTLDs are usually EBV negative and occur later than B-cell PTLDs. PTLDs of NK-cell lineage are rare and include T/NK-cell

**TABLE 10-1**

**Classification and Key Features of PTLD**

| Type of PTLD | Histology | Phenotype | Molecular |
|---|---|---|---|
| ***Early lesions*** | | | |
| Plasma cell hyperplasia–IM-like | Variable follicular hyperplasia; numerous plasma cells; immunoblastic proliferation in background of small T lymphocytes and plasma cells | Polyclonal plasma cells T cells; most EBV positive | Polyclonal or oligoclonal |
| ***Polymorphic*** | | | |
| | Effacement of architecture; mixed population of lymphocytes, plasma cells, immunoblasts | B cells and T lymphocytes admixed with immunoblasts and plasma cells | Clonal Ig gene rearrangements; usually EBV positive; plasma cells may show light chain restriction |
| ***Monomorphic*** | | | |
| DLBCL, Burkitt, myeloma, plasmacytoma, classical Hodgkin lymphoma, T-cell lymphoma | Resembles lymphoma type in nonimmunosuppressed population | Similar to lymphoma subtype; most EBV positive; large cells may express EBER and LMP1 | Clonal Ig gene rearrangements; often have other molecular abnormalities (e.g., *MYC, BCL6, NRAS,* p53) |

*DLBCL,* Diffuse large B-cell lymphoma; *Ig,* immunoglobulin; *IM,* infectious mononucleosis; *PTLD,* posttransplantation lymphoproliferative disorder.

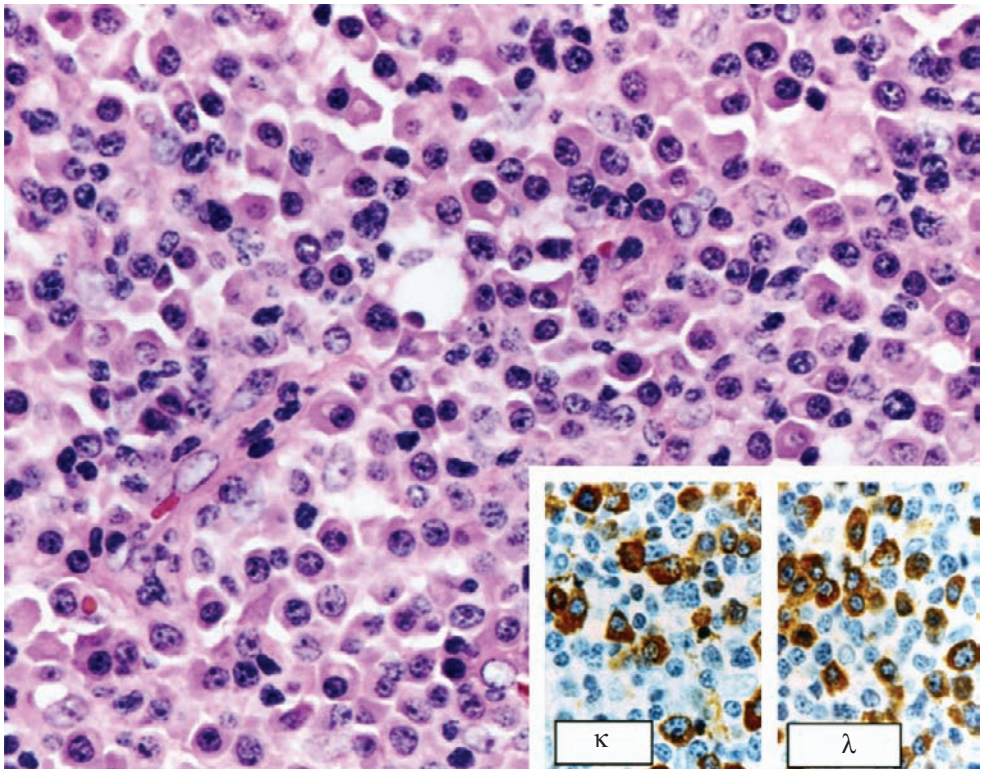


**FIGURE 10-1**

Posttransplantation lymphoproliferative disorder plasma cell hyperplasias. There are sheets of plasmacytic cells with minimal atypical and polyclonal staining for immunoglobulin light chains.

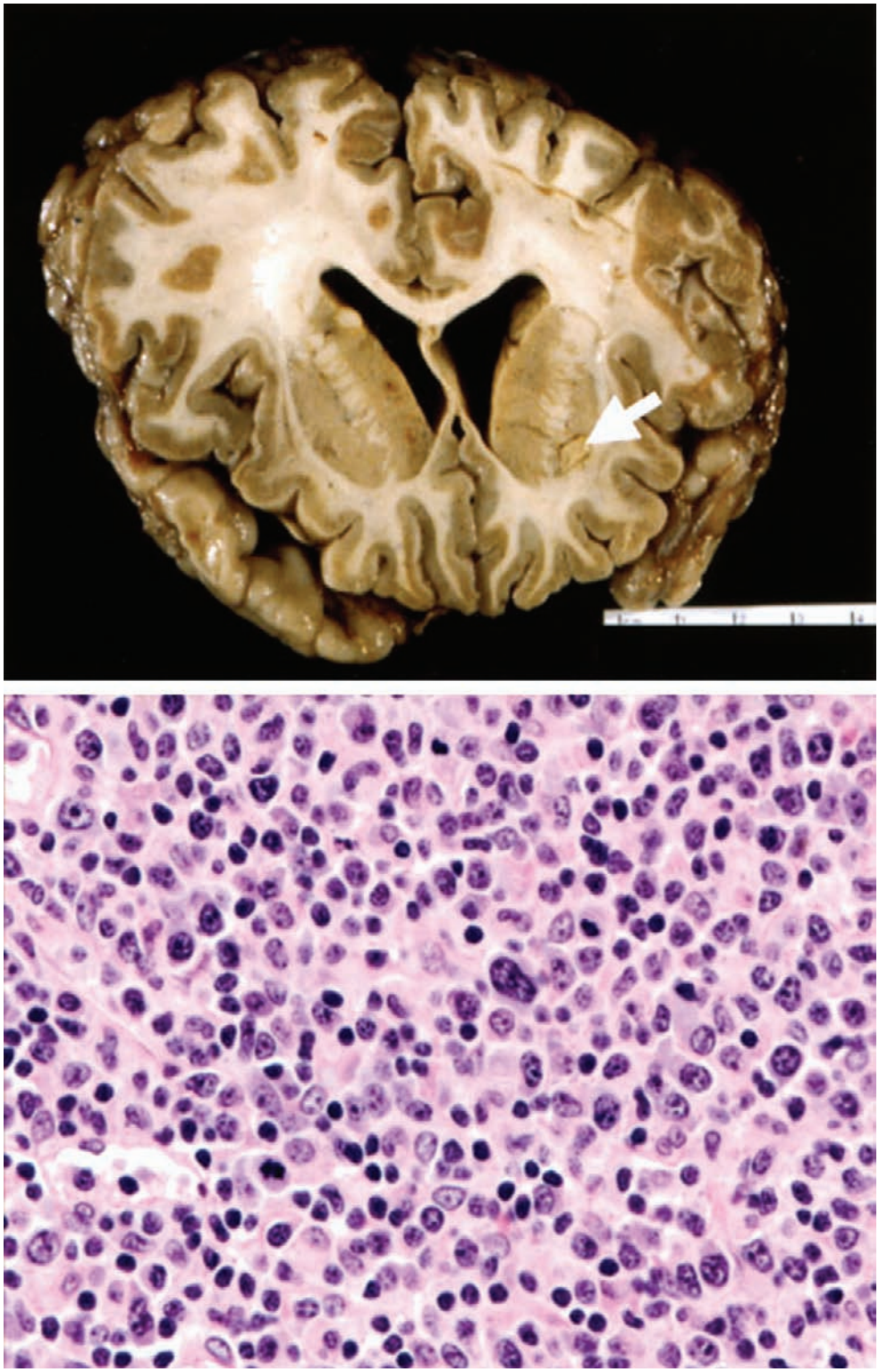

**FIGURE 10-2**

Orthotopic liver transplant patient treated initially for plasma cell hyperplasia but recurred with central nervous system involvement by polymorphic PTLD. There is a partially necrotic deep-seated lesion in the region of the basal ganglia *(arrow).* Histologic section reveals a polymorphous proliferation including plasma cells and large immunoblasts.

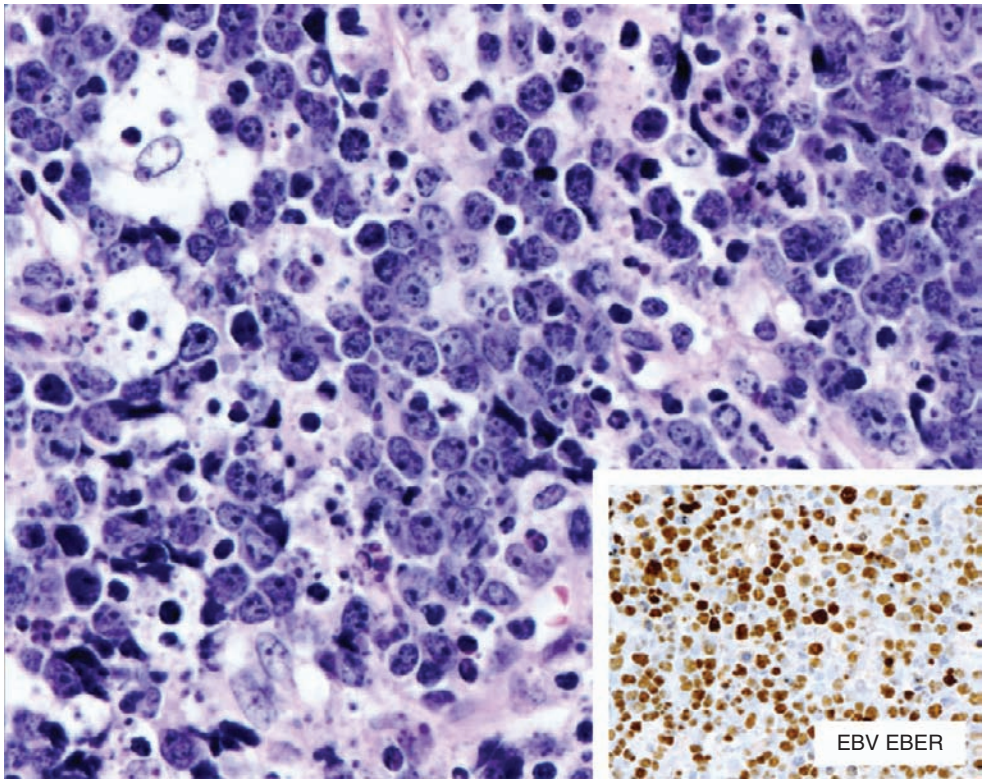


**FIGURE 10-3**

Burkitt lymphoma post transplant with intermediate sized noncleaved lymphoma cells and a background, which includes numerous phagocytic macrophages. The in-situ hybridization for Epstein-Barr virus encoded RNA (EBER) is strongly positive.

lymphoma nasal type and large granular lymphocytic leukemia. T-cell PTLDs can occur subsequent to other types of PTLD.

#### Immunophenotype

With the exception of the early plasma cell hyperplasia and IM-like lesions, PTLD cases often reveal clonally restricted surface or cytoplasmic immunoglobulin light chain expression. Despite their polymorphous histology, most polymorphic PTLDs are clonal in terms of immunoglobulin gene rearrangements and expression of EBV.

#### Genetic Features

PTLDs are monoclonal in regard to immunoglobulin gene rearrangements, with the exception of the early lesions, which are germline. Approximately 90% of cases of PTLD are associated with EBV, which can easily be demonstrated with in situ hybridization for Epstein-Barr virus encoded RNA (EBER). PTLD expresses EBNA-2 and LMP1, corresponding to EBV latency patterns II or III.

In plasmacytic hyperplasia–IM-like PTLD, there are no abnormalities in oncogenes or tumor suppressor genes. Polymorphic PTLD usually lacks abnormalities in oncogenes, with the exception of *BCL6*. Monomorphic PTLD usually demonstrates structural abnormalities involving oncogenes and tumor suppressor genes such as *MYC, NRAS,* and *TP53.*

## HUMAN IMMUNODEFICIENCY VIRUS-RELATED LYMPHOPROLIFERATIVE DISORDERS

### INTRODUCTION AND DEFINITION

This chapter will restrict discussion to neoplastic lymphoproliferative disorders, because the spectrum of lymphoid hyperplasia associated with HIV infection is discussed in Chapter 4. AIDS-related lymphomas account for approximately 10% of all lymphomas from the United States and Europe. The introduction of highly active antiretroviral multiple-agent therapy for HIV (HAART) has significantly affected the incidence of AIDS lymphoma and has resulted in a 75% decline in mortality. Before HAART, lymphoma was a late manifestation of HIV infection, occurring in severely immunocompromised individuals with extremely low $CD4^+$ T-cell counts. Tumor pathobiology at the time of clinical presentation is becoming a critical determinant of outcome rather than the immune environment during

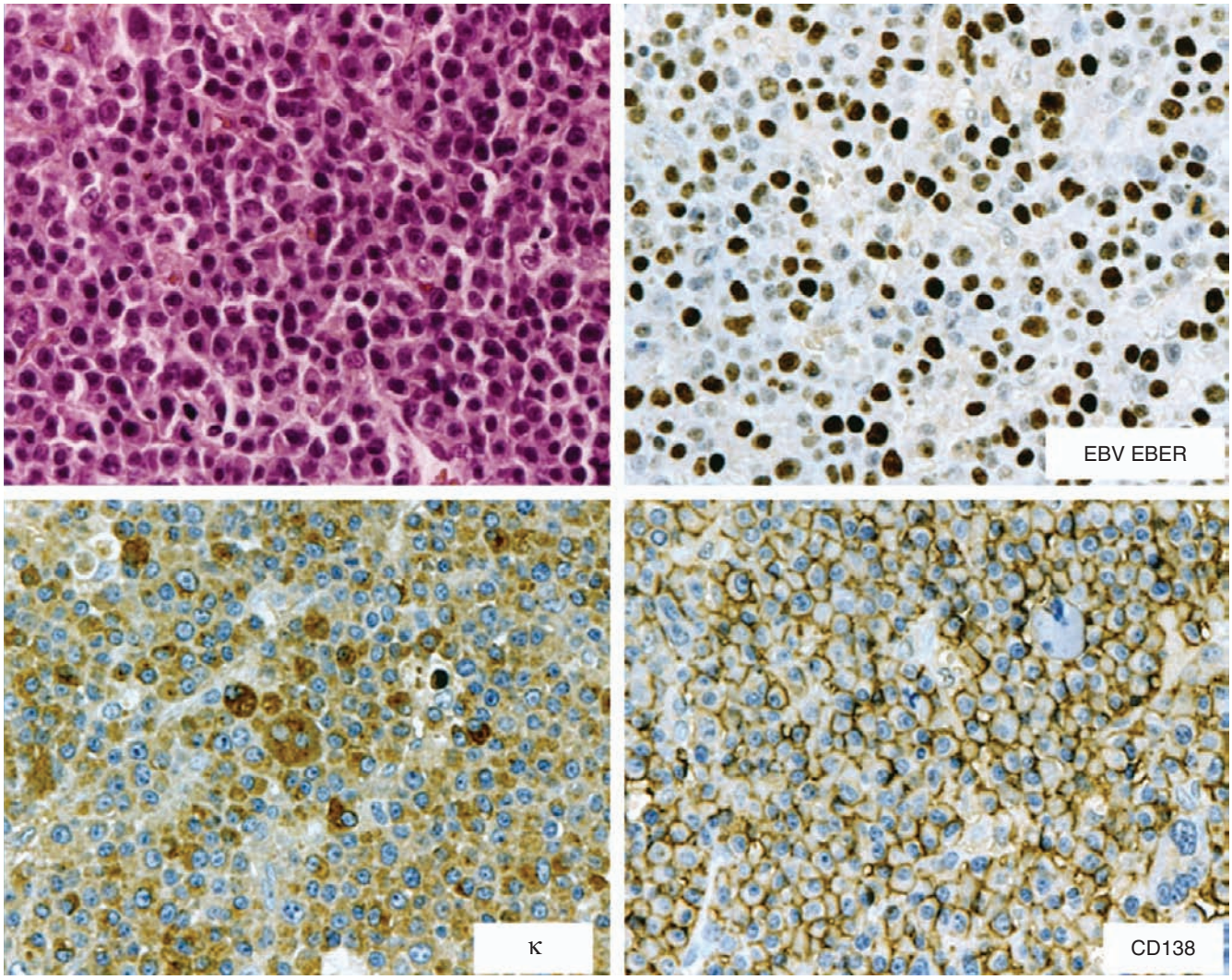


**FIGURE 10-4**

Plasmacytoma-like posttransplantation lymphoproliferative disorder with sheets of uniform plasmacytic cells, positive for CD138, κ light chains, and Epstein-Barr virus. These disorders resemble extramedullary plasmacytomas and are not associated with lytic bone lesions.

treatment. The incidence of low-grade lymphomas in HIV-infected patients is also increasing.

The pathogenesis of AIDS lymphomas most likely relates to chronic antigen stimulation and EBV infection resulting in B-cell overproduction and secondary genetic abnormalities in the background of immune deficiency. Polyclonal and oligoclonal B-cell expansion frequently precedes the development of lymphoma. Unlike lymphomas in the transplant setting, AIDS-related lymphomas (ARLs) are associated with both A- and B-type EBV. Viral infection (EBV and human herpesvirus 8 [HHV8]) may be associated with cytokine production including IL6, IL10, and IL13, which may also contribute to lymphoid proliferation and plasmacytoid differentiation of neoplastic cells.

EBV can be demonstrated in approximately 40% of cases of HIV-related lymphoma and is highest in cases with immunoblastic morphology and lymphomas involving the central nervous system (CNS; approaching 100%), compared with BL, where the incidence of infection of tumor cells with EBV resembles sporadic rather than endemic cases (approximately 40%, slightly higher in cases with atypical or plasmacytoid morphology). The incidence of EBV infection in AIDS-related centroblastic lymphoma is 20% to 30%. Cases of primary effusion lymphoma in AIDS patients contain both EBV and HHV8. Plasmablastic lymphomas associated with HIV are usually EBV positive, as are many cases of myeloma, including the plasmablastic variant.

Chromosomal translocations can result in the activation of oncogenes, including *MYC,* and less commonly other oncogenes, including *RAS*, *BCL2,* and *TP53*. *MYC* activation and *TP53* inactivation occurs more often

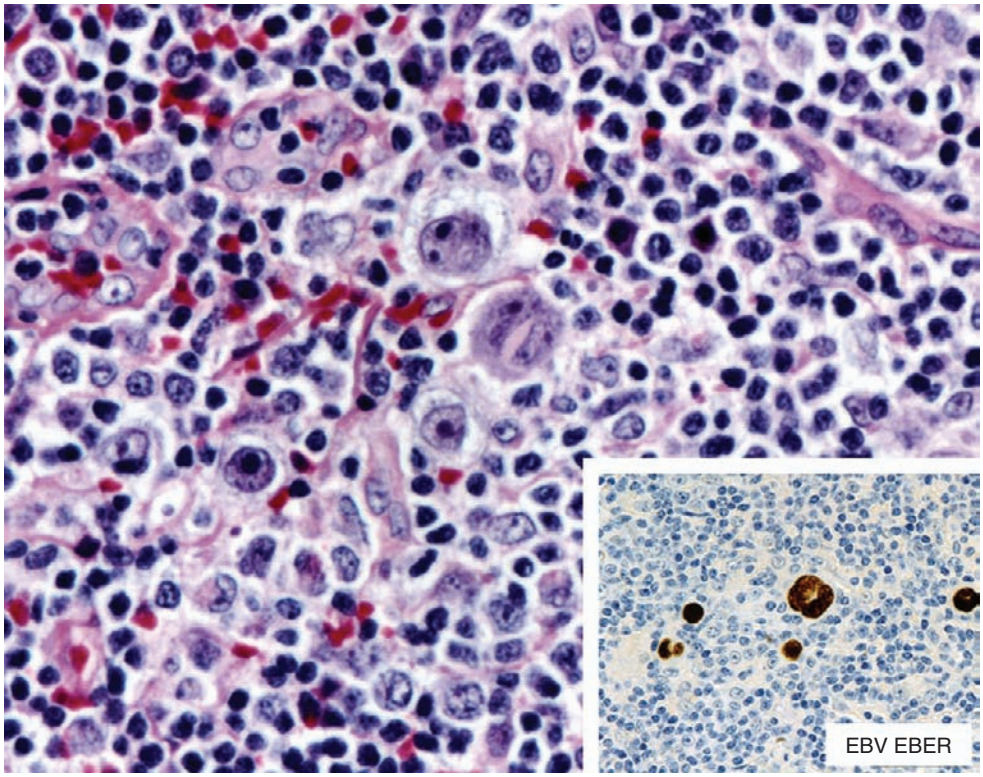


**FIGURE 10-5**

Classical Hodgkin lymphoma, mixed cellularity type post transplant. Numerous Reed-Sternberg and Hodgkin cells are present, with strong localization of EBERs.

in cases of BL. MYC has also been associated with plasmablastic differentiation in cases of EBV-positive plasmablastic lymphoma.

## Clinical Features

Systemic B symptoms are usually present in patients with AIDS lymphomas, and these include unexplained fever, drenching night sweats, and weight loss greater than 10% of normal body weight. Patients frequently have extensive disease at diagnosis, including involvement of the bone marrow, cerebrospinal fluid, and gastrointestinal tract. Any site can be involved by lymphoma, and unusual sites include the oral cavity, adrenal, heart, kidney, and gallbladder. Multiple tumor masses may be present and can represent synchronous clonal neoplasms. There may be extensive organ infiltration leading to organ failure, particularly in the bone marrow and liver.

The incidence of primary CNS lymphomas has fallen dramatically since the onset of HAART. These patients usually have one or more space-occupying lesions in the brain that can be deep seated. A computed tomographic scan of the brain may be confused with cerebral toxoplasmosis, although in the latter condition the lesions are usually multiple and smaller. In children with AIDS, primary lymphoma is the most common cause of focal or multifocal brain masses.

## Pathologic Features

The World Health Organization has classified AIDS-related lymphomas into three categories. The first category, lymphomas that also occur in immunocompetent patients, includes BL, diffuse large B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, and classical HL. Cases of peripheral T-cell lymphoma have been reported, as well as rare natural killer cell lymphomas.

In the second category of tumors, which occur more specifically with HIV, there is HHV8-related primary effusion lymphoma (PEL) and its extracavitary variant, plasmablastic lymphoma of the oral cavity type, and lymphoma arising in the setting of HHV8-associated multicentric Castleman disease. The third category, limited to lymphomas also occurring in other immunodeficiency disorders, contains a single-entity, polymorphic B-cell or PTLD-like lymphoma.

Immunohistochemical features vary in different forms of AIDS-related lymphomas. In general, patients

**AIDS-RELATED LYMPHOMAS CLASSIFICATION—FACT SHEET**

**Lymphomas Also Occurring in Immunocompetent Patients**

- Burkitt lymphoma
- Diffuse large B-cell lymphoma
  - Centroblastic
  - Immunoblastic (greater than 90% of the cells resemble immunoblasts)
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- T/NK-cell lymphomas including peripheral T/NK-cell lymphoma NOS, anaplastic large-cell lymphoma, cutaneous T-cell lymphoma, and nasal type NK/T-cell lymphoma
- Classical HL

**Lymphomas Occurring More Specifically in HIV-Positive Patients**

- Primary effusion lymphoma
- Plasmablastic lymphoma of the oral cavity type

**Lymphomas Also Occurring in Other Immunodeficiency States**

- Polymorphic lymphoma (PTLD-like)

**Diffuse Large B-Cell Lymphomas (30% ARL Cases)**

- Heterogeneous morphology, most centroblastic or immunoblastic (greater than 90% of the cells resemble immunoblasts in the immunoblastic variant)
- EBV variable but 100% in the CNS

**Burkitt Lymphomas (30% ARL)**

- Present at peripheral sites (e.g., the gastrointestinal tract) or with lymphoma-like packing of the marrow
- Subtype with plasmacytoid cytoplasm characteristic of AIDS
- EBV in about 30% to 40% of cases
- Have *C-MYC* translocations and frequent p53 mutations

**Primary Effusion Lymphomas**

- Present in body cavities without tumor masses
- Have an immunoblastic or anaplastic large cell appearance
- Infected with HHV8 and EBV
- Phenotype negative for CD45 (leukocyte common antigen) and T- and B-cell markers, but express activation antigens and exhibit immunoglobulin gene rearrangements
- Rare solid lymphomas with similar phenotype and appearance to PEL

**Plasmablastic Lymphomas of the Oral Cavity Type**

- First described in the jaw but present at other extranodal sites
- Positive for EBV but negative for HHV8
- May be associated with deregulation of *c-MYC*
- Phenotype showing strong plasmacytoid differentiation, including expression of CD138

**Classical Hodgkin Lymphoma**

- More aggressive than classical HL in the general population
- B-symptoms, early dissemination, and bone marrow involvement common
- Hodgkin and Reed-Sternberg cells positive for EBV

with AIDS-related lymphomas have lower BCL-2 and higher CD10 expression compared with the general population. They also have higher Ki67 scores and express p53 in a greater percentage of cases. Immunoglobulin gene rearrangement studies reveal monoclonal patterns in almost all cases of HIV-related lymphoma. Rearrangements of the *BCL6* gene are associated with diffuse large B-cell lymphoma (DLBCL). CNS lymphomas are monoclonal B-cell neoplasms positive for EBV, and lack *MYC* rearrangements. Abnormalities in the tumor suppressor gene *TP53* occurs in approximately 60% of cases of BL and 40% of DLBCL cases. Translocations involving the *MYC* locus also occur in approximately 20% of DLBCL cases.

## DIFFUSE LARGE B-CELL LYMPHOMAS

DLBCL forms approximately 30% of cases of ARL, and this subtype is becoming relatively more common since the widespread use of HAART antiretroviral therapy. Morphologically these lymphomas are heterogeneous and include cases with centroblastic morphology (Figure 10-6), as well as variants with plasmacytoid differentiation (immunoblastic or plasmablastic lymphomas) and anaplastic lymphomas (Figure 10-7). The majority of cases of HIV-related DLBCL are of the nongerminal center or activated B-cell type.

The immunoblastic variant of DLBCL (approximately 20% of cases) is more characteristic of ARL and is composed almost entirely of immunoblasts. These cells are large with single central macronucleoli and amphophilic or plasmacytic cytoplasm. Multinucleated and Reed-Sternberg-like cells may also be present. Staining of the lymphoma cells for EBV (EBER and latent membrane protein LMP-1) can be demonstrated frequently, and in immunoblastic lymphomas of the brain the incidence of EBV infection approaches 100%. Characteristically in biopsies from the CNS, the large neoplastic cells are clustered around blood vessels in the brain (Figure 10-8), and tumor necrosis is frequent.

## BURKITT LYMPHOMA

The incidence of BL has decreased relative to large-cell lymphoma since the use of HAART antiretroviral therapy. Patients are usually young age and are less

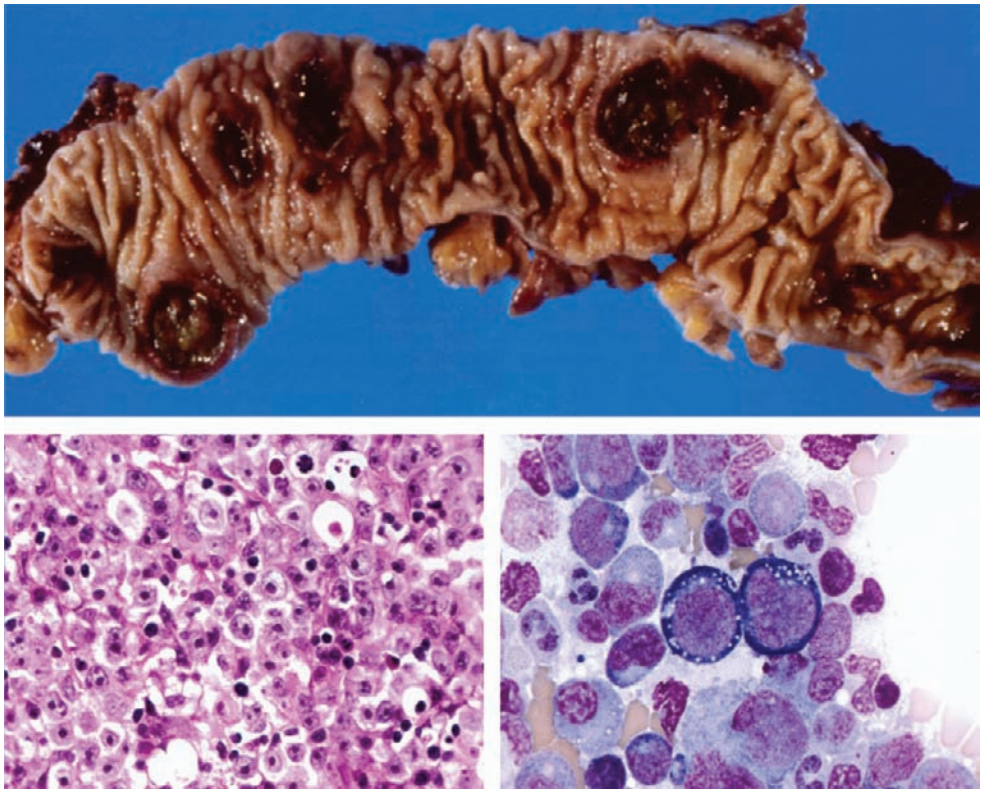

**FIGURE 10-6**

Multifocal diffuse large B-cell lymphoma of the bowel with multiple ulcerated masses. A histologic section reveals diffuse proliferation of large lymphoid cells. Touch imprints reveal large round or oval cells with vacuolated cytoplasm.

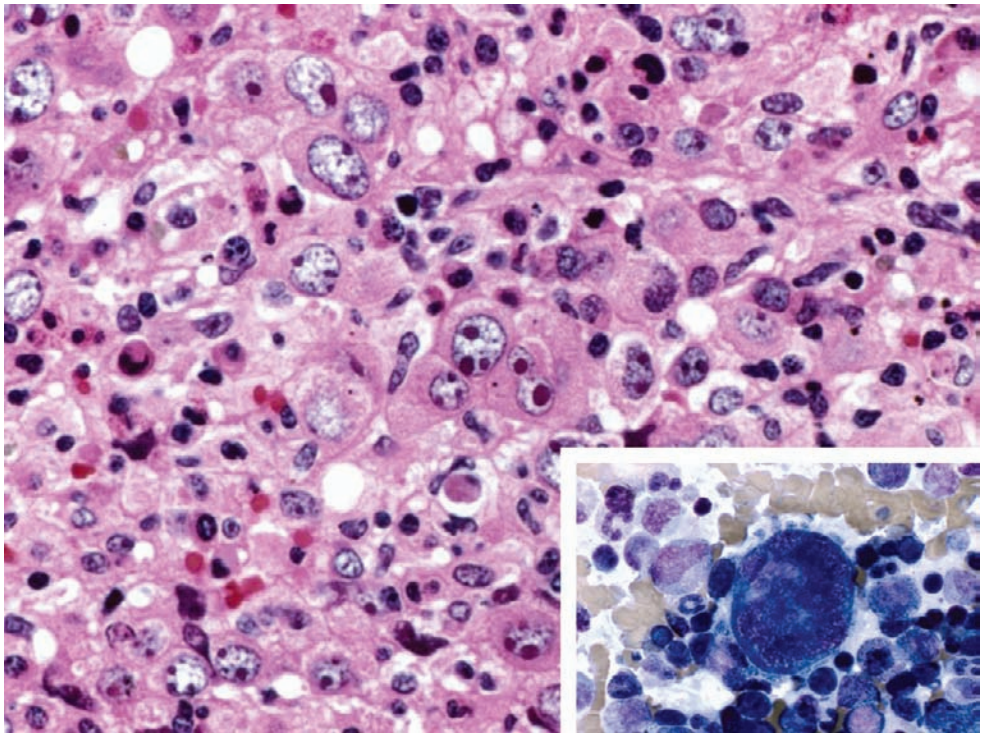

**FIGURE 10-7**

Anaplastic diffuse large B-cell lymphoma with pleomorphic malignant cells with multiple macronucleoli.

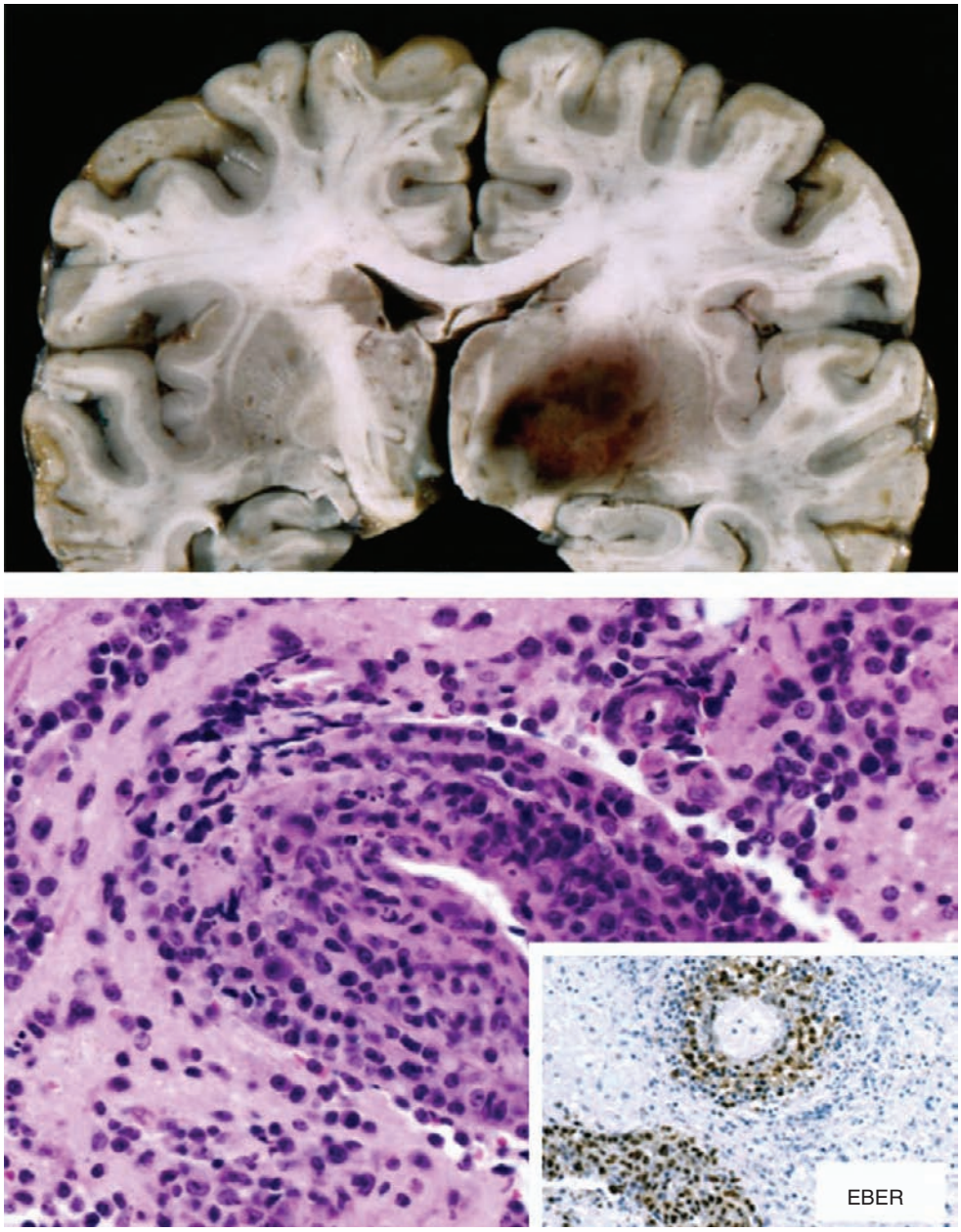


**FIGURE 10-8**

Immunoblastic large-cell lymphoma of the brain with a deep-seated hemorrhagic lesion. Histologic sections reveal perivascular cuffing by immunoblastic cells positive for EBER.

immunocompromised with peripheral CD4 cell counts exceeding 200/μL. Most cases of BL manifest at extranodal sites or bone marrow. The bone marrow may be heavily infiltrated at presentation, resembling lymphoblastic leukemia, but without spillage into the peripheral blood. The bowel is a common site, and symptoms can include abdominal pain, obstruction, bleeding, or syndromes resembling appendicitis or intussusception.

In BL there is a diffuse and cohesive proliferation of intermediate-sized, noncleaved cells with basophilic cytoplasm often containing lipid droplets. There is a high proliferation rate, numerous mitoses, and tingible body or starry-sky macrophages. There are usually few admixed reactive T cells. The nuclei are round or oval with multiple nucleoli (Figure 10-9). Associated with HIV infection, there may be a spectrum of cytologic appearance in BLs. The cells may be more pleomorphic in size and shape with fewer nucleoli, and they often have more cytoplasm and a plasmacytoid appearance (Figure 10-10). This plasmacytoid variant of BL is unique to the setting of HIV. In some cases, features may be intermediate between BL and DLBCL, making pathologic classification difficult. EBV infection is present in 30% to 40% of cases of BL with classical

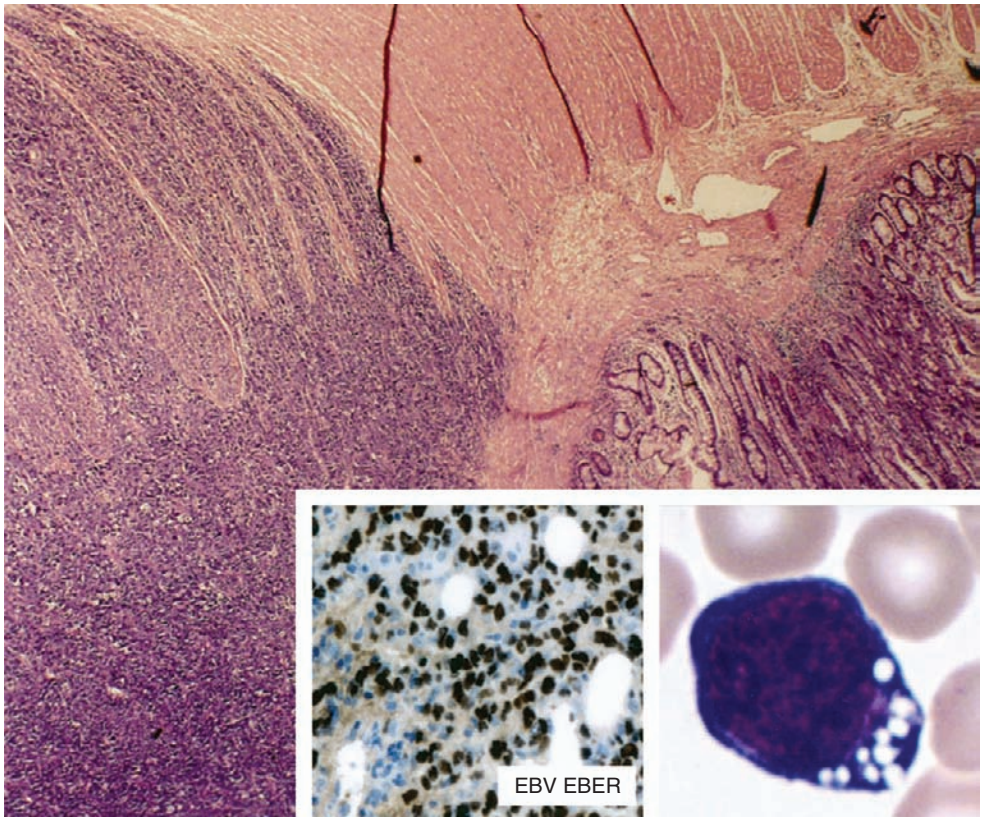


**FIGURE 10-9**

Burkitt lymphoma of the bowel forming a large mass in the bowel wall. Imprints reveal noncleaved cells with lipid vacuoles in the cytoplasm. EBER stain is positive.

morphology and 40% to 50% in cases of the plasmacytoid variant.

Eighty percent of BLs have translocations t(8;14), which involves the juxtaposition of the *MYC* gene on chromosome 8q24 and the heavy chain locus on chromosome 14 q32. In the remaining 20%, the translocations with the *MYC* gene involve the κ or λ light chain loci on chromosomes 2 and 8, respectively, resulting in t(2;8)(p12;q24) or t(8;22)(q24;q11). Deregulation of *MYC* may also arise from point mutations in the first intron-first exon regulatory regions and amino acid substitution in the second exon. In both HIV-associated and sporadic BL, and unlike endemic cases, the breakpoints occur between exons 1 and 2 of the *MYC* gene and within the Sμ switch region of the *IgH@*. *TP53* point mutations are also relatively common in BL (approximately 60% of cases).

## EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA)

There appears to be an increasing incidence of low-grade lymphomas, particularly MALT lymphomas in patients with AIDS. This incidence may relate to the use of HAART antiretroviral therapy and prolonged survival in patients infected with HIV. MALT lymphomas have been reported in the lung, in which patients have multiple pulmonary nodules consisting of centrocyte-like or monocytoid lymphocytes, and there is infiltration into the bronchiolar epithelium forming characteristic lymphoepithelial lesions (Figure 10-11). Some of these cases were preceded by lymphocytic interstitial pneumonitis.

## PERIPHERAL T/NK-CELL LYMPHOMAS

T/NK-cell lymphomas are rarely associated with AIDS, but when they occur they are usually associated with involvement of extranodal sites, particularly the skin. The full spectrum of T/NK-cell lymphomas has been associated with AIDS, including $Ki1^+$ anaplastic large-cell lymphoma (ALCL) and neutrophil-rich ALCL, as well as EBV-positive T/NK-cell lymphoma of the nasal type.

Peripheral T-cell lymphomas are usually aggressive neoplasms with pleomorphic large cells (Figure 10-12). They frequently invade blood vessels and are associated with necrosis. T/NK-cell lymphomas in patients with AIDS are usually positive for CD2, CD3, CD5, CD8, and CD16 and CD56 in the case of NK-cell phenotype. As with other peripheral T-cell lymphomas, there is

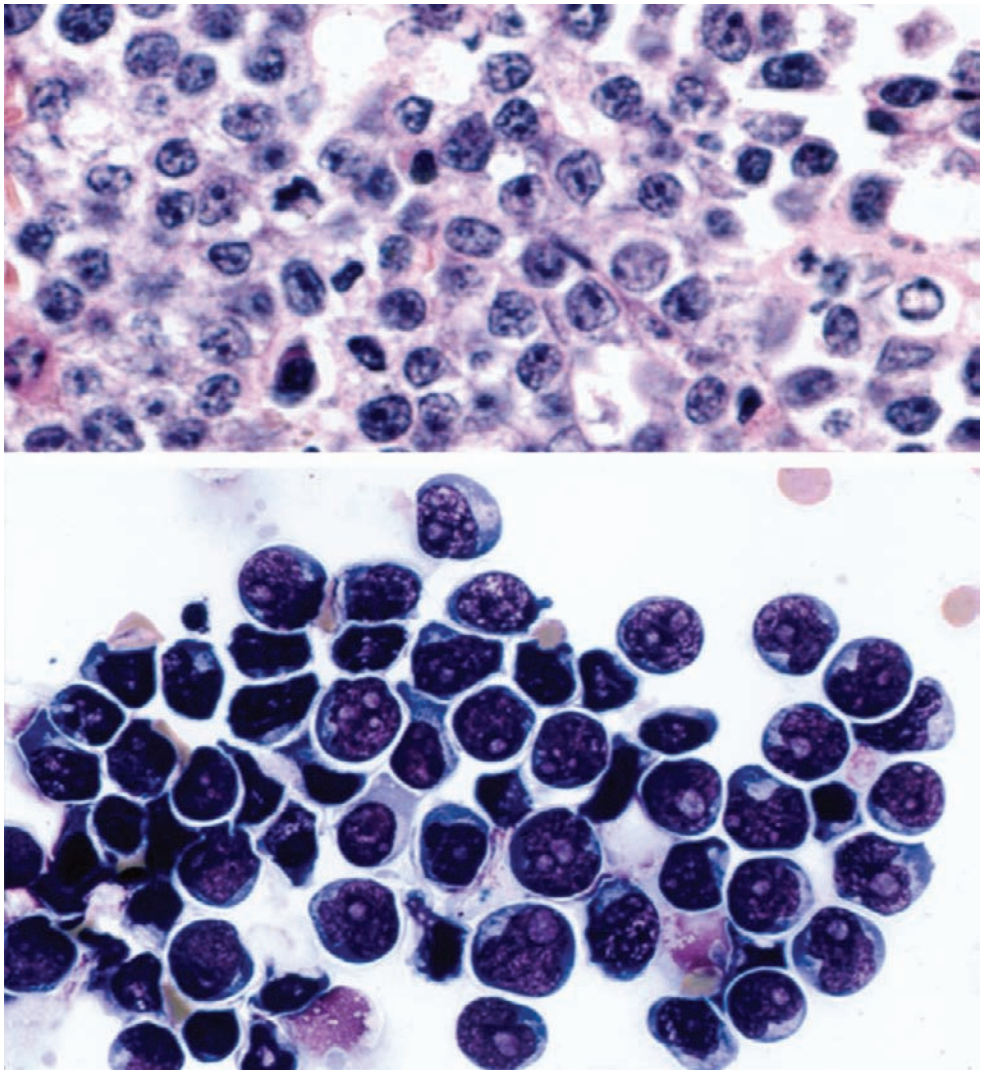

**FIGURE 10-10**
Burkitt-like lymphoma showing cytoplasmic or plasmacytoid Burkitt cells densely packing the bone marrow.

frequent loss of T-cell antigens, particularly CD7 and CD5. Most large-cell lymphomas in the skin of patients with HIV express CD30, but are negative for the ALK protein.

## MYCOSIS FUNGOIDES

Mycosis fungoides (MF) occurs in the setting of HIV infection and AIDS, and criteria for the diagnosis resemble that in the general population, notably the presence of cerebriform lymphocytes infiltrating the epidermis and the formation of small clusters or Pautrier microabscesses. The cerebriform lymphocytes or Sézary cells are frequently positive for CD8, unlike MF in the general population, which is usually $CD4^+$. The disease may be more aggressive in AIDS patients and may be associated with involvement of lymph nodes and bone marrow. Difficulties may occur in differentiating MF from numerous dermatologic conditions associated with AIDS.

## PRIMARY EFFUSION LYMPHOMA AND OTHER KAPOSI SARCOMA–ASSOCIATED, HERPESVIRUS-RELATED NON-HODGKIN LYMPHOMAS

PEL is a rare form of lymphoma that occurs usually but not exclusively in patients with AIDS and is characterized by presentation in body cavities (e.g., pleural, pericardial, abdominal), usually without an associated solid tumor mass. Malignant cells in PEL are large and pleomorphic, often with plasmacytoid features resembling immunoblasts, or more bizarre and anaplastic in appearance resembling ALCL (Figure 10-13). Some cells may resemble Hodgkin or Reed-Sternberg cells. Because of

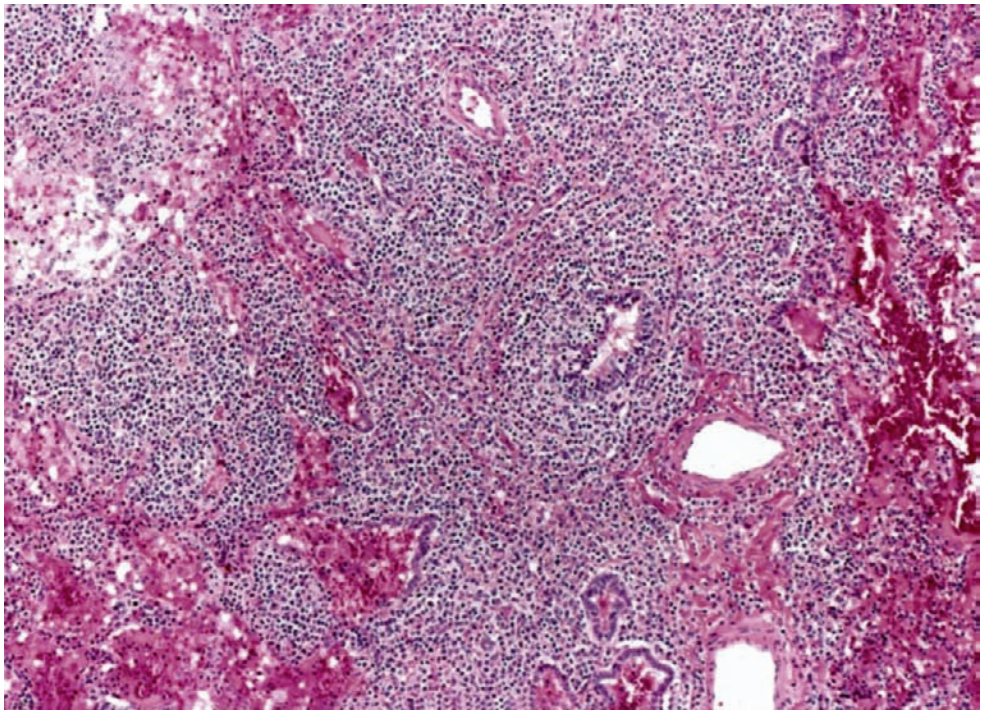

**FIGURE 10-11**

MALT lymphoma of the lung showing small cytoplasmic lymphoid cells surrounding and infiltrating the walls of the bronchioles forming lymphoepithelial lesions.

the presentation in body cavities, diagnosis is often made in cytologic preparations. With disease progression there may be an associated solid mass, usually involving adjacent soft tissues, and the cells have a morphologic appearance similar to those in the fluids with sheets of large anaplastic or plasmacytoid cells.

Cells from PEL are productively infected with the Kaposi sarcoma–associated herpesvirus, also called HHV8. This virus also causes Kaposi sarcoma (KS), although KS lesions are absent from most patients with PEL. The presence of HHV8 may be readily demonstrated by electron microscopy (Figure 10-14), but is most commonly demonstrated with immunohistochemistry using antibodies to the HHV8 latency-associated nuclear antigen LANA. In patients with AIDS, PEL cells are also infected with EBV, as can be demonstrated by EBER in situ hybridization. Most patients with PEL are severely immunocompromised with extremely low CD4 cell counts. The prognosis is poor, with most patients surviving less than 1 year from diagnosis.

In addition to characteristic clinical and cytologic features, cells in PEL also have a distinctive phenotype. Although they are B cells based on molecular studies, they usually are negative for common leukocyte antigen and lack conventional T- and B-cell markers such as CD19, CD20, and surface immunoglobulins. The cells exhibit features of postgerminal center B-cell differentiation, however, and may express activation antigens including HLA-DR, EMA, CD30, CD38, and CD77. Molecular analysis of PEL reveals clonal rearrangements and somatic mutations of the immunoglobulin genes, features of late (postfollicular) B-cell differentiation, and no involvement of *MYC*. PELs also lack *BCL6* gene rearrangements and ras oncogene or p53 tumor-suppressor gene mutations. Cytogenetic studies reveal multiple chromosomal abnormalities. At least two potential viral oncogenes have been identified, one a cellular type D cyclin similar to the *CCND1* oncogene involved in mantle cell lymphomas, and one homologous to the cellular G protein–coupled receptor family of proteins.

Although HHV8-related lymphomas usually manifest with involvement of body fluids, the virus is rarely associated with solid lymphomas in patients who do not have effusions (called *extracavitary PEL*). These lymphomas usually occur at extranodal sites, including the gastrointestinal tract. In other respects they are similar to PEL, with similar morphologic features (Figure 10-15). The phenotype is also similar, although the solid lymphomas more often express B-cell–associated markers such as CD20 and CD79a.

HHV8 is also associated with multicentric Castleman disease (MCD) in patients with HIV infection and AIDS (discussed in Chapter 4). In this setting, monotypic plasmablasts expressing immunoglobulin M λ can develop and are associated with B-cell follicles. They may form lymphoma-like clusters inside and outside of follicles

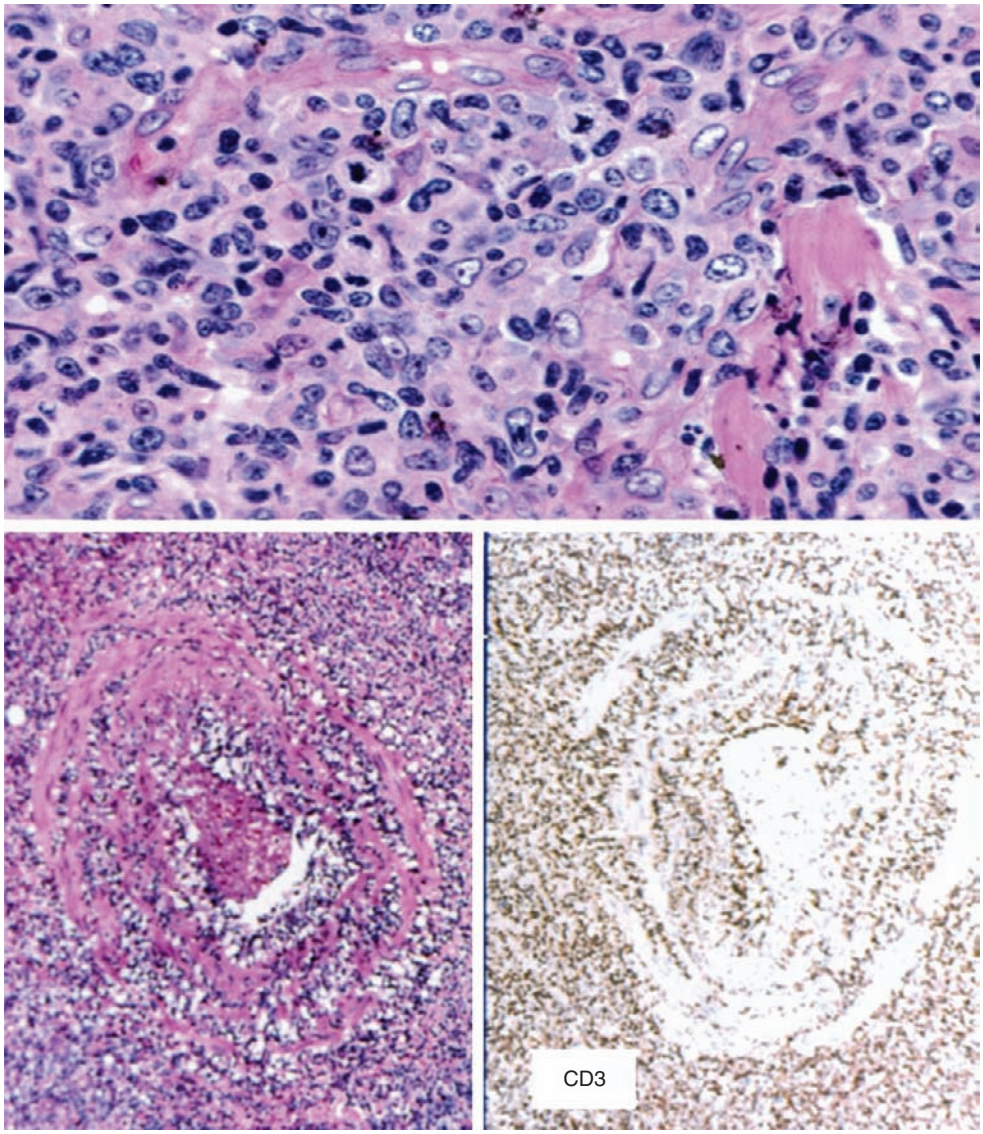


**FIGURE 10-12**

T/natural killer–cell lymphoma with large pleomorphic cells infiltrating the walls of a blood vessel. The cells express pan–T-cell marker CD3.

(so-called microlymphomas) or develop into frank plasmablastic lymphomas (Figure 10-16). It is thought that activation of the interleukin-6 signaling pathway may be important in differentiation of HHV8-infected naive B cells into plasmablasts and development of plasmablastic lymphomas. Plasmablastic lymphomas associated with MCD occur in both HIV-positive and -negative patients, and unlike the plasmablastic lymphomas of the oral cavity type are negative for EBV.

## PLASMABLASTIC LYMPHOMA

Plasmablastic lymphoma was first described in the jaw and oral cavity of patients infected with HIV, but subsequently have been described at other predominantly extranodal sites (Table 10-2). Histologically, plasmablastic lymphomas are large cell lymphomas with plasmablastic differentiation evidenced by large blastic nuclei and prominent often central nucleoli, as well as amphophilic or plasmacytoid cytoplasm (Figure 10-17). The phenotype of plasmablastic lymphoma is also distinctive, with plasmacytoid differentiated evidenced by weak or absent expression of leukocyte common antigen CD45, and B-cell antigen CD20. Some cases are positive for pan B-cell marker CD79a. There may be cytoplasmic immunoglobulin, and gene rearrangement studies reveal a clonal B-cell population. The cells are positive for plasma cell antigens such as VS38c or CD138. There is no association with HHV8 in most cases, but the neoplastic cells are positive for EBV EBER and variably positive for EBV latent membrane protein (LMP1).

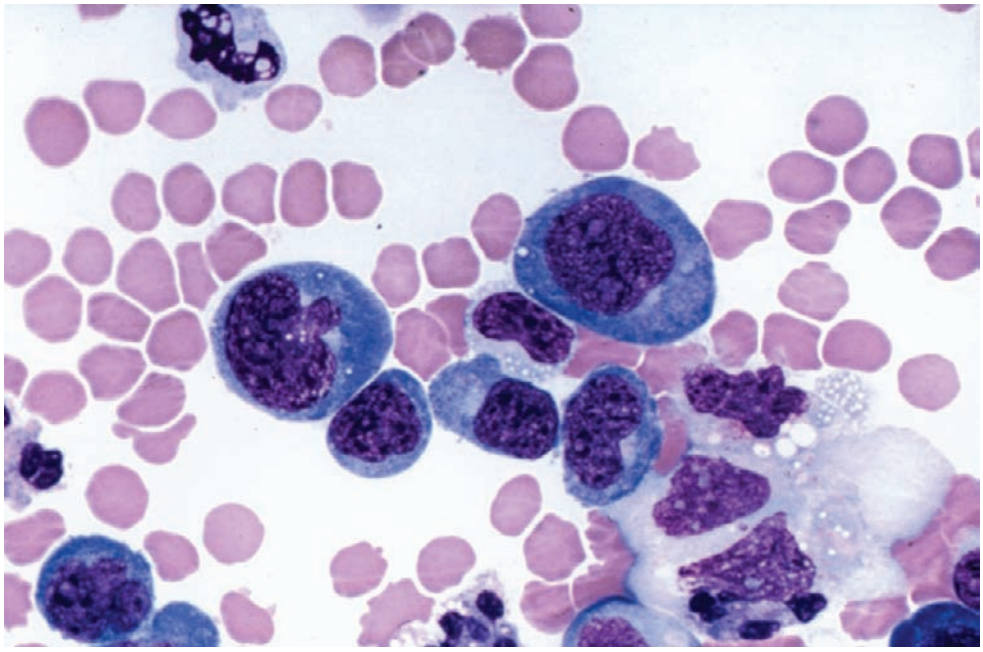

**FIGURE 10-13**

Pericardial fluid cytospin showing large anaplastic and plasmacytoid lymphoid cells characteristic of PEL.

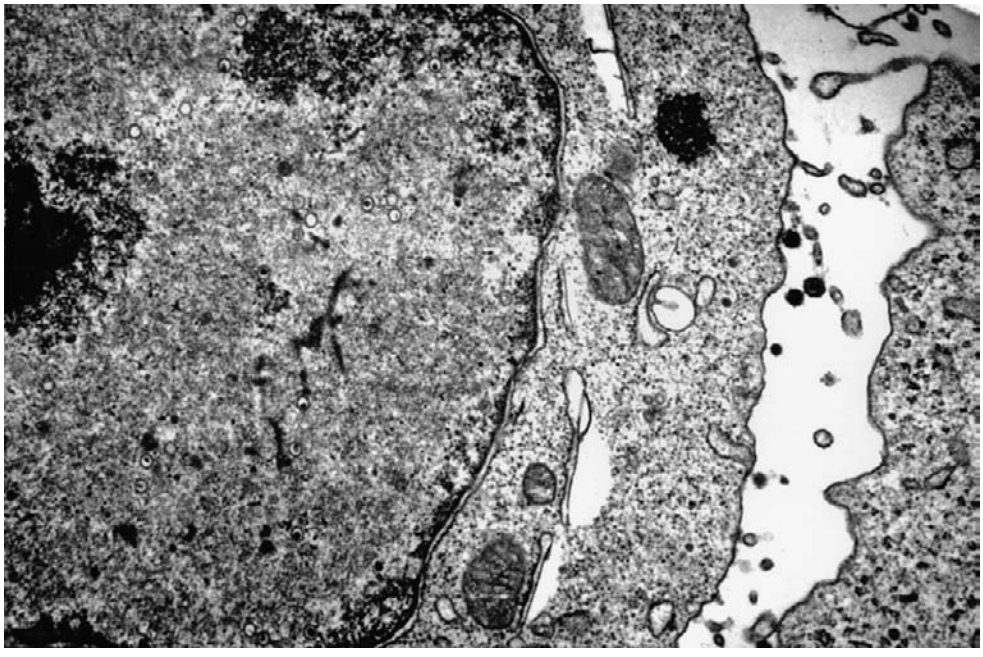

**FIGURE 10-14**

Ultrastructural study showing numerous herpesvirus 8 particles in the nucleus and being shed from the cell surface.

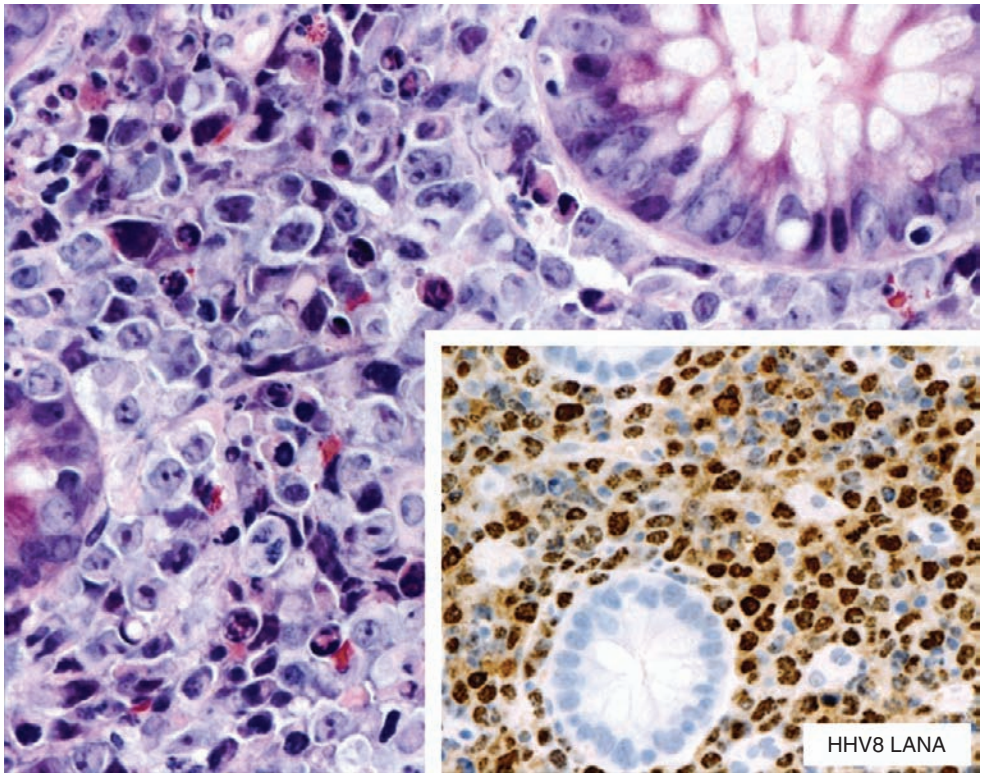


**FIGURE 10-15**

HHV8-positive solid lymphoma mass in the bowel wall. The cells are large and pleomorphic and are shown infiltrating the colon epithelium.

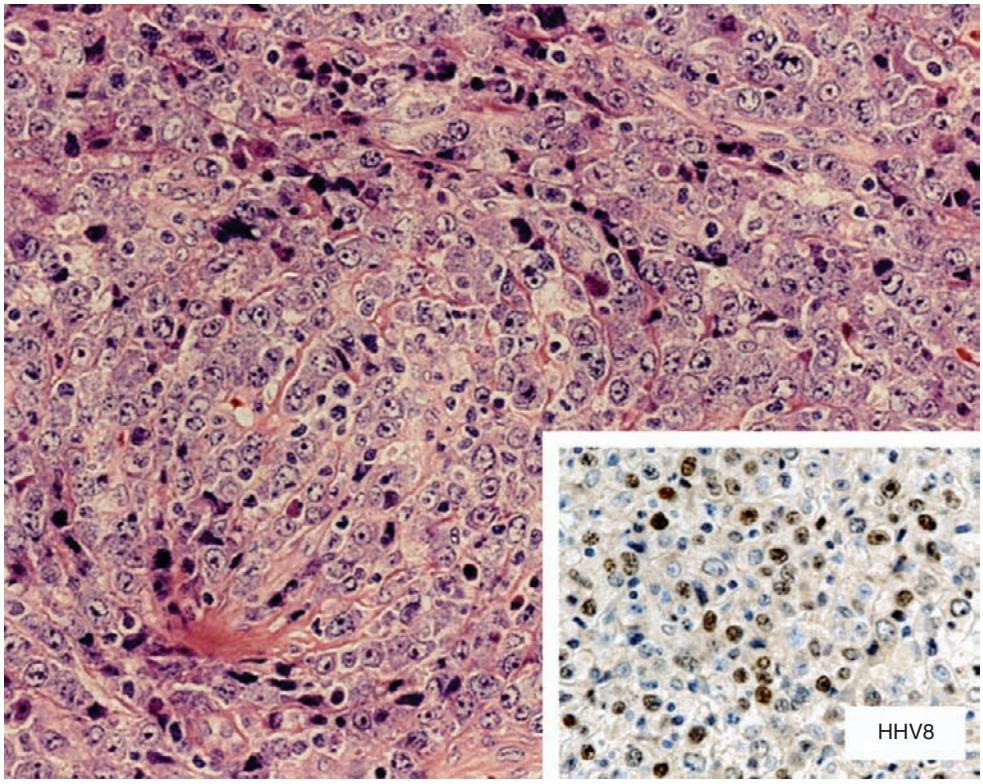


**FIGURE 10-16**

HHV8-positive plasmablastic lymphoma arising in the spleen in a patient with multicentric Castleman disease.

**TABLE 10-2**
**Differential Diagnosis of PEL and Plasmablastic Proliferations in HIV**

| Type | Plasmablastic Lymphoma of the Oral Cavity Type | Plasmablastic Myeloma | MCD-Associated Plasmablastic Proliferations | PEL |
|---|---|---|---|---|
| ***Clinical features*** | Extranodal mass | Features of myeloma including lytic bone lesions, paraproteinemia, plasma cells in the marrow and peripheral blood | Clinical features of MCD including malaise and adenopathy | Malignant effusions; may be an associated mass lesion |
| ***Histology*** | Sheets of cells with large blastic nuclei, prominent nucleoli, and plasmacytoid cytoplasm; some cases may have a more centroblastic appearance | Sheets of plasmablasts admixed with plasma cells | Clusters or sheets of plasmablasts in association with features of MCD; microlymphomas start in follicular mantles | Large cells with anaplastic and plasmablastic features |
| ***Phenotype*** | Weak expression of CD45 and CD20, may express CD79a and cytoplasmic immunoglobulin $CD138^+$ | Absent CD45 and CD20, cytoplasmic immunoglobulin, $CD138^+$ | Plasmablasts are EBV positive and express IgM λ | Absent CD45 and CD20; activation antigens including CD30, CD38, HLA-DR |
| ***EBV*** | EBV encoded RNA(EBER) positive EBV LMP1 variable | EBV positive or negative | Negative | EBER positive, EBV LMP negative |
| ***Molecular*** | MYC deregulation | May have myeloma associated abnormalities | Polyclonal or clonal | Clonal Ig gene rearrangements, complex cytogenetics |
| ***HHV8*** | Negative | Negative | Positive | Positive |

*EBV,* Epstein-Barr virus; *HHV8,* human herpes virus 8; *Ig,* immunoglobulin; *PEL,* primary effusion lymphoma.

It has been suggested that dysregulation of *MYC* may be responsible for the plasmablastic morphology in these cases.

## POLYMORPHIC B-CELL LYMPHOMA (PTLD-LIKE)

Patients with AIDS can develop polymorphic B-cell lymphomas, which resemble the lymphoid proliferations seen in the posttransplantation setting, but this is uncommon. Like cases of PTLD, these lymphomas are associated with EBV infection and often occur at extranodal sites including the lung, salivary glands, and skin, although they may involve lymph nodes. Morphologically, these lymphomas resemble polymorphic PTLDs, with a heterogeneous population of lymphocytes, plasma cells, and immunoblasts (Figure 10-18). Cytologic atypia and necrosis are variable. The proliferations are composed mainly of $CD20^+$ B cells admixed with lesser populations of T cells, and in most cases there is monoclonal expression of immunoglobulin light chains detectable with immunohistochemistry or standard molecular techniques. The rearranged bands in Southern blots may be faint and superimposed on a polyclonal background. EBV sequences in the cases studied have been of type A, which is the characteristic pattern in PTLDs.

## MULTIPLE MYELOMA

Patients with AIDS have an increased incidence of myeloma, which resembles myeloma in the general population but may be more aggressive with blastic or anaplastic appearance and involve extraskeletal sites. In patients with HIV the myeloma cells may be positive for EBV, and in cases with plasmablastic morphology it may be difficult to differentiate these cases from plasmablastic lymphomas. Clinical features indicating myeloma include paraproteinemia, lytic bone lesions, and neoplastic plasma cells in the marrow (generally 30% or more), peripheral blood, or extramedullary sites such as the liver or spleen.

## CLASSICAL HODGKIN LYMPHOMA

Although HL is not an AIDS-defining neoplasm, there is an increased risk for HL in patients with HIV. HIV-associated HL and NHL occur in patients with similar age, gender, HIV risk factors, degree of immunodeficiency, and incidence of previous acquired immunodeficiency syndrome. Patients with HIV tend to have more aggressive HL with early dissemination, often stage four at presentation (Figure 10-19). B symptoms are usually

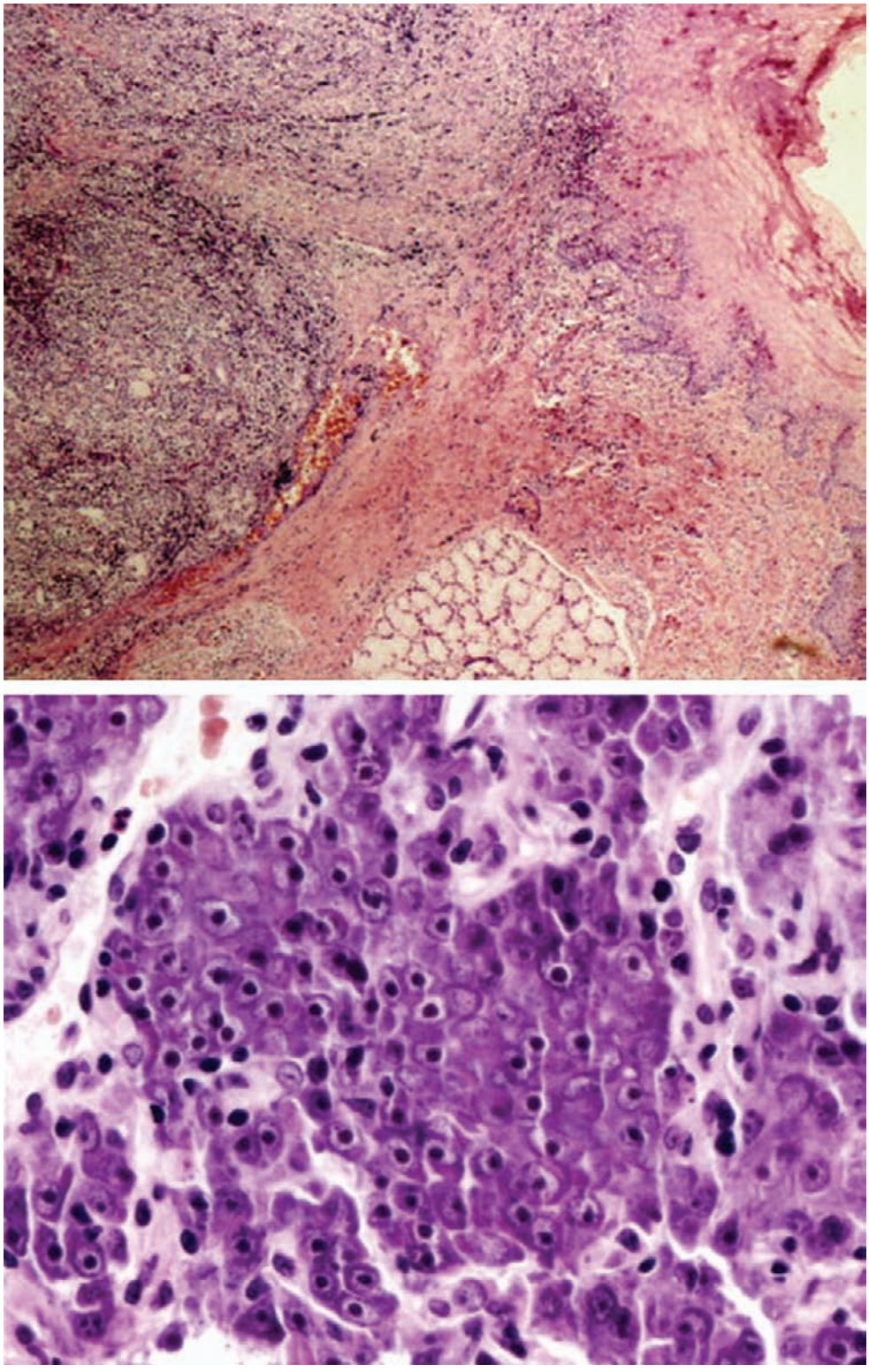

**FIGURE 10-17**

Plasmablastic lymphoma of the oral cavity showing a submucosal infiltrate of large plasmablastic cells.

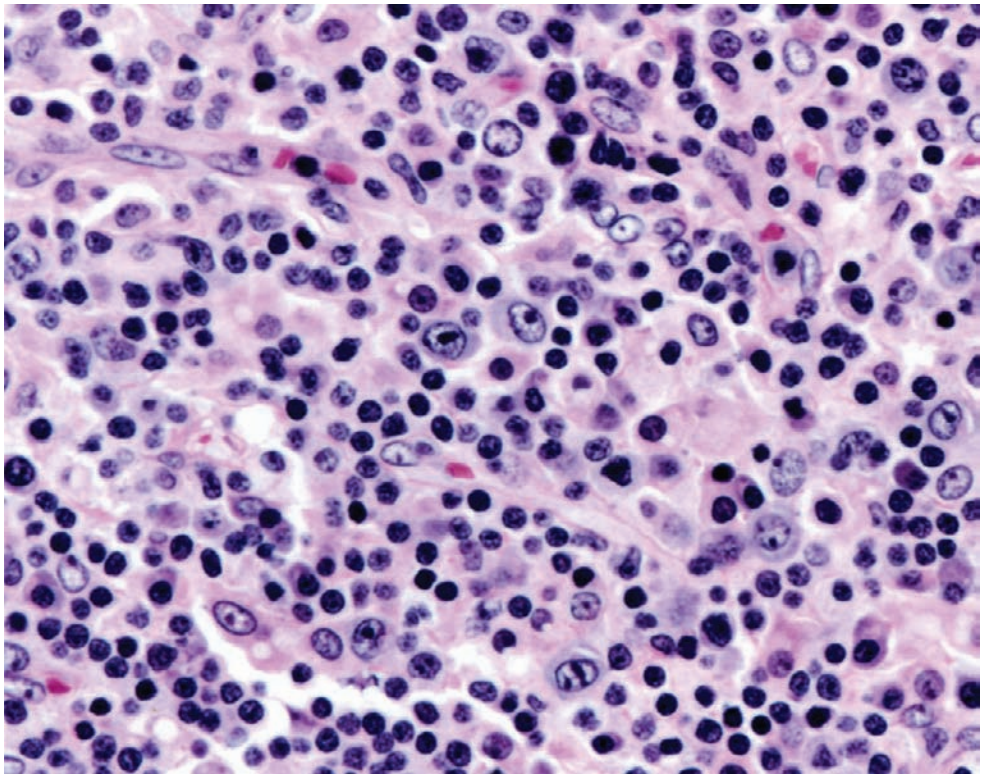

**FIGURE 10-18**

Polymorphous infiltrate resembling polymorphic PTLD in a patient with AIDS. There is a spectrum of lymphoid cells including frequent plasma cells and immunoblasts. Despite the polymorphous appearance, this lymphoma revealed monoclonal rearrangement of the immunoglobulin genes.

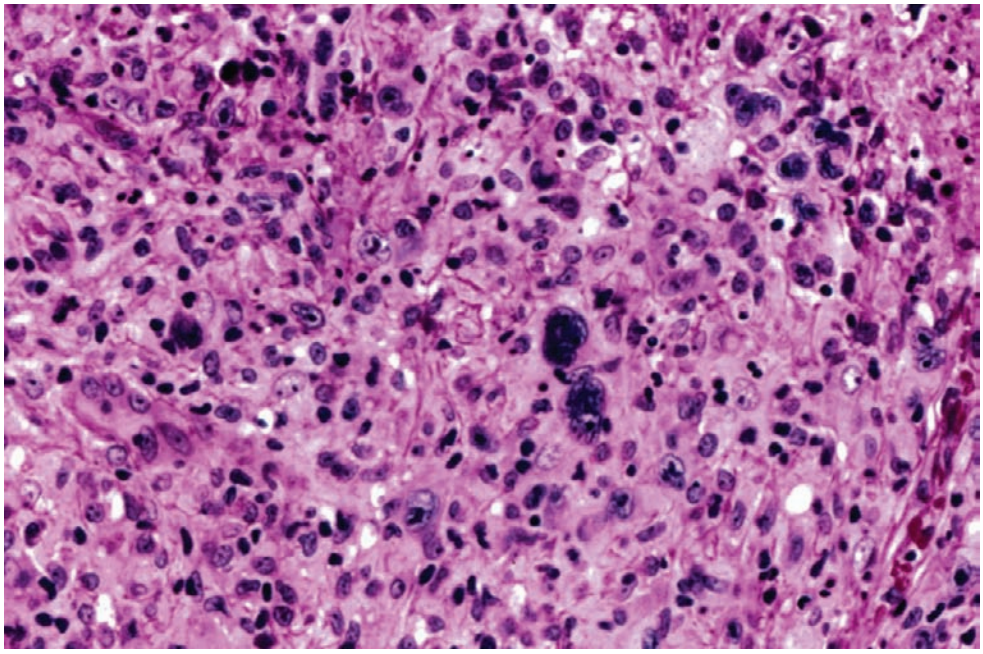

**FIGURE 10-19**

Hodgkin lymphoma presenting as stage-four disease with bone marrow and liver infiltrates. Section from the liver biopsy reveals pleomorphic Hodgkin cells in a mixed background including numerous histiocytes.

present, and bone marrow involvement is present at presentation in approximately half of the patients. The clinical presentation of HIV-associated HL is atypical and the disease is more aggressive than in HIV-negative patients. The most common subtype of HL in patients with HIV is mixed cellularity, although the full spectrum may be seen including nodular sclerosis.

The phenotype of HL in patients with AIDS is somewhat different in that the background lymphoid proliferation tends to be CD4 depleted, and the Hodgkin and Reed-Sternberg cells are invariably positive for EBV EBER or EBV-latent membrane proteins, or both. Patients may respond to conventional HL chemotherapy, but are at risk for opportunistic infections and other complications of AIDS. The overall 5-year survival in one series is estimated as 24%, but may be substantially better in the post-HAART era.

## BONE MARROW INVOLVEMENT IN AIDS-RELATED LYMPHOMA

Bone marrow involvement is common in all forms of ARL. Hemoglobin levels are similar in patients with ARL with and without bone marrow involvement, but thrombocytopenia of less than 100,000/μL is more common in patients with lymphoma in the marrow. Patients with marrow involvement have a higher incidence of lymphomatous meningitis and positive cerebrospinal fluid.

In DLBCL there may be tumorlike aggregates in the marrow or single-cell dispersion among the other hematopoietic elements. Extensive marrow infiltration is associated with impaired survival. BLs can appear with packing of the marrow resembling lymphoblastic leukemia (see Figure 10-10). Bone marrow involvement by HL is common at presentation, and criteria for diagnosis are the same as in the general population.

### Prognosis and Therapy

The patient's immune status at the time of diagnosis is an important factor in determining the outcome of ARL. Adverse prognostic factors include CD4 cell counts less than 100/mm$^3$, age greater than 35 years, stage III or IV disease, history of injection drug use, and high International Prognostic Index score. Therapy may involve chemotherapy and radiation, as well as antiretroviral therapy, hematopoietic growth factor support, and stem cell transplantation. Clinical outcomes from patients with DLBCL are currently approaching those of patients with de novo lymphoma.

## LYMPHOPROLIFERATIVE DISEASES ASSOCIATED WITH PRIMARY IMMUNE DISORDERS

This is a broad group of heterogeneous lymphoproliferative disorders that includes lymphoproliferative disease in association with ataxia telangiectasia, Wiskott-Aldrich syndrome, common variable immunodeficiency (CVID), severe combined immunodeficiency, X-linked lymphoproliferative disorder, and autoimmune lymphoproliferative syndrome (ALPS) among others. The majority arises in association with EBV infection in the setting of an underlying primary immune defect.

## ATAXIA TELANGIECTASIA

Ataxia telangiectasia (AT) is a rare autosomal recessive disorder caused by inheriting biallelic mutations of the ataxia telangiectasia mutated *(ATM)* gene. There is a strong association with lymphoid malignancies,

**LYMPHOPROLIFERATIVE DISEASES ASSOCIATED WITH PRIMARY IMMUNE DISORDERS—FACT SHEET**

**Ataxia Telangiectasia**
- Both T- and B-cell lymphomas and leukemias are associated with *ATM* mutations in patients with ataxia telangiectasia
- *ATM* gene alterations occur in sporadic lymphoproliferative disorders

**Combined Variable Immunodeficiency**
- There is a spectrum of lymphoid proliferations including atypical proliferation, which can be confused with lymphoma
- Granulomatous proliferation can be confused with sarcoid
- Lymphoma should be confirmed by immunohistochemistry and molecular studies
- MALT lymphomas occur in the lung and at other sites

**Autoimmune Lymphoproliferative Syndrome**
- Associated mostly with mutations in the *FAS* gene or its ligand
- Paracortical expansion of CD4–, CD8– T cells
- Lymphoid proliferations possibly confused with lymphoma, including HL

**Immunomodulator Agent-Related Lymphoproliferative Disorders**
- Occur in patients with severe autoimmune disease, usually rheumatoid arthritis treated with immunosuppressive drugs such as methotrexate and TNF-α antagonists
- Usually extranodal disease
- Spectrum of polymorphous lymphoplasmacytic infiltrates, which may resemble diffuse large B-cell lymphoma and classical Hodgkin lymphoma
- Usually regresses or resolves after withdrawal of methotrexate therapy
- May be associated with EBV

suggesting that this gene may be involved in lymphoma genesis. Lymphoid neoplasms in patients with ataxia telangiectasia are of both B- and T-cell origin, and include HL, non-Hodgkin lymphoma, and several forms of leukemia. *ATM* normally functions as a tumor suppressor, and it is activated primarily in response to double strand breaks. *ATM* alterations including loss of heterozygosity at 11q22-23 (location of the *ATM*) also occur in diverse sporadic lymphoproliferative disorders. These disorders include patients with T-cell prolymphocytic leukemia, B-cell chronic lymphocytic leukemia, and mantle cell lymphoma.

## AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME

ALPS is associated with germline mutations in the *Fas* gene (Type 1a) or its ligand (Type 1b). Both *Fas* (CD95) and its ligand play a pivotal role in apoptosis, and defective lymphocyte apoptosis caused by *Fas* gene mutations can result in a severe ALPS, manifested by moderate to massive splenomegaly and lymphadenopathy. Clinically there is hypergammaglobulinemia, autoimmunity, B-cell lymphocytosis, and the expansion of an unusual population of CD4-/CD8- T cells that express the alpha/beta T-cell receptor (TCR) (Figure 10-20). A related condition in patients with similar clinical symptoms due to a functional *FAS* deficiency without *FAS* gene mutations has been termed ALPS type 2 (ALPS 2). A third type of autoimmune lymphoproliferative syndrome (i.e., ALPS 3) has no known gene mutations (Table 10-3).

Lymph node biopsies in patients with ALPS reveal marked paracortical hyperplasia, with expansion of interfollicular areas by $CD3^+/CD4^-/CD8^-$ (double negative) T-cells, many of which stain for cytotoxic granules. The paracortical proliferation may be extensive enough to suggest a diagnosis of lymphoma (see Figure 10-20). In addition, there may be a polyclonal plasmacytosis and

**TABLE 10-3**

**Classification of Autoimmune Lymphoproliferative Syndrome**

| Type | Mutation |
|---|---|
| 1a | *Fas* gene |
| 1b | *Fas* ligand gene |
| 2 | *Caspase 8* or *Caspase 10* gene |
| 3 | No known gene mutations |

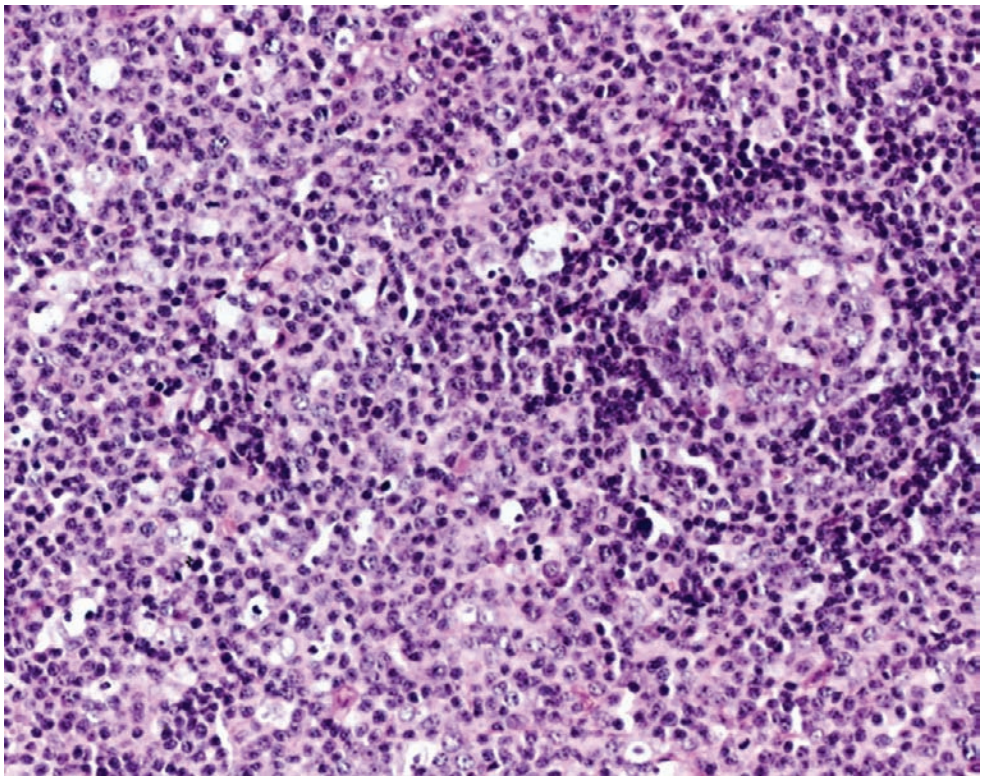

**FIGURE 10-20**

Lymph node from a patient with autoimmune lymphoproliferative syndrome type I reveals lymphoma-like paracortical expansion by T cells with $CD4^-CD8^-$ phenotype.

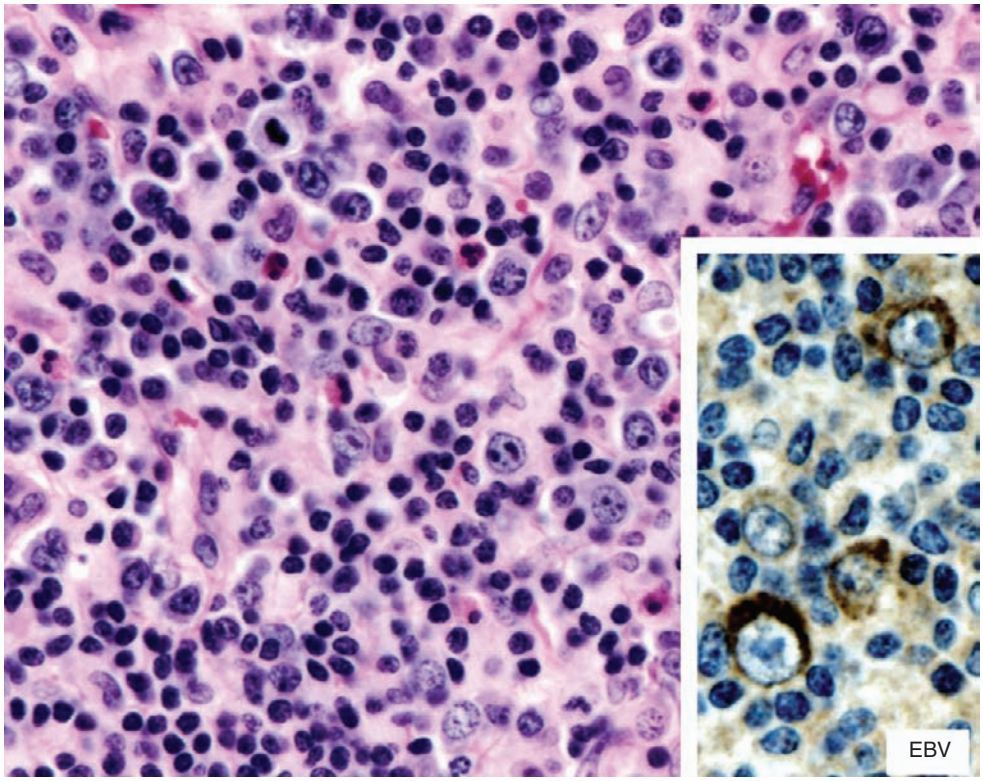


**FIGURE 10-21**

Polymorphous infiltrate effacing nodal architecture in a patient with severe rheumatoid disease treated with methotrexate. There are frequent EBV-positive immunoblasts in a mixed background.

florid follicular hyperplasia, follicular involution, and progressive transformation of germinal centers. Similar changes may be present in the spleen. Although some cases may resemble HL or EBV-related lymphoproliferative disease with increased large cells, caution should be used in the diagnosis because there may be spontaneous regression, even in the presence of a clonal lymphoid population. Lymphomas occurring in association with ALPS include HL, non-Hodgkin lymphomas including Burkitt and DLBCL, as well as peripheral T-cell lymphomas.

## LYMPHOPROLIFERATIVE DISORDERS IN PATIENTS TREATED WITH IMMUNOSUPPRESSIVE DRUGS FOR AUTOIMMUNE DISEASES OR RELATED CONDITIONS (IMMUNOMODULATOR AGENT-RELATED LYMPHOPROLIFERATIVE DISORDERS)

Immunomodulator agent-related lymphoproliferative disorders usually occur in patients with severe autoimmune disease (mostly rheumatoid disease) who are being treated with immunosuppressive drugs such as methotrexate. Cases are also described in association with newer immunomodulator agents such as TNF-α antagonists. Lymphoid proliferations are usually extranodal and involve the gastrointestinal tract, skin, lung, and soft tissues, among other sites. There is a broad spectrum of histologic appearances ranging from polymorphous lymphoplasmacytic infiltrates (Figure 10-21), diffuse large B-cell lymphoma (most common), classical HL, Hodgkin-like lymphoid proliferations, BL, and peripheral T-cell lymphoma, NOS. Follicular lymphomas are unusual.

Most of the lymphoid lesions have a morphology and phenotype similar to those in the general population. The Hodgkin-like lesion contains EBV-positive large cells resembling Hodgkin and Reed-Sternberg cells in a polymorphous background, which includes lymphocytes, plasma cells, and histiocytes. Immunohistochemical analysis may be helpful because the large cells express CD20 and CD30 but are negative for CD15. The importance of recognizing this entity in the setting of methotrexate therapy is that most cases show at least partial regression on withdrawal of methotrexate, particularly in the lymphoplasmacytic infiltrates, the Hodgkin-like lesions, and those that are positive for EBV.

## COMMON VARIABLE IMMUNODEFICIENCY

CVID is also known as *late-onset hypogammaglobulinemia* and is recognized clinically by variably reduced

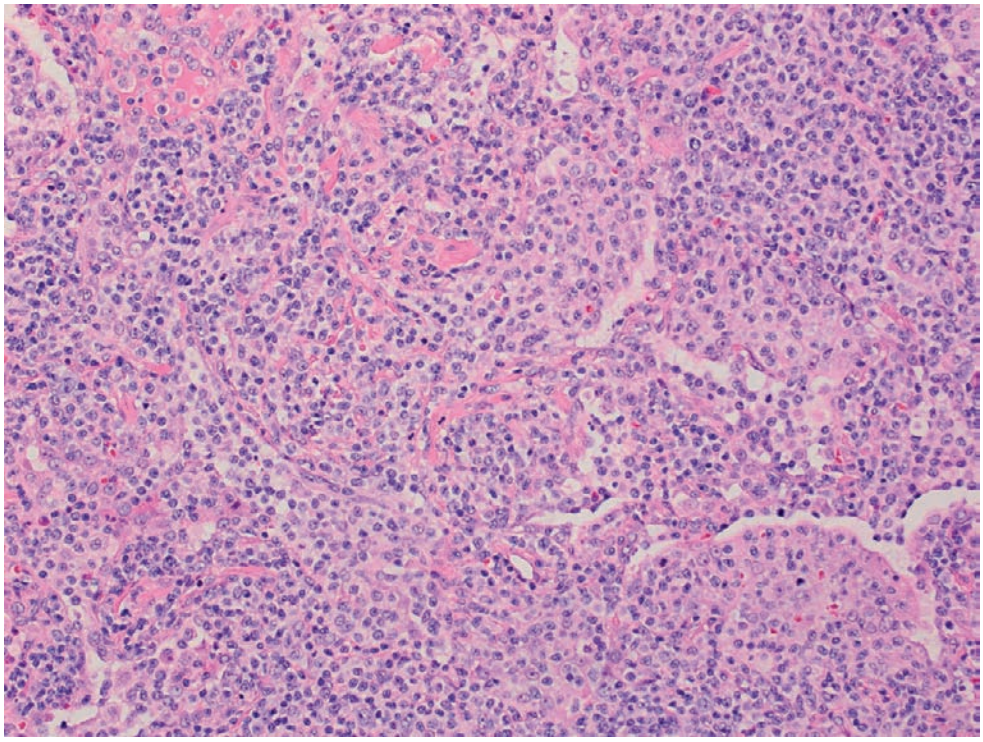

**FIGURE 10-22**

Pulmonary MALT lymphoma in a patient with CVID. There is a dense infiltrate of lymphoid cells, including nests of centrocyte-like cells with clear cytoplasm.

levels of serum immunoglobulin, loss of antibody production, and increased incidence of infections. Most patients are adults aged 20 to 40 years.

Localized or systemic granulomatous disease occurs in up to 20% of patients with CVID and should not be confused with sarcoidosis. A spectrum of lymphoproliferative disorders is encountered, with the majority of cases showing reactive or atypical lymphoid hyperplasia. Recognition of atypical lymphoid proliferations is particularly important, because these patients may have extensive or disseminated disease and may be misinterpreted as having lymphoma clinically and on pathologic examination. Lymphoid infiltrates in the lung can lead to interstitial pneumonia or follicular bronchitis or bronchiolitis.

Biopsy specimens of lymph nodes usually show lymphoid hyperplasia, which may be atypical, or granulomatous inflammation. In cases of atypical lymphoid hyperplasia, the nodal architecture may appear partially effaced in routine histologic sections, but compartmentalization into B- and T-cell zones and the presence of residual germinal centers can be identified with immunohistochemical studies. Some cases reveal chronic granulomatous inflammation, which may be necrotizing.

The incidence of malignancy is increased in CVID (up to 15% of subjects), and there is a 30-fold increase in non-Hodgkin lymphoma. Lymphomas are more common in older patients (range 13 to 88 years) and is more common in females. Lymphoma can arise in a background of lymphoid hyperplasia. Lymphomas of extranodal MALT-type can occur in the lung (Figure 10-22) and at other sites. Lymphomas in CVID are usually not associated with infection by EBV. Diagnoses of lymphoma in this setting is based on conventional criteria.

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The complete reference list is available online at www.expertconsult.com.

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# 11

# Hodgkin Lymphoma

**Bertram Schnitzer, MD**

## INTRODUCTION

In this chapter, we review the evolution of the classifications of Hodgkin lymphoma, emphasizing the histologic features of the various subcategories of this lymphoma, and we discuss the practical application of immunohistochemistry in the diagnosis and differential diagnosis of this intriguing disease.

## HISTORICAL PERSPECTIVE

In 1832, Thomas Hodgkin reported the gross findings of seven autopsy cases of the disorder that Sir Samuel Wilks then termed Hodgkin disease in 1865. The histologic features of this disorder were described by Langhans in 1872 and by Greenfield in 1878. It remained for Carl Sternberg in 1898 and Dorothy Reed of Johns Hopkins University in 1902 to describe the cytologic characteristics of the giant cells that must be present for a diagnosis of Hodgkin disease (HD) to be established. The latter two pathologists have also been credited with the initial definitive microscope description of the disorder. These giant cells, which are now known to be the malignant cells in HD, are called Reed-Sternberg (RS) cells, and, prior to the updated classifications of HD, they were also referred to as Sternberg-Reed cells.

Hodgkin lymphoma (HL), the official WHO designation, is an unusual malignancy, both in its clinical as well as its histologic features. Some patients initially have symptoms more consistent with an infectious process rather than a malignancy in that the patient presents with fevers and often drenching night sweats. Both Reed and Sternberg did not consider HL to be a malignancy. Sternberg thought it was related to tuberculosis, which was a common disease at the time and often accompanied HL, whereas Reed considered HL to be an inflammatory process unrelated to tuberculosis. The debate as to whether HL was an infectious or malignant disease lasted for many years, but despite an extensive search for a causative agent, none was found. It was not until the 1960s that cytogenetic studies demonstrated that RS cells were malignant because they were both aneuploid and clonal.

As far as therapy for HL is concerned, it has been a success story of modern medicine. HL was the first malignancy in which the curative potential of combination chemotherapy was demonstrated, and currently approximately 80% of patients can be cured. This cure rate is especially heartening because, in contrast to most other malignancies, HL is predominantly a disease of young individuals. However, with improved survival and long-term follow-up, late effect treatment-induced complications have become apparent, especially second malignancies. The challenge for the future is to devise treatment strategies that minimize toxicity and late complications without jeopardizing the high cure rates that are currently being attained.

### Clinical Features

HL accounts for approximately 1% of all malignancies and 30% of all lymphomas in the United States. About 8000 new cases of HL are diagnosed annually in the US, with a male to female ratio of 1.3 to 1.4:1. In industrialized nations, classical HL (CHL) has a bimodal age distribution, with the first peak occurring between ages 15 and 35 years and the second peak seen in approximately the sixth decade. The first peak is due to the nodular sclerosis subtype of HL, whereas mixed cellularity type occurs more often in the older age group. In developing countries, HL is less common but affects more children, mostly boys, and mixed cellularity is more often seen. Although the etiology of HL remains unresolved, Epstein-Barr virus (EBV) has been implicated in a subset of cases; however, proof that EBV is the cause of the disease is lacking.

Most patients with HL present with localized disease limited to one or two lymph nodes, most often in the cervical region (75% of cases). Mediastinal involvement may be the presenting sign with or without peripheral (particularly supraclavicular) lymphadenopathy.

Sometimes the patient is asymptomatic and a mass is detected on routine chest x-ray, or else the patient may complain of symptoms secondary to enlarged lymph nodes or to a mediastinal mass. Some patients present with weight loss, pruritus, and intermittent fevers, which may be accompanied by drenching night sweats (B symptoms), and occasionally the patient has cyclic bouts of a fever known as Pel-Ebstein fever, named after the two physicians who first described it. Such symptoms suggest an infectious rather than a malignant process. In rare cases, when a patient with HL imbibes alcoholic beverages, he/she may experience pain in the lymph nodes involved by the disease.

**HODGKIN LYMPHOMA—FACT SHEET**

**Definition**

- A monoclonal B-cell lymphoma is composed of a minority (<1%) of malignant cells called Reed-Sternberg (RS) and Hodgkin (H) cells admixed in an inflammatory cell infiltrate
- HL consists of two distinct clinical and biologic entities
  - Nodular lymphocyte predominant HL
  - Classical HL
- Classical HL consists of four histologic subtypes (nodular sclerosis, lymphocyte-rich, mixed cellularity, lymphocyte depleted). These subtypes are based on the characteristics of the background inflammatory infiltrate and the cytologic features of the H/RS cells

**Incidence and Location**

- Classical HL accounts for approximately 1% of all malignancies, 30% of all lymphomas
- Approximately 8000 new cases annually in the United States
- Arises in lymph nodes, most often cervical nodes (75% of cases)
- Mediastinal involvement common in nodular sclerosis, classical HL

**Mortality**

- Depends on extent (stage) of the disease at presentation

**Gender, Race, and Age Distribution**

- Male predominance (except for nodular sclerosis classical HL)
- Higher incidence in Caucasians
- Most cases occur in young adults but are seen in all age groups

**Clinical Features**

- Classical HL has a bimodal age distribution in industrial countries: first peak between ages 15 and 35 years, with a second peak in older age group
- Patients may be asymptomatic except for lymph node enlargement, or they may also have fatigue, weight loss, fever, and night sweats
- Greater than 60% of patients have localized disease (stages 1 and 2)

**Prognosis and Therapy**

- Prognosis is related to extent (stage) of disease, which also dictates the type of therapy to be given. The antibody-drug conjugate targeting CD30 (brentuximab vedoitin) has been approved for use in $CD30^+$ lymphomas including classical HL
- Presence of tumor-associated macrophages ($CD68^+$) is strongly associated with shortened survival in classical HL

Staging of patients with HL is important in determining the extent of the disease, which, in turn, dictates the type of therapeutic regimen that the patient will receive. At one time, bipedal lymphangiography was used to identify abdominal lymph nodes possibly involved by HL. This procedure was followed by staging laparotomy (pathologic staging) with biopsy of selected lymph nodes, and splenectomy was routinely carried out to determine the stage of disease. Lymphangiography not only identified nodes to be removed, but also led to the observation that HL spreads in a non-random fashion but that there is an orderly progression of disease with dissemination to contiguous lymph node groups including the spleen. For unknown reasons, contiguous spread does not occur in HIV-positive patients. With the advent of advanced diagnostic imaging techniques available for determining the extent of disease, pathologic staging is no longer necessary. In 1971, a clinical staging classification known as the Ann Arbor staging system was adopted, was subsequently modified at a meeting in Cotswold, England, and hence, was called the Cotswold revision of the Ann Arbor staging classification (Table 11-1). This revision included the new diagnostic techniques for evaluating of the extent of disease.

## Pathologic Features

### Histopathologic Classifications of Hodgkin Lymphoma

Not only are the clinical features of HL unusual for a malignant neoplasm, but the histologic features are also not typical of such other malignancies as

**TABLE 11-1**

**Cotswold Revision of the Ann Arbor Staging Classification**

| Stage | Definition |
|---|---|
| I | Involvement of a single lymph node region of lymphoid structure (e.g., spleen thymus, Waldeyer's ring) |
| II | Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes are lateralized); the number of anatomic sites should be indicated by suffix (e.g., $II_3$) |
| III | Involvement of lymph node regions or structures on both sides of the diaphragm |
| $III_1$ | With or without splenic, hilar, celiac, or portal nodes |
| $III_2$ | With paraaortic, iliac, or mesenteric nodes |
| IV | Involvement of extranodal site(s) beyond those designated E |

*A*, No symptoms; *B*, fever, drenching sweats, or weight loss; *X*, bulky disease: >⅓ widening of the mediastinum at T5-6, or maximum of nodal mass >10 cm; *E*, involvement of a single extranodal site, or contiguous or proximal to known nodal site of disease; *CS*, clinical stage; *PS*, pathologic stage.

non-Hodgkin lymphoma, epithelial neoplasm, or sarcoma. In malignancies other than HL as well as in non-Hodgkin lymphomas (NHL), the neoplasm is composed predominantly of malignant cells, and the tumor is named and classified according to the cell of origin. In contrast, the enlargement of lymph nodes in HL is caused by the overwhelming number of benign inflammatory cells, whereas the malignant cells are often fewer than 1% and thus may be difficult to identify. Until recently, the paucity of the malignant cells (RS cells) has hindered attempts to identify their cellular origin and lineage. Furthermore, the classification of HL is based primarily not on the malignant cell but on the benign inflammatory infiltrate and the presence of fibrosis, as well as on the immunomorphologic features of the RS cells.

Numerous classifications of non-Hodgkin lymphomas (NHL) have been proposed over the years, whereas relatively few schemes for subdividing HL have been devised. Also, in contrast to classifications of NHL, which are relatively short-lived, the organizational schemes for HL have remained remarkably stable for almost 40 years.

The first useful histologic classification of HL was the one proposed by Jackson and Parker in 1944. It divided HL into three types: paragranuloma, granuloma, and sarcoma (Table 11-2). This classification was not found to be clinically useful in predicting the outcome of the disease, because almost 90% of cases fell into the clinically and morphologically heterogeneous granuloma subtype. Some patients with this subtype fared fairly well, whereas in others, the disease had a more aggressive course. In 1956 Smetana and Cohen identified a subtype of the granuloma group, which was characterized by a nodular pattern; hence, it was named nodular granuloma and later renamed nodular sclerosis Hodgkin disease (HD) in the classification of Lukes and Butler. This subtype appeared to be associated with a better outcome than the nonnodular granuloma type.

In the classification of Lukes and Butler, the heterogeneous granuloma type of HL of the Jackson and Parker scheme was divided into the histologically descriptive mixed cellularity (mixture of inflammatory cells) and nodular sclerosis (lymph node divided into nodules by fibrocollagenous bands) subtypes (see Table 11-2). Paragranuloma, which consisted of small lymphocytes and varying numbers of epithelioid histiocytes, was appropriately renamed lymphocytic and/or histiocytic (L&H) type and was further divided into subtypes with either a nodular or diffuse growth pattern. The sarcomatous type was separated into reticular and diffuse fibrosis categories.

The six subtypes of the morphologically descriptive scheme of Lukes and Butler were felt to be too cumbersome for both clinicians and pathologists to use, and consequently, at a conference at Rye, New York in 1965, the six subtypes were simplified and condensed into four subgroups. This so-called Rye classification retained the prognostic significance of the Lukes and Butler classification (now made irrelevant by modern treatment protocols) and was based on the inverse relationship between the small lymphocytes and RS cells. The four histologic types in the Rye classification included: (1) lymphocyte predominance, in which there is a predominance of small lymphocytes, and few of the neoplastic RS cells; (2) nodular sclerosis, a disorder with frequent mediastinal disease and the only subtype found in almost equal distribution in young women and men; (3) mixed cellularity, which as the term implies, is characterized by a mixed inflammatory cell infiltrate including small lymphocytes, eosinophils, plasma cells, histiocytes and neutrophils; and (4) lymphocyte depletion, characterized by few lymphocytes and many RS cells (see Table 11-2). In the Lukes and Butler/Rye schemes, lymphocyte predominant (LP) HL was associated with an indolent course and a favorable prognosis (an observation made by Rosenthal in 1936), whereas the lymphocyte depletion type, in which there are many malignant (RS) cells and few lymphocytes, was found

**TABLE 11-2**
**Classifications of Hodgkin Lymphoma**

| Jackson and Parker (1944) | Lukes and Butler (1966) | Rye (1966) | REAL/WHO (1994, 2001, 2008) |
|---|---|---|---|
| Paragranuloma → | Lymphocytic and/or histiocytic, nodular | Lymphocytic predominance | Nodular lymphocyte predominant |
| | Lymphocytic and/or histiocytic, diffuse | Lymphocytic predominance | Lymphocyte-rich classical HL* |
| Granuloma → | Nodular sclerosis → | Nodular sclerosis → | Nodular sclerosis |
| | Mixed cellularity → | Mixed cellularity → | Mixed cellularity |
| Sarcoma → | Diffuse fibrosis, reticular → | Lymphocytic depletion → | Lymphocyte depleted |

*REAL,* Revised European American Lymphoma (classification); *WHO,* World Health Organization.
*Lymphocyte-rich classical was a provisional entity in the REAL classification.

to be aggressive with an unfavorable outcome. The prognostic significance of these histologic types has become less relevant with the advent of modern therapeutic regimens, and survival is now known to be associated with the stage of disease rather than with the histologic subtype of disease.

The Lukes and Butler/Rye classification remained unchanged until it was supplanted in 1994 by the Revised European American Lymphoma (REAL) classification and its updated version, the World Health Organization (WHO) scheme in 2001. In 2008, the revised fourth edition of the WHO classification was published (see Table 11-2). Two changes noted in this edition include: LP cells replaced the term lymphocytic and/or histiocytic (L & H) Reed-Sternberg cell variants in nodular lymphocyte predominant (NLP) HL, and cases that could not be classified into one of the subgroups were placed into the mixed-cellularity (MC) category. When the Lukes and Butler/Rye classifications were originally proposed, ancillary techniques including antibodies routinely used for immunophenotyping today, as well as modern cytogenetic and molecular genetics techniques, were not yet available, and hence these classifications were based on morphology alone.

Although the REAL and WHO schemes were founded on the Lukes and Butler/Rye classifications, several significant changes were made by virtue of the existence of ancillary techniques including: (1) a name change from Hodgkin disease (HD) to Hodgkin lymphoma (HL), because recent clinical and biological studies have demonstrated that HL is truly a lymphoma since its neoplastic cells are of lymphoid origin; and (2) HL was divided into two distinct clinical and biological entities: NLP HL and classical (C) HL. These two types of HL differ from each other in their natural history, epidemiology, clinical features (Table 11-3), immunophenotypes, preservation or downregulation of the B-lineage-specific program (preserved in NLP HL, downregulated in CHL), genetic features, and association with EBV. However, despite the fact that the two types of HL are clinically and biologically different, they share certain features. Both contain a paucity of malignant cells (Hodgkin [H] and RS cells) against a background of benign inflammatory cells. Both types of HL frequently occur in young adults, and both also frequently involve cervical lymph nodes at the time of presentation. In addition, recent studies have shown that in almost all cases of both NLP and CHL, the RS cells are B cells of germinal center origin; (3) diffuse (D) LP HL is not recognized as a separate or distinct entity, although it may be seen in conjunction with NLP HL (NLP HL with diffuse areas); (4) a new subtype of CHL, lymphocyte-rich (LR) CHL was added. This subtype was only a provisional entity in the REAL classification, whereas it has been accepted as a distinct subtype in the WHO schemes, the updated versions of the REAL

**TABLE 11-3**
**Clinical Features of NLP HL and CHL**

| | CHL | NLP HL |
|---|---|---|
| Age distribution | Bimodal | Unimodal |
| Percentage male sex | NS, 50%; MC, 70% | 70% |
| Sites involved | Peripheral lymph nodes, mediastinum, abdomen, spleen | Peripheral lymph nodes |
| Stage at diagnosis | Often II or III | Usually I |
| B symptoms | 40% | Rare |
| Association with EBV | 25% to 70% | None |
| Course | Aggressive, curable | Indolent, late relapses |
| Risk of large B-cell lymphoma | 1% | 2%-5% |

*CHL*, Classical Hodgkin lymphoma; *EBV*, Epstein-Barr Virus; *MC*, mixed cellularity; *NLP*, nodular lymphocyte-predominant; *NS*, nodular sclerosis.

**HODGKIN LYMPHOMA–PATHOLOGIC FEATURES**

**Microscopic Findings**
- Hodgkin/Reed-Sternberg (H/RS) cells or variant RS cells must be present to make a diagnosis of HL
- H/RS cells and variant RS cells are the malignant cells and constitute the minority (usually <1%) of the nodal infiltrate
- The vast majority of cells are responsible for lymphadenopathy are benign inflammatory cells
- Inflammatory cells and types of RS cells determine the histologic subtype of HL
- Classic H/RS cells are most abundant in MC CHL
- Lacunar cell variants of RS cells are characteristic of NS CHL
- LP or popcorn cell variants are found in NLP HL
- Pleomorphic RS cells are seen in LD CHL

**Immunohistochemical Findings**
- See Pathologic Features of various HL subtypes

**Genetics**
- H/RS and RS variant cells are monoclonal B cells of germinal center derivation
- LP cells have clonally rearranged IG genes
- In LP cells, the variable region of the IG heavy chain genes carry a high load of somatic mutations with signs of ongoing mutations
- LP cells have productive rearrangements of the IG heavy chain genes with detectable IG mRNA
- Classic H/RS cells contain clonally rearranged IG genes
- In classic H/RS cells, the variable region of the IG heavy chain genes carry a high load of somatic mutations without signs of ongoing mutations
- Classic H/RS do not have productive rearrangements and have no detectable IG mRNA

classification; and (5) nodular sclerosis has been divided into two histologic grades based primarily on the number of RS cells within the nodules.

### Prognosis and Therapy

Modern therapeutic regimens have transformed HL into a curable disease in the majority of cases. Prior to the time that adequate procedures for determining the extent or stage of HL were available and before modern chemotherapy and radiation therapy for treating HL were established, prognosis was related to the histologic subtype. Lymphocyte predominant HL was associated with the best prognosis (as it is today), whereas lymphocyte depleted with the worst. Nodular sclerosis was generally associated with a better outlook than the mixed cellularity subtype (because of lower stage disease). Stage I/II is associated with a better prognosis than advanced stage disease (III/IV) in all types of HL. The prognosis of NLP HL in stage I/II is the best of all cases, having a 10 year overall survival of greater than 80%. Patients with localized NLP HL and favorable prognostic features are usually treated with involved field radiotherapy without chemotherapy. Rituximab (anti-CD20) has also been used in clinical trials in *de novo* as well as relapsed disease with favorable results. In patients with advanced disease, treatment similar to that for CHL is generally recommended. It is not entirely clear whether or not treatment following excisional biopsy of stage I NLP HL is required to achieve this prolonged survival. A watch and wait approach following lymph node excision has been advocated in low stage disease prior to treatment. The cause of death of patients with NLP HL is often not related to the disease but is secondary to coronary heart disease or to secondary neoplasm. In CHL, stage and B symptoms (fever and weight loss) are also much more relevant for predicting outcome and dictating the type of therapy required. In contrast to patients with NLP HL, patients with CHL require multiagent chemotherapy, radiation therapy, or both. Such therapy has made CHL curable in greater than 85% of cases.

BCL2 expression in RS cells appears to be an independent poor prognostic marker in CHL. Recent studies have shown that the presence of macrophages (histiocytes) in CHL is important in predicting prognosis. The presence of more than 5% tumor-associated macrophages (identified by CD68) was strongly associated with shortened survival, thus providing a new biomarker for risk stratification.

## REED-STERNBERG CELLS

The origin and lineage of Hodgkin/Reed-Sternberg (H/RS) cells and L&H (or LP) cells have been controversial for decades. At different times the RS cell has been postulated to be a lymphocyte, interdigitating dendritic cell, histiocyte, or granulocyte. More recent molecular studies have conclusively shown that with rare exceptions the H/RS and LP cells are lymphocytes of B-cell lineage of germinal center cell origin. In rare cases, there is evidence that classic RS cells are of T-cell origin.

The presence of the Reed-Sternberg (RS) cell, or one of its variants (LP cells or lacunar cells), is required for a diagnosis of HL to be made. Not only must RS cells be found, but they must also be present in a cellular environment of one of the five subtypes of HL. The background milieu is of utmost importance not only in establishing a diagnosis of HL but also in subclassifying HL. In addition, RS cell impostors may be found not only in some non-Hodgkin lymphomas but also in some benign lymphoid proliferations. However, with a few exceptions such as peripheral T-cell lymphomas and T-cell histiocyte-rich large B-cell lymphomas, the accompanying inflammatory cells characteristic of HL are not found. Three types of RS cells are recognized: (1) classic, (2) lacunar cells, and (3) lymphocyte predominant (LP) cells, formerly called L&H cells (from the Lukes and Butler classification) (Figure 11-1). Classic and lacunar cells are found in classical HL, whereas LP cells are characteristic of NLP HL. Because of its resemblance to an exploded kernel of corn, the variant RS cell seen in NLP HL is also referred to as a popcorn cell. The classic binucleate or owl eye cell is most abundant in MC HL, although it is also found in the most recently described LR classical types and occasionally in the nodular sclerosis (NS) and lymphocyte-depleted (LD) types. Lacunar cells predominate in NS HL (see Figure 11-1). The lacunar cell may also be seen in the LR classical type and in small numbers in the MC type. In the rare LD HL, the RS cells have been referred to as *pleomorphic, anaplastic,* or *sarcomatous*. Hodgkin cells have the cytologic features of classic RS cells but are mononuclear (see Figure 11-1). Like classic RS cells, Hodgkin cells are most abundant in the MC type, and they are also found in other CHL but not in NLP HL.

Classic RS cells are binucleated, multinucleated, or lobulated giant cells with prominent, inclusion-like eosinophilic nucleoli surrounded by a halo in each nucleus. The nucleoli are seen in at least two nuclei in multinucleated cells or in each of two lobes in lobulated RS cells (see Figure 11-1). These cells have abundant eosinophilic or amphophilic cytoplasm. The binucleated RS cells are also called *diagnostic* or *owl eye cells*. Apoptotic RS cells—referred to as *mummified* or *zombie cells* and characterized by pyknotic chromatin, barely recognizable nucleoli, and eosinophilic and occasionally retracted cytoplasm—are present in all types of CHL (see Figure 11-1). Lacunar cells have been so named because they are found in clear spaces or lacunae. These cells are formed by the retraction of cytoplasm around the nucleus, although some cytoplasmic strands may

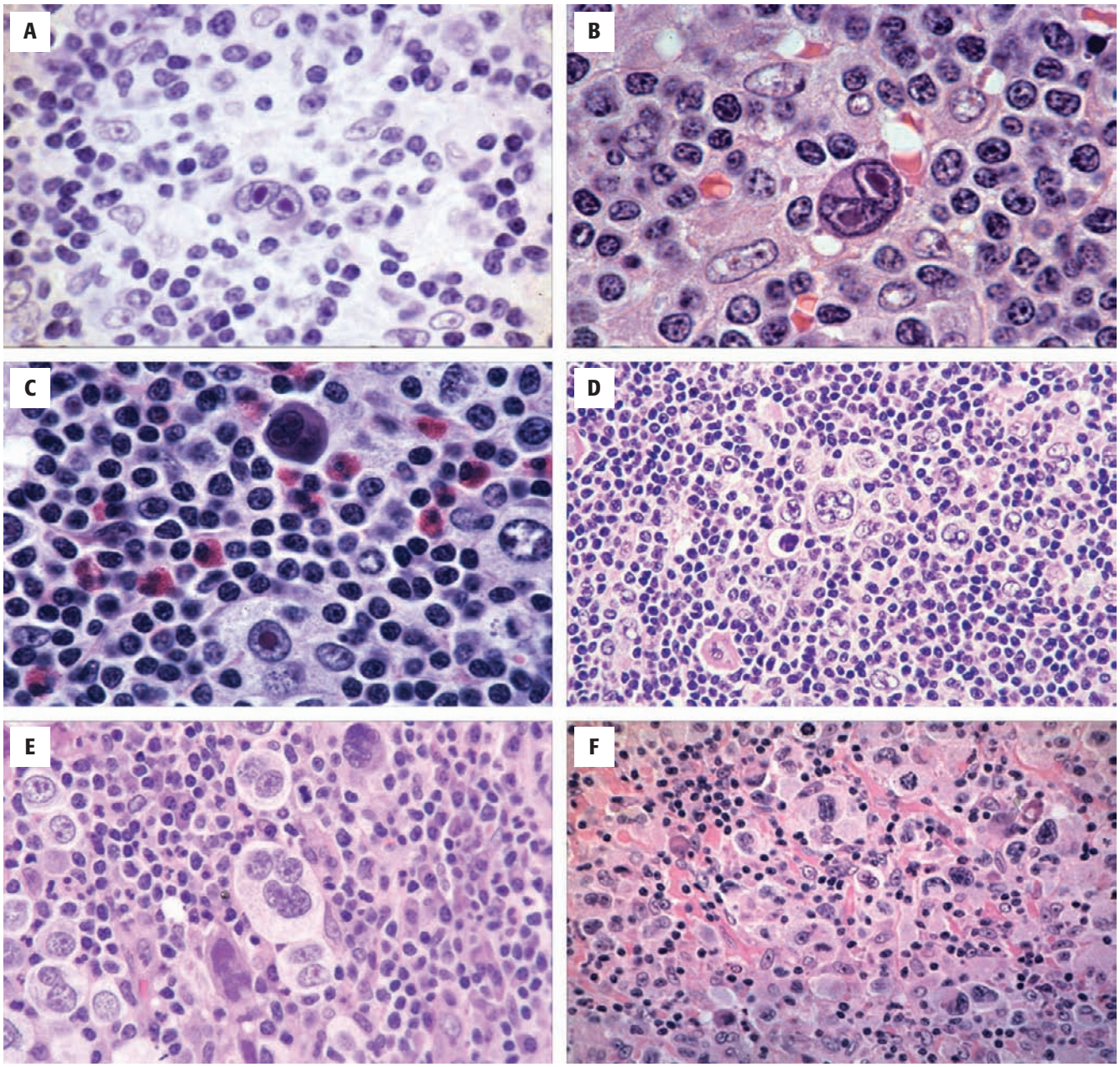



**FIGURE 11-1**

Reed-Sternberg cells and variants: microscopic features. **A,** Classic owl-eye cell. The cell is large and binucleate, with each nucleus containing a large inclusion-like nucleolus. **B,** Bilobed Reed-Sternberg cell. **C,** Hodgkin cell, which is a uninucleated cell with cytologic features of an Reed-Sternberg cell, and a mummified cell characterized by purple-staining cytoplasm and a pyknotic nucleus. **D,** LP (popcorn) cells with lobulated nuclei and small nucleoli, characteristic of the Reed-Sternberg variant of nodular lymphocyte predominant Hodgkin lymphoma. **E,** Lacunar cell variants of Reed-Sternberg cell with characteristic multilobate nuclei containing small nucleoli. **F,** Tissue fixed in B5 showing lacunar cells with ample cytoplasm.

*Continued*

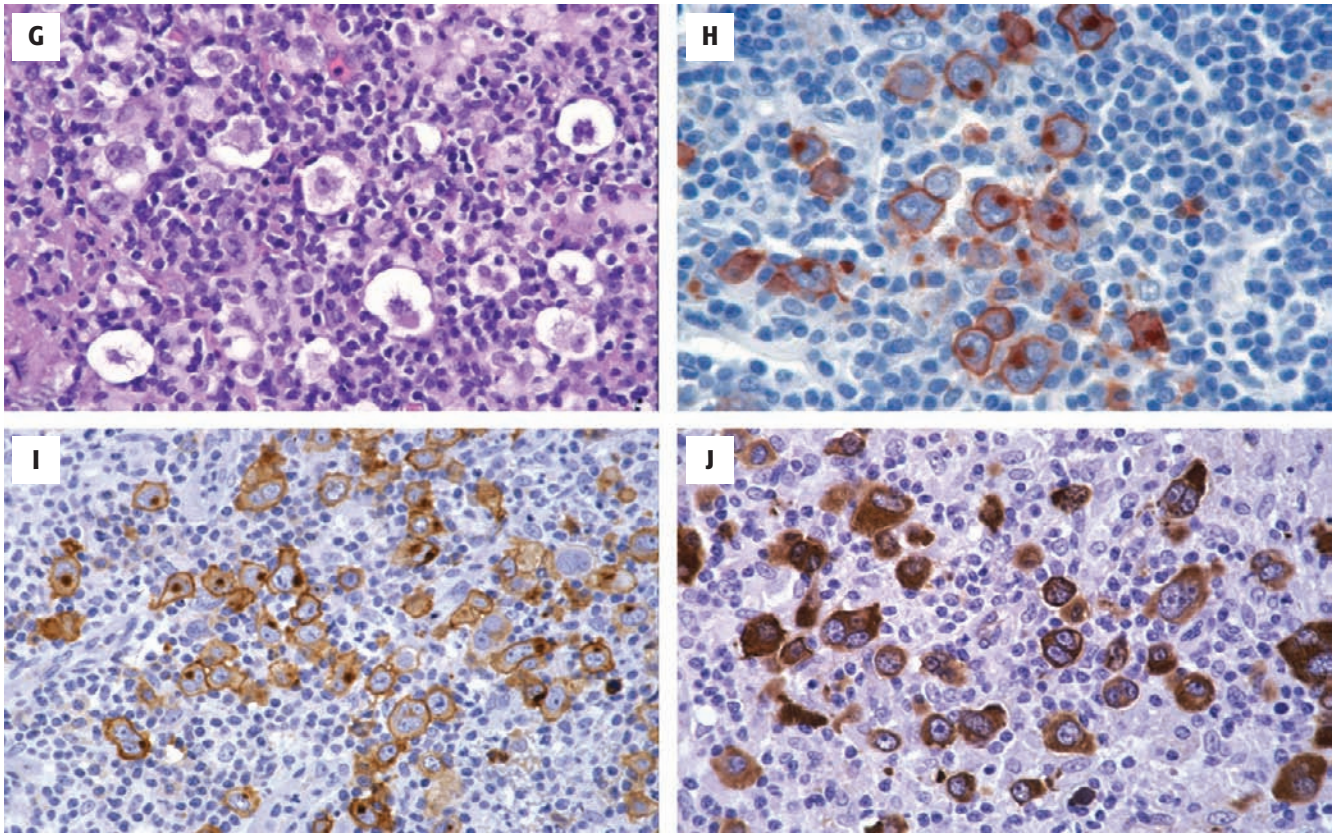



**FIGURE 11-1, cont'd**

**G,** Formalin-fixed tissue shows retraction of cytoplasm away from cell membrane of lacunar cells. **H,** Classic Reed-Sternberg cells stained with CD15. **I,** CD30. **J,** Fascin. CD15 and CD30 stain the cell membrane or Golgi region, or both, whereas fascin diffusely stains the cytoplasm. All images are high magnification.

still be attached to the cell membrane, thus leaving a space around the cells that is a useful artifact in establishing a diagnosis. Such lacunae are artifacts of formalin fixation and are not evident in tissue processed in metal-containing fixatives such as B5. In B5-fixed tissue, the lacunar cells are not surrounded by empty spaces, but the cells have ample, pale-staining cytoplasm (see Figure 11-1, F). Lacunar cells without lacunae are often, but not always, still identifiable by their characteristic cytologic features composed of hyperlobate, small nuclei and their nucleoli, which are smaller than those seen in classic RS cells. Lacunar cells seem to be more pleomorphic than has been appreciated in the past, and they may also have prominent nucleoli. The cells may appear bizarre, resembling cells of anaplastic large cell lymphoma, or they may be mononuclear and thus indistinguishable from cells of large cell lymphoma (see Figure 11-3). These pleomorphic cells are especially prominent in cases of the syncytial variant of NS HL. LP or popcorn cells of NLP HL are large, centroblast-like cells with complex lobulations of their nuclei. The nuclei contain peripherally placed basophilic nucleoli that are smaller than those of classic RS cells (see Figure 11-1). These cells have a thin rim of cytoplasm. In some cases of NLP HL, certain LP cells may have more prominent nucleoli that resemble those in classic RS cells. Some popcorn-type cells may resemble or be indistinguishable from lacunar cells; however, the cytoplasm is not as voluminous as that in lacunar cells.

## Ancillary Studies

### Immunophenotype

Immunophenotyping in paraffin sections is exceedingly useful in both the diagnosis and subclassification of HL. A panel of antibodies must be used to characterize RS cells, because no single marker is sufficiently sensitive or specific. Antibodies routinely used when a diagnosis of CHL is suspected include CD15, CD30, CD3, CD20, and sometimes CD45. In problematic cases, additional B- and T-cell antibodies, epithelial membrane antigen (EMA), BCL-6, fascin, EBV-latent membrane protein 1 (LMP1), and in situ hybridization for Epstein Barr virus–encoded RNA (EBER) may be added. Other markers that are not routinely used but are occasionally helpful include transcription factor OCT-2 with its cofactor BOB.1, PU.1, J-chain, and the gene product of PAX (PAX-5), also called *B-cell–specific activator protein* (Table 11-4).

**TABLE 11-4**

**Differential Diagnosis of Hodgkin Lymphoma: Immunophenotypes of Malignant Cells**

| | CHL | NLP | TC/HRLBCL | DLBCL | ALCL |
|---|---|---|---|---|---|
| CD45 | – | + | +/– | +/– | +/– |
| CD15 | +/– | – | – | – | – |
| CD30 | + | – | –/+ | –/+* | + |
| Fascin | + | – | – | –/+ | +/– |
| CD20 | –/+ | + | + | + | – |
| CD79a | –/+ | + | + | + | – |
| PAX-5 | +† | + | + | + | –‡ |
| OCT-2 | – (or weakly +) | + | + | + | – |
| BOB.1 | – (or weakly +) | + | + | + | – |
| PU.1 | – | + | – | + | – |
| CD3, CD7, CD8 | – | – | – | – | –/+ |
| CD2, CD4, CD5 | – | – | – | – | +/– |
| CD43 | – | – | – | – | +/– |
| EMA | – | + | –/+ | – | +/– |
| ALK | – | – | – | – | + (60%-80%) |
| BCL-6 | –§ | + | + | +/– | – |
| Clusterin | – | – | – | – | + |
| EBV(LMP1, EBER) | +/– | – | – | – | – |
| TIA-1, granzyme B, perforin | – | – | – | – | + |
| CD21 (FDC meshwork) | – | +¶ | – | – | – |

*ALCL,* Anaplastic large-cell lymphoma; *CHL,* classical Hodgkin lymphoma; *DLBCL,* diffuse large B-cell lymphoma; *NLP,* nodular lymphocyte predominant; *TC/HRLBCL,* T-cell/histiocyte–rich large B-cell lymphoma.

*Especially in primary mediastinal large-cell lymphoma, DLBCL with anaplastic features, and some cells of ordinary DLBCL.

§Occasionally positive.

†Negative in 10% of cases.

¶Absent in diffuse LP.

‡Rarely positive.

### Immunophenotype of the Reed-Sternberg Cell in Classical Hodgkin Lymphoma

Whereas the immunophenotypes of RS cells in all types of classical HL are the same, the presentation of various subtypes, the sites of involvement, the ages of the patients at time of presentation, and the natural history of the subtype often vary. In the majority of cases, classical RS cells express CD15, CD30, and fascin, and they are CD45 (common leukocyte antigen) negative (see Table 11-4). In addition, some RS cells in CHL may be BCL-6 positive, but in most cases they are negative. They also weakly express PAX-5 (pan–B-cell marker).

The immunophenotype of classic RS cells may differ slightly from case to case, and it is important to remember that not all RS cells in a given case will be positive for a specific marker. In a study of 1751 cases in which antigen retrieval and an immunoglobulin (Ig) M secondary antibody were used, 83% of cases of CHL revealed $CD15^+$ RS cells, whereas CD30 was expressed in 96% of cases, and the B-cell marker CD20 in 5% of cases. Other studies, as well as our own, indicate that CD15 is positive in fewer cases (less than 83% ), CD30 is positive in almost 100% of cases, and B-cell markers CD20 and CD79a expression have been reported in 60% of cases of NS and MC HL, a percentage that appears to be high in the experience of most authors. Such differences in antigen expression may be due to the particular fixation used (formalin, zinc formalin, alcohol-based fixatives, metal-based fixatives), the length of fixation, the antibody clone used, whether antigen-retrieval method was used, and if so which type. PAX5, a B-cell transcription factor, is usually expressed, albeit at lower levels than on normal mature B cells, and other transcription factors such as OCT2 and BOB.1 are weakly expressed or absent.

Immunophenotyping may also have an effect on the survival of patients with CHL. The German Hodgkin's Study Group reported that cases in which RS cells did not express CD15 (seen predominantly in older males

with advanced stage disease), but were CD30$^+$, had a significantly higher relapse rate and lower overall survival than cases in which RS cells were CD15$^+$. By multivariate analysis, absence of CD15 expression was found to be an adverse prognostic factor. In addition, this study showed that coexpression of CD15 and CD20 on RS cells had no effect on survival, but cases in which RS cells were CD20$^+$, CD15$^-$, and CD30$^-$ had poor survival rates. These cases (RS cells CD15$^-$, CD30$^-$, and CD20$^+$) are probably not classic HL but are most likely examples of T-cell/histiocyte–rich large B-cell lymphoma (TC/HRLBCL), which is an aggressive NHL. Absence of CD15 or CD30 positivity (but not absence of both) does not, therefore, exclude a diagnosis of HL. As has been mentioned previously, not all RS cells in a single case of CHL express CD15, but only a minor subset of RS cells may reveal this marker in a membrane or focal cytoplasmic (Golgi) staining pattern (see Figure 11-1). In contrast, almost all H/RS cells in a single case will be CD30 and fascin positive. The postgerminal center B cell marker IRF4/MUM1 is usually strongly expressed by classic RS cells. In addition, as mentioned previously, antigen expression is not specific for a definite cell lineage; CD15 is also expressed by myeloid cells, and care must be taken to determine that CD15 is not expressed on neutrophils or eosinophils that are often part of the inflammatory infiltrate. Adenocarcinoma cells, particularly primary pulmonary types, may also be CD15$^+$ but with diffuse cytoplasmic staining. When RS cells express CD20, it is usually only a minor subset of RS cells, and the intensity of staining with CD20 is generally variable, with most cells showing only weak expression. CD79a may also stain classical RS cells but does so less often than CD20. CD30 is also not specific for RS cells because this antibody is expressed by all cases of anaplastic large-cell lymphomas (ALCLs), activated B and T cells, plasma cells, and embryonal cell carcinomas. Like CD15, CD30 stains RS cells in a membrane or cytoplasmic pattern, or both. Fascin, in addition to staining RS cells in almost all cases of CHL, is an excellent marker for various types of dendritic cells (interdigitating and follicular) and endothelial cells (see Figure 11-1). It may also stain cells in some cases of ALCL and a variable number of large B- and T-cell lymphomas. The staining with fascin in all these cells is diffuse and cytoplasmic.

The frequency of EBV in H/RS cells in CHL varies according to the histologic subtype. It is most often (approximately 75%) associated with the MC type and least often (10% to 40%) with NS HL. Most cases of LD type, which are seen in HIV-positive individuals, are EBV positive.

### Immunophenotype of Reed-Sternberg Cell Variant in Nodular Lymphocyte Predominant Hodgkin Lymphoma

In contrast to the immunophenotype of RS cells in CHL, LP cells in NLP HL express a completely different set of markers (see Table 11-4). These cells are CD15$^-$, CD30$^-$, and fascin negative, but they consistently express B-cell markers CD20, CD79a, PAX-5, and CD45. In addition, LP cells express the nuclear protein encoded by the *BCL6* gene (attesting to their germinal center origin) and EMA in approximately 25% to 50% of cases (depending on the clone of the antibody) in a membranous or Golgi pattern, and they also synthesize J chains (a feature of B cells only). In addition, LP cells are positive for the transcription factors OCT-2, its cofactor BOB.1, and PU.1, the latter being necessary for B-cell development. These antibodies are not used routinely, but they are sometimes important in the differential diagnosis (e.g., nodular lymphocyte–rich [NLR] CHL) versus NLP type, in which classic RS cells in NLR CHL are almost always negative for all three of these markers. Clonality of RS cells can also be shown by molecular studies of isolated LP cells. In contrast to H/RS cells in CHL, EBV is negative in LP cells, with rare exceptions.

### Immunophenotype of Background Lymphocytes

The immunophenotypes of the background benign small lymphocytes are also different in NLP and CHL, with the exception of the NLR classical type in which they are the same as in NLP HL (Table 11-5). In both NLP and NLR classical, the nodules of small lymphocytes are polyclonal B cells of mantle cell origin expressing CD20, CD79a, IgM, and IgD, whereas the background small lymphocytes in all other types of CHL are T-cells (Figures 11-2 and 11-7). Another similarity between NLP and NLR CHL is the rosetting, collaretting, or ringing of T cells around LP and H/RS cells, which can be clearly seen in the B-cell–rich background in both subtypes and which is clearly evident on low magnification (see Figures 11-2 and 11-7). However, programmed death (PD)-1 positive T-cells form rings around RS cells

**TABLE 11-5**

**Immunophenotype of Small Lymphocytes in CHL, NLP HL, and NLR CHL**

| | CHL (Except NLR CHL) | NLP HL | NLR CHL |
|---|---|---|---|
| B cells | – | + | + |
| T cells | + | Ring RS cells | Ring RS cells |
| PD-1+ cells | – | Ring RS cells | Mostly in germinal center, rarely ring RS cells |
| CD57+ cells | – | Random distribution may ring RS cells | Mostly in germinal center, rarely ring RS cells |

*CHL,* Classical Hodgkin lymphoma; *HL,* hodgkin lymphoma; *NLP,* nodular lymphocyte-predominant; *NLR,* nodular lymphocyte-rich.

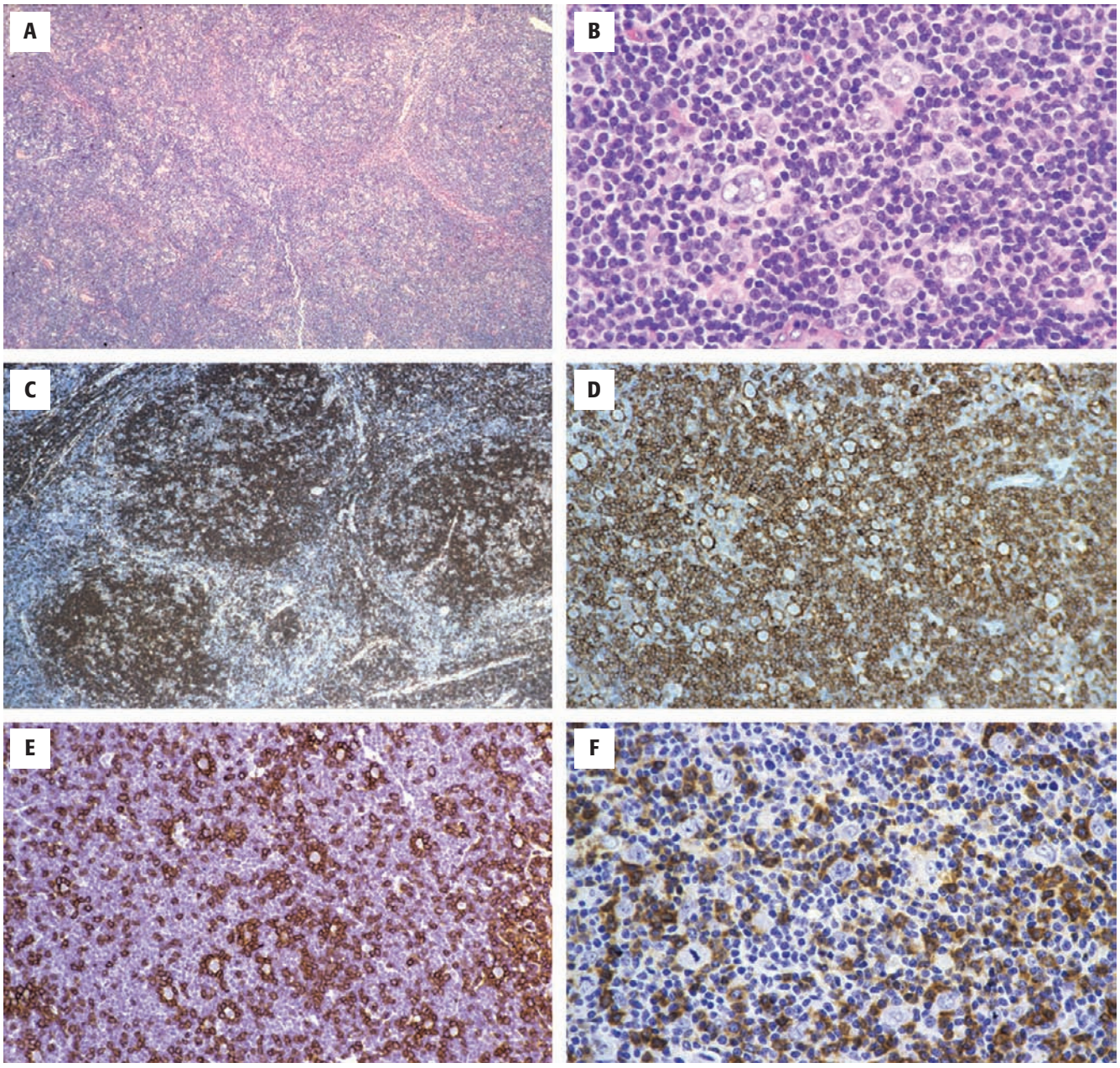
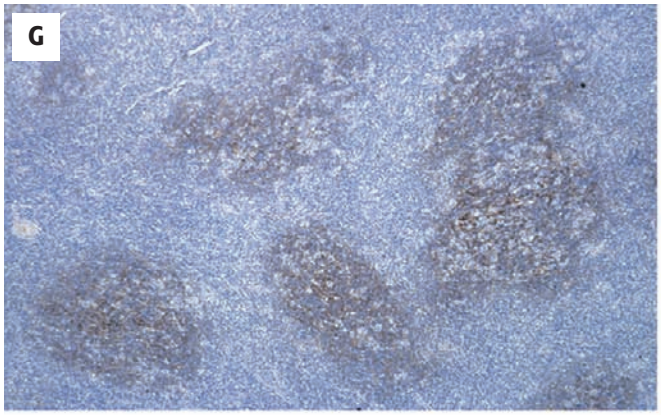
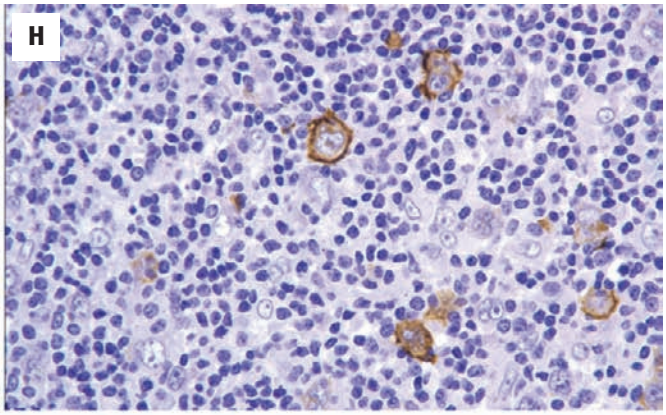



**FIGURE 11-2**

Nodular lymphocyte predominant Hodgkin lymphoma. **A,** At low magnification, the lymph node architecture is largely effaced by apposed macronodules (hematoxylin and eosin). **B,** High magnification of lymphocyte-predominant (LP) variants of RS cells within one of the nodules. **C,** CD20 stain outlines the large nodules and shows their irregular, moth-eaten borders and the small holes produced by the $CD20^-$ staining T cells (low magnification). **D,** CD20 outlines the membrane of the larger LP cells, which are stained more intensely than the surrounding B cells of the nodules (high magnification). **E,** High magnification showing $CD3^+$ rosettes around LP cells. **F,** $CD57^+$ lymphocytes are randomly scattered within the nodules and occasionally form rosettes around LP cells (high magnification).

*Continued*



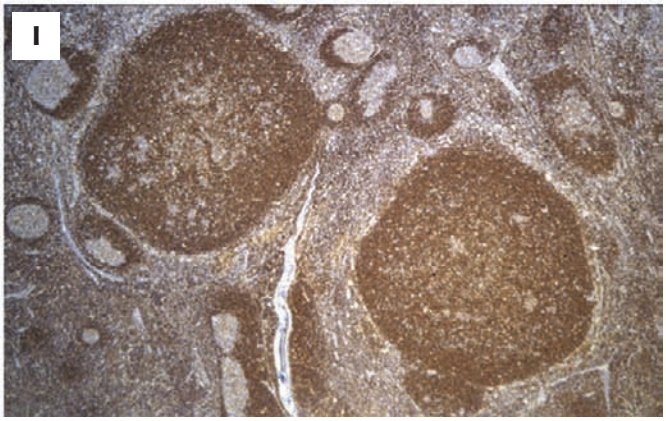




**FIGURE 11-2, cont'd**

**G,** CD21 shows an expanded follicular dendritic meshwork within nodules. **H,** EMA-positive LP cells (low magnification). **I,** Progressive transformation of germinal centers. Low magnification shows two CD79a$^+$ macronodules composed of small lymphocytes adjacent to reactive germinal centers (low magnification). Germinal centers are not seen among the macronodules of nodular lymphocyte predominant Hodgkin lymphoma.

in NL PHL. This is not a feature of NLR HL. Rosetting of T cells around RS cells is also present in all types of CHL but is not easily detectable because of all the background T cells. In addition, CD21 staining is similar in both subtypes. In NLP HL, CD21 outlines prominent, large, expanded spherical meshwork of follicular dendritic cells (FDCs), whereas in NLR CHL, CD21 highlights tightly arranged, concentric FDC meshwork within residual germinal centers (see Figures 11-2 and 11-7). These meshworks extend into the expanded mantle zones in a more loosely arranged fashion in NLR CHL.

### Genetic Features

The availability of laser capture techniques for isolating single RS cells from tissue sections and the application of polymerase chain reaction (PCR) to isolated RS cells in all subtypes of HL have unequivocally demonstrated that RS cells are clonal B cells. The clonal rearrangements are usually detectable only in DNA of isolated single RS cells rather than from whole tissue DNA. The rearranged IG genes of RS cells in both NLP and CHL carry a high load of somatic hypermutations in the variable region of the Ig heavy chain gene, indicating a germinal center origin. In NLP HL, ongoing mutations are found, in contrast to CHL where ongoing mutations are absent. The rearrangements in LP cells are usually productive with IG mRNA transcripts being detectable within them, whereas in RS cells of classic HL, rearrangements are not productive and IG mRNA is not found. It was initially believed that crippling mutations were responsible for the absence of Ig transcripts in classic RS cells. More recent studies of a large number of cases have shown that crippling mutations are absent from 75% of cases of CHL, indicating that such mutations are not responsible for the absence of Ig transcripts in most cases. However, the absence of these transcripts can possibly result from downregulation of synthesis of transcription factors BOB.1, OCT-2, and PU.1. Immunohistochemistry for the expression of the transcription factors BOB.1, OCT-2, and PU.1 showed decreased or lack of expression in H/RS cells of most cases of CHL, whereas these markers were consistently expressed by LP cells in NLP HL. Although cytogenetic abnormalities may be found in NLP HL as well as in CHL, specific abnormalities have not been reported.

The morphologic, immunophenotypic, and genetic features described previously all indicate a relationship between LP cells and centroblasts of germinal centers. Clinical features and the lack of CD15 and CD30

expression as well as the consistent expression of CD20 and the absence of EBV indicate that NLP is a different disorder from CHL. The clinical features and the absence of BCL-2 expression and rearrangement indicate that NLP HL is also not related to follicular center cell non-Hodgkin lymphoma. The probable relationship to progressive transformation of germinal centers (PTGCs; vide infra) suggests that NLP HL is a neoplasm of an abnormal germinal center reaction.

## NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA

NLP HL is defined as a monoclonal B-cell neoplasm characterized histologically by a nodular or, less commonly, a nodular and diffuse proliferation consisting of a predominance of small lymphocytes admixed with varying numbers of histiocytes and scattered large neoplastic cells known as *LP variants of RS*. Whether a purely diffuse form of LP HL exists is currently a matter of controversy, although a diffuse form is not accepted as a definite subtype in the 2008 WHO classification.

### Clinical Findings

The clinical features of NLP HL are well known and are distinct from those of CHL. They differ from CHL in clinical, morphologic, immunophenotypic, and molecular features. NLP HL is the second least common subtype, representing approximately 5% of all cases of HL. There is a male predominance (70% to 75% of cases), and the disorder occurs most frequently in the 30- to 50-year age group, although it is also commonly seen in children and occasionally in older adults. The vast majority of patients develop a single, enlarged peripheral lymph node, most often in the cervical, axillary, or inguinal region (stage I or II), and in contrast to NS HL, mediastinal involvement is extremely uncommon. Nodal spread is not contiguous, in contrast to the contiguous spread of CHL. Furthermore, NLP HL is the only subtype to occasionally involve mesenteric lymph nodes. Lymph nodes in patients with NLP HL enlarge slowly. Because the patients are otherwise asymptomatic, these nodes may reach a considerable size before medical attention is sought; therefore lymph nodes in patients with NLP HL are generally larger than the nodes from patients with CHL.

Although NLP HL is a B-cell lymphoma of follicle center origin with a prolonged clinical course and multiple relapses, similar to the picture seen in low-grade follicular lymphoma, the suggestion that NLP HL be classified as an NHL is unwarranted for a number of reasons. Unlike follicular lymphoma, which most often manifests with widespread disease, NLP HL is usually localized, and the paucity of neoplastic cells among the overwhelming number of benign lymphocytes is inconsistent with NHL. Furthermore, patients with follicular lymphoma do not respond to local radiation therapy, whereas patients with NLP HL do. In addition, BCL-2 is expressed by the neoplastic cells of large follicular lymphoma (FL), whereas LP cells are negative. In addition, in contrast to follicular lymphomas, the t(14;18)(q32;q31) involving the rearrangement of the *BCL2* gene is absent in NLP HL. Similarities between low-grade follicular lymphomas and NLP HL include clonally rearranged immunoglobulin genes with ongoing mutations, as well as transformation to large B-cell lymphoma, which is much more common in follicular lymphoma (approximately 25% versus 2% to 5% of cases in NLP HL).

**NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA—FACT SHEET**

**Definition**
- A monoclonal B-cell neoplasm characterized histologically by a nodular or nodular and diffuse proliferation of small, benign lymphocytes, and varying numbers of histiocytes admixed with scattered LP variants of Reed-Sternberg cells

**Incidence**
- Approximately 5% of all cases of Hodgkin lymphoma

**Gender and Age Distribution**
- Male predominance (70%)
- Most often seen in 30- to 50-year age group
- Age ranges from children to older adults

**Clinical Features**
- Indolent disease
- Localized peripheral lymph node enlargement (stage 1 or 2); cervical, axillary or inguinal nodes most often involved
- B symptoms uncommon
- Multiple relapses, including late relapses after prolonged disease-free intervals
- Advanced stage disease uncommon but more aggressive

**Prognosis and Therapy**
- Low-stage (I/II) associated with excellent prognosis (10-year survival greater than 80%)
- Relapses respond well to therapy
- Rarely fatal (patients die of unrelated disease: cardiac disease or unrelated malignancy)
- Standard treatment of low-stage disease is involved field radiotherapy or regional radiotherapy
- Treatment of stage I: surgical excision with watch-and-wait approach, or local radiation and/or anti-CD20 treatment; advanced stage, chemotherapy with or without radiotherapy
- Progression to large cell lymphomas in 3% to 5% of cases; if localized may be associated with good prognosis

## Pathologic Features

### Microscopic Features

A diagnosis of NLP HL can often be strongly suspected on a low-magnification examination of the lymph node section. The nodal architecture, in most instances, is totally effaced by large nodules or infrequently by nodules and diffuse areas. The nodules are often closely apposed and may be molded against one another, or in other instances there may be considerable space between them (see Figure 11-2). Occasionally, uninvolved parts of the lymph node remain as a rim of compressed tissue at the periphery of the node, usually in a subcapsular location. This uninvolved tissue may show normal or even hyperplastic follicles or, occasionally, progressively transformed germinal centers. Most often, the nodules are distinct and readily identifiable under low magnification, but occasionally they are not well circumscribed and difficult to identify on hematoxylin and eosin (H&E)-stained sections; therefore immunostaining with CD20 may be needed to highlight them. Rarely, diffuse areas may predominate; however, only a single nodule is required to establish a diagnosis of NLP HL.

The nodules are composed predominantly of small lymphocytes with scattered large cells. The large cells may be RS variants, epithelioid histiocytes, or both. The large cells, which have some resemblance to centroblasts of the germinal center, are RS variants called *LP* or *popcorn cells* (see Figures 11-1 and 11-2). Most LP cells are found within the nodules; however, a few may be outside the nodules in the internodular T-cell areas. The presence of numerous LP cells in internodular areas may represent evolution to a diffuse pattern. The LP cells are distinctive and differ from RS cells of classical HL. They also differ from normal germinal center centroblasts in that they have a large nucleus that is folded and multilobate with vesicular chromatin and multiple inconspicuous nucleoli often, as in centroblasts, located adjacent to the nuclear membrane (see Figures 11-1 and 11-2). The nucleoli are usually smaller than those found in classic RS cells, although occasional popcorn cells may have prominent nucleoli. A thin rim of cytoplasm surrounds the nucleus. The number of popcorn cells may be few, and a prolonged search for them may be necessary, or else they may be more plentiful and easily recognizable. When these cells are rare, immunostaining may help in identifying them. When popcorn cells are numerous (a rare occurrence), transition to large cell lymphoma, which occurs rarely in association with NLP HL, must be considered. Some clusters of LP cells may have the appearance of lacunar cells but have the immunophenotype of LP cells. Varying numbers of histiocytes of the epithelioid type are present within the nodules and at times may ring the nodules. Small epithelioid granulomas may also be present, usually outside the nodules. These granulomas can at times be so prominent that the HL is completely missed, and a diagnosis of granulomatous lymphadenitis is made erroneously. FDCs, usually in the vicinity of the popcorn cells, are often present. These cells may occasionally be confused with popcorn cells. FDCs are often binucleate cells, approximately the size of centroblast nuclei with the nucleus flattened against its partner, and they contain a single, small, inconspicuous nucleolus. These cells and the expansile large follicular dendritic meshwork present in the large nodules may be recognized by staining with anti-CD21/CD35, whereas popcorn cells do not react with these antibodies (see Figure 11-2). The dendritic cells, in contrast to popcorn cells, are $CD20^-$. A rare subtype called *syncytial variant of NLP HL* similar to

**NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA—PATHOLOGIC FEATURES**

**Microscopic Findings**

- Architecture of lymph node effaced by macronodules composed predominantly of small lymphocytes or macronodules with diffuse areas
- Scattered LP variants of RS cells (popcorn cells) and varying numbers of histiocytes within or surrounding nodules
- Classic RS cells absent
- Eosinophils and plasma cells absent or rare
- Necrosis and fibrosis absent
- Benign follicles not present between macronodules
- If uninvolved portion of node present, it is confined to periphery, usually in subcapsular region

**Immunohistochemical Findings**

- LP cells: $CD20^+$, $CD79a^+$, $CD45^+$, $EMA^{+/-}$, $BCL\text{-}6^+$, $CD15^-$, $CD30^-$, fascin negative
- Small lymphocytes within nodules: $CD20^+$, $CD79a^+$, $IgD^+$, $IgM^+$
- $CD3^+$ and $PD\text{-}1^+$ lymphocytes rosette around LP cells
- $CD57^+$ lymphocytes randomly scattered within nodules, occasionally ringing LP cells
- Expanded $CD21^+$ spherical follicular dendritic meshwork within nodules
- Absence of EBV infection in LP cells

**Differential Diagnosis**

- Progressive transformation of germinal centers
- Nodular lymphocyte-rich classical HL
- T-cell/histiocyte-rich large B-cell lymphoma
- Follicular lymphoma
- Mantle cell lymphoma with a nodular pattern

**Genetics**

- LP cells are B cells of germinal center cell origin
- LP cells have clonally rearranged immunoglobulin (IG) genes
- Clonal rearrangements are detectable only in DNA isolated from single LP cells
- Variable region of IG heavy chain genes carry a high load of somatic mutations and show ongoing mutations
- Rearrangements are functional, and IG mRNA transcripts are present in LP cells

syncytial NS has recently been described. It is characterized by small and large clusters of LP variants of RS cells embedded within an expanded follicular dendritic meshwork and is associated with internodular fibrosis and a predominant neutrophilic infiltrate in a subset of the nodules.

## Ancillary Studies

### Immunohistochemical Features

As stated earlier (see Reed-Steinberg Cells), LP variants in NLPH have an immunophenotype that is completely different from that in RS cells in CHL (see Table 11-4). Antibodies helpful in differentiating popcorn cells from classical RS cells include those against: CD20, CD3 (or other T-cell antibodies), PD-1, CD45, EMA CD57, BCL-6, CD21/CD35, CD15, CD30, and fascin. Popcorn cells are positive for CD20, CD45, and BCL-6; and for EMA in 25% to 50% of cases (depending on the antibody clone used; see Figure 11-2). They are negative for CD15, fascin, and, with rare exceptions, CD30. The latter three markers are usually expressed by H/RS cells in most cases of CHL.

The small lymphocytes within the nodules in NLP HL also stain with B-cell markers CD20 and CD79a, whereas the internodular areas are composed predominantly of small $CD3^+$ paracortical T cells (see Figure 11-2). It is of note that in longstanding and relapsed cases, the number of T cells within nodules may increase, and occasionally the T cells outnumber the small B cells in some or all of the nodules. In typical cases, however, both the small lymphocytes and the popcorn cells are B cells. CD20 expression on the popcorn cells is often more intense than on the small lymphocytes within the nodule (see Figure 11-2). The cells immediately surrounding the popcorn cells are $CD20^-$, making it easy to identify popcorn cells on low magnification. These $CD20^-$ cells are $CD3^+$ T cells that rosette or ring the LP cells, which again are easily seen on low magnification (see Figure 11-2). The PD-1 marker, which is expressed by germinal center–associated T cells is also an excellent immunomarker that forms rosettes around LP cells. Therefore, on low magnification of a CD20-stained section, the nodules appear to have little holes of negative areas represented by a corona of T cells around LP cells (see Figure 11-2). In addition, small clusters of T cells are found throughout the nodules. It is the presence of these small T cell clusters that is responsible for the irregular borders or the moth-eaten appearance of the nodules in CD20-stained sections (see Figure 11-2). This moth-eaten appearance is helpful in the differential diagnosis of NLP HL from progressive transformation of germinal centers, in which T cells are not found in clusters and the contours of nodules are not irregular but, rather, sharply circumscribed (see Figure 11-2). Varying numbers of CD57-positive lymphocytes are also randomly scattered throughout the nodules in NLP HL and occasionally form rings around popcorn cells (see Figure 11-2). CD21/CD35 demonstrates large, expansile, spherical follicular dendritic meshworks within the nodules (see Figure 11-2). These meshworks are not seen in diffuse areas. Other markers that are not routinely used for diagnosis include BCL-6, antibodies against J chains, and antibodies to B-cell transcription factors OCT-2, BOB.1, and PU.1, all of which are expressed by popcorn cells. RS cells in CHL usually lack J chains and are negative or only weakly positive for either OCT-2 or BOB.1 and rarely for both. They are consistently negative for PU.1. Rarely, a few popcorn cells may be $CD30^+$, although most $CD30^+$ larger cells present in NLP HL are immunoblasts found in internodular areas. Such immunoblasts are smaller than popcorn cells and do not have their complex lobulated nuclei.

Flow cytometry is usually not helpful in the diagnosis of CHL but in NLPHL, a population of dual $CD4^+/CD8^+$ T cells can sometimes be found and can be a clue to the correct diagnosis when present.

## Differential Diagnosis

The differential diagnosis of NLPHL includes benign lymphoid proliferations as well as CHL and non-Hodgkin lymphomas. These entities include PTGCs, NLR CHL, TC/HRLBCL, mantle cell lymphoma with a nodular pattern, and FL. Among these disorders, PTGC and NLR CHL are most problematic.

### Nodular Lymphocyte Predominant Hodgkin Lymphoma versus Progressive Transformation of Germinal Centers

PTGC is a benign disorder of unknown etiology associated with follicular hyperplasia. It occurs predominantly in children and young males, but no age is exempt. Like NLP HL, PTGC tends to recur, especially in the same site. A histogenetic relationship between PTGC and NLP HL has been suggested, and the two conditions may coexist. PTGC may occur before, simultaneously with, or after a diagnosis of NLP HL. Although individuals with PTGC rarely develop NLP HL, a repeated biopsy is warranted to exclude this possibility if lymphadenopathy recurs. Conversely, biopsy must be repeated in patients with NLP HL when lymphadenopathy recurs to determine which of the two disorders is present. The differential diagnosis between PTGC and NLP HL is made largely on low-magnification examination of the lymph node (see Figure 11-2). The presence of one or more, or even many (florid PTGC) large,

expansile nodules in a background of hyperplastic follicles is consistent with the diagnosis of PTGC. In contrast, NLP HL is characterized by the presence of numerous nodules, often back-to-back with no germinal centers among them. As previously mentioned, the only site in which follicles may appear in NLP HL is at the periphery of the node as a compressed peripheral rim, usually in a subcapsular location.

As the term PTGC implies, the transformation is progressive, and the composition of the nodules depends on the stage of transformation. Fully transformed germinal centers are threefold to fourfold the size of reactive follicles and are composed of small polyclonal lymphocytes of mantle cell origin ($IgM^+$, $IgD^+$) with rare remaining centroblasts and some residual follicular dendritic cells. Earlier in the course of transformation, varying numbers of follicle center cells remain, sometimes together with some tingible body macrophages. It appears that the large nodules are formed by a proliferation of mantle cells in an outward and an inward direction, gradually replacing the germinal center and thus resulting in the formation of the large macronodules. LP or popcorn cells are, of course, present only in NLP HL, although remaining centroblasts and FDCs (often binucleate cells) can mimic the RS variants. In contrast to LP cells, FDCs do not have lobulated nuclei and the cell membranes are flattened where they abut one another.

Immunohistochemistry also aids in the differential diagnosis, although the immunophenotypes of the two conditions are similar. In addition to the vast predominance of $CD20^+$, $CD79a^+$ small B cells, in both conditions varying numbers of T cells and $CD57^+$ cells are also present; however, the distribution differs. In PTGC, as in normal germinal centers, the T cells are scattered singly and not in clusters, as is seen in NLP HL, and only rarely do T cells form rosettes around remaining centroblasts, whereas the rosetting of T cells around popcorn cells is a prominent feature of NLP HL. In addition, the prominent PD-1–positive rosettes found in NLP HL are absent in PTGC, although numerous PD-1–positive calls are randomly scattered within the nodules of PTCC. As mentioned previously, CD20 staining in PTGC shows a smooth perimeter of nodules in contrast to the irregular, moth-eaten appearance seen in nodules of NLP HL (see Figure 11-2). Furthermore, most of the small lymphocytes that occupy the nodules in PTGC stain with CD20, whereas in NLP HL there are multiple unstained coronae surrounding large $CD20^+$ LP cells, the sites of T-cell ringing LP cells. Staining with CD57 is less helpful in making a differential diagnosis. This antibody shows a random distribution of cells in both conditions with rosetting around some popcorn cells in NLP HL, whereas the latter is not seen around residual centroblasts in PTGC. Follicular dendritic meshworks are seen in both conditions, attesting to the germinal center origin of both. EMA may also be helpful in the differential diagnosis. It may be expressed on popcorn cells but is not positive on residual centroblasts or follicular dendritic cells.

### Lymphocyte-Rich-Classical Hodgkin Lymphoma versus Nodular Lymphocyte Predominant Hodgkin Lymphoma

There are two histologic forms of LR CHL, the more common nodular type and the diffuse type. The nodular type may mimic NLP HL because both have a nodular growth pattern composed of small B-lymphocytes (see Figure 11-7). In fact, it is the nodular subtype of LR CHL that must be differentiated from NLP HL. In a review of cases of NLP HL performed by the European Task Force on Lymphoma, approximately 30% of cases initially diagnosed as NLP HL were reclassified as NLR CHL after immunohistologic examination. The nodules in both types of HL are composed of expanded mantle cell B lymphocytes ($IgM^+$, $IgD^+$). Like NLP HL, the nodules have irregular contours and usually do not contain eosinophils or plasma cells. In addition, necrosis or fibrosis is not found. In contrast to NLP HL, RS cells are of the classic type, and occasional lacunar-type RS cells or rare RS variants, morphologically indistinguishable from LP cells, may also be present. The diagnosis of NLP CHL is established by the immunophenotype of the RS cells regardless of their cytologic features ($CD15^{+/-}$, $CD30^+$, fascin positive, $CD20^{-/+}$, $CD45^-$). These RS cells are present within the expanded mantle zone of NLR CHL. Another important feature that may be helpful in the differential diagnosis is the presence of residual, most often atrophic, germinal centers that are usually placed eccentrically within some of the nodules. Atrophic germinal centers may occasionally be seen in a minority of nodules in NLP HL, in contrast to the majority of nodules containing germinal centers in NLR CHL. Although epithelioid histiocytes may be present in NLR CHL, they are much more often seen in NLP HL. The mantle cell lymphocytes are $CD20^+$, $IgD^+$, $IgM^+$, and $CD79a^+$ B cells. As in NLP HL, the RS cells are prominently rosetted by T cells. These rosettes are readily seen at low magnification, because, as in NLP HL, they stand out within the T-cell–negative B-cell areas. In contrast, PD-1–positive rosettes are infrequently found around classic RS cells in NLR CHL, whereas they are a prominent feature in NLP HL. CD21 stains show dense concentric rings of the follicular dendritic meshwork of the germinal centers, which extends into the expanded mantle zones in a more loosely woven meshwork, whereas in NLP HL the meshwork is uniformly and loosely woven and considerably larger. EBV latent membrane protein and EBER may be positive in the classic RS cells of NLR CHL; however, they are, with rare exceptions, negative in the popcorn cells of NLP HL. The internodular areas in both conditions are composed of T cells.

### T-Cell/Histiocyte-Rich Large B-Cell Lymphoma versus Nodular Lymphocyte Predominant Hodgkin Lymphoma

The distinction between TC/HRLBCL and NLP HL is extremely important, because TC/HRLBCL is an aggressive lymphoma that requires aggressive therapy; it often manifests with advanced-stage disease, sometimes involving lymph nodes in addition to liver, spleen, and bone marrow. There usually is no problem in separating TC/HRLBCL from NLP HL because of the distinctive nodular pattern in the latter. However, immunohistologic differentiation between TC/HRLBCL and NLP HL containing extensive, diffuse areas, or in smaller or needle core biopsies containing diffuse areas may be extremely difficult, or at times impossible. In the fourth edition of the WHO classification, TC/HRLBCL is defined as a variant of diffuse large B-cell lymphoma in which there are a limited number of scattered, large, atypical B-cells embedded in a background of numerous small non-neoplastic T-cell lymphocytes. Varying numbers of histiocytes (as in LP HL), some in small clusters, are often admixed. Therefore the limited number of large neoplastic cells among a vast predominance of small T cells mimics the histologic features of diffuse NLP HL. On morphologic grounds alone, a definitive differential diagnosis cannot be established in small biopsies composed of diffuse areas. A vaguely nodular pattern is occasionally present in TC/HRLBCL; however, the nodular areas are composed of T cells rather than B cells. The large cells may be centroblasts or immunoblasts, or they may mimic classic or LP variants of RS cells, thus suggesting the possibility of either LP or CHL. In addition, similar to diffuse areas in NLP HL, few small B-lymphocytes are present in TC/HRLBCL. If increased numbers of small B cells are found and a vaguely nodular pattern is seen, the possibility of NLP HL with diffuse areas must be entertained. The diagnosis of DLP HL may occasionally, perhaps erroneously, be made on a small, inadequate biopsy specimen in which a nodular component is not represented. Such a small biopsy specimen showing a vast predominance of small T cells with scattered large B cells will have the same immunophenotype in both DLP HL and TC/HRLBCL ($CD45^+$, $CD20^+$, $CD79a^+$, $EMA^{+/-}$, $CD30^{-/+}$, $CD15^-$; see Table 11-4). However, the T cells in TC/HRLBCL and diffuse areas of NLP HL also differ. In TC/HRLBCL the T cells have a cytotoxic phenotype ($CD8^+$ and $TIA\text{-}1^+$), whereas in NLP HL they are $CD4^+$. Furthermore, $PD\text{-}1^+$ cell rosettes present in NLP HL are absent in TC/HRLBCLL. EMA may be positive in the large cells of both disorders, whereas CD15, CD30, and fascin positivity would be consistent with CHL. Detection of EBV in the large cells would also favor CHL because EBV is found in neither NLP HL nor in TC/HRLBCL (see Table 11-4). Light chain restriction by immunostaining and clonal immunoglobulin heavy-chain gene rearrangement by PCR in whole tissue DNA would favor a diagnosis of TC/HRLBCL. Nodular meshworks of $CD21^+$ FDCs, $CD57^+$ cells, and PD-1–positive cells would favor NLP HL, while their absence would be more consistent with TC/HRLBCL. As alluded to previously, TC/HRLBCL is also a consideration in the differential diagnosis of CHL.

It is recommended in the fourth edition of the WHO classification that cases of NLP HL with a diffuse T-cell–rich pattern should be interpreted as NLP HL, TC/HRCLBCL-like in order to distinguish the latter from cases of primary (de novo) TC/HRLBCL. Furthermore the presence of small B cells and $CD4/CD57^+$ T cells would favor a diagnosis of NLP HL, whereas absence of small B cells and the presence of $CD8^+$ cells and $TIA\text{-}1^+$ cells favor primary TC/HRLBCL. Furthermore, IgD positivity in LP cells argues against TC/HRLBCL. IgD is not expressed by the large cells in NHL, but may be positive in LP cells in a minority of cases of NLP HL.

### Follicular Lymphoma versus Nodular Lymphocyte Predominant Hodgkin Lymphoma

The differential diagnosis of follicular lymphoma versus nodular lymphocyte predominant is usually not difficult. The follicles in FL are ordinarily considerably smaller than the nodules of NLP HL and are composed of at least a subpopulation of centrocytes that are not found within the macronodules of NLP HL. Extension of neoplastic follicles through the capsule into surrounding tissue, although common in FL, is not seen in NLP HL. CD20 staining usually highlights popcorn cells, whereas large cells in FL are usually not more intensely positive than surrounding B cells. CD3 and PD-1 do not show the prominent rosettes around large centroblasts in contrast to the rosettes around LP cells, although varying numbers of T cells are seen within neoplastic follicles of FL. BCL-2 is expressed by the neoplastic cells of FL but is absent in LP cells. The t(14;18) is found in most FL but absent in HL, and heavy-chain gene rearrangement is not found in tissue sections of NLP HL, but it can usually be demonstrated in FL. Finally, most patients with FL exhibit with advanced-stage disease, which is rare in patients with NLP HL.

### Mantle Cell Lymphoma versus Nodular Lymphocyte Predominant Hodgkin Lymphoma

Mantle cell lymphoma (MCL) is usually not confused with NLP HL. It is a B-cell lymphoma composed of small lymphocytes with a distinctive immunophenotype. The neoplastic cells coexpress CD5 and CD43 and are characteristically cyclin D1-positive. The monomorphous population of lymphocytes may have three different growth patterns: vaguely nodular, diffuse, or mantle zone. Cases with a nodular pattern may rarely resemble NLP HL. In most, but not all instances, however, the nodules are not as large as those found in NLP HL. The small lymphocytes in MCL usually, but not always, have

irregular nuclear contours in contrast to the round nuclei of small lymphocytes in NLP HL, and the nodules are better circumscribed than those of lymphocytes in NLP HL. In addition, large cells are absent within nodules except for some centroblasts remaining from germinal centers that have been overrun or colonized by the small neoplastic cells. Pink-staining histiocytes (not epithelioid histiocytes) are often scattered among the nodules, and hyalinized small vessels may be present, whereas both of these characteristics are absent in NLP HL. Immunostaining will easily differentiate the two disorders. NLP HL lymphocytes do not coexpress CD5, CD43, and are cyclin D1 negative. In fact, MCL is the only lymphoma whose cells express cyclin D1. The t(11;14) is seen in 70% to 75% of cases, and this translocation will be detected by fluorescence in situ hybridization in virtually all cases of MCL but not in NLP HL.

### Prognosis and Therapy

As stated earlier, the prognosis of all types of HL depends on the stage of the disease. NLP HL is the type of HL most often found in the low stage (I/II); therefore it is associated with the most favorable prognosis. Rarely, patients have advanced disease (stage III or IV), which is associated with a worse prognosis. The course of the low-stage disease is indolent, despite multiple relapses often following prolonged disease-free intervals. Such relapses usually respond well to treatment and are not associated with an adverse prognosis. In contrast to the patients with CHL, the relapses often occur after a long interval (10 to 13 years); therefore continued surveillance for late relapses is indicated. As stated earlier, it should be noted that the number of relapses may be overestimated when based on clinical and imaging studies alone, because patients with NLP HL frequently develop lymphadenopathy from follicular hyperplasia with PTGC; however, no morphologic evidence of HL is detected on microscopic examination. Another reason for a repeated biopsy of enlarged lymph nodes in NLP HL is that transformation to large cell lymphoma may occur, often many years after the diagnosis of NLP HL.

Currently, therapy consists of involved-field radiotherapy or regional radiotherapy for patients with limited-stage disease and combined modality therapy or chemotherapy for those with advanced-stage disease. A recent study by the French Society of Pediatric Oncology found that no further therapy for children with low-stage disease in complete remission following lymph node biopsy is a valid therapeutic approach and that complementary treatment diminishes relapse frequency but has no effect on survival. A watch-and-wait approach in both adult and pediatric patients has been suggested in this indolent type of HL, because most deaths in patients with NLP HL appear to be treatment related because of coronary artery disease or a second unrelated malignancy. In a 2010 report of 164 patients with NLP HL, the 10-year overall survival for patients who received specific therapy and those followed using the watch-and-wait strategy was 93% and 91%, respectively. In addition, immunotherapy with anti-CD20 in adults has been attempted with some success; therefore it is of utmost importance to make certain that the correct diagnosis of NLP HL is established before initiating immunotherapy.

#### Transformation of NLP HL to Large Cell Lymphoma

Approximately 3% to 5% of patients with NLP HL develop a large B-cell lymphoma, occurring simultaneously with, after, or, rarely, prior to development of NLP HL. The large cell lymphoma can appear in the same lymph node as the HL or in a different node, one unaffected by NLP HL. The presentation of the cells in the large cell lymphoma is variable, resembling the lobulated appearance of the LP cells or, less frequently, having features of centroblasts or immunoblasts or occasionally exhibiting anaplastic morphology. The neoplastic large cells express B-cell markers CD20 and CD79a and show light chain restriction. When large cell lymphoma is found in the same node as the NLP HL, the demarcation between the two is usually sharp, although in some cases it is difficult or impossible to clearly separate the HL in which there are clusters of LP cells from the large cell lymphoma. In fact, sometimes the differentiation between NLP HL with clusters of LP cells and the large cell lymphoma is not clear. As a general rule, large cell lymphoma should not be diagnosed unless areas of the node are effaced by large cell lymphoma. Although a clonal relationship between the neoplastic cells in both lymphomas has been demonstrated in most cases, it has not been reported in every instance. In the latter cases, it is presumed that the large cell lymphoma represents a de novo malignancy.

## Nodular Sclerosis Hodgkin Lymphoma

Nodular sclerosis Hodgkin lymphoma is a subtype of classical HL characterized by collagen bands that subdivide the lymph node into cellular nodules of inflammatory cells admixed with lacunar cell variants of RS cells.

### Clinical Features

NS HL is the most common subtype of HL in developed countries, accounting for approximately 60% to 80% of CHL cases. It is particularly common in

individuals of high socioeconomic status and is often found in adolescents and young adults. The median age at diagnosis is 28 years. NS HL is the only subtype of HL in which there is no male predominance, with the male to female ratio being about equal. Presentation with a mediastinal mass is common (approximately 80% of patients), and greater than 50% of patients with a mediastinal mass have bulky disease (an adverse prognostic factor). A typical clinical history is that of a young woman with an enlarged supraclavicular lymph node, with a chest radiograph showing a mediastinal mass. The majority of patients have stage II disease, and B symptoms are present is approximately 40% of cases. Lung involvement is found in approximately 10% of patients, usually by contiguous spread from mediastinal disease, or, less often, there may be miliary lung involvement secondary to vascular spread. The spleen is involved in approximately 10% of cases, bone marrow disease is found in 3% of cases and, the liver is affected in 2%.

**NODULAR SCLEROSIS CLASSICAL HODGKIN LYMPHOMA—FACT SHEET**

**Definition**

- A subtype of classical HL characterized by collagen bands that subdivide the lymph node into nodules of inflammatory cells admixed with lacunar cell variants of RS cells

**Incidence and Location**

- Approximately 60% to 80% of classical HL in the United States and Western Europe
- Mediastinal mass in approximately 80% of patients
- Supraclavicular or cervical lymph node involvement

**Gender and Age Distribution**

- Equal male-to-female ratio (only type of HL without male predominance)
- Median age is 28 years; may be seen in children and older adults

**Clinical Features**

- Majority of patients with stage II disease
- Approximately 40% of patients with B symptoms
- Mediastinal involvement with or without symptoms
- Bulky disease in approximately 50% of patients with mediastinal involvement
- Lung involvement in 10% of patients
- Spread to spleen in 10% of cases
- Spread to bone marrow in 3% of patients

**Prognosis and Therapy**

- Prognosis is related to stage of disease at clinical presentation.
- Bulky mediastinal disease is an adverse prognostic factor irrespective of stage
- Therapy (i.e., chemotherapy, radiation) depends on stage of disease and presence or absence of bulky mediastinal disease (an unfavorable prognostic sign)

## Pathologic Features

### Microscopic Features

The characteristic histologic features of NS HL include sclerosis, nodules, and lacunar cell variants of RS cells (Figure 11-3). The lymph node may show focal, partial, or total involvement. Sclerosis consists of sparsely cellular fibrocollagenous bands emanating from a thickened fibrous capsule dividing the lymph node into cellular nodules that are often visible at gross examination of the biopsy. The fibrous bands show green birefringence in polarized light, but polarization is not essential for diagnosis. The diagnostic classic RS cells may be present but are rare. To make a diagnosis of NS HL, at least one fibrocollagenous band forming a partial nodule must be seen. The degree of fibrosis varies from case to case or differs even in different lymph nodes in a single case. Fibrosis can range from thin and delicate lacing to thick fibrous bands, and in some cases there is almost complete obliterative sclerosis with only a small amount of cellular tissue remaining (see Figure 11-3). A variable inflammatory component, ranging from that seen in LP to that in LD, is present within the nodules, which contain variable numbers of lacunar cells (see Figure 11-3). Lacunar cells may occur singly, in small aggregates, or in sheets. As stated earlier under Reed-Sternberg cells, the appearance of lacunar cells depends on the type of fixative used (prominent lacunae appearing with formalin fixation and absent or less obvious lacunae with B-5 fixed tissue). The syncytial variant of NS causes the greatest problem in differential diagnosis (Figures 11-4 and 11-5). This variant is characterized by cohesive sheets or aggregates of lacunar cells that may be monomorphic, giving the appearance of large cell lymphoma, or they may be pleomorphic, mimicking cases of anaplastic large cell lymphoma, or else they may resemble metastatic neoplasms. The aggregates of lacunar cells often have admixed histiocytes and may contain areas of necrosis, which may be coagulative or suppurative, sometimes having a granulomatous appearance (see Figure 11-5). Such necrotic lesions are occasionally misinterpreted as representing infectious granulomas. A grading scheme for NS HL according to the proportion of H/RS cells or the characteristics of the background infiltrate has been devised in the REAL and WHO classifications, although at the present time, this scheme is not strictly required for routine clinical purposes. The two histologic grades are 1 and 2. The majority of cases (75% to 85%) are grade 1, and the remainder of cases are classified as grade 2. In grade 1, 75% or more of the nodules contain scattered lacunar cells in a background of a predominance of lymphocytes, or else the nodules contain a mixture of inflammatory cells (lymphocytes, eosinophils, histiocytes, plasma cells, and neutrophils)

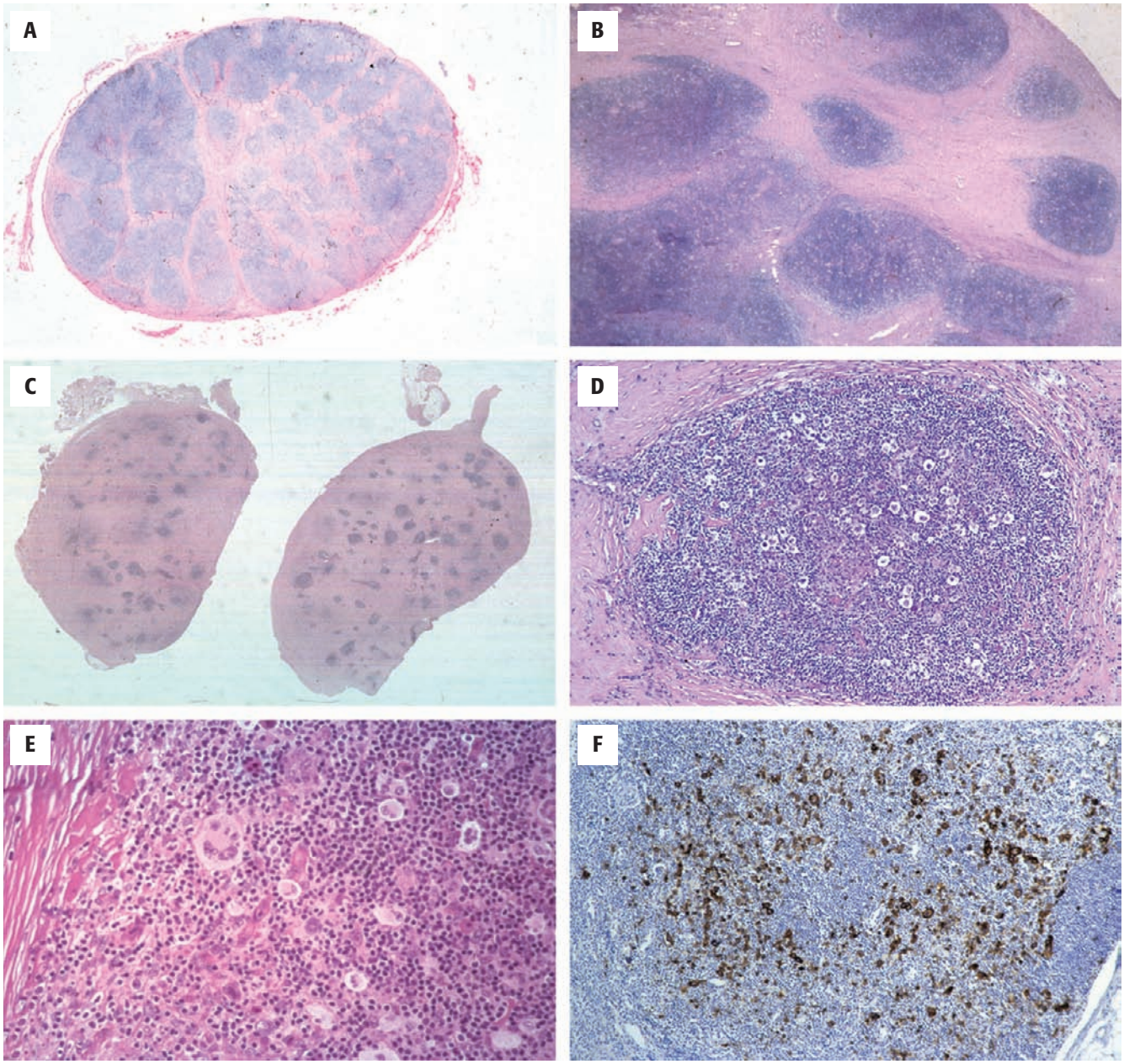



**FIGURE 11-3**

Nodular sclerosis classical Hodgkin lymphoma. Varying degrees of sclerosis. **A,** The capsule is slightly thickened, and the node is divided into cellular nodules by thin fibrotic bands (low magnification). **B,** Fibrotic bands are thicker. **C,** Obliterative sclerosis with little cellular tissue containing lacunar and inflammatory cells. **D,** A nodule formed by a fibrous band contains lacunar cells interspersed among inflammatory cells. **E,** High magnification showing edge of sclerotic band and scattered lacunar cells characteristic of grade 1 nodular sclerosis. **F,** Cellular phase of nodular sclerosis Hodgkin lymphoma. A vague nodular pattern containing $CD30^+$ lacunar cells but no sclerotic bands. Images in **A, B, C, D,** and **F** are low magnification.

in varying proportions. Cases that are classified as grade 2 contain increased numbers or sheets of lacunar cells, filling at least one 40× high power field (hpf) in at least 25% of nodules. Grade 2 appears to be similar or identical to the syncytial variant of NS and to cases in which there are sheets of lacunar cells and few lymphocytes present. The latter cases have, in the past, been referred to as the *lymphocyte depletion type of NS HL*. Although, it was initially believed that grade 2 NS was associated with a worse prognosis than grade 1, this finding is controversial and has not been substantiated in more recent studies.

Occasional cases have characteristic lacunar cells, sometimes arranged in a vague nodular pattern but with no fibrous bands. Such cases have in the past been referred to as the *cellular phase of NS HL* (see Figure 11-3). These cases are most likely true NS HL because when pathologic staging was performed, patients

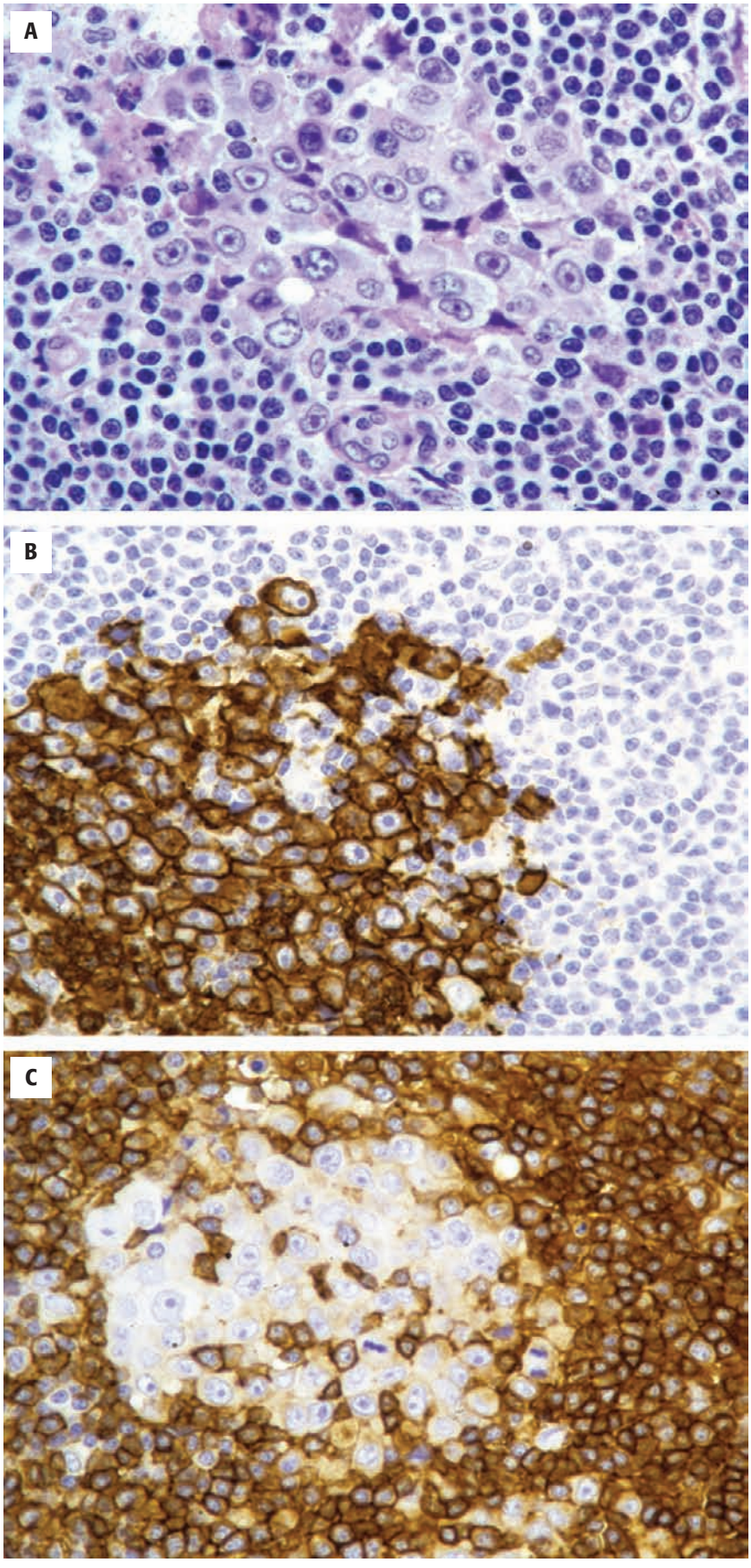



**FIGURE 11-4**

Syncytial variant of classical nodular sclerosis Hodgkin lymphoma (grade 2). **A,** Mediastinal biopsy specimen showing a cluster of large cells (lacunar cells). **B,** The cluster of cells shows intense membrane staining with CD15. **C,** The lacunar cells are negative with CD45, whereas the small background lymphocytes are $CD45^{+}$. All images are high magnification.

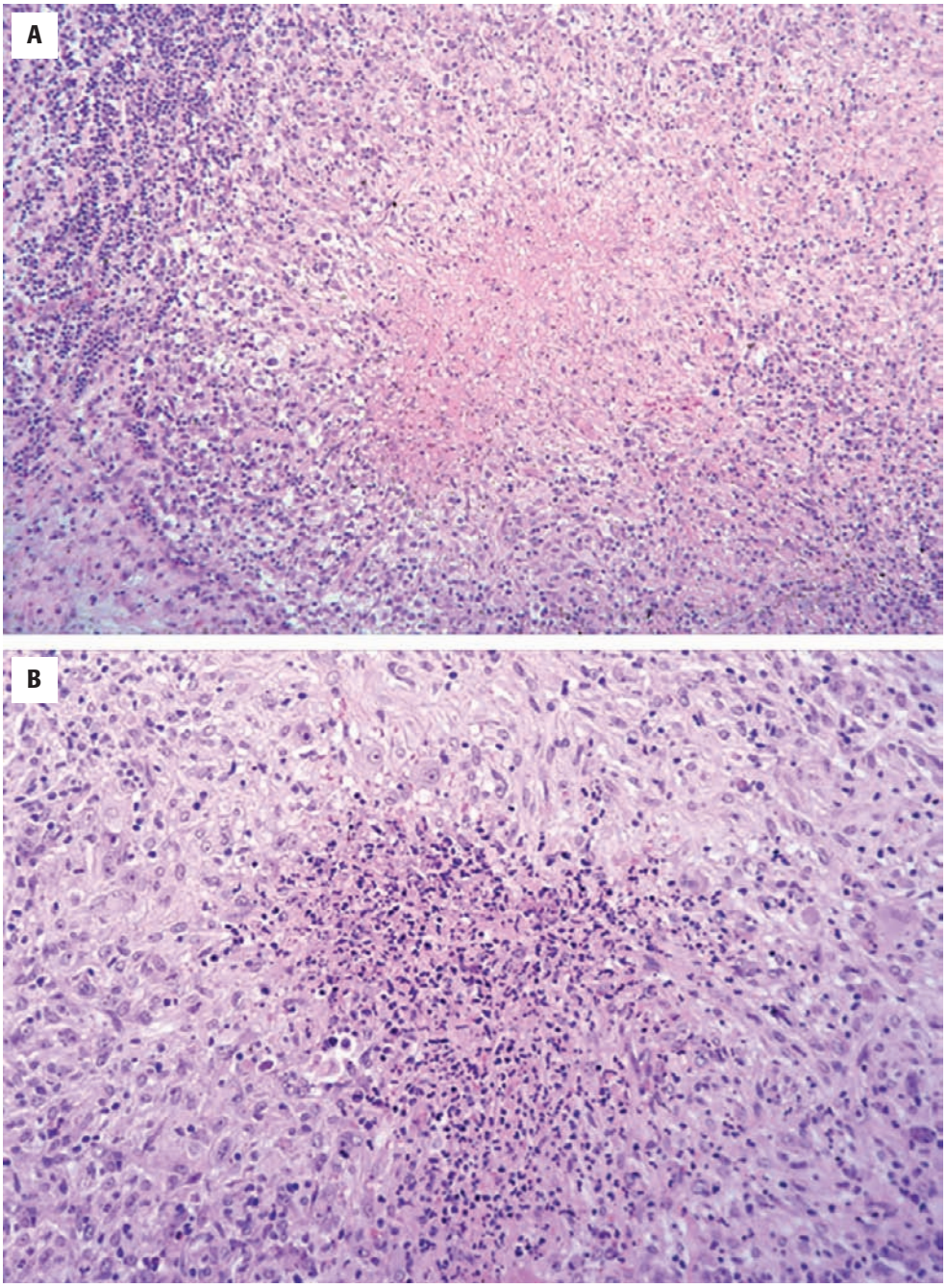



**FIGURE 11-5**

Syncytial variant of classical nodular sclerosis Hodgkin lymphoma with necrosis. **A,** Coagulative necrosis surrounded by lacunar cells resembling a granuloma (low magnification). **B,** Suppurative necrosis surrounded predominantly by epithelioid histiocytes resembling cat-scratch disease. Rare lacunar cells with prominent nucleoli are present (low magnification). The latter cells were $CD15^+$ and $CD30^+$ (not shown). Cells with prominent nucleoli are not found in cat-scratch disease.

with typical NS HL in cervical lymph nodes were sometimes found to have the cellular phase in abdominal nodes.

Rare cases of NS HL involve only interfollicular areas of lymph nodes with prominent follicular hyperplasia that can mask the focal involvement by HL. Such cases usually cannot be further subclassified and are called *interfollicular HL*. On subsequent biopsy specimens, such cases are found to be either NS or MC HL.

## Ancillary Studies

### Immunophenotype

The immunophenotype of the RS cells is identical to that of other types of CHL. EBV demonstrated by EBER or EBV-encoded LMP1 is associated with a subpopulation (10% to 40%) of NS HL. The background lymphocytes are T cells, usually CD4 predominant.

**NODULAR SCLEROSIS CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES**

**Gross Findings**

- Lymph node possibly showing varying numbers of nodules surrounded by sclerotic bands
- Thickened fibrous capsule

**Microscopic Findings**

- Lymph node may show focal, partial, or total involvement
- Nodules are composed of an inflammatory cell infiltrate with scattered lacunar cell variants of Reed-Sternberg cells (grade 1) in most cases (75% to 85%)
- Collagen bands (sclerosis) surround nodules
- Collagen bands show green birefringence under polarized light
- Syncytial variant of nodular sclerosis HL is characterized by cohesive sheets or aggregates of lacunar cell in background of inflammatory cell infiltrate
- Grade 2 shows sheets of lacunar cells and filling of at least one 40× hpf in at least 25% of nodules (similar to syncytial variant)
- Grading is not required for routine clinical purposes

**Immunohistochemical Features**

- H/RS cells have immunophenotype identical to that in other types of classical HL ($CD15^{+/-}$, $CD30^{+}$, fascin positive, $CD45^{-}$)
- EBV encoded LMP1 or EBER are expressed in H/RS cells in 10% to 40% of cases
- Background lymphocytes are T cells

**Pathologic Differential Diagnosis of Syncytial Variant of Nodular Sclerosis**

- Anaplastic large cell lymphoma
- Primary mediastinal large B-cell lymphoma
- Diffuse large B-cell lymphoma
- Mediastinal seminoma
- Metastatic melanoma or carcinoma
- Necrotizing granulomas (especially cat-scratch disease)

**Genetics**

- H/RS cells are B cells of germinal center cell origin
- H/RS cells contain clonal *IG* gene rearrangement
- Clonal rearrangement is detected only in DNA of isolated H/RS cells
- Rearranged *IGH* genes have a high load of somatic hypermutations in the variable region of the *IG* heavy chain genes without signs of ongoing mutations
- Rearrangements are not functional, and Ig mRNA transcripts are not present in H/RS cells

## Differential Diagnosis

The differential diagnosis of syncytial NS HL includes non-Hodgkin lymphomas, non-hematolymphoid neoplasms, and benign proliferations. The lymphomas include ALCL, primary mediastinal (thymic) large B-cell lymphoma (PMLBCL), and diffuse large B-cell lymphoma. The non-hematolymphoid neoplasms are mediastinal seminoma, metastatic carcinoma, or melanoma. The benign entities include necrotizing granulomas, especially cat-scratch disease. Some of the differential diagnostic considerations are especially ambiguous when areas characteristic of grade I NS HL are absent, which is often encountered in small biopsies and needle core biopsies.

### Anaplastic Large Cell Lymphoma

In the fourth edition of the WHO classification, ALCL ($ALK^{+}$ or $ALK^{-}$) is defined as a T-cell lymphoma strongly expressing CD30 and consisting of large lymphoid cells with ample cytoplasm and pleomorphic nuclei. Most cases in children and young adults express the ALK protein because of the translocation of ALK gene (ALCL, $ALK^{+}$). $ALK^{-}$ ALCL is found more often in adults (ALCL, $ALK^{-}$) and is associated with a worse prognosis than $ALK^{+}$ ALCL. Approximately 3% of adult and 10% to 20% of childhood non-Hodgkin lymphomas are ALCL.

In the vast majority of cases, ALCL is readily distinguished from HL, especially when a sinusoidal pattern of lymph node involvement is present. However, the distinction between ALCL and syncytial NS HL is not always sharp. In fact, a provisional entity in the REAL classification was named *ALCL-Hodgkin-like*. This entity was, on further investigation, found to be more closely related to HL and was not accepted as a distinct entity in both the third and fourth editions of the WHO classifications. It is well known that some cells in the syncytial variant of NS HL may closely resemble the hallmark cells of ALCL. The distinction between these two lymphomas is of utmost importance, because ALCL is treated like an aggressive lymphoma but can be cured in approximately 80% of cases with third-generation therapeutic regimes, whereas HL requires a different type of treatment. ALCL can share cytologic and architectural features with syncytial NS HL, including fibrotic bands and nodule formation (Hodgkin-like pattern). Although the inflammatory cell component is usually smaller than that in NS HL, eosinophils, small lymphocytes, histiocyte, plasma cells, and neutrophils may at times accompany the large cells in ALCL. Immunophenotyping is helpful in the differential diagnosis (see Table 11-4). Antibodies that are helpful in differentiating NS HL from ALCL include: B- and T-cell markers, CD15, PAX-5, CD25, ALK, EMA, MUM1, and EBER. CD2, CD4, CD5, and CD43 are the T-cell markers most often expressed by ALCL cells, although a minority of cases may not express any T-cell markers (null cell ALCL), and it is these cases that can cause the greatest confusion. If the large cells express CD20, CD79a, and CD30 and have morphologic features of ALCL, then a diagnosis of diffuse large B-cell lymphoma with anaplastic features should be made. In such cases, the large cells express PAX-5, which is almost always negative in ALCL. Clusterin, which is positive in almost all ALCL and, with rare exceptions, negative in RS cells, would favor ALCL, whereas positivity for EBV LMP1, and/or EBER, which is not associated with ALCL, would be consistent with

CHL. T-cell receptor gene rearrangement analysis may be helpful even in cases in which the neoplastic cells fail to express T-cell markers, because such cases often show clonal T-cell receptor gene rearrangement, thus being consistent with a diagnosis of ALCL. Cases in which a combination of morphology, immunophenotyping, or molecular analysis is equivocal are rare.

### Primary Mediastinal Large B-Cell Lymphoma and Other Larger B-Cell Lymphomas

Another potentially difficult differential diagnostic dilemma relates to PMLBCL, which is recognized as a distinct entity in the WHO classifications and accounts for approximately 2% to 4% of non-Hodgkin lymphomas. In contrast to nonmediastinal B-cell lymphomas, the male-to-female ratio is 1:2, and this disorder is found in a younger age group more than other large cell lymphomas (37 versus 55 years). Patients with PMLBCL, unlike patients with NS HL, often have superior vena cava syndrome or dyspnea and cough. Both lymphomas share a number of clinical and morphologic features. Both have a female predominance and both manifest with a mediastinal mass that is often bulky, and extension to supraclavicular lymph nodes may be found in both. PMLBCL and syncytial NS HL both consist of large pleomorphic cells, and both usually have background sclerosis (finely banded compartmentalizing in PMLBCL and bands of collagen with nodule formation in NS HL). Many cells in PMLBCL may be indistinguishable from H/RS cells as well from lacunar cells. Immunophenotypic similarities include lack of surface immunoglobulin and CD30 positivity. CD15 is occasionally expressed weakly in some large cells in PMLBCL. Cases in which it is difficult or impossible to distinguish between the two lymphomas have been encountered. Such cases with transitional or overlapping features have been referred to as *gray zone lymphoma*. In the 2008 edition of the WHO classification, gray zone lymphoma has been replaced by the long and rather cumbersome name of *B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma* (BCLU). In contrast to both PMLBCL and NS HL, BCLU is most often seen in young males aged 20 to 40 years. Most but not all such cases exhibit mediastinal disease. BCLU may have the appearance of diffuse large B-cell lymphoma but with admixed H/RS or lacunar type cells, whereas other cases resemble syncytial NS HL but have only a sparse inflammatory background. The immunophenotype of BCLU may also overlap between the two entities. CD45 is usually expressed by the large cells in both BCLU and PMLBCL, but not by H/RS cells in CHL. Cases morphologically more consistent with NS HL have large cells strongly expressing CD20 and CD79a, whereas cases with features of PMLBCL often only weakly express or are negative for CD20 but are weakly positive for CD15 and CD30, the reverse of what would be expected. CD23 expression, characteristic of thymic medullary B cells and PMLBCL may be positive in NS HL. Expression of B-cell transcription factors PAX-5, BOB.1, and OCT-2 found in PMLBCL is also positive in BCLU, in contrast to NS HL where only PAX-5 is consistently expressed. EBV expressed by H/RS cells in some cases of NS HL is not seen in BCLU or PMLBCL. The gene expression profile of PMLBCL bears striking similarities to the expression profile of NS HL. It has been suggested that PMLBCL and NS HL arise from a common precursor cell or that the two entities represent opposite ends of a biologic continuum with the intermediate form manifesting as mediastinal BCLU.

Cases of BLCU usually have a more aggressive clinical course than either CHL or PMLBCL. A consensus regarding therapy for BCLU has not been determined, although aggressive treatment (chemotherapy plus radiation therapy) appears to be effective.

Composite lymphomas consisting of both NS HL together with PMLBCL may also be found in the mediastinum. In addition, cases of NS HL may be followed by PMLBCL, or the reverse may occur. In such cases, each component has its own characteristic immunophenotype, and the composite and sequential lymphomas are not considered to be examples of BCLU.

### Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphomas can be difficult to differentiate from NS HL, especially on a small or needle core biopsy (see Figure 11-4). If the large cell lymphoma cells express CD45, are strongly positive with CD20 and CD79a, and are negative with CD15, a diagnosis of large B-cell lymphoma can be made; whereas if the large cells are $CD15^+$, $CD30^+$, and $CD45^-$, a diagnosis of HL is almost certain (see Table 11-4). Even if CD45 staining is negative, as it may be on large cell lymphomas, the strong expression of the B-cell antigens is still consistent with LCL.

### Metastatic Carcinomas, Melanoma, Primary Mediastinal Seminoma

Undifferentiated nasopharyngeal carcinoma is among the metastatic carcinomas that may closely resemble syncytial NS HL. This carcinoma occurs in young patients, especially males, and often metastasizes to laterocervical lymph nodes. The metastasis is often recognized before the primary carcinoma, because the latter is usually small and occult. The neoplastic cells may be in small clusters or individually dispersed and may closely resemble H/RS/lacunar–type cells. Sclerosis and a vaguely nodular pattern may accompany the nodal deposits. Eosinophils are commonly found among tumor cells, and plasma cells are also often present. This tumor is readily differentiated from HL by staining for

cytokeratin. The malignant cells are also positive for EMA and LMP1 and EBER by in situ hybridization, but they are negative for CD15, CD30, and fascin.

Metastatic melanoma can be differentiated from syncytial NS HL immunohistochemically. Melanoma cells are positive for S-100 and melan A, and they are negative for CD15 and CD30.

Primary mediastinal seminoma can also superficially resemble syncytial NS HL; however, immunophenotyping will distinguish these two neoplasms. Periodic acid-Schiff and placental alkaline phosphatase will stain seminoma cells, whereas seminoma cells are negative with CD15, CD30, and CD20.

#### Granulomatous Lymphadenitis

Rare cases of syncytial NS HL in which there are central areas of suppurative necrosis within the sheets of lacunar cells may be misinterpreted as cat-scratch disease. Although lacunar cells are usually abundant and easily recognizable, there are occasional cases in which the cells around the necrosis are predominantly epithelioid histiocytes (see Figure 11-5). However, careful examination will usually show an occasional cell with a prominent nucleolus not seen in histiocytes. This observation should raise the suspicion that HL may be present. Staining with CD15, CD30, CD45, and CD68 will confirm the histologic impression of HL. A similar differential diagnostic consideration holds true for cases of granulomas with coagulative necrosis.

### Prognosis and Therapy

As in other types of CHL, the prognosis depends on the stage of the disease at presentation. The prognosis is generally better than that of the MC and LD types and is related to the low stage rather than to the histologic type. Further refinement in prognosis and treatment selection is the subject of ongoing studies, particularly using functional imaging modalities (e.g., fludeoxyglucose [$^{18}$F] positron emission tomography [FDG-PET]) to guide risk-adapted therapy. The antibody-drug conjugate brentuximab vedotin, consisting of an anti-CD30 antibody with monomethyl auristatin E (an antimicrotubule agent), has recently been approved for use in the relapsed/refractory setting for Hodgkin lymphoma and systemic anaplastic large cell lymphoma.

## MIXED CELLULARITY HODGKIN LYMPHOMA

A subtype of classical HL with scattered classical H/RS cells in a diffuse or rarely vaguely nodular growth pattern of a mixed inflammatory cell background without nodular sclerosing fibrosis. Cases that do not fit into the other subtypes are placed into this category.

### Clinical Features

Approximately 15% to 25% of all cases of HL are of the MC type. There is a male predominance (approximately 70%) with a median age at presentation of 38 years. It is uncommon in young adults, but is seen more frequently after 50 years of age. A bimodal age distribution is not present. Both MC and LD subtypes are found most often in nonindustrialized nations and are also the subtypes found most often in HIV-positive patients. Presentation with high-stage disease (stage III/IV) and B symptoms is common. Unlike NS HL, MC rarely involves the mediastinum. The spleen is involved in approximately 30% of cases, the bone marrow in 10%, and the liver in 3%. In HIV-positive patients, the bone marrow is positive in 15% to 30% of patients at the time of diagnosis. In the REAL classification and the third edition of the WHO schemes, cases that did not conform to any

**MIXED CELLULARITY CLASSICAL HODGKIN LYMPHOMA—FACT SHEET**

**Definition**
- A subtype of classical HL with scattered H/RS cells in a diffuse or rarely vaguely nodular growth pattern of a mixed inflammatory cell background without nodular sclerosing fibrosis
- Cases that do not fit into other subtypes

**Incidence and Location**
- Approximately 15% to 25% of all cases of CHL
- More often found in nonindustrialized nations
- Bimodal age distribution absent
- Peripheral lymph nodes involved
- Mediastinal involvement uncommon

**Gender and Age Distribution**
- Male predominance (approximately 70%)
- Median age at presentation, 38 years; uncommon in children and young adults

**Clinical Features**
- Presentation often with high-stage disease (stage III/IV)
- B symptoms common
- Splenic involvement and disease below the diaphragm more common than in nodular sclerosis

**Prognosis and Therapy**
- Prognosis similar to other types of CHL at same stage of disease
- Chemotherapy or radiation therapy, or both, depending on stage of disease

**MIXED CELLULARITY CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES**

**Microscopic Findings**

- Lymph node architecture effaced
- Mixed inflammatory cell infiltrate consisting of varying proportions of lymphocytes, eosinophils, plasma cells, epithelioid histiocytes, neutrophils, and fibroblasts
- Classic RS and Hodgkin cells readily found; occasional lacunar cells
- Interstitial fibrosis possibly present, but no band-forming sclerosis
- May focally involve interfollicular areas of lymph nodes with follicular hyperplasia (interfollicular HL)

**Immunohistochemical Features**

- H/RS cells have immunophenotype identical to that in other types of classical HL ($CD15^{+/-}$, $CD30^{+}$, fascin positive, $CD45^{-}$)
- Background lymphocytes are T cells
- EBV encoded LMP1 or EBER expressed in H/RS cells more often (75%) than other types of CHL

**Differential Diagnosis**

- Peripheral T-cell lymphoma
- T-cell/histiocyte-rich large B-cell lymphoma
- Infectious mononucleosis
- Hypersensitivity reactions (diphenylhydantoin)

**Genetics of Reed-Sternberg Cells**

- H/RS cells are B cells of germinal center cell origin
- H/RS cells contain clonal IG gene rearrangement
- Clonal rearrangement detected only in DNA of isolated H/RS cells
- Rearranged *IGH* genes have a high load of somatic hypermutations in the variable region of the *IG* heavy chain genes without signs of ongoing mutations
- Rearrangements are not functional, and Ig mRNA transcripts are not present in H/RS cells

subtype were called *unclassifiable,* whereas in the fourth edition, cases that did not fit into any other subtype were included in the MC category. The prognosis of MC HL is similar to that of other subtypes of CHL of similar stage, whereas before the use of current therapeutic regimens, MC had a prognosis worse than that of NS and a better one than that of LD.

## Pathologic Features

### Microscopic Features

As the name *MC* implies, there is a mixed inflammatory cell infiltrate composed of lymphocytes, eosinophils, granulocytes, plasma cells, epithelioid histiocytes, and fibroblasts in varying proportions admixed with classical H/RS cells, which can be found readily (Figure 11-6). In some cases there may be many epithelioid histiocytes that may form small granuloma-like clusters. The classical type of owl eye RS cells, Hodgkin cells, and occasional lacunar cells may be present. Band-forming sclerosis and a thickened capsule characteristic of NS HL are absent, although interstitial fibrosis may be present. The lymph node architecture is usually effaced, but both MC and NS HL may involve interfollicular areas of lymph nodes showing prominent follicular hyperplasia that can mask the HL. Such cases are called *interfollicular HL*. To avoid missing such cases, interfollicular areas of lymph nodes with follicular hyperplasia should be scrutinized for evidence of HL. Focal collections of histiocytes may be a clue to the presence of

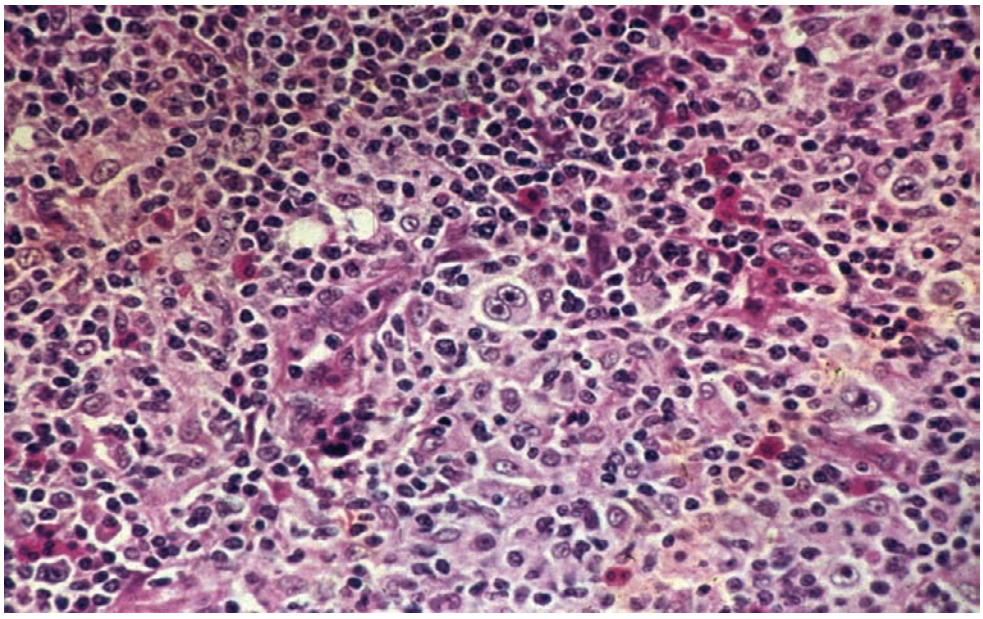


**FIGURE 11-6**

Mixed-cellularity classical Hodgkin lymphoma. At low magnification, classic Reed-Sternberg and Hodgkin cells are evident within a mixed inflammatory infiltrate including lymphocytes, histiocytes, eosinophils, and plasma cells.

interfollicular HL, and a search for classical RS cells should be undertaken. Immunostaining must always be performed in such cases to differentiate binucleate immunoblasts, which may be seen in viral infections such as infectious mononucleosis, that may resemble classic RS cells.

## Ancillary Studies

### Immunophenotype

The immunophenotype and molecular characteristics of H/RS cells are like those of other cases of CHL ($CD15^{+/-}$, $CD30^{+}$, $CD45^{-}$), and the background lymphocytes are T cells. EBV-encoded LMP1 and EBER are expressed much more frequently (in approximately 75% of cases) than in other types of CHL.

## Differential Diagnosis

The differential diagnosis of MC HL includes peripheral T-cell lymphoma (PTCL), not otherwise specified, TC/HRLBCL, and benign proliferations, including hypersensitivity reactions, especially to antiepileptic medications, the prime example being diphenylhydantoin. In addition, viral disorders such as infectious mononucleosis can mimic MC HL.

### Peripheral T-Cell Lymphoma

Immunophenotyping must always be performed before a diagnosis of MC is rendered, and in some instances it must be followed by molecular genetic studies to determine whether a clonal PTCL is imitating MC HL. MC HL and PTCL can be morphologically indistinguishable. The mixed inflammatory cell infiltrate may be identical in both, and classic H/RS imitators may be present in PTCL. Morphologic features that may be helpful in the differential diagnosis and those that favor PTCL include irregular nuclear contours of small lymphocytes and a spectrum of lymphocyte sizes ranging from small to medium to large. In addition, the presence of cells with clear cytoplasm is a feature of PTCL, especially angioimmunoblastic T-cell lymphoma. Another PTCL, one that has numerous histiocytes, known as *lymphoepithelioid cell lymphoma* or *Lennert's lymphoma* (a variant of PTCL, not otherwise specified), may contain RS-like cells and therefore imitate MC HL with its many histiocytes. A diagnosis of PTCL can be established with reasonable certainty when the majority of large cells, including RS-like cells, stain with T-cell markers (extremely rare in HL) and the cells are also $CD15^{-}$ and $CD30^{-}$. However, classic RS cells in CHL may be $CD15^{-}$, and RS-like cells in PTCL may rarely be CD15 positive. Because many small T cells surround H/RS-like cells, it might not be possible to determine whether these H/RS cells express T-cell markers. In such cases, features favoring a diagnosis of PTCL include: immunostaining with Ki-67, which shows a higher proliferative rate in PTCL than in HL, and loss of or significant decrease in the expression of one of the pan T-cell markers, by coexpression of both CD4 and CD8, or by loss of expression of both these markers on the neoplastic cells. This loss or decrease in expression of a T-cell marker is best quantitated with flow cytometry, but it can also be observed with immunostaining. Such aberrant expression of T-cell markers does not, however, indicate clonality, and it is not seen in all peripheral T-cell lymphomas (approximately 75% of cases). Currently, clonality in peripheral T-cell lymphomas can be detected only by gene rearrangement studies. Clonal rearrangement is, however, not found in all cases of peripheral T-cell lymphomas, and rarely a definitive diagnosis is not possible. The clinician must be made aware of this diagnostic dilemma and use his or her clinical judgment in choosing therapy. Peripheral T-cell lymphomas are one of the most aggressive non-Hodgkin lymphomas and must be differentiated from MC HL, because prognosis and therapy of these two disorders are completely different.

### T-Cell/Histiocyte-Rich Large B-Cell Lymphoma

TC/HRLBCL is discussed under the differential diagnosis of NLP HL. In some cases of TC/HRLBCL, a number of the large cells are binucleate with prominent inclusion-like nucleoli and are indistinguishable from classic RS cells. The cells are differentiated from true RS cells by their intense staining with CD20, CD79a, and usually CD45. The cells are always $CD15^{-}$ but may express CD30 (see Table 11-4). Eosinophils, often but not always present in MC HL, are usually absent in TC/HRLBCL. In addition, EBV may be present in H/RS cells in MC HL, but it is not found in the large B-cells of TC/HRLBCL.

### Benign Proliferations

Drug-induced hypersensitivity reactions, especially to diphenylhydantoin, often subtotally efface the nodal architecture and may provoke a mixed inflammatory infiltrate identical to that in MC HL, including eosinophils and immunoblasts, with some having the appearance of classical H/RS cells. A vascular proliferation (high endothelial venules) is often present in drug-induced hypersensitivity reactions and is usually not pronounced in MC HL. The H/RS-like cells are $CD15^{-}$, although they often express the activation antigen CD30. They may be positive or negative for CD45, and these

H/RS-like cells are often mixtures of B and T lymphocytes. A clinical history, which is often not immediately provided to the pathologist, is of the utmost importance in confirming a diagnosis. Lymphadenopathy generally recedes after cessation of the antiepileptic medication.

It has been established that patients with rheumatoid arthritis or other autoimmune disorders receiving long-term, low-dose methotrexate therapy can develop lymphoproliferative disorders, including those that resemble HL or those that are diagnostic of HL. Patients in the latter group are diagnosed as having HL based on immunomorphologic features that are characteristic of H/RS cells ($CD15^+$, $CD30^+$, $CD45^-$), whereas those in the former group have morphologic features but not the classic immunophenotype of HL ($CD45^+$, $CD15^-$, $CD30^+$, $CD20^+$). The latter disorder is referred to as *Hodgkin-like lesions* or *lymphoproliferative disorder with HL features*. In approximately 80% of patients with HL and HL-like proliferations, EBV is detected in H/RS cells. None of the cases of HL are of the NLP type. Regression of both HL and the mimicker of HL can follow withdrawal of methotrexate.

The great imitator of CHL, infectious mononucleosis, should also be considered in the differential diagnosis. If tonsils, which are frequently enlarged in cases of infectious mononucleosis, are removed, then caution should be exercised in making a diagnosis of CHL. As a rule, involvement of Waldeyer's ring by HL is extremely uncommon, and infectious mononucleosis in this instance should be strongly suspected, especially in a young individual. A clinical history, the results of agglutinin screening tests (MonoSpot or heterophil test), tests for EBV-specific antibodies (viral capsid antigen [VCA]-specific IgM), and examination of a peripheral blood smear for reactive lymphocytes are essential in confirming a diagnosis of infectious mononucleosis. The histologic features of infectious mononucleosis depend on the time during the course of the illness that the biopsy is performed. Follicular hyperplasia may be present, and there is, at best, subtotal effacement of the nodal architecture by a proliferation of immunoblasts among the small paracortical lymphocytes, resulting in a characteristic mottled appearance in the expanded paracortex. Some of the immunoblasts are binucleate and morphologically indistinguishable from classic RS cells, and mononuclear immunoblasts may mimic Hodgkin cells. The H/RS impostor cells express CD30 in a membrane or Golgi pattern, or both (like true RS cells), but not CD15. The cells consist of mixtures of EBV-infected B and T cells; however, the background cells are different from those seen in one of the subtypes of HL. The cells consist of varying proportions of small lymphocytes, plasmacytoid lymphocytes, plasma cells, and immunoblasts, some with plasmacytoid features. In addition, and again differing from HL, there is an increased number of high endothelial venules, and often the sinuses are focally distended with monocytoid B cells and some immunoblasts. The histologic findings may be similar to those found in drug-induced hypersensitivity reactions, except that the eosinophilia seen in the latter reaction is absent in infectious mononucleosis.

### Prognosis and Therapy

As in other types of CHL, the prognosis of MC HL depends on the stage of disease at presentation. The stage of the disease dictates the type of therapy to be given.

## Lymphocyte-Rich Classical Hodgkin Lymphoma

LR CHL is a subtype of CHL in which there is a predominance of small lymphocytes. It typically has a nodular or, less commonly, a diffuse cellular background. It is also characterized by the absence of eosinophils and neutrophils.

### Clinical Features

LR CHL shares many of the clinical aspects of NLP HL, features which are distinct from those of other forms of CHL. Approximately 5% of all cases of CHL are the LR classical type, although the number of cases diagnosed as LR CHL will probably increase as pathologists recognize this more recently described subtype. The median age of patients at diagnosis is similar to that of NLP HL, but significantly greater than that of other types of CHL, and there is a male predominance (approximately 70%). Disease-free survival and overall survival are similar for both NLR CHL and NLP HL, in addition to being similar to survival of other types of HL with limited stage disease. As in patients with NLP HL, patients with LR CHL usually lack B symptoms, lack bulky disease, have mediastinal involvement in only approximately 15% of cases, and have low-stage disease (usually stage I/II). In contrast to NLP HL, LR CHL is less often associated with multiple relapses (27% versus 5%, respectively).

### Pathologic Features

#### Microscopic Features

As the term *LR CHL* implies, the histologic features are characterized by a vast predominance of small lymphocytes, which may be arranged in a diffuse, or much

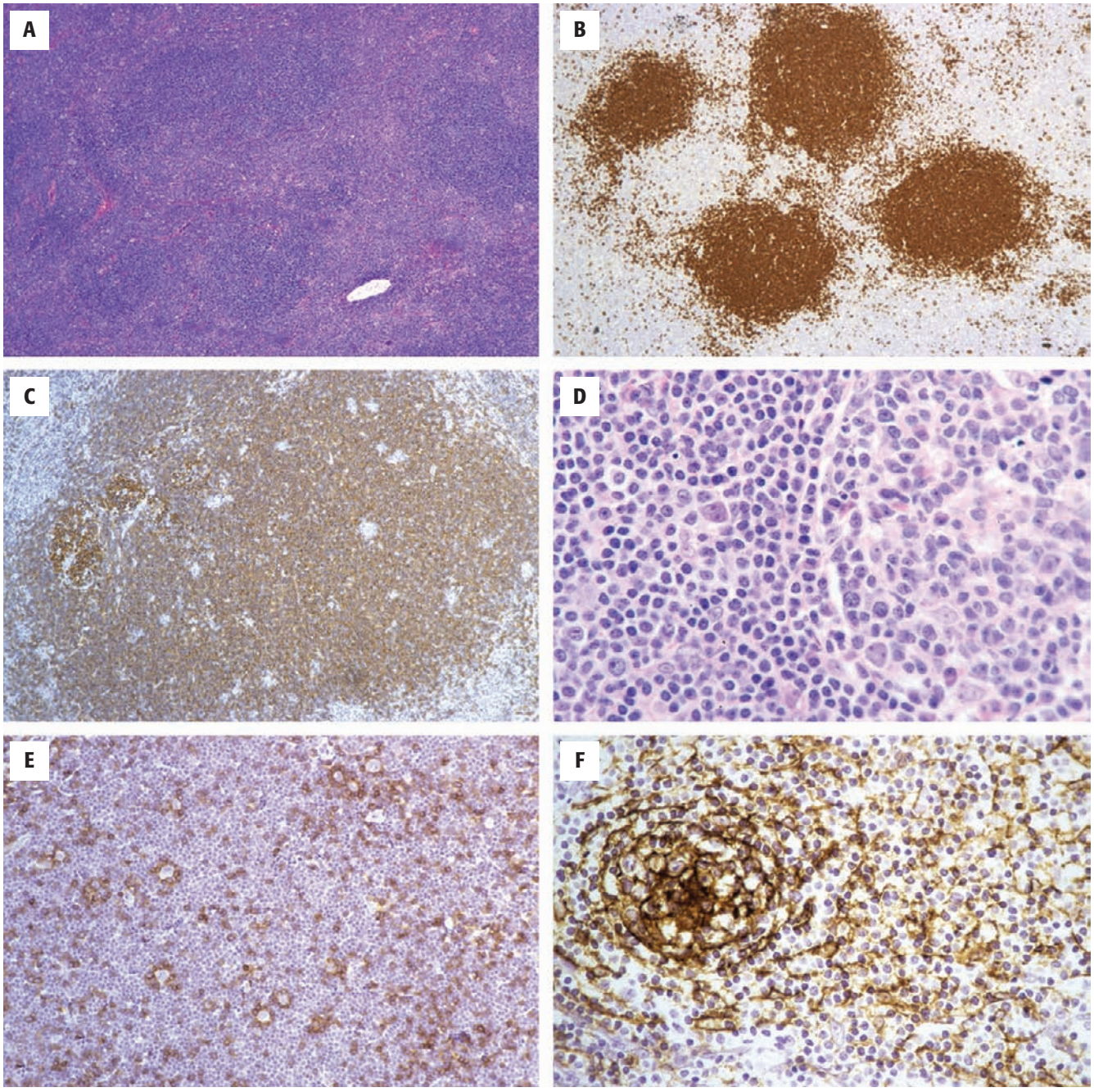



**FIGURE 11-7**

Nodular lymphocyte-rich classical Hodgkin lymphoma. **A,** At low magnification, multiple vague nodules are evident. **B,** At low magnification, the CD79a-stained sections show three nodules of expanded mantle cell lymphocytes. The nodules have irregular borders. **C,** CD79a-stained section with eccentrically placed germinal center with surrounding mantle zone (low magnification). **D,** Germinal center with adjacent classic Reed-Sternberg cells within the mantle zone (high magnification). **E,** $CD3^+$ rosettes encircle classic Reed-Sternberg cells (low magnification). **F,** CD21 stain shows a concentric dense follicular dendritic meshwork within a residual germinal center extending into surrounding mantle zone in a more loosely arranged fashion (low magnification).

more frequently a nodular growth pattern, along with a paucity of other inflammatory cells. Necrosis and fibrosis are absent. The nodular type of LR CHL was first described in 1992 and called *follicular HL;* subsequently, the term follicular was changed to nodular in the REAL and WHO schemes. The nodules, which are expanded mantle zones, have irregular borders and contain a predominance of small lymphocytes, thereby resembling nodules in NLP HL (Figure 11-7). Some nodules contain eccentrically placed, regressed germinal centers, or less often they contain normal-sized or hyperplastic germinal centers (see Figure 11-7). In contrast, germinal

### LYMPHOCYTE-RICH CLASSICAL HODGKIN LYMPHOMA—FACT SHEET

**Definition**
- A subtype of CHL in which there is a predominance of small lymphocytes admixed with scattered H/RS cells
- The growth pattern either nodular or less often diffuse
- Eosinophils and neutrophils rare or absent

**Incidence and Location**
- Approximately 5% of all cases of CHL
- Peripheral lymph nodes, most often cervical, involved

**Gender and Age Distribution**
- Median age at diagnosis greater than that of other types of classical HL and similar to that of NLP HL
- Male predominance (approximately 70%)

**Clinical Features**
- Features similar to those of NLP HL except that multiple relapses less common
- Peripheral lymphadenopathy, most often cervical
- Mediastinal disease uncommon (15%)
- Stage I/II disease at presentation
- B symptoms usually absent

**Prognosis and Therapy**
- Survival and progression-free survival is slightly better than other types of classical HL and similar to those of NLP HL
- Prognosis depends on stage of disease at presentation
- Therapy (chemotherapy or radiation therapy, or both) depends on stage of disease

### LYMPHOCYTE-RICH CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES

**Microscopic Findings**
- Lymph node architecture effaced
- Nodular (more common) or diffuse growth pattern
- Vast predominance of small lymphocytes
- Paucity of other inflammatory cells
- Absence of necrosis and fibrosis
- Nodular growth pattern
  - Composed of small mantle cell lymphocytes
  - Nodules may contain small, residual germinal centers
  - Nodules contain scattered classic RS or lacunar cells or cells morphologically indistinguishable from L&H cells
  - Nodules have irregular borders
- Diffuse growth pattern
  - Composed of small T lymphocytes with or without histiocytes
  - Scattered classic RS cells

**Immunohistochemical Features**
- Nodular growth pattern (more common)
  - Nodules composed of B-mantle cells ($CD20^+$, $CD79a^+$, $IgD^+$, $IgM^+$)
  - T-cell rosettes encircle RS cells
  - $CD57^+$ cells in germinal center and mantle zone, rarely encircling RS cells
  - RS cells with immunophenotype identical to that in other types of classical HL ($CD15^{+/-}$, $CD30^+$, fascin positive, $CD20^{-/+}$, $CD45^-$)
  - $CD21^+$ follicular dendritic meshwork in germinal center extending into mantle zone
- Diffuse growth pattern (rare)
  - Background lymphocytes are T cells
  - RS cells have immunophenotype identical to that in other types of classical HL
  - Absence of $CD57^+$ cells and no $CD21^+$ follicular dendritic meshwork

**Differential Diagnosis**
- Nodular LR CHL
  - Nodular LP HL
- Diffuse LR CHL
  - TC/HRLBCL

**Genetics of Reed-Sternberg Cells**
- H/RS cells are B cells of germinal center cell origin
- H/RS cells contain clonal IG gene rearrangements
- Clonal rearrangement is detected only in DNA of isolated H/RS cells
- Rearranged IGH genes have a high load of somatic hypermutations in the variable region of the *IG* heavy chain genes without signs of ongoing mutations
- Rearrangements are not functional and Ig mRNA transcripts not present in H/RS cells

centers are infrequently found within nodules of NLP HL, and the RS cells in NLR HL are of the classical type. Some lacunar cell variants may be seen, and occasionally cells with morphologic features of LP cells may also be present. Most of the RS cells are found within the mantle zone, some at the junction of mantle and internodular areas, whereas a few RS cells may be present in the internodular areas that are composed predominantly of residual paracortical T cells. Occasional eosinophils and neutrophils may be present in the internodular areas.

## Ancillary Studies

### Immunophenotype

The immunophenotype of the H/RS cells is essential in differentiating NLR CHL from NLP HL and in establishing the correct diagnosis. The H/RS cells have the immunophenotype of other CHLs ($CD15^{+/-}$, $CD30^+$, $CD20^{-/+}$, $CD45^-$), and the H/RS cells are surrounded by a collarette of $CD3^+$ T cells identical to that found in NLP HL (see Figure 11-7). PD-$1^+$ cells are present in germinal centers, and only rare rosettes encircling the H/RS cells may be found. CD21/CD35 shows the FDC meshwork, which is dense in residual germinal centers

but loose in the surrounding expanded mantle zone (see Figure 11-7). The nodules are composed of $CD20^+$ and $CD79a^+$ mantle cell ($IgM^+$, $IgD^+$) lymphocytes with or without residual germinal centers (see Figure 11-7). The internodular areas contain mostly small paracortical T cells.

### Differential Diagnosis

The major differential diagnostic consideration is NLP HL. Under low magnification on H&E-stained sections and in sections immunostained with B-cell markers CD20 and CD79a and with IgD, the nodularity may be indistinguishable between the two types of HL, except that nodules in NLP HL tend to be larger and often more closely apposed and there is usually less internodular tissue than in NLR CHL. The difficulty in distinguishing between NLR CHL and NLP HL is exemplified by a study of 379 cases initially diagnosed as NLP HL that were then immunophenotyped and reviewed by the European Task Force on Lymphoma. Only 51% of the cases were found to be NLP HL, whereas 27% were reclassified as LR CHL, 5% as other types of CHL, 3% as non-Hodgkin lymphomas, and 4% as benign, reactive processes. The authors of these studies recommended immunophenotyping all cases morphologically consistent with NLP HL to exclude a diagnosis of NLR CHL. Another study of 208 cases considered to be NLP HL by at least one pathologist was found to have a similar rate of misdiagnosis; only 44% of cases had the immunophenotype of LP HL, whereas 56% were found to be CHL. If residual germinal centers are found within many nodules, although they are not always present or only seen in rare nodules, a diagnosis of NLR CHL is favored because germinal center are infrequently present in NLP HL. Immunostaining of the RS cells will definitively confirm the diagnosis. As in other types of CHL, the RS cells in NLR CHL are usually $CD15^+$, $CD30^+$, and fascin positive; they may be $CD20^+$ or $CD20^-$, and they do not express CD45. EMA may be helpful if positive; RS cells in CHL are, with rare exceptions, EMA negative. They may be EBV positive in NLR CHL but not in NLP HL. Similarities between the two types of HL consist of B-cell nodules, rosetting of T cells around RS cells, and the presence of a CD21/CD35 concentric meshwork of dendritic reticulum cells. As mentioned previously, the meshwork is usually less extensive in NLR CHL because the nodules usually, but not always, are smaller than in NLP HL. In addition, the meshwork in germinal centers, if present, stains more intensely in tight, round aggregates and extends in a less concentric, loosely spaced pattern into the mantle zone. In addition, $CD57^+$ lymphocytes are found scattered throughout the large nodules in NLP HL, occasionally ringing LP cells, whereas in NLR CHL they are concentrated in the germinal centers with fewer being present in mantle zones, rarely encircling RS cells. A striking difference between the two types of HL is the number of PD-$1^+$ cells within the nodules and especially the number of PD-$1^+$ rosettes around RS cells. The number of rosettes in NLP HL is a prominent feature, whereas rosettes are rare in NLR CHL. Almost all nonrosetting PD-$1^+$ cells in NLR CHL are present within germinal centers rather than within the mantle zone.

## DIFFUSE LYMPHOCYTE-RICH CLASSIC HODGKIN LYMPHOMA

### Pathologic Features

#### *Microscopic Features*

The less common type of diffuse lymphocyte rich (DLR) CHL is characterized by a diffuse proliferation of small lymphocytes with occasional admixed classic or lacunar type of H/RS cells or, rarely, variants with morphologic features of LP-type cells. Eosinophils and neutrophils are few or absent, and necrosis and fibrosis are absent. Varying numbers of epithelioid histiocytes are occasionally found among the lymphocytes. On morphologic grounds, the diagnosis of DLR CHL is occasionally, perhaps erroneously, made based on a small, inadequate biopsy specimen in which a nodular component of NLP HL may not be represented. Before the availability of immunohistochemistry, most cases of DLR CHL were diagnosed as the diffuse type of lymphocyte predominant HL (Lukes and Butler classification), which today is not considered to be a distinct entity in the WHO classifications but may be seen infrequently as part of NLP HL. Less often, DLR CHL was categorized as mixed cellularity HL.

### Ancillary Studies

#### Immunophenotype

Whereas the immunophenotypes of the H/RS cells are identical to those of the nodular type, the small lymphocytes are T cells, in contrast to the mantle cell B-lymphocytes in the nodular type. The $CD21/CD35^+$ FDC meshwork present in NLR CHL is absent. The T-cell rosettes around H/RS cells that are prominent in the nodular type are not readily identifiable in the diffuse type because of the multitude of background T cells present.

### Differential Diagnosis

TC/HRLBCL is the major consideration for differential diagnosis of DLCR HL, diffuse areas of NLP HL (see differential diagnosis of NLP HL) and MC HL. On purely morphologic grounds, DLR CHL may be indistinguishable from TC/HRLBCL; however, these two types of lymphomas are immunomorphologically different. The use of four antibodies—CD15, CD30, CD20, and CD45—will distinguish the two disorders in most instances. RS cells in DLR CHL are usually $CD15^+$, $CD30^+$, and fascin positive, most often $CD20^-$, and always $CD45^-$; however, the large cells in TC/HRLBCL strongly express CD20 and usually CD45, but are $CD15^-$, and most often $CD30^-$. Demonstration of EBV in the large cells would favor HL, because EBER and LMP1 are not expressed in TC/HRLBCL. Even when RS cells in DLR CHL express CD20, it is usually of variable intensity and only a minor subpopulation is $CD20^+$. In contrast, all the large cells in TC/HRLBCL strongly express CD20, and expression of BCL-6, CD45, and CD79a also favors that diagnosis. MC HL can usually be differentiated from DLR CHL by the absence of neutrophils, plasma cells, and eosinophils in the latter.

### Prognosis and Therapy

Overall survival is generally favorable in low-stage disease, being slightly better than in other subtypes of CHL, although similar to but not as favorable as survival of patients with the NLP type. Patients who have relapses do not fare as well as patients with relapsed NLP HL.

## LYMPHOCYTE-DEPLETED CLASSICAL HODGKIN LYMPHOMA

LD CHL is a rare diffuse type of classical HL depleted of small benign lymphocytes with numerous or, less often, few H/RS cells.

### Clinical Features

LD HL is rarely diagnosed in industrialized nations, except in HIV-positive patients, but it is reported more often in developing countries. The majority of LD HL cases were diagnosed before immunophenotyping became available and most are known to be examples of NHL, the syncytial variant of NS HL, or occasionally sarcomas, carcinomas, or melanomas. Because of the paucity of cases of true LD HL, reliable clinical data are sparse. The median age at diagnosis was reported to be 30 to 37 years, with 60% to 75% of the patients being male. Most of the patients had B symptoms with abdominal involvement and advanced-stage disease (splenic, liver, and bone marrow involvement), and peripheral lymphadenopathy was reported to be rare. In a recent study of LD HL in which immunophenotyping and molecular genetic analysis were applied, some of the clinical features previously reported have been refuted. In this report of eight patients only half were male, and there was a predilection for older individuals with the median age of the patients being 62 years. None of the eight patients were HIV positive. In contrast to previous reports, lymph nodes were the most common sites of involvement (seven of eight patients), but widespread disease involving liver, spleen, lungs, and mediastinum, as has been previously reported in the literature, was also observed in these patients. The sites of peripheral lymph node biopsies included cervical, supraclavicular, axillary, and inguinal regions.

**LYMPHOCYTE-DEPLETED CLASSICAL HODGKIN LYMPHOMA—FACT SHEET**

**Definition**
- A diffuse type of classical HL depleted of small benign lymphocytes with numerous or occasionally few H/RS cells

**Incidence and Location**
- Least common type of HL in industrialized countries (<1% of cases in Western countries)
- More common in nonindustrialized countries
- Often seen in HIV-positive individuals

**Gender and Age Distribution**
- Male predominance (65% to 75%)
- Median age range, 30 to 37 years

**Clinical Features**
- Reliable clinical data not available because cases that were diagnosed as this type of HL before the advent of immunohistochemistry were found, upon reexamination, to be mostly non-Hodgkin lymphomas or syncytial variant of nodular sclerosis HL
- Older patients (except for HIV-positive individuals)
- Advanced stage (III/IV)
- Frequent B symptoms
- Disease below diaphragm common
- Spleen, liver, and bone marrow involvement common

**Prognosis and Therapy**
- Prognosis depends on stage of disease at presentation
- Therapy (chemotherapy or radiation, or both) depends on stage of disease

## Pathologic Features

### Histologic Features

As the name implies, there is a depletion of small lymphocytes, and the two histologic types of LD HL described in the Lukes and Butler classification are the reticular and diffuse fibrosis types. These two histologic subtypes are still recognized in cases currently diagnosed as LD HL. The more common reticular type is hypercellular and consists predominantly of an abundance of pleomorphic large cells, some resembling or indistinguishable from classical H/RS cells and admixed histiocytes (Figure 11-8). Some cases with pleomorphic H/RS cells have a sarcomatous appearance. Few lymphocytes, eosinophils, histiocytes, and plasma cells are present. Necrotic areas are common, and disorderly fibrosis and a fibrillary matrix may be present as well. The other, less common, subtype is hypocellular and consists of disorderly fibrosis (but not a nodular sclerosis pattern) with amorphous pink-staining eosinophilic material containing few cellular elements in the background (see Figure 11-8). There may be many fibroblasts or reticulin fibrosis and few inflammatory cells. Classical H/RS cells are rare, and necrosis is often present. In some cases, there is a mixture of the reticular and the diffuse fibrosis types in the same lymph node. Fibrous bands are not present. It should be remembered that a diagnosis of LD HL should never be made without immunohistochemical confirmation.

**LYMPHOCYTE DEPLETED CLASSICAL HODGKIN LYMPHOMA—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Lymph node architecture effaced
- Depletion of small, benign lymphocytes
- Hypocellular or hypercellular with many bizarre, pleomorphic RS cells
- Disorderly fibrosis
- Background of fibrillary matrix or amorphous pink-staining material
- Necrosis common

**Immunohistochemical Features**
- RS cells have immunophenotype identical to that of other types of classical HL
- RS cells in most HIV-associated cases are EBV positive

**Differential Diagnosis**
- Anaplastic large cell lymphoma
- Diffuse large B- or T-cell lymphoma
- Syncytial variant (grade 2) of nodular sclerosis HL

**Genetics**
- H/RS cells are B cells of germinal center cell origin
- H/RS cells contain clonal IG gene rearrangement
- Clonal rearrangement is detected in DNA of isolated H/RS cells
- Rearranged IGH genes have a high load of somatic hypermutations in the variable region of the IG heavy-chain genes without signs of ongoing mutations
- Rearrangements are not functional, and Ig mRNA transcripts are not present in H/RS cells

### Immunophenotype

The immunophenotype of H/RS cells is the same as that of other types of CHL. In recently published cases, H/RS cells were cell positive for CD15, CD30, PAX-5, MUM1, fascin, and either BOB.1 or OCT-2 (or rarely both these transcription factors), but they were negative for CD45 and EMA. All the evaluated cases had clonally rearranged *IGH* genes. EBV was detected in H/RS cells in four of eight patients by in situ hybridization despite the fact that none of these patients were immunodeficient.

## Differential Diagnosis

As stated previously, the diagnosis of LD HL is rare, especially in immunocompetent patients. Most of the cases diagnosed before the use of immunohistochemistry are, on reassessment with immunophenotyping, examples of ALCL; diffuse, large B- or T-cell lymphoma; or the syncytial variant of NS HL. ALCL (see also under Differential Diagnosis of NS HL) is almost always $CD15^-$, usually expresses some T-cell markers (especially CD2, CD4, CD5, and CD43), may or may not be $CD45^+$, and is often ALK positive as well as $CD25^+$ and clusterin positive, whereas classical H/RS cells do not express the latter markers. On the other hand, PAX-5 is expressed by classic RS cells, whereas the cells of ALCL are almost always negative for this marker. In addition, ALCL may have a sinusoidal distribution in lymph nodes, which is usually not seen in HL. Other B-cell and large T-cell lymphomas express B- or T-cell markers and are $CD15^-$, although they may be $CD30^+$ (activation marker). Fascin, which is positive in almost all cases of CHL, may also be positive in a subset of B- and T-large cell lymphomas as well as in ALCL. Its presence, therefore, is not helpful in the differential diagnosis. Fascin negativity, however, would be evidence against LD HL. Syncytial NS HL or NS HL with few lymphocyte and numerous lacunar cells can mimic LD subtype; however, the presence of a nodular sclerosis pattern (even a single sclerotic band) or numerous lacunar cells is consistent with NS HL. In addition, the fibrocollagenous bands in NS HL are birefringent, whereas the fibrosis in LD is not. Diffuse large B-cell lymphoma in older patients (EBV+ diffuse large B-cell lymphoma of the elderly)

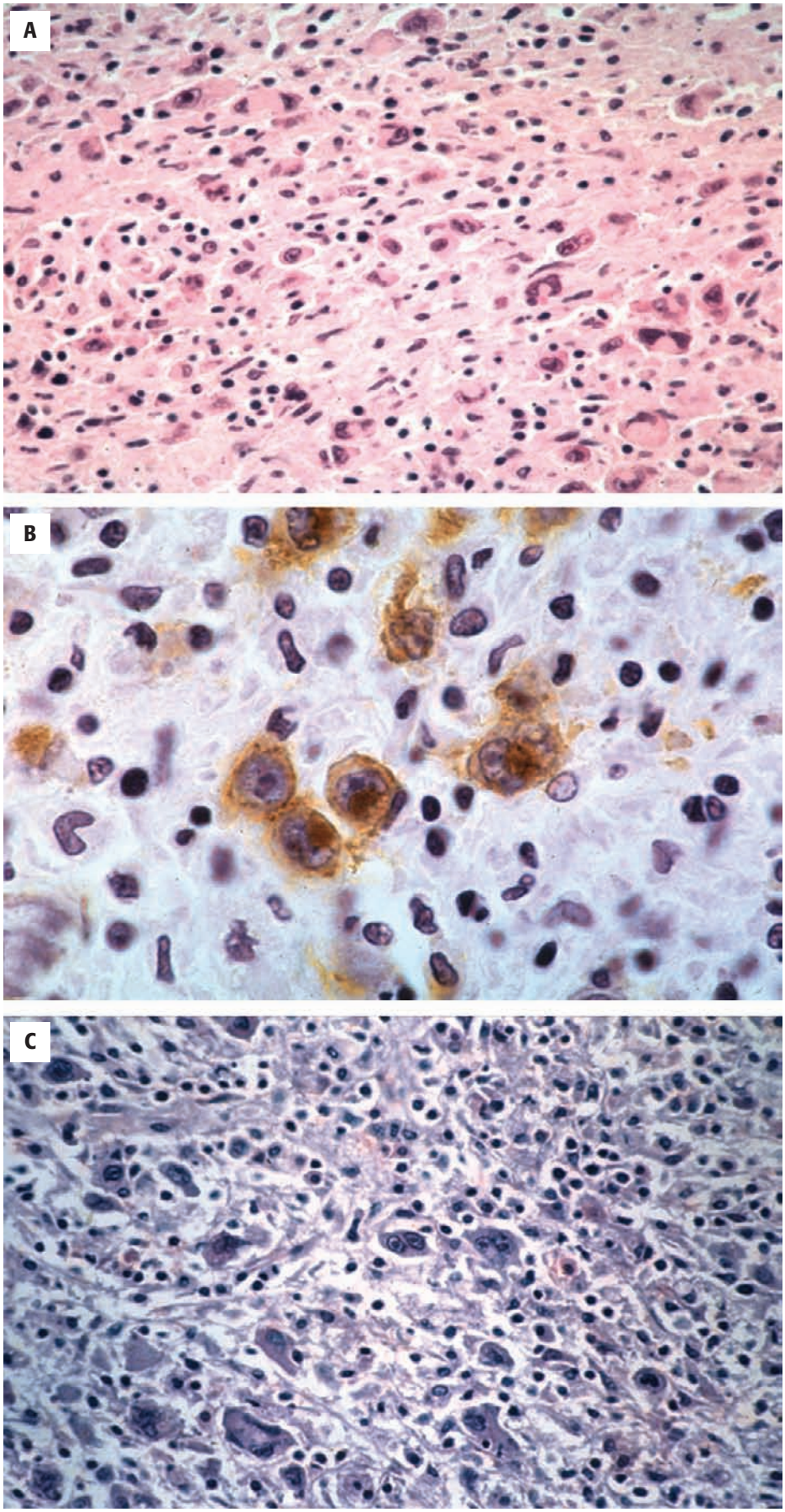



**FIGURE 11-8**

Lymphocyte-depleted classical Hodgkin lymphoma. **A,** There is a paucity of lymphocytes and other inflammatory cells among amorphous pink-staining material containing large pleomorphic Reed-Sternberg cells. **B,** The large cells are $CD15^{+}$. **C,** Another case showing bizarre Reed-Sternberg cells in a hypocellular, lymphocyte-poor background.

can resemble LD HL; however, the large cells, some of which resemble H/RS cells, strongly express CD20 but are $CD15^-$ and EBER-positive.

### Prognosis and Therapy

As in other types of CHL, the prognosis and the type of therapy depend on the stage of disease at presentation.

## HODGKIN LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA

The development of CHL in patients with chronic lymphocytic leukemia (CLL) has been described and has been considered by some investigators to be a type of Richter syndrome (originally described as a transformation of CLL to a large cell lymphoma). Two different histologic patterns have been reported. In the first pattern, RS cells and variant cells are scattered in a background of typical CLL without the cellular milieu of CHL. Such cases should not be diagnosed as HL. They have, in the past, been referred to as *H/RS–like cells in B-CLL* and are not regarded as a true form of Richter transformation. The RS cells reside in a B-cell background and usually express CD30 and CD15 and are variably $CD20^+$. The RS cells are ringed by T cells. The CLL cells have the typical immunophenotype of CLL ($CD20/CD79a^+$ with coexpression of CD5, CD43, and cyclin D1 negative). This first pattern is more common than the second, which consists of segregated areas of CHL and CLL. The second pattern, which has been diagnosed as HL in the setting of CLL, an HL variant of Richter syndrome, or Richter syndrome with HL features, may be present within a single node or involve several nodes. The cellular environment in which the RS cells are seen consists of T lymphocytes, eosinophils, plasma cells, and other inflammatory cells in varying proportions. The RS cells in both types may have the immunophenotype of classical RS cells ($CD15^+$, $CD30^+$, $CD45^-$), although RS cells in type 1 are often $CD15^-$. Some of the RS cells in both types may also be $CD20^+$. A clonal relationship between the CLL cells and microdissected RS cells (by PCR amplification) has been demonstrated in some cases, indicating that classical RS cells are derived from transformed B lymphocytes. In other cases, a clonal relationship between the two diseases could not be found. EBV in H/RS or H/RS-like cells has been reported in some cases but not in others. It appears that EBV positivity is found in H/RS-like cells predominantly, but not exclusively, in clonally unrelated cases. These findings suggest that underlying immunodeficiency associated with CLL, in concert with severe T cell lymphocytopenia associated with fludarabine treatment commonly used in CLL, increases the risk of development of an unrelated EBV-associated lymphoma. Clinically, both patients with the HL variant of Richter transformation and patients with CLL and scattered H/RS-like cells often have B symptoms and enlarged lymph nodes that are resistant to CLL type of treatment. The prognosis is unfavorable, with some patients dying within months of diagnosis.

## HODGKIN LYMPHOMA AND HIV INFECTION

An association between HL and HIV infection has been demonstrated in a number of studies from around the world. In fact, HL is the most common non–AIDS-defining tumor in HIV-infected patients. The relative risk of HL among HIV-positive patients ranges from 7.6 to 8.9. In contrast to immunocompetent individuals, HIV-positive patients have an increased incidence of unfavorable histologic subtypes (mixed cellularity and lymphocyte depleted), whereas the NS type is uncommon (Table 11-6). The incidence of NS HL in HIV-positive patients decreases with decreasing CD4 counts. CHL in patients with AIDS characteristically has a high frequency of EBV infection in RS cells. There is no increased incidence of NLP HL in HIV-positive patients.

### Clinical Features

The majority of HIV-positive patients with HL exhibit B symptoms and often widespread disease (stage III/IV). In addition, extranodal involvement is much more common than in HIV-negative patients. Extranodal sites include bone marrow, which may be the site on which the initial diagnosis is made, as well as liver and spleen. In contrast to HL in immunocompetent individuals, the

**TABLE 11-6**

**Features Characteristic of Hodgkin Lymphoma in HIV-Positive Individuals**

- Advanced stage disease at presentation (stage III/IV)
- Constitutional symptoms
- Noncontiguous pattern of spread
- Aggressive clinical course and often poor response to therapy
- Involvement of extranodal sites common
- Bone marrow involvement at presentation common
- Aggressive subtypes (mixed cellularity, lymphocyte depleted) more common than in immunocompetent patients
- Strong association with EBV (80% to 100%)

Background lymphocytes mostly $CD8^+$ cells (not $CD4^+$ lymphocytes as seen in immunocompetent patients).

spread of HL is often not contiguous. For example, liver without splenic involvement or lung without mediastinal involvement may be present in HIV-positive HL patients. There is a strong association with EBV (80% to 100%). Prognosis in these patients with high-stage HIV is unfavorable.

## Pathologic Features

### Histologic and Immunophenotypic Features

Histologic features are similar to those of MC and LD subtypes seen in immunocompetent patients; however, the background lymphocytes in HIV-positive individuals are mostly $CD8^+$ lymphocytes rather than the $CD4^+$ cells in immunocompetent individuals. The widespread use of HAART resulted in an increase in number of $CD4^+$ cells with concomitant increased immunity and a spectacular decrease in development of full-blown AIDS, together with a decline in mortality. Paradoxically, the number of cases of HL increased, including the NS type. It appears that improved immunity related to increased CD4 counts in patients treated with HAART results in increased numbers of cases of HL, especially the NS type. Therefore the institution of HAART may have placed AIDS patients at an increased risk for the development of HL. H/RS cells in most cases of MC and LD are EBV positive, but those in NS are usually negative. The prognosis in HIV-positive patients is not favorable, although it has improved in patients who receive combined antineoplastic therapy and HAART.

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SECTION 3

# Leukemias, Chronic Myeloproliferative Neoplasms, and Myelodysplasia

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12

# B-Cell Leukemias of Mature Lymphocytes

**Eric D. Hsi, MD**

## INTRODUCTION

The leukemias of mature B cells are a limited set of diseases in which blood and bone marrow are the primary sites of involvement. Although any lymphoproliferative disorder can eventually enter a leukemic phase, this chapter is limited to B-cell chronic lymphocytic leukemia (CLL), B-cell prolymphocytic leukemia (PLL), hairy cell leukemia (HCL), and HCL variant (HCLv). Burkitt leukemia/lymphoma and splenic marginal zone lymphoma (SMZL) are addressed in other chapters.

## CHRONIC LYMPHOCYTIC LEUKEMIA

CLL is the most common leukemia in the Western world. The age-adjusted estimated annual incidence in the United States is approximately 3.4/100,000. The median age at diagnosis is 65 years, with a 2:1 male-to-female ratio. CLL is an indolent leukemia with a disease course that can span decades. There are effective therapies, particularly when used early in the disease course, that can induce remissions; however, relapses inevitably occur. As a result, CLL is considered incurable in the vast majority of cases, and new therapies are needed.

### CHRONIC LYMPHOCYTIC LEUKEMIA—FACT SHEET

**Definition**

- Mature B-cell leukemia composed of small round lymphocytes
- Small lymphocytic lymphoma represents the tissue equivalent of CLL

**Incidence**

- Most common leukemia in the Western world (3.4/100,000 in the United States)

**Clinical Features**

- Older adult (median age 65), with male predominance (2:1)
- Patients may be asymptomatic or exhibit cytopenia and adenopathy or organomegaly

**Prognosis and Therapy**

- Indolent, incurable disease with prolonged clinical course
- Treatment varies depending on patient factors and includes watchful waiting; alkylating agents with or without corticosteroids; purine analogs, such as fludarabine; monoclonal antibodies; and multiagent chemotherapy

### CLINICAL FEATURES

Patients exhibit lymphocytosis (leukemic lymphocytes greater than $5.0 \times 10^9$/L) and are often asymptomatic. Others may have symptoms relating to organ involvement (e.g., splenomegaly, hepatomegaly) or lymphadenopathy. Anemia and other cytopenias are often present because of immune hemolysis related to the leukemia or simple bone marrow replacement by leukemic infiltrate. CLL can progress with increasing numbers of prolymphocytes in the blood, or patients may experience transformation to a large cell lymphoma (Richter syndrome) heralded by a sudden change in symptoms or rapid localized lymph node enlargement. Staging systems such as those of Rai and of Binet are used to predict prognosis in patients with CLL (Table 12-1). These systems are useful in stratifying patients, but predicting outcome in patients with intermediate-stage disease is still difficult, and biologic predictors are needed.

### PATHOLOGIC FEATURES

#### MORPHOLOGY

The peripheral blood smear of patients with CLL is characterized by a variable lymphocytosis that may be greater than $500 \times 10^9$/L. The lymphocytes are typically

**TABLE 12-1**

**Staging System for Chronic Lymphocytic Leukemia**

| Staging System | Stage | Clinical Features | Median Survival (yr) |
|---|---|---|---|
| ***Rai*** | | | |
| Low risk | 0 | Lymphocytosis | 14.5 |
| Intermediate risk | I | Lymphocytosis, lymphadenopathy | 7.5 |
| | II | Lymphocytosis, hepatomegaly, splenomegaly | |
| High risk | III | Lymphocytosis, anemia | 2.5 |
| | IV | Lymphocytosis, thrombocytopenia | — |
| ***Binet*** | | | |
| | A | Normal hemoglobin, normal platelet count, fewer than three node-bearing areas | 14 |
| | B | Normal hemoglobin, normal platelet count, three or more node-bearing areas | 5 |
| | C | Anemia (<10 g/dL), thrombocytopenia (<1 × $10^9$/L) | 2.5 |

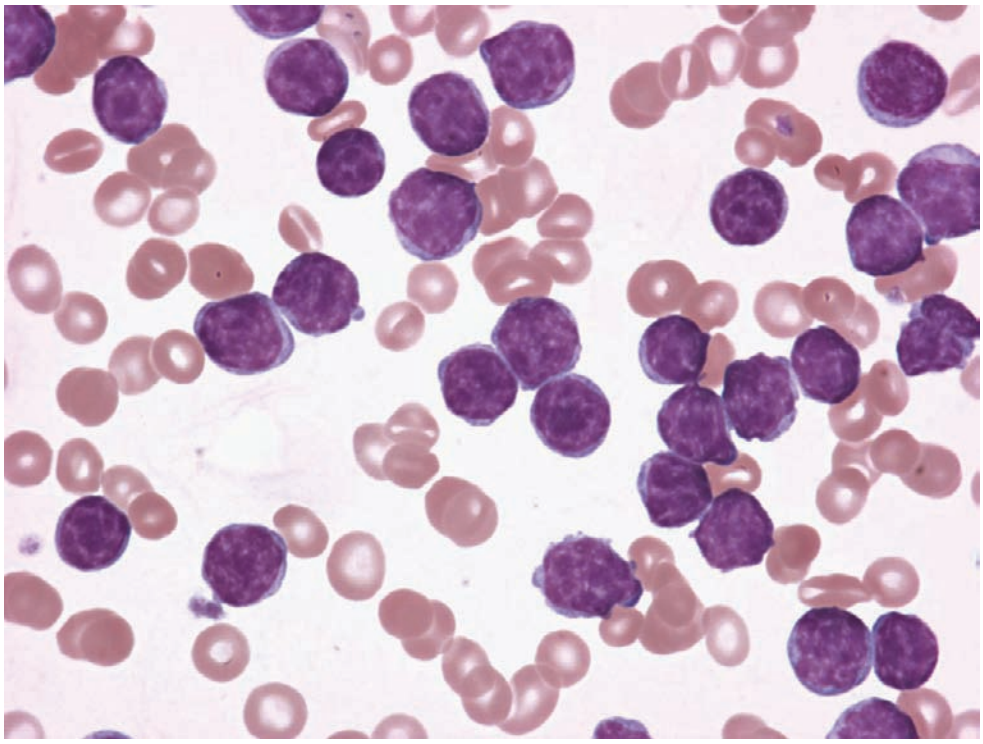


**FIGURE 12-1**

Peripheral blood smear of a case of chronic lymphocytic leukemia demonstrating a marked lymphocytosis. The lymphocytes are small with condensed chromatin, imparting a "soccer ball" pattern, and scant cytoplasm (Wright stain).

small and round with condensed chromatin alternating with lighter areas, imparting a "soccer ball" or "cracked" chromatin pattern (Figure 12-1). The cytoplasm is usually scanty. Variation from this typical form is acceptable within the spectrum of CLL, and some cases may have substantial numbers (more than 10%) of cells with nuclear irregularity or moderate amounts of pale blue cytoplasm (Figure 12-2). Some studies have associated this variant morphology with a worse outcome. Prolymphocytes are present in varying proportions. These cells are 1.5-fold to twofold larger than the typical CLL cells, with slightly open chromatin and a central nucleolus. Increased numbers of prolymphocytes (greater than 5 × $10^9$/L) have been associated with a poor prognosis and certain genetic alterations, such as *TP53* abnormalities and trisomy 12. Cases in which prolymphocytes constitute more than 10% but less than 55% of the lymphocytes have been termed *mixed cell CLL* or *CLL/PLL* in the original French-American-British classification scheme, but are not distinguished from CLL in the 2008

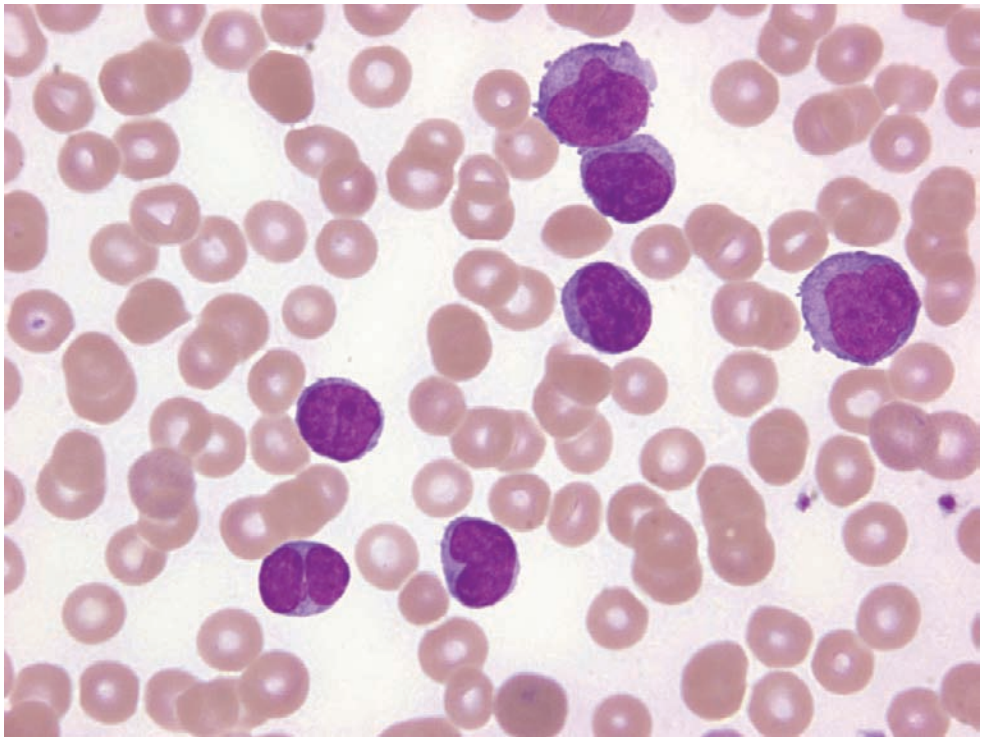


**FIGURE 12-2**

Chronic lymphocytic leukemia (CLL) with variant morphology with irregular nuclei and occasional prolymphocyte (Wright stain). Although unusual, cells with irregular nuclear contours can be seen in CLL. This particular case had an immunophenotype typical of CLL.

World Health Organization classification. The bone marrow may show variable involvement. Four major patterns are recognized: nodular, interstitial, mixed, and diffuse (Figure 12-3). The nodules tend to be nonparatrabecular. The cells are similar in appearance to those seen in lymph nodes: small and round with condensed chromatin. Proliferation centers can be seen. In the interstitial pattern, the lymphocytes infiltrate around preserved fat spaces, admixed with varying amounts of residual hematopoietic elements. The diffuse pattern, with areas totally replaced by sheets of leukemia cells, has been associated with a poor prognosis and more advanced disease.

Transformation to PLL is defined by more than 55% of lymphocytes being prolymphocytes and is usually characterized by worsening symptoms, loss of response to therapy, and poor prognosis. Richter syndrome (transformation to a diffuse aggressive B-cell lymphoma) occurs in approximately 3% to 4% of patients and survival is short. Unusual transformations or second malignancies such as Hodgkin lymphoma (HL), acute lymphoblastic leukemia, and multiple myeloma have been reported. Of note, Reed-Sternberg (RS)–like cells have been shown in some cases to be positive for Epstein-Barr virus and clonally related to the CLL. HL transformation should be reserved for cases demonstrating areas with all the histologic features of HL and not used for cases with scattered RS-like cells in a background of SLL.

**CHRONIC LYMPHOCYTIC LEUKEMIA—PATHOLOGIC FEATURES**

**Morphology**

- Peripheral blood: small, mature-appearing lymphocytes with condensed chromatin and round nuclei; variable numbers of prolymphocytes (less than 55%)
- Bone marrow: interstitial, nodular, or diffuse involvement; proliferation centers can be seen
- Spleen: white pulp predominant or diffuse involvement

**Immunophenotype**

- $CD5^{+}$, $CD10^{-}$, $CD19^{+}$, $CD20^{+}$ (dim), $CD23^{+}$, $sIg^{+}$ (dim), $FMC7^{-/+}$, $CD79b^{-/+}$
- CD38 and ZAP-70 expression associated with adverse outcome

**Genetics**

- 13q deletion as sole abnormality associated with good prognosis; 17p deletion associated with poor prognosis (see text)
- Unmutated immunoglobulin heavy chain gene variable region (greater than 98% homology to germline) associated with poor prognosis
- *NOTCH1* activating mutations in 12%

**Differential Diagnosis**

- Leukemic MCL
- Leukemic FL
- SMZL
- Acute lymphoblastic leukemia (L-1 morphology)
- PLL (B- or T-cell)
- Monoclonal B-cell lymphocytosis

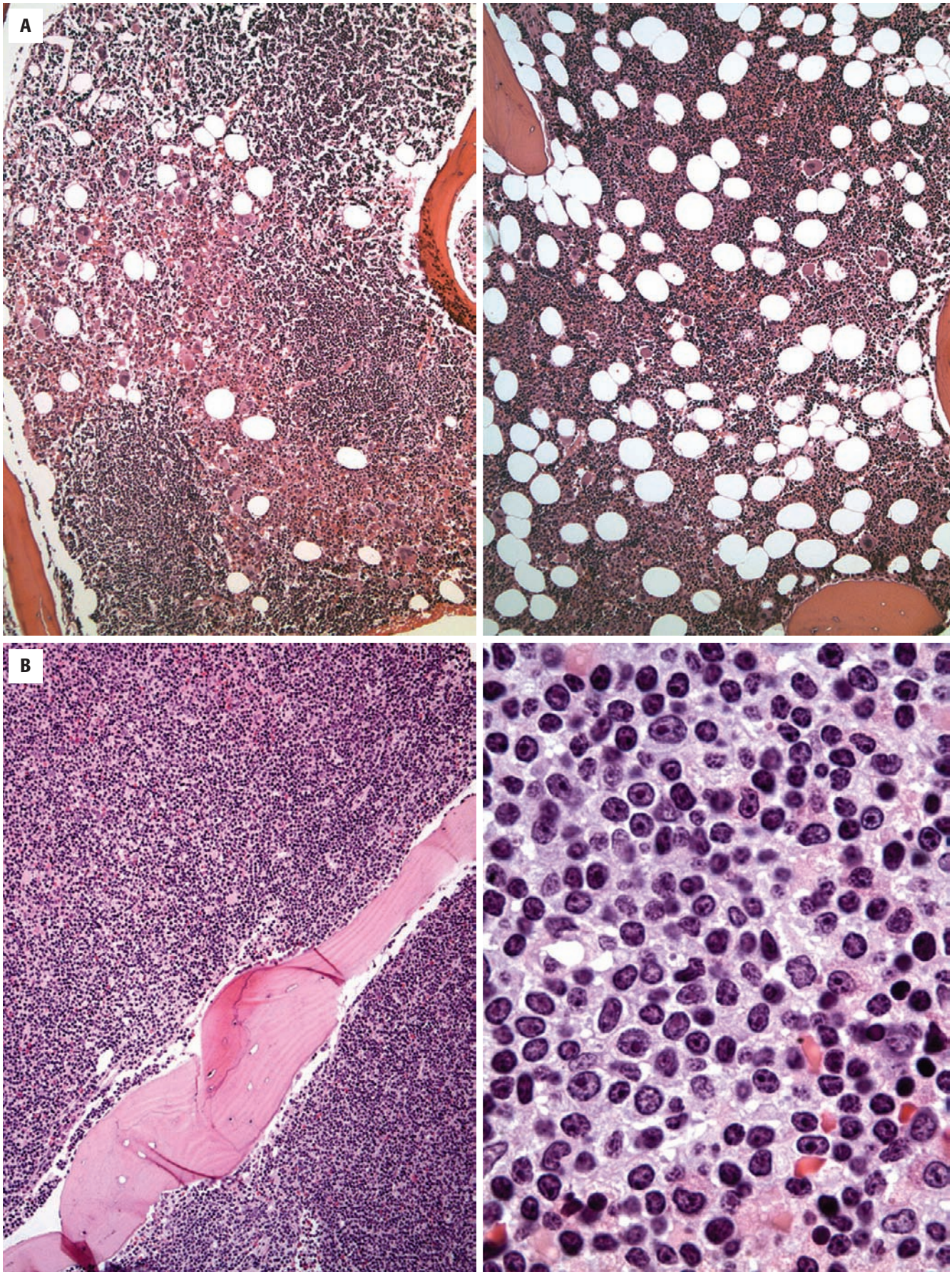



**FIGURE 12-3**

Bone marrow involvement by chronic lymphocytic leukemia **A,** Bone marrow biopsy specimen of patient with chronic lymphocytic leukemia demonstrating nodular *(left)* and interstitial *(right)* patterns. **B,** Diffuse pattern *(left)* with high magnification showing a collection of nucleolated prolymphocytes characteristic of a proliferation center (hematoxylin and eosin).

### Immunophenotype

Flow cytometric immunophenotyping is required to confirm the diagnosis of CLL. CLL cells express CD5, CD19, CD20, CD22, CD23, and restricted surface immunoglobulin (Ig) light chain. The expression of CD20 and immunoglobulin is usually dim compared with normal B cells (Figure 12-4). Heavy chain expression is usually of the IgM and IgD type. FMC7 and CD79b are absent or only dimly expressed. Deviation from this typical immunophenotype occurs, and a scoring system has been proposed to help quantify the likelihood that the diagnosis is CLL (Table 12-2). One caveat to the presence of restricted light chain expression is the existence of rare cases of biclonal CLL in which a κ CLL clone and a λ CLL clone are seen. Cases might seem polytypic but have the other immunophenotypic features of CLL and, on molecular analysis, show biclonal rearrangement patterns.

Two additional markers have been shown to be important in the prognosis of CLL. CD38 is a marker that initially was thought to correlate with *IGH* variable region (*IGHV*) mutational status (see following section). The correlation has subsequently been proved to be imperfect, but it has been shown in numerous studies that CD38 expression (a commonly used cutoff is 30%

**TABLE 12-2**

**Proposed Scoring System for Chronic Lymphocytic Leukemia**

| | No. of Cases (%) | | |
|---|---|---|---|
| Score* | CLL | Other B-Cell Leukemia | B-NHL |
| 5 | 209 (52) | 0 | 0 |
| 4 | 139 (35) | 0 | 1 (0.5) |
| 3 | 39 (10) | 1 (1) | 7 (4) |
| 2 | 11 (3) | 8 (10) | 43 (23) |
| 1 | 1 (0.2) | 25 (33) | 75 (39) |
| 0 | 1 (0.2) | 43 (56) | 63 (33) |

Data from Matutes E, Owusu-Ankomah K, Morilla R, et al: The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL, *Leukemia* 8:1640–1645, 1994.
*B-NHL,* B-cell non-Hodgkin lymphoma; *CLL,* chronic lymphocytic leukemia.
*Point system: surface Ig (weak = 1, moderate/strong = 0); CD5 (positive = 1, negative = 0); CD23 (positive = 1, negative = 0); FMC7 (negative = 1, positive = 0); CD22 (weak/negative = 1, moderate/strong = 0).

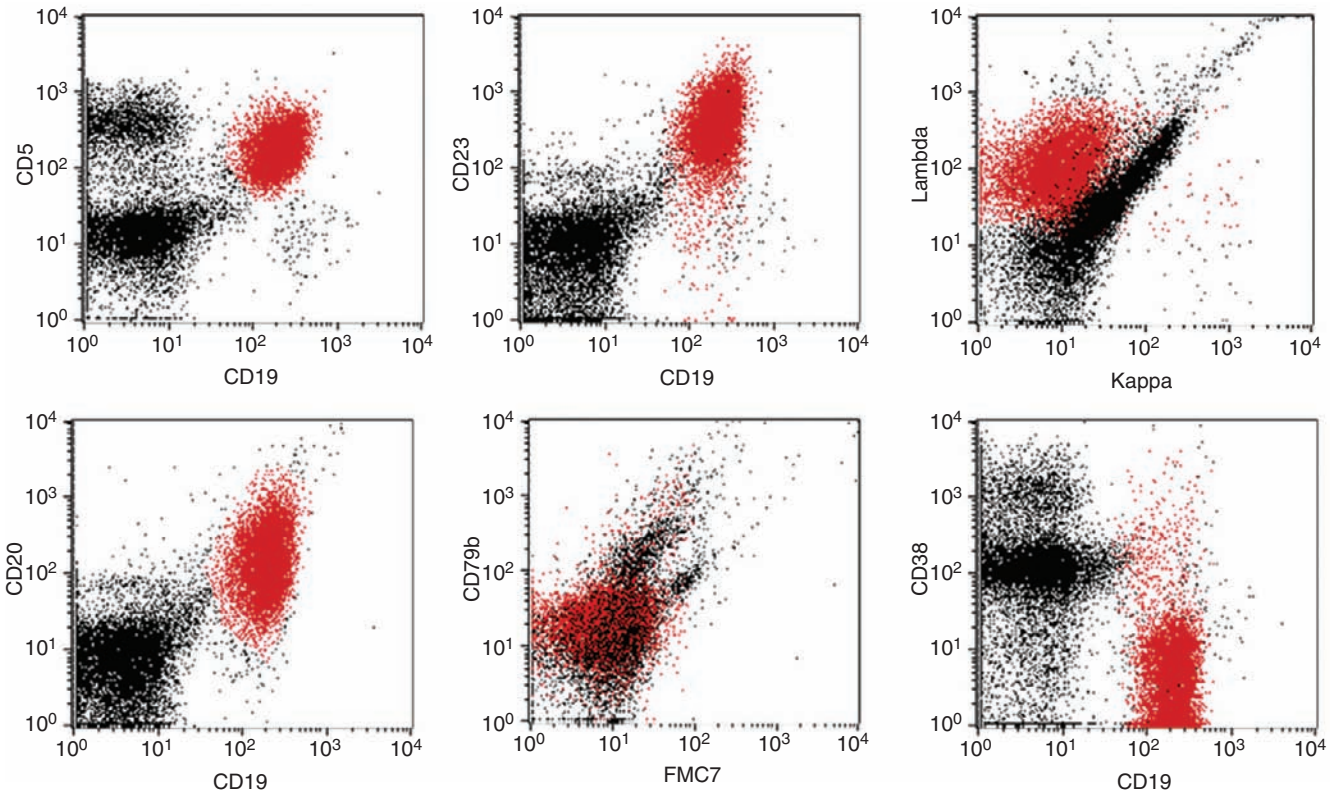



**FIGURE 12-4**

Flow cytometric immunophenotype of chronic lymphocytic leukemia. A prominent B-cell population (red) is present expressing CD5, CD19, CD20 (dim), CD23, and λ light chains. FMC7 and CD79b are not expressed to any appreciable degree. In this case, CD38 is not expressed, a feature suggesting a more favorable prognosis.

of CLL cells) is a poor prognostic indicator in CLL. Several studies have also shown it to be independent of other clinical parameters. The intracellular nonreceptor tyrosine kinase ZAP-70 correlates fairly well with *IGHV* gene mutational status and can also be assessed by flow cytometry or immunohistochemistry. Expression in more than 20% of CLL cells is associated with unmutated *IGHV* genes and poor prognosis.

### Molecular Genetics

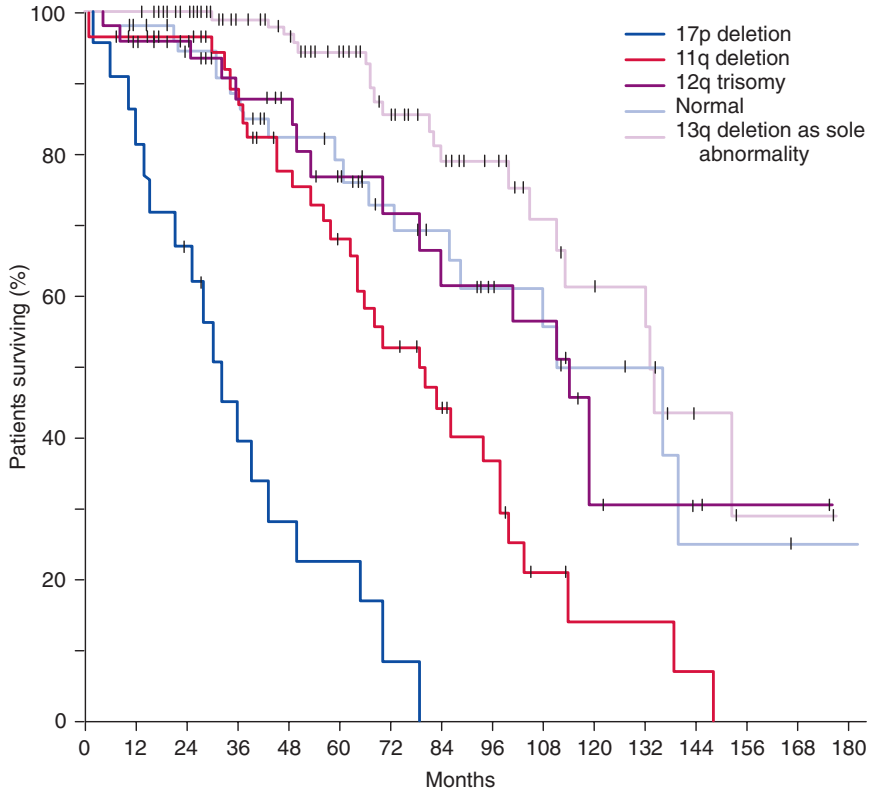
Interphase FISH studies have revealed many recurrent genetic abnormalities that also have clinical importance (Table 12-3). Deletion of 13q is the most common abnormality and is associated with typical morphology and good prognosis (Figures 12-5 and 12-6). Trisomy 12 is associated with atypical morphology and intermediate prognosis. Del 6q and del 11q22-23 have a poor prognosis. Deletion of 17p involving *TP53* is uncommon but is associated with poor prognosis. Assessment of CLL cases for these markers by FISH panels is

**TABLE 12-3**

**Frequency of Chromosomal Abnormalities in CLL***

| Aberration | Percentage of Cases |
|---|---|
| 13q deletion | 55 |
| 11q deletion | 18 |
| Trisomy 12 | 16 |
| 17p deletion | 7 |
| 6q deletion | 6 |
| Trisomy 8q | 5 |
| t(14q32) | 4 |
| Trisomy 3q | 3 |
| Normal | 18 |

*Defined by fluorescence in situ hybridization; some cases may have more than one abnormality.

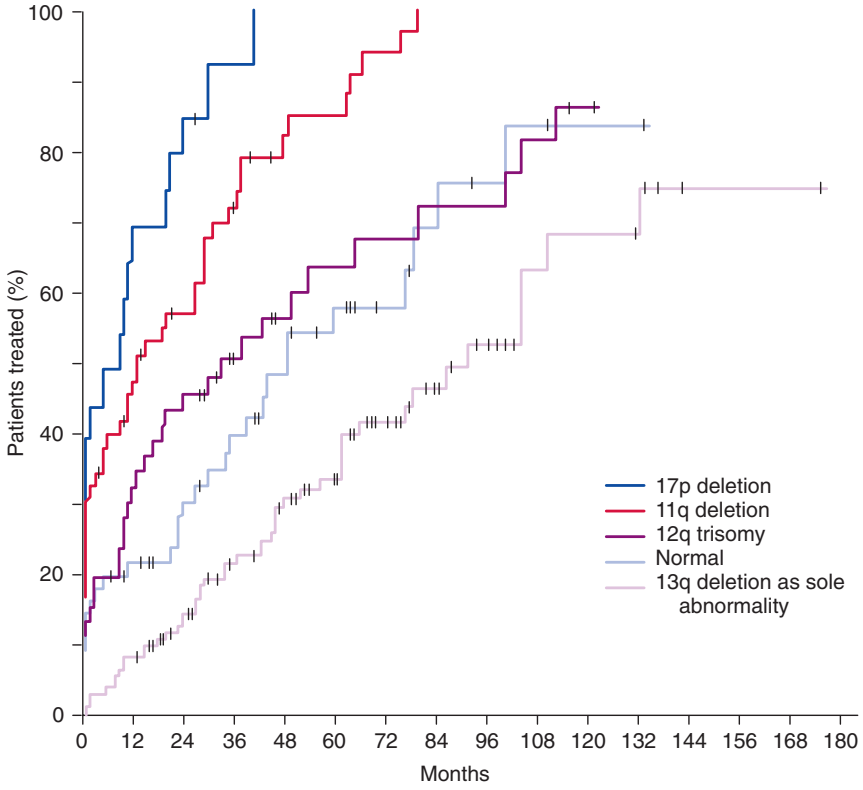



NO. AT RISK

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17p deletion | 23 | 18 | 13 | 8 | 5 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11q deletion | 56 | 53 | 47 | 43 | 33 | 27 | 20 | 15 | 10 | 4 | 2 | 2 | 1 | 0 | 0 | 0 |
| 12q trisomy | 47 | 44 | 41 | 29 | 24 | 17 | 14 | 13 | 12 | 11 | 4 | 3 | 2 | 1 | 1 | 0 |
| Normal | 57 | 51 | 45 | 37 | 30 | 27 | 20 | 17 | 12 | 11 | 6 | 5 | 2 | 2 | 1 | 1 |
| 13q deletion as sole abnormality | 117 | 117 | 106 | 91 | 80 | 63 | 45 | 36 | 24 | 16 | 12 | 11 | 3 | 1 | 1 | 0 |

**FIGURE 12-5**

Outcomes of chronic lymphocytic leukemia patients by fluorescence in situ hybridization abnormality. Karyotypic abnormality predicts survival. *(With permission from Dohner H, Stilgenbauer E, Benner A, et al: Genomic alterations and survival in chronic lymphocytic leukemia,* N Engl J Med *343:1910–1916, 2000.)*




NO. UNTREATED

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17p deletion | 23 | 7 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11q deletion | 56 | 29 | 20 | 13 | 7 | 5 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12q trisomy | 47 | 32 | 26 | 18 | 12 | 9 | 7 | 6 | 6 | 4 | 1 | 0 | 0 | 0 | 0 | 0 |
| Normal | 57 | 42 | 33 | 24 | 17 | 13 | 8 | 5 | 3 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| 13q deletion as sole abnormality | 117 | 108 | 94 | 72 | 58 | 45 | 28 | 21 | 13 | 7 | 6 | 6 | 1 | 1 | 1 | 0 |

**FIGURE 12-6**

Outcomes of chronic lymphocytic leukemia patients by fluorescence in situ hybridization abnormality. Karyotypic abnormality predicts requirement for treatment. *(With permission from Dohner H, Stilgenbauer E, Benner A, et al: Genomic alterations and survival in chronic lymphocytic leukemia,* N Engl J Med *343:1910–1916, 2000.)*

becoming routine. More than any other biomarker in CLL, deletion of 17p is beginning to affect the selection of therapy in individual CLL patients outside of clinical trials.

*IGHV* mutational status has been shown to be an independent predictor of outcome in CLL. *IGHV* mutational status can be characterized as unmutated based on 98% or greater homology to the germline sequence. Patients whose CLL cells have unmutated *IGHV* genes generally have a poorer prognosis than patients whose CLL cells have mutated *IGHV* genes. Approximately 40% to 50% of CLL cases will have unmutated *IGHV* genes. ZAP-70 was identified as one molecule that was important in distinguishing mutated (ZAP-70 negative) from nonmutated (ZAP-70 positive) CLL and was associated with shorter time to treatment from diagnosis. Newer markers such as lipoprotein lipase *(LPL), ADAM29,* and *FCRL2* may become important. *LPL* mRNA levels in particular have been shown in several studies to be associated with *IGHV* mutations and outcome, but these markers require additional validation.

MicroRNAs (miRNAs) have become the focus of intense investigation in CLL. These small noncoding RNAs are important in the regulation of gene expression. Two miRNA genes, *miR-16* and *miR-15,* have been mapped to the minimally deleted region in CLL at 13q14.3. These miRNAs control B-cell proliferation, and deletion in transgenic mice leads to monoclonal B-cell lymphocytosis and CLL-like disease.

Next generation sequencing of CLL has revealed a limited set of recurrent mutations in CLL. *NOTCH1, MYD88, XPO1, and KLHL6* were mutated in 12.2%, 2.9%, 2.4%, and 1.8% of cases, respectively. Differences are present between *IGHV* mutated and unmutated CLL cases. For example, *NOTCH1* mutations were found in 20% of unmutated cases but in only 7% of unmutated cases. Clinical correlates are emerging. *NOTCH1*

mutations are activating mutations and have been reported to be associated with poor survival, independent of *TP53* deletion and *IGHV* mutation.

## Differential Diagnosis

The differential diagnosis of CLL includes other B-cell leukemias and peripheralized lymphomas, particularly mantle cell lymphoma (MCL), marginal zone lymphoma, and follicular lymphoma (FL). Given the phenotypic and morphologic similarities, leukemic MCL is an important consideration. Morphologically, MCL cells can show slight nuclear irregularities and occasional intermediate-sized cells (Figure 12-7). Flow cytometry is extremely helpful. Whereas both CLL and MCL express CD5, CD23 is expressed in most cases of CLL and is absent in MCL. Other useful features include bright CD20 and surface immunoglobulin (sIg) expression in MCL and expression of CD79b and FMC7. The t(11;14)(q13;q32) involving *IGH@* and *CCND1* can confirm a diagnosis of leukemic MCL with the caveat that some cases of myeloma may also have this translocation. However, plasma cell leukemia would not coexpress CD20 and CD5. Table 12-4 shows the

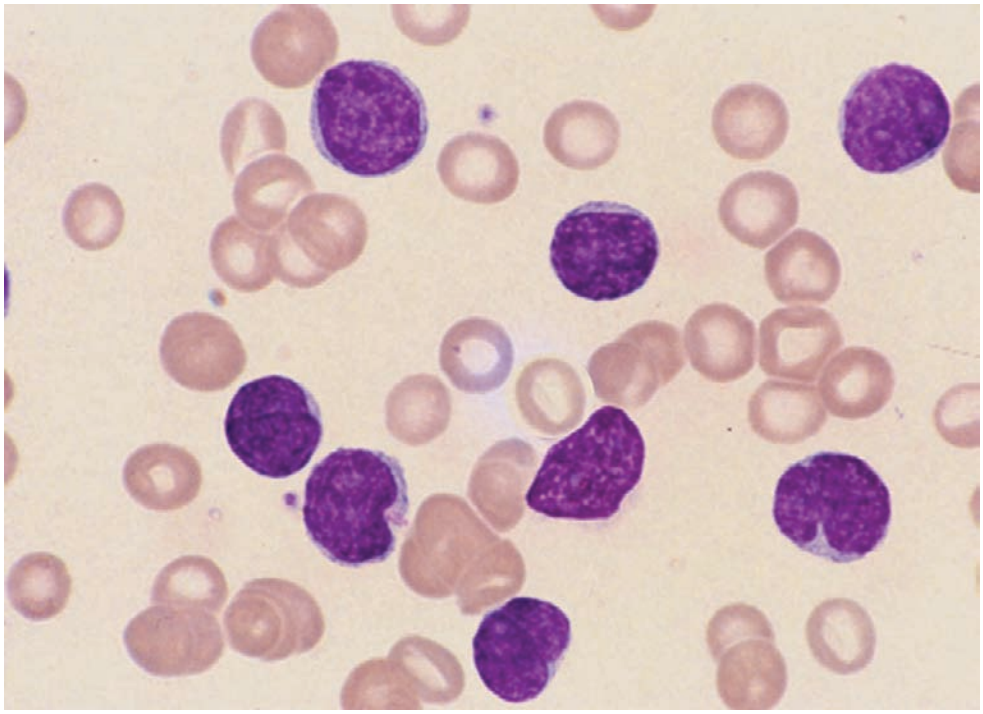


**FIGURE 12-7**

Leukemic mantle cell lymphoma. Note the small to intermediate-sized cells with nuclear irregularities. Some of the cells have small inconspicuous nucleoli that are not seen in the small lymphocytes of chronic lymphocytic leukemia (Wright stain).

**TABLE 12-4**

**Immunophenotype and Molecular Genetic Features of B-Cell Lymphoproliferative Disorders**

| | CD5 | CD10 | CD20 | CD23 | CD79b | FMC7 | CD103 | sIg | t(14;18) | t(11;14) |
|---|---|---|---|---|---|---|---|---|---|---|
| CLL | + | – | +(dim) | + | –/+(dim) | –/+(dim) | – | +(dim)/– | – | – |
| MCL | + | – | + | – | + | + | – | + | – | + |
| MZL | – | – | + | –/+ | + | + | – | + | – | – |
| HCL | – | –/+ | + | – | + | + | + | + | – | – |
| FL | – | + | + | –/+ | + | + | – | +/– | + | – |

*CLL,* chronic lymphocytic leukemia; *FL,* follicular lymphoma; *HCL,* hairy cell leukemia; *MCL,* mantle cell lymphoma; *MZl,* marginal zone lymphoma.

phenotypic and genetic features of the various B-cell lymphoproliferative disorders. Peripheralized FL may also have individual cells that resemble CLL cells but always has at least occasional, and often many, deeply clefted cells. Expression of CD10 and lack of CD5 is the rule in FL. The presence of a t(14;18)(q32;q21) is strong evidence against CLL and favors a diagnosis of FL. Splenic marginal zone lymphoma will typically have occasional cells that have abundant cytoplasm or cytoplasmic villous projections, or both. The immunophenotype is that of an immunoglobulin light chain–restricted, CD5-negative lymphoproliferative disorder. Rare cases of marginal zone lymphoma may express CD5. Some cases of CLL show atypical features such as irregular nuclei or mild deviation from the normal immunophenotype. However, elimination of other serious considerations using combined morphology, flow cytometry, and, when needed, molecular studies allows a confident diagnosis in most cases.

Occasional cases of T-cell PLL can mimic CLL, but attention to the morphologic features (small nucleoli) and flow cytometric immunophenotype makes distinction straightforward. Likewise, some cells of acute lymphoblastic leukemia (with a French-American-British classification L-1 morphologic subtype) might have small cells resembling CLL, but close attention to morphologic details such as chromatin pattern and immunophenotyping allows easy distinction from CLL.

Persistent polyclonal B-cell lymphocytosis might be mistaken for a B-cell leukemia. This disorder appears most commonly in female smokers with a modest lymphocytosis composed of polyclonal B cells, which may coexpress CD5. There is a strong association with HLA-DRB1*07. Deeply clefted lymphocytes are present, which makes confusion with CLL less likely than for other entities such as leukemic FL. In fact, *BCL2/IGH@* translocations have been detected in these cases but are also polyclonal. Isochromosome 3q has been reported in one-third of cases. Uncommonly (less than 5%), patients with persistent polyclonal B-cell lymphocytosis may develop subsequent B-cell lymphoma. CLL-phenotype monoclonal B-cell lymphocytosis should be distinguished from CLL based on absolute monoclonal B-cell count (see monoclonal B-cell lymphocytosis).

### Prognosis and Therapy

As noted previously, the prognosis varies depending on stage and biologic marker studies. Still, the disease course is measured in years to decades. A conservative approach to therapy is often taken (e.g., watch and wait), withholding treatment until the patient becomes symptomatic. Current approved therapies are generally aimed at symptomatic disease and are not curative. Recently, nucleoside analogues such as fludarabine, cyclophosphamide, and rituximab have become prominent in first-line therapies with alemtuzumab used in second line. Whether targeted therapies exploiting genetic abnormalities such as *NOTCH1* mutations are effective will require further study.

## MONOCLONAL B-CELL LYMPHOCYTOSIS

Monoclonal B-cell lymphocytosis (MBL) is a low level (less than $5 \times 10^9$/L monoclonal B-cells) asymptomatic proliferation of monoclonal B cells. Proposed criteria are in Table 12-5. MBL has been recognized in the past, but only recently have attempts been made to better define and characterize it. The most data exists for CLL phenotype MBL. It is known that 2% to 3% of healthy subjects have an MBL using sensitive multiparameter flow cytometry, most of which are $CD5^+$ CLL phenotype MBL. The incidence increases with age, reaching up to 5% in adults older than 60 years. Most patients remain asymptomatic, but there is a low incidence of progression to CLL. In fact, recent studies suggest that nearly

**TABLE 12-5**

**Diagnostic Criteria and Subclassification for MBL**

***Diagnostic Criteria***

- Documentation of clonal B-cell population by one or more of the following:
  - Light chain restriction
  - Overall κ:λ ratio >3:1 or <0.3:1 or >25% of B cells lacking or expressing low-level surface immunoglobulin
  - Heavy chain monoclonal *IGHV* rearrangements
- Presence of a disease-specific immunophenotype
  - Absolute B-cell count less than $5 \times 10^9$ cells/L
  - No other features of a lymphoproliferative disorder or autoimmune disease
  - Normal physical examination (no lymphadenopathy or organomegaly)
  - Absence of B symptoms (for example, fatigue, weight loss and night sweats) attributable to NHL
  - No autoimmune or infectious disease

***Subclassification***

- CLL-like phenotype
  - Coexpression of CD5 with CD19, CD20 (dim), and CD23
  - Light chain restriction with dim surface immunoglobulin expression (small MBL clones may be oligoclonal and thus not light chain restricted)
- Atypical-CLL phenotype
  - Coexpression of CD5 with CD19 but $CD23^-$ or CD20 (bright)
  - Light chain restriction with moderate-to-bright surface immunoglobulin expression
  - Exclude t(11;14) to rule out mantle cell lymphoma
- Non-CLL phenotype
  - $CD5^-$
  - $CD20^+$
  - Light chain restriction with moderate-to-bright surface immunoglobulin expression

Adapted from Marti GE, Rawstron AC, Ghia P, et al: Diagnostic criteria for monoclonal B-cell lymphocytosis, *Br J Haematol* 130:325–332, 2005.

all CLL is preceded by MBL. For patients with MBL that is discovered for clinical reasons such as modest leukocytosis, the rate of progression to CLL that requires therapy is 1% to 2% per year. Of note, the *IGHV* and cytogenetic features are that of good prognosis CLL. However, MBL found in population screening studies using high-sensitivity methods show a different *IGHV* gene repertoire from that found in CLL, and these patients only rarely progress to overt CLL. As a result, it may be necessary to distinguish between clinical MBL and population-screening MBL. The former is the type that may be seen in routine practice.

Bone marrow biopsy is not routinely done for MBL, but it can show variable infiltration by lymphocytes, usually 10% to 20%, but more extensive involvement can be seen. A higher absolute B cell count is associated with progressive lymphocytosis in CLL-type MBL, but there is currently no way to predict progression in individual patients. Patients are followed periodically to monitor for potential development of CLL or other lymphoma. Treatment is not required for MBL of any subtype.

## B-CELL PROLYMPHOCYTIC LEUKEMIA

PLL is defined as a B-cell leukemia that is composed of more than 55% prolymphocytes in the peripheral blood. PLL can arise from preexisting CLL (prolymphocytic transformation of CLL), in which case it should be noted in the diagnosis. The current concept of PLL is evolving, because cases of leukemia with more than 55% prolymphocytes appear to be a heterogeneous group of diseases encompassing transformed CLL, nucleolated variants of MCL, and de novo PLL. The de novo form is discussed in this section. De novo PLL is a rare disorder.

**PROLYMPHOCYTIC LEUKEMIA—FACT SHEET**

**Definition**
- Mature B-cell leukemia composed of prolymphocytes (>55% of lymphocytes)

**Incidence**
- Rare (<1% of lymphoid leukemias)

**Clinical Features**
- Older adults (median age, 67 years)
- Splenomegaly and marked leukocytosis, without lymphadenopathy

**Prognosis and Therapy**
- Median survival is 5 years
- Therapy is variable, consisting of purine analogs or multiagent chemotherapy. Monoclonal antibody therapy is being explored

### Clinical Features

Patients are usually older (median age, 70 years) with a male predominance. Patients have splenomegaly and a high leukocyte count (often more than $100 \times 10^9$/L), and usually lack significant lymphadenopathy. Cytopenias are common, usually resulting from marrow replacement.

### Pathologic Features

#### Morphology

The peripheral blood smear shows numerous prolymphocytes (more than 55% of lymphocytes) characterized by intermediate size, slightly dispersed chromatin, and a prominent central nucleolus. The cytoplasm is pale blue and moderate to abundant in amount. The nuclear contours are classically round, but indentations can be seen (Figure 12-8). The bone marrow often shows extensive involvement with intermediate-sized cells containing small nucleoli. The spleen may show white pulp involvement with or without red pulp involvement. Again the cells are round and intermediate in size and show small central nucleoli in tissue section.

#### Immunophenotype

The reported immunophenotype of PLL is variable, likely because previous reports have included several entities (listed previously). PLL expresses CD19, CD20 (bright), CD79b, FMC7, and sIg. CD5 may be expressed

**PROLYMPHOCYTIC LEUKEMIA—PATHOLOGIC FEATURES**

**Morphology**
- Peripheral blood: more than 55% of lymphocytes are prolymphocytes; usually a high white blood cell count
- Bone marrow: extensive involvement by prolymphocytes
- Spleen: white pulp predominant or diffuse involvement

**Immunophenotype**
- $CD5^-$, $CD10^-$, $CD20^+$ (bright), $CD23^-$, $sIg^+$ (bright), $FMC7^+$, $CD79b^+$

**Genetics**
- No specific abnormality
- Abnormalities of TP53, deletion 13q14, and deletion 11q23

**Differential Diagnosis**
- CLL with increased prolymphocytes
- MCL, nucleated variant
- Other non-Hodgkin lymphoma

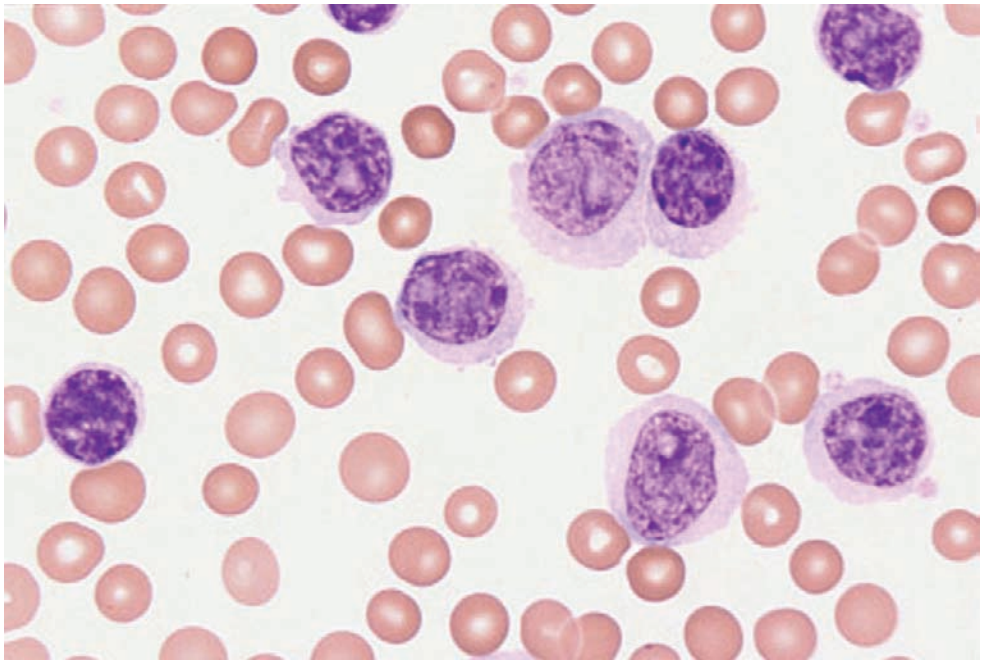


**FIGURE 12-8**
B-cell prolymphocytic leukemia. Numerous intermediate-sized prolymphocytes are present with prominent central nucleoli (Wright stain).

in some cases; however, prior data concerning CD5 expression may be inaccurate because of differences in inclusion criteria. Expression of cyclin D1 strongly suggests a nucleolated variant of MCL and essentially excludes PLL.

### Molecular Genetics

Rearranged immunoglobulin genes are present. Given the confusion in the precise definition related to MCL, the molecular genetic features of PLL are not yet precisely defined. The t(11;l4)(q13;q32) resulting in an *IGH@/CCND1* fusion is present in a minority (20% of some series) of previously reported PLL cases. However, these cases are best considered a variant of MCL. Other nonspecific abnormalities reported in PLL include *TP53* abnormalities (deletion or mutation in 75% of cases) and deletions of 13q14 (55% of cases) and 11q23 (39% of cases).

## Differential Diagnosis

The differential diagnosis of a leukemia composed of prolymphocytes includes transformed CLL, MCL variant, T-cell PLL, and HCLv. A history of CLL allows the reasonable diagnosis of a case of B-cell leukemia with more than 55% prolymphocytes as prolymphocytic transformation of CLL. The presence of t(11;14)(q13;q32) can be used to confirm a diagnosis of MCL. T-cell PLL cells may resemble B-cell PLL cells morphologically because of the presence of nucleoli, although the T-PLL cells tend to have more nuclear irregularities and the chromatin may be more condensed. Immunophenotyping easily identifies a T-cell process in T-cell PLL. HCLv cells may have small nucleoli, but still have some cytoplasmic features of HCL that are not seen in PLL. The presence of CD103 also favors HCLv.

## Prognosis and Therapy

PLL is usually an aggressive disease, although a subset of cases will not follow a rapidly progressive course. Older age, anemia, and presence of TP53 abnormalities may indicate more aggressive disease. Therapy has not been uniform for this uncommon disease and has varied from low-grade alkylator-based therapy to more aggressive anthracycline-containing regimens. More recently, nucleoside analogues and monoclonal antibodies (rituximab and alemtuzumab) have been used. PLL is difficult to treat, and median survival is 3 to 4 years.

# HAIRY CELL LEUKEMIA

HCL is an uncommon mature B-cell leukemia composed of cells with cytoplasmic projections when examined on Wright-stained smear. It represents 2% of lymphoid leukemias.

## Clinical Features

HCL appears in middle-aged to older patients (median age, 50 years), with a 4:1 male-to-female predominance. Patients typically exhibit cytopenias, including monocytopenia and splenomegaly. Infectious complications also occur, particularly with bacterial organisms.

### HAIRY CELL LEUKEMIA—FACT SHEET

**Definition**
- Mature B-cell leukemia composed of cells with characteristic cytoplasmic hairy projections

**Incidence**
- Uncommon (<2% of lymphoid leukemias)

**Clinical Features**
- Presents in middle age (median age, 50 years), with male predominance
- Cytopenias, including monocytopenia and splenomegaly, are common

**Prognosis and Therapy**
- High rate of durable remission with purine nucleoside analog therapy

## Pathologic Features

### Morphology

On peripheral blood smear, HCL cells have a characteristic appearance. The chromatin is slightly less condensed than a mature non-neoplastic lymphocyte with a reticulated pattern. The cytoplasm is moderate in amount and stains pale gray-blue. It has a textured or lacy quality to it. Fine cytoplasmic projections can be seen around the circumference of the cell (Figure 12-9). The bone marrow aspirate may be hemodiluted, because reticulin fibrosis is usually present and accounts for frequent "dry

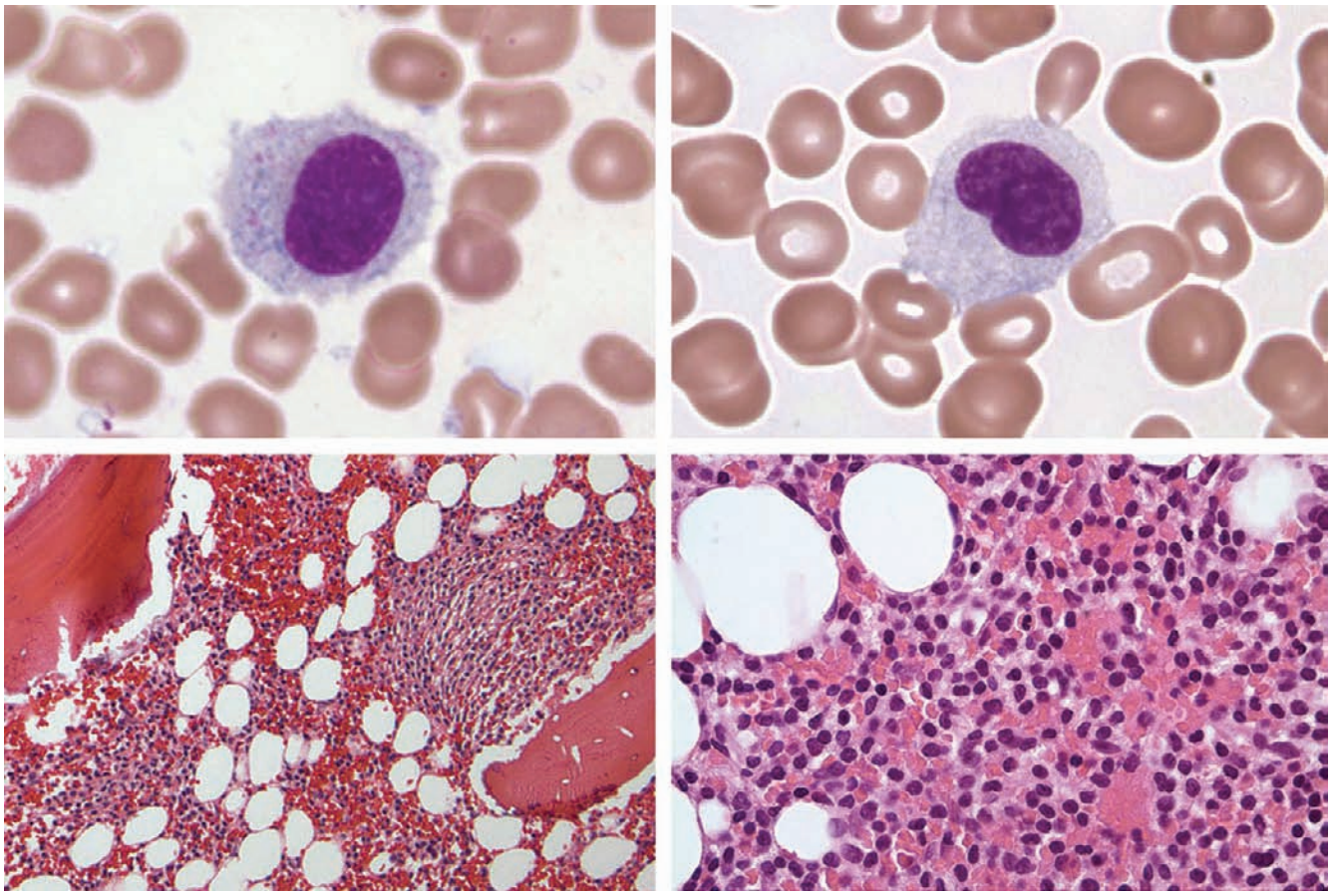


**FIGURE 12-9**

Hairy cell leukemia. *Top*, Typical morphology. Small cells with round to bean-shaped nuclei with reticulated chromatin pattern and abundant lacy cytoplasm. Some cells may have ragged circumferential cytoplasmic borders imparting a hairy appearance (Wright stain). *Bottom*, Aggregates of hairy cells that at higher magnification show the characteristic morphology with cells spaced apart from one another because of the abundant cytoplasm (hematoxylin and eosin).

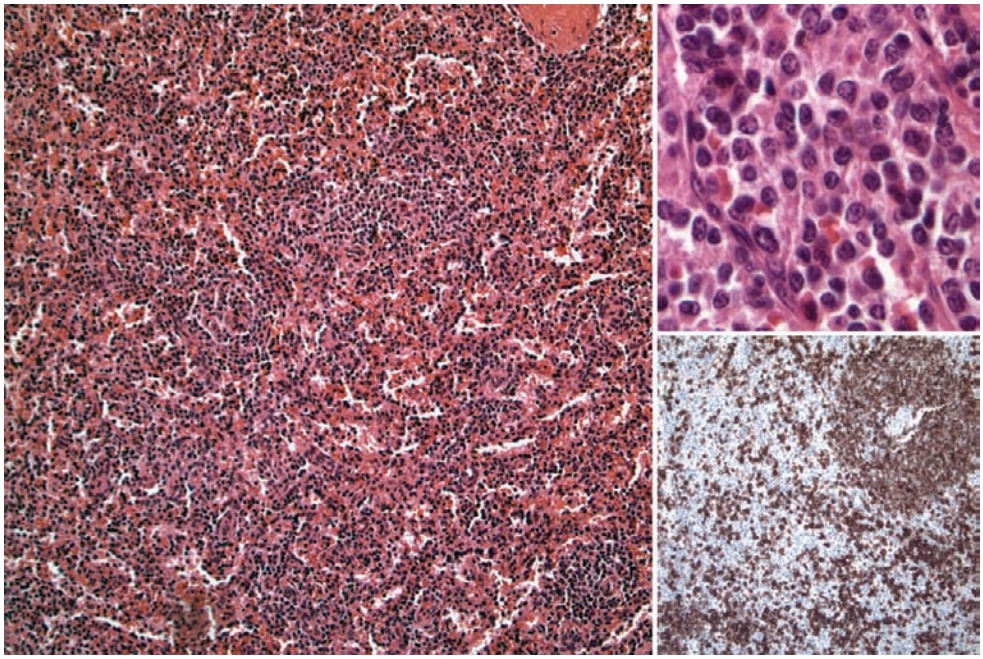


**FIGURE 12-10**

Hairy cell leukemia involving spleen with red pulp. *Left*, low magnification; *top right*, high magnification. CD20 immunostain (*bottom right*) highlights the B-cell red pulp infiltrate.

taps" in HCL patients. Trephine biopsy is therefore important. The infiltrate can be nodular, interstitial, or paratrabecular (see Figure 12-9). The collections of lymphocytes may have a "fried egg" appearance, imparted by the moderate amounts of cytoplasm. Nuclei are usually round to oval, but the cells may also have a spindled quality. Involvement may be subtle, and immunostains for B-cell markers such as CD20 or DBA.44 can highlight the cells, often showing many more cells than suspected on hematoxylin and eosin–stained sections. Careful examination of a CD20-stained section may show the cytoplasmic projections. Mast cells are increased in bone marrow of HCL patients and may be seen on aspirate smear and in tissue section. In the spleen, HCL involves the red pulp preferentially (Figure 12-10), which is in contrast to the white pulp involvement seen in other low-grade B-cell lymphoproliferative disorders. Blood lakes are seen. These are non–endothelium-lined spaces filled with erythrocytes and surrounded by leukemia cells. A tartrate-resistant acid phosphatase stain is strongly positive in HCL cells, but is not required for diagnosis in the era of multiparameter flow cytometry.

**HAIRY CELL LEUKEMIA—PATHOLOGIC FEATURES**

**Morphology**

- Peripheral blood: circulating hairy cells
- Bone marrow: interstitial, nodular, or diffuse infiltrate of cells (fried egg appearance or spindling); reticulin fibrosis
- Spleen: red pulp involvement

**Immunophenotype**

- $CD5^-$, $CD10^-$, $CD11c^+$ (bright), $CD19^+$, $CD20^+$, $CD22^+$ (bright), $CD25^+$, $CD103^+$, $CD123^+$, annexin $A1^+$, $sIg^+$, phospho-ERK

**Genetics**

- No specific karyotypic abnormality
- *BRAF* V600E mutation*

**Differential Diagnosis**

- SMZL
- HCLv

*Initial report demonstrated *BRAF* V600E in virtually all cases of HCL.

### Immunophenotype

Flow cytometric analysis shows the cells express CD19, CD11c, CD20, CD22, CD25, CD103, CD123, FMC7, annexin A1, and monotypic immunoglobulin light chains. Strong coexpression of CD11c, CD22, CD25, and CD103 is characteristic of this leukemia. CD103, CD123, and annexin A1 are fairly sensitive and specific. Cyclin D1 can be weakly expressed in HCL, and CD10 can be seen in approximately 10% of cases. Phospho-ERK can be detected by immunohistochemistry in bone marrow biopsy sections and is likely a consequence of the BRAF V600E mutation.

### Molecular Genetics

Immunoglobulin genes are monoclonally rearranged. There is no specific karyotypic abnormality; however, recent studies show that gain of 5q13-31 and loss of

7q32 occur in a minority of cases and that the region at 7q32 is the same area also deleted in some cases of SMZL. Expression array studies have shown that HCL cells are related to postgerminal center memory B cells, but they differ from memory cells in expression of chemokine and adhesion molecules. As mentioned, although cyclin D1 is overexpressed in some cases, the t(11;14)(q13;q32) seen in MCL is absent.

Next generation sequencing in HCL has led to the discovery of *BRAF* V600E mutations in HCL. At the time of this writing, virtually all cases of HCL have been reported to harbor this mutation. Among the peripheral B-cell leukemias and lymphomas, *BRAF* V600E appears to be specific to HCL. Confirmatory studies will be required, but this mutation may be diagnostically useful and a potential therapeutic target.

### Differential Diagnosis

The characteristic morphologic and immunophenotypic features usually allow easy separation from other B-cell lymphoproliferative disorders such as CLL, PLL, and the leukemic phase of FL or MCL. The main diagnostic considerations are SMZL and HCLv. Morphologically, HCL cells have cytoplasmic projections around the circumference of the cell, whereas SMZL cells usually have their cytoplasmic projections only around part of the cells, often at opposite ends of the cells. The cells in SMZL are often more heterogeneous appearing, with some containing nucleoli and others more closely resembling CLL cells. HCLv cells have a more condensed chromatin pattern than do typical HCL cells, and they also have a small visible nucleolus. Immunophenotypically, the expression of bright CD11c, CD22, CD25, and CD103 is characteristic of HCL. CD25 is absent in HCLv. SMZL usually lacks these markers but may express them in a minority of cases. For example, CD103 can be expressed in approximately 15% of cases. When dealing with lymphocytes with villous, hairy projections, CD123 appears useful in distinguishing HCL (positive) from HCLv and SMZL (negative). This latter marker is not specific because it can be seen in some cases of CLL, acute myeloid leukemia, and B-cell precursor acute lymphoblastic leukemia. Annexin A1 is also reported to be seen in essentially all cases of HCL and is specific (approaching 100%).

### Prognosis and Therapy

With recent therapies using nucleoside analogues such as pentostatin or cladribine, the prognosis of HCL is excellent with high complete response rates and excellent long-term survival. Current efforts are aimed at eradication of minimal residual disease to prevent relapse. Immunostaining of trephine biopsy specimens using B-cell markers has identified minimal residual disease that was associated with relapse.

## HAIRY CELL LEUKEMIA VARIANT

HCLv is uncommon, representing 0.4% of chronic lymphoproliferative disorders. It differs from HCL in several clinical, morphologic, and immunophenotypic features. Currently, HCLv is a provisional World Health Organization entity considered under the rubric of splenic B-cell lymphoma–leukemia, unclassifiable, in order to reflect our incomplete understanding of the definition and uncertain relationship to other ill-defined splenic lymphomas–leukemia, including so-called splenic diffuse small B-cell lymphoma.

### Clinical Features

The median age at onset is 71 years, older than in typical HCL, and there is only a slight male predominance (gender ratio of 1.6). Splenomegaly and cytopenias are common, as in typical HCL, but monocytopenia is not. There is usually a leukocytosis instead of leukopenia.

**HAIRY CELL LEUKEMIA VARIANT—FACT SHEET**

**Definition**

- Unusual B-cell leukemia with features resembling typical HCL but having higher white blood cell count (WBC) than HCL; cytologic variation with more condensed chromatin and nucleoli

**Incidence**

- Rare (<1% of lymphoid leukemias, estimated to be 10% of HCL)

**Clinical Features**

- Presents (median age, 71 years) with leukocytosis and lacks monocytopenia
- Splenomegaly and cytopenias usually are present
- A monoclonal serum paraprotein is often found

**Prognosis and Therapy**

- Treatment varies according to patient features; HCL variant will not respond to typical HCL treatment
- Splenectomy results in durable partial responses and is of value in palliation
- Median survival is 9 years

## Pathologic Features

### Morphology

Morphologically, the cells resemble typical HCL cells in blood, but have a more condensed chromatin pattern and a nucleolus (Figure 12-11). The cytoplasmic projections are less prominent than in typical HCL. Bone marrow infiltration appears similar in pattern to typical HCL, with an interstitial or sinusoidal pattern commonly seen. Some cases may show a fried egg appearance. In the spleen, as in typical HCL, there is a predilection for diffuse red pulp involvement (Figure 12-12). Portal and sinusoidal infiltrates are seen in the liver.

### Immunophenotype

Immunophenotyping shows some similarities to typical HCL. HCLv cells express CD19, CD20, CD22, and CD11c. CD103 is expressed in the majority of cases

**HAIRY CELL LEUKEMIA VARIANT—PATHOLOGIC FEATURES**

**Morphology**
- Peripheral blood: cells resembling HCL cells but with more condensed chromatin pattern and small nucleoli (prolymphocytoid variant of HCL)
- Bone marrow: sinusoidal or interstitial
- Spleen: red pulp involvement

**Immunophenotype**
- $CD5^-$, $CD10^-$, $CD19^+$, $CD20^+$, $CD22^+$, $CD25^-$, $CD103^+$, $CD123^-$, annexin $A1^-$, $sIg^+$

**Genetics**
- No specific abnormality

**Differential Diagnosis**
- SMZL
- HCL
- Splenic diffuse red pulp small B-cell lymphoma (relationship between this and HCLv under investigation)

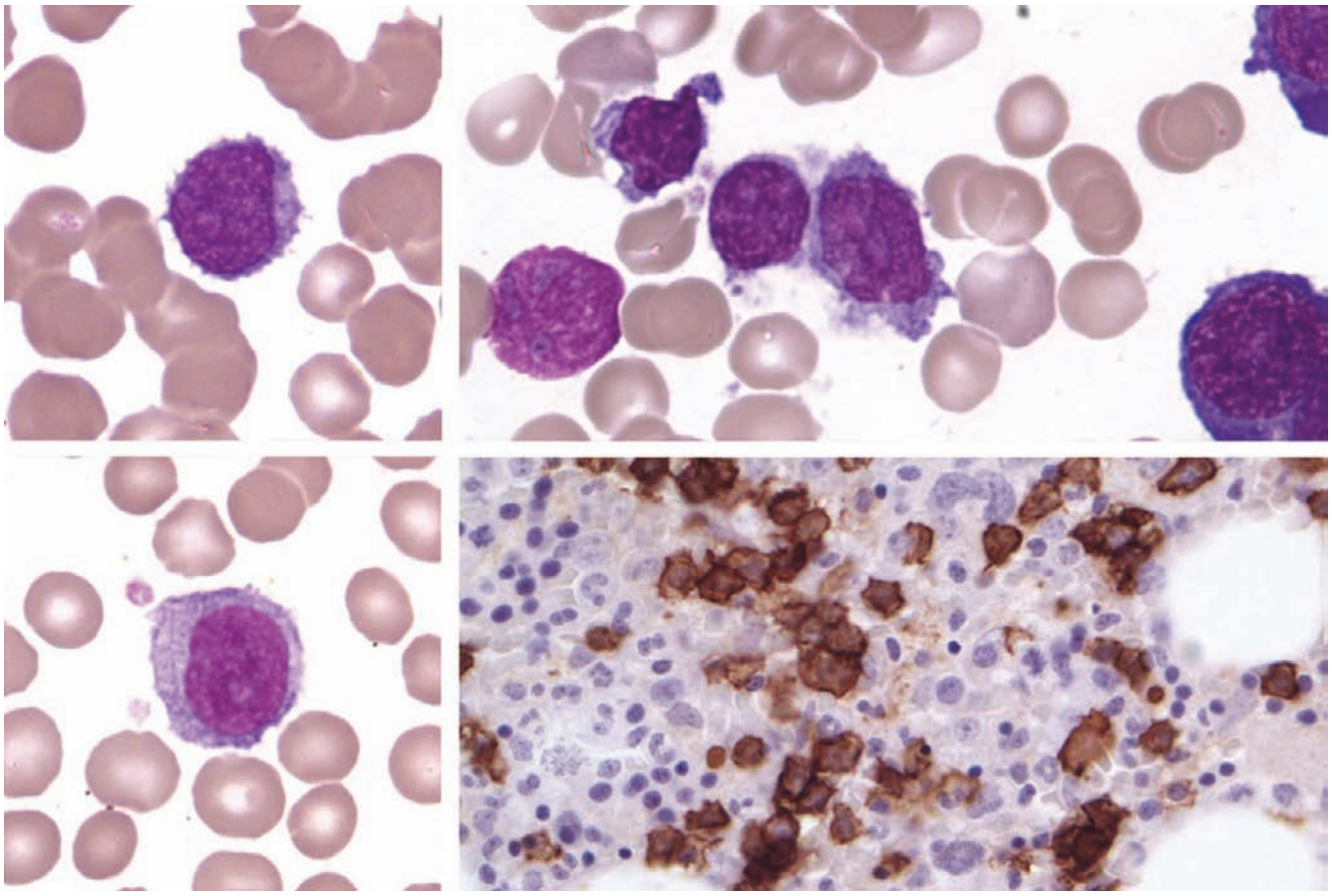


**FIGURE 12-11**

Hairy cell leukemia variant. *Left*, Cells with scant to moderate amounts of cytoplasm with variable cytoplasmic projections and small nucleoli (Wright stain). Bone marrow aspirate shows similar cells *(top right)*. Immunostains in the bone marrow show that a subtle $CD20^+$ B-cell infiltrate is present. Flow cytometry showed an immunophenotype of $CD19^+$, $CD20^+$, $CD5^-$, $CD10^-$, $CD25^-$, $CD103^+$. Tartrate-resistant acid phosphatase stain was negative.

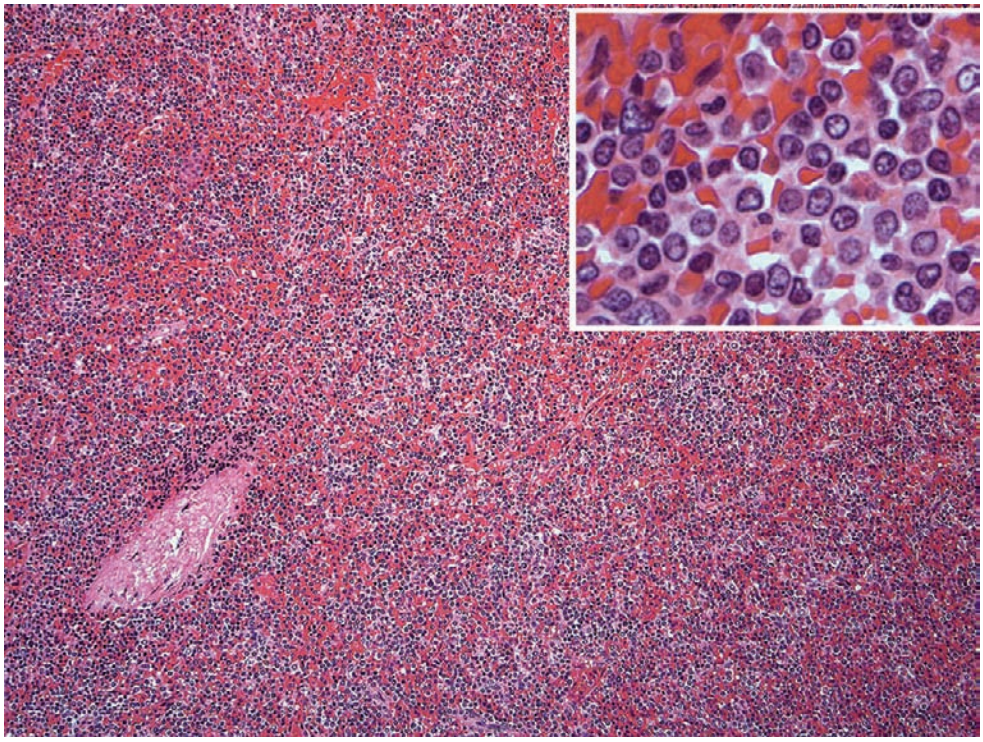


**FIGURE 12-12**

The same patient as in Figure 12-11 underwent splenectomy, which demonstrated a marked red pulp leukemic infiltrate similar to typical hairy cell leukemia (hematoxylin and eosin).

(60%), but CD25, CD123, and annexin A1 (ANXA1) are absent. Unlike typical HCL, HCLv cells are negative for tartrate-resistant acid phosphatase.

### Molecular Genetics

No specific chromosomal abnormalities have been described. However, studies examining *IGH@* mutational status and repertoire show that HCLv cases have characteristics close to SMZL and diffuse splenic red pulp lymphoma rather than HCL. Similarly, expression of *AID*, required for *IGH@* somatic hypermutation and class switch recombination, is higher in HCL compared with HCLv and SMZL. These data suggest that HCLv may be more closely related to SMZL.

## Differential Diagnosis

The main differential diagnostic considerations are HCL and SMZL. In addition to the classic morphologic features described earlier, flow cytometry can be helpful. HCL expresses CD11c, CD25, CD103, CD123, and ANXA1, whereas the variant form usually lacks CD25, CD123, and ANXA1. SMZL cells also lack CD103 in most cases. CD123 and ANXA1 are also absent in SMZL. Recently, it has been suggested that expression of preswitched immunoglobulin (IgM/IgD) or both preswitched and postswitched immunoglobulin (IgG/IgA) heavy chains are characteristic of HCLv. SMZL characteristically lacks IgG or IgA.

## Prognosis and Therapy

Distinguishing HCLv from HCL is of clinical importance because of the lack of response to standard HCL therapy. Monoclonal antibody therapy against CD20 and CD22 shows promise. Splenectomy has been suggested as an alternative therapy in these patients.

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13

# Mature T-Cell and Natural Killer Cell Leukemias

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## INTRODUCTION

The mature T-cell and natural killer (NK) cell leukemias form a heterogeneous group of diseases with diverse etiologies and markedly varied clinical behavior. When applied to T cells, the term mature or peripheral is often used to describe nonblastic T-cell neoplasms marked by their resemblance to post-thymic T lymphocytes and characterized by the absence of terminal deoxynucleotidyl transferase (TdT) expression and usually by the presence of surface CD3 expression (except in those cases in which CD3 is aberrantly deleted). CD3 is a structure of several polypeptides that is intimately associated with the surface T-cell receptor complex. It is present in the cytoplasm of early T-cell precursors but is present on the cell surface only in the post-thymic stage of T-cell maturation. The diagnosis and appropriate classification of T-cell and NK cell leukemias rely heavily on the correlation of morphologic, immunophenotypic, genetic, and clinical data.

## EVALUATION OF PERIPHERAL BLOOD IN MATURE T-CELL LEUKEMIAS

### MORPHOLOGIC EVALUATION

Thorough morphologic assessment of a carefully prepared peripheral blood smear can yield valuable clues in the diagnosis of mature T-cell leukemias. As with other lymphoproliferative disorders, the peripheral blood morphology of various T-cell leukemias is often not specific as to subtype; however, specific morphologic findings can be of use in the differential diagnosis and subclassification of these disorders. For example, the distinct morphology of large granular lymphocyte (LGL) leukemias helps to set them apart from other T-cell leukemias or lymphomas (e.g., hepatosplenic T-cell lymphoma) that can manifest with similar clinical patterns of anatomic distribution. Likewise, the bizarre, flowerlike appearance of the neoplastic cells in human T-lymphotropic virus type 1 (HTLV-1)–associated adult T-cell leukemia–lymphoma (ATLL) can be contrasted with the more subtle, delicate nuclear folding of cerebriform lymphocytes in Sézary syndrome (SS)–mycosis fungoides (MF).

Although peripheral blood smear examination is an indispensable part of the diagnostic evaluation in T-cell leukemias, it is important not to get dogmatic regarding morphologic correlates in these diseases. The abnormal lymphocytes in a variety of T-cell lymphomas can mimic the cells of more specific entities such as SS or ATLL. Likewise, the LGLs synonymous with LGL leukemia can be present in a variety of reactive lymphocytoses. As with other diagnostic modalities, examination of the blood smear should be considered necessary but not sufficient for the specific diagnosis and classification of mature T-cell leukemias.

### FLOW CYTOMETRIC IMMUNOPHENOTYPING

The value of flow cytometric immunophenotyping in the diagnosis and monitoring of T-cell leukemias and lymphomas is generally underappreciated, in part due to long-heralded truisms. The common wisdom states that because T cells do not have an easily assessable phenotypic measure of clonality (analogous to immunoglobulin light chain expression on B cells), flow cytometry is of limited value in the diagnosis and management of T-cell leukemias and lymphomas. This concept would be true if clonality were the only important determinant in diagnosis. In fact, flow cytometry is highly useful in the workup of T-cell neoplasms for two main reasons. First, a majority (greater than 90% in one study) of T-cell neoplasms show at least one demonstrable immunophenotypic aberrance on flow cytometric analysis (e.g., deletion of normally expressed antigens,

differences in intensity of antigen expression compared to normal T cells), providing immunophenotypic evidence of malignancy. Second, the specific pattern of aberrance is often characteristic of a particular type of T-cell lymphoma or leukemia and therefore can be of help in subclassification. Most recently, the use of large T-cell receptor Vβ family–specific antibody panels that cover most of the T-cell receptor family repertoire has made immunophenotypic analysis for restricted T-cell receptor expression (as a surrogate for T-cell monoclonality) possible. However, antibodies to some families are not yet commercially available, and interpretation of a clonal population requires marked skewing of the normal T-cell repertoire distribution. Nonetheless, a high percentage of T-cell neoplasms show some immunophenotypic aberrancy. With these concepts and the fact that peripheral blood and bone marrow are excellent substrates for flow cytometry, there is a strong case for the routine flow cytometric analysis of either blood or bone marrow in the diagnostic evaluation of T-cell leukemias.

NK-cells and some cytotoxic T-cell subsets also express a class of receptors known as killer-associated immunoglobulin-like receptors (KIRs). Recently, the assessment of KIR isoforms has been used in the assessment of clonality of NK or T-LGL proliferations, analogous to the use of κ and λ light chain for clonality detection in B cells, or the use of Vβ isoforms for clonality in T cells.

The effective application of flow cytometry to the diagnosis of T-cell leukemias also requires knowledge of the array of normal T and NK lymphocyte subsets that may be found in peripheral blood and bone marrow. A lack of knowledge of physiologic immunophenotypes of these subsets may lead to overdiagnosis of abnormal T cell populations. For example, the normal (usually minor) subset of T cells that expresses the T-γδ receptor complex manifests as a population of T cells with modally brighter CD3 than other T cells and the absence of CD4, CD5, and CD8. Physiologic expansions of this normal subset may be interpreted erroneously as immunophenotypically aberrant T cell populations. Similarly, dermal-derived T lymphocytes may show downregulation of CD7 that could be erroneously interpreted as aberrant.

## ANTIGEN RECEPTOR GENE REARRANGEMENT STUDIES

In the case of B-cell leukemias and lymphomas, clonality can be inferred by the restriction of B-cell populations to the expression of a single class of immunoglobulin light chain (either κ or λ). Unfortunately, the molecular components of the T-cell receptor complex come not in two types but in dozens, making their routine assessment challenging for diagnostic laboratories. As a result, the DNA-based assessment for clonal patterns of T-cell antigen receptor gene rearrangement has become the standard for assessment of T-cell clonality.

As noted in the previous discussion of flow cytometry, assessment of clonality per se is not always necessary in the diagnosis of either B- or T-cell neoplasms. However, because T-cell leukemias and lymphomas often present diagnostic challenges and non-neoplastic conditions may enter the differential diagnosis, gene rearrangement studies are a relatively common part of their workup. With advances in polymerase chain reaction–based T-cell gene rearrangement studies, this once cumbersome analysis has evolved to become relatively simple, while preserving high levels of sensitivity and specificity.

## CYTOGENETIC ANALYSIS

The diagnostic contribution of karyotypic analysis to mature T-cell leukemias varies depending on the specific disease entity, as noted in the individual descriptions that follow. In general, the cytogenetic analysis of mature, nonblastic leukemias is challenging because the cells in these disorders often do not have high proliferative rates, and standard cytogenetic analysis relies on the isolation of dividing cells. As a result, cells in such disorders can be analyzed using either cultures stimulated by mitogens or interphase methods (i.e., fluorescence in situ hybridization) that do not require the presence of dividing cells. In some instances, such as T-cell prolymphocytic leukemia (T-PLL), cells have a fairly high proliferative rate and yield characteristic karyotypic abnormalities, making cytogenetic analysis an important aspect of diagnosis and classification.

# SPECIFIC TYPES OF MATURE T-CELL AND NATURAL KILLER CELL LEUKEMIAS

## LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

### Clinical Features

In the mid 1970s, there were reports of a distinct type of lymphoproliferative disorder marked by the leukemic proliferation of T cells containing prominent azurophilic cytoplasmic granules and expressing Fc receptors for the γ heavy chain of the immunoglobulin G molecule. This disorder has since been known by many names, including T-gamma lymphocytosis and large granular lymphocytosis, and it is now classified as T-cell LGL leukemia (T-LGL leukemia; Figure 13-1). Although generally a leukemia of cytotoxic T-lymphocytes, a

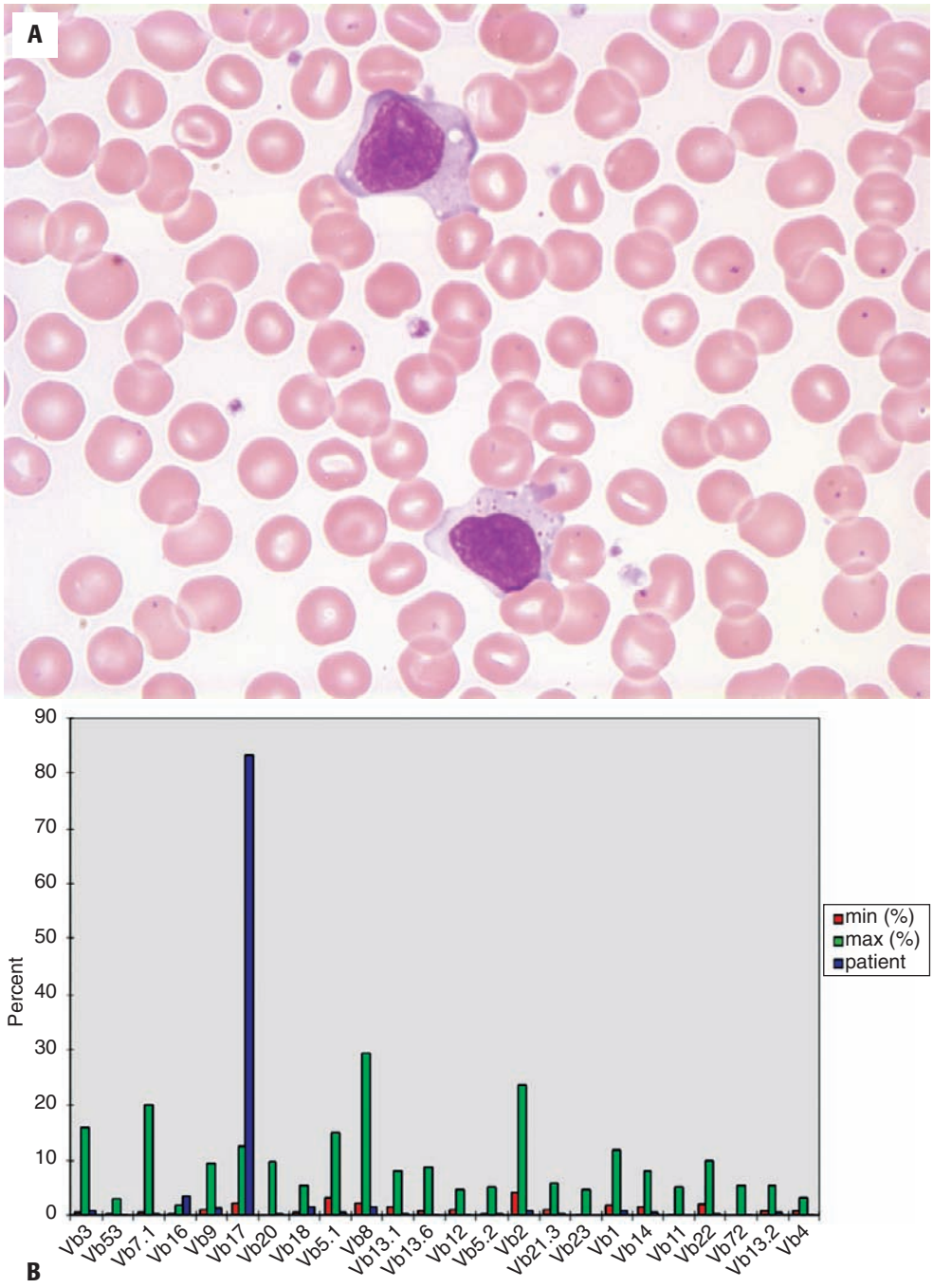


**FIGURE 13-1**

T-cell large granular lymphocyte leukemia. **A,** Circulating large granular lymphocytes. **B,** Preferential V-β expression as a marker of clonality in circulating T cells. *(**B,** Courtesy Eric Hsi.)*

subset of LGL leukemias may show a true NK-cell immunophenotype as discussed in more detail later in this section.

LGL leukemia in its typical form is a clinically indolent disorder and usually manifests with a distinct array of clinical and laboratory findings. Median age at diagnosis is 60 years, with a relatively even prevalence in men and women. Although many reports stress that absolute peripheral blood lymphocyte counts may be normal at diagnosis, the median lymphocyte count in one large series was $8 \times 10^9$/L (approximately twice the upper limit of normal for adults), and most patients exhibit peripheral blood absolute lymphocytosis. Splenomegaly in the absence of lymphadenopathy is common at clinical presentation. Although approximately 60% of patients with LGL leukemia have a positive rheumatoid

**LARGE GRANULAR LYMPHOCYTIC LEUKEMIA—FACT SHEET**

**Clinical Features**
- Splenomegaly (common)
- Rheumatoid factor positive in most patients, with frank rheumatoid arthritis in approximately one fourth of patients
- Infectious complications related to neutropenia

**Peripheral Blood Morphology**
- Variable absolute lymphocyte count (but usually more than $4 \times 10^9$/L)
- Predominance of LGLs
- Neutropenia (common)
- May be associated with pure red cell aplasia

**Bone Marrow Morphology**
- Lymphoid infiltrates may be scant or subtle
- Despite peripheral neutropenia, marrow generally shows normal granulopoiesis

**Immunophenotype**
- Usually the neoplastic cells are cytotoxic T cells positive for CD2, CD3, CD5, CD7, CD8, CD16, and CD57 and negative for CD4 and CD56
- Sometimes it is $CD56^+$; this has been associated with more aggressive behavior in some studies
- Occasionally, patients with this specific clinical syndrome will show a true NK-cell phenotype ($CD2^+$, $CD3^-$, $CD5^-$, $CD7^{-/+}$, $CD8^{+/-}$, $CD16^+$, $CD56^+$, $CD57^{-/+}$)

**Genetics**
- Clonal cytogenetic abnormalities may be present, but no single abnormality is characteristic
- Molecular studies show clonal T-cell antigen receptor gene rearrangement pattern in T-LGL leukemia. Cases with a true NK immunophenotype will not show clonal T-cell gene rearrangement pattern

factor, frank rheumatoid arthritis is present in approximately one fourth of cases. Neutropenia is also common at presentation in LGL leukemia and is a major cause of morbidity in this disorder. Interestingly, bone marrow biopsy specimens from patients with LGL leukemia generally show adequate granulocyte precursors. The mechanism of neutropenia in this disorder has not been fully characterized, but recent data suggest increased peripheral neutrophil apoptosis, possibly linked to increased levels of circulating Fas ligand (part of the Fas apoptotic pathway) in these patients. The complications of chronic neutropenia are commonly the reason for eventual therapeutic intervention in this otherwise generally nonaggressive neoplasm. LGL leukemia also has been linked to pure red cell aplasia in some patients. In fact, some studies indicate that large granular lymphocytic leukemia may be the most common underlying disease in patients with noncongenital pure red cell aplasia.

Following the initial descriptions of this disorder, LGL leukemia was often referred to as large granular lymphocytosis, because the disease typically follows an indolent clinical course (median survival greater than 13 years in one series), and clonality was, at the time, difficult to determine in T-cell or NK-cell populations. Since then, however, the detection of clonal T-cell receptor gene rearrangements and clonal cytogenetic abnormalities in patients with the classic clinical and laboratory features of large granular lymphocytosis has established that this syndrome generally represents a clonal neoplastic disorder. Recent reports have indicated a potential association between LGL leukemia and subclinical detectable populations of clonal B-cells (so-called monoclonal B-cell lymphocytosis) or monoclonal gammopathies.

### Morphologic Findings

The leukemic cells of T-LGL are large lymphocytes with mature chromatin and moderate amounts of pale cytoplasm. Some cases may show mild nuclear indentation, but deep clefts or lobulations are not seen. The cytoplasm contains variably prominent azurophilic granules. Ultrastructurally, the azurophilic granules consist of parallel tubular arrays containing cytotoxic enzymes such as TIA-1, perforin, and granzyme. Bone marrow biopsy and immunostaining for CD3 or cytotoxic molecules characteristically shows a sinusoidal infiltrate.

### Immunophenotype

A majority of LGL leukemias consist of cytotoxic T-lymphocytes that express pan-T-cell–associated antigens (e.g., CD2, CD3, CD5) and generally express the cytotoxic T-cell marker CD8, but are negative for the T-helper antigen CD4. Expression of CD5 is often decreased compared with normal T-cell populations, and in some cases CD5 may be absent. T-LGL leukemia cells also typically express the NK-cell–associated antigens CD16 (the Fc receptor-γ noted in original descriptions of LGL leukemia) and CD57, but are usually negative for the NK-cell–associated antigen CD56 (the neural cell adhesion molecule). Expression patterns of other markers not typically used in diagnostic immunophenotyping—specifically the absence of CD27, CD28, CD45RO, and CD62L (L-selectin) and the expression of CD45RA—are consistent with a constitutively activated terminal-effector memory T-cell phenotype, which may explain at least in part some of the autoimmune phenomena observed in many cases of LGL leukemia. The immunophenotype in LGL leukemia is characteristic but may not show frank antigenic aberrance compared with physiologic subsets of cytotoxic T lymphocytes. For that reason, T-cell antigen receptor gene rearrangement analysis should be used to confirm the diagnosis.

A minority of LGL leukemia cases consist of true NK lymphocytes. Indolent forms of NK-LGL leukemia may

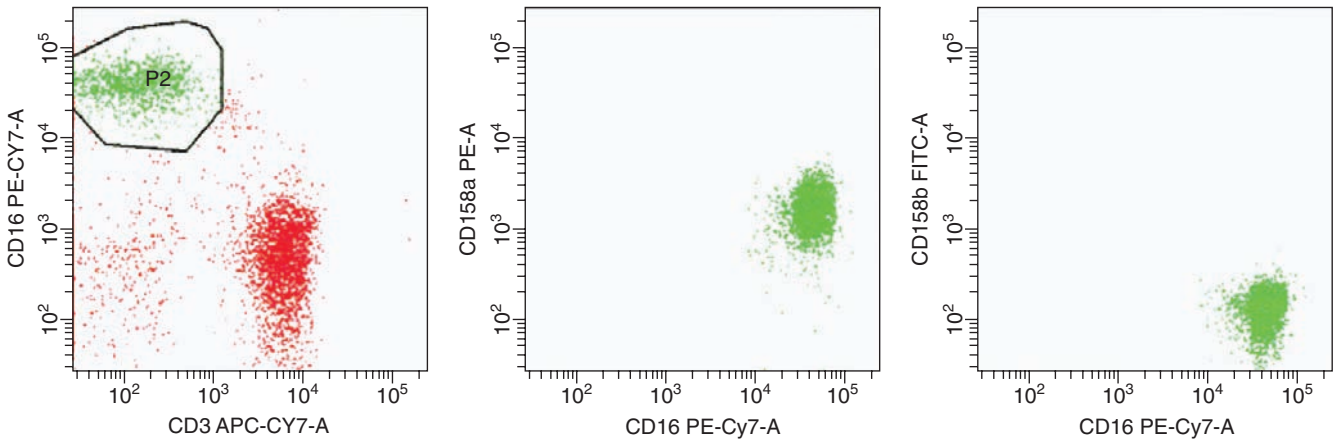


**FIGURE 13-2**

Large granular lymphocyte leukemia of NK cells. Flow cytometry histogram of a peripheral blood sample shows that all NK cells in the sample ($CD3^-$, $CD16^+$, highlighted in green) are restricted to the expression of a single killer immunoglobulin-like receptor (KIR), in this case CD158a. *(Courtesy Curtis Hanson.)*

appear as a clinicopathologic syndrome indistinguishable from T-LGL leukemia. However, there are also forms of indolent NK LGL lymphocytosis that lack the full spectrum of LGL leukemia features such as organomegaly. True NK-LGL leukemias do not express surface CD3 but do express the NK-cell–associated antigens CD16 and CD56. In contrast to aggressive NK cell leukemia, indolent NK cell LGL proliferations are Epstein-Barr virus negative. Clonality has been demonstrated in a few cases by human androgen receptor assay, and more recently by demonstration of monotypic restriction of KIR subtype expression (Figure 13-2). In addition, aberrant expression patterns of NK-associated antigens such as CD94 or CD161 have been cited as evidence of neoplasia in NK cell population in lieu of markers of clonality. Some investigators believe that at least some cases of chronic NK-cell lymphocytosis represent a chronic reactive or perhaps a clonal nonmalignant condition.

### Genetic and Cytogenetic Findings

T-LGL leukemia is a monoclonal disorder and, therefore, T-cell receptor gene rearrangement studies show clonal rearrangement patterns. Both the TCR-β and TCR-γ loci can usually be shown to be rearranged. The neoplastic nature of T-LGL leukemia was first revealed by the discovery of clonal cytogenetic abnormalities in this disorder. The initial report of clonal cytogenetic abnormalities in T-LGL leukemia described two cases: one with clonal trisomy 8 and one with clonal trisomy 14. Since then, numerous cytogenetic abnormalities have been described in T-LGL leukemia, but none are considered a unique or defining abnormality. These abnormalities include, among others, structural abnormalities of chromosomes 7 and 14 that are common to other T-cell neoplasms.

NK-LGL leukemia does not show clonal T-cell receptor gene rearrangements, but clonality or aberrancy can be demonstrated in many cases by immunophenotypic assessment of KIR expression or abnormal deletion of NK-associated antigens.

## Differential Diagnosis

### Reactive Lymphocytosis

Some types of transient or non-neoplastic lymphocyte proliferations may be marked by a relative or absolute increase in circulating LGLs. The characteristic reactive T lymphocytes of infectious mononucleosis may occasionally harbor cytoplasmic granules; however, infectious mononucleosis typically is marked by striking morphologic heterogeneity among circulating lymphocytes, in contrast to the monotonous LGL proliferations typical of T-LGL leukemia.

A common but underrecognized form of reactive peripheral blood lymphocytosis, known as stress lymphocytosis, may occur following marked tissue injury (e.g., myocardial infarct, cerebrovascular accident, pulmonary embolus, physical trauma). Stress lymphocytosis generally consists of a mixture of lymphocyte types, but circulating LGLs may be increased. However, stress lymphocytosis is typically transient, subsiding within hours or days and giving way to absolute peripheral neutrophilia. Some patients with HIV infection may demonstrate a transient CD8+ T-cell lymphocytosis. A subset of these patients may develop persistent T-cell clones that phenotypically mimic T-LGL leukemia but lack typical clinical features of T-LGL leukemia. These appear to represent immune response to viral infection, and one should avoid over-interpretation of T-cell clones in the blood of such patients. If CD56 is expressed in a potential case of T-LGL leukemia, the leukemic phase of hepatosplenic T-cell lymphoma should be excluded.

Features supporting the latter include hepatosplenomegaly, aggressive clinical course, hemophagocytosis, and presence of isochromosome 7q.

#### Posttransplantation Large Granular Lymphocyte Proliferations

Persistent T-cell LGL proliferations have been described in recipients of allogeneic hematopoietic stem cell transplants. These proliferations may be polyclonal, oligoclonal, or monoclonal. Some data indicate that a relatively common immunophenotype in such cases includes expression of CD57 in the absence of either CD16 or CD56, in contrast to the $CD16^{+}CD57^{+}$ phenotype typical of de novo T-LGL leukemias. Rare reports suggest that some of these posttransplant LGL proliferations represent true neoplasms; however, the bulk of evidence seems to support that, despite the clonal nature of some of these cases, they likely represent nonneoplastic graft-versus-host phenomena in bone marrow or stem cell transplant recipients. Recent data suggest lower relapse rates of primary diseases in patients with more robust LGL responses after hematopoietic stem cell transplant, further supporting the notion that this represents a graft-versus-host phenomenon. Longer term follow-up, even in cases with substantial lymphocytosis and documented clonality, supports classification of this phenomenon as reactive rather than neoplastic. Similar LGL proliferations have also been described in some solid organ transplant recipients.

## SÉZARY SYNDROME AND MYCOSIS FUNGOIDES

### Clinical Features

Mycosis fungoides (MF) is a clinicopathologically distinct form of primary cutaneous T-cell lymphoma. A minority of patients with MF develop a syndrome of diffuse erythroderma, diffuse lymphadenopathy, and leukemic involvement by the lymphoma. This constellation of findings is termed *Sézary syndrome* (SS), and the circulating lymphoma cells are termed *Sézary cells* (Figure 13-3). The International Society for Cutaneous Lymphomas has set forth a proposed hematologic definition of Sézary syndrome that includes erythroderma plus one or more of the following:

- Minimum of $1000/mm^3$ Sézary cells
- CD4:CD8 ratio greater than 10, owing to an increase in $CD4^{+}$ or $CD3^{+}$ cells by flow cytometry
- Aberrant expression of pan–T-cell antigens by flow cytometry
- Increased lymphocyte count with T-cell monoclonality by molecular techniques
- Abnormal clonal karyotype

MF has a variable clinical course, with numerous emerging therapeutic options. True SS is clinically aggressive. A recent study evaluating International Society of

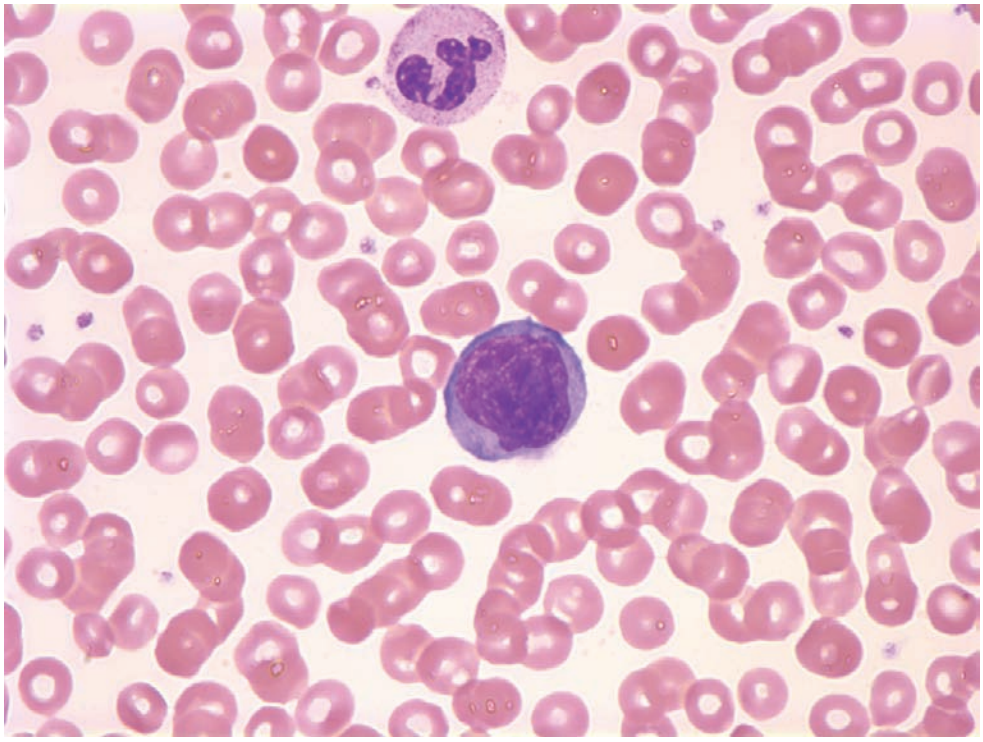

**FIGURE 13-3**
Characteristic cerebriform nuclear morphology of circulating Sézary cells in a patient with Sézary syndrome–mycosis fungoides.

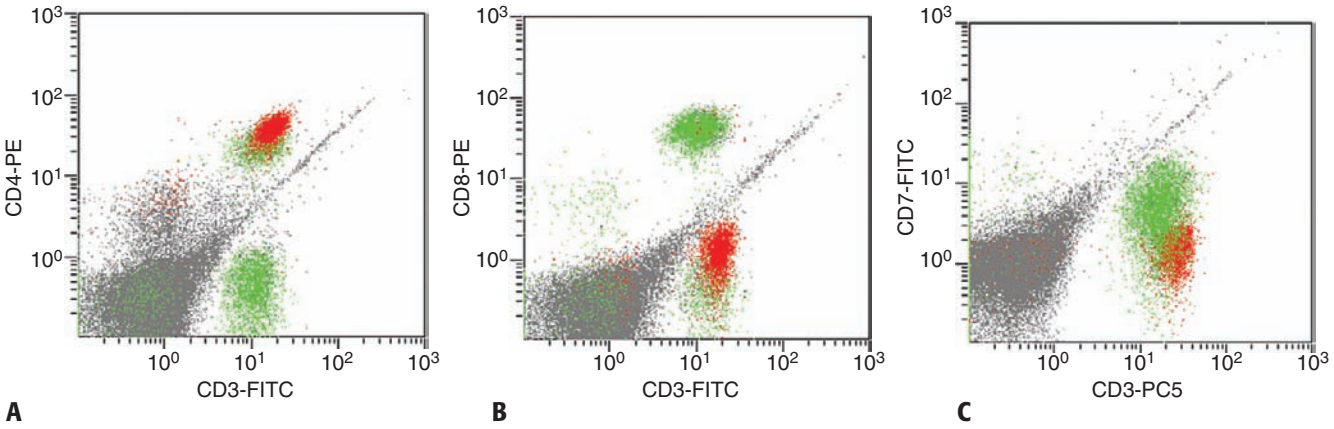


**FIGURE 13-4**

Flow cytometric histograms displaying typical immunophenotypic findings in Sézary syndrome–mycosis fungoides. Background normal T cells *(green)* are contrasted with Sézary cells *(red),* which exclusively express CD4 **(A)** but not CD8 **(B)** and show deletion of the CD7 antigen **(C).** *FITC,* Fluorescein isothiocyanate; *PE,* phycoerythrin; *PC5,* phycoerythrin Cy5.

**SÉZARY SYNDROME—FACT SHEET**

**Clinical Features**

- Leukemic phase of MF (cutaneous T-cell lymphoma)
- Marked by diffuse erythroderma, lymphadenopathy, and circulating lymphoma cells (Sézary cells)

**Peripheral Blood Morphology**

- Circulating Sézary cells: morphologically distinct, convoluted (cerebriform) cells

**Immunophenotype**

- Typical immunophenotype is T-helper cell with deletion of expression of CD7 (CD2+, CD3+, CD4+, CD5+, CD7–, CD8–), but other T-cell antigen deletions may be seen
- Expression of CD25 (IL-2 receptor) is variable or absent despite this being a therapeutic target for denileukin diftitox

**Genetics**

- Clonal cytogenetic abnormalities are common, but no single abnormality is characteristic

Cutaneous Lymphomas/European Organization for Research and Treatment of Cancer (ISCL/EORTC) staging criteria observed a median survival of true SS of 3 years, with 26% survival at 5 years and 12% at 10 years.

### Morphologic Findings

The diagnosis of circulating Sézary cells relies heavily on careful microscopic examination of a routinely stained peripheral blood film. Classic Sézary cells present as distinctly abnormal, delicately convoluted cells that are generally larger than background benign lymphocytes (see Figure 13-3). Smaller variants of Sézary cells are sometimes referred to as *Lutzner cells*. There may be histologic differences in skin biopsy specimens of patients exhibiting primarily SS versus those who develop SS after an established phase of MF limited to the skin. Primary SS may lack the characteristic epidermotropic skin infiltrates of MF, and instead may show more subtle perivascular infiltrates. Some consider primary SS to be a distinct clinicopathologic entity to secondary SS evolving in patients with established MF.

### Immunophenotypic Findings

Although peripheral blood morphology remains a staple of assessment and staging in SS/MF, flow cytometric immunophenotyping can be helpful. Classically, Sézary cells are T-helper cells and will therefore express CD4 in the absence of CD8. Furthermore, deletion of expression of the pan–T-cell–associated antigen CD7 is noted in the Sézary cells of a large majority of patients (Figure 13-4). Note, however, that physiologic (i.e., nonneoplastic) dermal T lymphocytes can display this same immunophenotype (including absence of CD7 expression), and these cells can occasionally be detectable in circulation. Therefore the combined evaluation of peripheral blood morphology and immunophenotype in difficult cases may be more useful than either evaluation alone.

Many clinical laboratories rely on the detection of the basic $CD3^+$, $CD4^+$, $CD7^-$, $CD8^-$ immunophenotype in the documentation of circulating Sézary cells; however, there are additional immunophenotypic studies that can be of help. Previous studies indicated that Sézary cells tend to express the immunophenotype of memory T-helper cells (also called *helper-inducer* or *inducer of help cells*)—that is, T-helper cells that assist in B-cell activation. These cells express CD29 and CD45RO but do not express CD45RA. This immunophenotype

is in contrast to the CD45RA$^+$, CD29$^-$, CD45RO$^-$ immunophenotype characteristic of naive T cells (also called *suppressor-inducer* or *inducer of suppression cells*) and may be of help diagnostically. More recently, there is evidence that assessment of CD26 (dipeptidyl aminopeptidase IV) may be of diagnostic use in SS/MF. Previous studies have indicated a CD4$^+$, CD26$^-$ immunophenotype in a large majority of samples with morphologic evidence of circulating Sézary cells, and that this absence of CD26 expression can be useful in distinguishing Sézary cells from background T lymphocytes.

The assessment of CD25 expression in SS/MF has gained attention in recent years because CD25 is an interleukin-2 receptor molecule that is a target of denileukin diftitox, a diphtheria toxin conjugated form of interleukin-2 approved for use in patients with MF whose malignant cells express CD25. However, most cases of MF do not express high levels of CD25 and many appear CD25$^-$ on routine flow cytometric evaluation. Furthermore, there is evidence that the efficacy of denileukin diftitox may not necessarily depend on CD25 expression on the neoplastic cells of SS/MF. To date, there has not been a clear consensus on the need for routine assessment of CD25 expression status in patients with SS/MF.

#### Cytogenetic Findings

Cytogenetic analysis is not a mainstay of diagnosis in SS/MF. Nonetheless, studies have shown high rates of clonal cytogenetic abnormalities in these disorders. Numerous recurring abnormalities have been identified, involving numerous different chromosomes. These karyotypic abnormalities may be complex, and no single abnormality is consistently associated with SS/MF.

### Differential Diagnosis

SS/MF may show considerable overlap with HTLV-1–associated ATLL, and this differential diagnosis is discussed in the next section of this chapter. T-PLL may also show clinical and morphologic overlap with SS/MF, including skin involvement and circulating convoluted lymphocytes. However, marked nuclear convolution, while sometimes present, is less common in the cells of T-PLL than in the cells of SS/MF, and the characteristic (albeit not entirely specific) immunophenotype of SS/MF may help to distinguish it from T-PLL. Furthermore, the inv(14) (q11q32) on cytogenetic analysis is more characteristic of T-PLL than of SS/MF. Ultimately, however, careful correlation with the clinical pattern may be necessary for reliable distinction between these two disorders. In actual practice, a diagnosis of MF has often been made by skin biopsy and clinical assessment before the evaluation of peripheral blood, aiding considerably in differential diagnosis.

## ADULT T-CELL LEUKEMIA–LYMPHOMA ASSOCIATED WITH HUMAN T-LYMPHOTROPIC VIRUS 1

### Clinical Features

HTLV-1 is a retrovirus, endemic in many areas worldwide, particularly southwestern Japan, central Africa, and the Caribbean. HTLV-1 has been linked to the development of a specific clinicopathologic syndrome known as ATLL. Although the link is well established, epidemiologic studies from high-prevalence regions indicate a long latency period for HTLV-1, with a lifetime ATLL risk of less than 5% in infected individuals.

ATLL occurs in adults (median age, 56 years; range, 27 to 82 years) and has four major clinical presentations: acute, lymphomatous, chronic, and smoldering. The acute subtype (55% to 60% of cases) is marked by severe peripheral blood lymphocytosis (often greater than $100 \times 10^9$/L), numerous "flower" cells in the peripheral blood (Figure 13-5), hypercalcemia, hepatosplenomegaly, elevated serum lactate dehydrogenase, and a rapidly progressive course with a median survival of 6 months despite multiagent chemotherapy. The acute variant may also present with lytic bone lesions, likely because of a release of cytokines that accelerate bone resorption—the

**HUMAN T-LYMPHOTROPIC VIRUS 1 ASSOCIATED ADULT T-CELL LEUKEMIA–LYMPHOMA—FACT SHEET**

**Clinical Features**
- Often divided into four clinical subtypes: acute, chronic, smoldering, and lymphomatous
- Long latency period between HTLV-1 infection and development of ATLL
- Low overall lifetime risk of ATLL in HTLV-1–infected individuals
- Virally encoded Tax protein may be important in leukemogenesis
- May show substantial clinical overlap with MF/SS
- Confirmation of HTLV-1 infection (by serology or otherwise) required for diagnosis

**Peripheral Blood Morphology**
- Bizarre flowerlike lymphocytes

**Immunophenotype**
- Usually T-helper phenotype, CD3$^+$, CD4$^+$, CD8$^-$; cells generally CD7$^-$, CD25$^+$

**Genetics**
- Clonal cytogenetic abnormalities may be seen, but no single abnormality is linked to this disease
- Clonal integration of HTLV-1 genome can be demonstrated

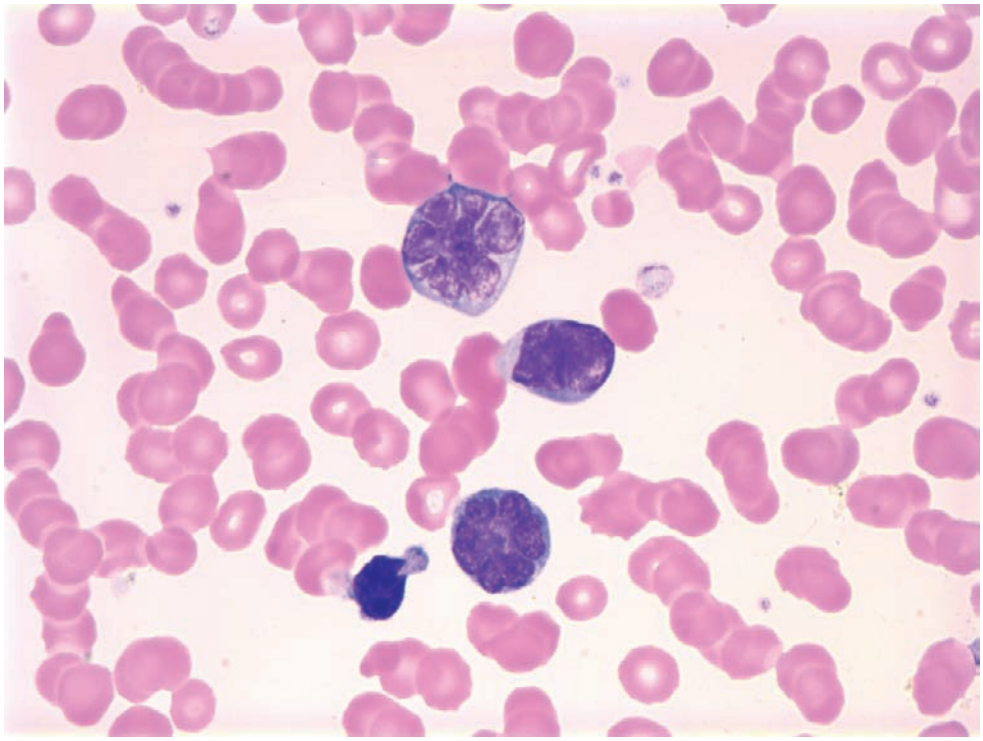

**FIGURE 13-5**

Adult T-cell leukemia–lymphoma (ATLL) associated with the human T-lymphocytic virus type 1. Flower-cell morphology of circulating neoplastic cells in a patient with ATLL associated with the human T-lymphocytic virus type 1.

same process that results in hypercalcemia at presentation. The chronic subtype (15% to 20% of cases) can manifest with a peripheral blood absolute lymphocytosis and circulating flower cells, but generally the lymphocyte count is much lower than that seen in the acute variant. Exfoliative skin rashes may be seen in chronic ATLL, but hypercalcemia is generally absent. The median survival for chronic ATLL is more than 2 years, but most patients die within 5 years because of progression to acute or lymphomatous ATLL. Smoldering ATLL (approximately 5% of cases) is marked by a normal peripheral blood leukocyte count, with circulating neoplastic cells constituting fewer than 5% of total leukocytes. Although skin rashes can develop in this variant, smoldering ATLL patients do not have hepatosplenomegaly, hypercalcemia, or lymphadenopathy. The median survival for smoldering ATLL is also more than 2 years but less than 10 years because of the progression to acute ATLL or to infectious complications. Finally, presentation as lymphoma without leukemia occurs in approximately 20% to 25% of patients and also has an aggressive clinical course with a median survival of approximately 1 year. These patients have generalized lymphadenopathy but generally do not have hepatosplenomegaly, skin involvement, or hypercalcemia; some may develop leukemia during the course of disease.

Lymphomagenesis in HTLV-1 infection is a complex, multiple-step process. This concept is manifest in the long latency period between HTLV-1 infection and onset of ATLL, and in the low lifetime risk of ATLL in HTLV-1–infected individuals. HTLV-1 randomly integrates into the genome of infected cells; in other words, the pattern of HTLV-1 integration is distinct in every infected cell, except in cells that are clonally related. The virally encoded protein Tax is thought to have a key role in the development of ATLL in infected individuals. Tax appears to act in a complex and pleiotropic manner that involves the activation of numerous transcription factors and the repression or functional inactivation of cell cycle–active proteins, the result of which is the persistent clonal proliferation of HTLV-1–infected cells and the eventual promotion of ATLL in some individuals. The constitutive activation of JAK/STAT signal transduction pathways in HTLV-1 associated ATLL has led to the use of JAK2 kinase inhibitors in some clinical trials of ATLL.

### Morphologic Findings

The most characteristic morphologic feature of ATLL is the presence of medium to large multilobate lymphocytes or flower cells in the peripheral blood. These cells are larger than normal peripheral blood lymphocytes and have moderately condensed chromatin, absent or small nucleoli, scant slightly basophilic cytoplasm, and nuclear multilobation (see Figure 13-5). The leukemic

cells are distinguished from Sézary cells by nuclear outpouching rather than complex nuclear infolding. The white blood cell count ranges from 25,000 to 450,000 in acute ATLL. In contrast, chronic ATLL tends to have only mildly to moderately elevated white blood cell counts with more than 10% leukemic cells. Leukemic cells may be seen in the peripheral blood in smoldering ATLL, but do not give rise to peripheral lymphocytosis (less than 3% leukemic cells).

Lymph nodes in acute ATLL and lymphomatous ATLL are virtually always involved. The low-magnification growth pattern is always diffuse, usually with totally effaced architecture except for occasional dilated sinuses containing individually scattered tumor cells. At high magnification, the prominent multilobation so typical of leukemic cells in the peripheral blood may be inconspicuous. However, tumor cells in lymph nodes are invariably pleomorphic cells, and most cases have a mixture of small to large pleomorphic cells with medium to large cells predominating. Occasional lymph nodes may have a predominance of small pleomorphic cells. Nuclear contours usually appear irregular, cleaved, or lobated but can occasionally appear cerebriform. Occasional lymphomas may have Reed-Sternberg–like cells.

Bone marrow biopsy specimens are usually involved by ATLL, but the degree of marrow involvement is often sparse or mild in comparison to the degree of peripheral blood involvement. Occasionally marrow involvement may be extensive, but marrow infiltration usually does not lead to hematopoietic abnormalities. The pattern of marrow involvement is diffuse and interstitial or sinusoidal and may be subtle.

Cutaneous involvement by ATLL occurs in 25% to 60% of patients with acute ATLL. Other sites commonly involved by ATLL include the liver, spleen, lungs, and central nervous system.

#### Immunophenotypic Findings

Immunophenotyping by flow cytometry may be helpful in further evaluating the abnormal cells in ATLL. By definition, ATLL cells are post-thymic T cells and will therefore express surface CD3. They are also generally positive for various pan–T antigens such as CD2 and CD5 and display a T-helper phenotype (positive for CD4, but negative for CD8); however, aberrant loss of the normally expressed T-cell antigen CD7 is a consistent finding in ATLL. Finally, ATLL cells generally express the α-chain of the interleukin-2 receptor CD25.

#### Cytogenetic Findings

As with SS/MF, cytogenetic analysis of cases of ATLL have revealed numerous and complex chromosomal abnormalities; however, no single karyotypic abnormality is diagnostically linked to HTLV-1–associated ATLL. Therefore cytogenetic analysis is not essential in the workup of this disorder.

### Differential Diagnosis

To the diagnostic pathologist, the differential diagnosis of ATLL includes several other T-cell neoplasms that can involve the blood at presentation, including T-PLL, SS/MF, and the leukemic phase of other peripheral T-cell lymphomas. The cells of T-PLL can often show some degree of nuclear irregularity, but the frank flower-cell morphology is somewhat unusual for T-PLL. Because ATLL often involves the skin at presentation, it can mimic SS/MF closely in both its morphologic and clinical features. Both ATLL and SS/MF are marked by the presence of circulating convoluted lymphocytes. However, the convolutions seen in SS/MF often impart an inwardly folded, creased, cerebriform look to the cells (see Figure 13-3), whereas ATLL cells tend to have more outward nuclear projections (see Figure 13-5). ATLL and SS/MF also frequently share the immunophenotypic finding of loss of the CD7 antigen. However, CD25 expression is almost always seen in ATLL, whereas it may be variable or absent in SS/MF and can therefore be a useful marker in the differential diagnosis of these entities. Although these differences can be helpful, there remains significant overlap clinically and diagnostically between ATLL and SS/MF. Therefore, ultimately, the distinction of ATLL from other T-cell neoplasms depends on documenting infection of the patient by HTLV-1, by either serologic analysis or detection of clonal integration of the HTLV-1 genome into neoplastic cells by molecular techniques.

## T-CELL PROLYMPHOCYTIC LEUKEMIA

### Clinical Features

Prolymphocytic leukemia (PLL) was originally described in 1973 and was thought at the time to be a variant of chronic lymphocytic leukemia. Because the neoplastic cells in this disorder displayed prominent single nucleoli, the term *prolymphocytic* was used to describe them, despite subsequent studies that confirmed these cells were mature and did not arise from lymphoid precursor cells. Subsequent studies showed that a subset of cases classifiable as PLL by morphologic criteria were of the T-cell type (with the majority being of the B-cell type). Since then, there has been a substantial reappraisal of B-cell PLL as a distinct diagnostic entity, with many reported cases of B-cell PLL appearing to represent mantle cell lymphoma or other distinct B-cell lymphoma

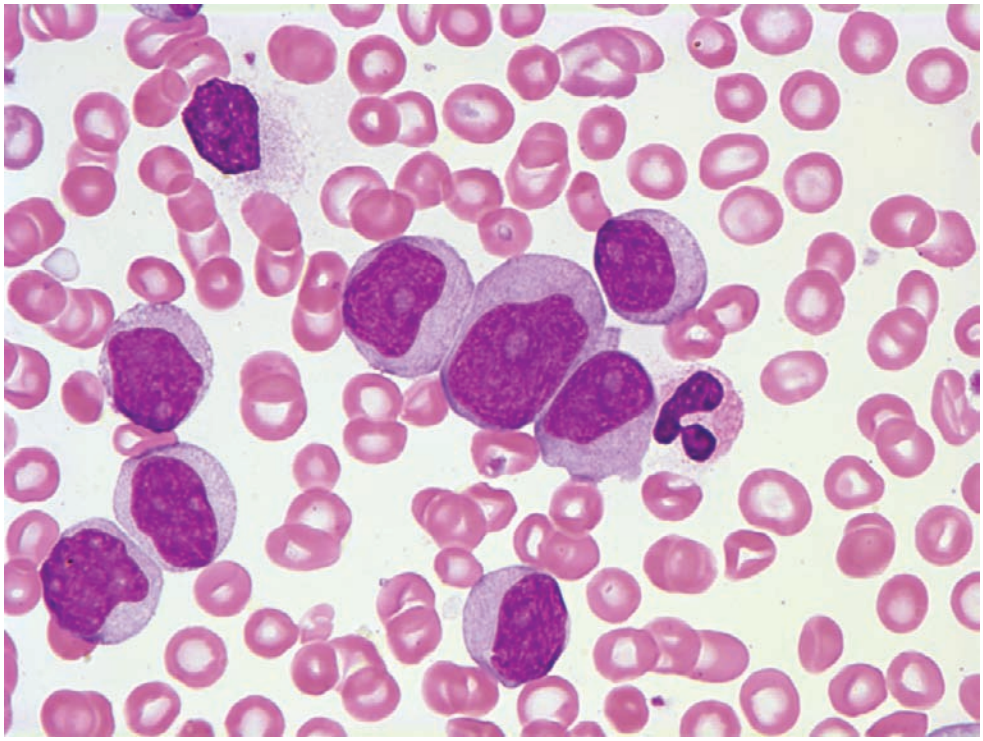

**FIGURE 13-6**

Circulating neoplastic prolymphocytes in a patient with T-cell prolymphocytic leukemia.

subtypes. However, T-PLL remains a well-defined disease entity, with distinct clinical, morphologic, immunophenotypic, and genetic features.

Clinically, T-PLL manifests with profound leukocytosis, often in excess of $100 \times 10^9$/L, because of the proliferation of circulating prolymphocytes (Figure 13-6). T-PLL typically also appears with splenomegaly, which is often marked. T-PLL may also appear with diffuse lymphadenopathy, a finding that is rare in its B-cell counterpart. T-PLL may occur more commonly in patients with ataxia-telangiectasia, possibly as a result of abnormalities in the *ATM* gene associated with that disorder. The disease course of T-PLL has generally been considered aggressive, with median survivals of 12 months or less. A subgroup of patients with an initial indolent course has been described. This form had a median survival of 51 months in one study; however, many patients progressed to an aggressive phase with

**T-CELL PROLYMPHOCYTIC LEUKEMIA—FACT SHEET**

**Clinical Features**

- T-PLL often develops with blood leukocyte counts in excess of $100 \times 10^9$/L
- Splenomegaly is common; lymphadenopathy may also be present
- Median survival less than 1 year, even with treatment
- Some cases are associated clinically with ataxia-telangiectasia; these cases may involve the ATM gene on chromosome 11q23

**Peripheral Blood Morphology**

- Larger cells with distinct central nucleoli (prolymphocytes)
- Small cells with indistinct or absent nucleoli (small-cell variant of T-PLL, sometimes reported as T-cell chronic lymphocytic leukemia)

**Immunophenotype**

- Usually (more than 60%) a T-helper phenotype: $CD3^+$, $CD4^+$, $CD8^-$
- May show aberrant deletion of one or more T antigens
- Minority of cases (approximately 20%) shows CD4/CD8 coexpression (more common in the small-cell variant, one of the rare instances in which mature T cells coexpress CD4 and CD8)
- An even smaller minority (10% to 15%) showing cytotoxic–suppressor phenotype: $CD3^+$, $CD4^-$, $CD8^+$
- TCL-1 expressed in 70% of cases

**Genetics**

- T-PLL is often associated with a specific cytogenetic abnormality: inv(14)(q11;q32), which juxtaposes the T-cell $\alpha/\delta$ gene complex at 14q11 with the TCL-1 gene at 14q32. (Note: This is not to be confused with the 14q32 breakpoint common in B-cell lymphomas, which involves the immunoglobulin heavy chain joining region.)
- Other cytogenetic abnormalities may be seen, including trisomy for the long arm of chromosome 8

short survival, similar to that classically recognized. Treatment with a monoclonal antibody to CD52 (alemtuzumab) has been reported to be capable of inducing remission and shows promise in improving survival.

#### Morphologic Findings

Morphologically, T-PLL comes in two forms. In its more typical form, the circulating neoplastic cells display single prominent central nucleoli. These cells are often round or oval but may show irregular nuclear contours (see Figure 13-6). A second morphologic variant, referred to as the *small-cell variant of T-PLL,* is marked by the proliferation of small round lymphocytes lacking visible nucleoli on light microscopic evaluation, although distinct single nucleoli are visible in these cells by electron microscopy. The small-cell variant does not appear to be a clinically or biologically distinct disease, but it is important to the diagnostic hematopathologist for purposes of differential diagnosis and classification. Involvement of extramedullary sites occurs with skin, lymph node, and liver being common sites. The morphologic findings are not specific, and clinical history is often needed to arrive at the correct interpretation. Fortunately, the diagnosis of T-PLL has usually been established in blood or bone marrow.

#### Immunophenotypic Findings

The immunophenotype of T-PLL is somewhat variable. In addition to expressing major pan–T- cell–associated antigens, a majority of cases (greater than 60%) express CD4 in the absence of CD8. Approximately 20% coexpress CD4 with CD8, and this subgroup of dual $CD4^+/CD8^+$ cases is weighted toward the small-cell variant of T-PLL. Finally, a smaller minority (approximately 10% to 15%) are positive for CD8 in the absence of CD4. Although aberrance in the level of expression of pan–T-cell antigens may be seen, there is no single pattern of antigen deletion that characterizes T-PLL. However, the finding of dual CD4 and CD8 expression in approximately 20% of cases is important because this is one of the rare instances in which a nonblastic (post-thymic) T-cell neoplasm expresses this immunophenotype. TCL-1 expression may be diagnostically useful. It is expressed in most cases of T-PLL (70% to 80% of cases) but not other T-cell malignancies; however, it is also expressed in some B-cell neoplasms. Therefore, in the setting of T-cell malignancy, it appears to be a relatively specific but not completely sensitive marker for T-PLL.

#### Cytogenetic Findings

Specific cytogenetic abnormalities are characteristic of T-PLL, although not exclusive to it. The most commonly cited recurring abnormality is a paracentric inversion of chromosome 14, inv(14)(q11q32), that involves juxtaposition of the T-cell receptor α gene (*TRA@*) complex and the *TCL1* oncogene. It is important to note that the band (q32) on the long arm of chromosome 14 associated with the *TCL1* gene is the same as that harboring the immunoglobulin heavy chain joining region that is involved in many different types of B-cell lymphoma. Therefore it is important not to infer lineage based solely on the presence of a chromosome 14q32 abnormality on karyotypic analysis. Other abnormalities, including trisomy for the long arm of chromosome 8, have also been associated with T-PLL.

### Differential Diagnosis

The differential diagnosis of T-PLL is discussed in the sections describing other T-cell leukemias. One semantic point, however, warrants discussion. The term *T-cell chronic lymphocytic leukemia* (T-CLL) is sometimes used to describe the entity now generally classified as the small-cell variant of T-PLL. However, the term *T-CLL* is discouraged for two important reasons. First, clinical and cytogenetic data, including documentation of the inv(14)(q11q32) in many reported T-CLL cases, support the concept that small-cell T-PLL is in fact the same biologic entity as T-PLL. Second, the term T-CLL has been used somewhat loosely in the literature, and some reports of T-CLL describe what is known as *T-LGL leukemia*. Because T-LGL leukemia is an indolent condition and T-PLL is highly aggressive, confusion of these two entities could have profound clinical ramifications.

## AGGRESSIVE NATURAL KILLER–CELL LEUKEMIA

### Clinical Features

Some cases of LGL leukemia that show a true NK phenotype are associated with a highly aggressive clinical course. Aggressive NK-cell–LGL leukemia is rare in North America but is prevalent in Japan and other Eastern nations. This disease entity generally follows a fulminant clinical course and is therefore classified in the World Health Organization (WHO) classification of hematopoietic neoplasms as aggressive NK cell leukemia.

Because of its rarity, most of the clinical information about this disorder is based on fairly small case series. However, typical reported signs and symptoms at presentation include fever, lymphadenopathy, and hepatosplenomegaly. Peripheral blood counts vary widely at presentation, ranging from frank leukopenia to leukocyte counts in excess of $200 \times 10^9$/L. As is the case with

**AGGRESSIVE NATURAL KILLER–CELL LEUKEMIA—FACT SHEET**

**Clinical Features**

- Rare disorder, more prevalent in Eastern hemisphere
- Aggressive clinical course with survival often measured in weeks to months
- Develops with fever, hepatosplenomegaly, lymphadenopathy
- May represent the leukemic form of extranodal NK-cell–T-cell lymphoma of nasal type, but some clinical differences reported (e.g., prevalence of organomegaly and lymphadenopathy in aggressive NK-cell leukemia, but not commonly in the nasal type NK-cell–T-cell lymphoma)
- Strong association with Epstein-Barr virus infection

**Morphology**

- Atypical lymphocytes (slightly larger than LGLs), variably prominent nucleoli, cytoplasmic granules

**Immunophenotype**

- Can be distinguished from mimics based on clinical presentation and on true NK immunophenotype (surface $CD3^-$, cytoplasmic $CD3\varepsilon^+$, $CD2^+$, $CD4^-$, $CD5^-$, $CD7^{-/+}$, $CD8^{-/+}$, $CD16^+$, $CD56^+$, $CD57^{-/+}$, cytotoxic molecules [TIA-1, granzyme B, perforin] positive)

**Genetics**

- Clonal T-cell antigen receptor gene rearrangements are absent
- Epstein-Barr–encoded RNA positive

certain peripheral T-cell lymphomas, patients may suffer from a concomitant hemophagocytic syndrome and coagulopathy, but these findings are not consistently reported across all series. Regardless of therapeutic intervention, clinical progression in aggressive NK cell leukemia is usually rapid, with median survival in larger retrospective series reported as less than 2 months from the time of diagnosis.

Like the nasal-type T-cell–NK-cell lymphomas prevalent in the Eastern hemisphere, the cells of aggressive NK cell leukemia are commonly positive for the Epstein-Barr virus; therefore in situ hybridization for the Epstein-Barr–encoded RNA may be of diagnostic utility. Indeed, aggressive NK-cell leukemia is regarded by some investigators as representing the leukemic phase of extranodal T-cell–NK-cell lymphoma of the nasal type, based on similar epidemiologic distribution and Epstein-Barr virus association. Differences in clinical presentation (including higher prevalence of lymphadenopathy and organomegaly in aggressive NK cell leukemia) and recent data suggesting differences in genomic alteration patterns based on comparative genomic hybridization have left open the possibility that aggressive NK cell leukemia and T-cell–NK-cell lymphoma are distinct entities.

### Morphologic Findings

Aggressive NK cell lymphoma generally manifests as a leukemia of LGLs. Examination of the peripheral blood smears of patients with NK-cell–LGL leukemia often reveals markedly pleomorphic lymphoid cells in contrast to the relatively monotonous, mature-appearing cells of T-LGL leukemia. Bone marrow involvement can be a subtle interstitial infiltrate or obvious diffuse involvement (Figure 13-7).

### Immunophenotypic Findings

As noted, aggressive NK-cell leukemias generally display a true NK-cell immunophenotype: surface CD3-negative (but positive for the cytoplasmic CD3ε), positive for CD2, CD16, and CD56, variably positive for CD7 and CD8, and negative for CD4. CD57 may be expressed in a minority of cases but is usually negative. Other pan–T-cell–restricted antigens are generally negative. Epstein-Barr virus infection can be demonstrated by immunohistochemistry or Epstein-Barr–encoded RNA in situ hybridization.

### Cytogenetic Findings

There is no consistent cytogenetic abnormality associated with aggressive NK cell leukemia, although abnormalities of chromosome 6 (both the long arm and the short arm) appear relatively common. Numerous clonal abnormalities, including some also described in other T-cell or NK-cell disorders, have been reported. T-cell antigen receptor gene rearrangement studies will not show evidence of clonal rearrangement in this disorder.

## Differential Diagnosis

The presenting signs and symptoms of aggressive NK-cell leukemia may overlap with other T-cell and NK-cell neoplasms. As noted previously, this disorder may simply represent the leukemic phase of extranodal NK/T-cell lymphoma of the nasal type and therefore may show substantial clinical overlap. Aggressive NK cell leukemia, however, generally shows a greater propensity for lymph node involvement and organomegaly. Furthermore, the skin involvement commonly reported in nasal type NK-cell–T-cell lymphomas is unusual in aggressive NK cell leukemia.

The organomegaly and constitutional symptoms at presentation overlap considerably with hepatosplenic T-cell lymphoma. However, hepatosplenic T-cell lymphoma will usually not have the lymphadenopathy associated with aggressive NK-cell leukemia. Furthermore, the neoplastic cells of hepatosplenic T-cell lymphoma have a distinct T-cell immunophenotype, including expression of surface CD3 (not seen in aggressive NK-cell leukemia) and deletion of numerous T-cell lineage antigens. Furthermore, although neoplastic cells

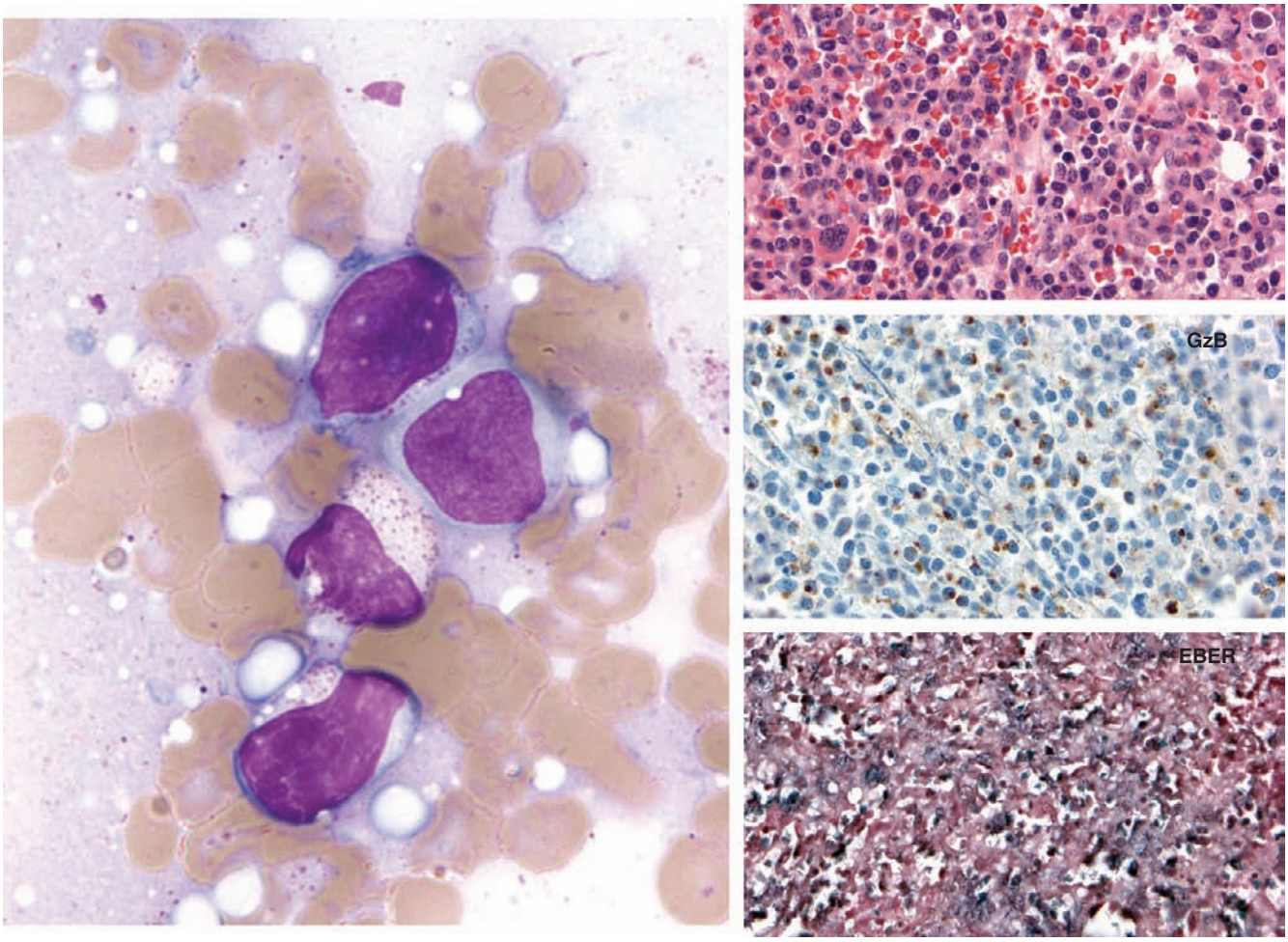


**FIGURE 13-7**

Aggressive natural killer–cell leukemia–lymphoma. The disease manifested in this patient with blood, bone marrow, and liver involvement. Flow cytometry showed an immunophenotype of $CD2^+$, cytoplasmic $CD3^+$, $CD5^-$, $CD7^+$, $CD16/56^+$, $CD57^-$. The bone marrow aspirate smear shows atypical lymphoid cells with cytoplasmic granules *(left)*. The trephine biopsy specimen showed an interstitial infiltrate of neoplastic lymphocytes that also expressed cytotoxic proteins such as granzyme B and were positive for Epstein-Barr–encoded RNA. *(Courtesy Eric Hsi.)*

may be seen in the peripheral blood in cases of hepatosplenic T-cell lymphoma, a true leukemic phase of this disorder is rare.

## LEUKEMIC PHASE OF OTHER PERIPHERAL T-CELL LYMPHOMAS

This chapter has highlighted T-cell neoplasms that tend to manifest clinically as leukemia. However, many different types of peripheral T-cell lymphoma can involve the peripheral blood at some point in their clinical course. Because some of these lymphomas may show considerable morphologic and immunophenotypic overlap with the entities described in this chapter, it is important for diagnostic pathologists to interpret the presence of abnormal circulating T cells in the appropriate clinical context. Precise classification of peripheral T-cell lymphomas that do not commonly manifest in the leukemic phase generally depends on examination of an enlarged lymph node or other primary site of tissue involvement.

## LYMPHOCYTIC VARIANT HYPEREOSINOPHILIC SYNDROMES

### Clinical Features

The term *hypereosinophilic syndrome* was used historically to describe a diverse set of clinicopathologic conditions marked by persistent unexplained peripheral blood eosinophilia and evidence of end-organ damage by eosinophilic infiltration (see Chapter 17). More recently, many processes previously classifiable as hypereosinophilic syndrome have been identified as true eosinophilic neoplasms linked to genetic abnormalities

**LYMPHOCYTIC VARIANT HYPEREOSINOPHILIC SYNDROMES—FACT SHEET**

**Clinical Features**

- Skin manifestations (e.g., eczema, erythroderma, urticaria)
- Increased absolute eosinophil count
- Absolute lymphocyte count usually normal or mildly elevated
- Increased serum IgE levels

**Morphology**

- Peripheral blood eosinophils appear mature, typical
- No specific peripheral blood lymphocyte morphology

**Immunophenotype**

- Most commonly associated with abnormal $CD3^-$, $CD4^+$ T cells detectable in peripheral blood
- Minority of cases may show variant T-cell immunophenotypes such as $CD3^+/CD7^-$, or $CD3^+/CD4^-/CD8^-$

**Genetics**

- Clonal T-cell antigen receptor gene rearrangement detectable in most patients
- No specific or defining cytogenetic abnormalities

affecting specific loci encoding for growth factor receptors, particularly platelet-derived growth factor receptor, α subunit (PDGFRA), platelet derived growth factor receptor, β subunit (PDGFRB), and fibroblast-derived growth factor (FGFR1). Eosinophilic neoplasms linked to these growth factor receptor genes are currently classified under the WHO category of "myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB, or FGFR1," and many hypereosinophilic syndromes that lack these abnormalities remain idiopathic. A subset of clinical hypereosinophilic syndromes, however, appear to involve the non-neoplastic proliferation of eosinophils in response to cytokine release from abnormal (and usually clonal) T-cell subsets. Such processes have been termed *lymphocytic variant hypereosinophilic syndromes* (L-HESs), and their study has been focused on the nature of the abnormal T-cell proliferations that underlie these disorders.

L-HES appears fundamentally to represent an abnormal T-lymphoid proliferation. However, there is no clear consensus as to whether L-HES represents a truly neoplastic process, a preneoplastic clonal proliferation, or a distinct pattern of immune dysregulation. Regardless, the clinical manifestations appear to be linked to the secretion of interleukin (IL) 5 (a potent mediator of eosinophil activation) by the abnormal T-cell clone, with resultant non-neoplastic stimulation of eosinophil proliferation. Some have proposed that the propensity of these abnormal T cells to overproduce IL-5 may be related to their generally $CD3^-$ immunophenotype, with activation of alternative T-cell activation pathways (independent of the CD3-anchored T-cell receptor) resulting in overproduction of Th-2 type cytokines, including IL-5.

Although the exact pathogenesis of the abnormal T-cell proliferation in L-HES is unknown, these disorders are marked by a common pattern of clinicopathologic signs and symptoms. Males and females appear to be affected in equal proportion (in contrast to the marked male predominance of eosinophilic myeloproliferative neoplasms). Patients tend to exhibit chronic skin manifestations (e.g., pruritus, erythroderma, eczema, urticaria) and, in contrast to eosinophilic myeloproliferative neoplasms, elevated levels of serum IgE. Complete blood count in these patients uniformly reveals absolute eosinophilia (eosinophils in excess of $5 \times 10^9$/L). Most patients easily exceed the minimum diagnostic criterion of a sustained eosinophil count of at least $1.5 \times 10^9$/L, but eosinophil counts vary widely and some patients might not exceed the $1.5 \times 10^9$/L threshold. The peripheral absolute lymphocyte count is often normal, but some patients demonstrate high-normal or frankly elevated absolute lymphocyte counts (greater than $4 \times 10^9$/L). The diagnosis requires confirmation of an abnormal T-cell population with characteristic immunophenotypic aberrancies as detailed below (Immunophenotypic Findings).

The clinical behavior of L-HES is generally indolent. Some patients do not require specific therapy, but corticosteroids are generally the first-line approach for those who require therapy. There are several individual reports of patients with L-HES developing overt peripheral T-cell lymphomas over time (often after many years); however, the actuarial risk of the development of frank T-cell lymphoma in L-HES patients is unknown.

#### Morphologic Findings

The main morphologic finding in L-HES is a peripheral blood eosinophilia. Eosinophils are generally mature, without other morphologic stigmata of myeloproliferative neoplasms (e.g., circulating blasts, immature granulocytes, leukoerythroblastic changes). There is not a specific lymphocyte morphology described in association with L-HES and, as noted previously, many patients exhibit nonelevated absolute lymphocyte counts.

#### Immunophenotypic Findings

In a majority of patients with L-HES, flow cytometric immunophenotyping of the peripheral blood reveals a distinct pattern of $CD4^+$ T-helper cells with deletion of the pan–T-restricted surface antigen CD3 ($CD3^-$, $CD4^+$; Figure 13-8). A minor subset of patients with L-HES show variant immunophenotypes, including a double-negative $CD3^+$, $CD4^-$, $CD8^-$ pattern, or deletion of the pan–T-associated antigen CD7. The double-negative immunophenotype resembles that seen in the

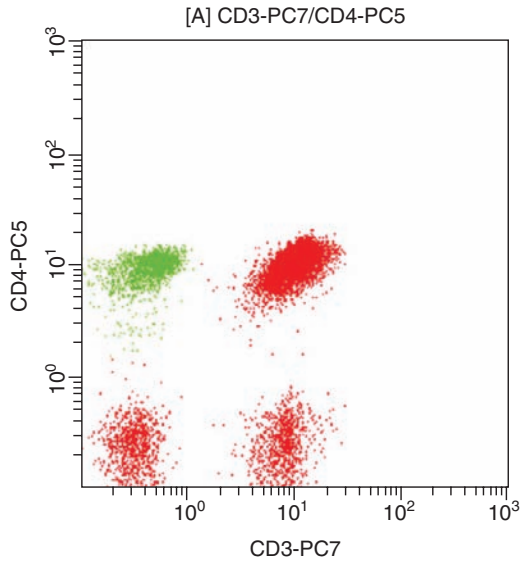


**FIGURE 13-8**

Flow cytometric histogram of peripheral blood lymphocytes showing the $CD3^-$, $CD4^+$ abnormal T-cell immunophenotype *(green)* typical of lymphocytic variant hypereosinophilic syndrome (L-HES).

physiologic subset of γδ T cells, but it has been shown that in association with L-HES the double negative cells express the T-αβ receptor complex. Whether these two less common immunophenotypic variants represent a truly pathogenic aberrant T-cell population is not entirely clear in all cases, because these immunophenotypes resemble those seen in normal subsets of dermal-derived T lymphocytes, and because many reported cases with these less common variant immunophenotypes have not included documentation of T-cell clonality.

### Genetic and Cytogenetic Findings

Sporadic cytogenetic abnormalities, including abnormalities of chromosome 6q, have been reported in association with L-HES. However, most cases are not associated with clonal cytogenetic abnormalities, and there is no cytogenetic abnormality specific or predictive for L-HES.

In general, clonality of T cells can be demonstrated in L-HES by T-cell antigen receptor gene rearrangement studies. T-cell clonality is not universal, however, and there are reported cases of L-HES that do not have demonstrable T-cell clones. It is possible that at least a subset of the nonclonal cases reported do not represent the true clinicopathologic syndrome of L-HES.

## Differential Diagnosis

A key differential diagnosis of L-HES is the true eosinophilic myeloproliferative neoplasms associated with abnormalities of *PDGFRA, PDGFRB,* or *FGFR1*. In general, the workup for suspected HES should include assessment for these abnormalities. Details regarding the cytogenetic and molecular genetic workup for these abnormalities can be found in Chapter 17.

Many peripheral T-cell lymphomas or leukemias can develop with the aberrant T-cell immunophenotypes detectable in L-HES (including aberrant deletion of CD3 or CD7, or double-negative $CD4^-$, $CD8^-$ immunophenotypes), and these lymphomas can sometimes develop with skin manifestations and marked peripheral blood eosinophilia. L-HES generally does not manifest with substantial lymphadenopathy or organomegaly. If these abnormalities are present, then biopsy of a lymph node or other primary site may be helpful in this differential diagnosis. L-HES is not an appropriate diagnosis if another well-defined PTCL subtype can be diagnosed. Biopsy of skin lesions may also be helpful in certain cases because the clinical presentation may mimic primary cutaneous T-cell lymphoma and mycosis fungoides. Finally, a markedly elevated peripheral absolute lymphocyte count should evoke a workup for a peripheral T-cell neoplasm other than L-HES. Although absolute lymphocyte counts may be modestly increased in some L-HES patients, most have normal absolute lymphocyte counts, and lymphocyte counts in excess of $10 \times 10^9$/L are rare in cases reported in the medical literature.

In the flow cytometric evaluation of suspected L-HES, it is important to note that other hematologic cell types, particularly monocytes, express CD4 in the absence of CD3, and careful steps should be taken to assure that analysis of CD3 expression is restricted to the T-lymphocyte population in the sample.

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The complete reference list is available online at www.expertconsult.com.

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# 14

# Acute Myeloid Leukemia

■ Amy E. Heerema-McKenney, MD ■ Daniel A. Arber, MD

## ■ INTRODUCTION AND CLASSIFICATION

Expanding knowledge of acute myeloid leukemia (AML) over the last 4 decades has resulted in major advances in the understanding and classification of this disease. AML is known to represent a number of distinct but related diseases, some of which require unique therapeutic approaches. The 2008 World Health Organization (WHO) classification integrates blast morphology and immunophenotype with cytogenetics and molecular studies to establish diagnoses with prognostic relevance. Risk stratification systems use these data to identify which patients may benefit from allogeneic bone marrow transplantation or novel therapeutics. The number of recurrent genetic abnormalities has been expanded from four to seven, and two provisional categories for subtypes of AML with gene mutations have been proposed. A new category, AML with myelodysplasia-related changes, is established that includes AML with multilineage dysplasia, as well as cases with a myelodysplasia-related karyotype or history of myelodysplasia. Patients with Down syndrome are classified separately, as are patients with a history of prior cytotoxic therapy (Table 14-1). This chapter uses the 2008 WHO classification and elaborates on diagnostic difficulties that persist, as well as the differential diagnosis for many of these entities.

## ■ DE NOVO ACUTE MYELOID LEUKEMIAS WITH RECURRENT GENETIC ABNORMALITIES

### ACUTE MYELOID LEUKEMIA WITH t(8;21)(q22;q22) *RUNX1-RUNX1T1*

The t(8;21)(q22;q22) is present in 8% to 13% of pediatric and adult AMLs. Some patients may exhibit extramedullary tumors (myeloid or granulocytic sarcomas). The t(8;21) results in fusion of the *RUNX1* gene (or *AML1*) on chromosome 21 with the *RUNX1T1* (or *ETO*) gene on chromosome 18, and the resulting *RUNX1/RUNX1T1* chimeric protein disrupts normal function of the core-binding factor, a transcription factor complex that regulates normal hematopoiesis. Despite disruption of the core binding factor by *RUNX1/RUNX1T1,* this genetic abnormality alone is not sufficient to cause leukemic transformation.

Blast cells with abundant pink granules and slightly basophilic cytoplasm are characteristic of this disease. The blasts usually show perinuclear clearing, or hofs, and a subset of the blasts have large, irregular pink (often termed *salmon-colored*) cytoplasmic granules (Figure 14-1). Thin Auer rods may also be present, but may be difficult to identify with some Wright stains. Eosinophils that are morphologically normal are often increased in number in the marrow, but this feature is not specific. Mature granulocytes may show dysplastic changes with nuclear pseudo–Pelger-Huët anomaly. This finding should not be interpreted as evidence of multilineage dysplasia, which is not seen in most cases of de novo AML with t(8;21). The large number of

**ACUTE MYELOID LEUKEMIA WITH t(8;21)—FACT SHEET**

**Definition**
- AML with bone marrow blasts showing distinctive morphologic and immunophenotypic features
- Presence of translocation diagnostic of AML regardless of blast count

**Frequency**
- 8% to 13% of all AMLs

**Cytogenetic Event**
- t(8;21)(q22;q22)

**Molecular Event**
- *RUNX1/RUNX1T1* fusion

**Prognosis**
- Intermediate to good with high-dose cytarabine regimens
- *KIT* mutations unfavorable in adults, not in children
- *FLT3* ITD mutations uncommon

**TABLE 14-1**

**WHO 2008 Classification of Acute Myeloid Leukemia**

AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22); *(RUNX1-RUNX1T1)*
- AML with inv(16)(p13.1q22) or t((16;16)(p13.1;q22); *(CBFB-MYH11)*
- APL with t(15;17)(q24;q21); *(PML-RARA)*
- AML with t(9;11)(p22;q23); *(MLLT3-MLL)*
- AML with t(6;9)(p23;q34); *(DEK-NUP214)*
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *(RPN1-EVI1)*
- AML (megakaryoblastic) with t(1;22)(p13;q13); *(RBM15-MKL1)*
- Provisional entity: AML with mutated *NPM1*
- Provisional entity: AML with mutated *CEBPA*

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML, not otherwise specified
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic/monocytic leukemia
- Acute erythroid leukemias
  - Pure erythroid leukemia
  - Erythroleukemia, erythroid/myeloid
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome
- Transient abnormal myelopoiesis
- Acute myeloid leukemia associated with Down syndrome

Data from Arber DA, Brunning RD, Le Beau MM, et al: Acute myeloid leukemia and related precursor neoplasm. In Swerdlow SH, Campo E, Harris NL, et al, editors: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue,* ed 4, Lyon, 2008, IARC, pp 109–147.
*AML,* Acute myeloid leukemia; *APL,* acute promyelocytic leukemia.

**ACUTE MYELOID LEUKEMIA WITH t(8;21)—PATHOLOGIC FEATURES**

**Blast Cell Morphologic Features**
- Abundant, large pink granules
- Basophilic cytoplasm
- Perinuclear hofs
- Thin Auer rods
- Admixed, normal eosinophils

**Blast Cell Immunophenotypic Features**
- Myeloid antigen positive (CD13, CD33)
- $CD34^+$
- Aberrant CD19 and PAX5 expression
- Subset of cases are $CD56^+$

**Differential Diagnosis**
- Growth factor effects
- Acute promyelocytic leukemia
- Myelodysplasia
- Mixed phenotype acute leukemia

cytoplasmic granules in the neoplastic cells of this leukemia subtype may raise concern for making a diagnosis of acute leukemia, because the cells might not be considered true blasts by some, but the WHO classification considers all cases with this cytogenetic abnormality to represent AML regardless of blast count. Cases of this type are usually classified as AML-M2 in the French-American-British (FAB) scheme, but represent a small subset of FAB M2 AMLs.

AML with t(8;21) has a characteristic immunophenotype that can be helpful in making the diagnosis. The blast cells characteristically express CD13 and CD33, similar to other types of AML, but also usually express CD34 and the B-cell associated antigens CD19 and PAX5. A subset of cases may also express CD56, which is associated with a worse prognosis in some studies. Rare cases will not express CD13 or CD33, but will be positive for other myeloid-associated markers, such as myeloperoxidase. Some cases may show expression of antigens associated with monocytic differentiation. The detection of the distinctive granular blast cell population with perinuclear clearing and large pink cytoplasmic granules in the setting of myeloid antigen expression and CD34 and CD19 expression is highly predictive of the t(8;21), and molecular studies are warranted in this setting if the initial karyotype is normal. AML with t(8;21) is considered to have an intermediate to good prognosis with therapeutic regimens that include high-dose cytarabine.

Low levels of the t(8;21) *RUNX1-RUNX1T1* transcript can persist in patients with clinical remission and increases of fusion transcript evident only on serial quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assays appear more predictive of recurrence. In some studies, eradication of molecular disease is necessary for long-term remission in AML with t(8;21).

The differential diagnosis of AML with t(8;21) includes growth factor changes, acute promyelocytic leukemia, myelodysplasia, and mixed-phenotype acute leukemia. Growth factor changes are described in more detail later, but the reactive promyelocytes seen after growth factor administration often show perinuclear hofs that can be confused with t(8;21) AML blasts. The detection of an aberrant immunophenotype, Auer rods, or the large pink granules of t(8;21) AML are useful in ruling out a growth factor change. Neoplastic promyelocytes of acute promyelocytic leukemia (see acute promyelocytic leukemia with *PML-RARA* below) tend to show more abundant Auer rods, finer cytoplasmic granules and more folded nuclei than are seen with t(8;21) AML. Because some of the blast cells in AML with t(8;21) have abundant granules, blast cells may appear to be less than 20%, suggesting myelodysplasia. However, as mentioned, cases with the characteristic features of t(8;21) AML do not usually show multilineage dysplasia and should be considered as acute

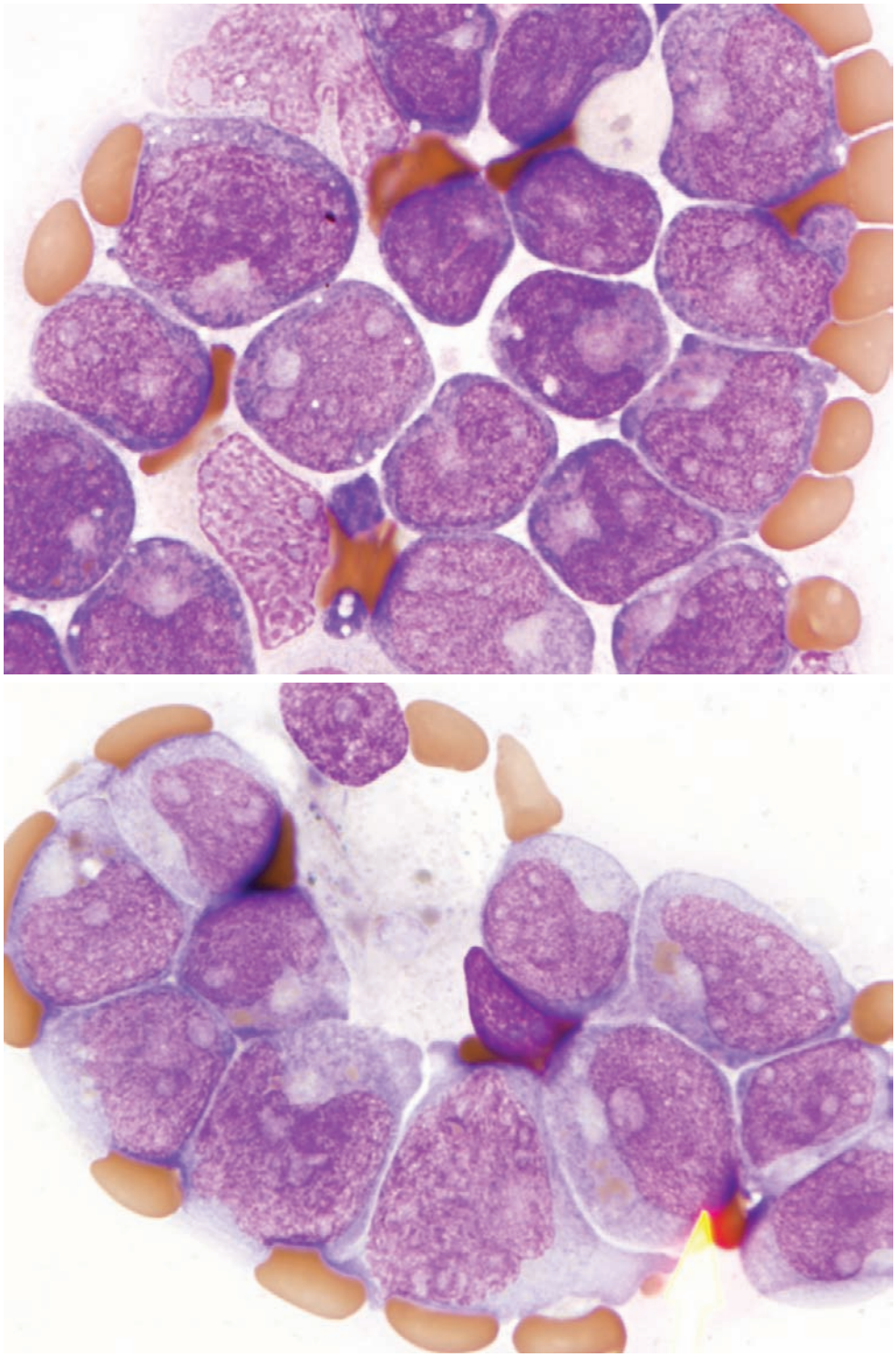

**FIGURE 14-1**

Acute myeloid leukemia with t(8;21). The blasts show characteristic perinuclear clearing or hofs with large pink or salmon-colored cytoplasmic granules.

leukemias rather than myelodysplasia. Finally, the aberrant expression of CD19 and PAX5 suggests an acute leukemia of ambiguous lineage. The 2008 WHO criteria for mixed phenotype acute leukemia, B-myeloid, require strong CD19 together with expression of CD79a, cytoplasmic CD22 or CD10, or weak CD19 with at least two of the additional B-cell markers noted above (strong CD79a, cytoplasmic CD22, and CD10). AML with t(8;21) typically has weak CD19 and does not commonly express CD22 or CD10. Demonstration of the t(8;21) by cytogenetics, fluorescence in situ hybridization (FISH), or PCR always trumps a diagnosis of acute

leukemia of ambiguous lineage. Patients with t(8;21) AML have a favorable prognosis with appropriate therapy. In contrast, patients with ambiguous-lineage leukemia often have a poor prognosis, and an ideal therapy has not been defined.

Core binding factor AML (those with RUNX1-RUNXT1 or CBFB-MYH11 fusion) commonly harbors mutations of *KIT* (20% to 25%). In adults, *KIT* mutations in exons 8 and 17 appear to be associated with a worse prognosis. In children, they do not have a significant prognostic effect. It is unclear whether adults with *KIT* mutated AML with t(8;21) benefit from allogeneic stem cell transplantation in first remission or tyrosine kinase inhibitor therapy. Rarely, patients with *KIT* mutated AML with t(8;21) have systemic mastocytosis. Those patients are reported to have a poor prognosis. Most AMLs with t(8;21) have additional cytogenetic abnormalities commonly involving loss of a sex chromosome or partial deletion of the long arm of chromosome 9 (i.e., del[9q]). The presence of additional cytogenetic abnormalities does not have prognostic significance. *FLT3* internal tandem duplication (ITD) mutations are uncommon in AML with t(8;21), as are mutations of *NPM1* or *CEBPA*.

## ACUTE MYELOID LEUKEMIA WITH inv(16) (p13.1q22) OR t(16;16) (p13.1;q22); *CBFB-MYH11*

AML with inv(16)(p12q22) or t(16;16)(p13;q22) occurs in 5% to 10% of all AML cases and tends to occur in children or young adults, whereas approximately 1% to 3% of older adult AMLs have this abnormality. Both cytogenetic abnormalities result in a fusion of the *CBFB* and *MYH11* genes on chromosome 16, which, similar to the t(8;21), disrupts the core binding factor transcription factor complex.

The bone marrow usually shows a proliferation of blasts with monocytic features and cytoplasmic granules as well as a population of abnormal eosinophils. This category generally corresponds to the AML M4Eo designation in the FAB classification. Not all cases, however, will show monocytic differentiation either by morphology or cytochemistry. The presence of an increase in normal-appearing eosinophils is not sufficient to suggest this diagnosis, because it can occur in a variety of AML subtypes. The maturing eosinophils must show abnormal basophilic granules that are large and coarse, which may give the appearance of basophils (Figure 14-2); however, there are readily identifiable background eosinophilic granules present in the cells as well. Whereas the abnormal eosinophils are classically described as positive for chloracetate esterase by cytochemistry, as opposed to normal eosinophils that are negative for chloracetate esterase, this test is often only weakly positive and not usually necessary for the diagnosis. Rare cases will have numerous eosinophils and maturing monocytes, making the blast cell count less than 20%; however, the WHO scheme classifies this molecular genetically defined disease as acute leukemia regardless of blast cell count. Multilineage dysplasia is uncommon in this subtype. The detection of abnormal eosinophils is highly predictive of a chromosome 16 abnormality, which may be subtle on routine karyotype. Therefore additional studies, such as RT-PCR or FISH, should be performed on cases with abnormal eosinophils and an apparently normal karyotype. In a significant percentage of cases (up to 20%), the abnormal eosinophils can be scarce or absent. The eosinophils with large basophilic granules are often not evident in the peripheral blood. In cases without abnormal eosinophils, the precise diagnosis can only be made after cytogenetic or molecular genetics detection of an inv(16) or t(16;16).

**ACUTE MYELOID LEUKEMIA WITH inv(16) OR t(16;16)—FACT SHEET**

**Definition**
- AML with bone marrow blasts showing distinctive morphologic and immunophenotypic features
- Presence of translocation diagnostic of AML regardless of blast count

**Frequency**
- 5% to 10% of all AMLs

**Cytogenetic Event**
- inv(16)(p12q22) or t(16;16)(p13;q22)

**Molecular Event**
- *CBFB/MYH11* fusion

**Prognosis**
- Good with high-dose cytarabine regimens
- *KIT* mutations impart poor prognosis in adults, not demonstrated in children
- *FLT3*-ITD mutations uncommon

**ACUTE MYELOID LEUKEMIA WITH inv(16) OR t(16;16)–PATHOLOGIC FEATURES**

**Blast Cell Morphologic Features**
- Often myelomonocytic appearance
- Abnormal eosinophils with large, basophilic granules

**Blast Cell Immunophenotypic Features**
- Myeloid or myelomonocytic antigen positive (CD13, CD33, CD14, CD64)

**Differential Diagnosis**
- AML, not otherwise categorized
- Myelodysplasia

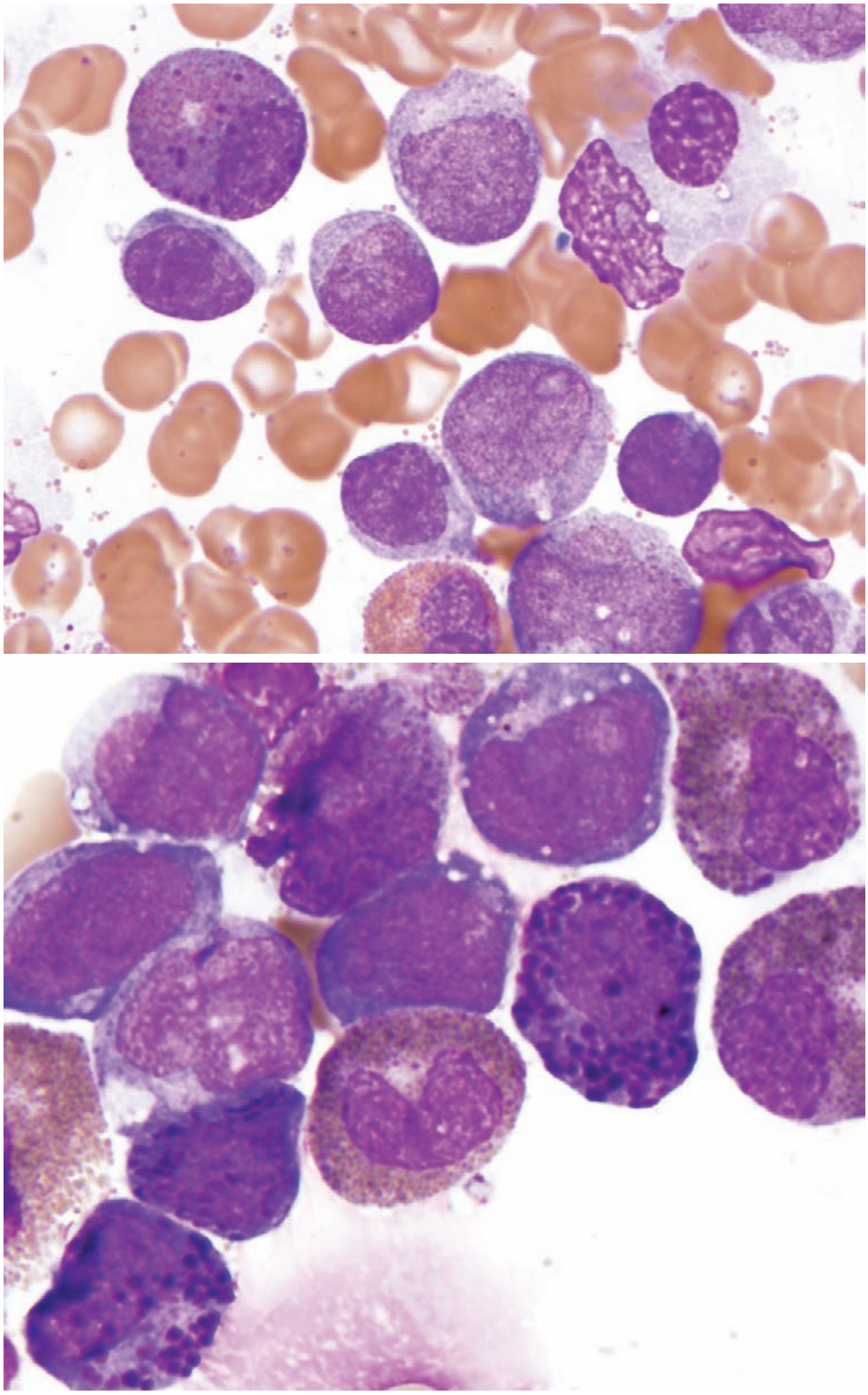

**FIGURE 14-2**

Acute myeloid leukemia with inv(16) or t(16;16). The blasts may have a myeloid or myelomonocytic appearance. They are accompanied by eosinophil precursors, some of which have large basophilic granules.

AML with inv(16) or t(16;16) shows expression of the expected myeloid antigens (CD13 and CD33) and often monocyte-associated markers, such as CD4, CD14, and CD64. Immunophenotyping may show a single population, or two distinct cell populations: one myeloblastic and the other monocytic. A subset of AML with inv(16) or t(16;16) cases will show aberrant expression of the T-cell associated marker CD2, but it is not specific for this disease.

AML with inv(16) or t(16;16) is generally associated with a favorable prognosis with high-dose cytarabine. Serial quantitative RT-PCR monitoring for these genetic abnormalities is useful in identifying patients at high risk for relapse.

Because subsets of cases will not show abnormal eosinophils, especially if diagnosed in peripheral blood, or will have less than 20% blast cells, the differential diagnosis of AML with inv(16) or t(16;16) includes AML, not otherwise specified, and myelodysplasia. Upon identification of the *CBFB-MYH11* fusion by cytogenetics, FISH, or PCR, the designation of AML with inv(16) or t(16;16) is established regardless of the blast count.

Like AML with t(8;21), this core binding factor leukemia has a favorable prognosis. *KIT* mutations are present in approximately 30% of cases and negatively affect prognosis in adults, but not in children. It is not yet clear whether adult cases of AML with an inv(16)(p13;q22) or t(16;16)(p13;q22) and mutated *KIT* benefit from allogeneic stem cell transplantation or tyrosine kinase inhibitor therapy.

**ACUTE PROMYELOCYTIC LEUKEMIA—FACT SHEET**

**Definition**
- A neoplastic proliferation of bone marrow blast cells with features of promyelocytes
- Presence of translocation diagnostic of AML regardless of blast count

**Frequency**
- 9% to 12% of all AMLs

**Most Common Cytogenetic and Molecular Events**
- t(15;17)(q24;q21) *PML/RARA* fusion

**Variant RARA Fusion Partners (Chromosome/Gene)**
- 5q35/*NPM1*
- 11q13/*NUMA*
- 11q23/*ZBTB16*
- 17q11/*STAT5B*

**Prognosis**
- Good with all-*trans* retinoic acid or arsenic trioxide regimens for cases with *PML/RARA*
- *FLT3* ITD mutations seen in a subset, associated with high WBC and inferior prognosis

## ACUTE PROMYELOCYTIC LEUKEMIA WITH t(15;17)(q24;q21); *PML-RARA*

Acute promyelocytic leukemia (APL) represents 9% to 12% of AMLs and is frequently associated with disseminated intravascular coagulopathy (DIC). Indeed, the DIC may be fatal if not recognized early in the management of these patients. Early recognition of this subtype of AML by the characteristic morphologic and phenotypic features is important to alert the clinician to the potential for DIC and to initiate specific therapy with all-*trans* retinoic acid (ATRA). All molecular subtypes contain mutations of the retinoic acid receptor alpha *(RARA)* gene on chromosome 17. The t(15;17)(q24;q21) is the most common genetic aberration in APL and results in fusion of *RARA* with the *PML* gene on chromosome 15. This category generally corresponds to AML-M3 in the FAB classification. Of note, the chromosomal breakpoints of APL, which were considered to be t(15;17)(q22;q12-21), have been recently updated and are now recognized to be t(15;17)(q24;q21).

A variety of morphologic types of APL are described, but they are most easily subdivided into hypergranular and hypogranular (or microgranular) forms of the disease (Figure 14-3). The classic hypergranular APL has blast cells that somewhat resemble normal promyelocytes with abundant cytoplasmic granules. Numerous Auer rods are present in individual blasts (termed *faggot cells*). In contrast to the blasts of AML with t(8;21) or reactive promyelocyte proliferations, APL cells do not show differing stages of maturation or perinuclear clearing. Microgranular APL is usually more difficult to recognize as a type of APL and may be mistaken for myelomonocytic leukemia. The blasts characteristically have folded or bilobed nuclei ("butterfly" nuclei) with very fine to undetectable cytoplasmic granules. The hypogranular appearance is due to the submicroscopic size of granules present in the cytoplasm of blasts. Rarely, hypergranular cells may be present with Auer rods, but they often require an extensive search to be identified. Both types of APL show strong myeloperoxidase positivity in virtually all blast cells, by both cytochemistry and flow cytometry, which is often helpful in differentiating hypogranular promyelocytes from blasts with monocytic differentiation. Some cases of APL contain a subset of blasts that are positive for nonspecific esterase by cytochemistry; this could lead to confusion with an acute myelomonocytic leukemia using FAB criteria. Multilineage dysplasia is not usually present in APL with t(15;17). Although there are no well-defined morphologic features to differentiate most of the cytogenetic variants of APL, patients with the *ZBTB16/RARA* molecular fusion are described as

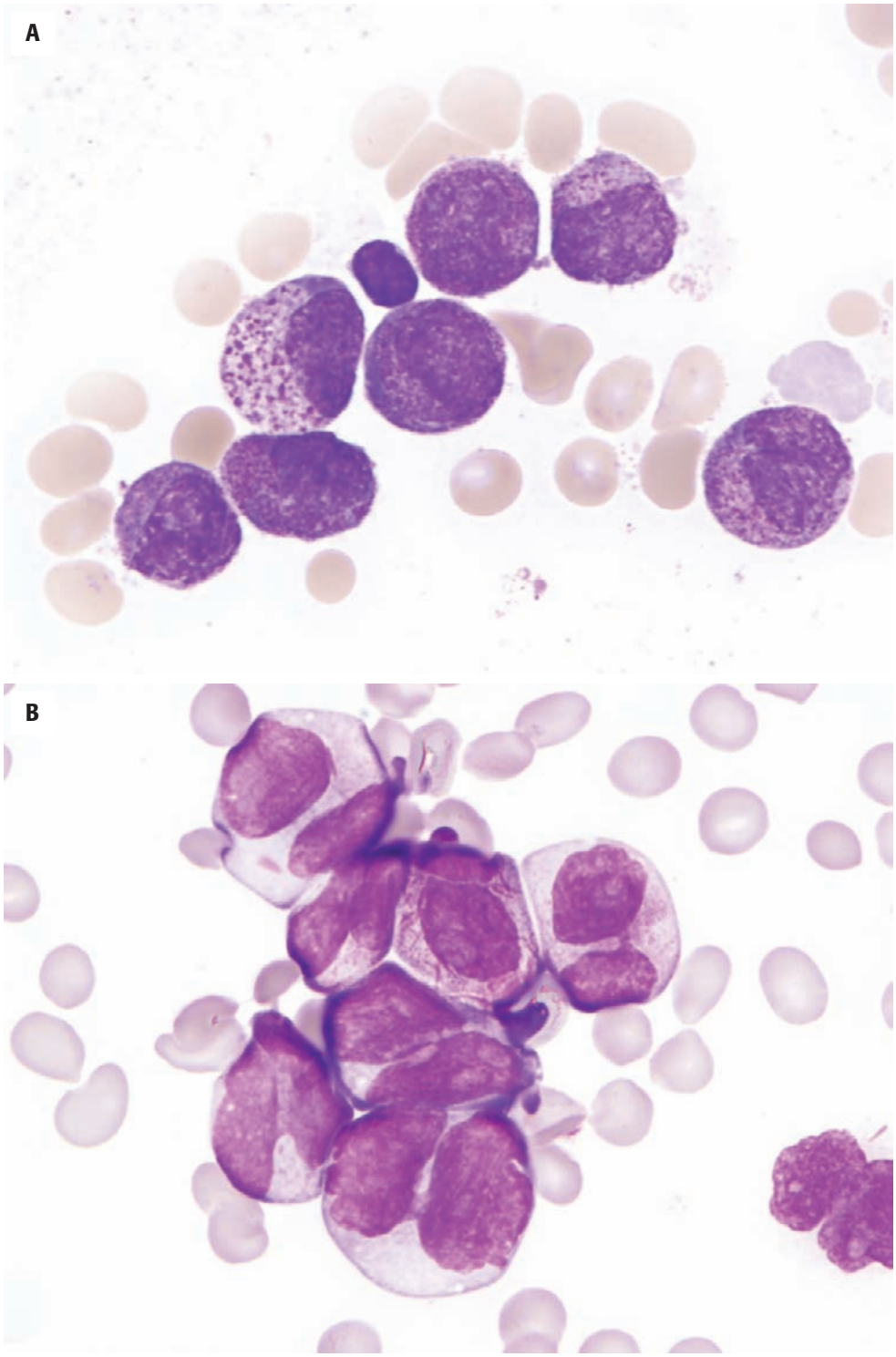


**FIGURE 14-3**

Acute promyelocytic leukemia. **A,** The granular type shows abundant cytoplasmic granules similar to normal promyelocytes. **B,** The microgranular variant has fine cytoplasmic granules and folded nuclei. Despite the hypogranular appearance of most cells, scattered cells with granules and numerous Auer rods can be seen.

having distinctive features. These features include more round blast cell nuclei with more variably sized cytoplasmic granules than in other types of APL. In addition, circulating Pelger-like neutrophils are described with clumped chromatin and hyposegmented or unsegmented nuclei. However, this molecular subtype of APL is extremely uncommon, and the predictive value of these morphologic features is unclear.

APL cells express CD33 and CD13, although the latter may be weak and heterogenous. With CD45

**ACUTE PROMYELOCYTIC LEUKEMIA—PATHOLOGIC FEATURES**

**Blast Cell Morphologic Features**

- Folded, bilobed nuclei
- Abundant cytoplasmic granule
- Numerous Auer rods

**Blast Cell Immunophenotypic Features**

- Myeloid antigen positive (CD13, CD33)
- Weak or absent CD34 expression
- Aberrant loss of HLA-DR
- Aberrant expression of CD2 (subset)

**Differential Diagnosis**

- AML, not otherwise categorized, acute monocytic leukemia (can resemble microgranular variant)
- Growth factor changes
- Myelodysplasia

gating, the blast cell area is expanded because of an increase in side scatter secondary to the cytoplasmic granularity of the leukemic cells. Hypergranular APL is typically human leukocyte antigen (HLA)-DR and CD34 negative. Microgranular APL may show weak or partial expression of these antigens. This immunophenotype is not unique to APL, as a subset of AML, not otherwise specified (NOS), cases without differentiation similarly lack CD34 and HLA-DR. Aberrant expression of CD2 is more commonly observed in microgranular APL. CD56 expression is described in 15% to 20% of patients and has been associated with shorter complete remission and poorer overall survival in some studies.

The *PML-RARA* fusion can arise from one of three breakpoint regions on *PML* at 15q24. Two (bcr1, bcr2) lead to long transcripts, and the third (bcr3) leads to the short transcript. Microgranular APL typically has the short transcript. Cytogenetics, FISH, or RT-PCR are necessary for genetic confirmation of the *PML-RARA* fusion. FISH, RT-PCR, and immunofluorescence for the microspeckled nuclear distribution of the *PML* protein all may aid in rapid diagnosis. RT-PCR is the only technique that can identify the *PML-RARA* transcript isoform useful for minimal residual disease monitoring. *FLT3* ITD mutations are seen in approximately 40% of patients, more commonly in the microgranular variant, with high white blood cell counts.

Atypical promyelocytes can persist in the marrow for up to several weeks after induction chemotherapy, as can detection of *PML-RARA* by karyotype, FISH, or RT-PCR. These findings do not necessarily indicate resistant disease. Detection of *PML-RARA* by RT-PCR after induction does not affect subsequent clinical outcome; however, detection of *PML-RARA* after complete remission is obtained strongly predicts risk of relapse.

The majority of mutations of *FLT3* in APL are *FLT3* ITDs. *FLT3* ITD in APL is strongly associated with the microgranular subtype, high white blood cell counts in peripheral blood, and the bcr3 breakpoint in *PML*. In one retrospective study, patients with mutant *FLT3* had a higher rate of death during the period of induction chemotherapy, but no significant difference in relapse rate or 5-year overall survival.

PML-RARA is the most common fusion product in APL. *RARA* is a nuclear hormone receptor that binds to specific DNA sequences referred to as retinoic acid responsive elements in a heterodimeric complex with retinoid-X receptors *(RXRA)*. The *RARA-RXRA* complexes can repress transcription through a variety of mechanisms, but normal physiologic concentrations of retinoic acid in the body control this activity, allowing for normal activation of gene transcription. With the *PML-RARA* fusion, physiologic levels of retinoic acid are no longer sufficient to control the effects of *RARA*, and gene transcription is blocked. Therapeutic strategies that include ATRA provide pharmacologic concentrations of retinoic acid that allow for the return of normal transcription. Less commonly, chromosomal translocations of *RARA* on chromosome 17 can occur with other genes such as *ZBTB16* (or *PLZF*) at 11q23, *NUMA* at 11q13, *NPM1* at 5q35, and *STAT5B* at 17q11. Patients that lack *RARA* translocations or harbor *RARA* translocations involving *ZBTB16* or *STAT5B* might not respond to ATRA and thus require a different therapeutic approach. With current therapy, using ATRA or arsenic trioxide, APL is considered to have a favorable prognosis.

The t(15;17) is usually detectable by karyotype analysis at diagnosis. Molecular detection of *PML-RARA* is usually performed by RT-PCR or FISH analysis, and these tests may be useful to rapidly confirm the initial diagnosis. However, the combined morphologic and immunophenotypic features can be extremely reliable in detecting this disease. The *PML-RARA* RT-PCR test has been shown to be useful in the early detection of residual disease in acute promyelocytic leukemia and as a predictor of relapse of that disease. Most assays for *PML-RARA* detect one translocated cell in 10,000 to 100,000 cells. The RT-PCR test appears to be less useful when more sensitive assays that detect one abnormal cell in one million are used. Results of these ultrasensitive tests will be positive in patients that are in long-term remission and do not appear to be clinically relevant. Recent studies suggest that serial quantitative assays may be useful to follow patients with low levels of disease.

The differential diagnosis of APL includes AML, NOS, myelodysplasia, and growth factor changes. As mentioned previously, the morphologic features of the microgranular variant may suggest a myelomonocytic type of AML. Because of the predominance of cells with promyelocyte features, the blast cell count may appear low and suggest a myelodysplastic syndrome, but these

cases are considered as APL without regard to the blast cell count. The predominance of promyelocytes might also suggest early growth factor changes. The characteristic perinuclear hof or reactive promyelocytes, however, is not seen in acute promyelocytic leukemia and cytogenetic abnormalities are not seen in those reactive proliferations.

## ACUTE MYELOID LEUKEMIA WITH t(9;11)(p22;q23) *MLLT3-MLL*

Rearrangement of the *MLL* gene at 11q23 is seen in 4% to 5% of de novo adult AMLs, and in a much larger percentage of pediatric patients (up to 22%), particularly infants. Patients are at increased risk for DIC and have a propensity for extramedullary disease, such as myeloid sarcoma or tissue infiltration, commonly in the gingiva or skin. The 2008 WHO classification limits the recurrent genetic abnormalities categorization to AML with t(9;11)(p22;q23), because this subtype appears to be the most clinically homogeneous. Other rearrangements of 11q23 are diagnosed as AML, NOS, with note of the translocation in the diagnostic line. In the 2008 WHO classification, the t(2;11)(p21;q23) and t(11;16)(q23;p13.3) are designated myelodysplasia associated karyotypes, and a diagnosis of AML with myelodysplasia related changes is given (see AML with myelodysplasia related changes and Table 14-2). *MLL*-rearranged AML most commonly shows monocytic differentiation, with or without nonspecific esterase cytochemical reactivity. AML with t(9;11) usually displays monocytic features, although other morphologies are observed rarely. In the bone marrow, the blast features are usually nonspecific but may have round to folded monocytoid nuclei with usually abundant, slightly basophilic, and vacuolated cytoplasm (Figure 14-4). Promonocytes are often present, with more mature-shaped nuclei but retaining immature nuclear chromatin. More mature cells may predominate in the blood, which suggests a chronic monocytic proliferation. Multilineage dysplasia is usually not present in the blood or marrow. Because the morphologic features are not specific for this molecular subtype of AML, there is no way to reliably predict an *MLL* abnormality without performing the appropriate cytogenetic and molecular assays.

The blasts may variably express myeloid-associated antigens CD13 and CD33, are usually CD34 and myeloperoxidase negative, and often express monocyte-associated markers such as CD4, CD14, and CD64. They may also express CD56.

*MLL* is a large, 90-kb gene containing 36 exons coding for a 431-kDa protein. The protein contains AT-hooks, which are known to regulate transcription by inducing changes in DNA conformation that permit the association of transcription factors with regulatory regions of DNA. These AT hooks recognize specific DNA structures. Other regions of *MLL* may repress transcription from reporter constructs, mediate homodimerization, mediate interactions with nuclear proteins, contain transcriptional activating activity, and are involved with ATP-dependent chromatin remodeling. Partial tandem duplication mutations of *MLL* also occur in AML and are more common in patients with normal karyotypes or trisomy of chromosome 11.

Despite the numerous translocation partners for *MLL*, the most common in AML are located on chromosomes 6q27 (*MLLT4*, or *AF6*), 9p22 (*MLLT3*, or *AF9*), 19p13.3 (*MLLT1*, or *ENL*), 19p13.1 (*ELL*), 19p13.3 (*SH3GL1*, or *EEN*), 16p13 (*CREBBP*, or *CBP*), and 22q13 (*EP300*, or *p300*). The t(9;11) conveys intermediate risk disease, with a complete remission rate similar to that of normal karyotype AML. In contrast, AML

**ACUTE MYELOID LEUKEMIA WITH t(9;11)(p22;q23)—FACT SHEET**

**Definition**
- AML with bone marrow blasts showing distinctive morphologic and immunophenotypic features
- Presence of translocation alone *not* diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

**Frequency**
- Approximately 10% of all pediatric AMLs
- 4% to 5% of de novo adult AMLs

**Cytogenetic Event**
- AML with t(9;11)(p22;q23)

**Molecular Event**
- *MLLT3-MLL* fusion

**Prognosis**
- Intermediate
- *FLT3*-ITD uncommon

**ACUTE MYELOID LEUKEMIA WITH t(9;11)(p22;q23)—PATHOLOGIC FEATURES**

**Blast Cell Morphologic Features**
- Usually monoblastic

**Blast Cell Immunophenotypic Features**
- Myeloid or myelomonocytic antigen positive (CD13, CD33, CD11b, CD11c, CD4, CD14, CD64)
- CD34 and myeloperoxidase negative
- Often $CD56^+$

**Differential Diagnosis**
- AML, not otherwise categorized
- Chronic myelomonocytic leukemia
- Juvenile myelomonocytic leukemia

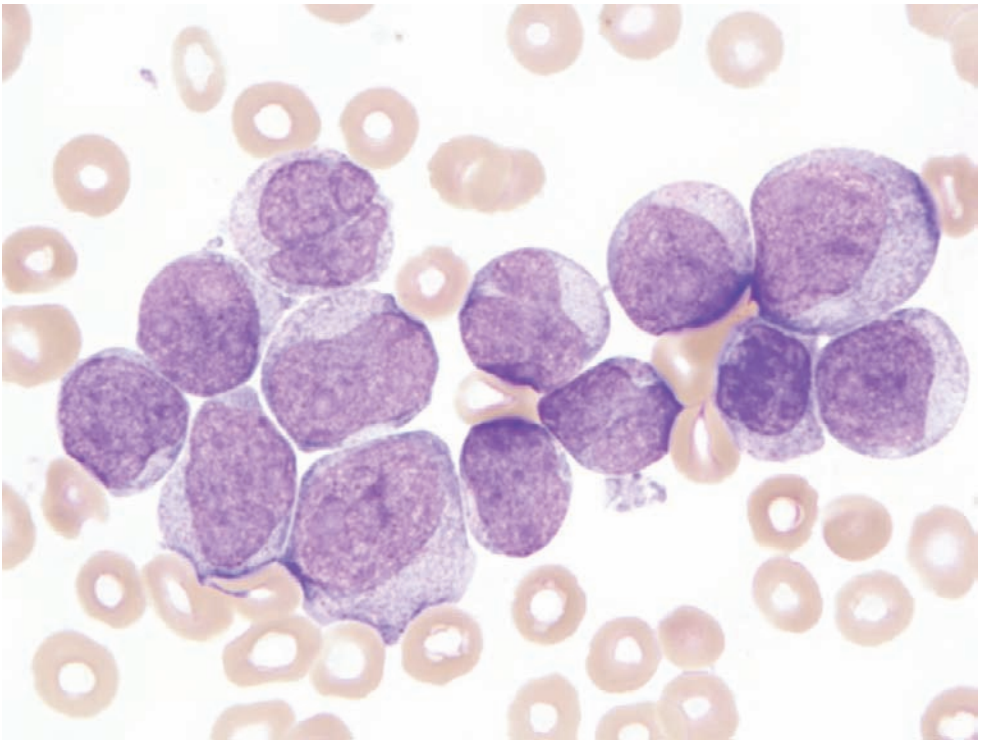

**FIGURE 14-4**
Acute myeloid leukemia with 11q23 abnormality showing a monocytic blast cell population.

with t(6;11)(q27;q23), AML with t(10;11)(p11 ~ 13;q23), and other 11q23 translocations with exception of the t(9;11) and t(11;19) are considered poor risk AMLs with shorter survival.

AML with t(9;11) lacks specific morphologic or immunophenotypic features and can be easily mistaken for various other types of AML in the not otherwise specified group. These cases are only clarified with cytogenetic or molecular genetic studies. Because many monocytic leukemias show maturation of neoplastic cells in the peripheral blood, which can be interpreted as mature monocytes, these cases could be misdiagnosed as chronic myelomonocytic leukemia or juvenile myelomonocytic leukemia. Neither of these diagnoses should be made on peripheral blood samples alone. The threshold of 20% blasts must be met to confer a diagnosis of AML with t(9;11). If fewer than 20% blasts are present, a diagnosis of myelodysplastic syndrome (MDS) is more appropriate and frequent monitoring for the development of AML is required. Any history of cytotoxic or radiation therapy would designate an AML with 11q23 rearranged as a therapy-related myeloid neoplasm.

## ACUTE MYELOID LEUKEMIA WITH t(6;9)(p23;q34) *DEK-NUP214*

Acute myeloid leukemia with t(6;9)(p23;q34) is a new addition to the recurrent genetic abnormality category in the 2008 WHO classification. AML with t(6;9) comprises less than 2% of AML in children and adults. Patients may have anemia, thrombocytopenia, and modestly elevated white blood cell counts. The marrow typically shows erythroid hyperplasia, dysplasia, and bone marrow basophilia (Figure 14-5). Although erythroid dysplasia appears to be the most common, a recent report has also noted at least some granulocyte and megakaryocyte dysplasia with this abnormality. Bone marrow basophilia is unusual in other AML types other than rare t(9;22) AMLs or myeloid blast crisis of chronic myelogenous leukemia. By flow cytometry, blasts typically express CD45, CD13, CD33, HLA-DR, and intracytoplasmic myeloperoxidase (MPO), with variable expression of CD34, CD15, and CD11c. Terminal deoxynucleotidyl transferase (TdT) may be positive in some cases by flow cytometry or immunohistochemistry. The t(6;9)(p23;q34) results in a fusion of *DEK* gene on chromosome 6 with the *NUP214* (or *CAN*) gene on chromosome 9. *NUP214* is a nucleoporin and one of several components of nuclear pore complexes, which are structures that allow macromolecules to move in and out of the cell nucleus. It appears that nucleoporin fusion proteins act as aberrant transcription factors as well as altering nuclear transport by binding to soluble transport factors. *FLT3* ITD mutations are common in this type of AML, with a reported frequency of 70%. Although this leukemia has a generally poor prognosis in the literature, some patients appear to do well with aggressive therapy that includes hematopoietic stem cell transplantation.

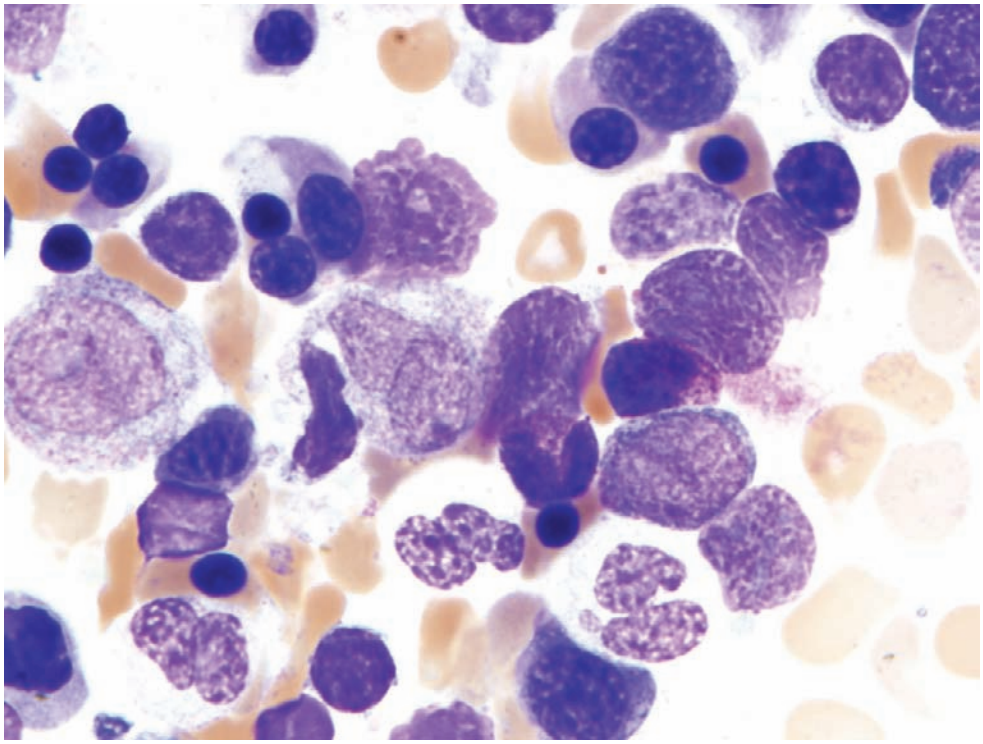

**FIGURE 14-5**

Acute myeloid leukemia with t(6;9). Myelomonocytic blasts are present with admixed small basophils, erythroid hyperplasia with dyserythropoiesis, and hypogranular neutrophils.

**ACUTE MYELOID LEUKEMIA WITH t(6;9)(p23;q34)—FACT SHEET**

**Definition**
- AML with common morphologic features and prognosis
- Presence of translocation alone not diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

**Frequency**
- Approximately 2% AMLs

**Cytogenetic Event**
- t(6;9)(p23;q34)

**Molecular Event**
- *DEK-NUP214* fusion

**Prognosis**
- Poor
- *FLT3*-ITDs commonly present

**ACUTE MYELOID LEUKEMIA WITH t(6;9)(p23;q34)—PATHOLOGIC FEATURES**

**Blast Cell Morphologic Features**
- Nonspecific myeloid phenotype, commonly associated with erythroid hyperplasia, multilineage dysplasia, and basophilia

**Blast Cell Immunophenotypic Features**
- Myeloid antigen positive (CD13, CD33)

**Differential Diagnosis**
- AML with myelodysplasia related changes (AML-MRC)
- Acute erythroid leukemia (erythroid/myeloid)
- Myelodysplastic syndrome
- Blast transformation of chronic myelogenous leukemia

The differential diagnosis of AML with t(6;9) includes AML with inv(16) or t(16;16). The abnormal eosinophils of the latter disease may be mistaken for basophils, but the detection of background eosinophil granules in these cells is helpful in this differential diagnosis. AML with t(6;9) is in the differential diagnosis of AML with myelodysplasia-related change because of the common presence of multilineage dysplasia. Presence of the translocation allows more precise classification. Rarely, patients have fewer than 20% blasts; the presence of the translocation in this setting is not diagnostic of AML and close follow-up is required.

## ACUTE MYELOID LEUKEMIA WITH inv(3)(q21q26.2) OR t(3;3)(q21;q26.2); *RPN1-EVI1*

AML with inv(3) or t(3;3) is a new component of the recurrent genetic abnormality category in the 2008

**ACUTE MYELOID LEUKEMIA WITH inv(3)(q21q26.2) OR t(3;3)(q21;q26.2)—FACT SHEET**

**Definition**
- AML with common morphologic features and prognosis
- Presence of translocation alone not diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

**Frequency**
- 1% to 2% of AML

**Cytogenetic Event**
- inv(3)(q21q26.2) or t(3;3)(q21;q26.2), with frequent additional monosomy 7

**Molecular Event**
- *RPN1-EVI1* fusion

**Prognosis**
- Poor
- *FLT3* ITDs uncommon

**ACUTE MYELOID LEUKEMIA WITH inv(3)(q21q26.2) OR t(3;3)(q21;q26.2)—PATHOLOGIC FEATURES**

**Blast Cell Morphologic Features**
- Myeloid phenotype, sometimes with megakaryocytic differentiation

**Non–Blast Cell Morphology**
- Multilineage dysplasia, small bilobed megakaryocytes

**Blast Cell Immunophenotypic Features**
- Myeloid antigen positive (CD13, CD33, CD34, CD117, HLA-DR), variable CD61, CD41

**Differential Diagnosis**
- AML with myelodysplasia related changes (AML-MRC)
- AML, NOS, acute megakaryoblastic leukemia
- Myelodysplastic syndrome, 5q- syndrome

WHO classification. This subtype is rare, representing 1% to 2% of AML in adults and less than 1% of AML in children. The inv(3) or t(3;3)(q21;q26) disrupt the ecotropic virus insertion site 1 (EVI1) gene on 3q26. This disease is associated with multilineage dysplasia and thrombocytosis (Figure 14-6). Giant platelets may be seen in the peripheral blood that may not be counted by automated methods. The most distinctive bone marrow feature is the presence of small, unilobate or bilobate megakaryocytes. The megakaryocytes are similar to those seen in the 5q-minus syndrome of myelodysplasia, but are smaller and are accompanied by the presence of multilineage dysplasia and an increase in blast cells in inv(3) AML—features that are not seen with 5q-minus syndrome. This disease is usually associated with a poor prognosis. Additional poor-prognosis cytogenetic abnormalities are common, monosomy 7 (66%) in particular, and this appears to worsen the already poor prognosis. *NPM1* mutations are rarely seen in this category, and *FLT3* ITDs are uncommon (7%). *CEBPA* mutations are not reported. Other translocations of 3q26 are not included in this category, and they appear to have a less severe but poor prognosis.

## ACUTE MYELOID LEUKEMIA (MEGAKARYOBLASTIC) WITH t(1;22)(p13;q13); *RBM15-MKL1*

AML (megakaryoblastic) with t(1;22) is a rare form of AML affecting infants almost exclusively. The t(1;22)(p13;q13) results in an *RBM15/MKL1* (or *OTT/MAL*) fusion product (Figure 14-7). Eighty percent of cases are diagnosed in the first year of life. AML with t(1;22) comprises approximately 1% of childhood AML. AML with t(1;22) may mimic a solid tumor at diagnosis with hepatosplenomegaly, skeletal lesions, or both. The diagnosis can be particularly challenging in cases presenting as myeloid sarcoma with no involvement of the bone marrow. The lesions may show cohesive nests of small, round, blue cells also suggestive of a childhood solid tumor. Blasts in the blood and bone marrow show typical features of megakaryoblasts, with a modest amount of agranular cytoplasm that may show blebs or budding of platelets. The nuclear chromatin may be more condensed than myeloid blasts and infrequently nucleolated. The bone marrow aspirate may be hemodilute or aparticulate owing to marrow fibrosis. Unlike acute megakaryoblastic leukemia of Down syndrome, or acute megakaryoblastic leukemia in adults with inv(3), multilineage dysplasia is not described. Some cases may show atypical megakaryocytic maturation with micromegakaryocytes, and many may show myelofibrosis. Few cases have a reported flow cytometry immunophenotype. CD45 and CD34 may be negative, as in other AML with megakaryoblastic morphology. The myeloid antigens CD13 and CD33 are expressed inconsistently, as is HLA-DR. Immunoreactivity for megakaryocytic antigens CD41 and CD61 is commonly seen by flow cytometry, but it may be difficult to prove immunohistochemically. CD4 and CD56 may also be expressed, bringing blastic plasmacytoid dendritic cell tumor into the differential diagnosis. Additional complex karyotypic abnormalities are common in older patients (greater than 6 months of age). The frequency of *FLT3* mutations is unclear, given the rarity of acute megakaryoblastic leukemia with t(1;22). In one study of pediatric AML (excluding patients with Down syndrome) none of the morphologic M7 cases had *FLT3* ITD

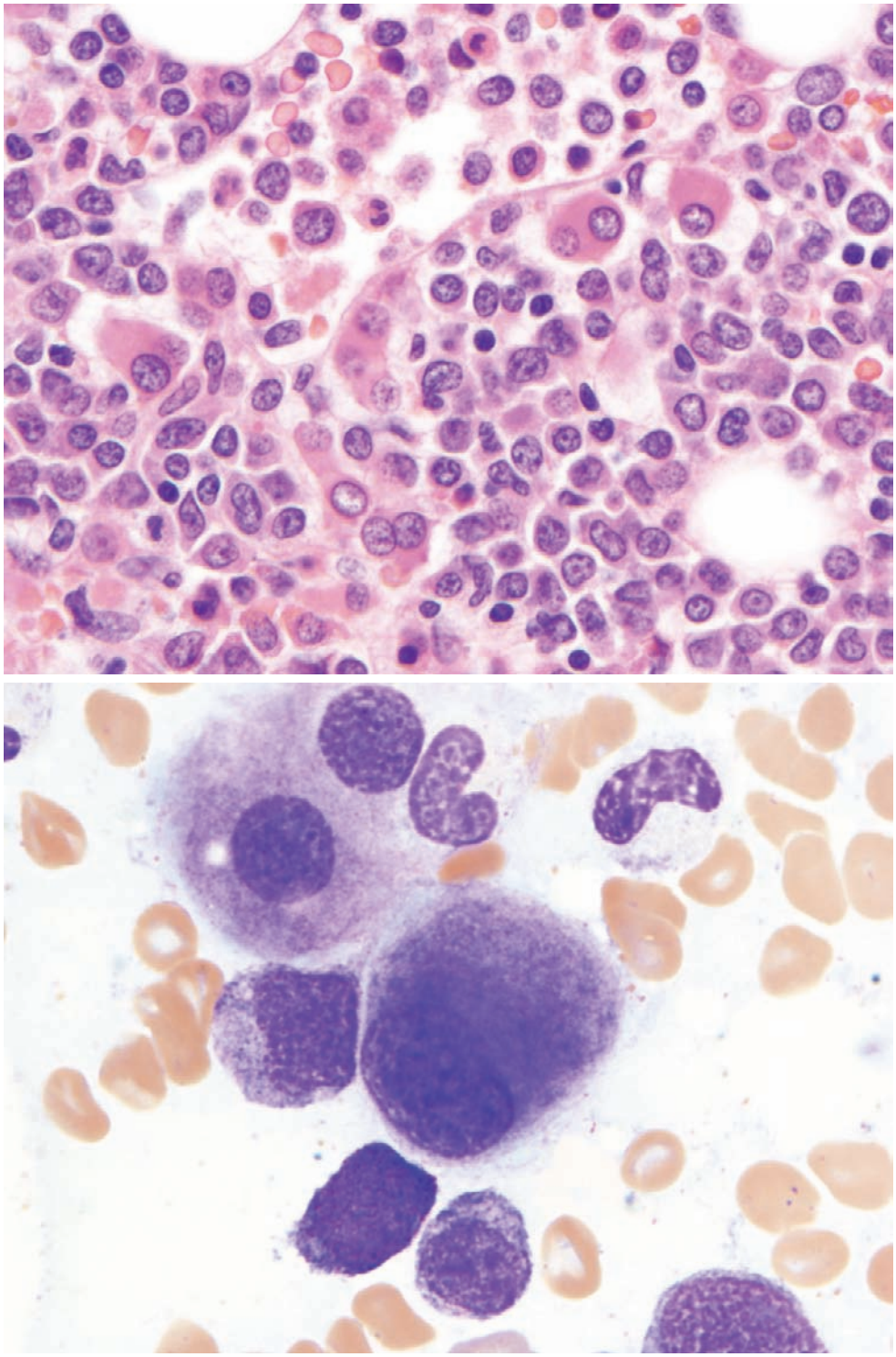

**FIGURE 14-6**

Acute myeloid leukemia with inv(3) or t(3;3). Small megakaryocytes with unilobate or bilobate nuclei are present.

mutations, although one patient had an activation loop domain point mutation. Diagnosis may be delayed in these patients because of the difficulties described previously. AML with t(1;22) was associated with poor survival in earlier studies, but more recent studies suggest they respond well to intensive AML therapy. Cases commonly present with fewer than 20% blasts in the blood or marrow, or both. Bone marrow core biopsies are often essential in the diagnosis, revealing extensive involvement when aspirates are aparticulate. Careful clinical evaluation is warranted to assess for extramedullary disease diagnostic of AML when blasts are less

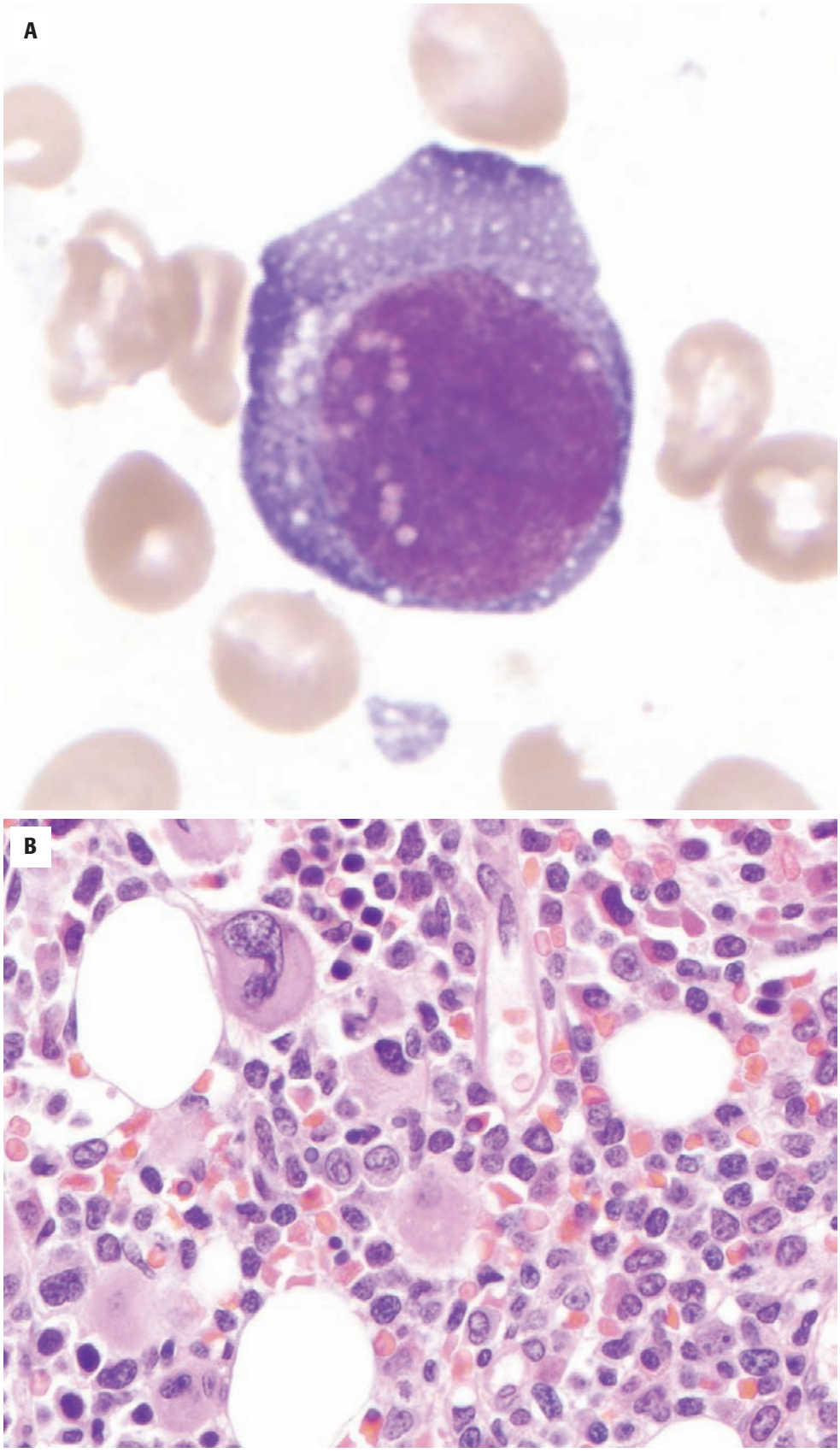


**FIGURE 14-7**

Infant acute megakaryoblastic leukemia with t(1;22). **A,** The blasts have basophilic, finely granular cytoplasm, similar to platelets. **B,** The biopsy material shows atypical megakaryocytes, blasts, and open sinuses resulting from marrow fibrosis.

**ACUTE MEGAKARYOBLASTIC LEUKEMIA WITH t(1;22)—FACT SHEET**

**Definition**
- A neoplastic proliferation of bone marrow blast cells with features of megakaryocytes, occurring almost exclusively in infants
- Presence of translocation alone not diagnostic of AML
- Mandatory threshold of 20% blasts or presence of myeloid sarcoma

**Frequency, Gender, and Age**
- Less than 1% of all AMLs
- Females more often than males
- Infants, usually younger than 6 months

**Cytogenetic Event**
- t(1;22)(p13;q13)

**Molecular Event**
- *RBM15-MKL1* fusion

**Prognosis**
- Intermediate

**ACUTE MEGAKARYOBLASTIC LEUKEMIA WITH t(1;22)—PATHOLOGIC FEATURES**

**Blast Cell and Bone Marrow Morphologic Features**
- Nonspecific cytoplasmic blebs
- Variable marrow fibrosis

**Blast Cell Immunophenotypic Features**
- Variably myeloid antigen positive (CD13, CD33)
- Aberrant CD7 commonly positive
- Megakaryocyte antigen positive (CD41, CD42, CD61)

**Differential Diagnosis**
- AML, not otherwise categorized
- Myeloid proliferations of Down syndrome
- Acute panmyelosis with myelofibrosis

than 20%. In the absence of extramedullary disease, careful close clinical follow-up is indicated for evolution of AML.

## ACUTE MYELOID LEUKEMIA WITH GENE MUTATIONS

AML with gene mutations is a new provisional entity in the 2008 WHO classification. A wide number of genetic abnormalities occur in de novo acute myeloid leukemia, but the significance of many is not yet well understood. Some are thought to ensure proliferation and survival of the abnormal clone (type I), others are thought to impair differentiation (type II). Type II abnormalities include those that characterize distinct subtypes of AML with recurrent genetic abnormalities (e.g., *PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, MLL* fusions). Type I mutations include receptor tyrosine kinase or GTPase alterations such as mutations of *FLT3, KIT, RAS, PTPN11,* and *JAK2*.

In recent years, it has become clear that mutations of the *FLT3* gene are the most common genetic abnormality in AML. *FLT3* encodes a receptor tyrosine kinase that is constitutively activated by either ITD of the juxtamembrane region or by point mutations in the second tyrosine kinase domain (D835) in AML. The FLT3 protein is expressed in early hematopoietic progenitor cells and is involved in early stem cell survival and myeloid differentiation. *FLT3* mutations occur in 20% to 28% of adult AMLs and 11.5% of all de novo pediatric AMLs. The mutations occur with multiple entities of the 2008 classification. Among AML with recurrent translocations, they are frequent in APL with t(15;17) and AML with t(6;9). *FLT3* ITD mutations are associated with shorter complete remission duration and overall survival. The ratio of mutant *FLT3* to wild type allele can identify patients with poor outcomes. FLT3 inhibitor drugs are under study for these patients. Because of the relatively high frequency and clinical significance of these mutations in AML, *FLT3* mutation testing is performed commonly.

## ACUTE MYELOID LEUKEMIA WITH MUTATED *NPM1*

Mutation of the nucleophosmin gene *(NPM1)* is seen in approximately 35% of primary adult AML. Mutations occur in exon 12 and affect the C-terminal portion of the protein, thus altering its subcellular localization. The mutations occur most frequently in cases of AML with normal karyotype (85%). They are rare in AML with the recurrent genetic abnormalities listed previously. Approximately 2% to 3% of AML with mutated *NPM1* have karyotypic abnormalities now classified as myelodysplasia related (del 9[q], complex karyotypes and –7), and some cases have multilineage dysplasia. Some studies report no significance to these associations, but additional study is warranted. Overall, AML with mutated *NPM1* in the absence of a *FLT3* mutation appears to have a favorable prognosis similar to core binding factor AML. The mutation leads to aberrant cytoplasmic localization of the nucleophosmin protein. Initial studies suggested that immunohistochemistry was an appropriate screening tool for this diagnosis; however, subsequent studies have shown discordance between immunostaining results and *NPM1* mutation status. Molecular assessment is the preferred method for assessing this mutation.

## ACUTE MYELOID LEUKEMIA WITH MUTATED *CEBPA*

Mutations of the *CEBPA* gene occur in 7% to 11% of acute myeloid leukemia and 15% to 18% of normal karyotype AML. More than 100 different non-silent mutations are identified. *CEBPA* encodes the basic zipper transcription factor CCAAT/enhancer binding protein α (C/EBPα), which is critical for normal differentiation of mature granulocytes. Mutations may occur in both the C- and N-terminal of the gene. C-terminal mutations of *CEBPA* disrupt the basic zipper region that contains both DNA-binding and dimerization domains. N-terminal mutations disrupt the reading frame and result in premature termination and truncation of the normal 42-kDa protein and enhanced translation of 30-kDa protein initiated downstream. N-terminal mutations of *CEBPA* result in inhibition of myeloid and erythroid cells in humans. Mutations in the *CEBPA* gene are reported in 10% of human patients with AML. Although the *CEBPA*-mutants are known to display distinct biologic function during leukemogenesis, the molecular basis for this subtype of AML remains elusive. Mutation of *CEBPA* appears to disrupt the regulation of microRNA control of granulocytic differentiation in AML, and these microRNAs may represent a novel therapeutic target in AML with *CEBPA* mutations.

Acute myeloid leukemias with *CEPBA* mutations are associated with intermediate risk cytogenetics and are more commonly associated with FAB M1 or M2 morphology and less commonly with monocytic features. Some authors suggest *CEBPA*-mutant patients should be considered a favorable risk category, not requiring transplant in first remission. Recent studies suggest that only mutation of both *CEBPA* alleles is associated with favorable outcomes. Unlike *NPM1* mutated AML, *FLT3* ITDs are uncommon in *CEBPA* mutated AML. However, when present, it abrogates the favorable prognosis.

## ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES

The 2008 WHO classification expanded the category of AML with multilineage dysplasia to AML with myelodysplasia-related changes (AML-MRC), now encompassing cases with multilineage dysplasia as defined in the previous classification, a myelodysplasia associated karyotype, or a history of myelodysplastic syndrome or myelodysplastic–myeloproliferative neoplasm. Dysplastic changes may be evident in the non–blast marrow and peripheral blood elements. To meet the morphologic criteria for AML-MRC (multilineage dysplasia), there must be 20% or more bone marrow or peripheral blood blast cells and 50% or more dysplastic cells in at least two cell lines (erythroid, granulocytic, or megakaryocytic). The 50% cutoff for dysplastic changes is arbitrary and excludes some cases with definite associated dysplastic changes.

**ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA RELATED CHANGES—FACT SHEET**

**Definition**

- AML with 20% blasts and at least one of the following:
  - Dysplastic changes in two or more bone marrow cell lines
  - Myelodysplasia syndrome-related karyotypic abnormality
  - History of myelodysplastic syndrome or myelodysplastic–myeloproliferative neoplasm, excluding cases with a recurrent genetic abnormality, history of cytotoxic therapy, or Down syndrome

**Frequency and Age Distribution**

- Up to 50% of adult AML, approximately 20% of pediatric AML
- Most common in elderly

**Most Common Cytogenetic and Molecular Events**

- Complex karyotypes, balanced and unbalanced abnormalities (see Table 14-3)

**Prognosis**

- Poor
- *FLT3*-ITDs common
- *NPM1* and *CEBPA* mutations possibly present; additional significance unclear

Dysplastic changes may be seen in both peripheral blood and bone marrow samples (Figure 14-8). Red cell changes manifest as anisopoikilocytosis of peripheral blood red cells, including hypochromic teardrop-shaped cells and macrocytes, dimorphic red cell populations of the blood, nuclear-cytoplasmic asynchrony of red cell precursors, megaloblastic changes, and irregularities of red cell precursor nuclei. Megaloblastic changes differ from a left shift of erythroid cells by the presence of more immature nuclear chromatin, often associated with more mature, red-staining erythroid cell cytoplasm (dyssynchrony of nuclear and cytoplasmic maturation). Nuclear irregularities, including multinucleation, nuclear blebs, and irregular nuclear contours are commonly seen in dysplastic erythroid precursors. Granulocyte dysplasia is most easily recognized in the more mature granulocyte forms of the blood and marrow. These changes include uneven cytoplasmic granulation or completely agranular mature neutrophils. Nuclear changes include clumping of nuclear chromatin that is usually associated with abnormalities of nuclear lobation, particularly monolobate or bilobate nuclei (pseudo–Pelger-Huët cells). Some nuclear abnormalities may be seen with drug therapy and do not constitute true dysplasia. These abnormalities are usually not accompanied by hypogranulation; therefore a requirement of both features in the granulocyte line for dysplasia seems warranted. Peripheral blood platelets may show variation in

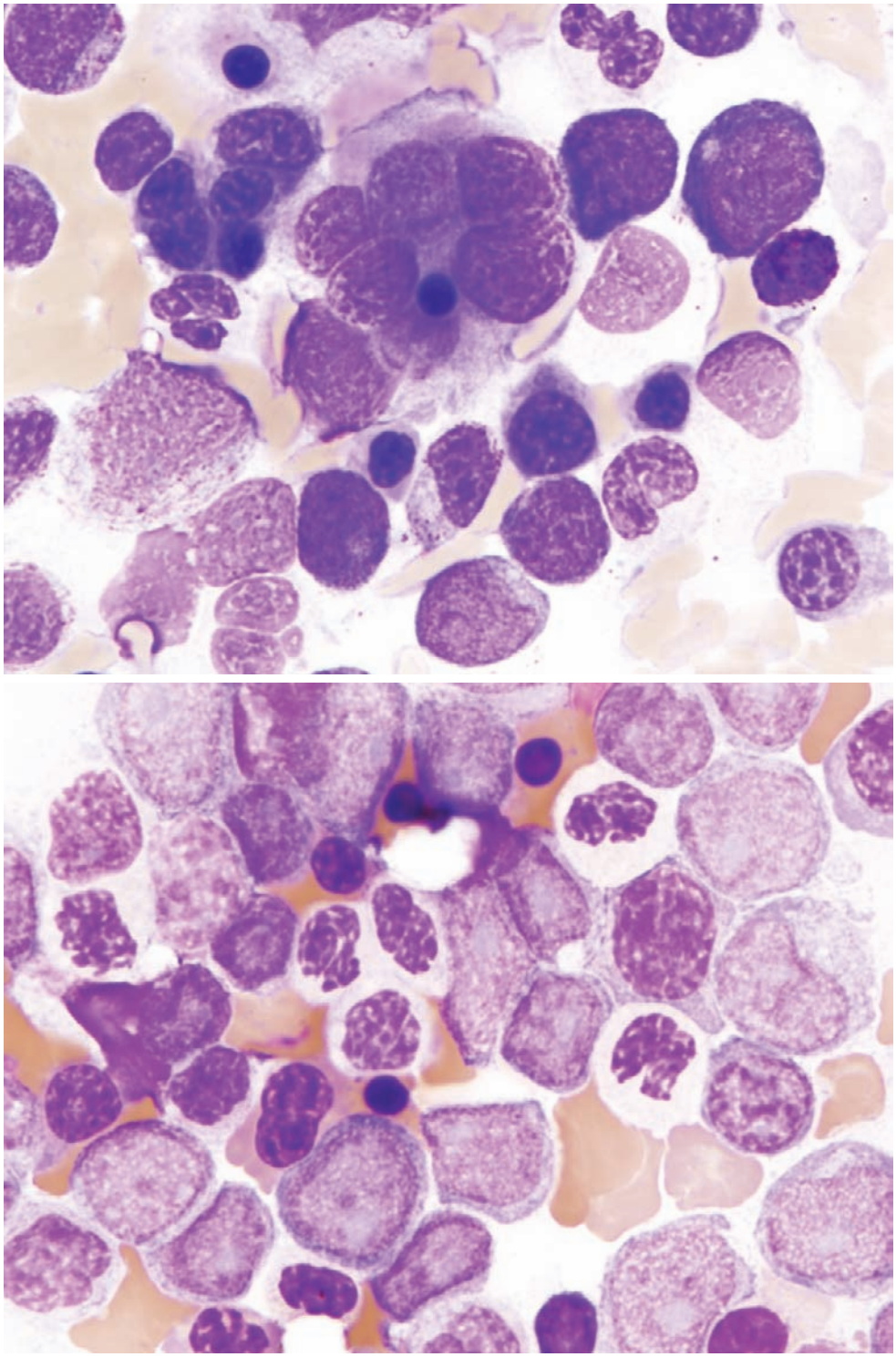

**FIGURE 14-8**

Acute myeloid leukemia with myelodysplasia-related changes (multilineage dysplasia). The blasts are associated with trilineage dysplasia that includes erythroid precursors with irregular nuclear contours, hypolobated neutrophils with hypogranular cytoplasm, and a megakaryocyte with irregular nuclear lobation.

platelet size with hypogranular platelets. Megakaryocytes may show great variation in size with detached, hyperlobate nuclei or hypolobate or monolobate forms with hyperchromatic nuclei.

Some cases have insufficient numbers of nonblast elements to make a morphologic assessment of multilineage dysplasia. A diagnosis of AML-MRC may be rendered based on a set of myelodysplasia-associated karyotypic abnormalities, including a complex karyotype (three or more unrelated abnormalities), certain unbalanced abnormalities, and a set of nine balanced translocations (Table 14-2). In addition, the history of

**ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA RELATED CHANGES—PATHOLOGIC FEATURES**

**Blast Cell Morphology**

- Heterogenous blast features

**Non–Blast Cell Morphology (Usual Types)**

- Erythroid cells
  - Nuclear-cytoplasmic dyssynchrony
  - Irregular nuclear contours
- Granulocytes
  - Hypolobated nuclei with clumped nuclear chromatin
  - Hypogranular cytoplasm
- Megakaryocytes and platelets
  - Hypogranular platelets
  - Megakaryocytes with hyperchromatic, hypolobated nuclei
  - Megakaryocytes with detached nuclear lobes

**Immunophenotypic Features**

- Nonspecific myeloid or myelomonocytic lineage

**Differential Diagnosis**

- AML with t(6;9)
- AML with inv(3), t(3;3)
- Myeloid proliferations of Down syndrome
- Therapy-related myeloid neoplasms
- AML, not otherwise categorized, acute erythroid–myeloid leukemia
- Myelodysplastic syndrome

**TABLE 14-2**

**Criteria for Diagnosis of AML with Myelodysplasia-Related Changes**

1. Greater than 20% myeloid blasts in the blood or bone marrow
2. No history of prior cytotoxic therapy for other disease
3. Absence of one of the seven recurrent genetic abnormality translocations [t(8;21), inv (16)t(16/16), t(15;17) or variants, t(9;11), t(6;9), inv(3)/t(3;3), t(1;22)] and one or more of the following features:
   1. Previous history of myelodysplastic syndrome or myelodysplastic–myeloproliferative neoplasm
   2. Multilineage dysplasia (dyspoiesis in at least 50% of the elements from two or more lineages)
   3. Presence of an MDS-associated cytogenetic abnormality, defined as the following:
      - Complex karyotype (three or more unrelated abnormalities)
      - Unbalanced abnormalities:
        - -7/del(7q)
        - -5/del(5q)
        - i(17q)/t(17p)
        - -13/del(13q)
        - del(11q)
        - del(12p)/t(12p)
        - del(9q)
        - idic(X)(q13)
      - Balanced abnormalities:
        - t(11;16)(q23;p13.3)
        - t(3;21)(q26.2;q22.1)
        - t(1;3)(p36.3;q21.1)
        - t(2;11)(p21;q23)
        - t(5;12)(q33;p12)*
        - t(5;7)(q33;q11.2)*
        - t(5;17)(q33;p13)*
        - t(5;10)(q33;q21)*
        - t(3;5)(q25;q34)

*AML,* Acute myeloid leukemia; *MDS,* myelodysplastic syndrome.
*These four translocations are *PDGFRB* rearrangements. The diagnosis should include the overlap of AML-MRC with myeloid neoplasms with *PDGFRB* rearrangement, because there may be a role for tyrosine kinase inhibitor therapy.

a prior myelodysplastic syndrome or myelodysplastic–myeloproliferative neoplasm is sufficient for the diagnosis of AML-MRC.

Of the nine MDS-associated translocations, four involve 5q33, the locus of platelet-derived growth factor receptor B *(PDGFRB)*. Imatinib is approved for treatment of MDS and CMML with translocations involving 5q33, but efficacy in AML-MRC with this rearrangement is unclear. Other rearrangements include two involving 3q26 (*EVI*1 locus) and 3q21 (*RPN*1 and *GATA*2 loci), both involved in the recurrent genetic abnormality AML with inv(3)(q21q26.2), t(3;3)(q21;q26.2) and two involving the *MLL* locus at 11q23. The final rearrangement, t(3;5)(q25;q35), results in a fusion of nucleophosmin *(NPM)* with myeloid leukemia factor 1 *(MLF1)*. Cases with this *NPM-MLF1* translocation tend to occur in young adult males and may respond well to stem-cell transplantation (Figure 14-9).

AML-MRC does not show specific blast cell morphologic features and can include all types of FAB AMLs, although FAB-M3 leukemia with multilineage dysplasia is unusual. Therefore there are no specific cytochemical or immunophenotypic features of the blast cells for this disease category. The AML-associated karyotypes, particularly the chromosomal monosomies, generally have a poor prognosis. Multilineage dysplasia without an MDS-associated cytogenetic abnormality is also associated with a poor prognosis. *FLT3, NPM1,* and *CEBPA* mutations have been observed in cases of AML-MRC, and their presence should be noted in the diagnosis. Additional study is required to know the prognosis of AML-MRC with these gene mutations.

The differential diagnosis of AML with multilineage dysplasia includes the more specific diagnoses of AML with t(6;9) and AML with inv(3)/t(3;3). Correlation with cytogenetics is always necessary because these specific karyotype abnormalities trump the category of AML-MRC. In addition, the diagnoses of therapy-related myeloid neoplasm or myeloid proliferation of Down syndrome are preferred in these clinical contexts, which commonly have multilineage dysplasia. Cases of AML-MRC may have erythroid hyperplasia with erythroid cells comprising greater than 50% of the marrow elements. If greater than 20% myeloblasts are present (out of all nucleated cells), a diagnosis of AML-MRC is preferred over the diagnosis of AML, NOS, acute erythroid–myeloid leukemia (Figure 14-10).

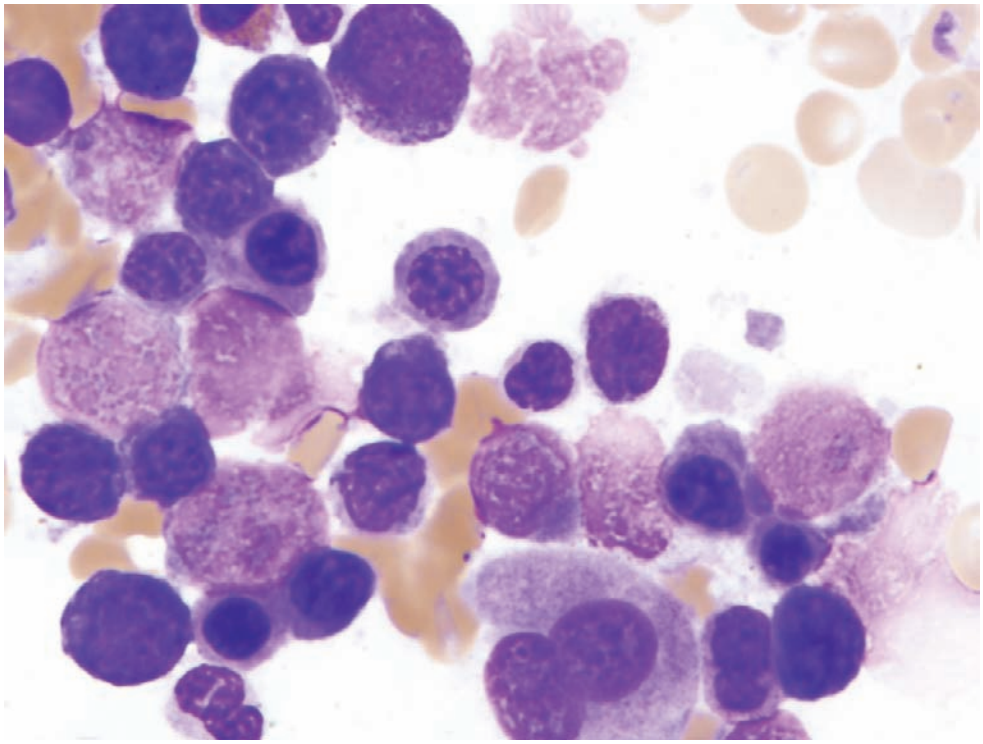

**FIGURE 14-9**

Acute myeloid leukemia with t(3;5). Blasts with associated multilineage dysplasia (erythroid and megakaryocytic in this field) are present.

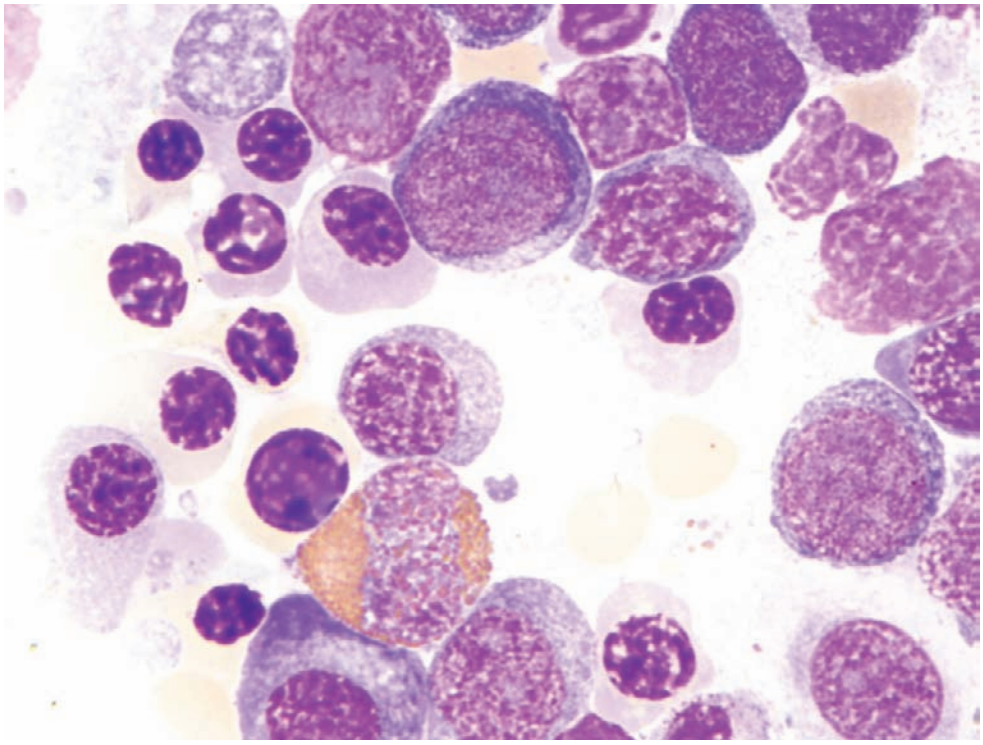

**FIGURE 14-10**

Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) with erythroid hyperplasia. Dysplastic erythroid cells represent more than 50% of the marrow cells. Because blast cells compose more than 20% of the total cells (erythroid and nonerythroid nucleated cells) and multilineage dysplasia is present, this case is best classified as AML-MRC instead of acute erythroid leukemia, erythroid–myeloid.

Some patients with multilineage dysplasia and MDS-associated karyotypic abnormalities have erythroid or megakaryocytic blast phenotypes. It is not clear whether there is a meaningful prognostic distinction of these entities from other cases of AML-MRC with myeloid blasts. Recognition of the blast phenotype is certainly important for monitoring response to therapy. The marked dysplastic changes in some cases of myelodysplasia and AML with multilineage dysplasia can make enumerating blast cells difficult. Careful, high-power examination of the blast cell nuclei is often necessary to separate dysplastic, hypolobated granulocytes from blasts in these cases, because both may contain cytoplasmic granules. The more mature dysplastic granulocyte population has clumped nuclear chromatin compared with the fine nuclear chromatin of a blast cell.

## THERAPY-RELATED MYELOID NEOPLASMS

The 2008 WHO classification expands the concept of therapy-related AML (a category in the 2001 WHO classification) to therapy-related myeloid neoplasms, recognizing that myelodysplasia, AML, and myelodysplastic–myeloproliferative neoplasms all may occur after cytotoxic therapy, with similar generally poor prognosis. Therefore these neoplasms are considered together as a distinct clinical syndrome. The distinction into alkylating agent-related and topoisomerase II inhibitor-related types is no longer necessary. It is currently recognized that radiation therapy, as well as cytotoxic therapy for non-neoplastic disease, can be an inciting factor. In some patients, myeloid disease as a second malignancy may follow a primary diagnosis of cancer treated with surgery alone. These cases are not truly therapy-related, but may be related to the primary neoplasm in other ways such as host or environmental factors. Transformation from myeloproliferative neoplasms (such as polycythemia vera or primary myelofibrosis) are excluded from this category because it is difficult to separate disease progression and evolution from therapy-related disease.

Therapy-related myeloid neoplasms may occur with a short latency (e.g., 2 years), often with abnormalities of *MLL* at 11q23 or *RUNX1* at 21q22 (Figure 14-11) and associated with topoisomerase II inhibitors. Longer-latency, therapy-related disease (e.g., 5 or more years) often has myelodysplastic features including a prolonged MDS phase, multilineage dysplasia, and MDS-associated karyotypic abnormalities such as loss of genetic material of chromosomes 5 or 7, or both (Figure 14-12). These features are more likely to be related to alkylating agents or ionizing radiation. A diagnosis of AML-MRC should not be made in this setting, because a history of prior therapy trumps a diagnosis of AML-MRC.

The pathologic features are not specific and are those of an MDS with multilineage dysplasia, MDS-myeloproliferative neoplasm (MPN) overlap such as CMML, or AML. It appears that the exact blast percentage, marrow cellularity, or pathologic subclassification are less important than the history of prior therapy. However, from a practical aspect, it is appropriate to report such cases as therapy-related(t)-AML/t-MDS or t-AML/t-MDS/MPN with a description of the findings and blast percentage.

A number of recurrent genetic abnormalities can occur in therapy-related myeloid neoplasms, including the more favorable subtypes of inv(16) and t(15;17). Some studies demonstrate cytogenetic prognostic significance similar to that of de novo AML. Treatment of therapy-related disease is challenging as a result of drug resistance and limited tolerance to chemotherapy because of previous exposure.

**THERAPY-RELATED ACUTE MYELOID LEUKEMIA—FACT SHEET**

**Definition**
- Acute myeloid leukemias that occur after chemotherapy or radiation therapy for other malignancies or chronic diseases

**Long Latency: Clinical Features**
- Occurs 5 to 7 years after therapy, classically after alkylating agent exposure

**Short Latency: Clinical Features**
- Occurs 2 to 3 years after therapy, often topoisomerase II inhibitor therapy

**Prognosis**
- Poor

**THERAPY-RELATED ACUTE MYELOID LEUKEMIA—PATHOLOGIC FEATURES**

**Long Latency**

Bone marrow morphologic features
- Similar to AML with multilineage dysplasia

**Cytogenetic Features**
- Complex karyotypes
- Abnormalities of chromosomes 5 and 7

**Short Latency**

Morphologic features
- Usually monoblastic or myelomonocytic without associated dysplasia

**Cytogenetic Features**
- Abnormalities at 11q23 or 21q22

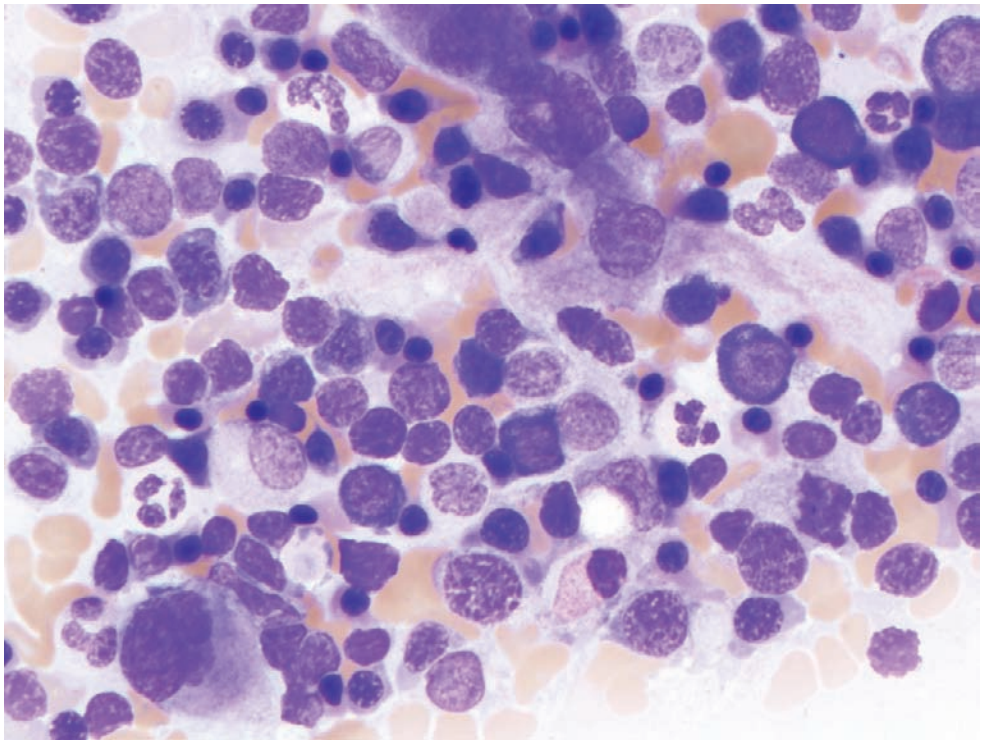

**FIGURE 14-11**

Therapy-related acute myeloid leukemia following alkylating agent therapy. There is multilineage dysplasia with erythroid hyperplasia.

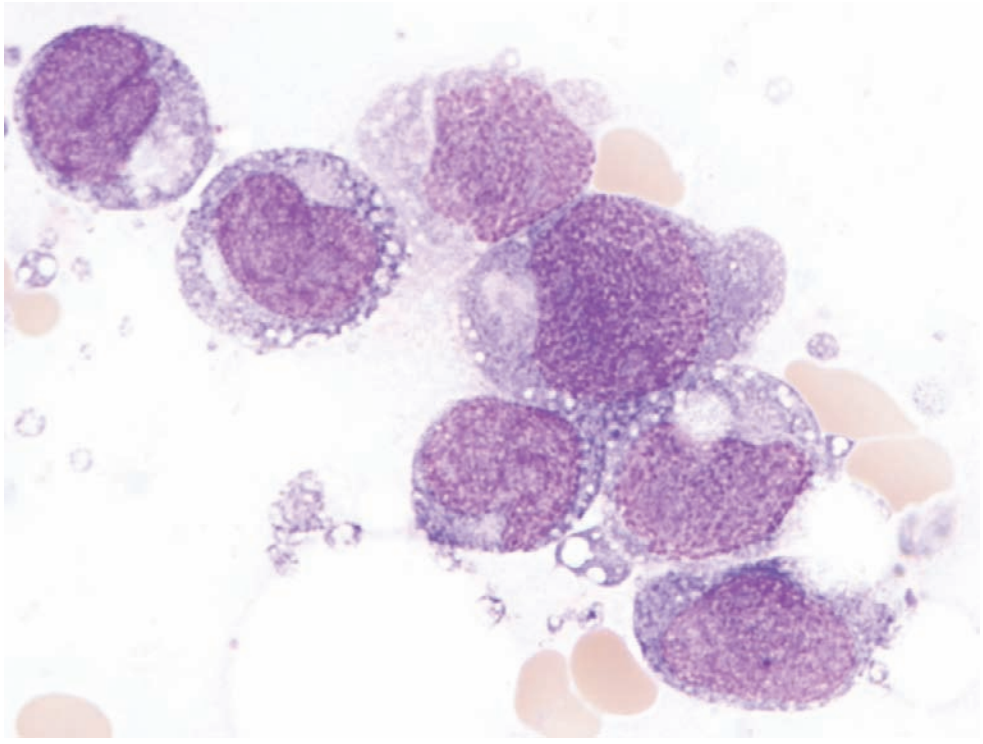

**FIGURE 14-12**

Therapy-related acute myeloid leukemia following topoisomerase II inhibitor therapy. The blast cells have a monoblastic appearance.

## ACUTE MYELOID LEUKEMIA, NOT OTHERWISE SPECIFIED, AND THE FRENCH-AMERICAN-BRITISH COOPERATIVE GROUP CLASSIFICATION OF ACUTE MYELOID LEUKEMIA

The WHO classification of AML includes an NOS disease group that includes nine different subgroups. Many of these subgroups have similarities to the original FAB disease groups, but differ in several ways. The WHO AML, NOS, category does not include cases with the seven recurring cytogenetic abnormalities mentioned previously, cases meeting criteria for AML-MRC, or those occurring in patients with a history of cytotoxic therapy or Down syndrome. As in the 2001 WHO classification AML, NOS, defines acute leukemia as bone marrow proliferations with 20% or more blasts as opposed to a 30% or higher cutoff for the FAB classification.

The category of AML, NOS, AML with minimal differentiation is similar to the FAB category of minimally differentiated acute myeloid leukemia (M0). This leukemia is acute with uniform blast cells without cytoplasmic granules, with no Auer rods, and negative blast-cell cytochemical reactions for myeloperoxidase, Sudan black B, and nonspecific esterase (Figure 14-13). Many laboratories today no longer perform cytochemical stains, making it difficult to distinguish this subtype from other AML. The lineage of the blast cells is only recognizable by immunophenotyping methods, which usually show the blast cells to express two of the more common AML markers, including myeloperoxidase, CD13, CD33, or CD117. This is considered evidence of myeloid lineage commitment. Antigens associated with monocytic or myeloid maturation, or erythroid or megakaryocytic lineage are not expressed. Myeloperoxidase may be positive by flow cytometry, but the WHO does not describe a threshold for "positive." The FAB classification required 3% MPO reactivity by cytochemistry, and the EGIL classification required 10% positivity by flow cytometry to designate a case as MPO positive. It can be difficult to differentiate minimally differentiated AML from acute undifferentiated leukemia in the absence of immunophenotypic evidence for MPO positivity. Acute leukemias with only one myeloid lineage marker, and no definitive evidence of erythroid, megakaryocytic, lymphoid, or blastic plasmacytoid dendritic cell lineage are best characterized as acute undifferentiated leukemia. AML with minimal-differentiation blast cells may be TdT positive and frequently express CD7. There may be rearrangements of T- and B-cell receptor genes, and these gene rearrangements are not to be interpreted as evidence of lymphoid lineage. Specific evidence of lymphoid lineage must be excluded by immunophenotyping. The WHO definition of lymphoid lineage is detailed in Chapter 16. The most specific marker of T-cell lineage is CD3. B-cell lineage is determined by a combination of antigens, including CD19,

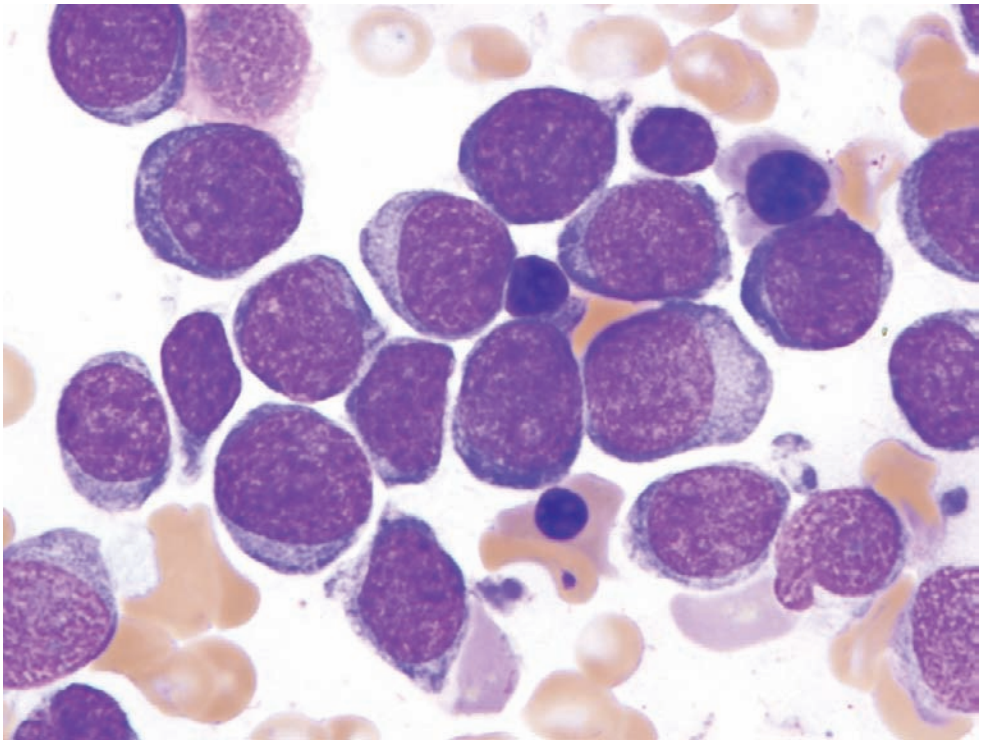

**FIGURE 14-13**

Minimally differentiated acute myeloid leukemia (FAB-M0). The blasts mark with CD13 and CD33 by flow cytometry, but are myeloperoxidase and nonspecific esterase negative by cytochemical study.

cytoplasmic CD22, CD10, and CD79a. Blastic plasmacytoid dendritic cell (BPDC) tumor is detailed below in a separate section. Expression of CD4, CD56, and CD123 are hallmarks of this entity, and it must be excluded by further immunostains before a diagnosis of AML with minimal differentiation is established. *RUNX1* (or *AML1*) gene mutations are more common in this FAB type than others. The largest series of FAB M0 cases with karyotype suggest that occasional cases (0.5%) will be diagnosed with a specific recurrent genetic abnormality (inv [3]/t[3;3] and t[9;11]), and approximately 22% will be reclassified as AML-MRC based upon the frequently complex karyotype. *FLT3* ITD mutations were reported in 22%.

Acute myeloid leukemias that are myeloperoxidase positive and nonspecific esterase negative by cytochemistry and that show less than 10% bone marrow cells with maturation to or beyond the promyelocyte level of differentiation are designated AML, NOS, without maturation in the 2008 WHO classification or FAB M1 (Figure 14-14). In the absence of cytochemical stains, this diagnosis is based on the flow cytometric detection of MPO-positive blasts lacking evidence of monocytic maturation. Without cytochemical stains, no criteria exist to distinguish flow cytometry MPO-positive blasts of AML with minimal differentiation that efface the marrow from AML without maturation. AML, NOS, with maturation (FAB M2) is defined as cytochemically similar to AML M1, but with 10% or more bone marrow cells showing maturation to or beyond the promyelocyte level (Figure 14-15).

Acute promyelocytic leukemia corresponds to FAB M3 and has no corresponding subcategory in the WHO AML, NOS, group. All such cases would fall into the category of AML with recurrent cytogenetic abnormalities (see previous discussion). Rarely, a case of AML, NOS, will exhibit granular blasts resembling promyelocytes, but lack cytogenetic or molecular evidence of an *RARA* rearrangement. Such cases are best designated AML with maturation.

AML, NOS, acute myelomonocytic leukemia (FAB M4) shows substantial myeloid (granulocytic) and monocytic differentiation. There are 20% or more blasts that may be the sum of myeloblasts, monoblasts, and promonocytes. Monoblasts are large with round nuclei, fine chromatin, variably prominent nucleoli, and moderate amounts of pale gray-blue cytoplasm with sparse azurophilic granules. The cytoplasm may also show vacuoles and blebs (pseudopods). Promonocytes have nuclear chromatin similar to monoblasts, with slightly indented nuclei with nuclear creases or folds and moderate amounts of gray-blue cytoplasm with more noticeable granules and vacuoles. Auer rods can be seen (Figure 14-16). A subset of the blast cells are myeloperoxidase positive, but the strong positivity of virtually all cells seen in the microgranular variant of acute promyelocytic leukemia, which may mimic acute myelomonocytic leukemia, is not usually present. The blast cells of

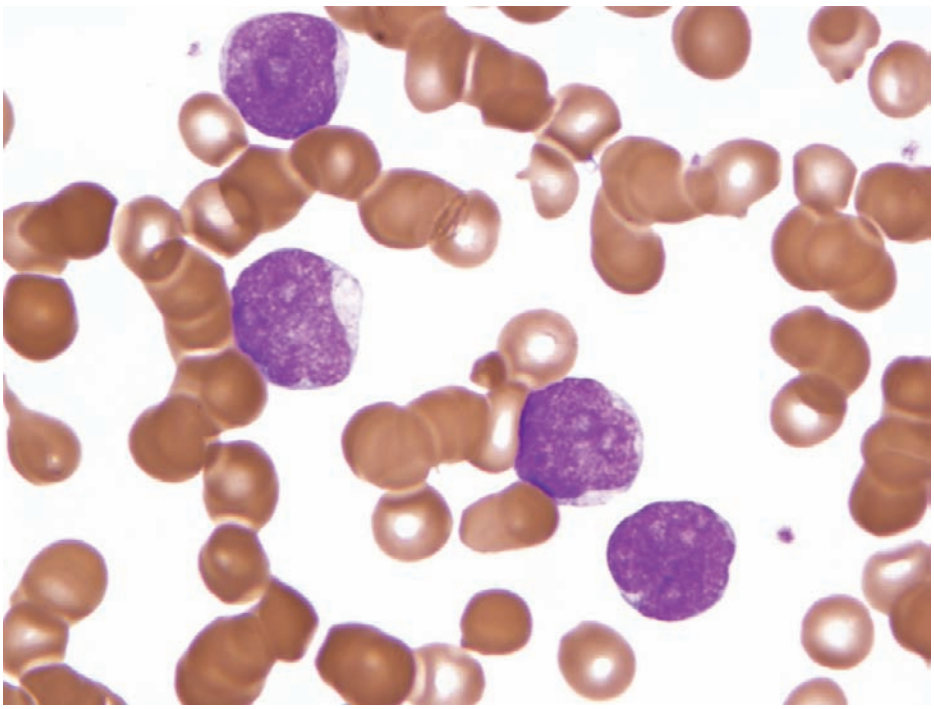

**FIGURE 14-14**

Acute myeloid leukemia without maturation (FAB-M1). The blasts are similar in appearance to those in Figure 14-13, but were myeloperoxidase positive and nonspecific esterase negative.

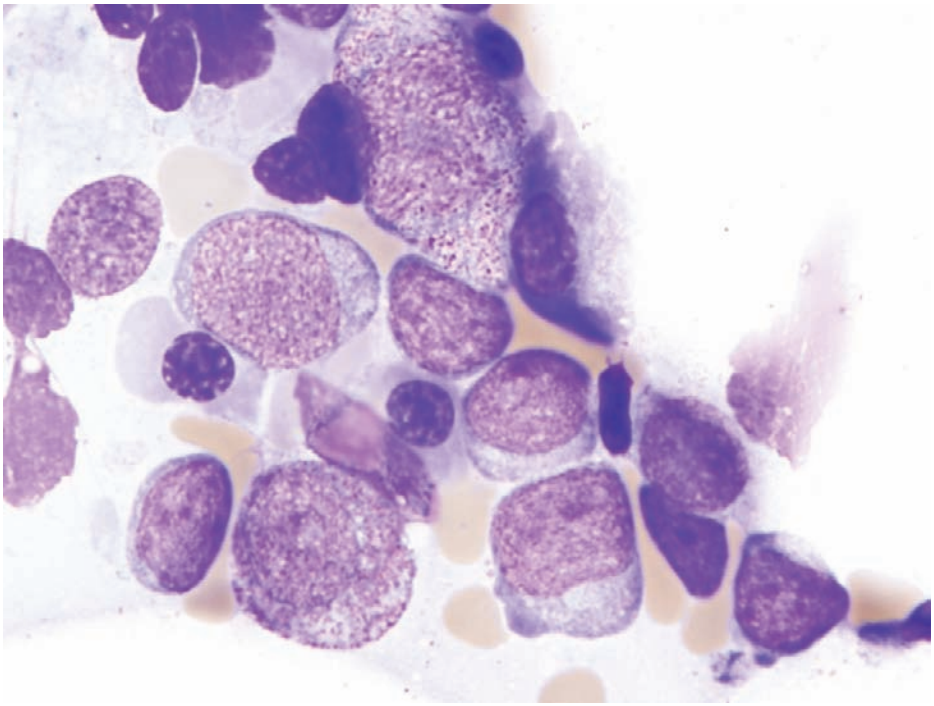

**FIGURE 14-15**

Acute myeloid leukemia with maturation (FAB-M2). More than 10% of marrow cells contain sufficient granules to be considered at the promyelocyte stage of maturation.

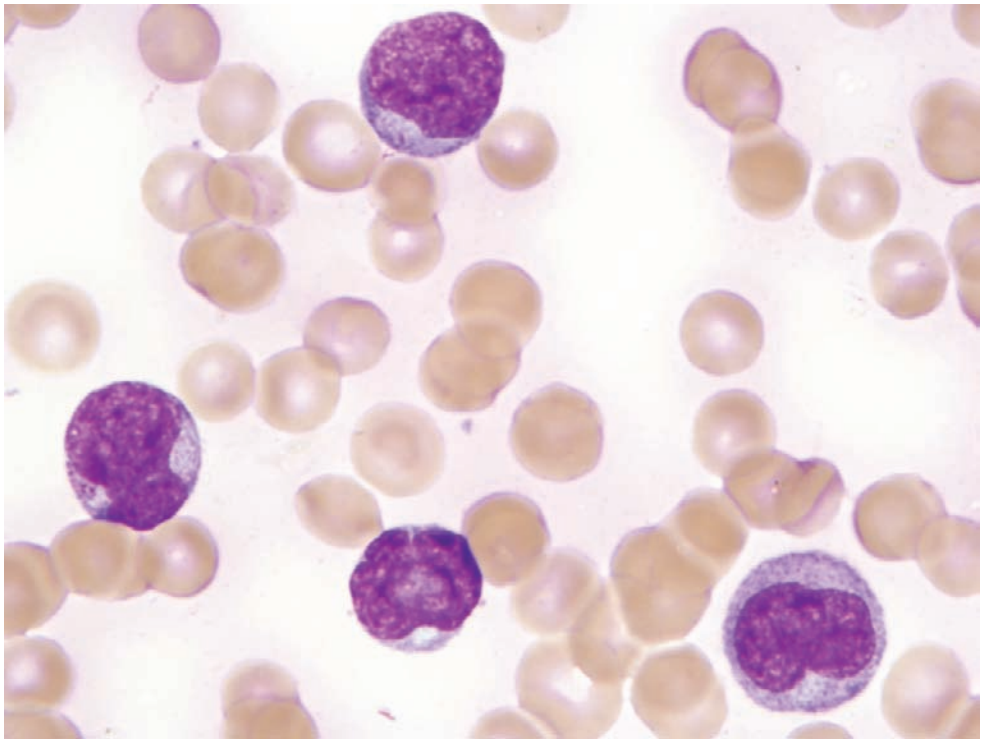

**FIGURE 14-16**

Acute myelomonocytic leukemia (FAB-M4). Some blasts have granules and others have folded nuclei suggestive of monocytic differentiation. These cells are partially myeloperoxidase positive and show more than 20% nonspecific esterase positivity by cytochemistry.

acute myelomonocytic leukemia are usually nonspecific esterase positive, reflecting monocytic differentiation. By definition, 20% to 79% of bone marrow neoplastic cells show monocytic differentiation by morphology or cytochemistry. Indeed, double staining for chloracetate esterase and nonspecific esterase may show dual-positive cells in some cases. A subtype of M4 AML (acute myelomonocytic leukemia with eosinophilia or M4Eo) is included in the FAB classification and corresponds to AML with inv(16)(p13q22) or t(16;16)(p13;q22) of the WHO AML with recurrent cytogenetic abnormalities (see Figure 14-2).

When there are more than 20% bone marrow or blood blasts (again a combination of myeloblasts, monoblasts, and promonocytes) and 80% of neoplastic cells are nonspecific esterase positive or are morphologically monoblasts with immunophenotypic evidence of monocytic differentiation, a diagnosis of AML, NOS, acute monocytic and monoblastic leukemia (FAB M5) is made (Figure 14-17). These cases may be myeloperoxidase negative, but differ from minimally differentiated AML by their positivity for nonspecific esterase. The blasts of acute monocytic leukemia have abundant, often agranular cytoplasm that may contain vacuoles. The blast nuclei may be round and uniform or folded. Cases with more immature nuclear features in the bone marrow (typically when the monocytic cells are 80% or more true monoblasts) are termed *monoblastic* (FAB M5a), whereas those with evidence of monocytic maturation in the bone marrow are termed *monocytic* (FAB M5b). Monocyte maturation may be prominent in the peripheral blood in either acute monocytic or myelomonocytic leukemia, and the subclassification is best made on bone marrow specimens. However, the blood may help to confirm the monocytic component of the leukemia. A subset of FAB M4 and M5 AMLs will have 11q23 abnormalities. Only the t(9;11) is recognized as a recurrent genetic abnormality. The variant 11q23 translocation should be specified in the diagnosis, because some are now recognized to have some specific prognostic importance as well (see previous discussion).

Acute erythroid leukemia consists of at least two different categories in the WHO classification that roughly correspond to the original Di Guglielmo syndrome and disease. Erythroleukemia or erythroid–myeloid leukemia of the 2008 WHO classification is similar to FAB M6. It is a myeloid blast cell proliferation that occurs in association with erythroid hyperplasia. By definition, erythroid precursors must represent 50% or more of the bone marrow cells, and blast cells are 20% or more of the nonerythroid elements for this diagnosis. Therefore cases with high numbers of bone marrow erythroid precursors and relatively low blast cell counts may be diagnosed as acute leukemia; some authors prefer to consider these cases as myelodysplastic syndromes. Caution must be made not to overinterpret erythropoietin therapy in a regenerating marrow as erythroid–myeloid leukemia. Most cases of AML with erythroid hyperplasia fulfill criteria for AML-MRC by virtue of greater than 20% myeloblasts (out of all nucleated cells) and multilineage dysplasia, or this blast percentage and an MDS-associated karyotype; these cases should not be called AML, NOS. As described previously, AML with t(6;9) commonly has erythroid hyperplasia. Pure erythroid leukemia is a second type of acute erythroid leukemia in the WHO AML, NOS, category. It is defined as a bone marrow proliferation of more than 80% neoplastic, immature erythroid cells without a significant myeloblast component (Figure 14-18). These cases are also frequently associated with multilineage dysplasia and myelodysplasia-related karyotypes. It is not clear whether these cases have a clinical behavior distinct from other cases of AML-MRC, or whether they should be classified together.

Acute megakaryoblastic leukemia corresponds to FAB M7. The WHO defines acute megakaryoblastic leukemia as an acute leukemia in which 50% or more of the blasts are of megakaryocytic lineage. Excluding cases with t(1;22), inv(3)/t(3;3), and Down syndrome, this morphology remains more common in children. The majority of cases of adult acute megakaryoblastic leukemia are associated with multilineage dysplasia and could be considered as AML-MRC. The morphologic, immunophenotypic, and ultrastructural features are similar to those described previously for other types of megakaryoblastic leukemias, with detection of CD41 and CD61 expression being the most common means of defining megakaryocytic lineage. These cases are commonly associated with marked marrow fibrosis and an inaspirable marrow ("dry tap"), making diagnosis difficult in many cases.

The WHO AML, NOS, group includes two additional subcategories that do not correspond to previous FAB categories. The first is acute basophilic leukemia, which is defined as an AML with primary differentiation to basophils. It is unclear whether this subcategory represents a distinct entity, because some specific AML types are associated with basophilia and should not be placed in this category, such as t(9;22) AML, blast crisis of chronic myelogenous leukemia, t(6;9) AML, t(3;6) AML, and AMLs with 12p abnormalities. In addition, cases of AML with the abnormal eosinophils of inv(16) and cases of mast cell leukemia may be mistaken for basophilic leukemias. Therefore the lack of specificity of this diagnosis should be recognized, and attempts to diagnose cases with increased basophils or basophil-like cells into more specific disease categories should be made.

Acute panmyelosis with myelofibrosis is defined by the WHO as an acute panmyeloid proliferation with accompanying bone marrow fibrosis (Figure 14-19). Some authors suggest that this disease may be a subtype of acute megakaryoblastic leukemia, because they commonly share marrow fibrosis, multilineage dysplasia, and complex cytogenetic abnormalities. Most cases of acute panmyelosis with myelofibrosis have 20% to 25%

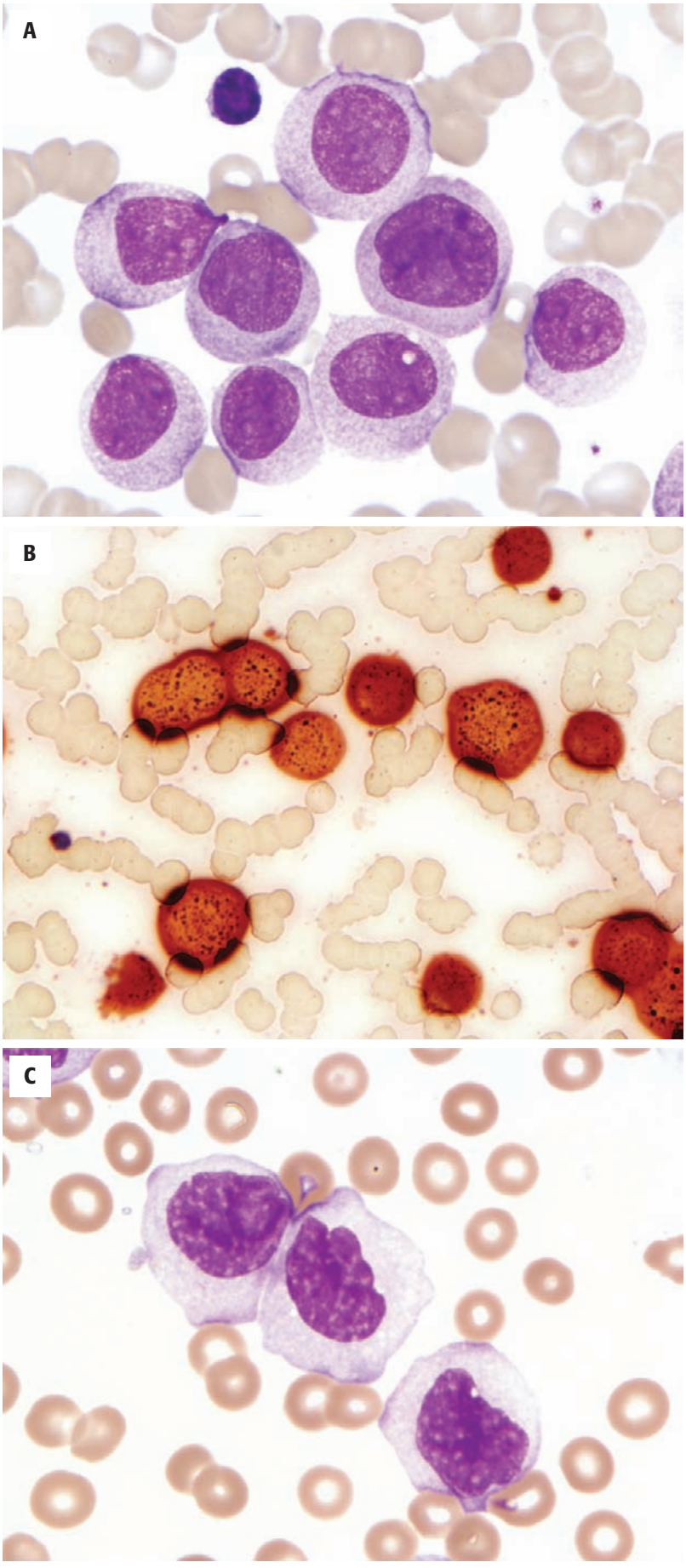


**FIGURE 14-17**

Acute monocytic leukemia (FAB-M5). **A,** Cases of M5a are more immature without nuclear folds. **B,** More than 80% of the blasts are nonspecific esterase positive. **C,** Cases of M5b show more monocytic maturation (promonocytes) with more irregular nuclear contours. These cells are also more than 80% nonspecific esterase positive.

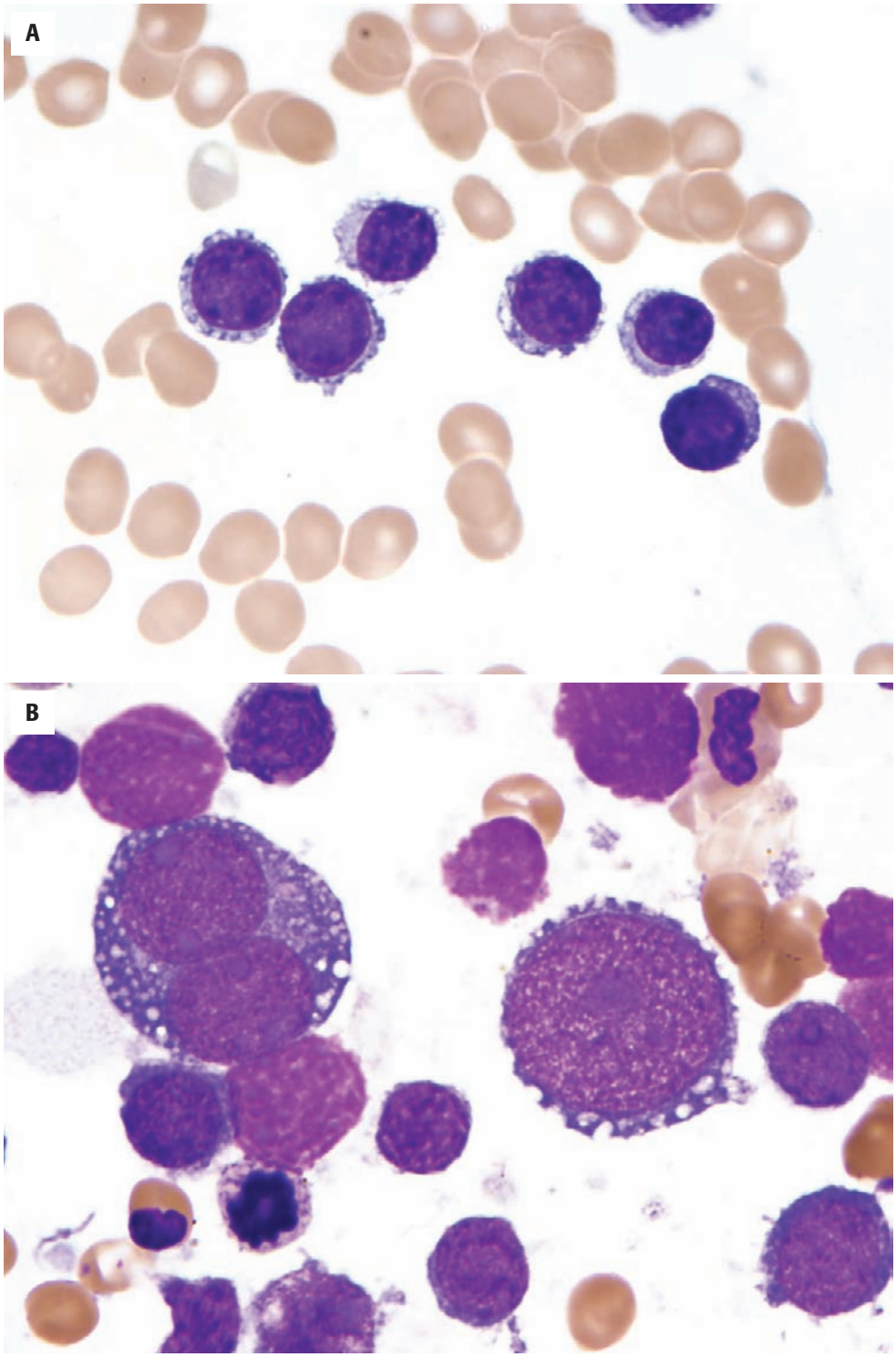


**FIGURE 14-18**

Pure erythroid leukemia. **A,** Rare cases show a monotonous population of immature erythroid cells with cytoplasmic vacuoles. **B,** Most cases, however, show more variation in erythroid cells and are associated with multilineage dysplasia.

blasts; however, unlike the other entities on the differential diagnosis (e.g., AML-MRC, acute megakaryoblastic leukemia, spent phase of a myeloproliferative disorder), there is a prominent proliferation of myeloblasts, proerythroblasts, and megakaryoblasts (panmyelosis).

## MYELOID SARCOMA

Myeloid sarcoma (also known as *granulocytic sarcoma, chloroma,* and *extramedullary myeloid tumor*) is defined as an extramedullary mass-forming lesion of immature

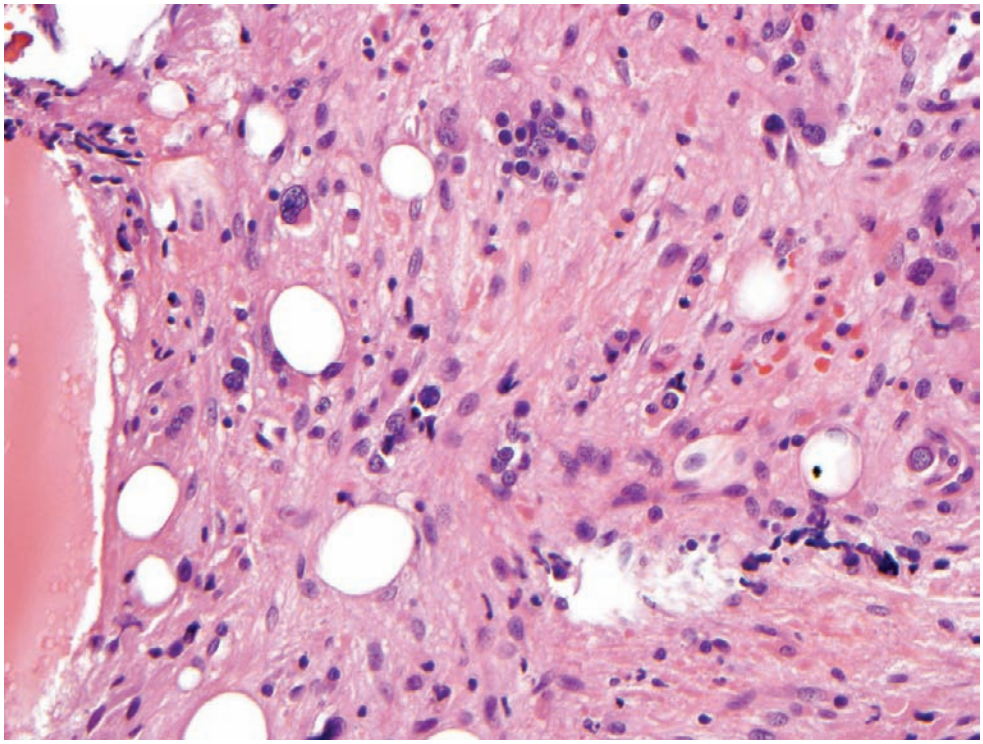

**FIGURE 14-19**

Acute panmyelosis with myelofibrosis. The marrow is inaspirable with marked marrow fibrosis, dysplastic megakaryocytes, and scattered immature-appearing cells. This appearance is similar to many cases of adult megakaryoblastic leukemia, but with less than 20% megakaryoblasts.

myeloid cells (Figure 14-20) and is diagnostic of AML, even without 20% blood or marrow blasts. Myeloid sarcoma can occur before, simultaneously with, independent of, or as a relapse of an acute or chronic myeloproliferative disorder. Although myeloid sarcoma has its own category, this type of myeloid proliferation is commonly associated with one of the AMLs with recurrent cytogenetic abnormalities, particularly in children. In adults, it can accompany AML-MRC, MDS, accelerated or blast phase of a chronic myeloproliferative neoplasm, or myelodysplastic or myeloproliferative neoplasm. Myeloid sarcoma is classically described as being associated with eosinophilic myelocytes, a clue to a myeloid proliferation. Cases with this morphology are often recognized as extramedullary presentations of AML with t(8;21). Other cases have a monotonous blastic or large cell appearance. Such cases may mimic lymphoblastic, Burkitt, or diffuse large cell lymphomas. Correlation with peripheral blood or bone marrow samples to evaluate for acute leukemia is usually helpful, but some cases represent isolated presentation or relapses of myeloid tumors. Myeloid sarcomas are usually diagnosed using paraffin section immunohistochemistry; they fail to react with specific lymphoid markers, such as CD2, CD3, CD20, or CD79a, and are usually immunoreactive for myeloperoxidase and CD33 or express monocyte markers such as CD163 or CD68. CD68 expression is nonspecific and should not be used alone for diagnosis of monocytic lineage. AML with t(8;21) commonly develops as myeloid sarcoma in the head and neck region of children. It should be remembered that this entity shows aberrant expression of B-cell markers such as PAX-5 and CD79a in some cases. In addition, AML with t(1;22) may develop as myeloid sarcoma and be negative for B- and T-cell markers, MPO, CD33, and CD68. CD43 will often be expressed, and megakaryocytic antigens such as CD61, CD41, or factor VIII–related antigen are diagnostic of the megakaryocytic lineage. Blastic plasmacytoid dendritic cell tumor commonly develops as a skin lesion and should be remembered in the differential diagnosis. These lesions share CD123 with many other types of AML, but express TCL1, commonly lack CD13 and CD33, and do not express MPO. Repeated biopsy for cytogenetics and molecular studies should be strongly considered if the marrow is not informative in cases of myeloid sarcoma, because a more specific diagnosis is necessary for optimal risk stratification.

## MYELOID PROLIFERATIONS OF DOWN SYNDROME

Approximately 10% of neonates with Down syndrome, as well as rare fetuses, develop a hematopoietic disorder

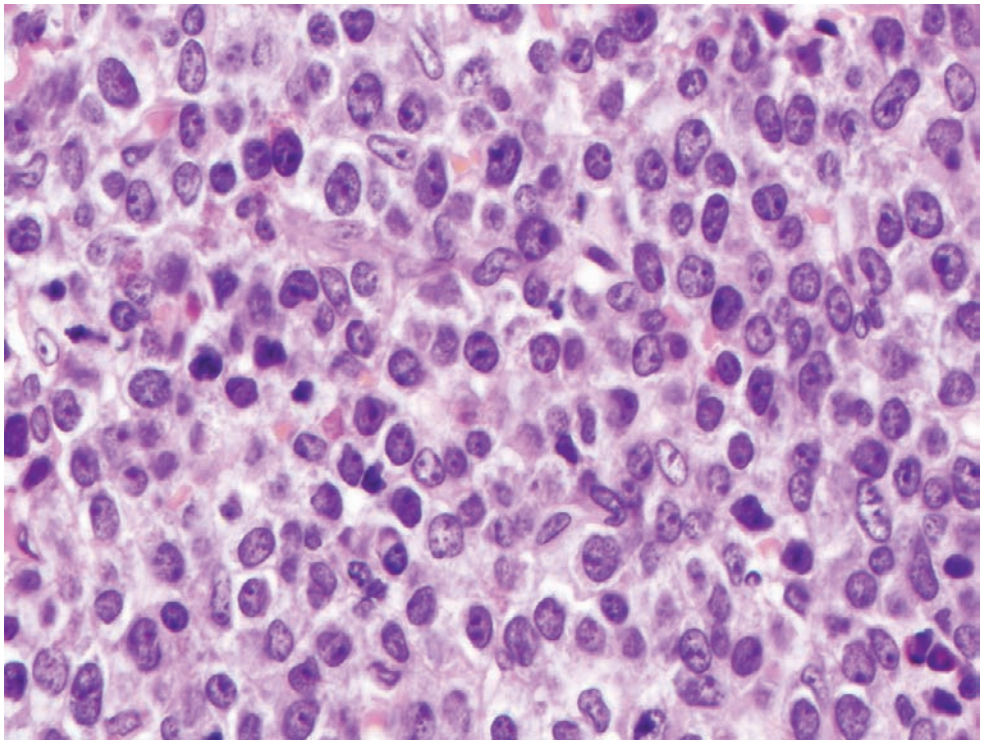

**FIGURE 14-20**

Extramedullary myeloid cell tumor (myeloid sarcoma) involving a lymph node. The tissue is infiltrated by a monotonous population of mononuclear cells with a subset showing granular cytoplasm.

**MYELOID PROLIFERATIONS OF DOWN SYNDROME—FACT SHEET**

**Definition**

- Myeloid proliferations in infants and children with Down syndrome

**Transient Abnormal Myelopoiesis**

Frequency and age distribution

- Fetus and neonates
- Approximately 10% of Down syndrome patients

**Clinical Features**

- Leukocytosis with resolution in 1 to 3 months

**Cytogenetic and Molecular Features**

- Trisomy 21 (constitutional), random nonclonal abnormalities, *GATA1* mutations, *JAK3* mutations

**Prognosis**

- Good, if not complicated by hepatic fibrosis

**Myeloid Leukemia of Down Syndrome**

Frequency and age distribution

- 2 years of age or older
- Approximately 2% of Down syndrome children

**Clinical Features**

- Leukocytosis without resolution until treated, typically from TAM which does not resolve

**Cytogenetic and Molecular Features**

- Trisomy 21 (constitutional), additional unbalanced clonal abnormalities (trisomy 1, trisomy 8, monosomy 7); *GATA1* mutations, *JAK2 and JAK3* mutations

**Prognosis**

- Generally good in children less than 4 years of age

that is morphologically indistinguishable from acute megakaryoblastic leukemia or a traditional myelodysplastic syndrome termed *transient abnormal myelopoiesis* (TAM). These infants have a marked leukocytosis with numerous peripheral blood and bone marrow megakaryoblasts (Figure 14-21). Some patients will also show peripheral blood basophilia and erythroid dysplasia. As in acute megakaryoblastic leukemia, the blasts are negative for myeloperoxidase and express myeloid and megakaryocyte-associated antigens. These transient proliferations may show karyotypic abnormalities in addition to trisomy 21, but they do not have the t(1;22)

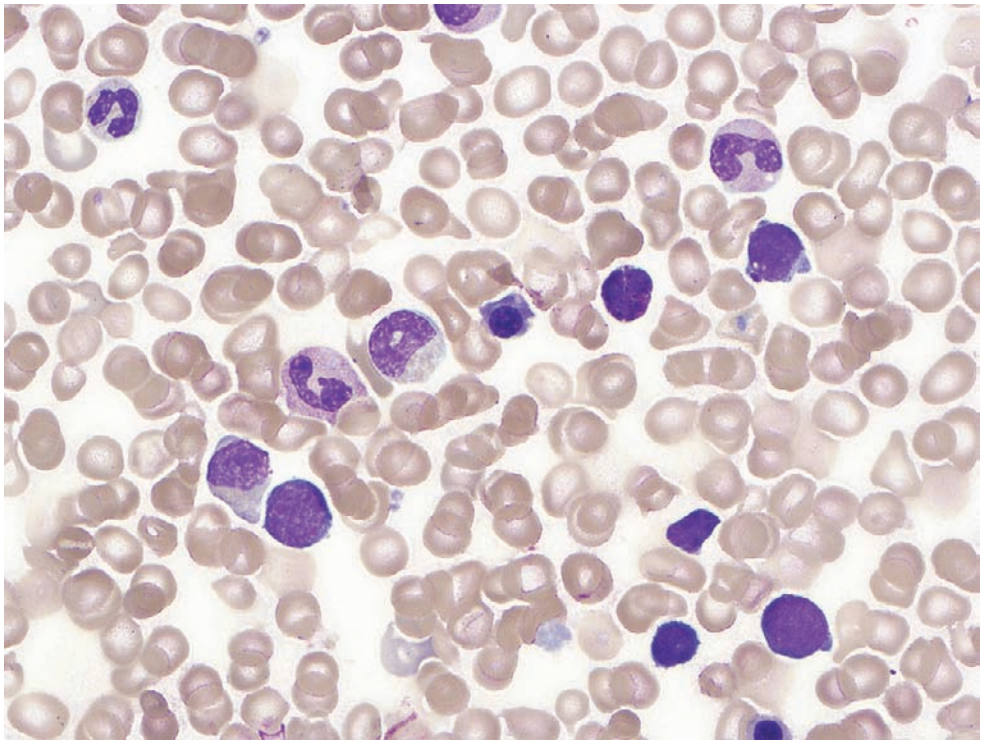

**FIGURE 14-21**

Transient abnormal myelopoiesis of Down syndrome. The peripheral blood of this infant shows an elevated white blood cell count with circulating blast cells. Some blasts show cytoplasmic blebs, suggestive of megakaryoblasts. This lineage was confirmed by detection of CD61 and CD41 on the blast cell population.

of infant acute megakaryoblastic leukemia, and they differ from true acute megakaryoblastic leukemia by clinical resolution in 1 to 3 months. Therefore caution should be used in diagnosing a proliferation of this type as acute leukemia in a neonate with Down syndrome. Although cases may spontaneously remit, they can be associated with severe morbidity and mortality when associated with hepatic fibrosis and occlusion of small vessels of vital organs by the megakaryoblasts. Of note, TAM may be the first clinical indication of Down syndrome in patients with mosaicism for the trisomy 21 who do not display the typical physical features of Down syndrome. Therefore awareness of this possibility in the neonatal setting and correlation with cytogenetic findings may prompt further investigation of the germline karyotype in nonmyeloid tissues.

Acute megakaryoblastic leukemia of Down syndrome (Figure 14-22) is clinically distinct from TAM of Down syndrome, and it can occur 1 to 2 years after the resolution of a TAM or follow neonatal presentations of TAM that never completely abates. The vast majority of Down syndrome-associated AML are acute megakaryoblastic leukemias. These leukemias usually occur around 2 years of age and have a generally favorable prognosis until the age of four years, after which the prognosis worsens with advancing age.

Both acute megakaryoblastic leukemia and TAM of Down syndrome are characterized by acquired mutations of the *GATA1* gene. A single gene array study identified three transcriptional differences between the two entities: *CDKN2C* and *PRAME* transcripts were increased in acute megakaryoblastic leukemia, while expression of the *MYCN* gene was increased in TAM. Confirmation of these transcriptional differences may provide useful future molecular markers to distinguish these related but prognostically distinctive diseases.

**TRANSIENT MYELOPROLIFERATIVE DISORDER AND ACUTE MEGAKARYOBLASTIC LEUKEMIA OF DOWN SYNDROME—PATHOLOGIC FEATURES**

**Blast Cell Features**

- Often megakaryoblastic with cytoplasmic blebbing

**Non–Blast Cell Morphology**

- Associated multilineage dysplasia

**Blast Cell Immunophenotype**

- Myeloid antigen positive (CD13, CD33)
- Megakaryocyte antigen positive (CD41, CD42, CD61)

**Differential Diagnosis**

- AML with t(1;22)
- AML-MRC
- AML, NOS, acute megakaryoblastic leukemia
- Myelodysplastic syndrome

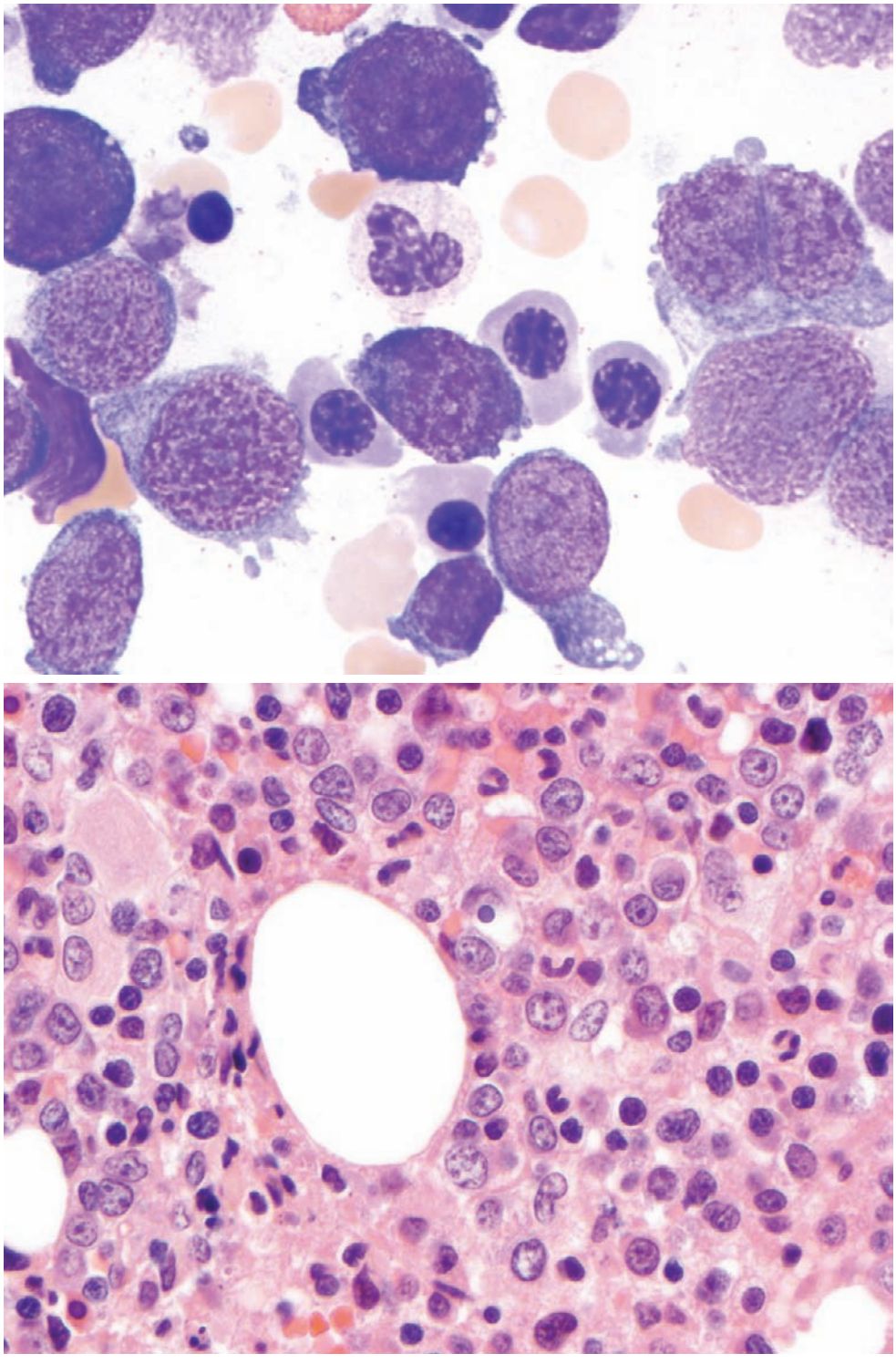

**FIGURE 14-22**

Acute megakaryoblastic leukemia of Down syndrome. The bone marrow is infiltrated by cells similar to the blasts seen in Figure 14-21, but this occurred in an older child.

## BLASTIC PLASMACYTOID DENDRITIC CELL TUMOR

BPDC is the entity previously known as *agranular CD4$^+$CD56$^+$ hematodermic neoplasm,* and *blastic NK cell leukemia–lymphoma*. Since the 2001 WHO classification was published, the morphologic and immunophenotypic resemblance of this neoplasm to plasmacytoid dendritic cells has been recognized, and the lineage has been established. Plasmacytoid dendritic cells are the major type 1 interferon producing cells in the body in response to the binding of nucleic acid type ligands on the cell surface. The precise pathway of derivation from a hematopoietic stem cell is not known.

BPDC is most commonly reported in adults, although cases in infants and children are seen. BPDC is an aggressive neoplasm that typically develops as a skin lesion, with subsequent rapid marrow dissemination. Spreading to lymph nodes and spleen is commonly reported. Up to 20% of patients will have an associated myeloid neoplasm (e.g., myelodysplasia, myeloproliferative–myelodysplastic neoplasm, or AML) recognized either before or after the diagnosis of BPDC.

Skin lesions show dermal infiltrates of the blasts with periadnexal accentuation and sparing of the epidermis. In the peripheral blood, blasts are intermediate in size with minimal cytoplasm. On bone marrow aspirate smears, they display a variable amount of grey-blue cytoplasm. Distinctive elongate pseudopodia, often with accumulation of small vacuoles, are a helpful clue to the diagnosis (Figure 14-23). The marrow may be effaced or minimally and interstitially involved. Nodules of plasmacytoid dendritic cells in the marrow or lymph nodes can be seen in other myeloid conditions; these are distinguished from involvement by BPDC by their focal nature and more mature appearance with abundant cytoplasm, unlike the blastic morphology of BPDC.

**BLASTIC PLASMACYTOID DENDRITIC CELL TUMOR—FACT SHEET**

**Frequency and Age Distribution**
- Uncommon, less than 1% of acute leukemias
- Older adults, less commonly infants and young children

**Clinical Features**
- Usually develops as skin lesion
- Common association with other myeloid proliferations (MDS, myeloproliferative, myeloproliferative/myelodysplastic, AML)

**Prognosis**
- Poor

**BLASTIC PLASMACYTOID DENDRITIC CELL TUMOR—PATHOLOGIC FEATURES**

**Morphology**
- Skin: dermal medium-sized blast infiltrates with periadnexal accumulation and sparing of the epidermis
- Bone marrow: on smears, blasts showing a variable amount of blue-grey cytoplasm, frequently with pseudopodia and small polarized vacuoles; irregular nuclei and one to several small nucleoli; aggregates or sheets of monomorphic-appearing blasts on core biopsy

**Immunophenotype**
- CD4$^+$, CD43$^+$, CD56$^+$, CD123$^+$, TCL-1$^+$, CD68$^+$, HLA-DR$^+$ with variable CD7, CD2, and rarely dim CD33

**Cytogenetic Features**
- Complex karyotype, (5q-, 12p-, 6q abnormalities; deletions of 13, 9, and 15)

BPDC commonly expresses CD4, CD56, CD123, CD43, CD45RA, and TCL-1. Variable expression of CD7, CD33, CD38, CD71, TdT, and a dot-like cytoplasmic reactivity for CD68 are also reported. Rare cases may lack either strong CD4 or CD56 reactivity. CD34, CD117, CD14, CD15, CD57, and MPO are not expressed. Granzyme B, but not TIA-1 or perforin, is detected by flow cytometry. Immunohistochemistry for granzyme B in paraffin section is usually negative. The immunophenotype CD4$^+$CD56$^+$CD123$^+$ lacking CD34 or CD117 overlaps significantly with AML with monocytic differentiation. BPDC lacks cytochemical esterase reactivity and will not express lysozyme. The presence of small vacuoles, with expression of CD71, may suggest acute erythroleukemia. Erythroblast vacuoles are usually PAS positive. Expression of TCL-1 is largely restricted to B-cell neoplasms and T-cell prolymphocytic leukemia. CD123 is widely expressed in other types of AML, as well as cases of B- and T-acute lymphoblastic leukemia. Newer markers such as CD303 (BDCA-2) and CD2AP may be useful in difficult cases. Some cases may harbor a normal karyotype, but most have a complex karyotype, including 5q, 12p, and 6q abnormalities, as well as deletions of chromosomes 13, 9, and 15.

## GROWTH FACTOR CHANGES THAT CAN MIMIC ACUTE MYELOID LEUKEMIA

Growth factors are administered for a variety of reasons, including enhancing bone marrow recovery after chemotherapy and priming the marrow or peripheral blood prior to stem cell collection. These agents can cause

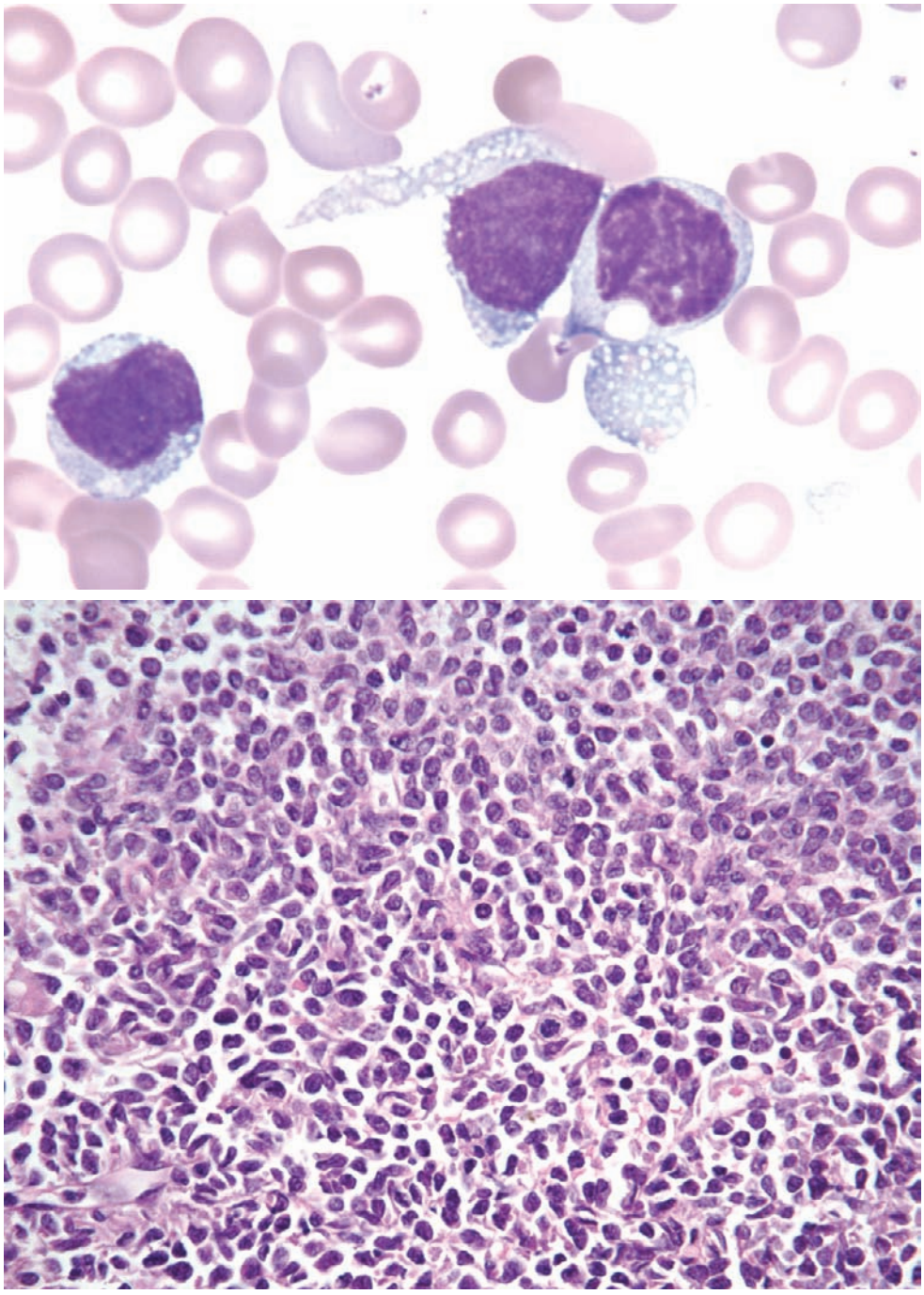

**FIGURE 14-23**

Blastic plasmacytoid dendritic cell tumor. Blasts of blastic plasmacytoid dendritic cells have oval, variably folded nuclei. The cytoplasm is pale grey-blue with elongate pseudopodia and small vacuoles in many forms. Sheets of immature cells with folded nuclei efface the marrow on the core biopsy.

changes that might suggest a new diagnosis of AML or relapsed AML. The most commonly used agents in this setting are human recombinant granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), which cause both peripheral blood and bone marrow changes (Table 14-3). Both agents cause a peripheral blood leukocytosis with a granulocyte left shift. The common presence of toxic granulation and Döhle bodies give the appearance of a reactive proliferation. Enlarged neutrophils or neutrophils with vacuolated cytoplasm can also occur.

The bone marrow shows a granulocytic hyperplasia (Figure 14-24). Depending on the timing of bone marrow examination, the marrow may show the complete spectrum of granulocytic maturation, give the appearance of maturation arrest, or show a predominance of segmented neutrophils. The maturation arrest-type changes usually occur just after administration of the growth

**TABLE 14-3**

**Peripheral Blood and Bone Marrow Changes Associated with Growth Factors (G-CSF and GM-CSF)**

- Peripheral blood changes
  - Circulating nucleated red blood cells
  - Neutrophilia
  - Vacuolated and giant neutrophils
  - Granulocyte left shift
  - Toxic granulation and Döhle bodies
  - Hypogranular neutrophils
  - Increase in large granular lymphocytes
  - Eosinophilia
  - Transient blast cells
- Early bone marrow changes
  - Granulocytic hyperplasia with increase numbers of promyelocytes and myelocytes
  - Toxic granulation of granulocytes
  - Enlarged promyelocytes and myelocytes
  - Increased mitotic activity of granulocyte precursors
  - Transient blast cell increase
  - Biopsy hypocellularity with left-shifted granulocytic precursors
- Late bone marrow changes
  - Binucleated promyelocytes
  - Marrow neutrophilia
  - Marrow eosinophilia
  - Toxic granulation
  - Variable biopsy cellularity

*G-CSF,* Granulocyte-colony stimulating factor; *GM-CSF,* granulocyte-macrophage colony-stimulating factor.

factor and offer the most diagnostic problems, including features worrisome for AML or myelodysplasia. A predominance of promyelocytes and myelocytes is usually present. In some cases, bone marrow and peripheral blood blast cells may exceed 5%, but this increase is usually accompanied by an even greater increase in promyelocytes. Blast proliferations that are not accompanied by an increase in promyelocytes should be considered highly suggestive of leukemia and not simply attributed to growth factor changes.

In a patient with a history of acute myeloid leukemia, it might not be possible to entirely exclude the possibility of leukemia in the setting of an increase in blast cells and cytogenetic studies, or evaluation for a prior aberrant leukemia immunophenotype may be useful in that setting. The promyelocytes that occur with G-CSF and GM-CSF therapy usually have prominent perinuclear hofs, which should be a clue to the possibility of growth factor administration. These cells differ from those of acute promyelocytic leukemia, which usually do not show perinuclear cytoplasmic clearing and demonstrate Auer rods that are not present in reactive promyelocytes. A repeated bone marrow examination 1 to 2 weeks after cessation of the growth factor will usually demonstrate more complete granulocyte maturation, and such a study is advisable in cases that are worrisome for residual leukemia. Less common changes that have been reported after G-CSF and GM-CSF therapy include marrow necrosis and marrow histiocyte proliferations.

## POST-THERAPY CHANGES IN ACUTE MYELOID LEUKEMIA

### CHANGES ASSOCIATED WITH MYELOABLATIVE THERAPY

Several studies have evaluated the marrow changes after high-dose chemotherapy, or chemotherapy with radiation, including regimens used for hematopoietic stem cell transplantation, approaches used for many types of acute myeloid leukemia. The changes are similar to the toxic changes of other drug injuries of the marrow. Complete marrow aplasia is the immediate expected change in the first week after myeloablative therapy (Table 14-4, Figure 14-25). The marrow cellularity is essentially zero with an absence of normal marrow fat. There is edema with dilated marrow sinuses and scattered stromal cells, histiocytes, plasma cells, and lymphocytes. Normal maturing granulocytes, nucleated red blood cells, and megakaryocytes are usually not identifiable. Histiocytes containing cellular debris are often present, and acellular areas of fibrinoid necrosis often predominate. The development of mild reticulin fibrosis and the reappearance of fat cells follow these initial changes. The early fat in a regenerating marrow is loculated. While the marrow remains markedly hypocellular, the loculated fat is accompanied by focal areas of early hematopoiesis in the second week after treatment; this may be represented by islands of erythroid cells alone or in combination with areas of left-shifted granulocytes. Both elements are usually present after 2 weeks. Megakaryocytes, often occurring in clusters with hypolobated nuclei, occur later in this process, but are usually easily identified by the third week. In some patients, particularly children, early regeneration may be accompanied by an increase in precursor B cells or hematogones.

The expected later changes after high-dose therapy usually include a loss of the mild reticulin fibrosis of early regeneration, and a return to normal or even slightly increased marrow cellularity. All three normal marrow cell lines are present, although a left shift of granulocytes and erythroid cells and atypical megakaryocyte clustering may persist for some time.

### MARROW AFTER RECOVERY FROM ACUTE MYELOID LEUKEMIA THERAPY

Guidelines for the definition of morphologic remission in patients treated for acute myeloid leukemia require a morphologic leukemia-free state (defined as less than

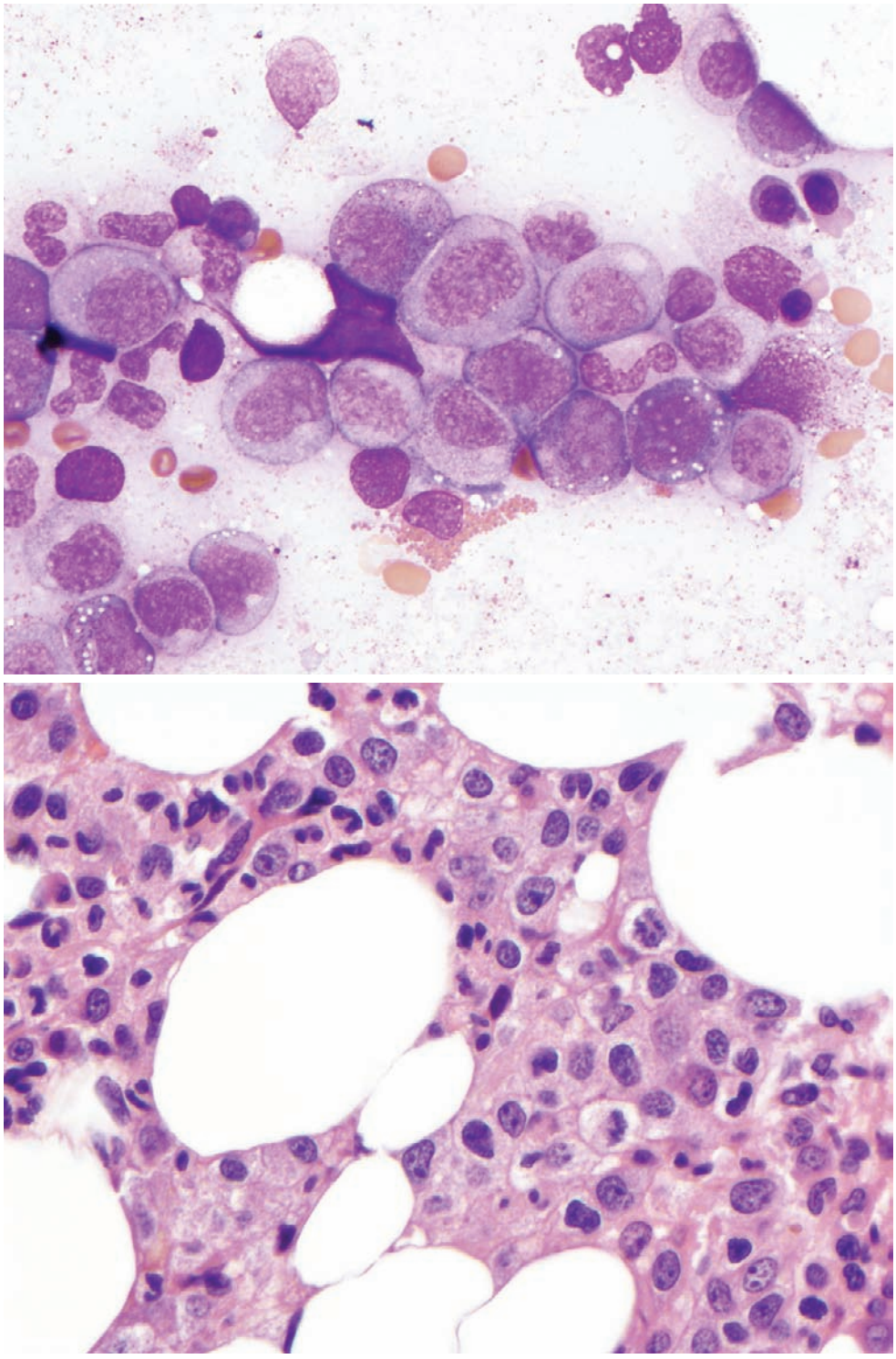

**FIGURE 14-24**

Growth factor changes. This patient received growth factor after therapy for lymphoma. The marrow shows an increase in promyelocytes with perinuclear hofs and a paucity of erythroid precursors.

5% bone marrow blasts, absence of Auer rods, or extramedullary disease, and the absence of a unique leukemia immunophenotype by flow cytometry), as well as an absolute neutrophil count of greater than 1000/μL and platelet count of 100,000/μL or greater. Before demonstrating these changes, however, several morphologic features of the peripheral blood and bone marrow have prognostic significance. Failure to demonstrate a reduction in blast cells and cellularity at day 6 of induction chemotherapy will usually result in a

change or augmentation of induction chemotherapy. Not surprisingly, the presence of residual leukemic cells at the end of induction chemotherapy is a poor prognostic indicator. Even after meeting the criteria for remission, patients with bone marrow hypercellularity, anemia, bone marrow blast cell counts of 1% or more, or peripheral blood blast cell counts of over 3% have a shortened duration of remission and shortened survival. Therefore more detailed evaluation of bone marrow and peripheral blood samples is needed than is suggested from most remission criteria.

**TABLE 14-4**

**Bone Marrow Changes Immediately Following Myeloablative Therapy**

- Initial changes (approximately 1 week)
  - Marrow aplasia
  - Absence of fat cells
  - Edema and fibrinoid necrosis with or without tumor necrosis
  - Dilated sinuses
  - Rare stromal cells, histiocytes, lymphocytes and plasma cells
- Intermediate changes (approximately 2 weeks)
  - Reappearance of fat, often showing lobulated cytoplasm
  - Mild reticulin fibrosis
  - Left-shifted erythroid and granulocyte islands
  - Increase in precursor B cells
- Late changes (approximately 3 to 4 weeks)
  - Resolution of reticulin fibrosis
  - Clusters of small megakaryocytes
  - Normal or slightly increased marrow cellularity

The blast cells in patients with a history of acute myeloid leukemia should be compared to the patient's original disease material. The presence of an increased number of cells with features similar to the original material should be regarded with suspicion (Figure 14-26). Auer rods, rod-shaped cytoplasmic aggregates of granules, are not a feature of regenerating or nonneoplastic myeloblasts, and should be considered as evidence of residual disease. Auer rods may be encountered rarely in maturing granulocytes, but are still considered an abnormal finding. Regenerating blast cells are usually admixed with promyelocytes and maturing granulocytes, and the presence of sheets of blasts on a smear is generally a sign of recurrent disease. In contrast, specimens with blast cells in numbers equal to or lower than promyelocytes usually represent regeneration. Clustering of blast cells is often difficult to interpret on hematoxylin and eosin (H&E)–stained biopsy material, and aggregates of regeneration may be difficult to differentiate from leukemic blast cell aggregates. Regeneration usually occurs adjacent to bony trabeculae, and the presence of immature cell aggregates away from the

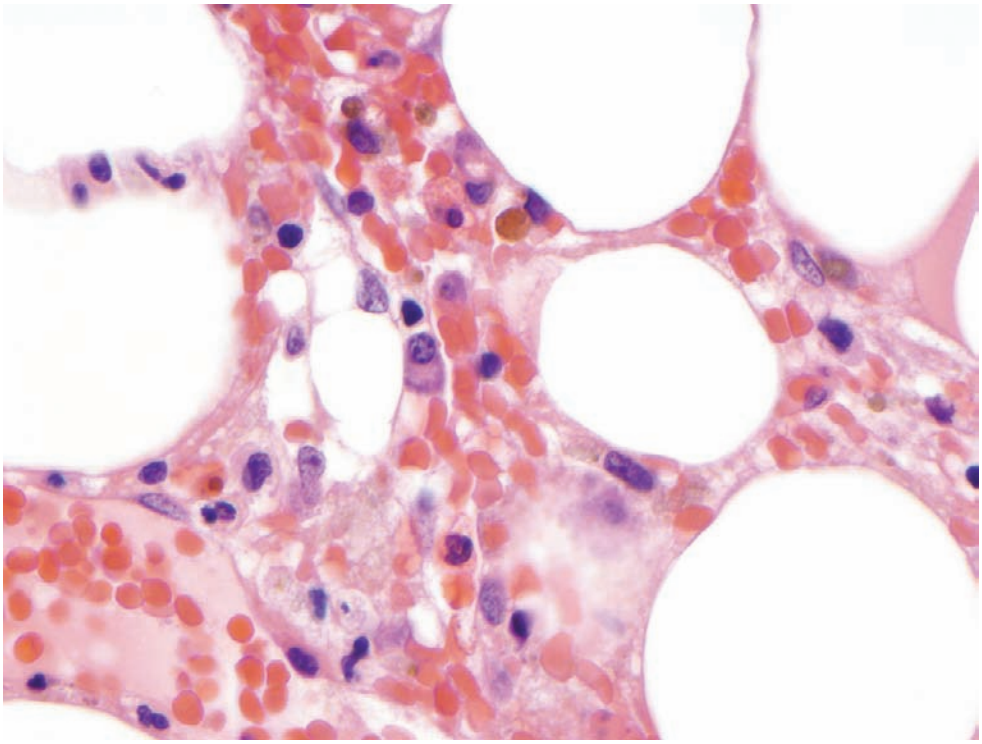

**FIGURE 14-25**

Changes immediately after myeloablative therapy for acute myeloid leukemia. The marrow shows hemorrhage and fibrin deposition without scattered lymphocytes, plasma cells, and histiocytes. Maturing myeloid, erythroid, and megakaryocytic cells are essentially absent.

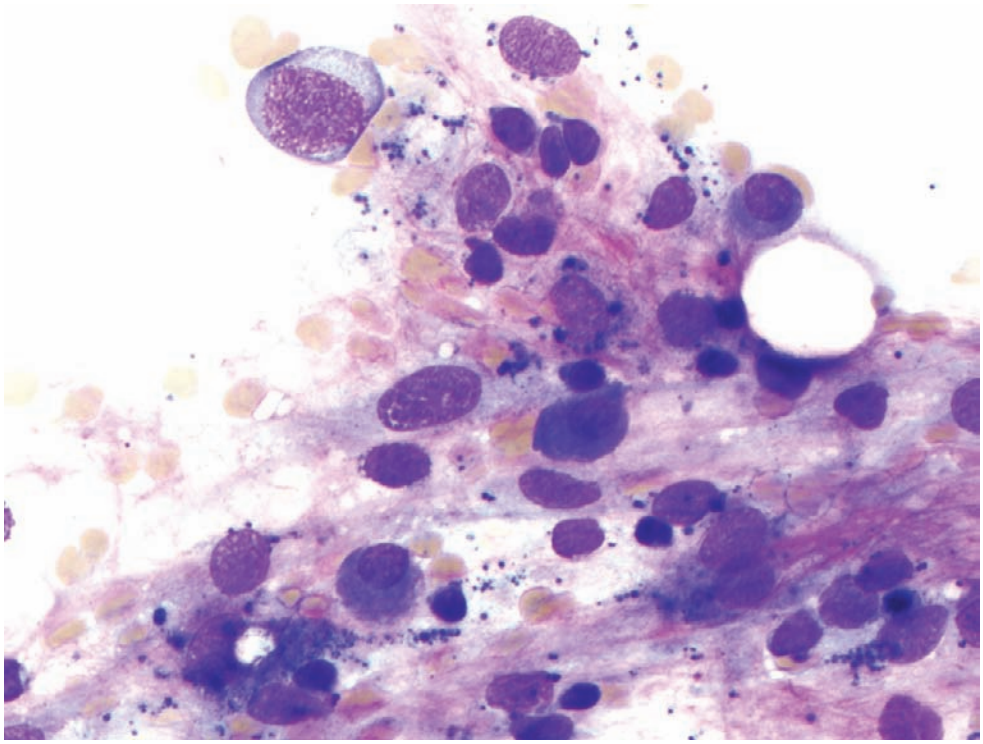

**FIGURE 14-26**

Post-therapy bone marrow aspirate showing evidence of ablation, but a few blast cells are present. These cases should be correlated with prior blast cell morphology, immunophenotyping, and cytogenetic studies to resolve the differential diagnosis of regeneration versus residual disease. A biopsy should be repeated in 1 to 2 weeks if these additional studies are not available or are ambiguous.

bone is generally considered abnormal. This abnormal localization of immature cell precursors has been used as a sign of a myelodysplastic syndrome, but caution should be used in applying these criteria in patients after hematopoietic stem cell transplantation. After transplantation, the normal bone marrow architecture of regeneration may change, and regenerating immature-appearing cells on H&E-stained sections may be present away from the bone. Paraffin section immunohistochemistry may also be of value in selected cases, particularly in the presence of left-shifted cell aggregates on H&E-stained sections. By immunophenotyping, the immature cell aggregates of regeneration can be shown to represent a spectrum of left-shifted cells that are not exclusively blast cells, whereas recurrent leukemia blast cell aggregates are a more uniform population of neoplastic cells. Therefore the identification of clusters of cells expressing the immature cell antigen CD34 in biopsy material is a feature favoring residual or recurrent disease. Correlation with cytogenetic, molecular genetics, or fluorescence in situ hybridization (FISH) studies may also be useful to search for a characteristic genetic abnormality associated with a given patient's original disease.

Acute myeloid leukemia with myelodysplasia-related changes may show the presence of multilineage dysplasia before an increase in blast cells at relapse. Again, the features of the original multilineage dysplasia should be reviewed with the possible relapse sample. Caution should be used to not overcall multilineage dysplasia during or immediately following therapy. Dyserythropoietic changes are common during chemotherapy and often include a left shift of erythroid precursors and multinucleation of erythroid cells. In addition, regenerating megakaryocytes often cluster and are small during or immediately after chemotherapy. Granulocyte changes after therapy are usually restricted to a left shift without the hypogranulation commonly seen in association with myelodysplasia. Therefore dysplastic changes of maturing granulocytes are probably more reliable than erythroid abnormalities alone for identifying recurrent AML with myelodysplasia-related changes during or immediately after chemotherapy.

Currently, most patients with acute promyelocytic leukemia are treated with both standard chemotherapy and ATRA, and they demonstrate bone marrow changes that are usually similar to other AML samples. However, some patients treated with ATRA or combination chemotherapy without ATRA mighty not show an initial marrow aplasia. The bone marrow in these patients may remain hypercellular with markedly elevated numbers of promyelocytes (Figure 14-27). These cells will usually slowly undergo maturation secondary to

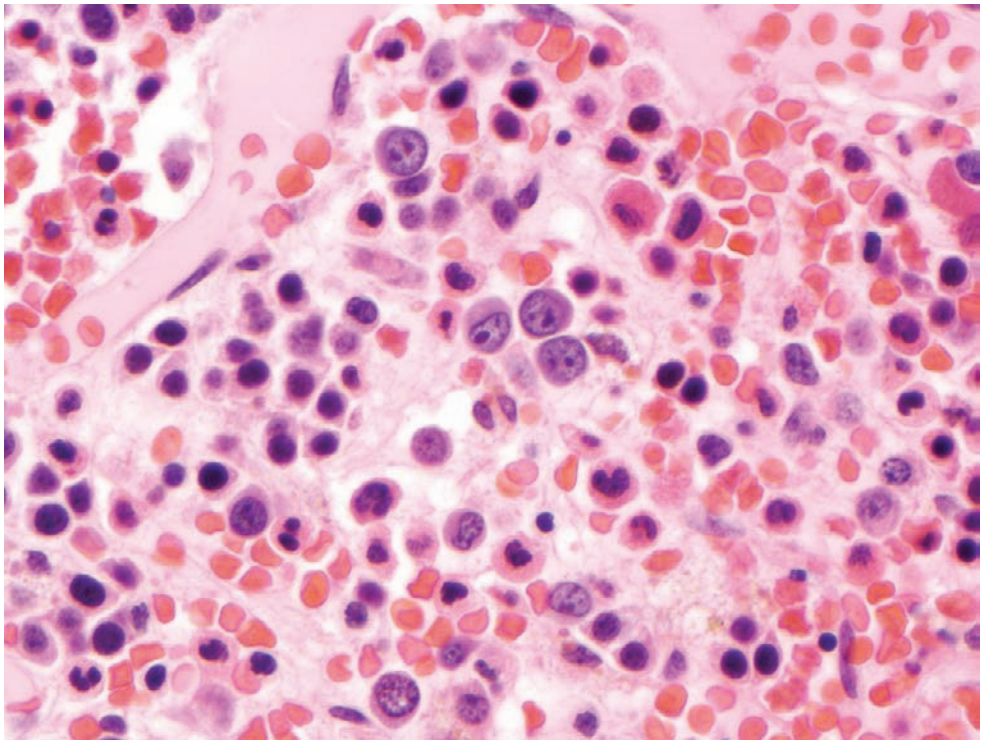

**FIGURE 14-27**

Acute promyelocytic leukemia post all-*trans* retinoic acid therapy. Residual promyelocytes, including degenerating promyelocytes may be present after therapy. These patients should be followed closely. This finding in an early bone marrow specimen is not sufficient for an interpretation of relapse or recurrent disease.

the therapy with loss of the t(15;17) cytogenetic abnormality associated with acute promyelocytic leukemia. In this subgroup of patients, it should be understood that the presence of sheets of promyelocytes may not indicate treatment failure, and the patients should be followed closely with additional marrow examinations to confirm that the maturational changes are occurring.

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The complete reference list is available online at www.expertconsult.com.

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15

# Precursor Lymphoid Neoplasms

**Mihaela Onciu, MD**

## INTRODUCTION

Acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL) constitute a biologic continuum of neoplastic lymphoid disorders characterized by the proliferation of immature (blast) cells of precursor B-cell or precursor T-cell lineage. For clinical purposes, an arbitrary cutoff has been used for many years to separate lymphoblastic leukemias and lymphomas. Cases with tissue involvement and less than 25% replacement of the marrow cellularity by lymphoid blasts have been designated as *lymphoblastic lymphomas*. Cases with 25% or greater marrow involvement have been designated *acute lymphoblastic leukemias*. For practical purposes, this separation is a measure of disease burden or disease stage, but there are no known biologic differences that correlate with this cutoff. However, when defined in this manner, it is notable that most of the cases of LBL (90%) are of T lineage, whereas true precursor B-cell LBL is extremely rare. This distinction appears to result from the fact that most of the B-lineage lymphoblastic neoplasms originate in the bone marrow, whereas most of the T-lineage tumors originate in extramedullary lymphoid organs, most frequently in the thymus. In the past, the original French-American-British (FAB) classification included in the category of ALL cases that represented the leukemic phase of Burkitt lymphoma, a neoplasm with mature B-cell immunophenotype. These cases were classified as L3 ALL. In the current classifications, this category no longer belongs with ALL and will be addressed in this chapter only as it applies to the differential diagnosis.

## Clinical Features

ALL and LBL can occur at any age, but the majority of the cases arise in children, constituting the most common type of childhood malignancy. The disease has a bimodal age distribution, with a higher incidence peak in early childhood (with median ages of 4 years for precursor B-cell ALL and 10 years for precursor T-cell ALL) and a second lower incidence peak in older adults. In the Western countries, approximately 80% to 85% of the cases are of B lineage, whereas only 15% to 20% are of T lineage. In the Middle East, North Africa, and Sicily, the proportion of T-cell ALL is higher (up to one third of all ALLs) for reasons that are unclear. T-cell ALL also occurs with increased frequency in patients with ataxia telangiectasia. ALL can occur as de novo disease or, rarely, as a secondary neoplasm following chemotherapy for a different malignancy.

ALL may present clinically as an acute illness or with symptoms that develop slowly and persist for months. The most common findings include fever, fatigue, bone or joint pain, bleeding, anorexia, abdominal pain, and hepatosplenomegaly. Approximately 50% to 60% of the patients with T-cell ALL develop an anterior mediastinal mass that can lead to superior vena cava syndrome.

ALL is a highly aggressive neoplasm that requires intensive chemotherapy. The modern approach to the treatment of ALL involves tailoring the intensity of the chemotherapy to risk groups defined by the presenting clinical features, lineage, cytogenetics, and molecular findings, as well as by the early response to therapy.

## Pathologic Features

### Gross Findings

When involving lymph nodes or other extramedullary tissues, LBL has the characteristic pale, pink, "fish-flesh" appearance typically associated with lymphomas.

### Microscopic Findings

LBL involving lymph nodes has a diffuse growth pattern with subtotal or complete effacement of the normal lymph node architecture. When partially involving the lymph node, T-cell LBL usually has a predominantly interfollicular distribution. There may be a

**ACUTE LYMPHOBLASTIC LEUKEMIA—FACT SHEET**

**Definition**
- Malignant lymphoid neoplasm composed of blastic cells of precursor B-cell or precursor T-cell lineage

**Incidence and Location**
- Incidence in United States (1997 to 2001): 1.4 per 100,000 population (all ages); 6.9 per 100,000 in children 1 to 4 years old
- 3200 new cases reported in the United States in the year 2000
- Frequently involves bone marrow and peripheral blood, as well as liver, spleen, lymph nodes, and mediastinum (the latter in T-cell ALL)

**Morbidity and Mortality**
- 80% to 90% cure rate in children treated with modern intensive chemotherapy, depending on prognostic features
- 30% to 40% 5-year survival in adults treated with dose-intensive regimens
- Less than 10% 3-year survival in patients older than 60 years

**Gender, Race, and Age Distribution**
- Slight male predominance (male-to-female, 1.2:1 to 1.8:1)
- Median age at diagnosis: 11 years
- Bimodal age distribution with an incidence peak in children up to 18 years of age and a lower incidence peak in adults older than 65 years

**Clinical Features**
- Most common presenting signs: fever, fatigue, bone or joint pain, bleeding, anorexia, lymphadenopathy, hepatosplenomegaly
- T-cell ALL: anterior mediastinal mass with or without superior vena cava syndrome

**Prognosis and Therapy**
- Poor prognostic factors in adults: age >35 years, leukocyte count >30,000, >4 weeks of therapy required to achieve complete remission, Philadelphia chromosome present in the leukemic blasts
- Poor prognostic factors in children: age ≥10 years, leukocyte counts >50,000, ≥5% bone marrow blasts at day 22 of induction therapy, Philadelphia chromosome or infant B-lineage ALL with *MLL* gene rearrangements
- All patients require intensive risk-adapted multiagent chemotherapy
- Prolonged continuation therapy (up to 3 years) is critical in preventing disease relapse

focal associated "starry-sky" appearance imparted by the presence of tingible-body macrophages. In paraffin-embedded tissue sections of lymph nodes and bone marrow biopsy samples, the lymphoma cells are typically intermediate in size, with oval, often indented nuclei, finely dispersed nuclear chromatin, and small inconspicuous nucleoli (Figure 15-1). Fewer cases are composed of large blasts with prominent centrally located nucleoli. In Wright-Giemsa–stained cytologic preparations, including smears of blood, bone marrow, and fine-needle aspiration samples, the ALL blasts are usually of intermediate size (typically up to twice the size of a small lymphocyte). Several cytologic subtypes of ALL, designated L1, L2, and L3, have been defined by the French-American-British (FAB) classification in the 1970s and early 1980s. The L3 subtype is known to correspond to the leukemic phase of Burkitt lymphoma, and therefore will be addressed in this chapter mainly as it pertains to the differential diagnosis of ALL. The L1 and L2 subtypes do not accurately correlate with disease subtypes or prognosis and therefore are mostly of descriptive importance at the current time. The most common cytologic subtype is L1 (Figure 15-2), characterized by relatively uniform, intermediate-sized blasts, with scant lightly basophilic cytoplasm, homogeneous, somewhat condensed chromatin, and inconspicuous or absent nucleoli. The L2 subtype (Figure 15-3) consists of more heterogeneous blasts that include larger forms, moderate amounts of lightly basophilic cytoplasm, finely dispersed nuclear chromatin, and prominent nucleoli. In any of these variants the blast cytoplasm may contain clear vacuoles (Figure 15-4) or granules (Figure 15-5), or rarely large unusual inclusions. The presence of granules, which are typically azurophilic or pale pink on the Wright-Giemsa stains, has been associated with the so-called granular ALL. Although the granules are of no prognostic consequence, they correlate closely with a precursor B-cell immunophenotype. In addition, the granules may be positive for Sudan Black B or nonspecific esterase on cytochemical staining, characteristics usually associated with acute myeloid leukemia. Rare cases of ALL, usually of T-cell lineage, may have a predominance of hand-mirror–shaped blasts that have an eccentric nucleus and a pale basophilic cytoplasmic tail. This finding is likewise of no prognostic significance. Last, the L3 ALL (Figure 15-6) is characterized by moderate-to-large blasts with regular nuclear outline, fine to slightly clumped nuclear chromatin, prominent and frequently multiple nucleoli, and moderately abundant deeply basophilic vacuolated cytoplasm.

## Ancillary Studies

### Immunophenotypic Analysis

Immunophenotypic analysis of ALL should provide at a minimum a lineage (T or B cell) that is of major importance for guiding the further work-up of

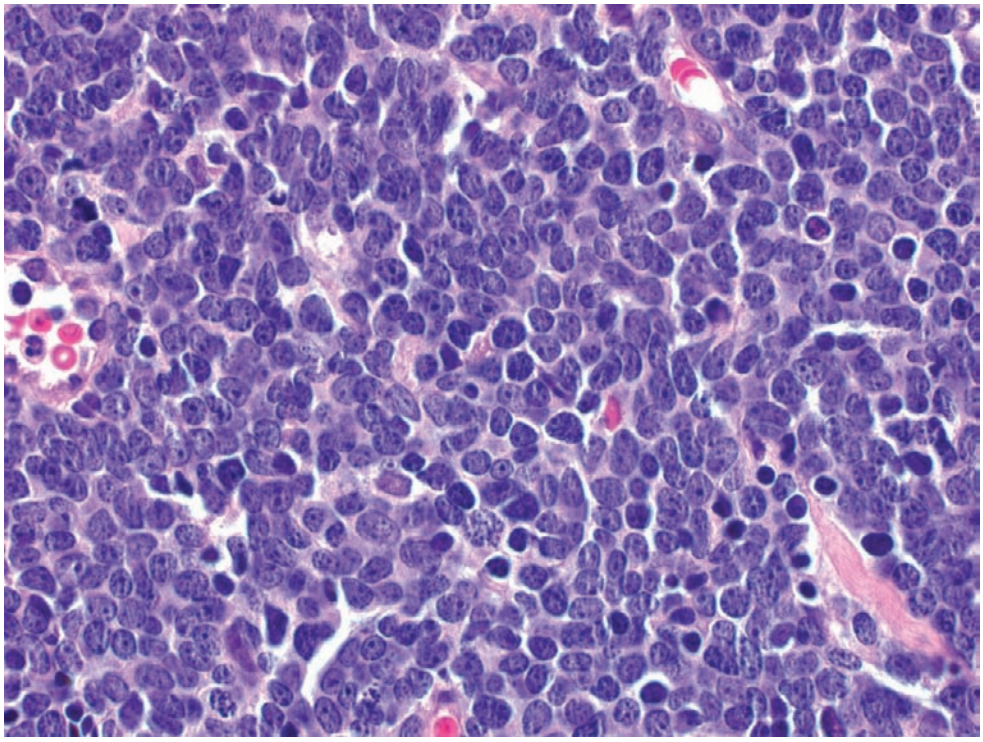

**FIGURE 15-1**

Lymphoblastic lymphoma. Morphologic features in paraffin-embedded tissue sections. The tumor is characterized by a diffuse growth pattern and is composed of relatively uniform blasts that are 1.5-fold to twofold the size of small lymphocytes; they have finely dispersed chromatin and small or absent nucleoli. Original magnification, ×60; oil immersion.

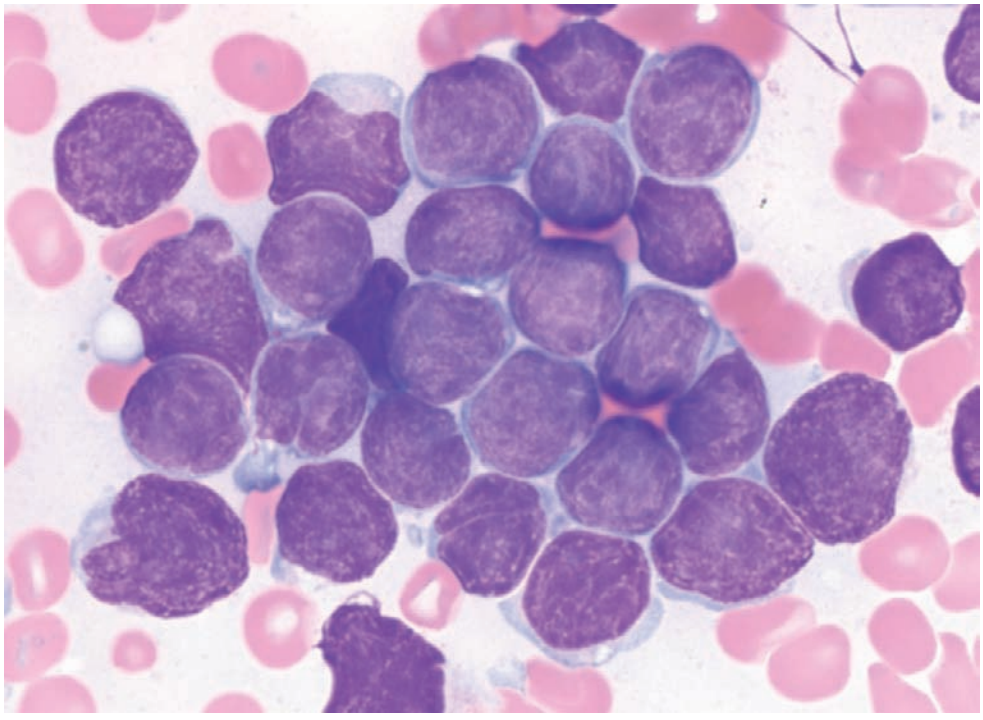

**FIGURE 15-2**

Acute lymphoblastic leukemia: the most common cytologic appearance of lymphoblasts in cytologic preparations (FAB L1 ALL). The blasts are intermediate in size and monotonous, with scant cytoplasm, coarsely clumped nuclear chromatin, and indistinct nucleoli. Some of the blasts have cleaved and folded nuclear outlines. Wright-Giemsa stain; original magnification, ×100; oil immersion.

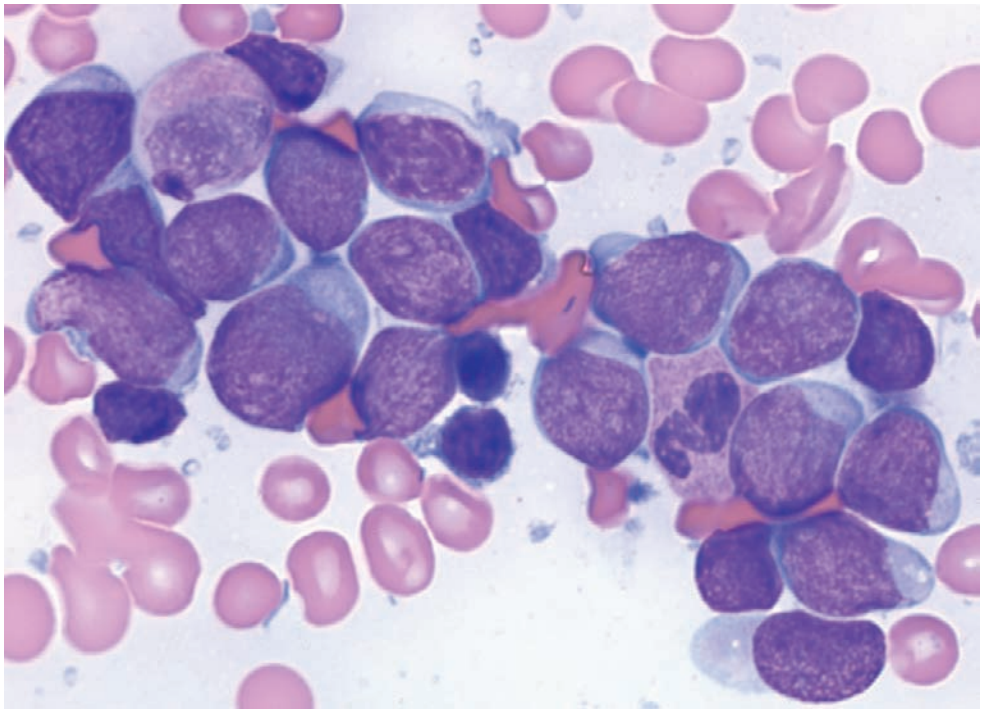

**FIGURE 15-3**

Acute lymphoblastic leukemia: cytologic appearance. A smaller number of cases may show the FAB L2 acute lymphoblastic leukemia blast morphology, which raises the differential diagnosis with acute myeloid leukemia. The blasts are typically large, with variable size and shape, moderate amounts of cytoplasm, finely dispersed nuclear chromatin, and one to several prominent nucleoli. Wright-Giemsa stain; original magnification, ×100; oil immersion.

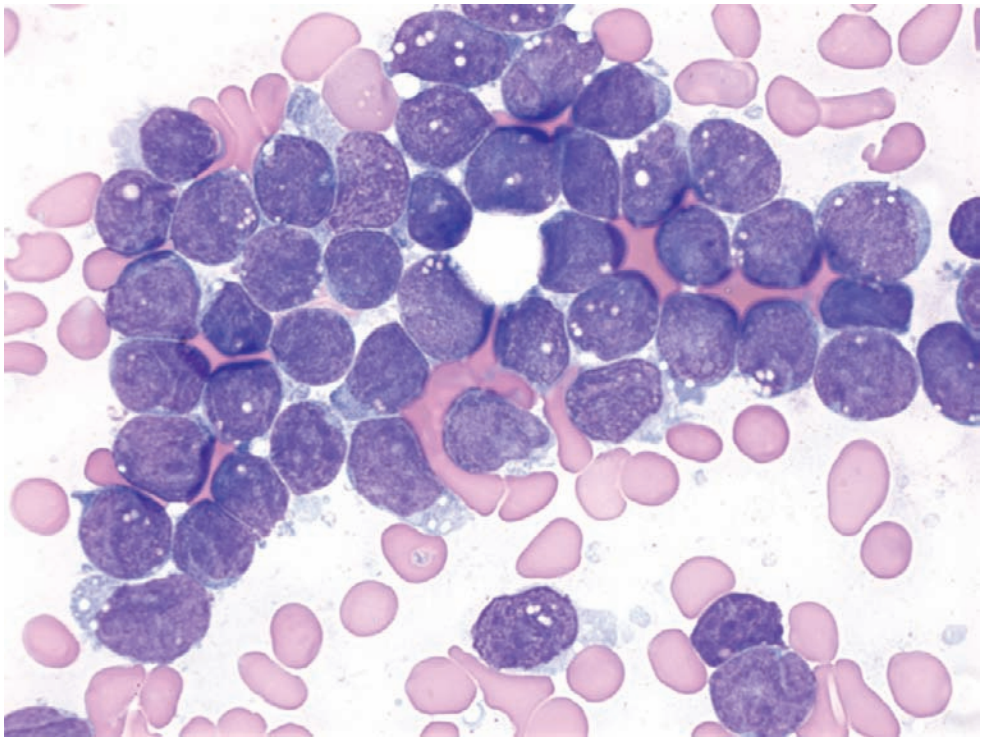

**FIGURE 15-4**

Acute lymphoblastic leukemia (ALL). Blasts with cytoplasmic vacuolization. This case of L1 ALL shows prominent clear vacuoles present in the cytoplasm of the blasts and occasionally overlying the blast nuclei. Vacuolization is not unusual in ALL and should be differentiated from that seen in Burkitt leukemia–lymphoma. Wright-Giemsa stain; original magnification ×60; oil immersion.

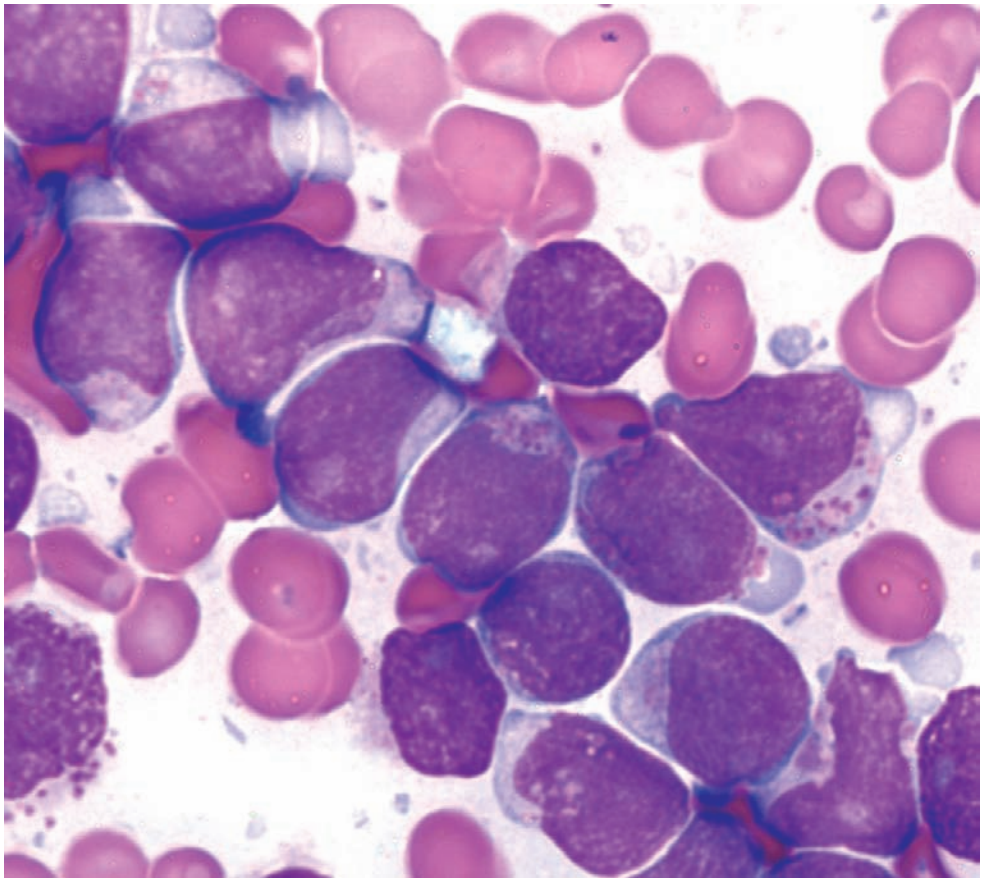

**FIGURE 15-5**

Acute lymphoblastic leukemia (ALL). Blasts with prominent cytoplasmic granules (i.e., granular ALL). In the cases of granular ALL, the blasts contain pale pink to magenta cytoplasmic granules. This morphologic finding correlates closely with a precursor B-cell immunophenotype and has no prognostic implications. Wright-Giemsa stain; original magnification, ×100; oil immersion.

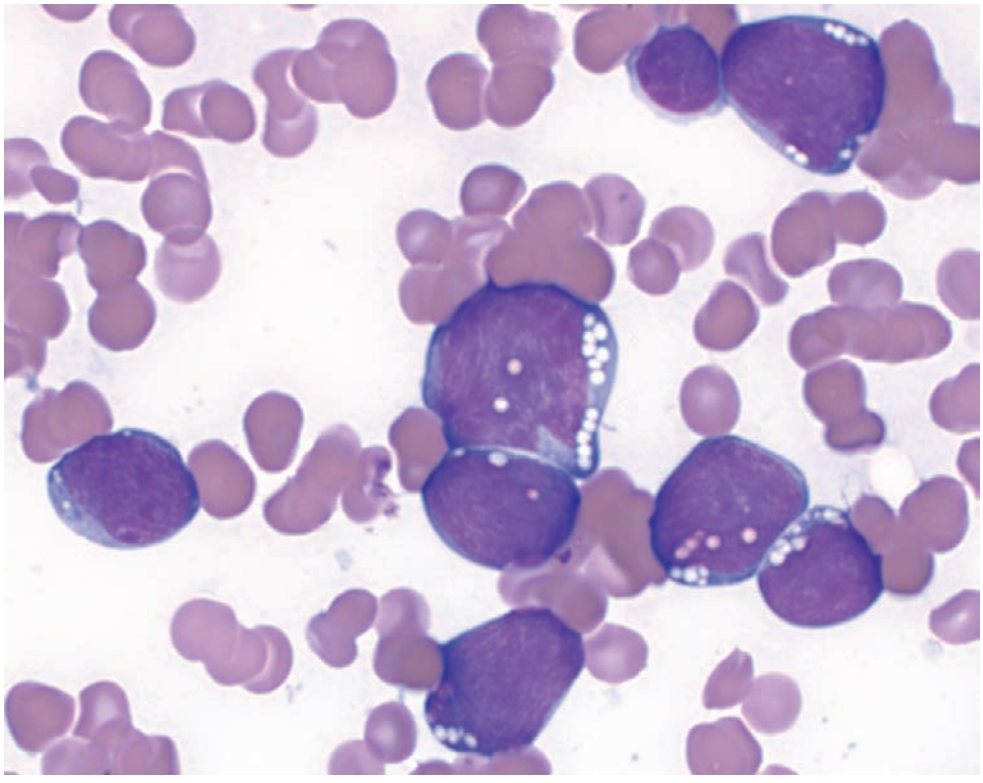

**FIGURE 15-6**

Burkitt lymphoma/leukemia (previously designated as FAB L3 acute lymphoblastic leukemia): cytologic features. The blasts are intermediate to large in size, with finely clumped nuclear chromatin, several small nucleoli, and moderate amounts of deeply basophilic cytoplasm with prominent vacuolization. Wright-Giemsa stain; original magnification, ×60; oil immersion.

**ACUTE LYMPHOBLASTIC LEUKEMIA—PATHOLOGIC FEATURES**

**Gross Findings**

- When involving lymph nodes: homogeneous, pale pink, "fish-flesh" appearance of cut sections

**Microscopic Findings**

- In tissue sections, there is a diffuse growth pattern (sheets of cells); there may be focal "starry-sky" appearance
- On Wright-Giemsa-stained smears:
  - L1 blasts:
    - Cells are small to intermediate in size (~twice the size of normal lymphocytes) and uniform in appearance
    - Nuclei are round or ovoid, sometimes cleaved, with homogeneous, often condensed chromatin
    - Nucleoli are small and inconspicuous or not visible
    - Cytoplasm is scant and lightly basophilic
  - L2 blasts:
    - Cells are heterogeneous in size, including intermediate and large forms
    - Nuclei are round, with finely dispersed chromatin
    - Nucleoli are large and prominent
- 4.5% to 7% of cases may have slightly pink or orange cytoplasmic granules (visible on Wright staining); some cases contain cytoplasmic vacuoles

**Ultrastructural Findings**

- Cytoplasmic organelles are sparse and include small mitochondria, a Golgi region, scattered polyribosomes, and rarely strands of rough endoplasmic reticulum
- In cases with cytoplasmic granules, these are 0.2- to 1.5-μm electron-dense structures clustered near the Golgi region or 1.5- to 2.5-μm membrane-bound cytoplasmic inclusions; some may show ultrastructural peroxidase positivity

**Immunophenotypic Features**

- B-ALL: vast majority positive for CD19, CD22, CD24, CD79a, CD34, TdT, HLA-DR; frequently positive for CD10, CD20 (weak), and myeloid-associated antigens such as CD13 and CD33; negative for surface immunoglobulin and lacking immunoglobulin light chain restriction
- T-ALL: vast majority positive for CD2, cytoplasmic CD3, CD5, CD7; frequently positive for CD1a, surface CD3 (dim), CD4, CD8, CD10, CD21, TdT, CD34, HLA-DR
- Most ALL are $CD45^{+}$; rare cases of B-ALL are $CD45^{-}$

**Pathologic Differential Diagnosis**

- Reactive expansions of benign B-cell precursors (hematogones)
- Normal cortical thymocytes (for mediastinal masses)
- Burkitt lymphoma
- Blastic variant of mantle cell lymphoma
- Peripheral T-cell non-Hodgkin lymphoma
- Acute myeloid leukemia or granulocytic sarcoma

these neoplasms. The modalities involved typically include flow cytometry (requiring fresh cell suspensions) and immunohistochemistry (when fixed tissue is available).

When evaluated by flow cytometry, the immunophenotype of precursor B-cell ALL typically includes the following surface antigens: CD10, CD19, CD20 (may be very weak or absent), CD22, CD24, CD34, CD45 (weaker than normal lymphocytes), and HLA-DR (Figure 15-7). Terminal deoxynucleotidyl transferase (TdT) is an intranuclear enzyme that is an excellent marker of precursor B lymphoid cells (although a minority of acute myeloid leukemias may express TdT) and more than 90% of precursor B-cell ALL express this marker. Cytoplasmic antigens include CD22, CD79a and, in a subset of ALL, μ immunoglobulin heavy chains (Igμ). Some of the cases that have cytoplasmic Igμ expression may also express weak surface Igμ (so-called transitional pre-B ALL). However, ALL typically lacks surface immunoglobulin light chain restriction, and the rare cases that show restriction should be interpreted with great caution. Of note, some subtypes of ALL, in particular those with hyperdiploid karyotypes may lack CD45 expression. Awareness of this fact becomes important when using CD45 alone to rule out a hematopoietic neoplasm in the evaluation of a small blue cell tumor in tissue sections.

Certain genetic subtypes may be associated with specific immunophenotypic patterns. Some common associations include B-ALL with *AF4/MLL* ($CD19^{+}$, $CD10^{-}$, $CD15^{+}$, $CD34^{+}$, $TdT^{+}$), *TEL/AML1* (*ETV6/RUNX1*; $CD19^{+}$, $CD10^{+bright}$, $CD34^{+}$ [variable intensity], $CD45^{+very\ dim}$, $HLA\text{-}DR^{+\ bright}$, $TdT^{+}$, cytoplasmic $Ig\mu^{-}$, frequent expression of myeloid markers such as CD13 and CD33), *BCR/ABL1* ($CD19^{+}$, $CD10^{+}$, $CD34^{+}$, $CD45^{dim+/-}$, $TdT^{+}$, $CD13^{+}$, $CD33^{+}$), and *E2A/PBX1* (TCF3-PBX1; $CD19^{+}$, $CD10^{-/dim+}$, $CD34^{-}$, $CD45^{dim+/-}$, cytoplasmic $Ig\mu^{+}$). These associations are not sufficiently specific, and final genetic subtyping requires confirmation by karyotype or molecular genetic testing.

T-cell ALLs are more heterogeneous in their pattern of T-antigen expression. All T-cell ALLs express CD45 and cytoplasmic CD3, and many cases may show weak surface CD3 expression. Other T-cell antigens that are consistently expressed include CD2, CD5, and CD7. Commonly, T-cell ALL blasts have a cortical thymic immunophenotype that also includes expression of CD1a, CD4, CD8, and CD21 (Figure 15-8). Other antigens that may be expressed in these cases are CD10 and CD56. Notably, as many as 10% to 20% of T-cell ALLs may be negative for CD34, TdT, and HLA-DR. Careful exclusion of other T-cell non-Hodgkin lymphomas may be required in these cases.

In addition to immunophenotype, cell cycle analysis for the DNA content (DNA index) of the leukemic blasts is used for risk stratification in some centers. A DNA index greater than 1.15 correlates with high hyperdiploidy and a good prognosis, whereas a DNA index less than 1.00 indicates hypodiploidy and a poor prognosis.

Finally, in many cases of ALL (B-cell and T-cell) there is lineage-inappropriate expression of one or several myeloid-associated antigens. These most

commonly include CD11b, CD13, CD33, and CD66c. The myeloid antigen expression has no prognostic significance and does not warrant a diagnosis of mixed-phenotype acute leukemia, unless specific criteria required for that entity are met. These criteria will be reviewed in Chapter 16 (mixed phenotype leukemias). Importantly, immunophenotypic aberrancies of ALL have provided a basis for monitoring minimal residual disease using flow cytometry, as part of some treatment protocols.

Immunohistochemical analysis is usually more limited in the number of antigens that can be evaluated. B-cell LBL and ALL are typically positive for CD10, CD34, CD45, CD79a, PAX5, and TdT. As mentioned previously, some of these tumors may be negative for CD45; therefore it is recommended that TdT also be assessed in the differential diagnosis with nonhematopoietic tumors. In addition, because many cases are negative for CD20, CD79a and/or PAX5 staining are mandatory in correctly identifying these tumors as B-cell LBL. The immunohistochemical evaluation of a potential T-cell LBL also includes CD10, CD34, CD45, and TdT (Figure 15-9). Typically, a CD3 stain is sufficient to complete the evaluation because the great majority of T-cell ALLs will express this lineage-specific marker. However, staining for CD1a, CD2, CD4, CD5, CD7, and CD8 may also be useful in assessing these tumors.

### Molecular and Cytogenetic Features

B-cell ALL includes several clinically important subgroups defined by recurrent cytogenetic and molecular abnormalities (Table 15-1). These groups have distinct gene expression profiles and are associated with different response to various chemotherapy agents and with different therapy outcomes. In most therapeutic protocols, the molecular and cytogenetic information is used to assign patients to distinct risk groups that receive chemotherapy regimens of different intensities. In most centers high-risk groups, such as Philadelphia-positive or *MLL* translocation–positive infant ALL, will also require bone marrow transplantation. Some of the translocations associated with a major prognostic effect, such as the *TEL-AML1* fusion, may not be detected by conventional cytogenetics, and additional techniques such as FISH or RT-PCR may be required for their detection (Figures 15-10 and 15-11). It is therefore important to characterize the genetic abnormalities of ALL by more than one technique. The molecular genetics of T-cell ALL are less well understood. It appears that abnormalities of genes such as *TAL1, LYL1,* and *HOX11* may correlate with significant disease subtypes and may have prognostic significance, but additional research is required at this time to confirm these results. Assays for

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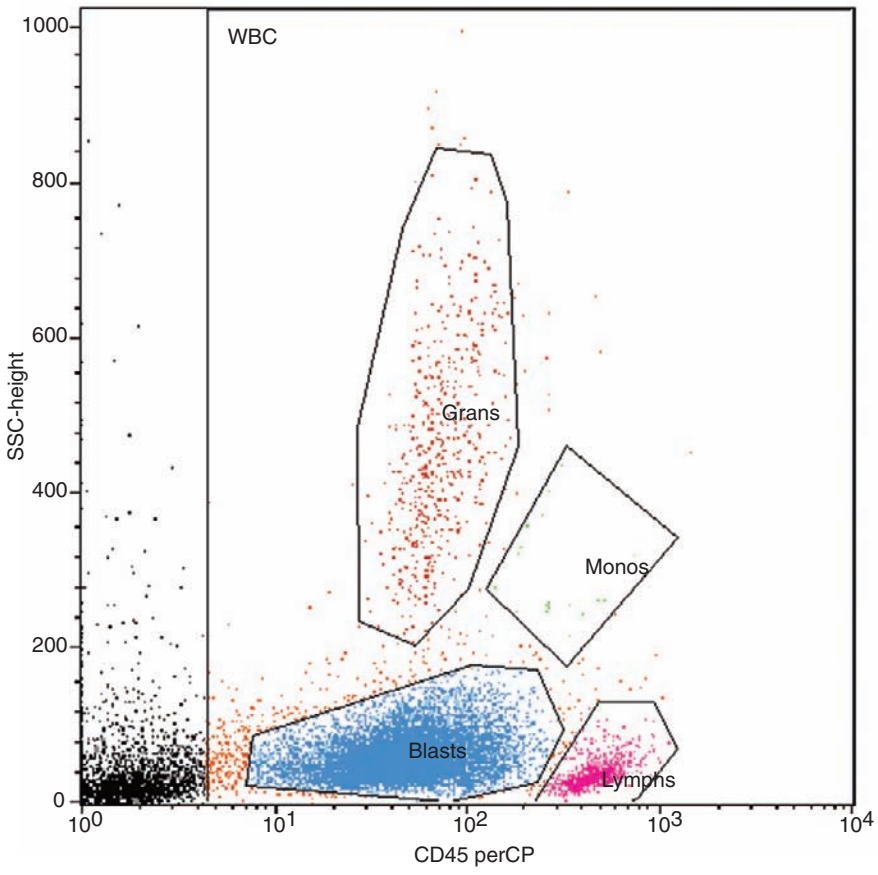
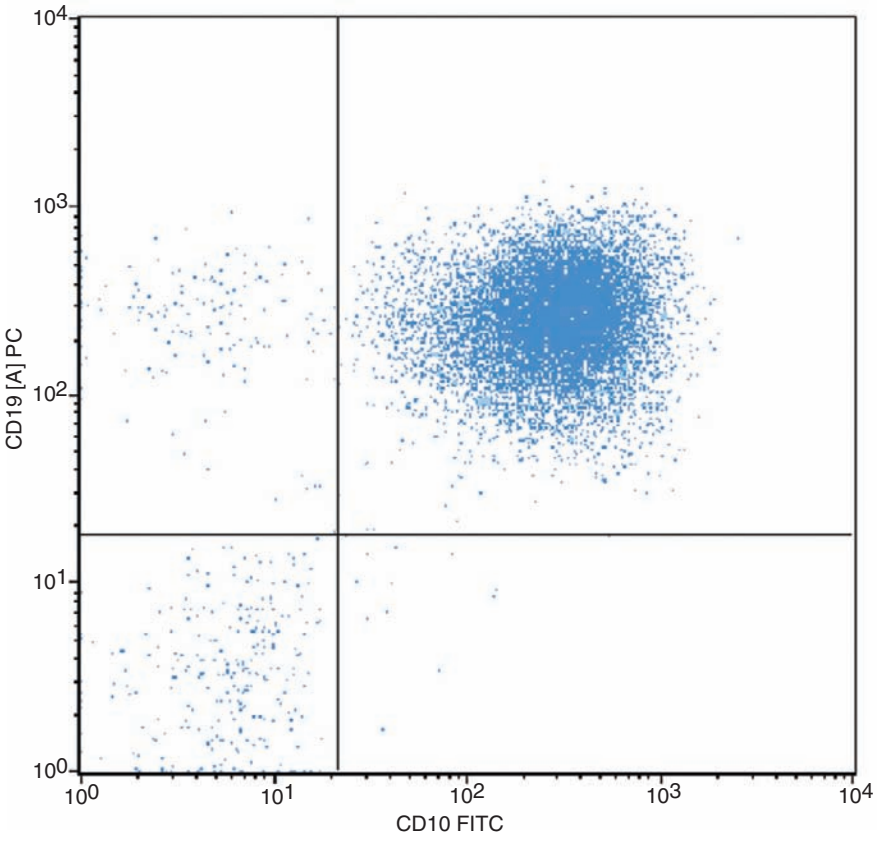



**FIGURE 15-7**

B-lymphoblastic leukemia: typical immunophenotypic findings. The blast population (depicted in blue) has a low side-scatter similar to the normal lymphocyte population (depicted in pink), and CD45 expression that is weaker than that of the lymphocytes. This blast population expresses CD10, CD19, CD34, cytoplasmic CD79a, and TdT. *(Flow cytometry histograms courtesy of Frederick Behm, MD, Memphis, Tenn.)* *Continued*



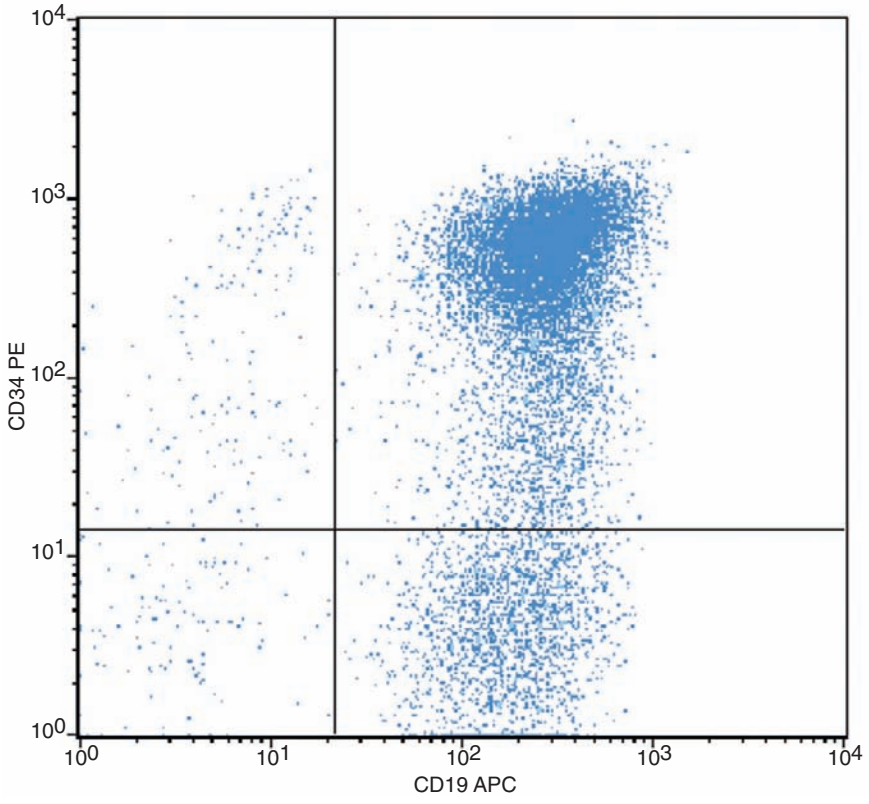




**FIGURE 15-7, cont'd**

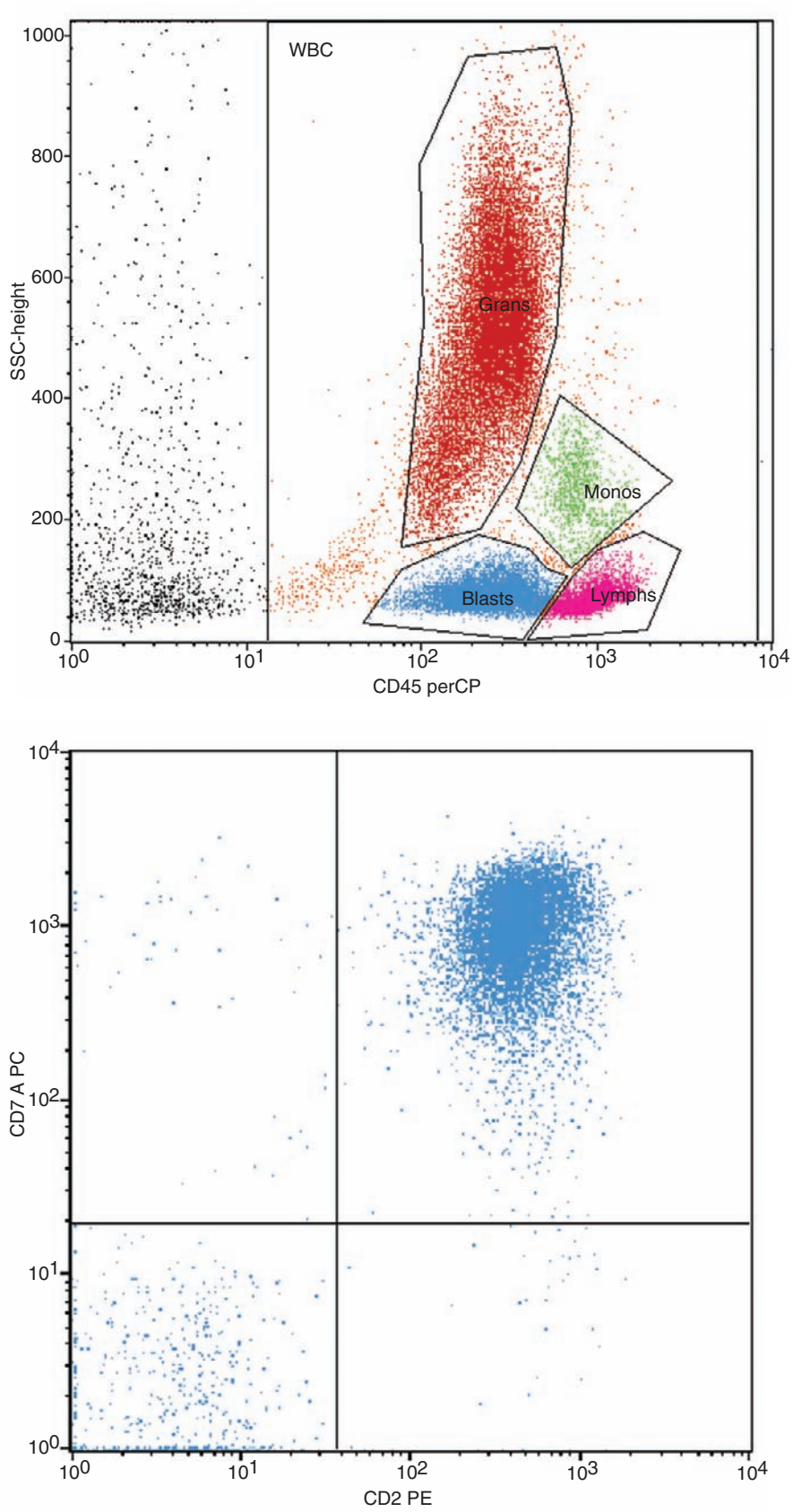



**FIGURE 15-8**

T-lymphoblastic leukemia: typical immunophenotypic findings. The blast population (depicted in blue) has a low side-scatter similar to that of the normal lymphocytes (depicted in pink) and CD45 expression that is weaker than that of the lymphocytes. The blasts express CD1a, CD2, cytoplasmic CD3, CD5, CD7, and TdT. The presence of CD1a expression is generally associated with the cortical thymic subtype of T-lineage ALL and leads to potential difficulties in the differential diagnosis with normal thymocytes in tissue obtained from the anterior mediastinum. *(Flow cytometry histograms courtesy of Frederick Behm, MD, Memphis, Tenn.)*

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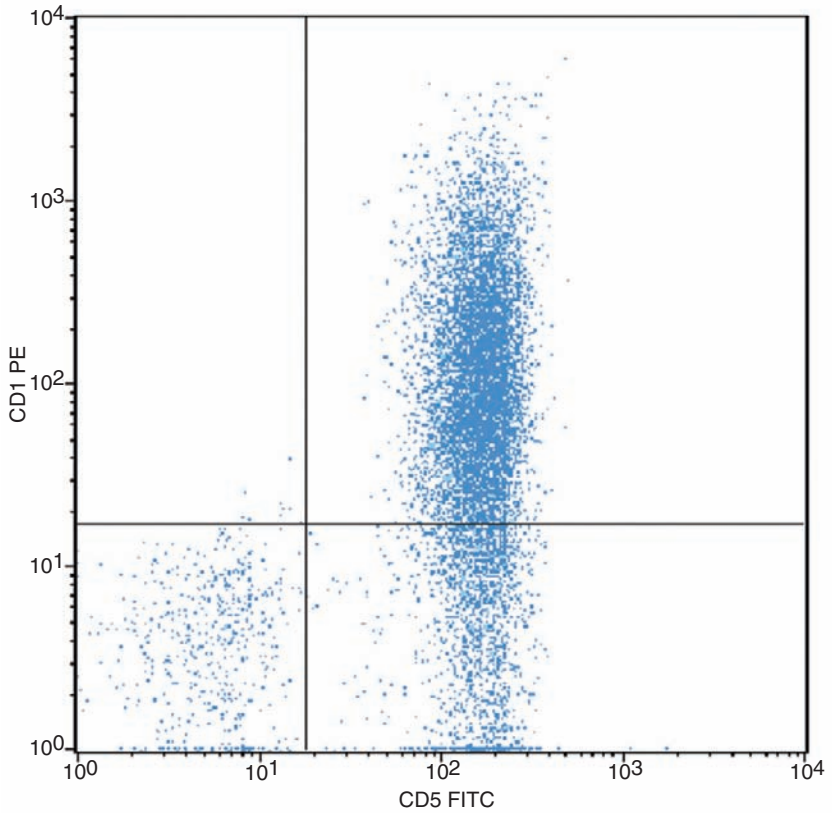


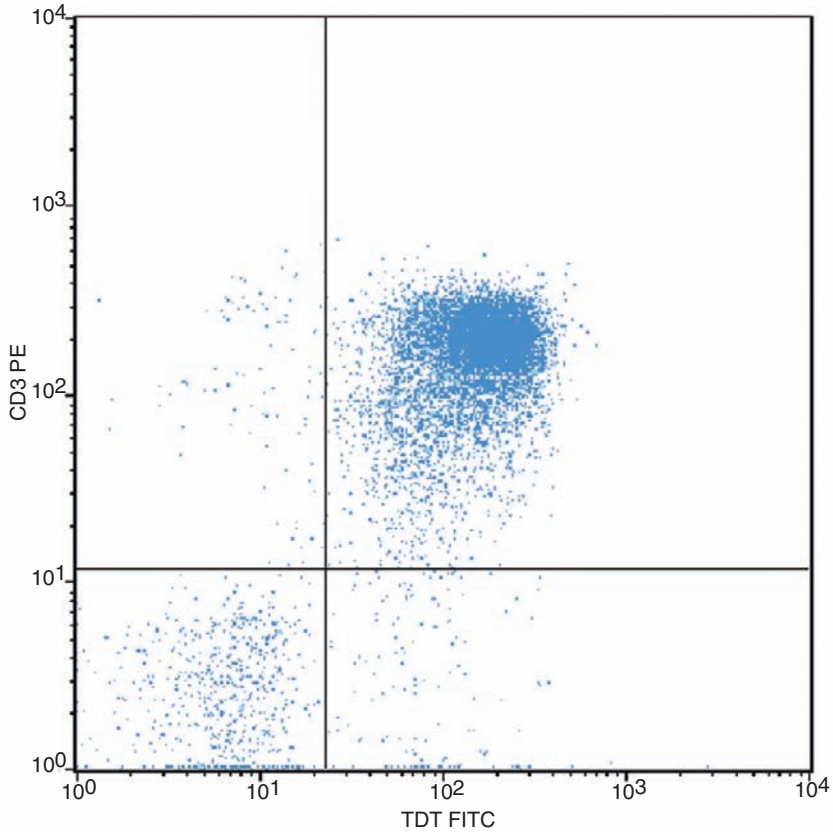


**FIGURE 15-8, cont'd**

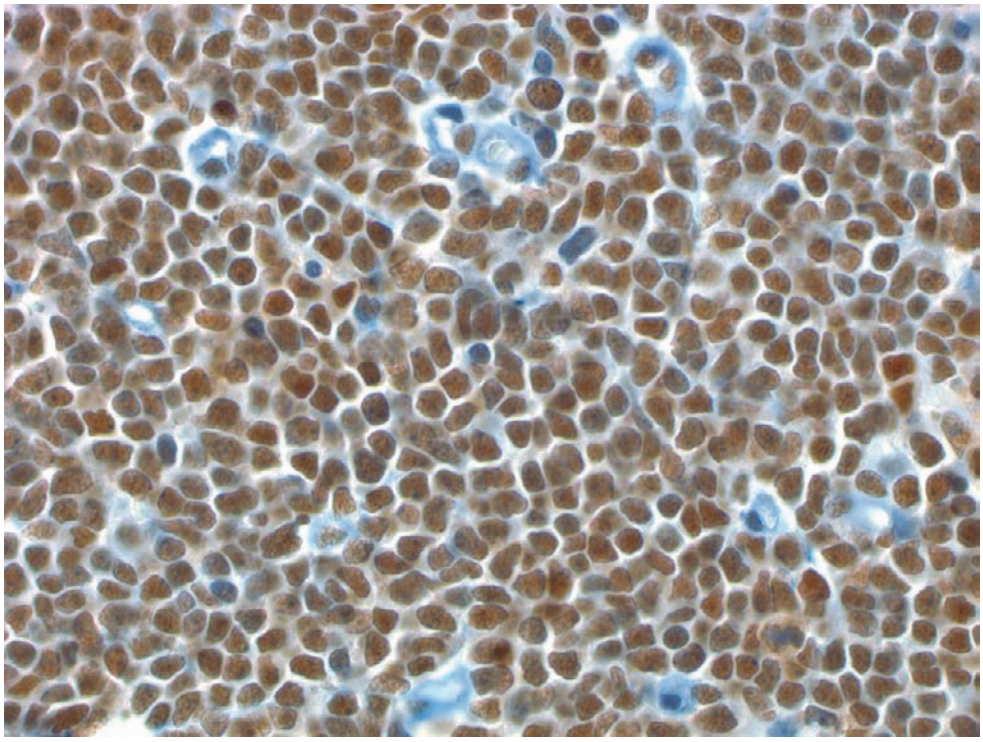

**FIGURE 15-9**

Lymphoblastic lymphoma: immunophenotypic findings. Most of the lymphoblastic neoplasms express the nuclear antigen terminal deoxynucleotidyl transferase, which can be detected using immunohistochemistry in paraffin-embedded tissue sections, as depicted here in a case of T-lymphoblastic lymphoma. Hematoxylin counterstain; original magnification, ×60; oil immersion.

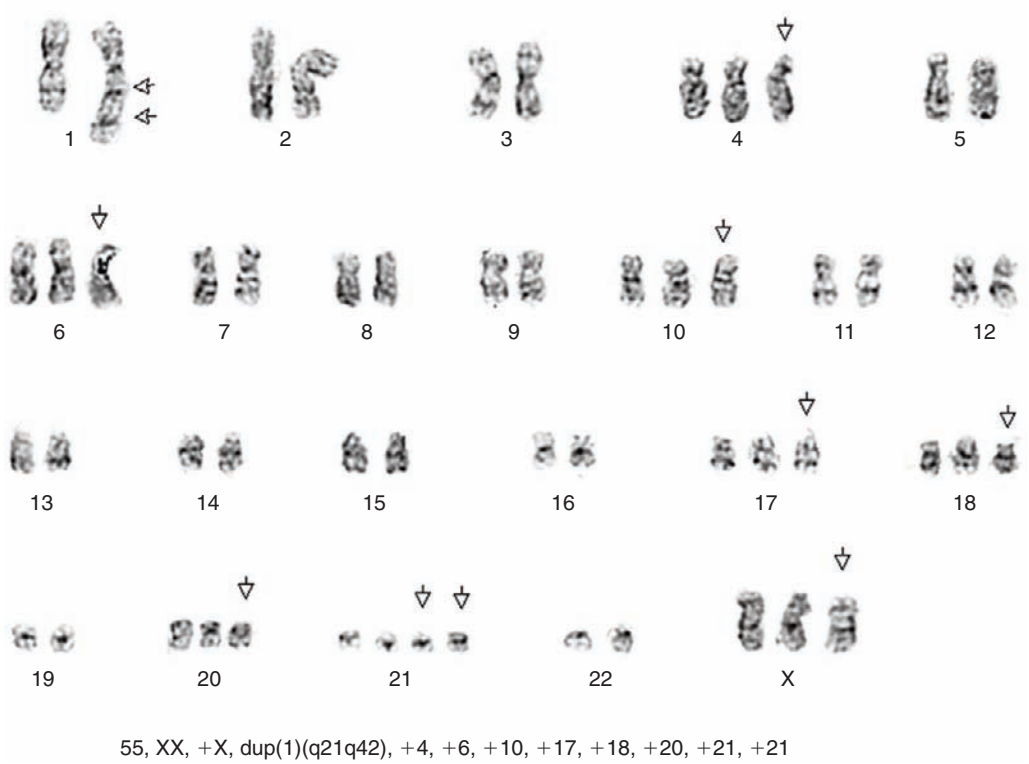


**FIGURE 15-10**

B-lymphoblastic leukemia: conventional cytogenetics. The most common cytogenetic abnormality in pediatric precursor B-cell acute lymphoblastic leukemia is the presence of high hyperdiploid karyotypes (greater than 50 chromosomes), associated with an excellent prognosis. Some of the abnormalities most commonly associated with these cases (and seen in this case with 55 chromosomes) include trisomies of the chromosomes 4, 6, 10, 21, and X. *(Courtesy of Susana Raimondi, PhD, Memphis, Tenn.)*

**TABLE 15-1**

**Molecular and Cytogenetic Subtypes of B-Lymphoblastic Leukemia**

| Chromosomal Abnormality | Fusion Gene | Frequency in Pediatric ALL | Prognostic Significance |
|---|---|---|---|
| Hyperdiploidy >50 (range 51-68) chromosomes | Not known | 25%-30% | Low risk |
| t(12;21)(p12;q22) | *TEL-AML1 (ETV6-RUNX1)* | 20% | Low risk |
| t(1;19)(q23;p13) | *E2A-PBX1 (TCF3-PBX1)* | 5% | Standard risk |
| t(9;22)(q34;q11) (Philadelphia chromosome) | *BCR-ABL1* | 4% | High risk |
| t(v;11q23)<br>t(4;11)(q21;q23) | *MLL gene*<br>*AF4-MLL* | 2% | High risk |
| Hypodiploidy (<46 chromosomes) | Not known | 4%-5% | High risk |
| t(5;14)(q31;q32) | *IL3-IGH* | <1% | Unknown (standard risk) |

*ALL,* Acute lymphoblastic leukemia.

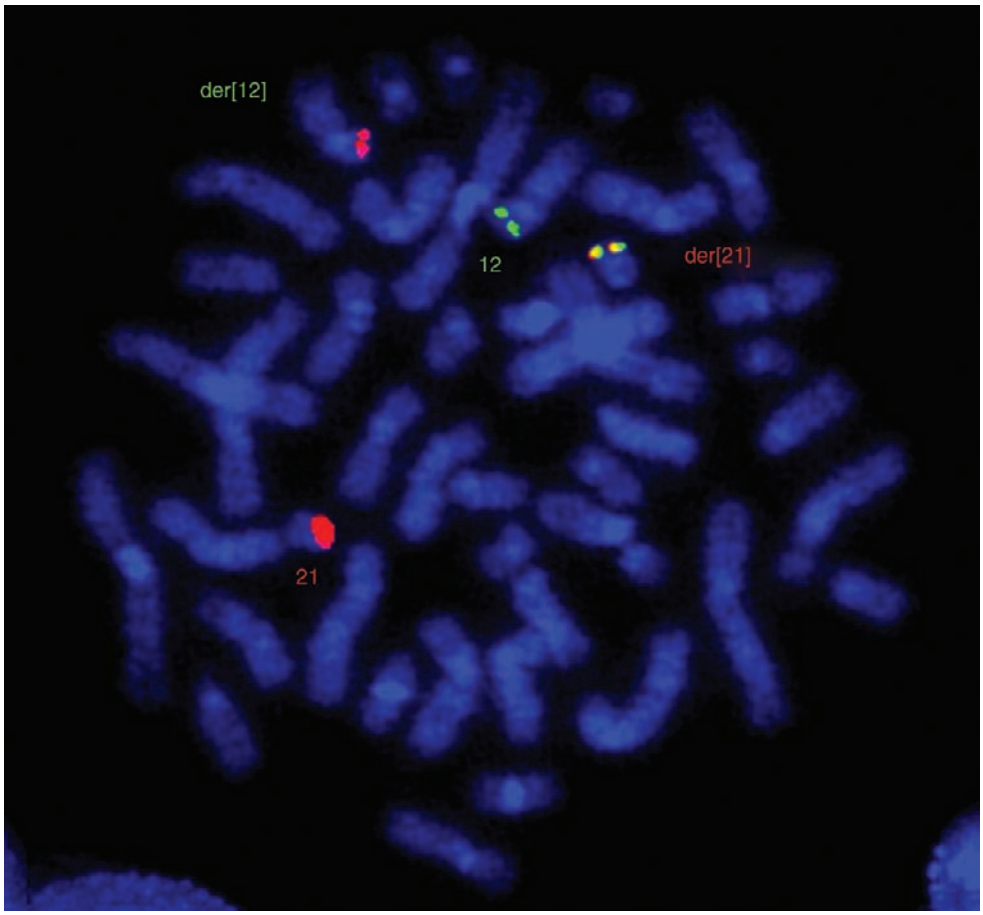


**FIGURE 15-11**

B-lymphoblastic leukemia: fluorescence in situ hybridization (FISH) for the *TEL-AML1(ETV6-RUNX1)* fusion gene associated with the translocation t(12;21)(p13;q22), the second most common genetic abnormality associated with pediatric B-cell ALL, which portends a very good prognosis. This metaphase FISH assay, using a probe for the *TEL* gene (green) and a probe for the *AML1* gene (red) demonstrates a fusion signal (yellow) present on the derivative chromosome 21. One copy of each gene is present on the normal chromosomes 12 and 21. This genetic abnormality is often too small to be detected by conventional cytogenetics and therefore FISH or reverse transcriptase polymerase chain reaction, or both, should be performed to rule it out in cases that appear diploid by the former technique. *(Courtesy of Susana Raimondi, PhD, Memphis, Tenn.)*

immunoglobulin and T-cell receptor gene rearrangements are seldom critical in establishing a diagnosis in ALL-LBL, but have been used successfully for minimal residual disease monitoring in these patients.

## Differential Diagnosis

ALL-LBL must be distinguished from benign (reactive) conditions characterized by the presence of precursor B-cell or precursor T-cell blasts and from a variety of malignant tumors with overlapping morphology. Benign expansions of precursor B-cells (so-called hematogones) may be seen in the bone marrow in a variety of reactive states, including infection, extramedullary tumors, and marrow regeneration following chemotherapy and bone marrow transplantation. Hematogones have a more heterogeneous morphology than the leukemic blasts and on flow cytometry are precursor B cells with a characteristic spectrum of antigen expression, including only partial expression of CD34 and CD20 and a range of CD10 expression. Hematogones lack myeloid antigen expression, clonal immunoglobulin gene rearrangements, and chromosomal abnormalities.

Benign thymocytes present in the thymic cortex have to be differentiated from T-cell ALL-LBL when the biopsy material originates in the anterior mediastinum of a child or young adult. Biopsies obtained from mediastinal tumors (e.g., thymoma, germ cell tumor, Hodgkin lymphoma) often include thymic tissue and therefore may lead to an erroneous diagnosis of T-cell LBL. Cortical thymocytes express TdT, CD34, CD1a, CD4, and CD8 (variable intensity), as well as other T-cell antigens. Similar to hematogones, cortical thymocytes lack immunophenotypic aberrancies and evidence of clonality. Morphologically, preservation of the normal thymic lobular architecture, including the presence of Hassall corpuscles, favors normal thymic tissue.

Burkitt lymphoma/leukemia has a characteristic morphology and a mature B-cell immunophenotype that includes strong CD20 expression and the presence of light chain-restricted surface immunoglobulin, lack of TdT expression, and a proliferation index (Ki-67 positivity) of greater than 95% of the tumor cells. The characteristic chromosomal translocation, t(8;14)(q24;q32), or its variants should be present. The blastic variant of mantle cell lymphoma is seen exclusively in adults and is characterized by a mature B-cell immunophenotype, immunohistochemical expression of cyclin D1, and the t(11;14)(q13;q32) chromosomal translocation.

Acute myeloid leukemias (AML) often show a spectrum of morphologic differentiation in addition to the presence of blasts, and they may show associated dysplasia in the maturing cells. Myeloid blasts may contain Auer rods and show cytochemical activity for myeloperoxidase and Sudan Black B. The differential diagnosis between ALL, myeloperoxidase-negative AML, and mixed phenotype acute leukemia usually requires extensive immunophenotypic characterization by flow cytometry. Tissue infiltrates of AML (myeloid or monoblastic sarcoma) may be positive for chloracetate esterase (Leder stain) and have immunohistochemical expression of myeloperoxidase or lysozyme, or both. Stains for TdT and lymphoid lineage antigens are negative in these blasts. Morphologically, the presence of immature myeloid elements and eosinophils in a blastic infiltrate suggest that it may represent myeloid sarcoma.

The differential diagnosis of T-cell ALL with T-cell non-Hodgkin lymphoma may be challenging, especially in cases of ALL that lack expression of TdT and CD34. Examples of T-cell lymphomas that may involve the bone marrow and peripheral blood in a leukemic fashion include T-cell prolymphocytic leukemia, mycosis fungoides, rare cases of ALK-positive anaplastic large cell lymphoma (small cell variant), and hepatosplenic T-cell lymphoma. For details regarding these lymphomas, the reader is referred to the chapters addressing these entities.

Last, small blue cell tumors such as neuroblastoma, medulloblastoma, or Ewing sarcoma can closely mimic ALL morphologically. This is particularly true on bone marrow aspirate smear or touch imprints when tissue sections are not available, as is common in pediatric marrows. It is important to remember here that some B-lineage ALLs may be negative for CD45 and that most lymphoblastic lymphomas as well as normal cortical thymocytes are strongly positive for CD99 (O13), an antigen often used in the diagnosis of Ewing sarcoma. In such tumors, a TdT stain should always be performed to rule out LBL.

## Prognosis and Therapy

The most important prognostic information provided by the pathologist in patients with ALL-LBL includes lineage (B-cell versus T-cell) and cytogenetic or molecular abnormalities (see previous discussion). Therefore it is crucial that material adequate for the corresponding techniques be obtained at the time of bone marrow or tissue sampling. In addition to these studies, the percentage of bone marrow involvement by blasts and the presence and percentage of blasts in the cerebrospinal fluid at the time of diagnosis may serve in further tailoring of therapy in various treatment protocols. Detection of minimal residual disease by molecular or flow cytometric methods has emerged as a strong prognostic factor in pediatric ALL. Flow cytometry–based methods use the ALL-specific immunophenotypic aberrancies to detect leukemic cells with a sensitivity of 1 in 10,000

cells and to differentiate these cells from normal B-lymphoid precursors. Polymerase chain reaction–based methods use patient- and clone-specific primers for the detection of immunoglobulin and T-cell receptor gene rearrangements, with a sensitivity of 1 in 100,000 cells. Several large-scale studies in pediatric ALL have demonstrated that minimal residual disease measured at specific time points during therapy is an important independent prognostic factor that can be used to adjust risk stratification and, therefore, treatment in these patients.

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16

# Acute Undifferentiated Leukemia and Mixed-Phenotype Acute Leukemias

**Mihaela Onciu, MD**

## INTRODUCTION

Acute leukemias of ambiguous lineage may be divided into acute undifferentiated leukemia (AUL) and mixed-phenotype acute leukemias (MPALs). AULs are leukemias with very primitive phenotypes with little evidence of lineage commitment. MPAL is a heterogeneous category that encompasses rare blastic hematopoietic cell neoplasms that express a mixture of myeloid and lymphoid (B- or T-lineage) antigens. Alternative names that have been used for these leukemias include acute leukemias of ambiguous lineage, hybrid acute leukemias, and acute leukemias of indeterminate lineage. The most recent World Health Organization (WHO) classification (2008) defines several categories of MPALs based on the presence of recurrent cytogenetic lesions and, when these lesions are not present, on the lineage of the leukemic blasts, defined as B-myeloid or T-myeloid. Any of these leukemias can develop as biphenotypic or bilineal (bilineage) acute leukemias. In the biphenotypic leukemias, blasts may be morphologically homogeneous or heterogeneous, but they uniformly express myeloid and lymphoid antigens. In the bilineal acute leukemias, two morphologically and immunophenotypically distinct blast populations (lymphoid and myeloid) can be discerned. The latter category also incorporates acute leukemias that switch lineage during chemotherapy.

## ACUTE UNDIFFERENTIATED LEUKEMIAS

### Clinical Features

AULs are diagnosed with decreasing frequency due to more stringent criteria and likely represent less than 1% of acute leukemias. Patients present with signs and symptoms of bone marrow failure, as with other acute leukemias. These include easy bruising, infection, and fatigue due to cytopenias such as thrombocytopenia, leukopenia, and anemia. Leukocytosis with blasts is also commonly seen.

### Pathologic Features

#### Microscopic Features

Blasts are the dominant cell population in bone marrow and are usually intermediate in size with round nuclei and fine chromatin containing visible nucleoli. Blasts may also be smaller, resembling morphologically lymphoblasts. Cytoplasm is scant without azurophilic granules or Auer rods. By definition they are negative for Sudan Black B (SBB) and myeloperoxidase (MPO) by cytochemistry. Trephine biopsy generally shows replacement of the marrow cellularity by sheets of blasts with little to no residual hematopoietic elements.

### Ancillary Studies

#### Immunophenotypic Findings

Flow cytometry shows only immature, primitive hematopoietic cells without lineage commitment, as evidenced in many cases by expression of CD7 and TdT and blast markers such as CD34. MPO is absent by definition. Expression of single non-specific lineage associated markers such as CD13, CD33, CD15, CD64, CD2, CD5, or CD10 can be seen, but combination of multiple markers in the same lineage, such as both CD13 and CD33, should not be present.

#### Molecular and Cytogenetic Findings

Little is known regarding the genetic findings in AUL. No specific genetic abnormalities have been associated with these leukemias. Clonal karyotypic abnormalities appear to be detected in most cases, but changes are not specific.

### Differential Diagnosis

The main differential diagnosis is with minimally differentiated acute myeloid leukemia. Both lack SBB and MPO. However, minimally differentiated acute myeloid leukemia will express a combination of myeloid-associated markers such as CD13, CD33, and CD117. Acute erythroid or megakaryoblastic leukemias may appear as AUL on initial work-up until blasts are studied for erythroid markers (glycophorin, hemoglobin A) or megakaryoblastic markers such as CD41 and CD61.

### Prognosis and Therapy

Optimal treatment for AUL is not yet known, but most patients are treated with AML-type therapy. Whether myeloablative therapy and stem cell transplantation is beneficial is also unknown. Reports of outcome are few, but some complete and durable remissions have been reported. Additional studies addressing modern therapies are needed but are difficult to undertake due to the rarity of these leukemias.

## MIXED-PHENOTYPE ACUTE LEUKEMIAS

### Clinical Features

MPALs represent 3% to 5% of all cases of acute leukemia (patients of all ages), and 8% of all cases of acute leukemia occurring in adults. These leukemias can occur at any age but are slightly more common in adults. In the largest study available, the median age for de novo acute biphenotypic leukemias is 25.5 years. Rare cases of MPAL secondary to chemotherapy for other neoplasms have also been reported.

The clinical presentation is similar to that of other acute leukemias, including symptoms secondary to anemia, thrombocytopenia, and neutropenia, such as fatigue, abnormal bleeding, and infections. Infants with acute bilineal leukemia may exhibit multiple skin lesions similar to those seen in the patients with acute monoblastic leukemia (so-called blueberry-muffin baby).

### Pathologic Features

#### Microscopic Findings

On Wright-Giemsa–stained smears, the MPALs overlap significantly with other acute leukemias. The acute biphenotypic leukemias and the undifferentiated acute leukemias may consist of morphologically homogeneous or heterogeneous blasts, with a spectrum that includes lymphoid-appearing cells and myeloid-appearing cells (Figure 16-1). The former cells are small, with scant cytoplasm and condensed nuclear chromatin, often with absent or inconspicuous nucleoli. The latter cells are typically larger, with more abundant cytoplasm, finely dispersed nuclear chromatin, and prominent nucleoli. Some of the cases of acute biphenotypic leukemia may show prominent morphologic differentiation toward myeloid lineage, with cytochemical myeloperoxidase positivity and even Auer rods. These cases may be classified as acute myeloid leukemia (AML) M1, M2, and, more rarely, M4 or M5 in the French-American-British classification. In the bilineal acute leukemias, two morphologically distinct populations of blasts are usually seen. These populations typically include, in variable proportions, small lymphoid blasts and large monoblasts, the latter similar to the neoplastic cells seen in acute monoblastic leukemia (Figure 16-2).

In hematoxylin and eosin–stained sections of bone marrow or other involved tissues, these leukemias consist of relatively monotonous blasts of intermediate

**MIXED PHENOTYPE ACUTE LEUKEMIA—FACT SHEET**

**Definition**
- Malignant blastic hematopoietic neoplasms that show differentiation along more than one lineage (lymphoid and myeloid)

**Incidence**
- Rare (3% to 5% of all acute leukemias); exact incidence unknown

**Morbidity and Mortality**
- Highly aggressive neoplasms
- Reported median survival 6 to 7 months in adult patients
- Probability of survival at 2 years: 39% in adults, 75% in children

**Gender, Race, and Age Distribution**
- Slight male predominance (male:female = 1.5:1)
- May occur at any age, but more frequent in adults
- Median patient age, 25 years

**Clinical Features**
- Symptoms secondary to anemia, thrombocytopenia, and neutropenia (fatigue, abnormal bleeding, infections)
- Multiple skin lesions in some infants with bilineal leukemia

**Prognosis and Therapy**
- Poor prognosis
- Intensive multiagent chemotherapy required, often as a combination of lymphoid and myeloid regimens

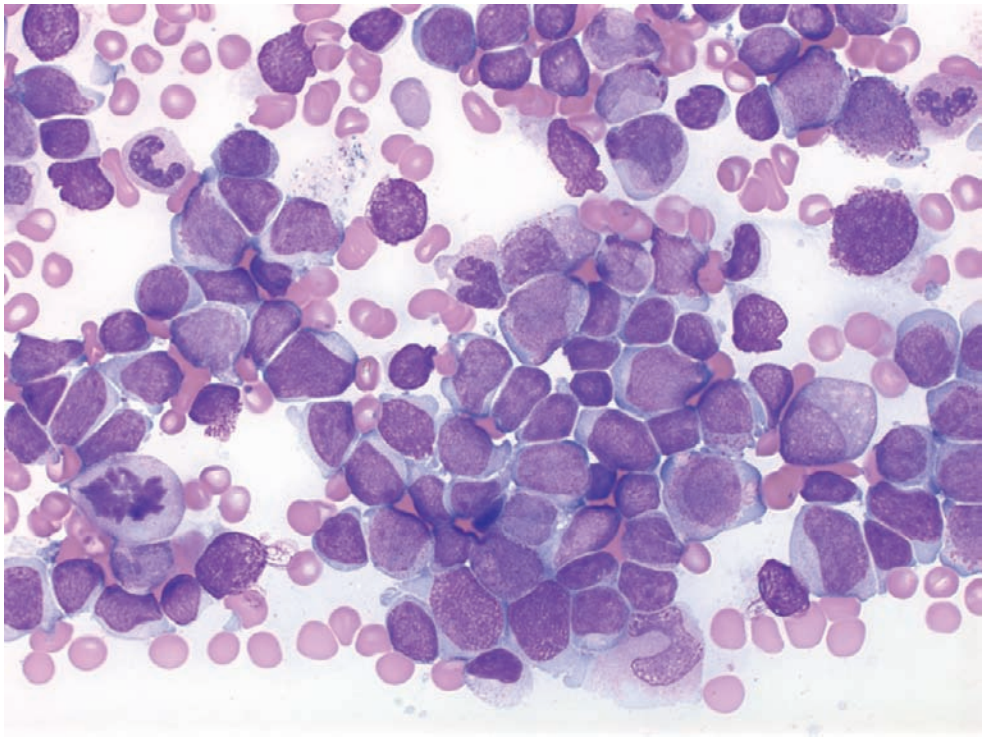

**FIGURE 16-1**

Mixed phenotype acute leukemia (biphenotypic type). Cytologic features on smear preparations. This leukemic process is composed of morphologically heterogeneous blasts, with a predominance of large cells with abundant cytoplasm and prominent nucleoli. These leukemic cells coexpressed T-lineage and myeloid antigens and contained the Philadelphia chromosome. Wright-Giemsa stain; original magnification, ×60; oil immersion.

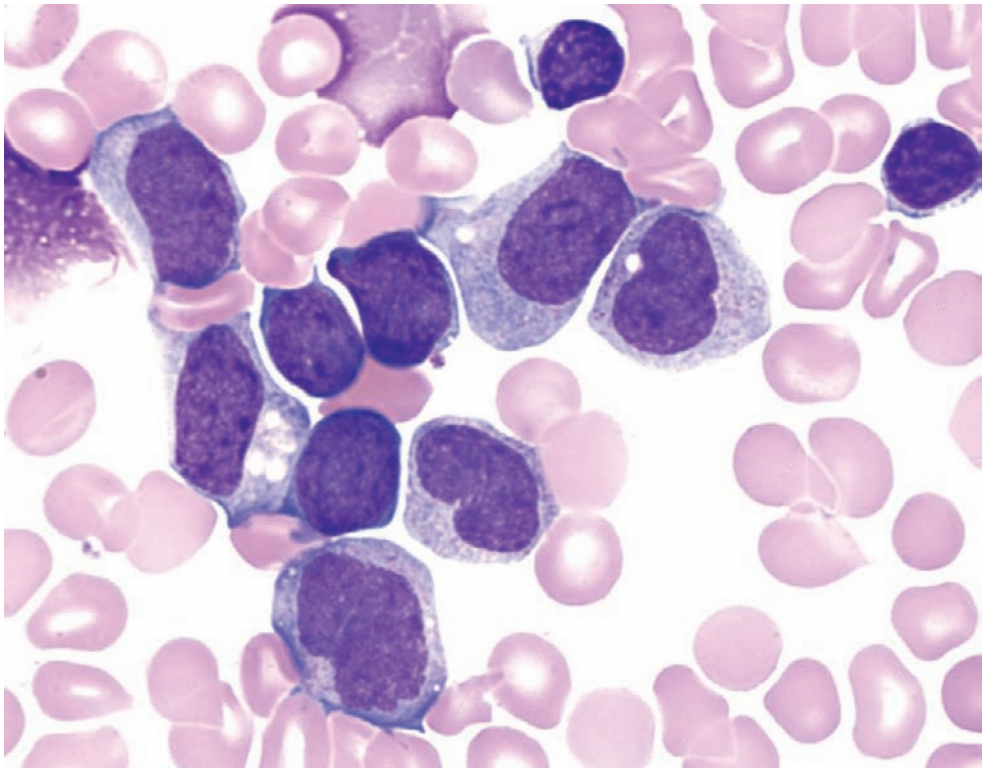

**FIGURE 16-2**

Mixed phenotype acute leukemia (bilineal type). Cytologic features on smear preparations. This leukemia consists of two morphologically distinct blast populations: lymphoid blasts (small, with very scanty cytoplasm and condensed nuclear chromatin) and monoblasts (large, with abundant granular cytoplasm, finer chromatin, and prominent nucleoli). Wright-Giemsa stain; original magnification, ×60; oil immersion.

size, with scant cytoplasm, vesicular chromatin, and often prominent nucleoli. Notably, in acute bilineal leukemia, only one of the two blast populations may be seen at a given anatomic site. For instance, leukemias with lymphoblastic and monoblastic differentiation may show only monoblastic infiltrates in the skin, whereas both blast populations are identified at other locations.

## Ancillary Studies

### Immunophenotypic Findings

Flow cytometric analysis is essential in the diagnosis of acute biphenotypic leukemias (Figure 16-3), which were initially defined based on their immunophenotypic

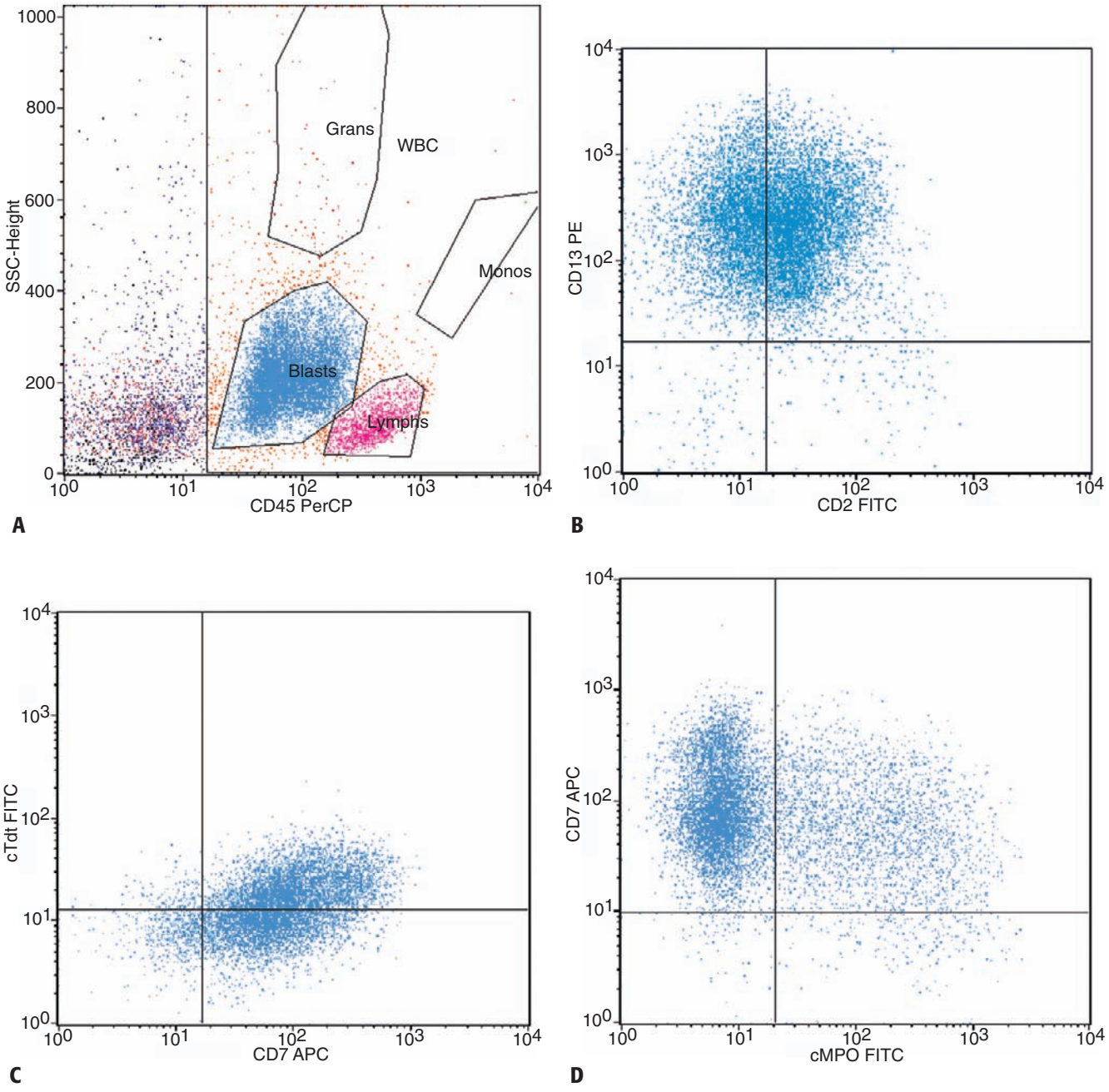


**FIGURE 16-3**

Mixed phenotype acute leukemia, T-myeloid. Immunophenotypic features. **A,** The blast population *(blue)* has a low side scatter (SSC) that is higher than that of the adjacent normal lymphocyte (lymphs) population *(pink),* and a level of CD45 expression that is lower than that of the latter cells, but similar to that of the granulocytes (grans). **B-D,** The blasts coexpress T-lymphoid–lineage antigens (CD2, CD7, and terminal deoxynucleotidyl transferase) and myeloid antigens (CD33 and myeloperoxidase [MPO]). This case was diagnosed prior to the 2008 WHO classification, and according to those criteria, surface or cytoplasmic CD3 would also need to be demonstrated. APC, Percp, FITC, and PE, fluorescent dyes used for flow cytometry. *Monos,* Monocyte gate; *WBC,* white blood cells. *(Flow cytometry histograms courtesy of Frederick Behm, MD, Memphis, Tenn.)*

**MIXED PHENOTYPE ACUTE LEUKEMIA—PATHOLOGIC FEATURES**

**Microscopic Findings**

- Blasts may be uniform or heterogeneous in appearance or typically dimorphic (lymphoid, myeloid, monoblastic)
- Lymphoid blasts are small, with scant cytoplasm, condensed chromatin, and inconspicuous nucleoli
- Myeloid blasts are larger with more abundant basophilic cytoplasm, finely dispersed chromatin, and prominent nucleoli
- Some cases may show blasts with Auer rods and myeloperoxidase positivity
- A maturing cell component is usually missing
- In bone marrow biopsy material, the marrow cellularity is increased and consists predominantly of blasts

**Immunophenotypic Findings**

- Biphenotypic leukemia: a single population of blasts is present that uniformly expresses a mixture of myeloid and lymphoid (B- or T-lineage antigens); diagnosis requires a weighted scoring system (e.g., European Group for the Immunological Classification of Leukemias scoring or requirements of WHO Classification)
- Bilineal (bilineage) leukemias: two morphologically and immunophenotypically distinct lymphoid and myeloid blast populations, usually precursor B-cell lymphoblasts and monoblasts
- Undifferentiated leukemias: poorly differentiated blasts that may express only HLA-DR, CD34, CD38, CD7, and TdT

**Differential Diagnosis**

- Acute lymphoblastic leukemia expressing myeloid antigens
- Acute myeloid leukemia expressing lymphoid antigens

**TABLE 16-1**

**Criteria Required by the Most Recent World Health Organization Classification for Assigning More Than One Lineage to a Leukemic Process**

| |
|---|
| ***Myeloid Lineage*** |
| Myeloperoxidase expression* |
| Monocytic antigen expression (at least two of the following: nonspecific esterase, CD11c, CD14, CD64, lysozyme) |
| ***T Lineage*** |
| Cytoplasmic CD3† or surface CD3 |
| ***B Lineage*** |
| Strong expression of CD19 and strong expression of at least one of the following: CD79a, cytoplasmic CD22, CD10 |
| Weak expression of CD19 and strong expression of at least two of the following CD79a, cytoplasmic CD22, CD10 |

*Myeloperoxidase may be detected by cytochemical or immunologic methods. Cytochemically positive myeloperoxidase should be present in at least 3% of the morphologically identified leukemic blasts. Immunophenotypically positive myeloperoxidase should be seen on a blast population that shows other immunophenotypic aberrancies (in order to exclude residual benign myeloblasts).
†Cytoplasmic CD3 expression should be detected by flow cytometry with antibodies to the CD3ε chain (immunohistochemistry using polyclonal anti-CD3 antibodies may detect CD3 zeta chain, which is not specific for T-lineage).

profile. Careful corroboration of all the antigens expressed is necessary in order to rule out acute lymphoblastic leukemia or AML showing aberrant lineage-inappropriate antigen expression (so-called lineage infidelity). For example, many of the precursor B-cell acute lymphoblastic leukemias may express the myeloid-associated antigens CD13 and CD33. In addition, certain subtypes of AML consistently express lymphoid-associated antigens (e.g., CD19 in AML with AML1-ETO and CD2 in M4Eo AML). In an attempt to better define these neoplasms, several classification systems have been devised. The one most widely used is the scoring system proposed and later revised by the European Group for the Immunological Classification of Leukemia. The newest WHO classification (2008) has abandoned this scoring system and has proposed a new set of more stringent requirements for assigning more than one lineage to a leukemic process (Table 16-1). In acute bilineal leukemia, flow cytometric analysis usually shows two distinct or only partially overlapping populations of blasts with respect to CD45 expression and side scatter (Figure 16-4). On further analysis, each of these populations of blasts has a distinct immunophenotypic profile, similar to that of acute lymphoblastic leukemia and of AML (often acute monoblastic leukemia), respectively. In such cases, one should ensure that the B-lymphoblast population shows immunophenotypic aberrancies compatible with a neoplastic cell population, in order to avoid overcalling benign precursor B-cell (hematogone) expansions associated with an otherwise typical AML.

It should be noted that the criteria in Table 16-1 are to be strictly applied only when describing the immunophenotype of a MPAL, and not for assigning lineage in otherwise typical ALL or AML, or in the differential diagnosis of acute undifferentiated leukemia and minimally differentiated AML (AML M0).

### Molecular and Cytogenetic Findings

The most common recurring genetic abnormality associated with biphenotypic leukemia is the t(9;22)(q34;q32) translocation resulting in the *BCR-ABL1* fusion transcript (the Philadelphia chromosome), which is present in approximately one third of cases. MPALs bearing this genetic lesion should be designated as MPAL with t(9;22)(q34;q11.2);*BCR-ABL1*. Most of these cases are of B-myeloid lineage. A much smaller number of cases have 11q23/*MLL* (mixed-lineage leukemia) gene abnormalities as part of the t(4;11)(q21;q23), t(9;11)(p13;q23), or t(11;19)(q23;p13.3) chromosomal translocations or as 11q23 deletions (Figures 16-5 and 16-6). MPALs containing such genetic lesions should be

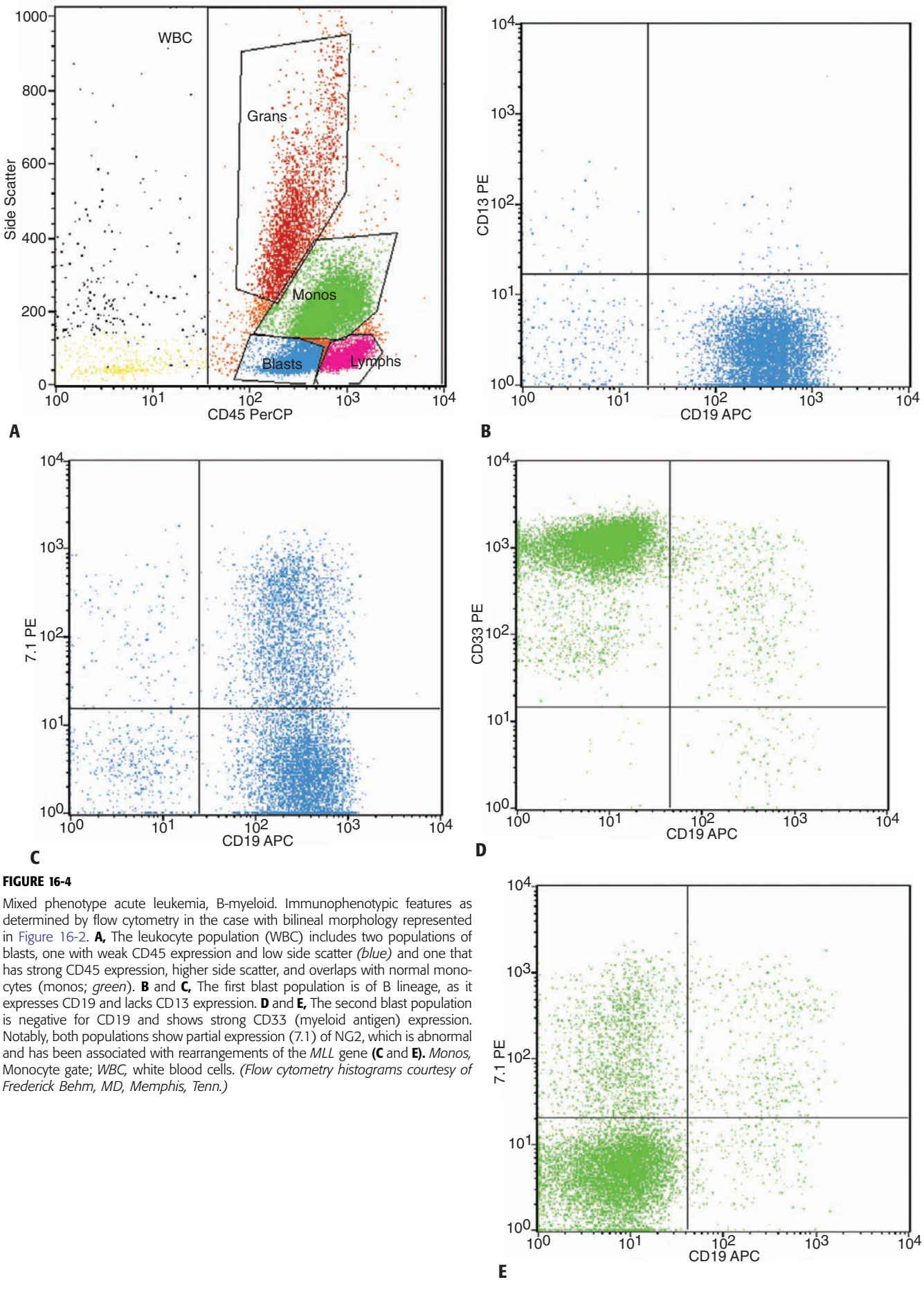


**FIGURE 16-4**

Mixed phenotype acute leukemia, B-myeloid. Immunophenotypic features as determined by flow cytometry in the case with bilineal morphology represented in Figure 16-2. **A,** The leukocyte population (WBC) includes two populations of blasts, one with weak CD45 expression and low side scatter *(blue)* and one that has strong CD45 expression, higher side scatter, and overlaps with normal monocytes (monos; *green*). **B** and **C,** The first blast population is of B lineage, as it expresses CD19 and lacks CD13 expression. **D** and **E,** The second blast population is negative for CD19 and shows strong CD33 (myeloid antigen) expression. Notably, both populations show partial expression (7.1) of NG2, which is abnormal and has been associated with rearrangements of the *MLL* gene **(C** and **E).** *Monos,* Monocyte gate; *WBC,* white blood cells. *(Flow cytometry histograms courtesy of Frederick Behm, MD, Memphis, Tenn.)*

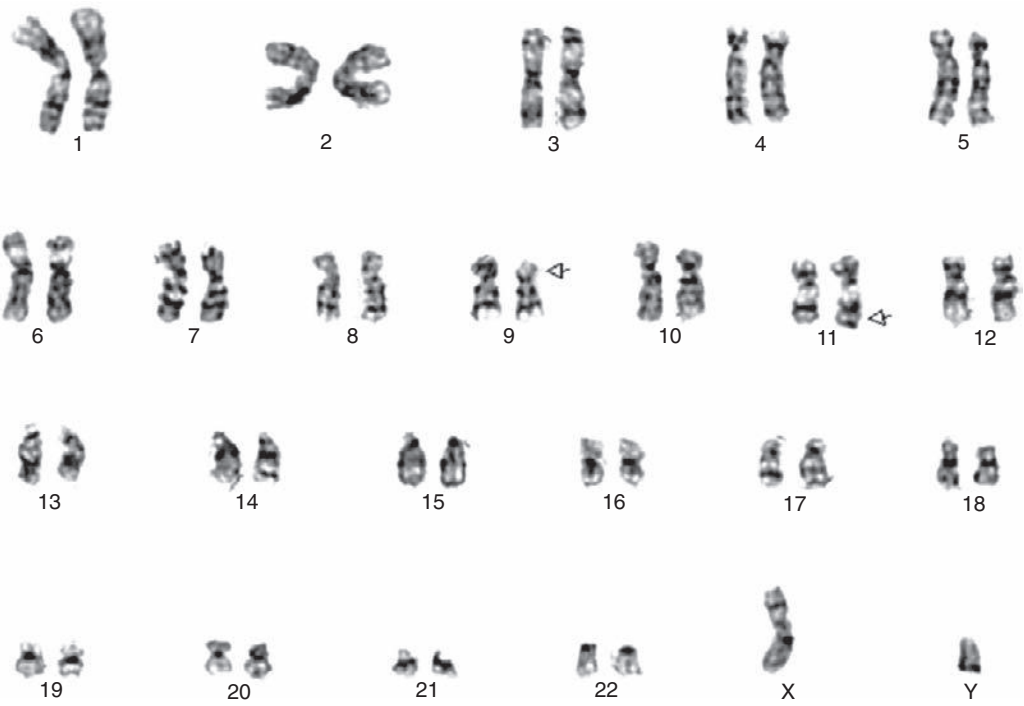


**FIGURE 16-5**

Cytogenetics of mixed phenotype acute leukemia with T(V;11q23); *MLL* rearranged. Conventional cytogenetics in the case of bilineal leukemia also presented in Figures 16-2 and 16-4. There is a balanced translocation T(9;11)(P22;Q23) that results in the *MLL-AF9* fusion product *(arrows)*. Rearrangements and deletions of 11q23 (*MLL* gene) are one of the most common genetic abnormality encountered in mixed phenotype acute leukemias. *(Courtesy of Susana Raimondi, PhD, Memphis, Tenn.)*

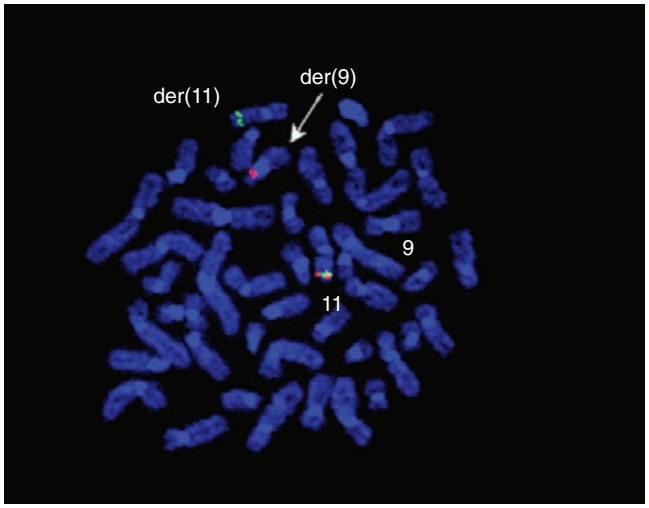


**FIGURE 16-6**

Genetic abnormalities in mixed phenotype acute leukemia with T(V;11q23); *MLL* rearranged. Fluorescent in situ hybridization in the case of mixed phenotype leukemia also depicted in Figures 16-2, 16-4, and 16-5. This assay was performed using a break-apart probe (split-signal fluorescent in situ hybridization) for the *MLL* gene, with green labeling the 5′ end and red labeling the 3′ end of the gene. In this case a fusion signal *(yellow),* here seen on the normal chromosome 11, corresponds to an intact gene, whereas the red signal present on the derivative chromosome 9 demonstrates that the 3′ portion of the *MLL* gene has been translocated onto the short arm of that chromosome, with the 5′ end of the gene *(red)* remaining on the long arm of the derivative chromosome 11. *(Courtesy of Susana Raimondi, PhD, Memphis, Tenn.)*

diagnosed as MPAL with t(v;11q23); *MLL* rearranged. These cases also have predominantly a B-myeloid immunophenotype. In bilineal cases, the B lymphoblasts show an immunophenotypic profile similar to ALL with *MLL* gene rearrangements ($CD19^+$, $CD10^-$, $CD15^+$, $CD65^+$, $TdT^+$). Other less frequent abnormalities include trisomy 4, deletions of chromosomes 5 and 7, trisomy 8, and rare translocations such as t(9;12), t(7;12), and t(2;7) in which the involved gene partners are unknown. Many of the MPAL have rearrangements or deletions of the immunoglobulin heavy chain gene or T-cell receptor genes.

## Differential Diagnosis

The main entities in the differential diagnosis of MPALs are acute lymphoblastic leukemia and AML, especially in cases in which the latter two show lineage-inappropriate antigen expression. Although in some cases the exact distinction between these types of leukemias remains controversial, the use of the recommended stringent diagnostic criteria should allow the accurate diagnosis of MPAL according to the most recent classifications.

## Prognosis and Therapy

MPALs are aggressive leukemias that have a poor prognosis, even in the setting of intensive multiagent chemotherapy. The chemotherapy involved often includes a combination of lymphoid and myeloid regimens. In most series, the median survival of adult patients with acute biphenotypic leukemia is 6 to 7 months, with a probability of survival at 2 years of 39% in adults and 75% in children. Poor prognostic factors include patient age older than 60 years, low patient performance status, and, in the pediatric age group, the presence of prominent myeloid differentiation (i.e., expression of myeloperoxidase, presence of Auer rods) in the leukemic process.

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The complete reference list is available online at www.expertconsult.com.

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17

# Myeloproliferative and "Overlap" Myelodysplastic/Myeloproliferative Neoplasms

**John Anastasi, MD**

## INTRODUCTION

The myeloproliferative neoplasms (MPNs), formerly referred to as the *myeloproliferative disorders,* are a group of clonal multipotential hematopoietic stem cell diseases that have a proliferative nature, frequently with hypercellular bone marrows, and an elevation of one or more cell types in the blood. These neoplasms are all insidious in onset and chronic in course, but have variable tendency to terminate in marrow failure or acute leukemia. The MPNs include the model disease, chronic myelogenous leukemia (CML). CML has become a prototype in medicine because it illustrates how the elucidation of pathways involved in the molecular pathogenesis of a process (specifically in this case the dysregulation of *ABL1* tyrosine kinase signaling) can lead to the rational development of targeted therapy for the disease (i.e., imatinib and other tyrosine kinase [TK] inhibitors).

The MPNs also include the rare myeloid neoplasms with eosinophilia. Although uncommon, these are noteworthy because, like CML, they have also been found to be due to dysregulation of TK signaling and, at least in part, can also be successfully treated with TK inhibition. The other MPNs include the more common non-CML entities: essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). These entities are related biologically, with multipotential hematopoietic stem cell origin, clonal proliferation, and chronic nature. Although they seem to be distinctive, as there is little transformation from one to another, early in their course they can present a diagnostic challenge and can be difficult to distinguish from one another because more characteristic features have not yet developed. Recent discoveries in the underlying molecular pathology of these entities have demonstrated that, like CML and the eosinophilic disorders, they too share TK signaling dysregulation, at least to some degree. This dysregulation is due to a mutation in the TK gene *JAK2,* which is present in approximately 50% to 95% of cases. This association has led to significant revisions in the criteria for diagnosis of these entities, but unfortunately has not yet led to a successful therapeutic approach in their treatment.

Last is chronic neutrophilic leukemia (CNL). Although it is surrounded by some controversy as to whether it is truly neoplastic, CNL is a rare entity that is included in the spectrum of this disease group. It is a poorly understood disease entity that has not yet been linked to dysregulation of a TK pathway.

It is important to recognize that the diagnosis of the MPNs does not rest solely with the routine microscopic examination of cells and tissues on slides. The diagnostic work-up is more far-reaching and must include reviewing the clinical history and pertinent physical findings, as well as obtaining and assessing laboratory values, including recent complete blood cell counts. Examination of a well-made peripheral blood smear and both bone marrow aspirate and biopsy specimens are still indeed crucial. However, certain ancillary studies, such as cytogenetic and molecular analysis as well as other more specific laboratory evaluations, might be just as important in formulating the correct diagnosis.

The overlap syndromes (i.e., the myelodysplastic syndromes/myeloproliferative neoplasms [MDS/MPNs]) are a relatively newly devised nosologic group of diseases that was created by the World Health Organization (WHO) committee (2001) writing on the hematopoietic tumors. This group of diseases was established to recognize the fact that some disorders share features of the myeloproliferative neoplasms and the myelodysplastic syndromes, but do not fit well into either group. The

overlap syndromes consist of chronic myelomonocytic leukemia (CMML), including the juvenile type, juvenile myelomonocytic leukemia (JMML), in addition to atypical chronic myeloid leukemia (atypical CML) and an unclassifiable category that includes the provisional entity refractory anemia with ring sideroblasts and thrombocytosis (RARS-T). The MDS/MPNs share proliferative features in some cell lineages, but also have dysplastic features, including ineffective hematopoiesis in others. Like the MPNs, the overlap syndromes require a full evaluation of clinical and morphologic findings and evaluation of ancillary studies before a firm diagnosis can be rendered.

## MYELOPROLIFERATIVE NEOPLASMS

### CHRONIC MYELOGENOUS LEUKEMIA

CML holds a unique place among the hematopoietic diseases and especially among the leukemias. It is remarkably associated with a long list of firsts. It was the first leukemia described and actually is the disease for which the term *leukemia* (meaning "white blood") was coined. CML was the first disorder found to be associated with a chromosomal abnormality, a smaller than normal G group chromosome that is referred to as the *Philadelphia* (Ph) *chromosome* for the city in which it was recognized. CML was among the first diseases for which a chromosomal abnormality was found to be due to a reciprocal translocation of genetic material from one chromosome to another. This translocation was the t(9;22)(q34;q11.2), where the derivative chromosome 22, the Ph chromosome, is fused with a portion of the long arm of chromosome 9 (Figure 17-1). CML was also one of the first diseases in which the chromosomal breakpoints were identified as genes disrupted by the translocation and giving rise to fusion products. These genes are the *ABL1* gene on chromosome 9, the *BCR* gene on chromosome 22, and the critical fusion gene *BCR-ABL1* on the Ph chromosome. CML was also one of the first diseases in which a fusion gene was studied to elucidate the molecular pathogenesis of the disorder, which proved to be increased ABL1 TK activity of the *BCR-ABL1* gene product. Most spectacularly, however, CML is the first disease for which an understanding of the underlying molecular pathogenesis has resulted in the development of a drug designed to counteract the molecular abnormality. The success of the drug, the TK inhibitor named imatinib mesylate, has made CML a model for understanding a disease at the molecular level, and for developing small molecule therapy to target the abnormal molecular pathway.

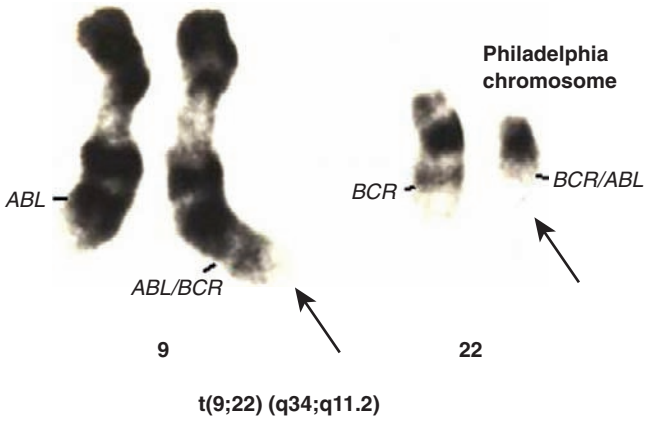


**FIGURE 17-1**

The t(9;22) involving the *ABL1* gene on the long arm of chromosome 9, and the *BCR* gene on the long arm of chromosome 22. The Philadelphia chromosome is the derivative chromosome 22 with the critical fusion gene *BCR-ABL1*.

CML is notable not just because of the consistent breakthroughs regarding its pathogenesis; it is also considered somewhat unique among the myeloproliferative disorders. It arises in the pluripotent hematopoietic stem cell and essentially affects all the hematopoietic cell lineages, both myeloid (including erythroid and megakaryocytic lineages) and lymphoid (including B, T and NK cell lineages). It has a chronic phase, which resembles the other myeloproliferative disorders; it has a blast phase, which resembles acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL); and it sometimes has a transitional or accelerated phase, which resembles, to some degree, a myelodysplastic syndrome or an MDS/MPN overlap disease. Therefore CML is a model for hematopoiesis, chronic leukemia, transformation to acute leukemia, and understanding molecular pathogenesis and developing targeted small molecule therapy. CML is also a model for the other MPNs, because it seems that they are also related to the dysregulation of TK signaling.

#### Clinical Features

CML is one of the most common leukemias, with an incidence of 12.8 cases per 100,000 persons per year, accounting for approximately 15% of all adult leukemias. The median age is between 46 and 53 years, with a male-to-female ratio of approximately 1.8:1. The median age has decreased over the years because of increased incidental diagnosis of early disease and due to the common use of the routine complete blood cell counts in well-patient examinations. Rare cases can be seen in children.

CML most frequently develops in a chronic phase, and patients are increasingly asymptomatic at diagnosis. When symptoms are present, they include fatigue, lethargy, bleeding, weight loss, and those related to splenomegaly. Less common symptoms include night sweats, bone pain, and symptoms related to hyperviscosity

**CHRONIC MYELOGENOUS LEUKEMIA—FACT SHEET**

**Definition and Other Names**

- CML is a chronic myeloid leukemia originating in a pluripotent hematopoietic stem cell causally associated with the *BCR-ABL1* fusion gene, and it is usually identified at the chromosome level as the Philadelphia (Ph) chromosome or t(9;22)(q34;q11.2). The process has a chronic phase in which the blood and marrow show a prominent proliferation of immature and maturing granulocytes, increased small (dwarf) megakaryocytes, and it has accelerated and blast phases that represent a terminal transformation resembling acute leukemia
- Chronic myeloid leukemia, chronic granulocytic leukemia

**Incidence, Gender, and Age Distribution**

- 12.8 cases per 100,000 population per year, 15% of all adult leukemia
- Median age at diagnosis, 46 to 53 years; occasionally seen in children
- Male:female = 1.8:1

**Clinical Features**

- Frequently asymptomatic
- Symptoms: fatigue, lethargy, bleeding, weight loss, full abdomen
- Physical findings: pallor, splenomegaly, occasionally lymphadenopathy

**Prognosis and Treatment**

- Prognosis
  - Before imatinib: 4 to 6 years for chronic phase followed by a terminal blast phase
  - Since imatinib: prolonged survival
- Treatment
  - TK inhibitor, imatinib (Gleevec)
  - Newer, more potent agents (second- and third-generation TK inhibitors)
  - Hematopoietic stem cell transplantation for younger patients with matched donor

owing to elevated cell counts. Physical findings include pallor, splenomegaly, lymphadenopathy, and occasionally bone tenderness and stigmata of thyrotoxicosis.

## Pathologic Features

### Chronic Phase

The diagnosis of CML requires the evaluation of blood, bone marrow, and ancillary studies, the most important of which is cytogenetic or molecular analysis to identify the Ph chromosome or the *BCR-ABL1* fusion.

#### Peripheral Blood

The laboratory evaluation plays a critical role in the diagnosis, and the peripheral blood findings are frequently highly suggestive, if not diagnostic in themselves. Although much emphasis is placed on the cytogenetic and molecular findings, without the initial peripheral blood findings, the confirmatory molecular or cytogenetic tests would not be obtained. Patients usually have a marked leukocytosis with white blood cell (WBC) count ranging from 20 to 500 $\times$ $10^9$/L, with a mean count somewhere between 134 and 225 $\times$ $10^9$/L. The peripheral smear shows mostly neutrophils at all stages of development. Segmented forms and band forms account for the majority of the cells, and there are usually few blasts (1% to 2%) and promyelocytes. The peripheral blood smear also shows a characteristic myelocyte bulge, where the myelocytes are greater in percentage than metamyelocytes (Figure 17-2). This finding is in contrast to the more common reactive granulocytic or leukemoid reaction where there is a progressive decrease in the number of bands, metamyelocytes, myelocytes, promyelocytes, and blasts. Dysplasia in the maturing granulocytic elements is usually mild, if present at all, and severe dysplasia should suggest a different diagnosis. Evaluation of the peripheral blood with the leukocyte alkaline phosphate (LAP) test, sometimes referred to as the *neutrophil alkaline phosphate* (NAP) *test,* yields a low score. The smear also shows an absolute basophilia in essentially 100% of cases. This finding may be difficult to appreciate because frequently the basophils are slightly hypogranular and not recognizable as basophils to the untrained eye (Figure 17-3). There may also be an eosinophilia, and some of the eosinophils may be immature with basophilic granules; these may resemble the abnormal eosinophils seen in AML with inv(16)/t(16;16), but are usually not as atypical (see Figure 17-3). Patients frequently have an absolute monocytosis (>1000 $\times$ $10^9$/L), but the percentage of monocytes is usually low and less than 3%. Patients also frequently have a moderate normochromic, normocytic anemia, and elevated platelets with counts as high as 1000 $\times$ $10^9$/L. Thrombocytopenia is rare, and, if present, another entity should be considered.

#### Bone Marrow

A bone marrow study is usually performed and is important to help exclude other entities and to obtain a specimen for cytogenetic analysis. The marrow is hypercellular, frequently approaching 100%. The marrow shows a marked proliferation of myeloid and megakaryocytic elements, with an elevated myeloid to erythroid ratio (approximately 10:1 to 20:1). Frequently the myeloid elements are expanded along the bony trabeculae, producing an expanded cuff of immature cells (Figure 17-4). In normal bone marrows, this cuff is approximately three cells thick, but in CML it can be 15 to 20 cells thick. The blast count is low, and the cellular features resemble those seen in the blood with a myelocyte bulge, basophilia, and eosinophilia. Megakaryocytes are frequently increased, although in some cases the megakaryocytic proliferation is not that prominent, whereas in others it is accentuated (Figure 17-5). The

## CHRONIC MYELOGENOUS LEUKEMIA—PATHOLOGIC FEATURES

### Microscopic Features

#### Blood

- Leukocytosis composed of granulocytes of all stages of maturation
- Myelocyte bulge, blasts usually less than 1% to 2%
- Absolute basophilia in all cases, absolute eosinophilia in 80% of cases
- Absolute monocytosis common, but monocytes less than 3% of leukocytes
- Thrombocytosis common, can be prominent; thrombocytopenia very rare

#### Marrow

- Hypercellular due to granulocytic and megakaryocytic proliferation
- Myeloid:erythroid = 10:1 to 20:1
- Widened paratrabecular cuff of immature granulocytes
- Basophilia, blasts less than 10%
- Small hypolobated (dwarf) megakaryocytes (not micro-megakaryocytes)
- Mild reticulin fibrosis
- Pseudo–Gaucher histiocytes (in approximately 20% to 40% of cases)

### Ancillary Studies

#### Blood

- LAP (NAP) score low
- $B_{12}$ increased

#### Marrow

- Cytogenetic analysis: t(9;22) or variant (more than 95% of cases)
- Molecular: *BCR-ABL1*$^+$ by FISH or PCR (100% of cases)
- Most cases: major *BCR-ABL1*, (e13 or14/a2 or 3), p210 protein
- Rare cases: minor *BCR-ABL1*, (e1/a2 or 3), p190 protein
- Rare cases: mu *BCR-ABL1*, (e19/a2 or 3), p230 protein

### Accelerated Phase Criteria (WHO, 2008)

#### Any of the Following

- Peripheral blood or marrow blasts 10% to 19%
- Persistent thrombocytopenia ($<100 \times 10^9$/L) unrelated to therapy
- Persistent thrombocytosis ($>1000 \times 10^9$/L) unresponsive to therapy
- Increasing WBCs and spleen size
- Peripheral basophilia greater than 20%
- Evidence of clonal evolution by cytogenetic analysis

### Blast Phase Criteria

- Twenty percent or more blasts in blood or bone marrow
- Myeloid blasts phase: 50% to 60% of cases
- Lymphoid blast phase: 16% to 30% of cases
- Bilineal or biphenotypic blast phase: rare
- Extramedullary blast phase: rare; lymph node, soft tissue, central nervous system

### Differential Diagnosis of Chronic Phase

- Leukemoid reaction
- CMML
- Atypical CML (Ph-neg, *BCR-ABL1*-neg)
- CNL
- Other MPNs

### Differential Diagnosis of Accelerated, Blast Phase

- Ph$^+$ ALL
- MDS/MPN
- AML

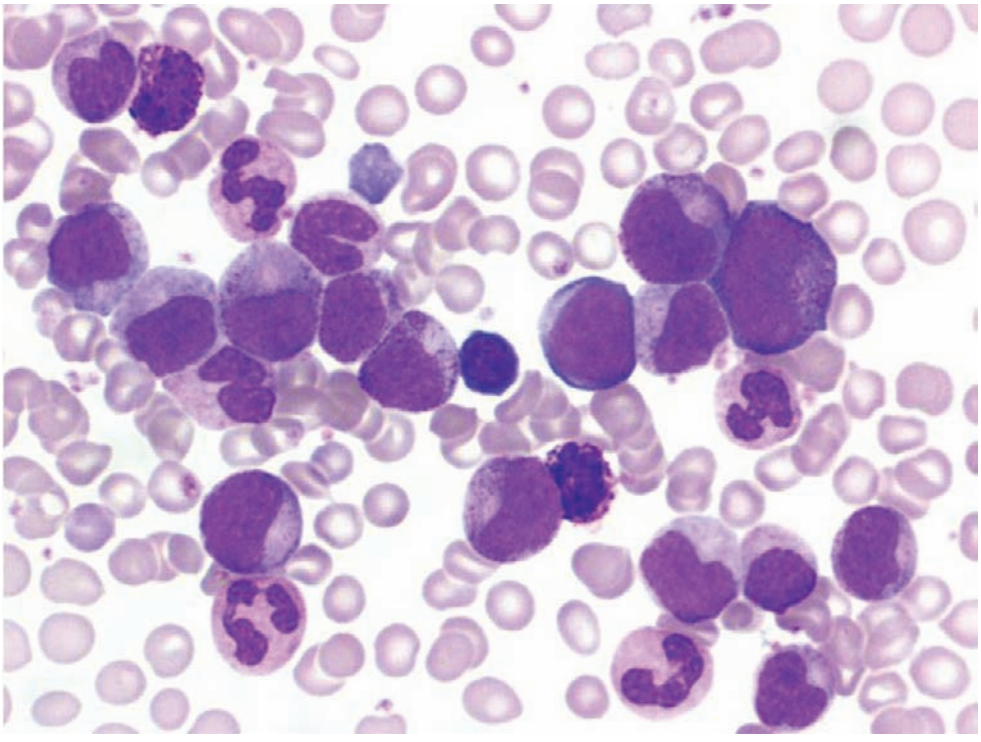

**FIGURE 17-2**

A peripheral blood smear from a typical case of CML in the chronic phase. There is a leukocytosis owing to granulocytes at all stages of maturation. Cells at the myelocyte stage outnumber metamyelocytes resulting in a myelocyte bulge. There is an absolute basophilia and only rare blasts.

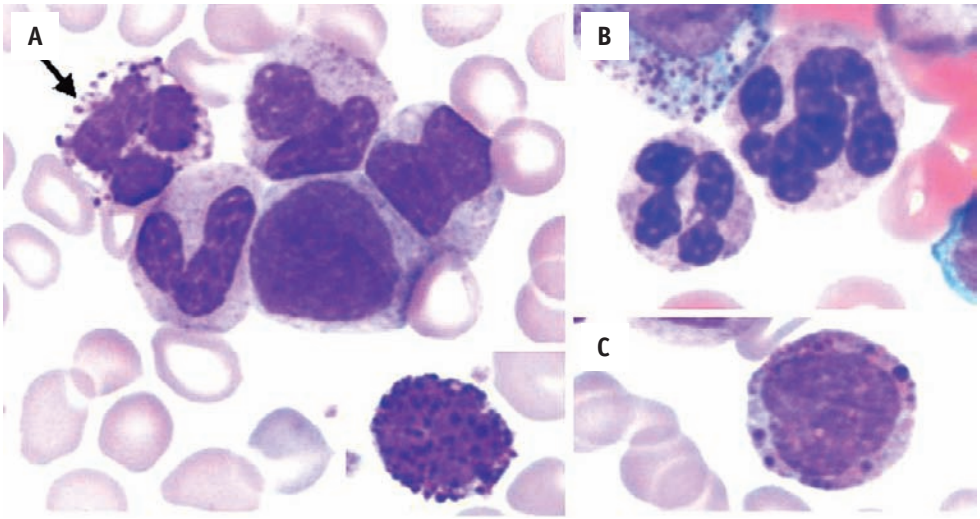


**FIGURE 17-3**

Sometimes the basophils **(A)** are hypogranular with fewer granules than normal basophils (*inset*). This can lead to underestimation of basophils, which should be increased in absolute number in all cases. The neutrophils do not show much dysplasia, although some hypersegmentation or nuclear twinning can be seen **(B).** Some immature eosinophils **(C)** can resemble the abnormal eosinophils seen in acute myelomonocytic leukemia with abnormal eosinophils, although they are usually less atypical.

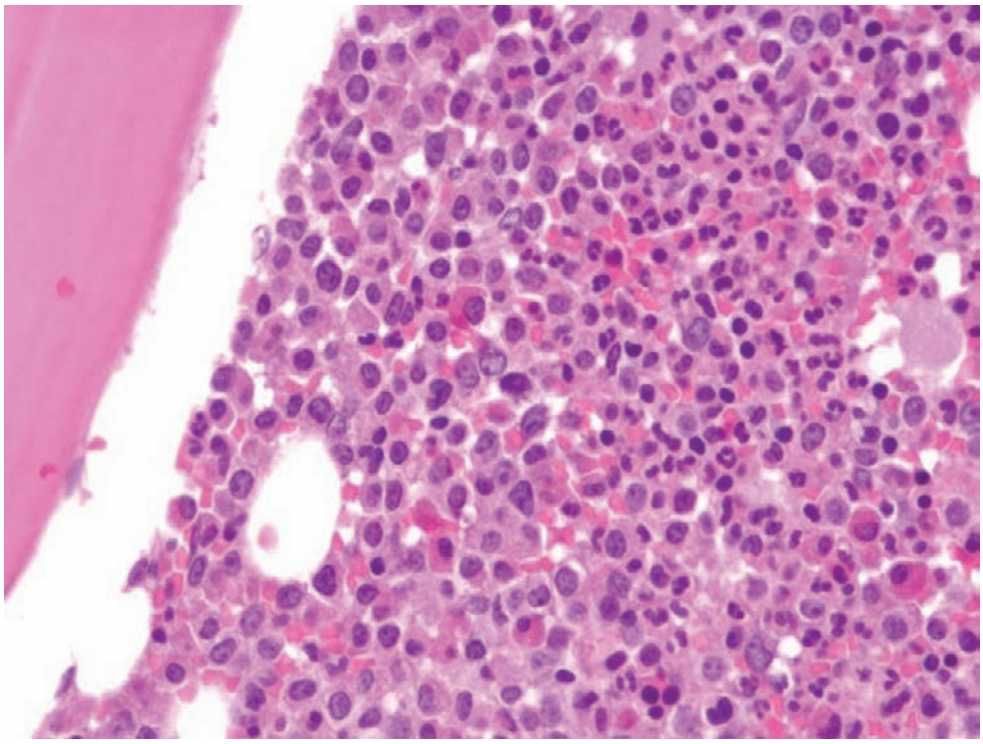

**FIGURE 17-4**

The marrow is hypercellular in chronic myelogenous leukemia, and the paratrabecular cuff of immature granulocytic elements is expanded from the normal three to four cells to approximately 15 to 20 cells.

megakaryocytes are characteristically small with hypolobated nuclei, which some refer to as *dwarf megakaryocytes*. They are not large and atypical, nor are they tiny micro-megakaryocytes. This feature is important to recognize, because it helps to distinguish CML from the other MPNs, which have larger than normal megakaryocytes, and from the myelodysplastic syndromes or MDS/MPNs, in which true micro-megakaryocytes are seen (Figure 17-6). Numerous micro-megakaryocytes in a suspected case of CML should make one consider another diagnosis. Frequently, in approximately 20% to 40% of cases, the marrow shows histiocytes that resemble Gaucher cells; these are referred to as *pseudo-Gaucher cells*. These cells have the characteristic crumpled tissue

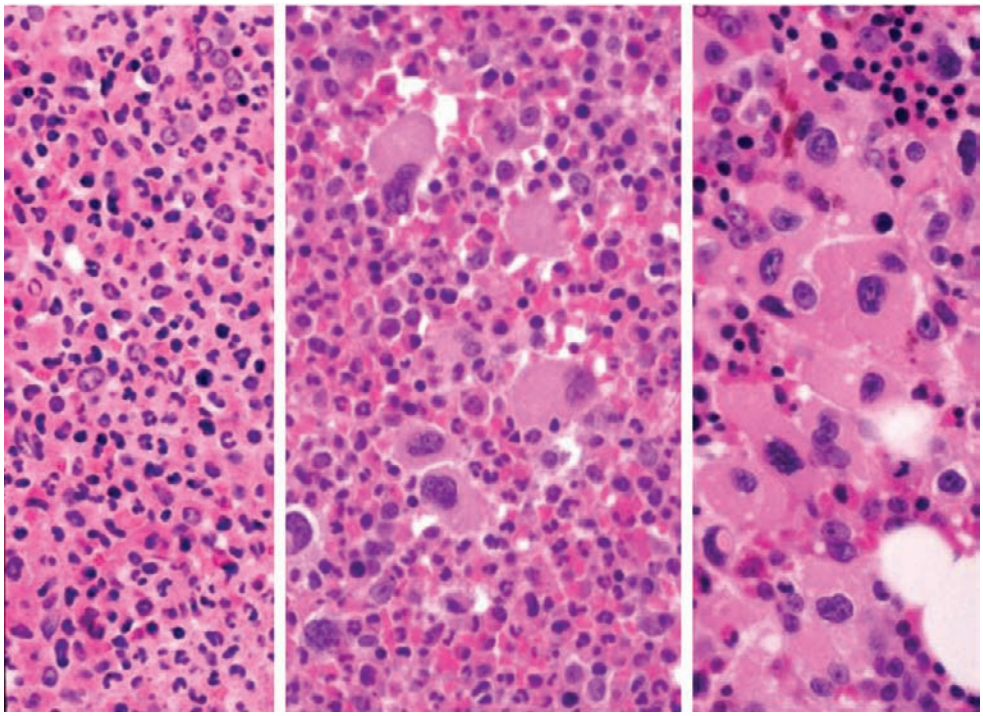

**FIGURE 17-5**

Most cases of chronic myelogenous leukemia show a granulocytic and megakaryocytic proliferation in the bone marrow *(center),* whereas rare cases have a granulocytic *(left)* or megakaryocytic *(right)* predominance.

paper–like cytoplasm and frequently show hemophagocytosis (Figure 17-7). The presence of these cells is not diagnostic of CML as they can be seen in any of a number of hematologic disorders. However, in CML they are derived from the neoplastic clone, as they have been shown to be *BCR-ABL1*$^{+}$ through fluorescence in situ hybridization (FISH) analysis.

### Ancillary Studies

As mentioned previously, the LAP or NAP score is usually below the normal range. Although not commonly used, this screening method is helpful but not absolute, because CML in the accelerated phase can show increased scores. Indeed, the LAP score is being replaced by more specific molecular testing for CML and other MPNs and is being discontinued by many laboratories. $B_{12}$ is increased by 10- to 20-fold the normal range, and uric acid is usually elevated.

Although the diagnosis of CML can usually be made with a great degree of certainty from the features in the blood and marrow, confirmation requires the demonstration of the characteristic t(9;22) (or variant) or the associated *BCR-ABL1* fusion gene. This demonstration can be accomplished by conventional cytogenetic analysis, by FISH with probes to *BCR* and *ABL1,* or through the use of polymerase chain reaction (PCR). The t(9;22) is seen in its characteristic form in greater than 95% of cases. In a small number of cases, however, there is a variant translocation involving the 9q34, the 22q11.2, and another involved chromosome (e.g., t[9;14;22]). In slightly less than 5% of cases, there is submicroscopic translocation, which cannot be identified by conventional cytogenetics, and the karyotype appears normal. However, with FISH probes or with PCR primers to identify the fusion gene, the underlying *BCR-ABL1* can be recognized. These cases are called *Ph-negative CML* and should probably be referred to as *Ph-negative, BCR-ABL1-positive CML* for clarity.

It is important to recognize that the *BCR* gene can be broken in three different regions, giving rise to three different *BCR-ABL1* proteins of different size. Almost all cases of CML are associated with the major breakpoint region, at exon 12-16 (formerly referred to as exon b1-b5) and fusion to the *ABL1* at its exon 2. This fusion is referred to as the *major BCR-ABL1* or *fusion associated with the p210 kilodalton (kd) BCR-ABL1 protein*. Rare cases of CML can have a fusion involving the first exon of *BCR*, e1-2, and this is referred to as the *fusion associated with the minor breakpoint* and a smaller fusion protein with 190kd size (p190). This fusion is far more common in Ph+ALL, but when associated with CML is associated with increased monocytes, which makes it difficult to differentiate from CMML. Last, rare cases of CML can have a fusion of *BCR* involving the regions around exons 17 to 20 (previously referred to as c1-c4) resulting in a μ breakpoint or a larger protein with 230kd weight (p230). This fusion is also rare, but may be associated with CML that has markedly increased platelets or CML with a predominance of mature neutrophils (CML-N). These can mimic either ET or CNL, respectively.

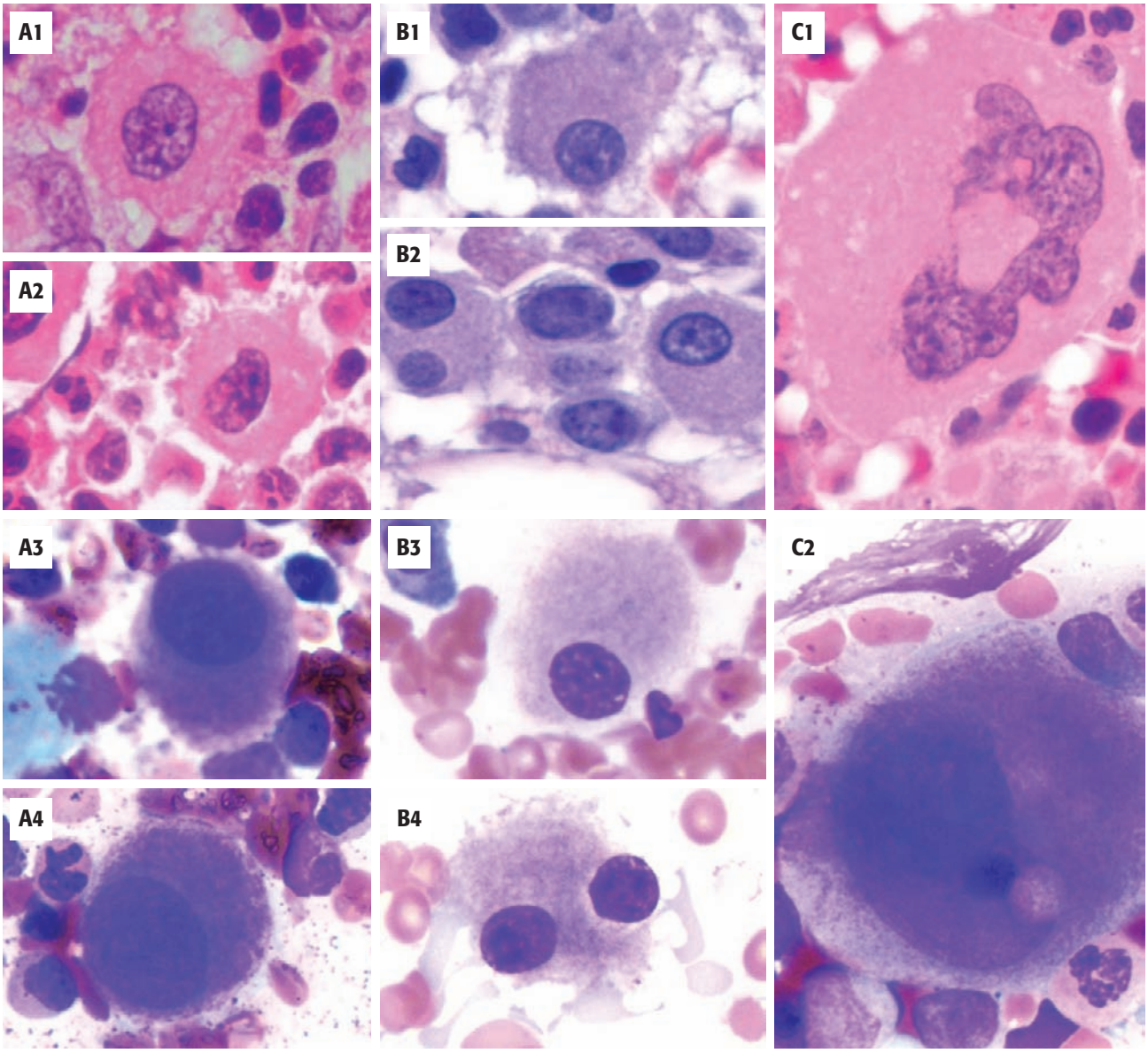


**FIGURE 17-6**

Comparison of megakaryocytes in CML, MDS, and MPN, BCR-ABL1-negative. CML megakaryocytes (from biopsy sections **A1, A2,** or from aspirate smears **A3, A4**) are considered "dwarf" forms that are small but not as small as the tiny micro-megakaryocytes seen in MDS (from biopsy sections **B1, B2,** or on aspirate smears **B3, B4**). The "dwarf" megakaryocytes in CML **(A1, A2,** and **A3, A4)** are also quite distinctive from the huge megakaryocytes seen in the other MPNs (from biopsy section **C1** or from aspirate smear **C2**).

### Accelerated Phase

CML sometimes progresses to a blast phase first through an accelerated phase. In fact, the concept of an accelerated phase was essentially developed to be a herald of blasts crisis. Because the accelerated phase was far more common before the era of TK inhibitor therapy, there has been some question as to whether the concept of the accelerated phase of the disease had outlived its usefulness. However, an accelerated phase is not synonymous with resistance to TK inhibitor therapy, and it still may have utility in identifying patients whose disease is progressing toward blast crisis as initially intended.

Accelerated phase is associated with worsening overall performance, fever, and night sweats, weight loss, bone pain, progressive splenomegaly, and loss of responsiveness to therapy. Although there had been different criteria used to diagnose accelerated phase, the WHO committee writing on hematologic disorders developed a list of six features, any of which would indicate accelerated phase. These features include peripheral blood or bone marrow blasts accounting for 10% to 19% of the cells (Figure 17-8), persistent thrombocytopenia ($<100 \times 10^9$/L) unrelated to therapy or thrombocytosis $>1000 \times 10^9$/L unresponsive to therapy, increasing WBC and spleen size, basophilia greater than 20% (Figure 17-9) and evidence of clonal evolution by

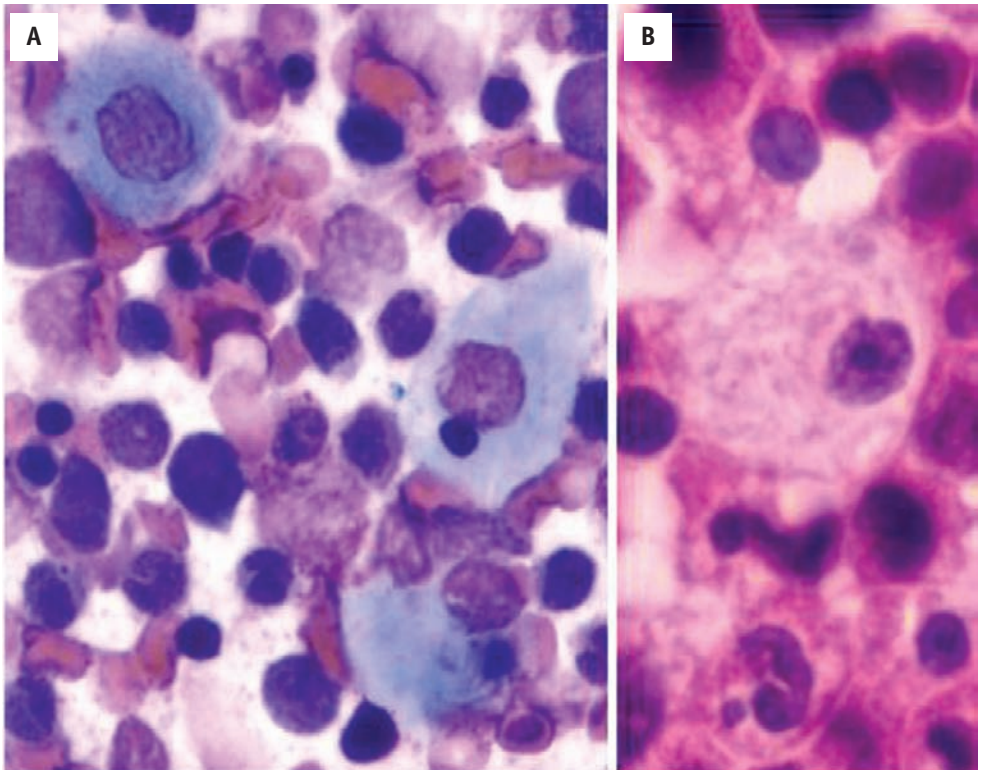


**FIGURE 17-7**

Up to 40% of cases of CML can show pseudo–Gaucher cells in the aspirate **(A)** and on the biopsy **(B).** Sometimes they can be seen undergoing phagocytosis. Although distinctive, they are not specific for CML as they can be seen in other entities.

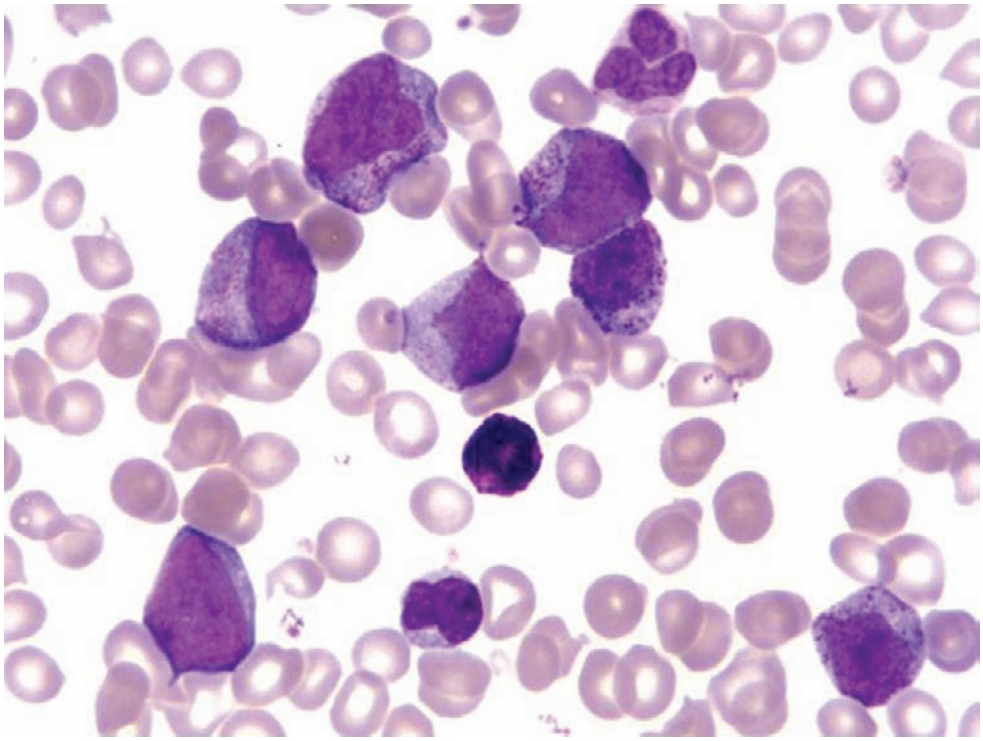

**FIGURE 17-8**

Chronic myelogenous leukemia in the accelerated phase showing more prominent left shift with increased immature cells. This patient case had greater than 10% circulating blasts.

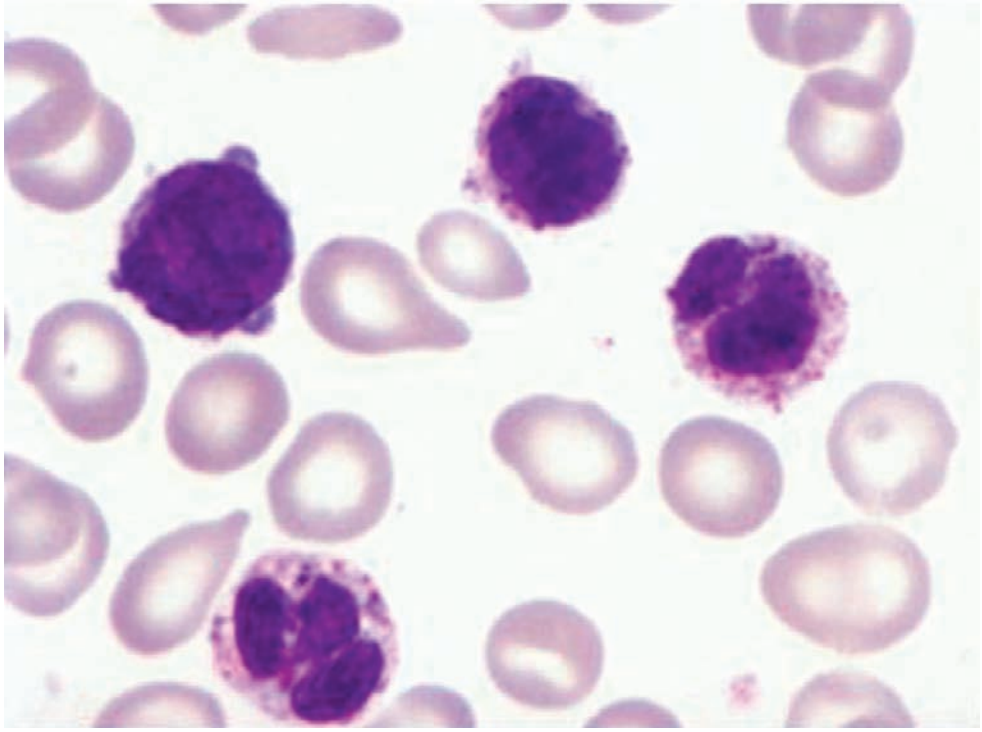

**FIGURE 17-9**

Chronic myelogenous leukemia in the accelerated phase. This patient had greater than 20% circulating basophils. There were also increased blasts.

cytogenetic analysis. Dysplasia and increased fibrosis are frequently seen in accelerated phase, but in themselves are not considered sufficient for a diagnosis according to the WHO guidelines.

Although almost any additional chromosomal change can be seen in accelerated phase, the most common changes include an extra Ph chromosome (+Ph or +der[22]), trisomy 8, isochromosome of the long arm of chromosome 17q [i(17q)], and +18. These abnormalities are seen singly or in combination in 81% of cases showing cytogenetic evolution. Other more common abnormalities include –7, –17, +17, +21 and –Y.

### Blast Phase

Blast phase, or blast crisis, occurs in virtually all patients with untreated CML. Before the use of TK inhibitor therapy, it usually occurred about 4 to 6 years after the initial diagnosis. In the era of imatinib and other TK inhibitors it occurs with much less frequently, sometimes either following an accelerated phase or suddenly without warning. Blast phase resembles an acute leukemia and is almost always a terminal event. Because the CML clone originates in the pluripotent stem cell, blast phase can occur in the myeloid series or the lymphoid series, or it can be biphenotypic or bilineal (two blasts phases at once). Blast phase is diagnosed when there are 20% or more blasts in the blood or marrow, but sometimes it is seen only focally on the biopsy as sheets of blasts (focal intramedullary blast transformation; Figure 17-10). Although most of the time blast phase is diagnosed from the blood and/or marrow, in some instances it can occur at an extramedullary site. In fact, the development of a mass lesion in a patient with CML should always warrant investigation with a biopsy.

#### Myeloid Blast Phase

Blast phase is of a myeloid type in approximately 50% to 60% of cases. Before TK inhibitor therapy, it commonly occurred following an accelerated phase and was seen more frequently in older patients with higher blood counts, more severe anemia, and larger spleens. Although blast phase responds to TK therapy, it still is refractory to therapy and has a poor survival. The myeloid blast crisis of CML is heterogeneous. In some cases it can resemble a de novo AML without maturation, with maturation, with a monocytic component, or it can even resemble erythroleukemia or megakaryoblastic leukemia (Figure 17-11). In rare cases, the blast phase can have a t(8;21), inv(16)/t(16;16), or t(15;17), cytogenetic abnormality usually associated with de novo AML. In these latter types, the blast phase component is morphologically and immunophenotypically identical to the de novo leukemia associated with these recurring chromosomal abnormalities (Figure 17-12). More frequently the myeloid blast phase can be of a mixed myeloid type in which the blastic element include myeloblasts, monoblasts, erythroblasts, megakaryoblasts and immature basophils (Figure 17-13). This type of blast phase is distinctive morphologically and does not

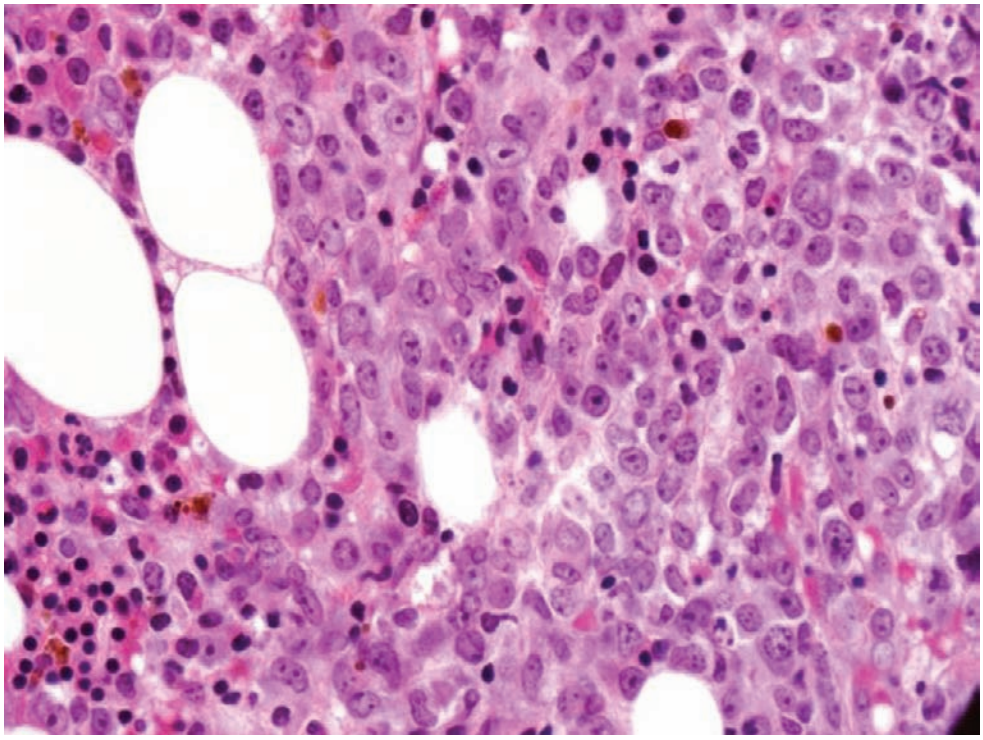

**FIGURE 17-10**

Focal blast phase. Sometimes the blast phase in chronic myelogenous leukemia is focal and only recognized in a localized area on the biopsy.

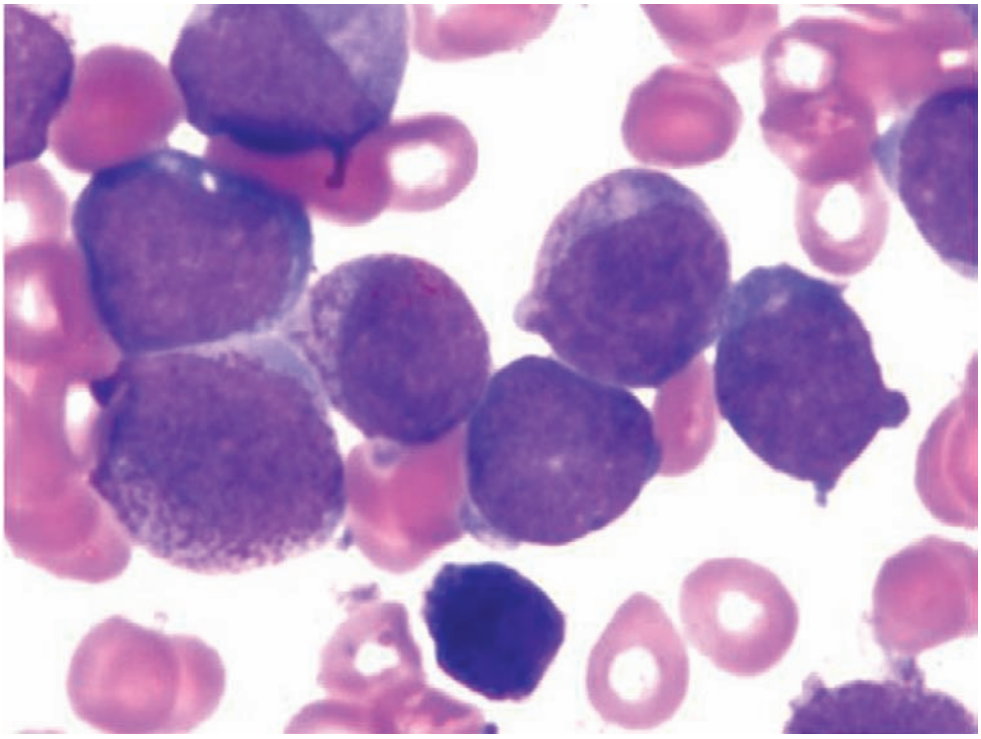

**FIGURE 17-11**

Chronic myelogenous leukemia in the myeloid blast phase. This case resembles acute myeloid leukemia without maturation.

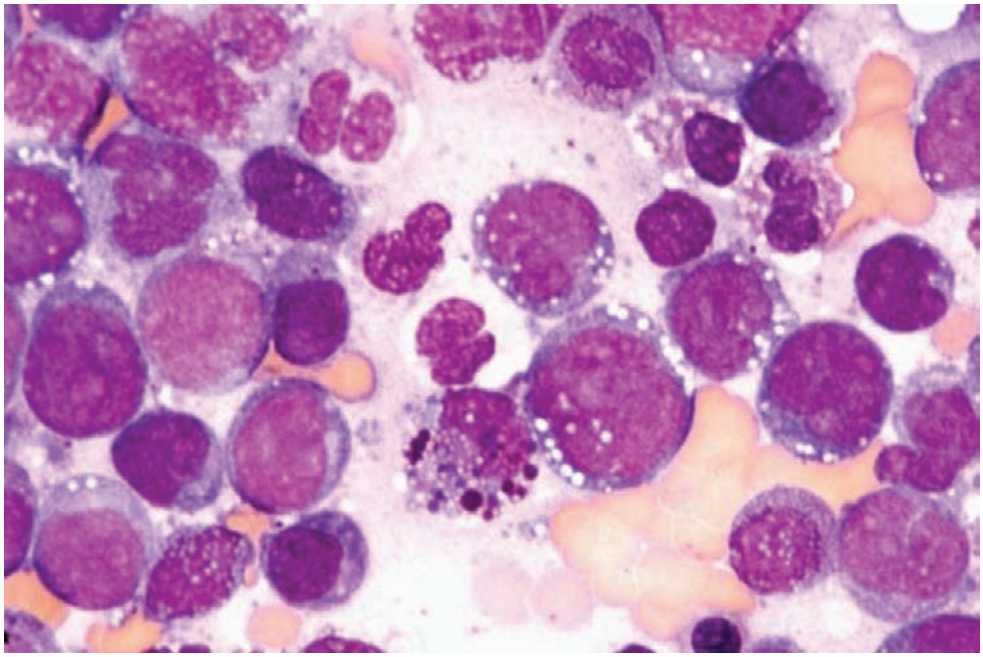

**FIGURE 17-12**

Chronic myelogenous leukemia in the myeloid blast phase. This patient had t(9;22) and inv(16) at the blast phase. Myelomonocytic blasts are seen along with an abnormal eosinophil (slightly below center).

have a well-known de novo AML counterpart, although a mixed myeloid blast population can be seen in some AMLs arising from MDS.

### Lymphoid Blast Phase

Before the use of TK inhibitor therapy, lymphoid blast phase accounted for 16% to 30% of cases of blast crisis and was more uniform morphologically and immunophenotypically than myeloid blast crisis. Clinically, lymphoid blast phase occurred in a younger patient with lower counts and less splenomegaly than in patients with myeloid blast phase. Interestingly, the lymphoid blast phase occurred abruptly and was not associated with a preceding accelerated phase. Thus, there is usually no gradual increase in the lymphoblasts of lymphoid blast phase. Morphologically the lymphoid blast phase shares features with ALL, although the background of CML is frequently still evident (Figure 17-14). Phenotypically, lymphoid blast phase is more commonly of a precursor B phenotype typically with CD19, CD10, and TdT expression and lack of cytoplasmic μ or surface immunoglobulin. Less commonly, the blast phase can be of a precursor T cell type. Although lymphoid blast phase also responds to second- and third-generation TK inhibitors and other chemotherapeutic agents, the overall survival is still poor and patients are frequently taken to stem cell transplant.

### Bilineal or Biphenotypic Blast Phase

In some cases the blasts in blast phase can be a mixture of lymphoblasts and myeloblasts. These bilineal processes may be associated with two distinct cytogenetic clones that have evolved separately from the $Ph^+$ clone. The clones likely represent separate lymphoid and myeloid blast phases occurring simultaneously. In the biphenotypic blast phase, the blasts show lymphoid and myeloid markers simultaneously on the same blasts. These blasts may be precursor B/myeloid or precursor T/myeloid. This type of blast phase requires the same diagnostic criteria as de novo acute leukemia with mixed phenotype for diagnosis.

### Extramedullary Blast Phase

In approximately 5% to 10% of cases, blast phase can manifest at extramedullary sites; therefore the development of a mass lesion in a patient with CML should prompt evaluation and an appropriate biopsy. The most common sites of extramedullary blast phase include lymph node, soft tissue and CNS. Extramedullary disease is usually of the myeloid type, but not always. Bone marrow involvement can be simultaneous, but if not it usually develops a short time after the extramedullary presentation.

## Differential Diagnosis

A discussion of differential diagnosis in CML needs to take into account at which stage the patient presents in. Although most patients present in chronic phase, some initially present in blast phase or accelerated phase, and the entities considered in the differential differ widely among these. Key to establishing the

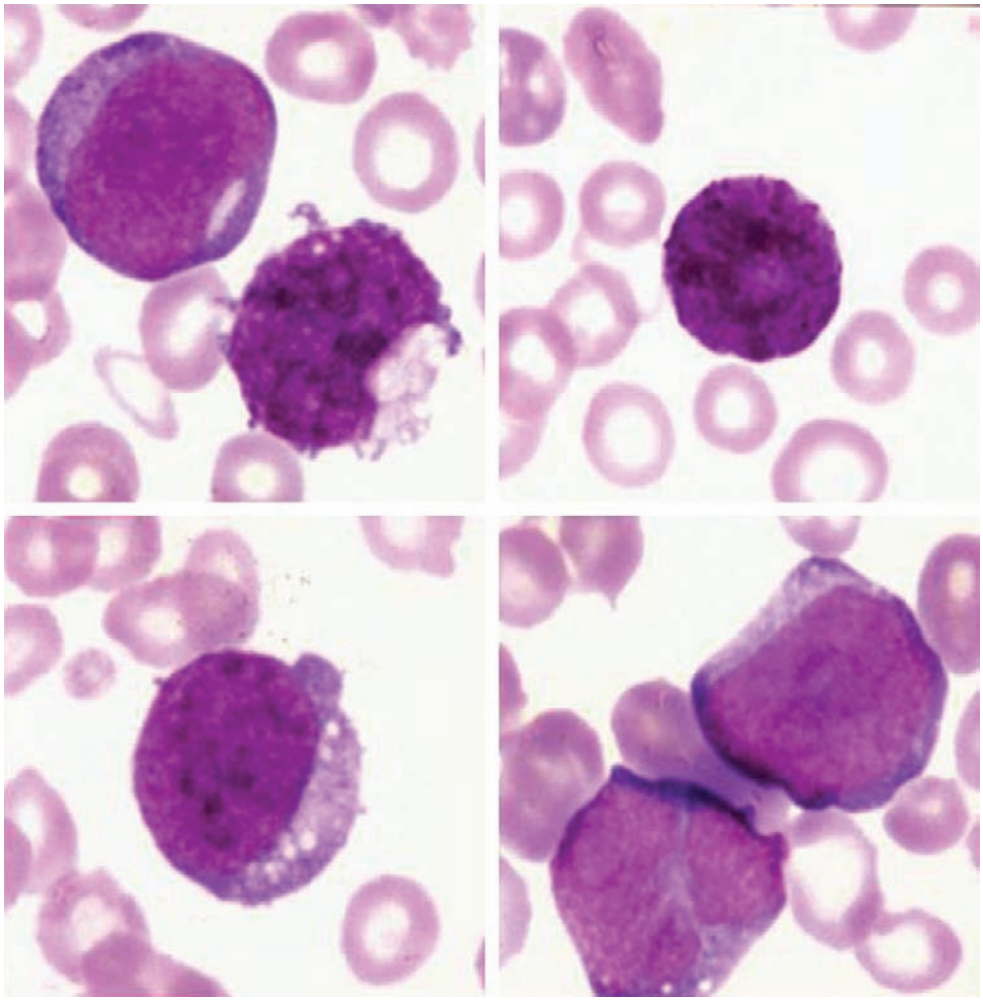

**FIGURE 17-13**

Chronic myelogenous leukemia in myeloid blast phase. The myeloid blasts are heterogeneous. Some resemble megakaryoblasts, myeloblasts, monoblasts, or immature basophilic cells.

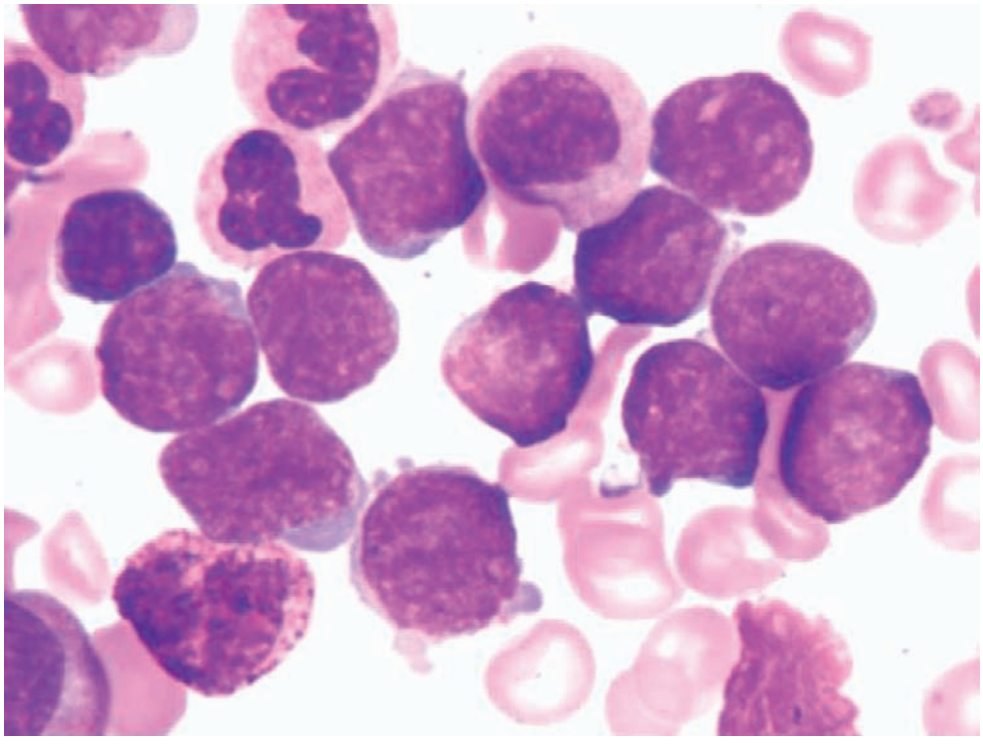

**FIGURE 17-14**

Chronic myelogenous leukemia in lymphoid blast phase. Lymphoblasts are present with some residual granulocytic elements of the chronic myelogenous leukemia.

differential diagnosis is the evaluation for t(9;22) or *BCR-ABL1*, or both. CML must be shown to have the t(9;22), a variant translocation or the *BCR-ABL1* by FISH or by molecular techniques.

#### Differential Diagnosis of Chronic Phase

Included in the differential diagnosis of chronic phase CML are a leukemoid reaction, CMML, atypical CML, and CNL. A leukemoid reaction is a normal response to infection or another disease process that resembles leukemia with high leukocyte counts in the blood. In some cases a leukemoid reaction can have counts as high as 30 to 100 $\times$ $10^9$/L. Although in a leukemoid reaction the granulocytes can show a significant left shift with circulating metamyelocytes, myelocytes, promyelocytes, and even blasts, the factors that help to distinguish it from CML include the lack of a myelocyte bulge, the presence of toxic granulation and Döhle bodies in the neutrophils, and the lack of absolute basophilia. In addition, there is usually a markedly elevated (not decreased) LAP score. However, identifying a cause of the underlying reactive granulocytosis is most helpful in considering a leukemoid reaction over CML. Most of the time this is obvious and usually an infectious process. Bone marrow evaluation has been shown to be of little help in distinguishing CML from a leukemoid reaction.

Chronic myelomonocytic leukemia is discussed in the Overlap Syndromes section. It figures prominently in the differential diagnosis of CML. Other than lacking the t(9;22) and *BCR-ABL1*, the key features that help to distinguish it from CML are the increased percentage (not absolute number) of monocytes (usually greater than 10%), the presence of dysplasia in the granulocytic and megakaryocytic cells (classic micro-megakaryocytes would be seen), less immaturity in the granulocytic precursors (usually less than 10% of the cells compared with CML, where they are usually greater than 20%), and fewer basophils. An absolute monocytosis alone is not helpful, because in many cases of CML the absolute number of monocytes is greater than 1000 $\times$ $10^9$/L.

Atypical CML (aCML) is also discussed in the Overlap Syndromes section. It can be distinguished from CML by the presence of marked dysplasia in the granulocytic, megakaryocytic, and erythroid series, by the presence of thrombocytopenia, and of course by the lack of t(9;22) and *BCR-ABL1*. Interestingly, a subset of cases of aCML or CMML has isochromosome 17 as a sole abnormality. In these cases, one must evaluate for a cryptic t(9;22) because i(17q) is a common secondary change in CML as mentioned previously.

Chronic neutrophilic leukemia is also discussed later. It must be considered in the differential, although it is a rare disorder. In most instances the peripheral blood features of CNL are distinctive, but care must be taken not to ignore the possibility of a type of CML referred to as *CML-N,* in which neutrophilic differentiation is more prominent than usual. Although CML-N is also rare, evaluation for t(9;22) associated with the μ breakpoint would be necessary to distinguish it from CNL, as this breakpoint can be seen in CML-N.

#### Differential Diagnosis of Accelerated Phase and Blast Phase

CML does not commonly manifest initially in accelerated phase, but in the rare case in which it does, the differential considerations would include entities in the MDS/MPN category as well as some of the MPNs, particularly PMF. Morphologic distinction from CML might be difficult, because the finding of dysplasia characteristic of MDS and MDS/MPN can also be seen in accelerated phase of CML. Cytogenetic and molecular studies are key to the correct diagnosis.

Patients with CML can initially present in the lymphoid blast phase. In some, the chronic phase may have gone unnoticed, but in others there may not have been a chronic phase at all. In either of these cases, the patient usually exhibits a leukocytosis with lymphoblasts and the background typical of CML (Figure 17-15). In other cases in which there is no recognizable CML in the background of the blastic process, the diagnosis can be made only after treatment because many patients revert to a chronic phase after therapy. Identification of t(9;22) or *BCR-ABL1* does not necessarily help in the initial evaluation because ALL can be t(9;22) and *BCR-ABL1*-positive. If a minor *BCR-ABL1* (p190) is present, then CML would be unlikely, but if the major *BCR-ABL1* (p210) is seen, either Ph+ ALL or CML presenting in lymphoid blast phase is possible. Identification of the *BCR-ABL1* specifically in the granulocytic, erythroid, or megakaryocytic components might be helpful to distinguish between these, because CML is a stem cell disorder involving all cell lineages, whereas Ph+ALL is believed to be a lymphoid-restricted process.

Rarely patients present with an acute myeloid leukemia that is shown to be t(9;22)$^+$ or *BCR-ABL1*$^+$. Whether these are truly Ph+AML or just CML presenting in the myeloid blasts phase is difficult if not impossible to determine. Many of these patients have an aggressive course with no reversion to chronic phase CML after therapy. However, in some patients the blastic proliferation is a mixed population of myeloblasts, monoblasts, erythroblasts, and megakaryoblasts similar to the mixed-myeloid blast phase of CML. This clue can be a strong indication that the patient may have the *BCR-ABL1* fusion and CML presenting in myeloid blast phase. Other patients may have t(9:22) in addition to a common recurring cytogenetic abnormality in AML, such as inv(16) or t(8;21), whereas other patients will exhibit a mixed or bilineal acute leukemia. Some reports have noted increased incidence of the p190 *BCR/ABL1* in cases presenting as AML or mixed lineage leukemia.

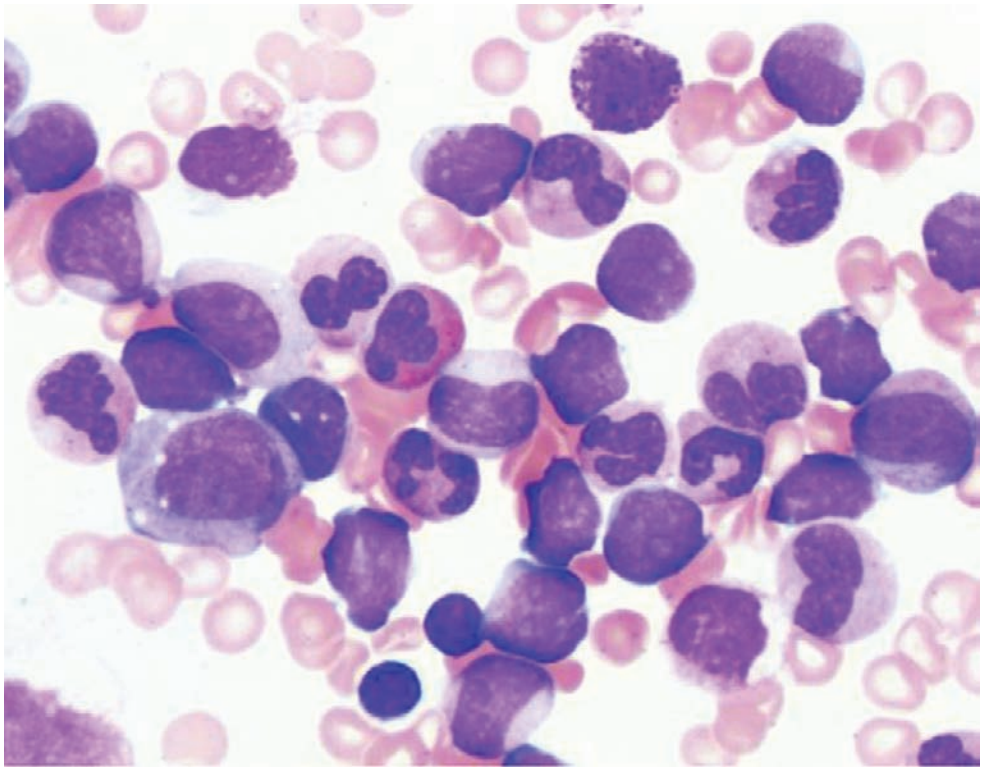

**FIGURE 17-15**

Chronic myelogenous leukemia initially presenting in the lymphoid blast phase. This patient had WBC count of 400 × $10^9$/L. Forty percent of the cells were lymphoblasts with a precursor B phenotype, but there was a significant granulocytic proliferation with left shift and basophilia, indicating CML in the lymphoid blast phase. The Ph+ chromosome, and transcripts for the p210 *BCR-ABL1* were identified.

## Prognosis and Therapy

The development of imatinib (Gleevec) in the late 1990s has revolutionized the treatment of CML and provides successful management for most patients. After trials of imatinib—first in patients who failed interferon therapy, then in patients with accelerated or blast phase, and finally in an up-front comparison to interferon—imatinib has become the treatment of choice because of its superiority to other therapies. A controversy that still exists is in the management of young patients with a suitable stem cell donor, and whether to first treat with imatinib or whether to transplant immediately.

Imatinib inhibits the constitutive phosphorylation activity of the *BCR-ABL1* TK. The drug sits in a pocket of the ABL1 portion of the *BCR-ABL1* fusion protein and blocks the adenosine triphosphate from binding. Usually after approximately 3 months of therapy there is normalization of blood counts, reduction of bone marrow cellularity with correction of the myeloid to erythroid (M:E) ratio, and normalization of megakaryocyte size (Figure 17-16). Frequently there are lymphoid aggregates composed of mostly small lymphocytes, which are a mix of B and T cells (Figure 17-17). These lymphocytes are reactive in nature. Many patients achieve a complete cytogenetic remission, but a molecular remission by PCR analysis for *BCR-ABL1* is relatively uncommon, occurring in only 10% to 15% of all patients.

There are no universally accepted recommended guidelines for monitoring patients undergoing therapy, and the optimal approach is subject to an ongoing debate. Patients are usually studied by conventional cytogenetic analysis of metaphase spreads until they have a complete cytogenetic response noted in the marrow. FISH evaluation for *BCR-ABL1* fusion can be used on peripheral blood or marrow cells for screening for residual clonal disease, but quantitative PCR provides the most sensitive means of detecting residual clonal cells. Efforts at standardizing sensitive quantitative PCR methods for monitoring patients on imatinib or other TK inhibitors are ongoing. Pivotal clinical trials with molecular monitoring of peripheral blood have shown the utility of assessing for minimal residual disease. Patients treated with imatinib who achieved at least a 3-log reduction in fusion transcript at 18 months of treatment have an extremely favorable (95%) event-free survival at 7 years and are at very low risk for progression to accelerated or blast phases.

Resistance to imatinib can occur because of mutations, overexpression of *BCR-ABL1,* or reduced cellular uptake of the drug. Although newer drugs with more powerful TK activity are being used, these still have

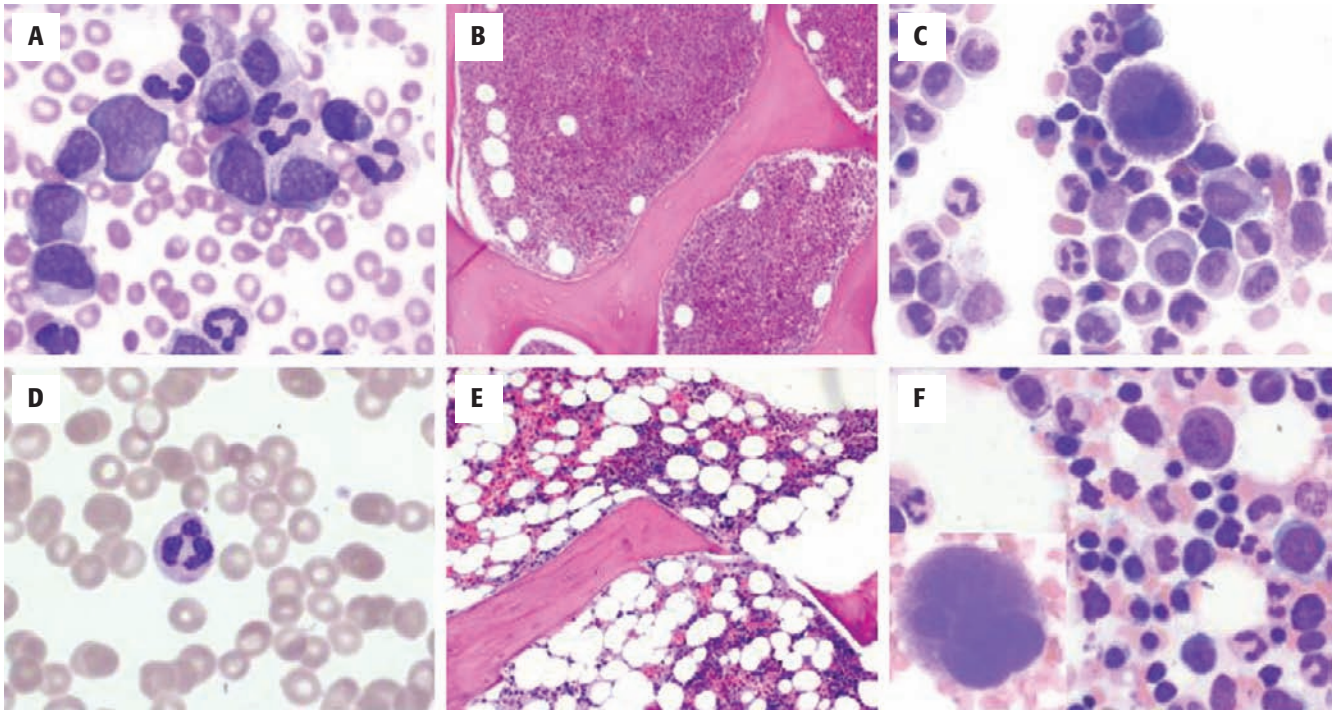


**FIGURE 17-16**

A comparison of before **(A-C)** and after **(D-F)** 3 months of imatinib therapy. There is a reduction in the number of circulating granulocytes **(A** and **D),** and bone marrow cellularity **(B** and **E),** and a reversion to normal megakaryocyte size **(C** and **F)** in the post-therapy specimen.

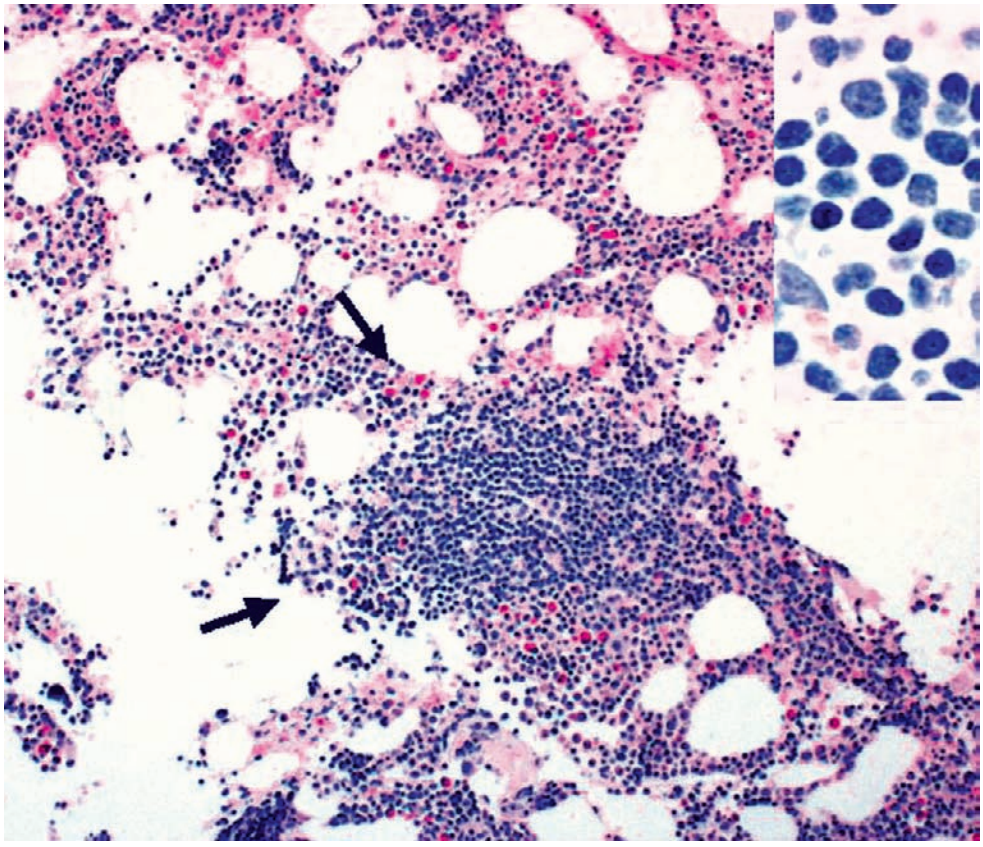

**FIGURE 17-17**

Atypical lymphoid infiltrate seen after imatinib therapy. The infiltrate is composed of mostly small lymphocytes, which on immunophenotyping were are a mix of B and T cells.

little effect in cases in which clonal evolution resulted in a stimulation of leukemogenic pathways that are independent of the *BCR-ABL1*–associated constitutive activity of TK. One mutation is particularly insensitive to second- and third-generation TK inhibitors. This T315I ABL1 kinase domain mutation is sometimes screened for early in the disease so that alternative therapy including possible stem cell transplantation can be considered. Currently, mutational testing is recommended by the European Leukemia Net based on suboptimal response or failure of imatinib.

The development of a t(9;22)-negative and *BCR-ABL1*–negative clonal proliferation can occur in patients undergoing TK inhibitor therapy. These proliferations can be associated with AML, MDS, MPN, or MDS/MPN. They are particularly intriguing because it seems possible that they arise from a *BCR-ABL1*-negative clonal expansion that was speculated to be the precursor of the *BCR-ABL1*-positive clone in CML. Whether this is proven to be the case or not, these patients can present a significant diagnostic challenge with an unexpected complication or progression developing in what is otherwise a successfully treated disease.

## MYELOID (AND LYMPHOID) NEOPLASMS WITH EOSINOPHILIA AND TYROSINE KINASE MUTATIONS, AND CHRONIC EOSINOPHILIC LEUKEMIA–HYPEREOSINOPHILIC SYNDROME

The myeloid neoplasms with eosinophilia are a heterogeneous group of rare diseases. They include the myeloid and lymphoid neoplasms with eosinophilia and TK mutations, chronic eosinophilic leukemia (CEL), and the hypereosinophilic syndrome (HES). In the 2008 WHO classification, the myeloid and lymphoid neoplasms with eosinophilia and TK mutations were segregated from the other MPNs but they are integrated in this discussion.

After CML, the myeloid neoplasms with eosinophilia were the next group of MPNs discovered to be related to TK signaling dysfunction. Compared to the hard-won inquiry in CML with its decades-long, logical scientific progression of knowledge, the discovery in the eosinophilic disorders came somewhat indirectly. After imatinib was approved for use, it was tried in a number of disorders other than CML, and in these trials, some of the eosinophilic disorders were found to be exquisitely sensitive. This finding implied that some of the eosinophilic diseases were due to overactivity of an inhibitable TK; these were later realized to be the receptor TKs, *PDGFRB,* or *PDGFRA* (platelet derived growth factor receptor, β and α genes, respectively). These genes had been shown previously to be altered in rare chromosomal translocations involving 5q31-3 (*PDGFRB*) and deletions of 4q (*PDGFRA*). In the earlier publications, the eosinophilic diseases with abnormalities of 5q31-3, and deletions of 4q were referred to as *chronic myelomonocytic leukemia with eosinophilia* or *mast cell disease with eosinophilia,* but in the current classification they are simply denoted as *myeloid neoplasms associated with eosinophilia* and *abnormalities of PDGFRB or PDGFRA*.

Another disease added to this group was the 8p11 myeloproliferative neoplasm. Although also rare, this disorder frequently has eosinophilia and is associated with rearrangement of the *FGFR1* (fibroblast growth factor receptor 1 gene), yet another TK receptor. Uniquely, the 8p11 usually develops as a myeloid proliferation, but subsequently can develop into or can initially present as a precursor lymphoid neoplasm, more frequently the precursor T-cell type than precursor B. As such, the 8p11 myeloid neoplasm resembles CML, in that it seems to be a stem cell disorder that can transform to or sometimes present in a lymphoid blast crisis. This stem cell nature of the process has led to the separation of these entities (as mentioned previously) and the use of the term *myeloid and lymphoid neoplasm,* but like CML (which arguably could also be considered myeloid and lymphoid), they are considered together with the other MPNs here. Unfortunately, and unlike CML or the other myeloid neoplasms associated with eosinophilia and *PDGFRA or B*, the 8p11 MPN is not responsive to imatinib.

CEL and the HES are proliferative disorders associated with marked eosinophilia in the blood and bone marrow that can infiltrate into other organs. By definition CEL is associated with an elevated blast count or is clonal by the finding of an associated clonal cytogenetic abnormality, a mutation of a gene (other than *PDGFRA/B* or *FGFR1*), or through such clonal evaluations as the human androgen receptor assay (HUMARA) test. However, HES is a disease diagnosed by exclusion when the process cannot be shown to be clonal or associated with increased blasts.

### Clinical Features

The myeloid neoplasms associated with eosinophilia (as defined above) are uncommon, and the exact incidence is not known. In general these diseases can occur at any age, but some are more common in the 4th decade and are more frequent in men. The eosinophilia is in the blood and bone marrow but there is also tissue infiltration which frequently results in end organ damage due to cytokines, enzymes and other proteins released from the eosinophils. The most serious complication of this is endomyocardial fibrosis that can result in a constrictive cardiomyopathy. Many patients also have splenomegaly. Transformation to acute leukemia is not common in most of the eosinophilic disorders, except in the 8p11 disorder. Patients with the 8p11 MPN frequently have lymphadenopathy, splenomegaly, or

**MYELOPROLIFERATIVE NEOPLASMS WITH EOSINOPHILIA—FACT SHEET**

**Definition**

- A myeloproliferative neoplasm characterized by a proliferation of eosinophils and their precursors in the blood and marrow, and infiltration into tissues
- Three categories
  - Myeloid and lymphoid neoplasms with eosinophilia and associated TK abnormalities or rearrangements
    - *PDGFRA* on 4q12
    - *PDGFRB* on 5q33
    - *FGFR1* on 8p11
  - Chronic eosinophilic leukemia (associated with increased blasts, or clonality)
  - Hypereosinophilic syndrome (no increase in blasts, nonclonal, idiopathic)

**Incidence**

- Rare, unknown

**Clinical Features**

- Mean age in fourth decade, more common in men
- Splenomegaly common
- End-organ damage owing to infiltration by eosinophils
- Endomyocardial fibrosis, most serious
- Lymphadenopathy in cases with *FGFR1* (8p11)

**Prognosis and Therapy**

- Chronic disease, rare transformation to acute leukemia, except for *FGFR1* (8p11)–associated cases, which can present in or transform to acute leukemia of myeloid or lymphoid type
- Subset of patients respond to imatinib (not those with *FGFR1* [8p11])

sometimes mediastinal masses. The disease is frequently aggressive with transformation to an acute process within 1 or 2 years.

## Pathologic Features

### General, Blood, and Bone Marrow

When considering a patient with eosinophilia for a possible myeloproliferative disorder, it is necessary first to rule out reactive causes of the eosinophilia, clinically and through the evaluation of the blood and bone marrow. This process is critical because reactive causes of eosinophilia owing to allergic processes, infectious agents, Hodgkin lymphoma, or T-cell lymphomas are far more common than any of the MPNs with eosinophilia. Patients with any of the myeloid neoplasms with eosinophilia have eosinophil counts greater than $1.5 \times 10^9$/L. The eosinophils are mature, but frequently they have fewer eosinophilic granules than normal (Figure 17-18). Although they may have other morphologic abnormalities, or be associated with a neutrophilia or monocytosis, none of these findings can accurately distinguish them from their reactive counterparts.

In the myeloid neoplasms with eosinophilia, the bone marrow is hypercellular because of a prominent proliferation of eosinophils. Blasts are usually less than 2% in the blood and less than 5% in the marrow, but can be elevated. Other hematopoietic cell lines may show some dysplasia, and patients can have monocytic proliferation or mast cell proliferations (as in the diseases first described). The marrow often is fibrotic and can show Charcot-Leyden crystals. The bone marrow assessment should carefully focus on ruling out CML, AML, and other MPNs associated with a substantial eosinophilic component. Tissue infiltration by the eosinophils is also often associated with fibrosis and Charcot-Leyden crystals.

In the 8p11 myeloproliferative syndrome, blood counts are variable, but 85% of patients have greater than $1.5 \times 10^9$/L eosinophils, and another 75% have an absolute monocytosis. The bone marrow is typically hypercellular frequently with increased eosinophils in addition to a myeloid proliferation. When blasts are increased sufficient to diagnose an acute leukemia, the blasts are more frequently myeloid (in two thirds of cases) than lymphoid. The lymphoid blasts are almost always of precursor T type. In patients who have lymphadenopathy, approximately 80% have a T-lymphoblastic process, whereas the remainder have a myeloid process.

### Molecular and Genetic

Molecular and genetic analyses play an important role in the definitive diagnosis and subclassification of the myeloid neoplasms with eosinophilia. When the clinical work-up and blood and marrow evaluation have ruled out reactive causes (infectious or malignancy related), and when they have also ruled out other myeloproliferative disorders associated with eosinophilia, an assessment for alterations of *PDGFRA, PDGFRB,* or *FGFR1* by cytogenetic, molecular or FISH analysis should be undertaken. Although abnormalities of *PDGFRB* and *FGFR1* are usually detected at the cytogenetic level with chromosomal abnormalities—t(5;12)(q31-q33;p12) and t(8;13)(p11;q12) are the most frequent, respectively—the abnormalities of *PDGFRA* involving 4q are cryptic. The molecular fusion between *PDGFRA* and another gene at 4q, *FIP1L1,* is usually due to a tiny deletion of an intervening gene called *CHIC2,* and this deletion cannot be readily seen microscopically at the level of the chromosomes. The deletion of *CHIC2* that can be identified by FISH has become a surrogate marker for the *FIP1L1-PDGFRA* fusion.

If the cytogenetic, molecular, or FISH studies are positive, one can render a diagnosis and subclassify the process by the particular molecular abnormality found. If negative, one would evaluate for clonality or for

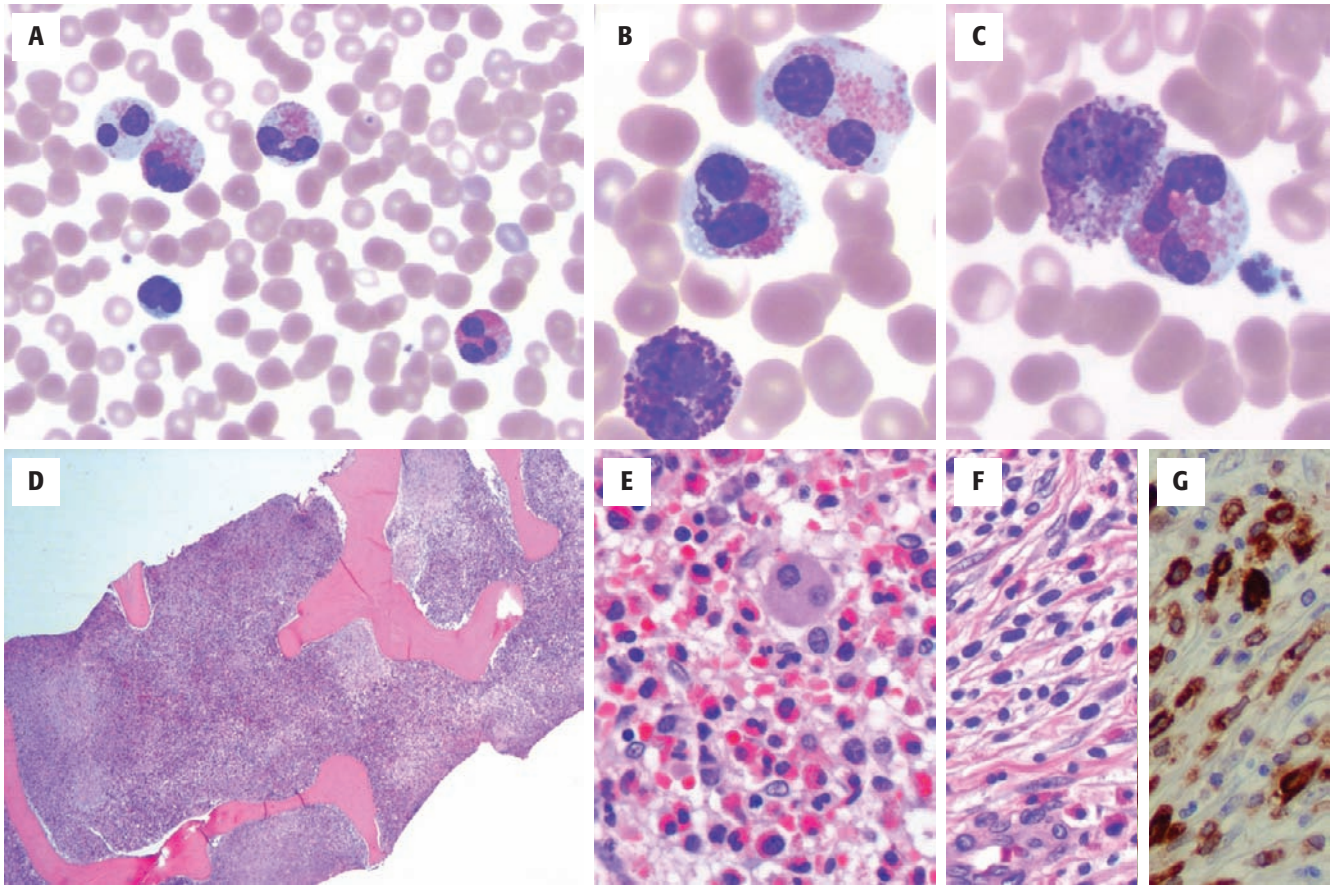


**FIGURE 17-18**

Myeloproliferative neoplasm with eosinophilia. This 32-year-old male patient had a sustained eosinophil count in the blood of greater than $15 \times 10^9$/L **(A).** Some of the eosinophils are hypogranular **(B),** but this is not sufficient to consider the eosinophils malignant, as this can be seen in reactive states. There was also a slight basophilia **(B).** Occasional eosinophils are immature with primary (blue) granules **(C),** but these are not the abnormal granules in the eosinophils of acute myeloid leukemia with inv(16). The biopsy was markedly hypercellular **(D)** with some areas of fibrosis. The increased cellularity was due to a proliferation of mature eosinophils, sometimes associated with abnormal or dysplastic cells such as the small megakaryocytes **(E).** In this patient, there were spindled cells in the areas of fibrosis **(F)** that were positive for mast cell tryptase **(G),** indicating a mast cell component to the process. This case was associated with *FIP1L1-PDGFRA* identified by a *CHIC2* deletion by fluorescence in situ hybridization.

## MYELOPROLIFERATIVE NEOPLASM WITH EOSINOPHILIA—PATHOLOGIC FEATURES

### Microscopic Findings

#### Blood

- Eosinophil count greater than $1.5 \times 10^9$/L
- Hypogranular eosinophils
- Neutrophilia or basophilia in some cases
- Monocytosis in some cases
- Blasts usually less than 2%

#### Marrow

- Hypercellular owing to infiltration by mature eosinophils
- Blasts usually less than 5%
- Some dysplasia in other hematopoietic elements
- Fibrosis present
- Charcot-Leyden crystals may be present
- Some cases have mast cell proliferations (usually those with *PDGFRA* abnormalities)
- Some cases have monocytosis

### Ancillary Studies

#### Cytogenetic Analysis

- del(4q12) cannot be detected on karyotype (cryptic)
- 5q33 translocations (many partners); t(5;12)(q31-q33;p12) most common
- 8p11 translocations (many partners); t(8;13)(p11;q12) most common

#### Molecular

- Deletion of *CHIC2* as a result of *FIP1L1-PDGFRA* (detectable by FISH)
- FISH probe sets available for *PDGFRB* and *FGFR1* molecular abnormalities

### Differential Diagnosis

- Reactive eosinophilia owing to allergy, parasitic disease, other infections, hypersensitivity, autoimmune disorders, neoplastic diseases (e.g., Hodgkin lymphoma, T-cell lymphoma, B-ALL with t[5;14]), aberrant T-cell proliferation
- Clonal eosinophilia associated with CML, AML, other MPNs

increased blasts. CEL is diagnosed if clonality is identified or if the peripheral blood or bone marrow blasts are greater than 1 or 5, respectively. The diagnosis reverts to HES if a reactive condition is excluded, if a specific myeloid disorder with eosinophilia is ruled out, if a TK abnormality is not detected, if there is no identifiable clonality, and if there is no increase in blasts.

### Differential Diagnosis

As mentioned previously, the myeloid neoplasms associated with eosinophilia must be distinguished from disorders in which there is a reactive or secondary eosinophilia, or from a clonal eosinophilia associated with other myeloid diseases. Frequent causes of reactive eosinophilia include allergy, parasitic disease, other infections, and hypersensitivity or autoimmune disorders. Reactive eosinophilia seen in association with neoplastic disorders, in which the eosinophils are not part of the neoplastic clone, must also be excluded. Some disorders frequently associated with eosinophilia include T-cell lymphoma, Hodgkin lymphoma, acute lymphoblastic leukemia (especially precursor B ALL with t[5;14]), and mastocytosis. The lymphocytic variant of HES should also be considered. This is discussed in Chapter 13. Disorders that may have an eosinophilia in which the eosinophils are part of the clone include, CML, AML, other MPN, and MDS. Obviously these too must be excluded.

### Prognosis and Therapy

Patients with abnormalities of *PDGFRA* on 4q and *PDGFRB* on 5q31-3 have been shown to respond to treatment with imatinib, because both the α and β subunits of *PDGFR* are TKs that are abnormally activated because of the respective fusions. This therapy can provide impressive resolution of the eosinophilia and of the harmful sequelae of the eosinophilic infiltration. Patients who are responsive to treatment may become resistant because of mutations that are analogous to the mutations in CML that result in a TK resistance.

Some patients with CEL and HES without proven *PDGFR* fusions and no recognizable molecular abnormality or evidence of clonality should probably be given a trial of therapy, because there can be difficulty in detecting the molecular abnormalities and treatment with a TK inhibitor has little toxic effect. If they respond, it must be assumed that they have clonal disease associated with an undescribed molecular change that is responsive to the TK inhibitor.

Patients with a diagnosis of 8p11 myeloproliferative syndrome are treated with a variety of therapies, including those for ALL, AML, or MPN. Overall, these therapies have been inadequate, as there are only a few long-term survivors, with a median survival of only 15 months. Stem cell transplantation is the only option that has produced better results.

## POLYCYTHEMIA VERA

PV is a myeloproliferative neoplasm that is characterized by a proliferation of erythroid cells that leads to marked erythrocytosis in the blood. However, the disorder is a stem cell disease that can involve other myeloid elements and, in some instances, might involve the lymphoid lineage as well. Although the exact genetic basis is still being worked out, the recent finding of a mutation in the TK *JAK2*, in a large percentage of cases of PV as well as in lesser numbers of PMF and ET, has linked PV and the other common MPNs to CML and the MPN with eosinophilia, as a group of diseases driven by dysregulated TK signaling.

In PV, the *JAK2* mutation, V617F, occurs in approximately 95% of cases, and mutations in another exon of the gene, exon 12, occur in another 3% to 4%. These findings have greatly simplified the diagnostic work-up for PV. In the past, the diagnosis was dependent on fulfilling a complex array of major and minor criteria made necessary to rule out reactive causes of erythrocytosis. In essence, the finding of a *JAK2* mutation readily rules out a reactive process.

PV has two distinct phases that include the polycythemic phase and the so-called spent phase or post-polycythemic myelofibrotic phase, which is a terminal event. There is likely a pre-polycythemic phase as well, although this is difficult to recognize. Rarely, patients with PV develop MDS or AML. The terminal MDS or AML is more frequent (3% to 5%) in patients treated previously with cytotoxic therapy administered for PV than in patients treated with phlebotomy alone (1% to 2%). However, whether some cases might represent a therapy-related process is difficult to prove. Typically the MDS or AML evolving in PV is considered a terminal phase of the disease.

### Clinical Features

The incidence of PV is approximately 1 to 3 per 100,000 persons per year. The incidence increases with age, with the median age at diagnosis being 60 years. There is a slight male predominance and an increase incidence in Ashkenazi Jews. Rare familial cases have been described, but the genetic basis for most of these is not known. Some familial cases have been shown to be associated with mutations in the erythropoietin (EPO) receptor that results in hypersensitivity to EPO.

Most of the presenting symptoms in PV are related to the increased red cell mass that is central to the

### POLYCYTHEMIA VERA—FACT SHEET

**Definition**

- PV is a myeloproliferative neoplasm arising in a pluripotential hematopoietic stem cell that is characterized by increased red blood cell production resulting in an elevated red blood cell mass. The process has a polycythemic phase and a terminal, or spent, phase characterized by marrow fibrosis. There may also be a pre-polycythemic phase, but this is difficult to recognize. Occasionally it may transform to acute leukemia

**Incidence, Gender, and Age Distribution**

- 1 to 3 cases per 100,000 population per year
- Slight male predominance
- Median age at diagnosis, 60 years; less than 5% younger than 40 years, rare cases in children
- Increased incidence in Ashkenazi Jews

**Clinical Features**

**Symptoms**

- Hyperviscosity-related headache, blurry vision
- Arterial thrombosis
- Hemorrhage
- Pruritus provoked by warm water
- Erythromelalgia
- Symptoms related to gout

**Physical Findings**

- Splenomegaly, hepatomegaly
- Plethora

**Prognosis and Therapy**

- Treatment: phlebotomy with or without myelosuppression
- Survival: 15-year survival is 65%
- Prognosis: poor prognosis with history of thrombosis

### POLYCYTHEMIA VERA—PATHOLOGIC FEATURES

**Diagnostic Criteria (WHO, 2008)**

- Diagnose PV when both major criteria and one minor are met or when the first major and two minor criteria are met

**Major Criteria**

- Hemoglobin greater than 18.5 g/dL in men, hemoglobin greater than 16.5 in women, or other evidence of increased red cell volume
- Presence of *JAK2* V617F or other functionally similar mutation, such as *JAK2* exon 12 mutations

**Minor Criteria**

- Bone marrow biopsy specimen showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
- Serum EPO level below the reference range
- Endogenous erythroid colony formation

**Microscopic Features (Polycythemic Phase)**

**Blood**

- Erythrocytosis (normochromic normocytic red blood cells)
- Granulocytes may be elevated
- Platelets elevated in ½ of patients

**Marrow**

- Usually hypercellular, maybe normocellular
- Panmyelosis: increased erythroid and megakaryocytic elements
- Megakaryocytes are large, clustered, but not bizarre
- Fe absent in 95% of cases
- Fibrosis not increased

**Ancillary Studies**

- EPO levels
- Red cell mass measurement (not widely available)
- Cytogenetic analysis: abnormalities in only 10% to 20%; +8, +9, del (20q), del(13p), del(1p)
- Evaluation of exogenous erythroid colony formation (in vitro; not widely available)

**Differential Diagnosis**

- Secondary erythrocytosis owing to appropriate or inappropriate excess EPO
- Spurious erythrocytosis
- Familial erythrocytosis
- Other MPN

disease process. Patients exhibit a hyperviscosity-related headache and blurry vision, or with arterial thrombosis and hemorrhage. There may also be symptoms related to gastrointestinal ulcers or bleeding. Many patients will have splenomegaly and pruritus, which is frequently provoked by warm water; this is referred to as *aquagenic pruritus*. Other common symptoms are those related to gout from hyperuricemia and erythromelalgia, which is reddening and painful swelling usually of the lower extremities.

## Pathologic Features

### Diagnosis

The discovery of an elevated red blood cell count, hemoglobin, or hematocrit first through clinical signs and symptoms and then through laboratory studies is the usual starting point in the diagnosis of PV. The major difficulty in distinguishing PV from reactive or spurious polycythemia has been overcome because *JAK2* mutations are common and sufficient to rule out a reactive process. Distinction of PV from the other MPN with *JAK2* mutations is still usually less of an issue, because the other types of MPN rarely manifest with elevated red cell mass.

The 2008 WHO diagnostic criteria have been revised significantly and are noted in the Pathologic Features box. To make a diagnosis of PV, it is necessary first to identify sufficiently elevated hemoglobin levels, hematocrit, or red cell mass above a set threshold and then demonstrate a normal or low EPO level to prove that

the erythroid proliferation is not related to physiologic causes. Next it is necessary to attempt to demonstrate the *JAK2* V617F or, if not found, a mutation in *JAK2* exon 12. A bone marrow evaluation is usually obtained as a baseline for comparison to future evaluations, or it might be necessary as a diagnostic criterion to illustrate a myeloproliferation if the EPO is not reduced. The criterion of illustrating exogenous erythroid colony formation is appropriate, because it is a direct manifestation of the abnormal TK signaling and fundamental to the disease; however, it is somewhat less relevant, because it is not a test that most laboratories have available. It should be noted that *JAK2* mutation-negative cases can still be diagnosed as PV by meeting the first criterion of illustrating sufficiently elevated hemoglobin and two of the minor criteria, which would likely include appropriate bone marrow findings and reduced EPO levels. It still should be remembered that *JAK2* mutation-negative cases are rare.

#### Blood and Bone Marrow: Polycythemic Phase

In the polycythemic phase, the peripheral blood shows erythrocytosis and the red blood cells are normochromic and normocytic with little poikilocytosis (Figure 17-19). Neutrophils may be elevated and there is often a basophilia. A significant left shift in the granulocytic elements is not common, although some immature cells may be seen. Platelets are elevated in at least half of the patients. The bone marrow cellularity is usually elevated, but some patients could have a normocellular marrow. When the marrow is hypercellular there is usually a panmyelosis, but the increase in erythroid precursors and megakaryocytes is most prominent. The erythropoietic cells are fairly unremarkable; however, the megakaryocytes are atypically large, but show variability in size, and are sometimes clustered around sinuses and close to the bone (Figure 17-20). They do not exhibit the bizarre features of the megakaryocytes seen in PMF, nor the prominent lobulated-nature of those in ET, and they are clearly different from the small hypolobated (dwarf) megakaryocytes in CML. Fibrosis is usually not increased, and stainable iron is low or absent in many if not most patients (95%). If the low iron results in reduced hemoglobin so that the patient does not meet the criterion for increased hemoglobin (hematocrit or red cell mass), the diagnosis is difficult to make and administration of iron supplementation is suggested. Some clinicians may hesitate to take this step. It should be recognized that although the blood and marrow findings are not at all diagnostic in themselves, they can be useful in helping to exclude entities in the differential diagnosis and can help to establish the diagnosis in association with the appropriate clinical and laboratory findings. In addition, although the bone marrow biopsy is not entirely necessary for a firm diagnosis in many cases, it is still useful as a baseline for assessing disease progression in the future.

Patients with *JAK2* exon 12 mutations have a somewhat different appearance in the bone marrow. These

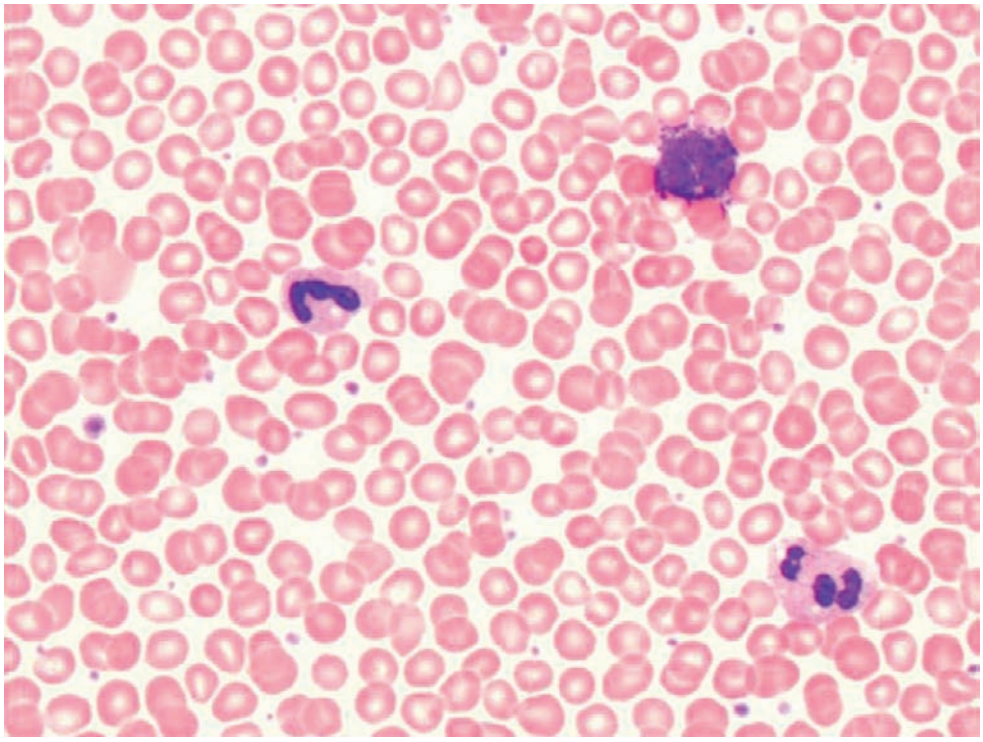

**FIGURE 17-19**
Peripheral blood from patient with polycythemia vera. Note the density of the red blood cells.

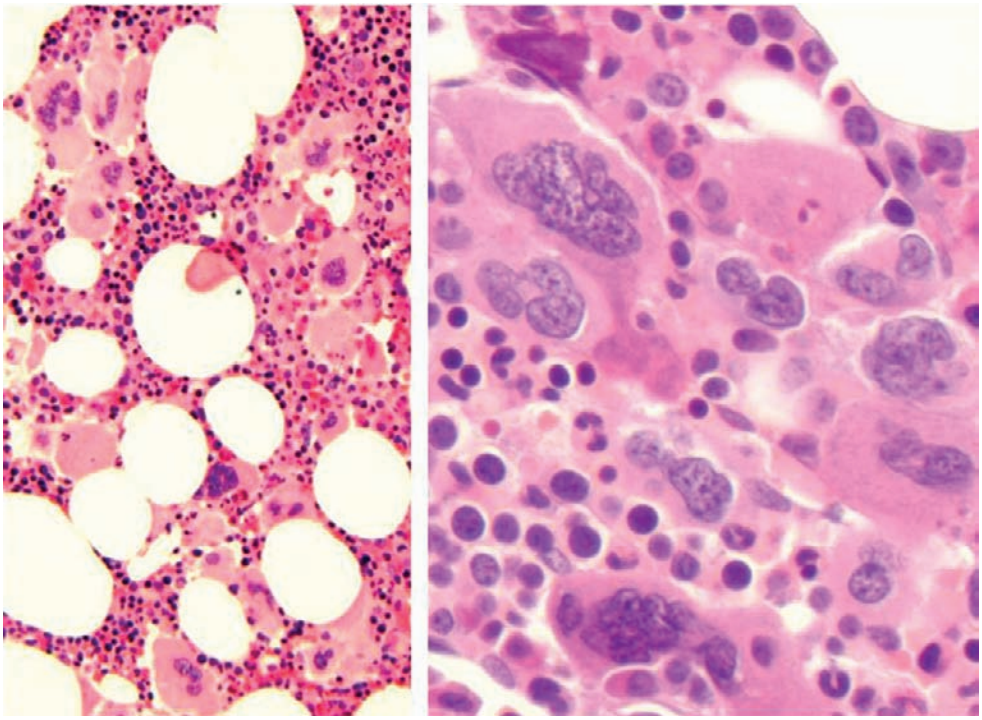

**FIGURE 17-20**
Polycythemia vera. Note the clustered and variably sized megakaryocytes.

patients have been reported to show more prominent erythroid proliferation without involvement of the megakaryocytic and granulocytic cell lines.

#### Molecular

As noted previously, the *JAK2* mutation is critical to the diagnosis in most cases because the *JAK2* V617F mutation is seen in approximately 95% of cases, and exon 12 mutations are seen in approximately 80% of the remaining cases. There are a number of methods available to assess the mutations, but allele-specific PCR is probably the most widely used. In this analysis a mutation specific primer is used in a PCR reaction, with a wild type sequence reverse primer labeled with a fluorescent tag. Because the forward primer is mutation-specific, only mutated DNA will be amplified and detected with the generation of fluorescence signal. The technique is sensitive to approximately 0.1%, but care must be taken not to overinterpret cases with low amplification. In fact, in PV the mutation is commonly homozygous and much higher values would be expected (frequently greater than 50%).

#### Cytogenetics

Although no specific cytogenetic changes are diagnostic of PV and although they occur in only 10% to 20% of cases at the time of diagnosis, their presence confirms clonality of the hematopoietic elements and essentially rules out a reactive condition. Trisomies 8 and 9 (sometime seen together), del(20q), del(13p) and del(1p) are the most frequent findings.

#### Blood and Marrow, Spent Phase, Post-Polycythemic Myelofibrosis

In a later stage of the disease, the red cell mass normalizes and even sometimes decreases. A leukoerythroblastic process is seen in the blood, resembling that associated with PMF (see PMF section below). There are teardrop red blood cells and immature granulocytic forms with nucleated red blood cells in the blood. The marrow becomes increasingly fibrotic and sometimes progresses to collagen fibrosis. Sinusoidal hematopoiesis is common, and osteosclerosis may develop. Immature elements also become more prominent. When patients initially present in the post-polycythemic phase, hemoglobin levels would have become normalized or even reduced. Thus, a diagnosis of MPN, unclassifiable, must be made because distinction from a *JAK2* mutation-positive PMF is not possible. Some patient may develop an acute leukemic transformation; however, this is seen more frequently in patients treated with chemotherapies than without.

### Differential Diagnosis

With the association of *JAK2* mutations in such a high percentage of cases of PV, there are fewer differential diagnostic considerations than in the past when a host

of apparent (spurious), secondary, and congenital causes of polycythemia had to be considered. These diagnoses must still be considered in patients with polycythemia to avoid costly work-up. Apparent polycythemia occurs because of hemoconcentration; secondary causes are hypoxia driven, owing to abnormal production of erythropoietin or to drugs; and the congenital form is sometimes caused by mutations in the erythropoietin gene, but more commonly it is of unknown causes.

The *JAK2* V617F mutation occurs in significant numbers of the other MPNs and in some MDS/MPNs, but these are much less likely to manifest with elevated hemoglobin or hematocrit, which are the key features for considering PV.

### Prognosis and Therapy

Most patients with PV are treated with phlebotomy with or without myelosuppressive agents. The 15-year survival for PV is approximately 65%, and an independent prognostic indicator is whether the patient has a history of thrombosis. Life expectancy is reduced, particularly when a diagnosis is made before the age of 50 years. This reduction is possibly caused by the longer disease course and more time for complications and natural evolution to the fibrotic stage or to acute leukemia.

Trials of TK inhibitor therapy are underway, but it does not appear that they will successfully change the course of the disease. Patients apparently have improvement of symptoms.

## ESSENTIAL THROMBOCYTHEMIA

Essential thrombocythemia (ET) is a myeloproliferative disorder that is largely characterized by a pronounced proliferation of megakaryocytes, resulting in a severe and sustained thrombocytosis, which is also referred to as *thrombocythemia*. However, since most of the other myeloproliferative neoplasms can have markedly elevated platelets and megakaryocytic proliferations, differential diagnostic considerations for the other MPNs are important. The differential diagnosis must also include other rare acute myeloid leukemias, MDS, or MDS/MPNs, associated with increased platelets (most notably AML with t[3;3] or inv[3], the 5q-minus syndrome, and RARS-T), and, of course, reactive conditions leading to elevated platelets.

The underlying pathogenesis of ET is not entirely clear, but in approximately 50% of cases there is a *JAK2* V617 mutation similar to that seen in PV and in PMF. In ET the dosage of the mutation is less and the stem cell affected is believed to be more lineage-restricted. Nevertheless, cases of ET with the *JAK2* mutation are believed to have some similarities to PV, in that there is a more panmyelosis with increased granulopoiesis and erythropoiesis compared to the mutation-negative cases in which thrombopoiesis is most prominent. Clinical differences in mutation-positive versus mutation-negative cases have not been obvious. In less than 1% of cases of ET, there is a mutation of the *MPL* gene, the thrombopoietin receptor (*MPL* W515L/K). However, this mutation is also not specific because it is seen in PMF and with a slightly higher frequency (5% to 9%).

The exact molecular pathology in mutation-positive or mutation-negative cases is not well understood, but it is believed that there is loss of control of the proliferative activity in the megakaryocytes, leading to autonomous platelet production. The megakaryocytes are believed to be hypersensitive to stimulation by one of a number of growth factors such as interleukin 3 or the megakaryocytic growth factor, thrombopoietin (TPO). However, it should be noted that TPO levels are not sufficiently different among ET, other myeloproliferative disorders and reactive conditions; therefore they cannot be used for diagnostic purposes. Mutations in TPO have not been found except in rare familial cases.

### Clinical Features

ET is an uncommon disorder with an incidence of approximately 1 to 2.5 cases per 100,000 persons per year. The median age is approximately 60 years, like in PV, and there may be a slight female predominance. In addition, there is an increased incidence in Ashkenazi Jews. Familial cases are extraordinarily rare, and some have been found to be due to mutations in the TPO gene that results in increased production of TPO.

Clinically, patients are usually asymptomatic and usually come to be evaluated for ET because of an elevated platelet count found on a routine complete blood cell count performed for a well patient check-up. Some patients exhibit symptoms believed to be related to thrombotic occlusion of the microvasculature. Such symptoms include headaches, lightheadedness, blurring vision and scotomata, palpitations, chest pain, and distal paresthesias. Erythromelalgia, characterized by erythema, warmth, and pain in the distal extremities is another symptom that is unusual, but not entirely specific for ET, as it is seen with some frequency in PV. More severe clinical features of ET include large vessel thromboses of either arterial or venous circulation. Pulmonary embolism and deep venous thromboses occur, but thrombotic events in less common sites such as hepatic or portal vein or retinal vein can also develop. Bleeding is another serious clinical manifestation and complication. Despite the high platelet count, patients are at risk for bleeding, which may be due to an acquired

**ESSENTIAL THROMBOCYTHEMIA—FACT SHEET**

**Definition**

- A chronic myeloproliferative neoplasm characterized by pronounced proliferation of megakaryocytes resulting a severe thrombocytosis (thrombocythemia)

**Incidence, Gender, and Age Distribution**

- 1 to 2.5 cases per 100,000 population per year
- Slight female predominance (male:female = 2:1)
- Median age, 60 years
- Increased incidence in Ashkenazi Jews
- Rare familial cases

**Clinical Features**

- Many patients are asymptomatic (one-quarter to one-third)
- Symptoms:
  - Headache, lightheadedness, blurry vision, scotomata, palpitations, chest pain, distal paresthesias, erythromelalgia, symptoms related to large vessel thromboses
  - Spontaneous abortions
- Physical findings: splenomegaly (20% to 50%), hepatomegaly

**Prognosis and Therapy**

- Very good prognosis, generally does not lower life expectancy
- Treatment aims are to lower platelet count and risk for thromboses
- Rare transformation to acute leukemia (1% to 2%)

von Willebrand factor deficiency related to platelet absorption. Hemorrhage can occur in mucocutaneous areas, and more seriously in the gastrointestinal tract. Splenomegaly is not as prominent as in the other MPNs, but it occurs in approximately 3% to 50%. Hepatomegaly is less common.

## Pathologic Features

### Diagnosis

The diagnosis of ET is made by identifying a sustained elevation in the platelet count; performing a bone marrow and identifying marrow findings consistent with the disease; excluding other MPN, MDS, or other myeloid neoplasm associated with elevated platelets; and showing *JAK2* or *MPL* mutations, cytogenetic clonality or in the absence of these, ruling out reactive thrombocytosis. These WHO 2008 criteria are listed in the Pathologic Features box.

The diagnostic criteria have been changed in two major ways compared to the previous criteria from the WHO publication of 2001. The threshold for the platelet elevation in ET has been lowered. In the earlier criteria it was required that the platelet count be 600 $\times 10^9$/L or higher, and in the current criteria the lower level is only 450 $\times 10^9$/L. The change was made in hopes of identifying cases earlier in their course for better intervention, mainly in an effort to avoid serious thrombotic complications that can occur with sustained thrombocytosis.

The second change was the inclusion of the finding of *JAK2* V617F or other clonal markers or in their absence, no cause for reactive thrombocytosis. Although seen in approximately 50% of cases, the presence of the *JAK2* or *MPL* mutation can be a tremendous help in ruling out a reactive process. Other evidence of clonality, such as a cytogenetically identified clonal abnormality, is rare in ET and thus less useful in this regard.

In the absence of the *JAK2* or *MPL* mutation, the diagnosis of ET is exclusionary of reactive conditions causing thrombocytosis and of other clonal myeloid disease with elevated platelets. However, even in the presence of either mutation, the diagnosis is still exclusionary, since other myeloid neoplasms that have *JAK2* or *MPL* mutations and elevated platelets must be ruled out. The exclusionary nature of the diagnosis in either regard makes negative findings critical. The absence of clinical features and situations that can give rise to reactive thrombocytosis, and the absence of clinical, morphologic, and cytogenetic or molecular features of the other myeloid neoplasms that can have elevated platelets play as important a role in making the diagnosis as the presence of positive criteria.

### Blood and Bone Marrow

The peripheral smear in ET shows marked thrombocytosis with a significant size variation (anisocytosis) of the platelets. Some giant platelets may be present (Figure 17-21). The platelets are usually fairly normally granulated. White blood cells are usually normal in number, with no left shift, and there is no dysplasia. There is usually no absolute or relative basophilia. Red blood cells are normocytic and normochromic, except in patients with significant hemorrhage and iron deficiency, in which case they may be hypochromic and microcytic. Red cell morphology should be otherwise unremarkable. A rare teardrop form and a mild leukoerythroblastic picture are reasons to consider PMF rather than ET.

The bone marrow is generally moderately hypercellular. Megakaryocytes are prominent and have a particular morphology that can help to distinguish them from those seen in PV, PMF and CML. The megakaryocytes are distributed throughout the marrow and are usually only slightly clustered, unlike the more intense clustering and perisinusoidal and paratrabecular distribution in PV. The megakaryocytes are generally large and have abundant cytoplasm frequently with cells within the cytoplasm (emperipolesis); however, the

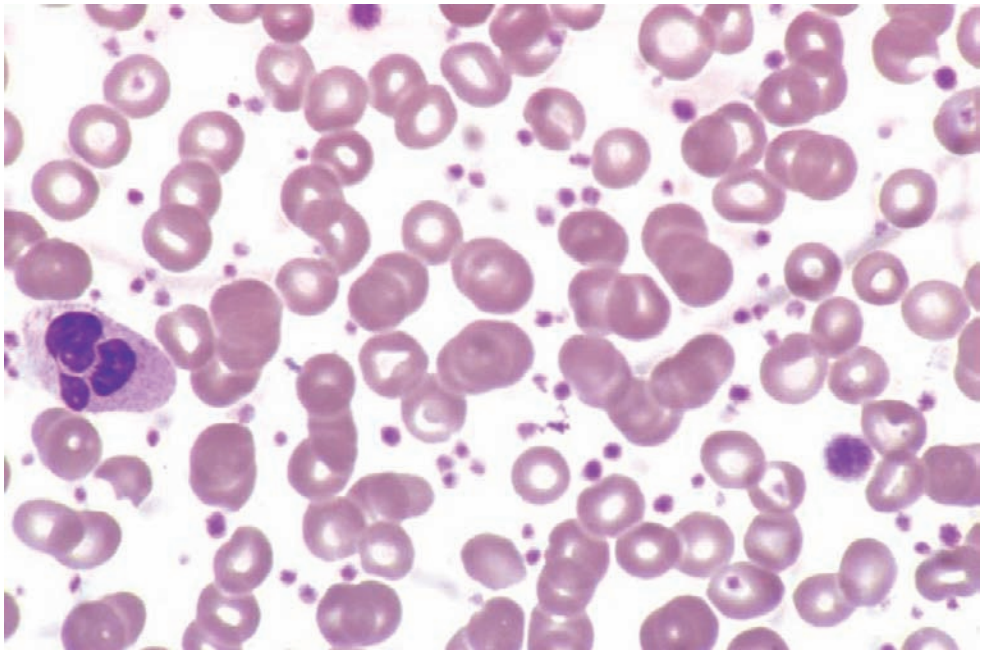

**FIGURE 17-21**
Elevated platelet count in essential thrombocythemia. The platelets exhibit moderate anisocytosis with occasional giant forms.

**ESSENTIAL THROMBOCYTHEMIA—PATHOLOGIC FEATURES**

**Diagnostic Criteria (WHO, 2008)**

- All four criteria must be met:
  - Sustained platelet count of 450 × $10^9$/μL
  - Bone marrow biopsy specimen showing proliferation mainly of megakaryocytes that are large and mature; no increase or left shift in granulocytes or erythroid elements
  - Not meeting criteria for PV, PMF, CML, MDS or other myeloid neoplasm
  - Demonstration of *JAK2* V617F or other clonal marker, or in the absence of *JAK2* V617F, no evidence of reactive thrombocytosis

**Microscopic Findings**

**Blood**

- Marked thrombocytosis with platelet anisocytosis
- Normal white blood cell count, no dysplasia
- Normal red blood cell morphology

**Marrow**

- Moderately hypercellular, in general
- Prominent megakaryocytic proliferation throughout, little clustering, large size, sometimes hyperlobulated (staghorn-like), not bizarre
- Minimal granulocytic proliferation
- Absent to minimal reticulin fibrosis
- Fe present

**Differential Diagnosis**

- Reactive thrombocytosis
- Early PV or early phase of PMF
- MDS associated with increased platelets
- CML with increased platelets
- MDS/MPN

latter finding is not diagnostic, because it is typical even in normal megakaryocytes. The nuclei are highly lobulated, a feature that has led some to refer to them as *staghorn-like* (Figure 17-22). Bizarre nuclear forms with hypercondensed chromatin are not as prominent as they are in PMF, and small megakaryocytes with hypolobated nuclei, typical of CML, are not seen. Granulocytic proliferation is usually absent or only minimal, and there is no left shift or increase in blasts. The mature granulocytes do not show dysplasia. Erythroid activity may be increased. Reticulin fibrosis may be minimally increased, but significant fibrosis should suggest another diagnosis. Stainable iron is usually present. When iron is absent, the possibility of PV should be considered. The absence of iron can mask PV by causing a reduction in erythropoietic activity and an increase in platelets, causing it to resemble ET. Some suggest that a trial of iron should be instituted in such patients.

## Ancillary Studies

Molecular analyses for *JAK2* V617F and *MPL* M515K/L are particularly helpful in ruling out a reactive process, as discussed previously. Like for PV, allele-specific PCR is one of the more common approaches to demonstrating the mutations. Other approaches include DNA melting curve analysis, pyrosequencing, and direct DNA sequencing. In ET the allelic burden of the *JAK2* mutation may not be as high as in PV, because in PV the mutation is frequently homozygous.

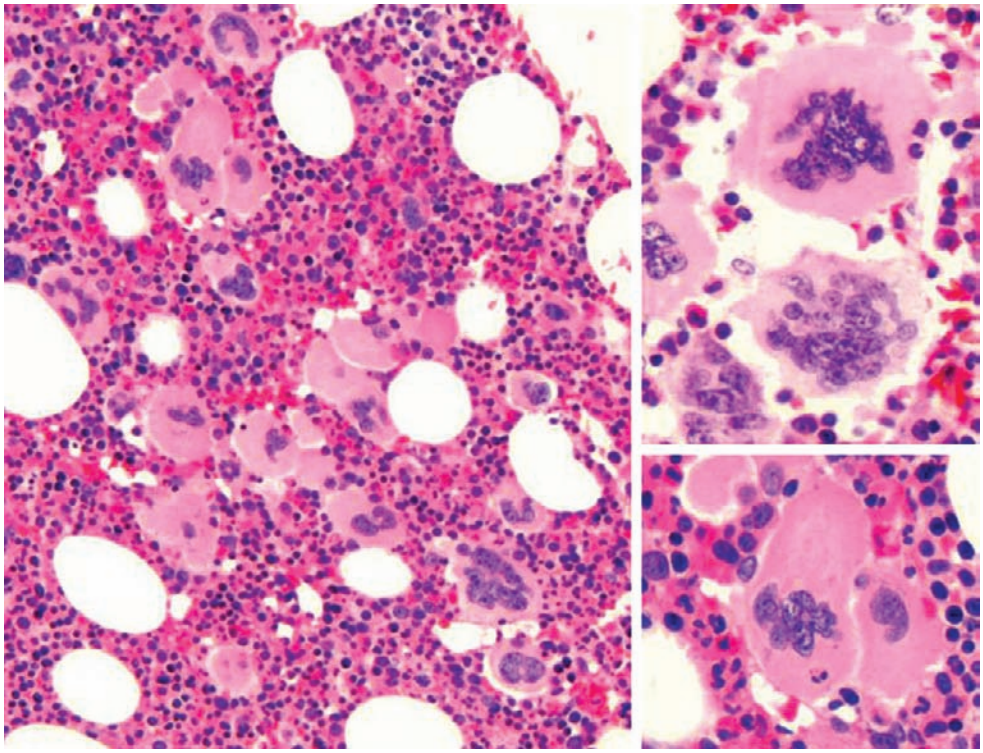

**FIGURE 17-22**

Essential thrombocythemia bone marrow. Note the large megakaryocytes with abundant cytoplasm and prominent nuclear lobulation.

Ruling out reactive conditions can be aided by evaluating for elevated C-reactive protein (C-RP), as increased levels may suggest an underlying reactive inflammatory process. However, an elevation of C-reactive protein does not entirely rule out ET. Immunohistochemical staining for MPL has been suggested as a means for helping to distinguish ET from a reactive process. In ET, MPL expression is low and exhibits a heterogeneous pattern in the megakaryocytes, whereas in reactive conditions there is normal expression homogeneously within the megakaryocytic elements. Reduced MPL expression is not diagnostic of ET, because it can be seen in other MPNs.

Cytogenetic analysis does not add much in resolving a differential diagnosis. Cytogenetic clones are rare in ET, as they are found in only 5% to 10% of cases. The cytogenetic findings can be used to support the diagnosis of a clonal MPN over a reactive thrombocytosis, but the genetic changes are nonspecific and do not help with cases that are difficult to distinguish from PV or early PMF. The abnormalities seen in ET include +8, +9, and del(13q). The finding of t(3;3) or inv(3) or of del(5q) should suggest AML or MDS associated with increased platelets. Of course the finding of t(9;22) in a case of suspected ET should permit the correct diagnosis of CML. Some patients with CML can have markedly elevated platelets, and some can have the p230 *BCR-ABL1* protein.

## Differential Diagnosis

As mentioned previously, the differential considerations must include reactive causes of thrombocytosis, other myeloproliferative diseases, or cases of AML, MDS, MPN, including CML, or MDS/MPNs associated with increased platelets. The most difficult differential is between ET and the prefibrotic phase of PMF (see PMF section below), and frequently this cannot be adequately resolved.

Reactive conditions causing thrombocytosis include infection, inflammatory diseases, blood loss and chronic iron deficiency, malignancy, trauma and surgery (especially splenectomy), and rebound following chemotherapy or replacement therapy for $B_{12}$ or folate deficiency. Reactive conditions are more frequently associated with elevated acute phase reactants like C-reactive protein. The reactive conditions should not be persistent or associated with splenomegaly, and they are not likely associated with a history of thrombotic episodes.

The myeloid disorders that are *JAK2* mutation negative but that can have elevated platelets are common, but they usually have some features that allow them to be recognized when considering ET in the differential diagnosis. For example, CML can frequently develop with thrombocytosis, but it shows the full spectrum of myeloid proliferation, with a myelocyte bulge and

basophilia in the peripheral blood that is usually quite distinctive and not to be expected in ET. In addition, the small, dwarf megakaryocytes in the bone marrow are also distinguished easily from the larger staghorn-like megakaryocytes in ET. Even in cases with prominent thrombocytosis and less easily distinguished blood or marrow morphology, the t(9;22) or *BCR-ABL1* will clarify any dilemma. In the older literature there is mention of t(9;22) or *BCR-ABL1*$^+$ cases of ET; however, these are now included as CML. Some cases might have been CML with p230 *BCR-ABL1* protein, which because of markedly elevated platelets can mimic ET, as mentioned previously.

The myelodysplastic disorder referred to as the *5q-minus syndrome* can develop with increased platelets, but typically the megakaryocytes are small, hypolobated, and distinctive from the large, hyperlobulated megakaryocytes seen in ET. In addition, the del(5q) would be unlikely seen in ET. AML can sometimes develop with elevated platelets; this is a feature of AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2) or in some megakaryoblastic leukemias. In addition to the abnormal cytogenetic finding, these patients will have elevated blasts and usually highly dysplastic megakaryocytes, including the classic micro-megakaryocytes that make them easily distinguishable from ET.

The other MPNs or MDS/MPNs with *JAK2* mutations that must be considered in the differential diagnosis include PV, RARS-T, and PMF; however, even these have features that make the distinction possible. The high hemoglobin–hematocrit of PV, the dysplasia and ring-sideroblasts of RARS-T, and the fibrosis of the fibrotic phase of PMF all provide ample clues of ET. Only the prefibrotic phase of PMF lacks specific clinical morphologic or molecular features that allow for a distinction; this is discussed in the PMF section below.

### Prognosis and Therapy

The prognosis in ET is good, and in the first decade the disease does not lower life expectancy. Treatment is aimed at lowering platelet counts and lowering the risk for thromboses. Acute leukemic transformation in ET is rare, occurring in less than 5%. Some of the cases, as in PV, may actually be t-AML owing to previous cytotoxic therapy.

A post-ET myelofibrotic transformation has been described, but it is difficult to determine whether these cases were misdiagnosed ET and actually the prefibrotic phase of PMF that naturally would proceed to fibrosis. However, in cases in which fibrosis occurs late in the disease course, many years or even decades after the initial diagnosis, this argument may not hold and may justify the existence of this transformation.

## PRIMARY MYELOFIBROSIS

PMF has been previously referred to by a number of terms, the most common of which include Agnogenic myeloid metaplasia, myelosclerosis with myeloid metaplasia (MMM) and idiopathic myelofibrosis. The WHO committee writing on hematopoietic tumors in 2001 developed the then recommended term of chronic idiopathic myelofibrosis (CIMF), but in keeping with the frequent name changing, altered it again in 2008 to PMF. The disorder is probably the most aggressive of the three common *BCR-ABL1*-negative MPNs (PV, ET, and PMF), because it is characterized by a bone marrow that becomes progressively fibrotic and even osteosclerotic; this results in a leukoerythroblastic process in the blood, marked extramedullary hematopoiesis with extensive hepatosplenomegaly, and in marrow failure. Sometimes there is also a transformation to acute myeloid leukemia. This transformation occurs in approximately 5% to 30% of cases, whereas the burnt-out phase of marrow fibrosis occurs in the majority. Although best characterized by this progressive fibrosis and osteosclerosis, the process likely begins with a more cellular phase with a proliferative process including a prominent megakaryocytic proliferation, which can be difficult or impossible to distinguish from ET and sometimes from PV. This cellular or prefibrotic phase frequently goes unnoticed as more patients (approximately 60% to 70%) are seen in the fibrotic phase.

The pathogenesis of PMF is probably the least understood when compared to the other types of MPN. Hematopoiesis (including lymphoid lineage cells in some cases) is clonal, but the proliferating fibroblasts, which play a major role in the pathology, are not. The exact nature of what is believed to be a "cytokine storm" responsible for the fibroblastic, osteoblastic, myeloid, and even vascular proliferation (leading to a neoangiogenesis), is not known, although in about 50% of cases there is a *JAK2* V617F mutation and in another 5% to 9% a mutation of *MPL* (*MPL* W515K/L). Some of the growth factors responsible for the disease are from the abnormal megakaryocytes, but others are likely from monocytes and macrophages. Growth factor pathway abnormalities have been detected, but they are not unique to PMF. CD34 cells, which are increased in the blood, may have a point mutation in the stem cell factor *KIT*. Serum VEGF is increased in most patients. Expression of the basic fibroblastic growth factor is increased, and transforming growth factor β, a negative regulated of hematopoiesis, is decreased. In addition, MPL is incompletely glycosylated and poorly expressed on platelets, megakaryocytes, and the stem cells. As mentioned previously, reduced expression of MPL is also seen in ET and in PV, and it is not unique to PMF. The extramedullary hematopoiesis in PMF is probably derived from marrow progenitor cells taking residence in the spleen, and not from reactivation of fetal splenic hematopoiesis as believed previously.

**PRIMARY MYELOFIBROSIS—FACT SHEET**

**Definition and Other Names**

- A progressive myeloproliferative neoplasm arising in a pluripotential hematopoietic progenitor, which is characterized by a proliferation of megakaryocytic and granulocytic elements and associated with marrow fibrosis
- Marrow fibrosis results in prominent extramedullary hematopoiesis and is commonly a progressive neoplasm with resulting marrow failure, in addition to transformation to acute leukemia
- Myelofibrosis with myeloid metaplasia, agnogenic myeloid metaplasia, chronic idiopathic myelofibrosis

**Incidence, Gender, and Age Distribution**

- 0.5 to 1 per 100,000 population per year
- Equal sex distribution
- Median age, 54 to 62 years
- Increased incidence in Ashkenazi Jews

**Clinical Features**

- Symptoms:
  - Thirty percent to 40% of patients are asymptomatic
  - Weight loss, constitutional symptoms
  - Symptoms related to anemia, splenomegaly, gout, renal stones
- Physical findings: splenomegaly, often massive

**Prognosis and Therapy**

- Progressive disease
- Poor prognosis: age (>70 years), low hemoglobin level, abnormal karyotype
- Accelerated phase when blasts are 10% to 19%
- Transformation to acute leukemia in 5% to 30% of cases
- Mean survival, 3 to 5 years from diagnosis
- No effective treatment

## Clinical Features

The incidence of PMF is approximately 0.5 to 1 per 100,000 persons. The average age is between 54 and 62 years, with an equal sex distribution. Occurrence is rare in children, and there is an increased incidence in Ashkenazi Jews.

Clinically, many patients are asymptomatic (30%). For those with symptoms, complaints are related to anemia, splenomegaly or constitutional symptoms. Other symptoms such as weight loss or gouty arthritis and renal stones from hyperuricemia may also be present.

## Pathologic Features

### Diagnosis, General

The diagnosis of PMF is fairly straightforward in the fibrotic phase, because the blood findings together with the bone marrow findings are distinctive and rarely seen in other entities. The prefibrotic phase is diagnostically difficult, but some morphologic clues have been developed. The WHO criteria are given in the Pathologic Features box that follows. The features include meeting three major criteria (including appropriate bone marrow findings, excluding other MPNs and other myeloid neoplasms, and demonstrating clonal markers or excluding other causes of marrow fibrosis) and two of four minor criteria (including finding leukoerythroblastosis, increased LDH, anemia, or splenomegaly). As with ET, the criteria are partially exclusionary in nature, indicating the importance of excluding other myeloid neoplasms (most notably CML, PV, and MDS) and excluding other causes of fibrosis.

### Diagnosis: Prefibrotic Phase

#### Blood

A minority of patients are first seen in the prefibrotic phase, which can present a diagnostic challenge. There will be a modest anemia and the white blood cell count will be moderately elevated. Platelets are often significantly elevated, with a mean platelet count of approximately $900 \times 10^9$/L. The more classic features of teardrop red blood cells and leukoerythroblastosis are usually absent, although if seen they can be of great help diagnostically.

#### Bone Marrow

The bone marrow is usually hypercellular and reticulin fibrosis is minimal if present at all. There is a proliferation of granulocytic and megakaryocytes (Figure 17-23). Blasts are not increased. The megakaryocytes might give a clue to the diagnosis because as they are clustered, and more importantly, atypical or even bizarre. The megakaryocytes are of variable size and have abnormal nuclei with disorganized lobulation and hypercondensed nuclear chromatin. Bare megakaryocyte nuclei might also be seen. Vascular proliferation may be present, and some cases may have reactive lymphoid nodules.

Some morphologic clues to help distinguish prefibrotic PMF from ET include cellularity (higher in PMF), megakaryocyte clustering (more prominent in PMF with tighter clusters), megakaryocytic size, shape, and maturation (more varied and defective in PMF), and background hematopoiesis (more granulocytic predominant in PMF; Figure 17-24, Table 17-1).

### Diagnosis: Fibrotic Phase

#### Blood

Most patients (approximately 70%) are in the fibrotic phase, at which time both the marrow fibrosis and extramedullary hematopoiesis are significant enough to produce findings that are characteristic and diagnostic of the process. Patients have mild leukocytosis, an anemia, and moderate thrombocytosis. The peripheral

## PRIMARY MYELOFIBROSIS—PATHOLOGIC FEATURES

### Diagnostic Criteria (WHO, 2008)

- Requires meeting all three major and two minor criteria

### Major Criteria

- Presence of megakaryocytic proliferation usually accompanied by reticulin or collagen fibrosis or in the absence of fibrosis the megakaryocytes must be accompanied by an increased marrow cellularity, characterized by granulocytic proliferation, and often decreased erythropoiesis
- Not meeting criteria for PV, CML, MDS or other myeloid diseases
- *JAK2* V617F or other clonal marker or in the absence of a clonal marker, no evidence that the marrow fibrosis or other changes are secondary (infection, autoimmune, chronic inflammation, hairy cell leukemia, other malignancies including metastatic tumor, or due to toxic myelopathies)

### Minor Criteria

- Leukoerythroblastosis
- Increased serum lactate dehydrogenase
- Anemia
- Splenomegaly

### Microscopic Features

#### Prefibrotic Phase

- Blood
  - Moderately elevated WBC count
  - Modest anemia
  - Elevated platelets, up to 900 × $10^9$/L
  - No teardrop cells, no leukoerythroblastic picture
- Marrow
  - Hypercellular
  - Proliferation of granulocytes and megakaryocytes
  - Megakaryocytes may be clustered or atypical
  - No significant fibrosis

#### Fibrotic Stage

- Blood
  - Mild leukocytosis
  - Anemia
  - Moderate, but variable thrombocytosis
  - Leukoerythroblastic picture
  - Relatively few blasts
  - Teardrop red blood cells (dacryocytes), other poikilocytic changes
- Marrow
  - Inaspirable, increased fibrosis progressing to collagen fibrosis
  - Osteosclerosis (sometime severe)
  - Progressive decreased in cellularity
  - Atypical megakaryocytic proliferation with bizarre features, hyper-condensed chromatin, naked megakaryocyte nuclei
  - Blasts less than 20%
  - Sinusoidal (extramedullary) hematopoiesis

### Differential Diagnosis

- Prefibrotic stage: difficult may not be resolvable; early PV, early ET
- Fibrotic stage
  - Myeloid disorders causing marrow fibrosis: postpolycythemic myelofibrosis, CML in accelerated phase, MDS with fibrosis, AML with fibrosis (including acute panmyelosis with myelofibrosis), mast cell disease
  - Other hematopoietic disorders causing marrow fibrosis: Hodgkin lymphoma, non-Hodgkin lymphoma, hairy cell leukemia
  - Other conditions causing marrow fibrosis: metastatic disease, certain infections, autoimmune myelofibrosis

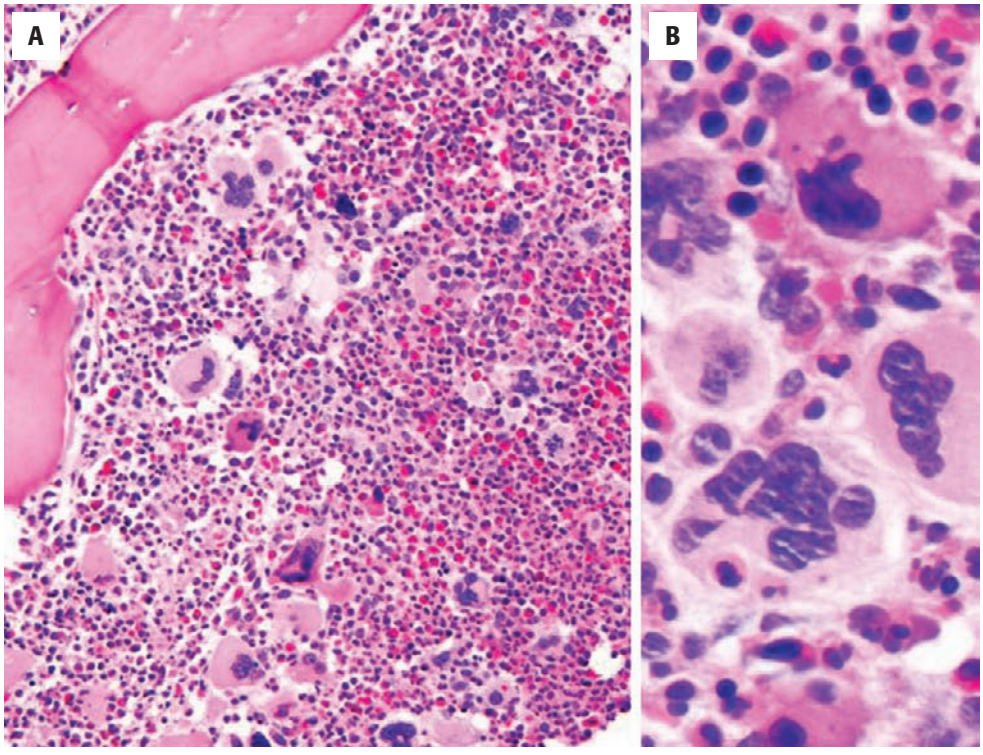


**FIGURE 17-23**

Primary myelofibrosis in the cellular phase. There is a granulocytic and megakaryocytic proliferation, but no fibrosis. Note the atypical megakaryocytes with hypercondensed nuclear chromatin. Diagnosis is difficult in such cases because the distinction from early polycythemia vera and essential thrombocythemia is difficult, if not impossible.

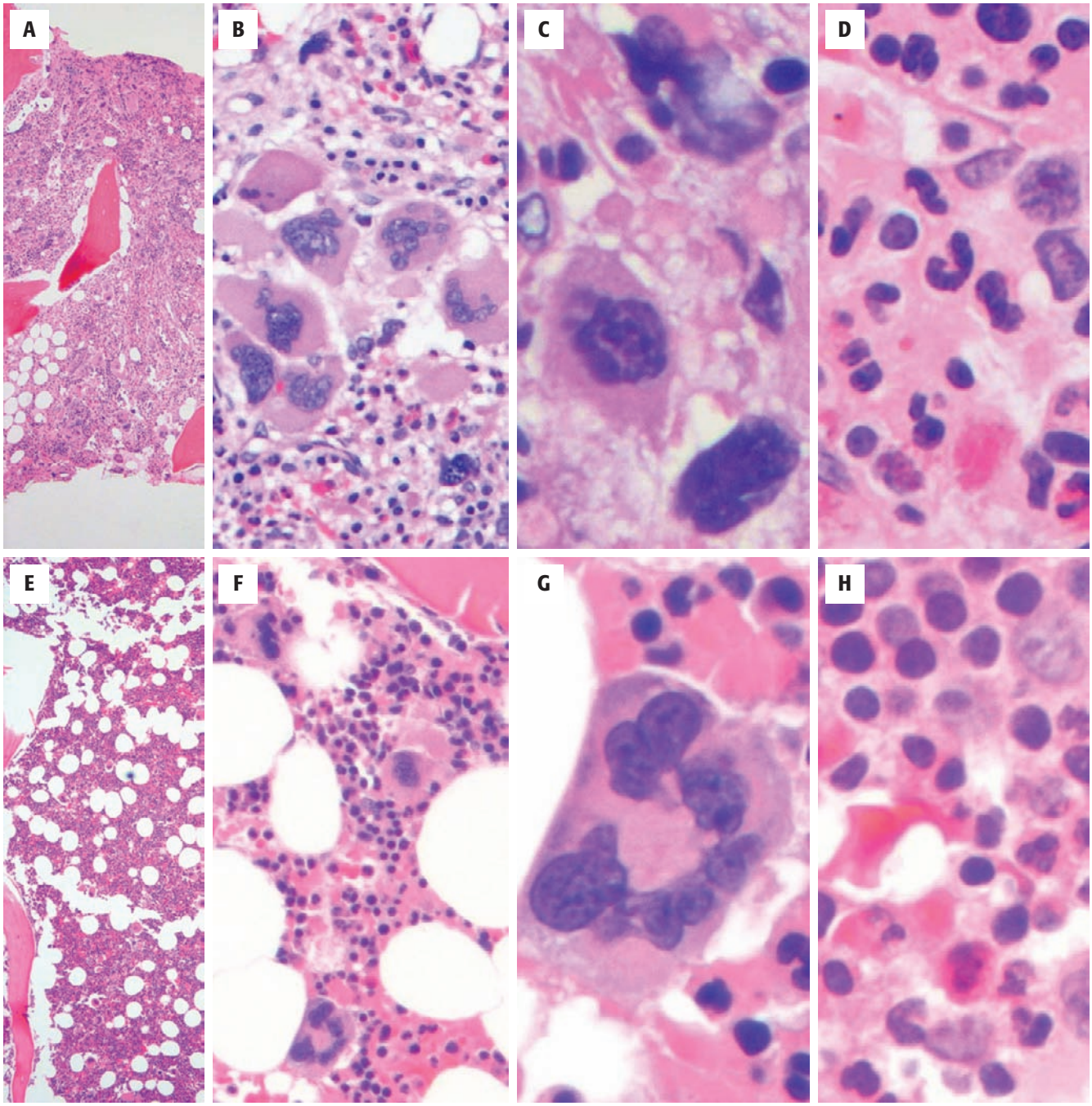


**FIGURE 17-24**

Some morphologic clues to distinguish the prefibrotic phase of primary myelofibrosis **(A-D)** from essential thrombocythemia **(E-H).** In prefibrotic primary myelofibrosis **(A-D),** the cellularity is usually high, the megakaryocytes are more tightly clustered, and they exhibit maturation abnormalities and nuclear atypia. The background hematopoiesis in prefibrotic primary myelofibrosis is also usually more granulocytic.

**TABLE 17-1**

**Morphologic Features Useful in Distinguishing Prefibrotic PMF from ET**

| Feature | Prefibrotic PMF | ET |
|---|---|---|
| Cellularity | Higher | Normal |
| Megakaryocyte clustering | Tighter clusters | Less clustering |
| Megakaryocyte size, maturation | Variable, small to large, immature to mature | Mostly large, mature |
| Megakaryocyte nuclear shape | Variable, bizarre | Hyperlobulated, staghorn |
| Background | Predominant granulocytic | Mixed |

*ET,* Essential thrombocythemia; *PMF,* primary myelofibrosis.

blood findings are fairly classic, with a leukoerythroblastic picture showing numerous teardrop red blood cells, immature granulocytes and nucleated red blood cells (Figure 17-25). The red blood cells can show significant poikilocytosis with numerous ovalocytes in addition to the dacryocytes or tear drop forms. Circulating myeloblasts are not uncommon and usually account for a low percentage of the cells. When blasts increase to greater than 10%, the process can be considered in the accelerated phase as mentioned later. Platelets can be significantly elevated, but the range is great. Some of the platelets may be large giant forms.

### Bone Marrow

In the fibrotic phase, the bone marrow is usually inaspirable because of increased reticulin fibrosis (Figure 17-26) and possibly collagen fibrosis. The cellularity varies from more cellular to hypocellular as the disease progresses. Atypical megakaryocytes are prominent and are present in sizable clusters; they are large and have large, bizarre nuclei frequently with hypercondensed nuclear chromatin (Figure 17-27). Naked megakaryocyte nuclei may be prominent. Myeloid and erythroid elements are fairly unremarkable, but may be decreased as the disease progresses. Blasts can be increased but should be less than 20%. Sinusoidal hematopoiesis is present and is a feature that some say should be present in all cases. It is fairly characteristic but not diagnostic in itself. It is identified by megakaryocytes or other hematopoietic elements within dilated sinuses.

## Ancillary Studies

Molecular analysis for *JAK2* V617F and *MPL* W515K/L is useful because the finding can be used to rule out a reactive cause of marrow fibrosis. These mutations are seen in approximately 50% and in 5% to 9% of cases, respectively.

Cytogenetic abnormalities are detected in 35% to 60% of cases of PMF, but they are not diagnostic and overlap with those seen in the other common non-CML MPNs. Although not diagnostic, they too can be important in ruling out reactive causes of marrow fibrosis. The more common findings include 20q-, 13q-, +8, +9, 12p- and abnormalities of chromosomes 1 and 7.

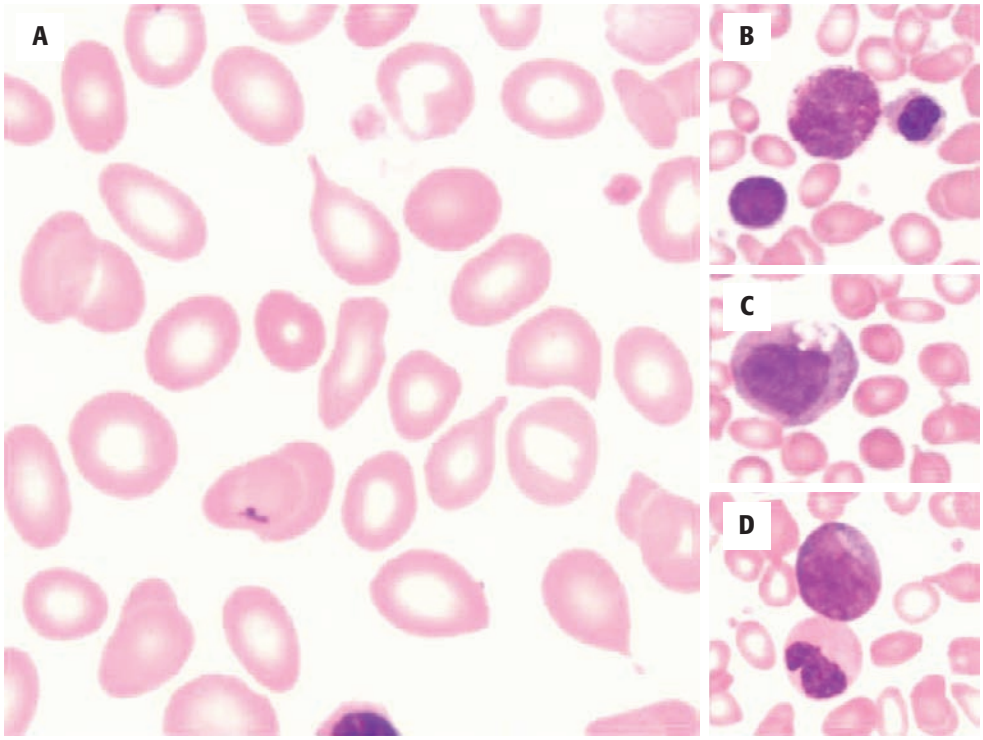


**FIGURE 17-25**

Peripheral blood smear from a patient with primary myelofibrosis. Numerous teardrop forms **(A)** are seen, and there is a leukoerythroblastic picture with nucleated red blood cells **(B),** immature granulocytes **(C),** including occasional circulating blasts **(D).**

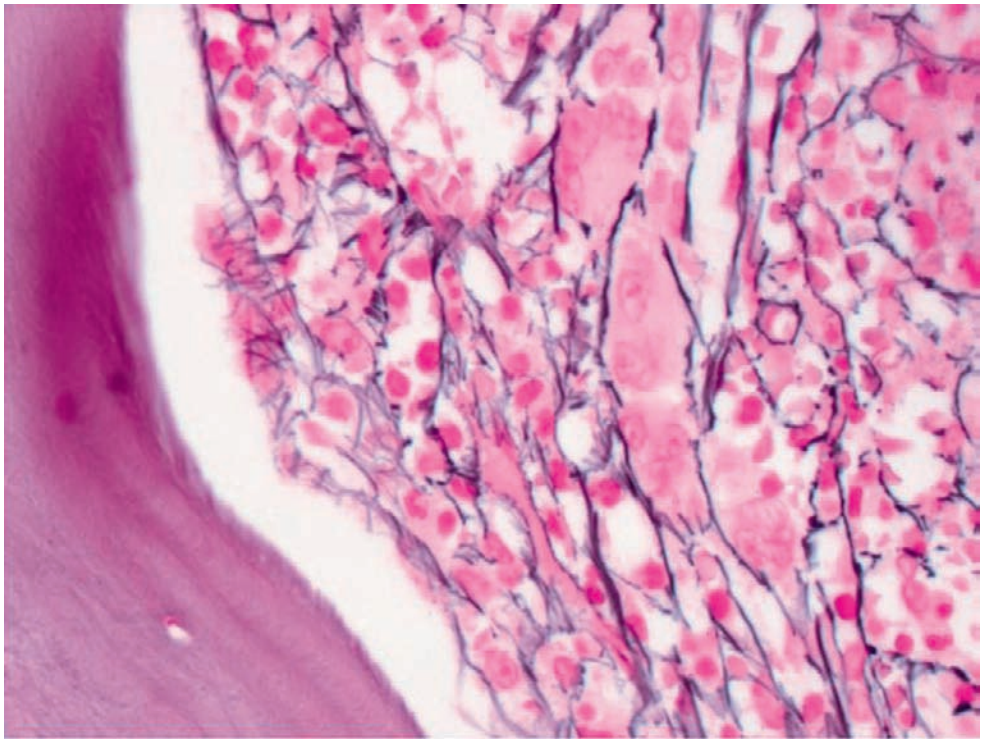

**FIGURE 17-26**

Reticulin stain in a case of fibrotic stage of primary myelofibrosis. Note the markedly increased reticulin fibrosis, particularly around megakaryocytes.

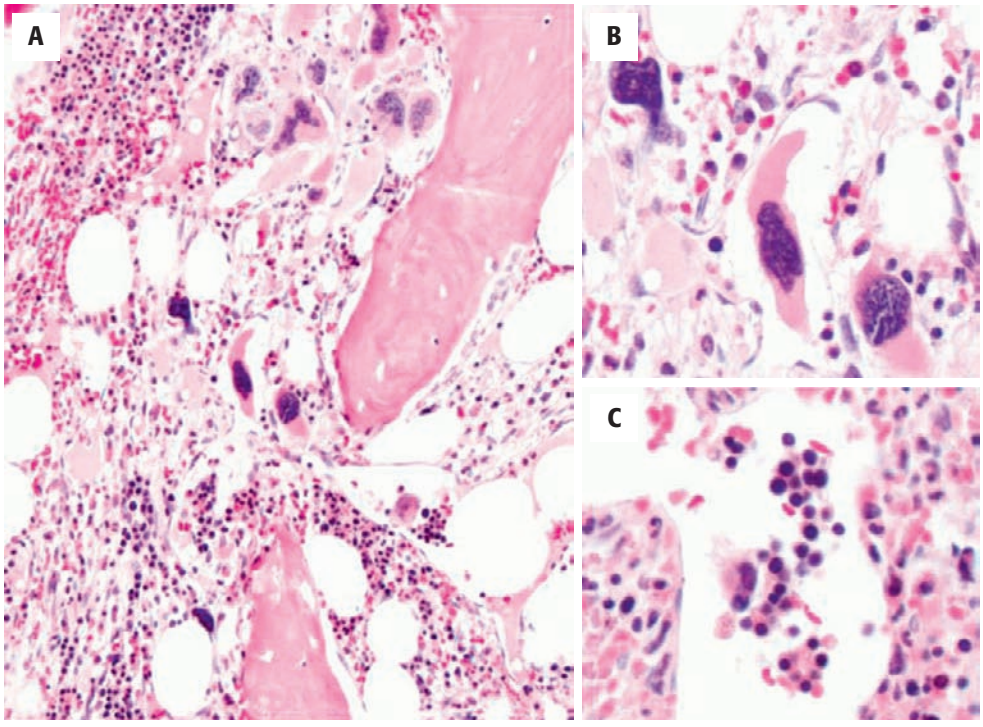


**FIGURE 17-27**

Fibrotic stage of primary myelofibrosis, bone marrow biopsy. **A,** Note the swirling effect of cells owing to underlying fibrosis. **B,** Megakaryocytes are atypical with hyperchromatic, almost pyknotic nuclei. **C,** Sinusoidal hematopoiesis is frequently if not always seen.

## Differential Diagnosis

The diagnosis in the prefibrotic phase can be difficult, especially if there are fewer abnormal megakaryocytes. The diagnosis of an MPN can usually be suggested because the cellularity is high for the patient's age, and there is a granulocytic and megakaryocytic proliferation. A high platelet count might suggest ET, but the megakaryocyte morphology differs, in that the megakaryocytes in ET are consistently larger, whereas those of PMF are variable in size and more bizarre.

Differential considerations of the pre-fibrotic stage include ET and PV, as discussed previously. Frequently the differential cannot be resolved, and only time and the development of fibrosis can resolve the issue. Until more successful therapy is developed, the inability to resolve this differential diagnostic problem remains more important for prognosis. Differential diagnostic considerations of the fibrotic stage include any disorders that cause marrow fibrosis, including postpolycythemic myelofibrosis, CML in accelerated phase, MDS with fibrosis, mast cell disease, and AML with fibrosis, including acute panmyelosis with myelofibrosis. The latter can be distinguished from PMF. In acute panmyelosis with myelofibrosis, there is an absence of both teardrop cells and a leukoerythroblastic picture in the blood because of the abrupt onset of the process. Other causes of marrow fibrosis must also be considered, including non-Hodgkin lymphoma, Hodgkin lymphoma, hairy cell leukemia, metastatic disease, and certain infections and autoimmune myelofibrosis. These causes are usually not a significant challenge because other malignant cells are usually present within the fibrosis, or large atypical megakaryocytes and sinusoidal hematopoiesis are lacking and make PMF less likely.

## Prognosis and Therapy

Survival times in PMF vary significantly; however, mean survival is usually 3 to 5 years from the time of diagnosis. Poor prognostic indicators include age greater than 70 years, anemia, and possibly abnormal karyotype. Deletion of 20q and 13q might be more specifically associated with poor outcome than some of the other associated abnormalities.

Whether the patient is in the prefibrotic or fibrotic stage, the disease is chronic and progressive. Over time, the marrow becomes more fibrotic and can become osteofibrotic with a marrow space "wiped out" by proliferating bone (Figure 17-28). The cellularity tends to

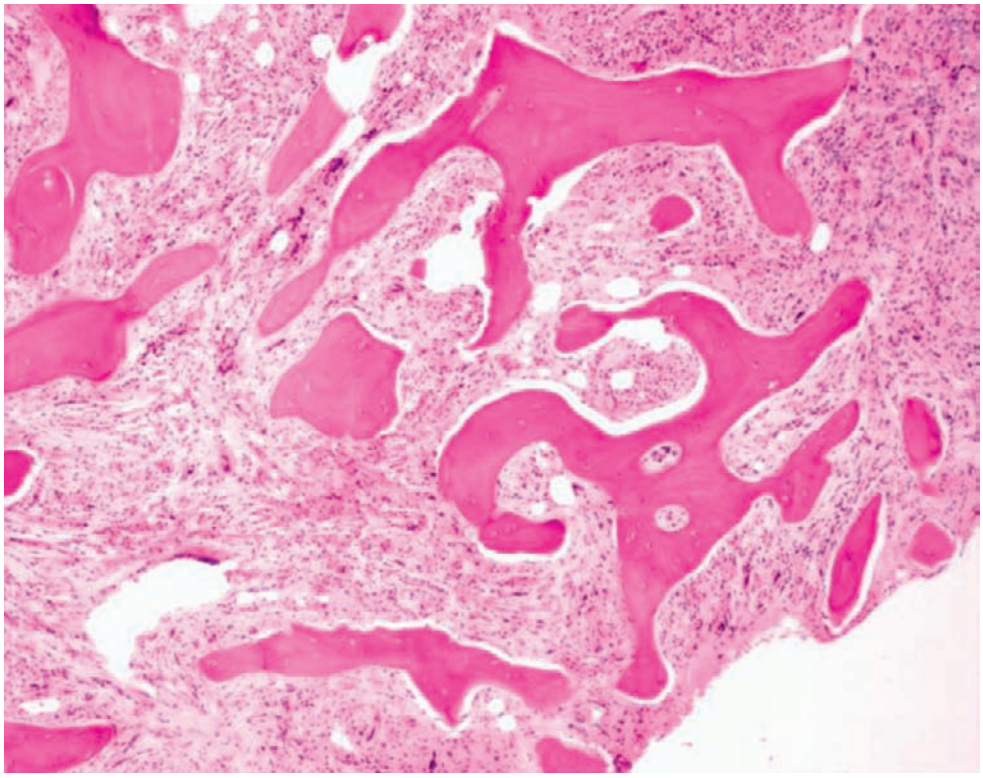

**FIGURE 17-28**

End-stage primary myelofibrosis with prominent osteosclerosis. Note the fibrotic marrow space with depletion of marrow elements.

reduce as the fibrosis increases, and this can be prominent and lead to a burnt-out appearance. Some patients will develop progression with myelodysplasia and an increase in blasts. When the blast count in the blood or marrow reaches 10% to 19%, the process should be considered in accelerated phase, which can signify impending acute disease. A transformation to acute leukemia ensues in approximately 5% to 30% of cases; this is higher than in PV or ET. The acute process is always myeloid and sometimes resembles acute megakaryoblastic leukemia, although acute disease with features of AML with minimal differentiation or AML with maturation is also common. Acute transformation in PMF is associated with dismal outcome and a life expectancy of only a few months.

## CHRONIC NEUTROPHILIC LEUKEMIA

### Clinical Features

CNL is an uncommon disorder with fewer than 150 cases reported in the literature. The disorder is characterized by a persistent neutrophilia in the blood and marrow associated with some infiltration of mature neutrophils into the tissues. It has been surrounded by some controversy as to whether it is truly a neoplastic and clonal disease. Many of the cases reported have been associated with concurrent plasma cell dyscrasias such as multiple myeloma, and some of the neutrophilic proliferations are believed not be clonal but secondary to cytokines produced from the plasma cells. Other cases have been shown to be clonal disorders, and some believe that these are the only true forms of CNL. There is no associated molecular abnormality associated with the leukemia.

Clinically, the leukemia occurs in older patients with a 2:1 male to female distribution. Patients frequently have splenomegaly, and some also have enlarged livers. Bleeding from mucous membranes is common, but bleeding elsewhere can also occur, as can infectious complications. Rarely, patients have been reported with chloromas or extramedullary tumor of mature neutrophils.

**CHRONIC NEUTROPHILIC LEUKEMIA—FACT SHEET**

**Definition**
- A chronic leukemic process characterized by a proliferation of mature clonal neutrophils in blood and marrow, and often infiltrating into tissues

**Incidence, Gender, and Age Distribution**
- Rare, fewer 150 cases reported
- Occurs in elderly
- Male : female = 2:1

**Clinical Features**
- Splenomegaly
- Bleeding
- Infectious complications

**Prognosis**
- Median survival less than 2 years
- Transformation to acute leukemia uncommon

### Pathologic Features

Patients with CNL must have a sustained neutrophilia greater than $25 \times 10^9$/L. The neutrophils of the segmented and band stages account for greater than 80% of the blood cells with no further left shift. The neutrophils characteristically have toxic granulation with Döhle bodies and some tendency for hypersegmentation (Figure 17-29). Outright dysplasia is not seen. Red blood cells and platelets are relatively normal. The bone marrow is hypercellular because of a proliferation of mature neutrophils. Blasts are not increased as they typically account for less than 5% of the marrow elements. Erythropoiesis and megakaryocytopoiesis are usually normal, and reticulin fibrosis is not increased. Evaluation for increased and clonal plasma cells should be undertaken. The WHO 2008 criteria are listed in the Pathologic Features box, and in large part are exclusionary.

### Ancillary Studies and Differential Diagnosis

CNL must be distinguished from a leukemoid reaction, from CML, and from other MPNs or MDS/MPNs. The search for a cause of neutrophilia must be undertaken with secondary causes ruled out. The presence of dysplasia should make the pathologist consider the diagnosis of a disorder with a myelodysplastic component rather than CNL. Cytogenetic and molecular studies should be undertaken to rule out CML. There should be no t(9;22) or *BCR-ABL1* and particularly no p230 *BCR-ABL1,* which is associated with CML with neutrophilia (CML-N). Cytogenetic findings occur rarely (10%) in CNL, but include +8, +9, del(20), and del(11q). These findings would be important as proof of clonality, but do not help in ruling out other clonal myeloid disorders. If no cytogenetic clone is identified then a clonality assay, such as the HUMARA assay, may be necessary to prove that a clone is present; this is particularly the case when there is an associated plasma cell dyscrasia, in order to rule out a reactive neutrophilia secondary to the plasma cells (Figure 17-30).

## CHRONIC NEUTROPHILIC LEUKEMIA—PATHOLOGIC FEATURES

### Diagnostic Criteria (WHO, 2008)

- Sustained neutrophilia greater than $25 \times 10^9$/L
- Hypercellular bone marrow with increased neutrophilic granulocytes, less than 5% blasts, normal pattern of neutrophil maturation, and normal megakaryocytes
- Hepatosplenomegaly
- No identifiable cause for neutrophilia (including no infection, no underlying tumor), or if present demonstration of clonality of myeloid cells by cytogenetics or molecular studies
- No Philadelphia chromosome or *BCR-ABL1*
- No rearrangement of *PDGFA, PDGFB, FGFR1*
- No evidence of PV, PMF, ET
- No evidence of MDS or MDS/MPN

### Microscopic Features

#### Blood

- Neutrophils greater than 80% of leukocytes
- Neutrophils have toxic granulation and Döhle bodies
- Neutrophils may show hypersegmentation of nuclei
- No outright dysplasia
- Red blood cells and platelets are normal

#### Marrow

- Hypercellular
- Proliferation of mature neutrophils
- Blasts generally less than 5%
- Erythropoiesis and megakaryocytopoiesis normal

### Ancillary Studies

- Cytogenetic and molecular studies to rule out t(9;22), *BCR-ABL1;* must rule out p230-related *BCR-ABL1* associated with CML with neutrophilia (CML-N)
- Cytogenetic or molecular genetic analysis to prove clonality
- HUMARA to demonstrate clonal nature of granulocytes

### Differential Diagnosis

- Reactive neutrophilia, including secondary neutrophilia due to plasma cell proliferation
- CML and CML-N
- Atypical CML

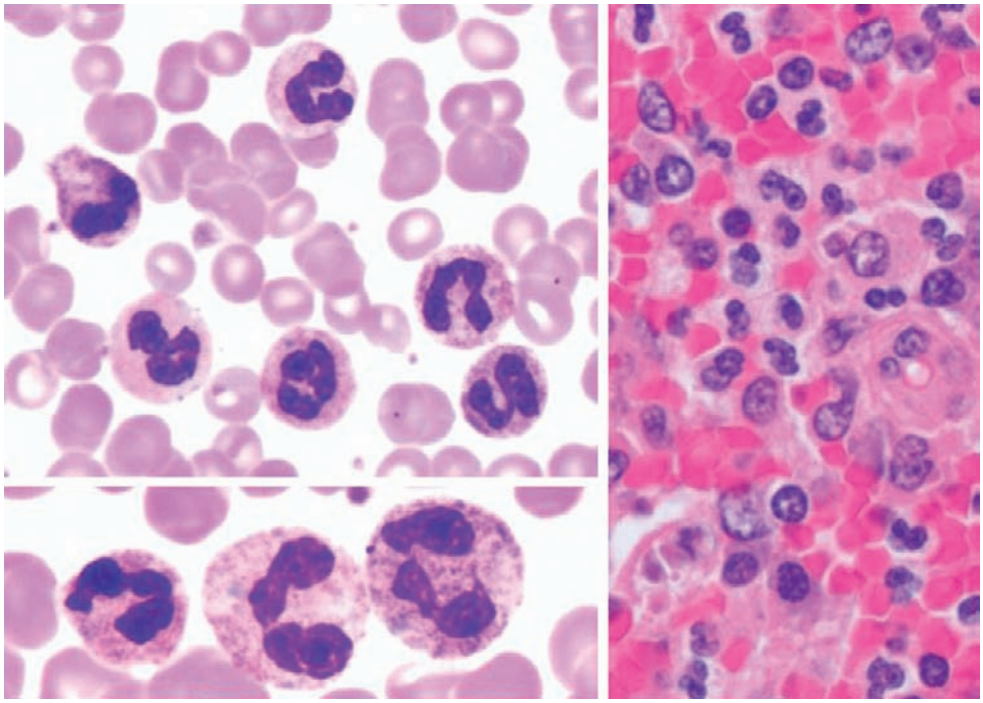

**FIGURE 17-29**

Chronic neutrophilic leukemia with leukocytosis in the blood *(right)*. The neutrophils show toxic granulation and Döhle bodies *(bottom left)*. The patient underwent a splenectomy (2200 g), and the neutrophils were found to be clonal using the human androgen receptor assay (HUMARA).

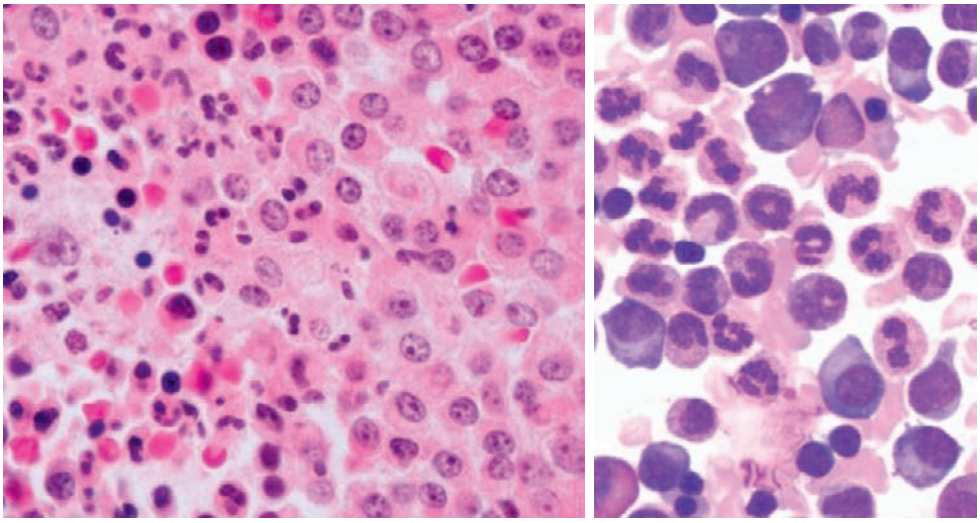

**FIGURE 17-30**
Suspected chronic neutrophilic leukemia in which the neutrophil proliferation were shown to be associated with a plasma cell myeloma.

### Prognosis and Therapy

The overall survival in CNL is low, with a median survival of less than 2 years in some studies. Patients develop progressive neutrophilia and worsening anemia and thrombocytopenia. A transformation to AML can occur but is not common.

## MYELOPROLIFERATIVE NEOPLASMS, UNCLASSIFIABLE (MPN-U)

The WHO committee writing on hematopoietic tumors included a category of MPN for cases that did not fit into the entities described previously. MPN-U was not intended for cases for which a better classification could not be made because of lack of information or clinical data. Most cases in the category are either early cases for which a more definitive diagnosis cannot be made or advanced cases in which a definitive diagnosis at an earlier stage was not made, and in which secondary changes have masked more typical features. The former situation commonly arises for patient with very early PV, ET, and PMF. Follow-up studies at 6-month intervals might be necessary before a better classification can be attempted. For the latter situation, when marked secondary changes such as fibrosis or osteosclerosis obscure the diagnosis, it is necessary to rule out CML with secondary changes as seen in the accelerated phase. This may be important because the correct diagnosis of accelerated phase of CML should initiate a trial of imatinib with adjuvant chemotherapy or one of the newer more potent TK inhibitors. A third category is for cases that are obviously a MPN, but in which the classification is complicated by a coexisting disease which obscures applying the diagnostic criteria.

## "OVERLAP" SYNDROMES (MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS)

CMML was classified as an MDS according to the French-American-British classification system; however, the entity was always somewhat perplexing, and in some cases there was a prominent proliferative component. This finding was not at all typical for a myelodysplastic process that is characterized by ineffective hematopoiesis with cytopenia in all cell lines. Over time, CMML was divided into two types—a dysplastic type with lower counts and a proliferative type with high counts ($13 \times 10^9$/L being the dividing line)—but these classifications appeared to have little clinical utility and probably had no basis biologically. Currently, CMML and its pediatric counterpart, JMML, are now the prototypes for a new nosologic group of disorders that recognizes the overlapping features of myeloproliferation and myelodysplasia. Atypical CML (aCML) and a provisional disorder of RARS-T, which also display proliferative and dysplastic features, are also included in this group.

## CHRONIC MYELOMONOCYTIC LEUKEMIA

CMML is a somewhat heterogenous disorder that has been difficult to study. It has no unifying or underlying molecular event and can sometimes be difficult to diagnose accurately. Although there has been progress in understanding the molecular basis of the pediatric form

of the disease JMML (see below), and although there are rare subtypes of CMML with recurring genetic abnormalities suggesting involvement of certain molecular pathways, the majority of cases are not well understood.

## Clinical Features

Because CMML was initially classified as an MDS, and grouped with those diseases, or alternatively grouped together with Ph-negative myeloid leukemias, there is little information on the epidemiologic features of the disease. Clinically, patients are usually older and complain of fatigue, weight loss, fever, night sweats, and symptoms related to splenomegaly.

## Pathologic Features

### Blood

The white blood cell count in CMML varies widely from 2 to 500 $\times$ $10^9$/L. The median counts are between 10 and 20 $\times$ $10^9$/L (Figures 17-31 and 17-32). Platelets can vary in number as well. Some patients have thrombocytopenia, but some may actually have normal or even slightly increased numbers. Anemia is common but usually mild.

A characteristic feature in the blood, and a necessary diagnostic criterion, is an absolute monocytosis of greater than 1 $\times$ $10^9$/L. The monocytosis can be minimal or rather dramatic with counts as high as 100 to 200 $\times$

**CHRONIC MYELOMONOCYTIC LEUKEMIA—FACT SHEET**

**Definition**

- A clonal leukemia disorder that is characterized by a proliferation of monocytes in the blood and marrow and by dysplasia in one or more cell lines
- Dysplasia frequently associated with ineffective hematopoiesis leading to anemia and or thrombocytopenia.

**Incidence, Gender, and Age Distribution**

- Unclear incidence
- Occurs in elderly

**Clinical Features**

- Symptoms: fatigue, weight loss, fever, night sweats, full abdomen
- Physical findings: splenomegaly

**Prognosis**

- Survival varies widely; overall median survival, 20 to 40 months
- Blast count predicts worse course: less than 10% blasts CMML-1; 10% to 19% blasts CMML-2

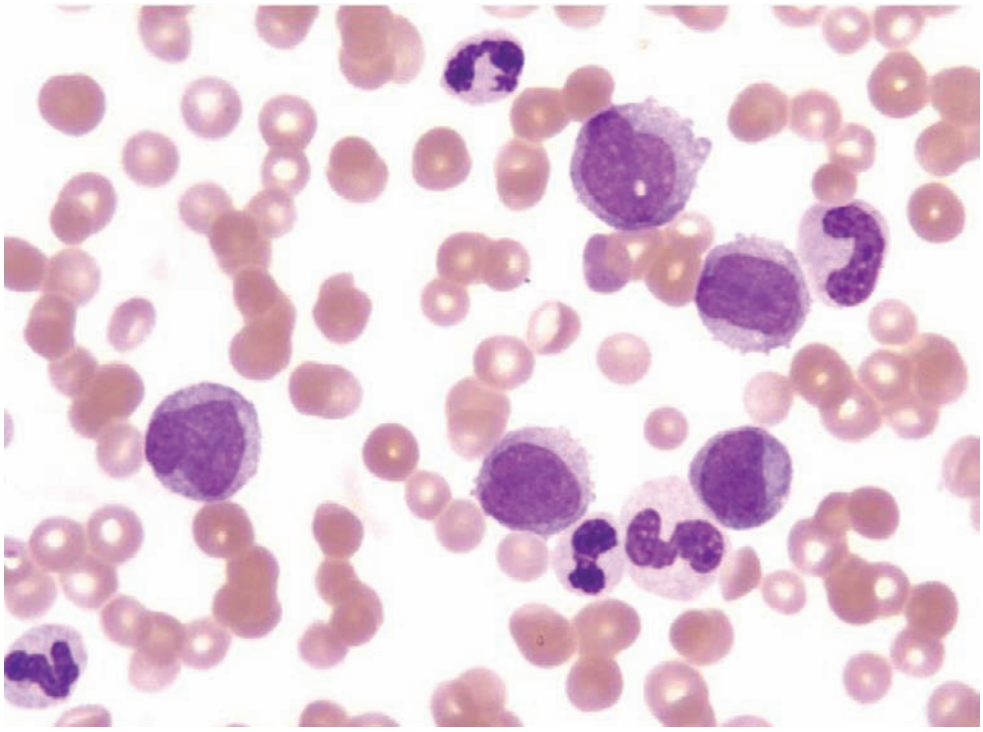

**FIGURE 17-31**
Peripheral blood smear in chronic myelomonocytic leukemia. Note the monocytes, dysplastic granulocytes, and absent platelets.

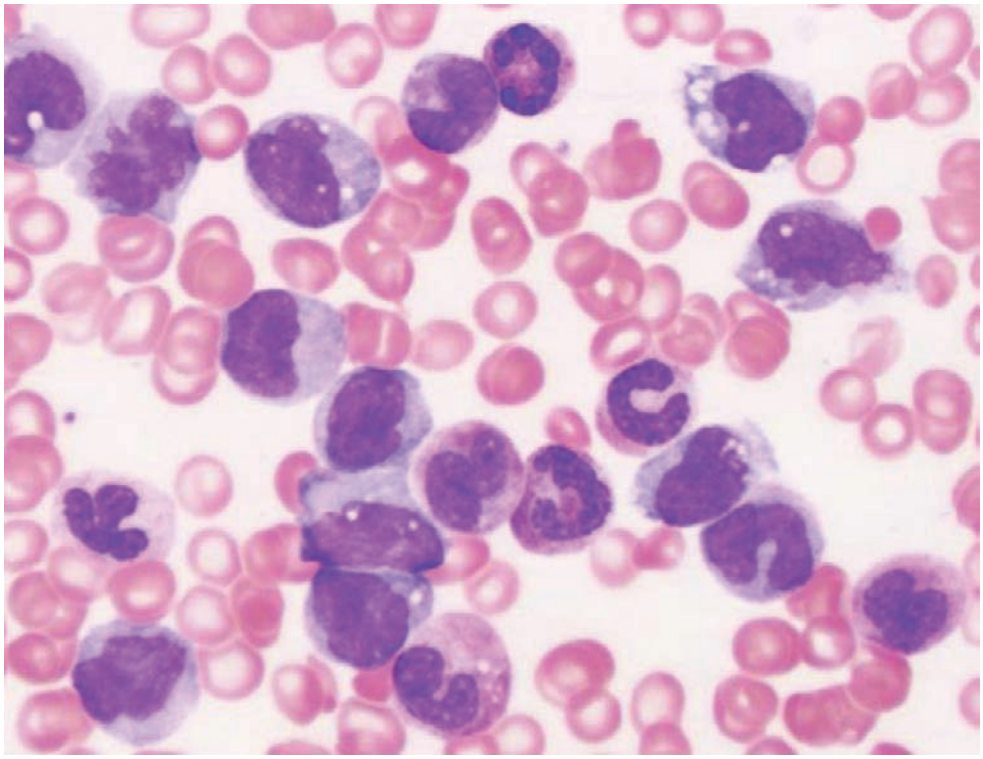

**FIGURE 17-32**

Peripheral blood smear in a case of chronic myelomonocytic leukemia with high count. Note the minimal dysplasia in the granulocytes.

## CHRONIC MYELOMONOCYTIC LEUKEMIA—PATHOLOGIC FEATURES

### Diagnostic Criteria (WHO, 2008)

- Persistent peripheral blood monocytosis greater than $1 \times 10^9$/L
- No Philadelphia chromosome or *BCR-ABL1*
- No rearrangement of *PDGFA, PDGFB, FGFR1*
- Fewer than 20% blasts in blood or marrow
- Dysplasia in one or more myeloid cell lines, or if absent or minimal the diagnosis can be made if the other requirements are met and:
  - An acquired clonal cytogenetic or molecular abnormality is present
  - The monocytosis has persisted for at least 3 months
  - All other causes of monocytosis have been excluded

### Microscopic Features

#### Blood

- WBC varies from 2 to $500 \times 10^9$/L
- Thrombocytopenia in some cases
- Mild anemia in most cases
- Monocytosis, relative and absolute, usually greater than 10% monocytes, and at least 1 but as high as $200 \times 10^9$/L; average, $5 \times 10^9$/L
- Blasts and promonocytes usually less than 10%; 10% to 19% = CMML-2
- Granulocytic dysplasia
- Basophilia common
- Eosinophilia (if associated with t[5;12][q33;p13] classify as MPN with eosinophilia)

#### Marrow

- Hypercellular, granulocytic proliferation most prominent
- Monocytes increased but sometimes difficult to appreciate
- Dysplasia in granulocytic, erythroid, and megakaryocytes
- Reticulin fibrosis usually increased

### Ancillary Studies

- Nonspecific esterase reaction useful to help identify monocytes
- Serum lysozyme may be increased
- Cytogenetic or molecular analysis to rule out t(9;22) and *BCR-ABL1* and other translocations associated with AML

### Differential Diagnosis

- CML
- Atypical CML (aCML)
- AML
- Reactive monocytosis
- MPN with eosinophilia and abnormalities of *PDGFB*—e.g., t(5;12)(q33;p13)

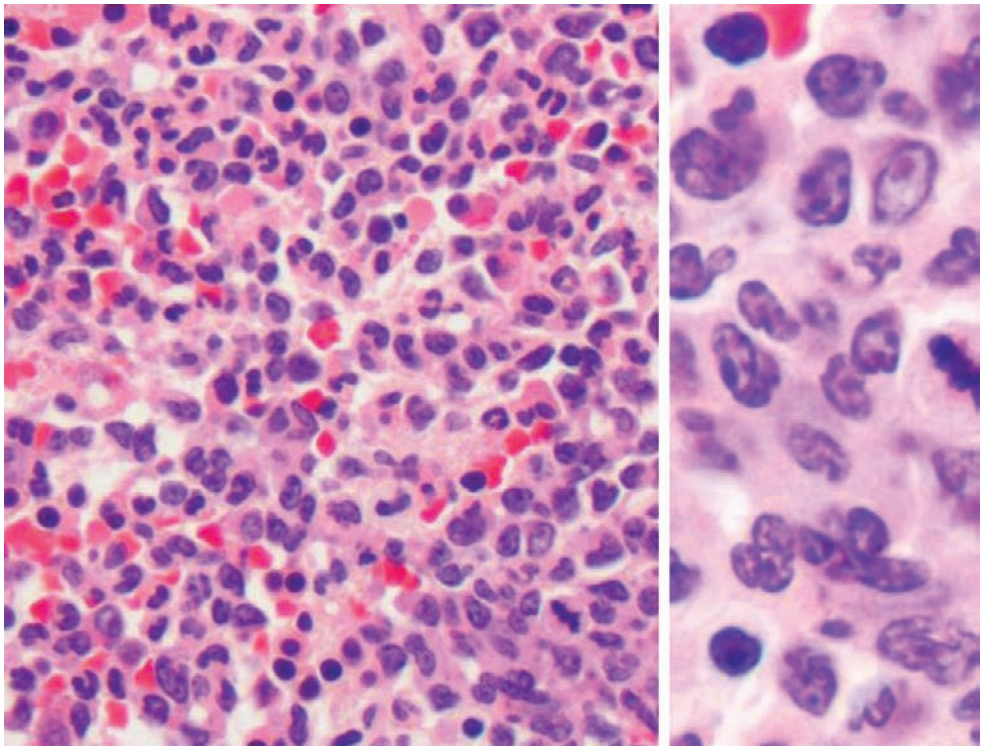

**FIGURE 17-33**

Bone marrow biopsy in chronic myelomonocytic leukemia showing proliferation of granulocytes and monocytic elements. The monocytes are prominent in this case and show clustering *(right),* but in some cases they are only slightly increased in the marrow.

$10^9$/L; however, most patients have modest numbers (approximately $5 \times 10^9$/L). Somewhat regardless of the absolute counts, the percentage of monocytes is consistently high and always greater than 10% of the circulating WBCs. This point is important, because there is commonly an absolute monocytosis in CML, but most of the time the monocytes are less than 3%. In CMML, the monocytes vary morphologically, but frequently are slightly immature. Blasts and promonocytes usually account for less than 10% of the cells, but are always less than 20%. Granulocytes also vary significantly in number but are usually not decreased. Immature granulocytes are usually not as prominent as in CML and account for less than 10% of the WBCs. The granulocytes typically exhibit dysplastic features, but in some cases the dysplasia is mild. Some patients have a basophilia, and others have a prominent eosinophilia; the latter are sometimes associated with a recurring cytogenetic abnormality, t(5;12)(q33;p13), which involves the *PDGFRB*. Such cases are considered myeloproliferative neoplasms with eosinophilia and associated TK mutation (see previous discussion).

#### Bone Marrow

The bone marrow is hypercellular in most patients, and the increased cellularity is usually due to a granulocytic proliferation and surprisingly not monocytes. In fact, the monocytic proliferation can sometimes be difficult to appreciate without the aid of a nonspecific esterase reaction, although sometimes it is obvious, even on the biopsy (Figure 17-33). The granulocytic precursors usually show dysplasia, although sometimes the dysplasia is mild. The granulocytes may be left shifted, but blasts and promonocytes are usually less than 10% and always less than 20% of the nonerythroid elements. Erythroid precursors are reduced and can show dysplasia. Megakaryocytes are usually abnormal and can include large atypical forms, sometimes with widely spaced small nuclei or small micro-megakaryocytes. Reticulin fibrosis is commonly increased, and lymphoid aggregates are seen in the biopsy specimen.

#### Other Extramedullary Tissues

The spleen is frequently enlarged and the abnormal myelomonocytic cells infiltrate the red pulp. Such infiltrates can be seen elsewhere, but lymph node involvement is rare. Transformation to acute leukemia can occur in the extramedullary sites.

## Ancillary Studies and Differential Diagnosis

A diagnosis of CMML requires the absolute monocytosis of greater than $1 \times 10^9$/L, and this may require the use of nonspecific esterase (α-naphthyl acetate esterase or

α-naphthyl butyrate esterase) reactivity to better identify the monocytes, as mentioned previously. Dual positivity for nonspecific esterase activity and chloracetate esterase is helpful in confirming an abnormal myelomonocytic component. The monocytosis will be associated with an elevated serum lysozyme, which can be useful to evaluate in some cases in which the monocytes are difficult to recognize. A diagnosis of CMML also requires ruling out CML with molecular or cytogenetic analysis for *BCR-ABL1* and t(9;22). Because rare cases of CML with increased monocytes can have a *BCR-ABL1* gene associated with the p190 protein, this fusion should be excluded as well. Cytogenetic findings are nonspecific in CMML and include +8, –7, –5, del(12p), del(20q), i(17q), and complex abnormalities. The finding of inv(16), or t(16;16), would indicate AML with abnormal eosinophils, and the finding of translocations involving 11q23 (MLL) would also suggest AML; both can develop with borderline blasts counts. The t(5;12)(q33;p13) can be seen in cases with the morphologic features of both CEL and in CMML with eosinophilia. Currently such cases would be best classified as myeloproliferative neoplasms with *PDGFRB* rearrangement. Some CMML cases can have an i(17q) as a sole abnormality and can be associated with prominent pseudo Pelger-Huët granulocytes. These cases were initially thought to be classified as aCML, but it seems that most have sufficient monocytes to categorize them as CMML.

*NRAS* or *KRAS* mutations occur in 20% to 60% of cases, and sometimes they are associated with alterations of *RUNX1.* Acquired somatic deletions and loss of function mutations in the *TET2* gene have also been observed in a large percentage of cases (up to 46%). *TET2* is a gene involved in the production of 5-hydroxymethylcytosine, and mutations appear to abrogate this function. *TET2* mutations are associated with DNA hypomethylation. Models in which *TET2* mRNA is depleted show skewed hematopoiesis toward the monocyte-macrophage lineage and provide supporting evidence that *TET2* is involved in the pathogenesis of myeloid malignancy such as CMML. The *JAK2* V617F is usually negative in CMML, but cases with the mutation have been reported rarely, although some cases might not have met diagnostic criteria or occurred as a secondary event.

### Prognosis

The survival in CMML varies widely, and accurate data are lacking because of difficulty in diagnosis and classification. Overall the median survival is 20 to 40 months after diagnosis. The blast count is an important predictor in CMML. When blasts are less than 10%, the case can be considered CMML-1. When blasts are greater than 10% but less than 20%, the case can be considered CMML-2, which indicates progression or more aggressive disease. The blasts count should include promonocytes and when greater than 20% indicates progression to AMLz; this occurs in approximately 15% to 30% of cases.

## JUVENILE CHRONIC MYELOMONOCYTIC LEUKEMIA

The disorder that is currently referred to as *juvenile myelomonocytic leukemia* had previously been called *juvenile CML*. Fortunately this term as been abandoned and should not be used. JMML does not have the t(9;22) nor does it have the *BCR-ABL1;* it essentially is a pediatric form of adult CMML. For children with $Ph^+$ and/or $BCR\text{-}ABL1^+$ CML, it is preferable to mention the translocation or the molecular fusion gene in the diagnosis for clarity.

JMML has an association with neurofibromatosis type 1 (NF1) and with Noonan syndrome in a small percentage of cases. Although uncommon, these associations have lead to an understanding of some of the underlying molecular pathology of the disease. In NF1 and Noonan syndrome, there are mutations in the genes *NF1* and *PTPN11*, respectively. These genes are important in the controls of the RAS/MAPK pathway. This pathway regulates a cell's proliferative response to granulocyte-macrophage colony-stimulating factor (GM-CSF) when GM-CSF is bound to the cell surface. In JMML, dysregulation of the RAS signaling pathway through the mutations associated with NF1 or Noonan syndrome, and through more recently described mutations in other regulators are believed to be underlying molecular events leading to the disease. Dysregulation of the RAS pathway leads to a marked hypersensitivity of the myeloid progenitor cell to GM-CSF and to the proliferation of the leukemic clone.

### Clinical Features

JMML is uncommon and accounts for only a small fraction of childhood leukemia. It shows a male predominance, is more frequent in children younger than 2 years and is more common in boys. The association with NF1 is seen in approximately 15% of cases, and children with NF1 have a 200- to 500-fold increased risk of this leukemia.

Children with JMML usually have impressive hepatosplenomegaly, lymphadenopathy, and an erythematous maculopapular rash on the face with a butterfly distribution. The rash can precede other symptoms by months. Fever associated with bronchitis or tonsillitis is seen in approximately half of the cases. Some patients present with hemorrhage. Café au lait spots are seen in the patients with NF1.

**JUVENILE MYELOMONOCYTIC LEUKEMIA—FACT SHEET**

**Definition and Other Names**
- A pediatric form of CMML: associated with neurofibromatosis type I and Noonan syndrome
- Sometimes referred to as JMML
- Referred to in the past as *juvenile CML,* but term should be avoided

**Incidence, Gender, and Age Distribution**
- Uncommon; accounts for only a small fraction of pediatric leukemia
- Male predominance
- More frequent in children younger than 2 years
- Associated with NF1 in approximately 15% of cases

**Clinical Features**
- Impressive hepatosplenomegaly, lymphadenopathy, erythematous maculopapular rash on face
- Fever with bronchitis or tonsillitis is common
- Café au lait spots in patients with NF1

**Prognosis**
- Variable course with disparate survival
- Lower age, high Hgb F levels may predict worse course
- Transformation to AML in approximately 10% to 15%

**JUVENILE MYELOMONOCYTIC LEUKEMIA—PATHOLOGIC FEATURES**

**Diagnostic Criteria (WHO 2008)**
- Peripheral blood monocytosis greater than $1 \times 10^9$/L
- Blasts (including promonocytes) less than 20% in blood and bone marrow
- No Philadelphia chromosome or *BCR-ABL1*
- Plus two of the following:
  - Hemoglobin F increased for age
  - Immature granulocytes in the peripheral blood
  - WBC count greater than $10 \times 10^9$/L
  - Clonal chromosomal abnormality
  - GM-CSF hypersensitivity of myeloid precursors (in vitro)

**Microscopic Features**

**Blood**
- Median WBC count, 25 to $35 \times 10^9$/L
- Absolute monocytosis
- Blasts usually less than 5%
- Dysplasia not common

**Marrow**
- Cellular
- Increased M:E ratio
- Increased monocytes
- Minimal dysplasia

**Ancillary Studies**
- Hgb F levels typically increased
- Cytogenetic analysis shows abnormal clones in 30% to 60% of cases
- Monosomy 7 in 10%, most frequently associated with NF1
- Polyclonal hypergammaglobulinemia in more than half of patients
- Hypersensitivity of myeloid precursors to GM-CSF in vitro

**Differential Diagnosis**
- Reactive monocytosis
- AML (acute myelomonocytic type)
- MDS

## Pathologic Features

### Blood and Bone Marrow

Pediatric patients with JMML have features similar to those seen in adult patients with CMML. There is leukocytosis, absolute monocytosis, and thrombopenia with or without anemia. The median WBC count is between 25 and $35 \times 10^9$/L. Granulocytic elements can include some immature forms, but blasts are usually less than 5% of the cells. Dysplasia is not prominent. The bone marrow is highly cellular, but this is normal in children younger than 2 years. The myeloid-to-erythroid ratio can vary but is usually increased. Dysplasia, like in the blood, is only minimal. Erythroid elements may be megaloblastoid. Megakaryocytes do not show prominent dysplasia.

## Ancillary Studies

Hemoglobin F levels are typically increased for the age of the patient, although this not the case in some patients. Cytogenetic studies are abnormal in only 30% to 60% of cases. Monosomy 7 is seen in 10%, and other abnormalities including trisomy 8 and 21 are not uncommon. Less frequent abnormalities include t(13;14)(q12.?2;q32.?3) and defects of 3q. The cases associated with NF1 more frequently have monosomy 7, which also may be acquired during the disease course. More than half of the patients have a polyclonal hypergammaglobulinemia, and 25% of patients have a positive direct Coombs test result.

Hypersensitivity of myeloid progenitors to GM-CSF in vitro studies is a characteristic finding related to the underlying molecular pathogenesis. This test is not a routine offering in most labs, although it is one alternative criterion among others.

## Differential Diagnosis

The diagnosis of JMML can be difficult, and differentiation must be made between it and leukemoid reactions and other myeloid diseases. Children with EBV, CMV,

and HHV-6 can present with similar clinical and morphologic features. The finding of a cytogenetic clone can be helpful in ruling out a reactive process. Cytogenetic analysis is also important in ruling out CML, which can develop occasionally in young children. The distinction of JMML from acute myelomonocytic leukemia must be made based on an accurate blast and promonocyte counts.

### Prognosis

JMML has a variable course with widely disparate survival times. Lower age, higher hemoglobin F, and lower platelet counts may all predict worse prognosis. The transformation to AML occurs in only 10% to 15% of patients.

## ATYPICAL CHRONIC MYELOID LEUKEMIA, *BCR-ABL1*-NEGATIVE

Atypical CML (aCML) is an uncommon disorder characterized by a myeloproliferation that is *BCR-ABL1*-negative, and associated myelodysplasia. The absence of a monocytosis separates it from CMMML. The process suffers from a confusing name, which always must be clarified to the clinician and be explained as being distinct from what might sound like an unusual case of CML that is merely atypical. It is always helpful to refer to it as *atypical CML, BCR-ABL1-negative* for clarity; the WHO 2008 recommendations designate this as formal designation for this disease. There is some question that some cases may have arisen from a preceding more typical myelodysplastic syndrome that initially was associated with cytopenia. In this regard, and in some cases, the process is likely a progression or an accelerated form of MDS. Some cases, however, do arise and present *de novo*. There is no known underlying molecular defect, and no distinctive cytogenetic abnormalities, although a number of common cytogenetic findings including +8, del(5q), del(7q), del(13q), del(17p), del(12q), and del(11q) are recurring changes.

A recently described entity with t(4;22)(q12;q11) might constitute an uncommon but distinctive subset involving the *BCR* gene on chromosome 22.

### Clinical Features

The incidence of aCML is not known, but it likely represents only a small fraction of all MPNs and MDS/MPNs. In fact, there are fewer than two cases of aCML for every 100 cases of *BCR-ABL1*$^+$ CML. Patients are usually older, with a median age between 65 and 74 years. The male-to-female ratio is about equal, but cases with i(17q) have a male predominance of 3 : 1. Patients usually exhibit symptoms related to anemia and thrombocytopenia, although some will also have splenomegaly and hepatomegaly and related symptoms.

**ATYPICAL CHRONIC MYELOID LEUKEMIA—FACT SHEET**

**Definition**
- A Ph-chromosome- and *BCR-ABL1*-negative leukemic process that has a proliferative component and a dysplastic component with ineffective hematopoiesis
- Proliferation of granulocytic elements, which are frequently dysplastic, and ineffective hematopoiesis usually manifest as anemia and thrombocytopenia

**Incidence, Gender, and Age Distribution**
- Incidence unknown, rare
- Male : female = 3 : 1
- Median age, 65 to 74 years

**Clinical Features**
- Symptoms related to anemia and thrombocytopenia most common
- Splenomegaly and hepatomegaly in some

**Prognosis**
- Severe thrombocytopenia or anemia at diagnosis associated with poor prognosis
- Some transform to AML; more die of marrow failure (60% to 75%)
- Median survival, 2 years

### Pathologic Features

#### Blood

Patients usually have a leukocytosis of approximately 35 to 100 $\times 10^9$/L. In one study, the median count was $36 \times 10^9$/L, although in some cases the counts can exceed 200 to 300 $\times 10^9$/L. Most of the leukocytes in the blood are granulocytes with a mild degree of left shift (10% to 20% immature forms). Circulating blasts may be present, but these are generally less than 5%. The granulocytes are characteristically dysplastic with pale cytoplasm, nuclear atypia, and hypolobation (Figure 17-34). Basophils are typically elevated but usually account for less than 2% of the cells. Hypogranular myelocytes can be difficult to distinguish from monocytic elements, but there is no absolute monocytosis and the percentage of monocytes is also low and less than 10% of the leukocytes. Nonspecific esterase may be helpful in the evaluation. There is commonly an anemia and the red blood cells frequently show anisopoikilocytosis, in keeping

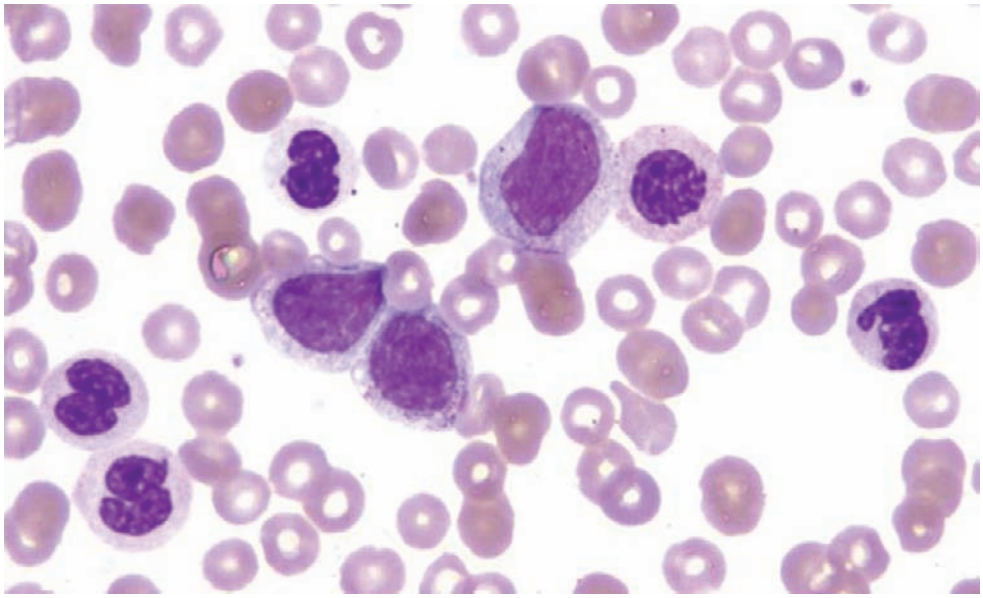

**FIGURE 17-34**

Peripheral blood smear in a case of atypical chronic myelogenous leukemia. Note the severe dysplasia in the mature granulocytes and the absence of platelets. The immature cells are dysplastic immature granulocytic elements. These could be distinguished from monocytes by a negative nonspecific esterase reaction.

**ATYPICAL CHRONIC MYELOID LEUKEMIA—PATHOLOGIC FEATURES**

**Microscopic Features**

**Blood**

- Leukocytosis of 35 to 100 × $10^9$/L
- Mostly granulocytes with mild left shift, blasts usually less than 5%
- Dysplasia usually marked, sometimes hypercondensed chromatin
- Basophilia in some cases
- No absolute monocytosis; less than 10% monocytes
- Anemia with anisopoikilocytosis
- Thrombocytopenia with hypogranular platelets in some cases

**Marrow**

- Hypercellular, granulocytic proliferation
- Blasts less than 20%
- Monocytes not increased
- Multilineage dysplasia frequently prominent
- Reticulin fibrosis may be increased

**Ancillary Studies**

- Nonspecific esterase useful to exclude increased monocytes
- LAP low or high (not useful)
- Cytogenetic and molecular analysis: must be t(9;22)- and *BCR-ABL1*-negative; some cases with t(4;22)(q12;q11)

**Differential Diagnosis**

- CML
- MDS
- CMML
- CNL
- AML

with the dysplasia that can be seen in the red blood cell precursors in the marrow. Platelets are decreased in approximately half of the patients. Hypogranulated forms might be seen.

### Bone Marrow

The bone marrow is usually hypercellular, mainly because of a granulocytic proliferation. The granulocytes may be left shifted with increased blasts, but these must account for less than 20% of the cells. As in the peripheral blood, dysgranulopoiesis is prominent. Monocytes are not increased, and both erythroid precursors and megakaryocytes show dysplasia. The finding of multiple distinct micromegakaryocytes can be a useful finding, as a sure indication of dysplasia (Figure 17-35). Reticulin fibrosis may be increased.

## Ancillary Studies and Differential Diagnosis

As mentioned previously, evaluation of nonspecific esterase activity may be useful in the blood and marrow cells for establishing whether there is a significant monocytic component. Increased monocytes (greater than 1 × $10^9$/L in the blood) would indicate the diagnosis might be CMML. LAP may be low or high and might not be useful when trying to rule out CML. However, cytogenetic or molecular findings must be performed to rule out t(9;22) or *BCR-ABL1*. Cases associated with i(17q) as a sole abnormality typically have sufficient monocytes and are best classified as CMML (see

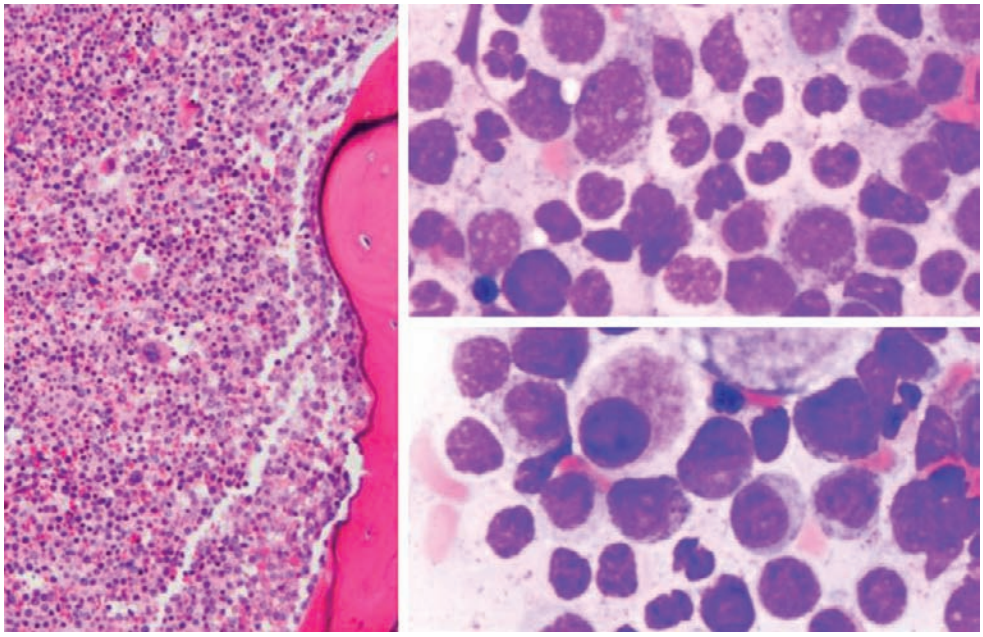

**FIGURE 17-35**

Bone marrow biopsy and aspirate in a case of atypical chronic myelogenous leukemia. Note the hypercellularity and marked dysplasia in mature granulocytes and megakaryocytes.

previous discussion). Cytogenetic clones are seen in approximately 80% of cases of aCML, and the common findings were listed previously. None are diagnostic or even suggestive of the diagnosis. The cases with t(4;22)(q12;q11) involve a fusion protein composed of *PDGFα* and *BCR*. Although these have been classified as aCML, it is not clear how well they meet the criteria for this entity.

### Prognosis

Patients with aCML have a poor prognosis, with a median survival of approximately 2 years (range, 11 to 25 months). Some patients (approximately 40%) transform to AML, whereas many more (60% to 75%) die of marrow failure. Thrombocytopenia and severe anemia at diagnosis may be a poor prognostic indicator.

## MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS, UNCLASSIFIABLE

As for the MPNs, the WHO classification allows for an unclassifiable type of MDS/MPN. The designation is not meant to be used for cases in which diagnostic data are lacking, but instead for when the entity, once fully characterized, does not fit the known types of disease. Cases usually have features of one of the myelodysplastic syndromes, but in addition have a myeloproliferative component with no history of a preceding MPN or MDS.

RARS-T remains a provisional entity that currently falls into the *unclassifiable* category. This process fulfills criteria for an overlap syndrome because it has both a dysplastic component, the anemia with ringed sideroblasts, and a proliferative component, the thrombocytosis. However, whether the process is a distinct entity has been debated. Some believe that it is a heterogenous mix of three things: cases of ET with ringed sideroblasts, cases of early or cellular phase of PMF, which later go on to develop overt myelofibrosis, and cases of MDS, which later develop an acute leukemia.

Patients have features of RARS but with thrombocytosis greater than $450 \times 10^9$/L. The lower limit for the platelet count was lowered from $600 \times 10^9$/L, as for ET. The bone marrow should have large megakaryocytes similar to those seen in other MPNs. Interestingly, approximately 60% have the *JAK2* V617F mutation, supporting the concept that the process belongs to the MPN and related disease category. Prognosis is good and better than for CMML1 and CMML2.

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The complete reference list is available online at www.expertconsult.com.

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18

# Myelodysplastic Syndromes

■ Adina M. Cioc, MD ■ Phuong L. Nguyen, MD

## ■ OVERVIEW

The myelodysplastic syndromes (MDSs) are a heterogeneous group of neoplastic stem cell disorders characterized by ineffective hematopoiesis. Clinically, this ineffective hematopoiesis manifests as progressive bone marrow failure with cytopenias and a variable probability of progression to acute myeloid leukemia (AML). Pathologically, MDSs are usually characterized by dysplastic morphologic features in one or more of the three hematopoietic lineages, with or without an accompanying increase in myeloblasts in the peripheral blood (PB) and bone marrow (BM), but at a lower percentage than the 20% threshold recommended for the diagnosis of AML. MDSs can arise de novo, or be therapy related, following exposure to cytotoxic chemotherapy, ionizing radiation, or both. Therapy-related MDSs are discussed with other therapy-related myeloid neoplasms in Chapter 14.

### Clinical Features

#### Epidemiology

MDS is a disease of elderly people. Approximately 80% of patients with MDS are older than 60 years at diagnosis, making MDS as common as chronic lymphocytic leukemia and multiple myeloma in this age group. The incidence rates for MDSs increase sharply with age, from 7.1 per 100,000 individuals between 60 and 69 years old, to 35.5 per 100,000 among those older than 80 years. MDS is extremely rare in children younger than 14 years, among whom the annual incidence rate is 1.8 per 1,000,000. Patients with therapy-related MDS tend to be younger than de novo MDS patients. Men are more likely to be diagnosed with MDS than women, and the incidence increases significantly with age in both groups.

Based on the 24,798 cases of MDS reported by the Surveillance, Epidemiology, and End Results program and the North American Association of Central Cancer Registries between 2001 and 2003, the average annual age-adjusted incidence rate for MDS in the United States is estimated to be 3.3 per 100,000 (4.4 per 100,000 men and 2.5 per 100,000 women), with a slight but statistically significant increase in the incidence rates with each calendar year. Based on these data, it is estimated that approximately 9000 to 10,000 MDS cases are diagnosed annually in the United States. The prevalence of MDS (the total number of people living with MDS) is unknown, because many patients with unexplained cytopenia never undergo a diagnostic BM evaluation. In one retrospective study performed at a North American tertiary care center, the BM reports of 322 patients who had undergone a BM evaluation for cytopenia were reviewed. The authors noted that the diagnosis of MDS was made in approximately 32% of patients over 65 years of age. Although it is difficult to extrapolate these findings to the general population given the particular design and patient selection of the study, these numbers suggest an estimated prevalence of approximately 90,000 cases of MDS among individuals older than 65 years in the United States.

Whereas the association between cytotoxic chemotherapy and ionizing radiation and therapy-related MDS is well established, risk factors for de novo MDS, which accounts for the large majority of cases, have not been fully elucidated. A variety of risk factors such as smoking and exposure to solvents (benzene and derivatives) and agrochemicals (e.g., insecticides, pesticides, fertilizers) have been reported, but the majority of cases remains unexplained. Among children with MDS, approximately 30% to 50% have a predisposing condition such as previous treatment for neoplasia, a congenital or acquired BM failure syndrome, or familial MDS. Childhood MDS in the setting of Down syndrome is currently classified with Down syndrome–associated myeloid leukemia and is excluded from population-based studies of MDSs.

#### Pathogenesis

The rate of cell proliferation in the BM of patients with MDS is increased compared to that in normal subjects,

**MYELODYSPLASTIC SYNDROMES—FACT SHEET**

**Definition**

- A heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis with a variable probability of progression to acute myeloblastic leukemia

**Incidence, Age Distribution, and Predisposition**

- Estimated 9000 to 10,000 newly diagnosed cases per year in the United States
- Eighty percent of MDS diagnoses are in individuals older than 60 years
- Incidence rates are age dependent, highest in older individuals (35.5 per 100,000 individuals per year among those older than 80 years) and lowest in children younger than 14 years of age (1.8 per 1,000,000 individuals per year)
- Predisposing risks: inherited or acquired bone marrow failure syndromes, previous exposure to alkylating or topoisomerase II inhibitor chemotherapy, ionizing radiation, and certain toxins

**Morbidity and Mortality**

- Related to the MDS subtype, extent of cytopenia, and type of cytogenetic abnormality

**Clinical Features**

- Symptoms are usually related to extent of cytopenias
- Some patients may be asymptomatic

**Prognosis and Therapy**

- Patients with normal blood and marrow blast count and with normal cytogenetics or with 5q– as the sole chromosomal abnormality have the best prognosis
- Patients with increased blood or marrow blasts, complex chromosomal abnormalities, or secondary MDS have the worst prognosis
- Current standard curative treatment: allogeneic hematopoietic stem cell transplantation

but the maturation of such hematopoietic precursors is impaired with increased apoptosis because of overexpression of the apoptosis receptor CD95/Fas on hematopoietic precursors in the early stages of the disease. This proapoptotic milieu appears to be driven also in part by increased production of the proinflammatory cytokine tumor necrosis factor (TNF) α, with reports of elevated BM and plasma levels of TNF-α and elevations of the TNF-related apoptosis-inducing ligand and interferon γ on peripheral blood lymphocytes in patients with MDS. A more recent study of 328 patients with de novo MDS with or without increased blasts showed statistically significant associations between the high-expressing AA *TNF*-308 and GA or AA *TNF*-238 genotypes and neutropenia and anemia, respectively. There may be also participation of other proinflammatory cytokines such as interferon γ and interleukin 6. This apparently proapoptotic BM environment could serve to explain the paradox of ineffective hematopoiesis in MDSs, in which there is increased marrow cellularity because of increased cell proliferation, but in which increased apoptosis of BM hematopoietic progenitors results ultimately and paradoxically in cytopenias (Figure 18-1).

In studies of patients with MDS and trisomy 8, among whom there is overexpression of Wilms tumor protein WT1, increased programmed cell death appears to be immunologically mediated: T cells from certain expanded Vβ subfamilies and specific for WT1 selectively killed trisomy 8 cells in vitro, whereas depletion of cytotoxic T cells enabled growth of these trisomy 8 colonies. Moreover, these WT1-specific $CD4^+$ and $CD8^+$ T cell responses appeared to be associated with clinical responsiveness to immunosuppressive therapy, with the greatest WT1-specific T-cell responses noted among those patients who were responders compared with nonresponders.

Although this model of autoimmunity may account for the increased apoptosis and ultimately cytopenia in this particular subset of MDS patients with trisomy 8, the pathogenesis of MDSs as a group is still elusive. Since the first edition of this chapter, the list of single gene mutations has continued to grow (see Molecular Genetics). However, the finding that each accounts for no more than 10% to 15% of MDS cases suggests that these genetic aberrations represent only events in a multistage process, and MDSs remain a heterogeneous group of disorders with more than one possible pathogenetic pathway. For example, recent evaluation by global gene expression profiling of hematopoietic stem cells in patients with MDS followed with pathway analysis identified not one but several frequently deregulated pathways. Moreover, when patients with MDS of the cytogenetic subgroup del(5q), –7/del(7q), or +8 were compared with healthy controls, distinct gene expression profiles and associated pathways also emerged. For example, among patients with del(5q), the most deregulated pathways included those involved in cell cycle regulation, integrin signaling, and chromatin assembly, whereas pathways involving cell survival appeared to be most heavily deregulated among patients with MDS and –7/del(7q). Interestingly, in view of the reported responsiveness to immunosuppressive therapy among some patients with MDS and trisomy 8 as discussed previously, the most significantly deregulated pathways among this group of patients were those associated with the immune response, as assessed by this approach.

As inferred from the apparently diverse backgrounds of patients with MDSs, there may be more than one pathogenetic pathway for MDSs—in a manner not too dissimilar from acute myeloid leukemias (AMLs) in which there is no one genetic abnormality or one single pathway to account for all AMLs. Somatic mutations seem to be a common mechanism, as judged by the

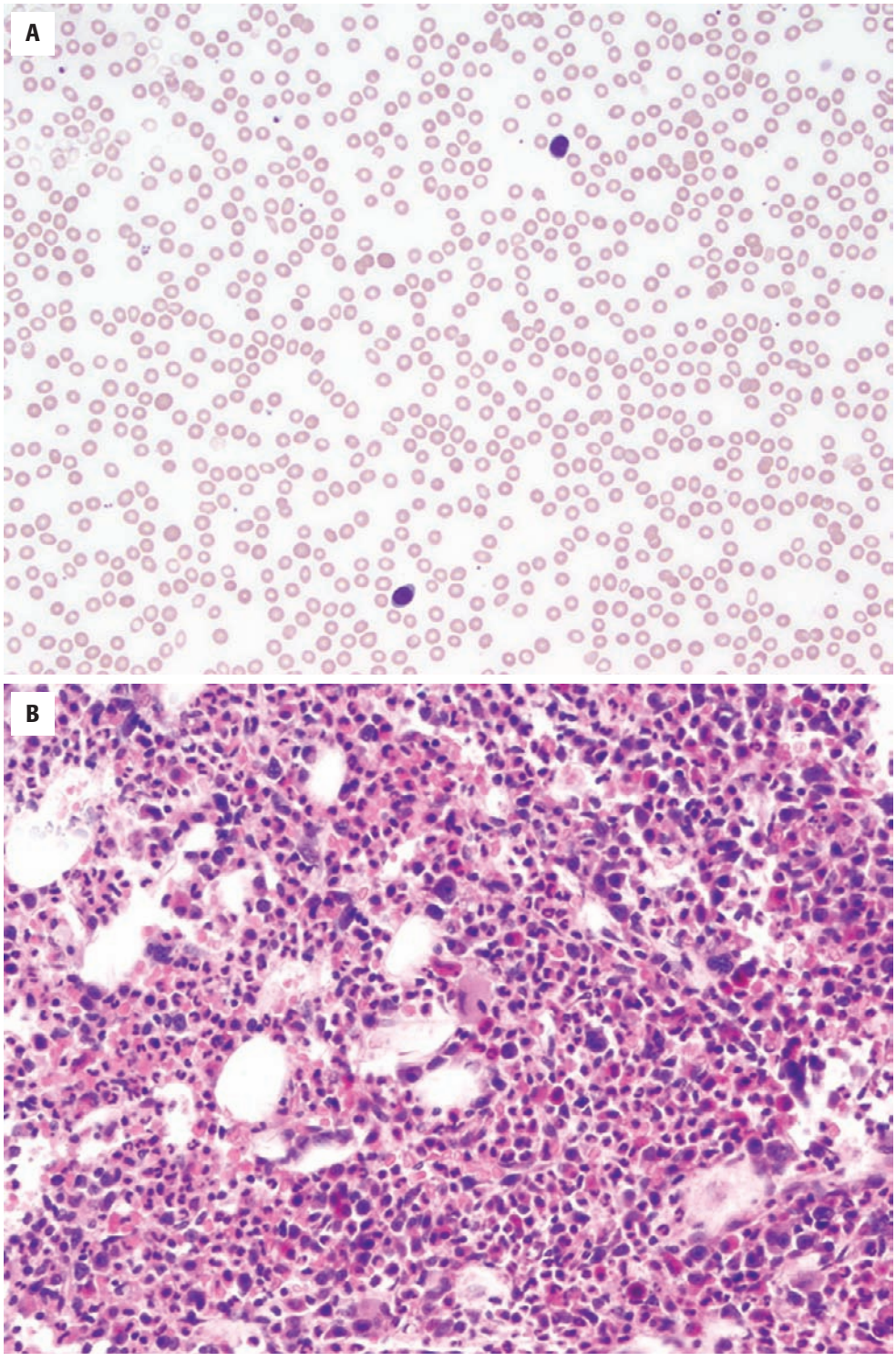


**FIGURE 18-1**

Ineffective hematopoiesis in myelodysplastic syndromes. **A,** The peripheral blood of this patient with myelodysplastic syndrome shows pancytopenia (Wright-Giemsa stain). **B,** In contrast, the marrow is hypercellular with increased hematopoietic precursors (hematoxylin and eosin).

growing list of single-gene mutations reported in patients with MDS within the last several years. It is less clear whether defects in DNA replication and/or DNA repair—common in secondary or therapy-related MDSs—may also play an equally major role in primary or de novo MDSs.

## Clinical and Laboratory Features

Patients with MDS usually exhibit symptoms related to cytopenia, most frequently fatigue, but they may also have bleeding problems, increased susceptibility to

infections, or both. Other patients may have a diagnosis of MDS made in an asymptomatic phase after detection of an abnormal complete blood cell count during health screening or as part of a medical evaluation for other unrelated symptoms. Organomegaly and lymphadenopathy are usually absent. Laboratory evaluation commonly reveals anemia with reticulocytopenia, neutropenia, or thrombocytopenia. The thresholds for cytopenias as recommended in the International Prognostic Scoring System (IPSS) for risk stratification in MDSs are hemoglobin less than 10 g/dL, absolute neutrophil count less than $1.8 \times 10^9$/L and platelets less than $100 \times 10^9$/L. However, the diagnosis of MDS can be made in the absence of a specific cytopenia if unequivocal morphologic or cytogenetic features of MDS are present.

For patients clinically suspected of having MDS, the National Comprehensive Cancer Network (NCCN) recommends that the minimal initial evaluation include a comprehensive history and physical examination, hemogram with leukocyte differential, reticulocyte count, erythropoietin levels, iron studies, and a BM aspiration and biopsy with iron stain and cytogenetic studies.

## Pathologic Features

### Morphology

Because there is currently no single biologic or genetic marker that reliably identifies all cases of MDS, morphologic and cytogenetic evaluation remains the cornerstone of diagnosis. For optimal morphologic evaluation of cellular details, it is essential to obtain fresh and well-prepared blood and BM aspirate smears stained with Wright-Giemsa or May-Grunwald-Giemsa stains. Poor technical quality of the blood or BM smears is a common obstacle in reaching the accurate diagnosis of MDS, because informative differences in the degree of neutrophil granulation and in other nuclear and cytoplasmic details may be obscured or distorted. A good quality BM biopsy (at least 1.5 cm in length and at a right angle to the cortical bone) is invaluable for an accurate histologic assessment of cellularity and cell distribution and for immunohistochemical studies.

### Peripheral Blood

In addition to the quantitative abnormalities mentioned previously, microscopic examination of the blood can reveal abnormalities involving one or more of the hematopoietic lineages. Dysplastic changes in the red blood cells can manifest as macrocytosis unrelated to nutritional deficiencies, increased anisopoikilocytosis, or a

**MYELODYSPLASTIC SYNDROMES—PATHOLOGIC FEATURES**

**Laboratory Findings**
- Anemia with reticulocytopenia, neutropenia, or thrombocytopenia
- Macrocytosis, dimorphic blood pattern, or circulating blasts (less than 20%) may be present

**Microscopic Findings**
- Accurate morphologic evaluation of blood and bone marrow slides is essential in the diagnosis of MDS
- In the 2008 WHO classification of MDSs, an accurate and exact count of circulating blasts matters: 0% versus 1% versus 2%-4% versus >5%
- The marrow is typically hypercellular with morphologic features of dysmyelopoiesis; increased blasts (less than 20%) or Auer rods, or both, may be present

**Cytochemistry**
- Iron stains of bone marrow slides to identify ring sideroblasts
- Globular or punctate periodic acid-Schiff reactivity in vacuolated erythroblasts
- Increased dual butyrate esterase and chloracetate esterase reactivity

**Immunohistochemistry**
- Increased numbers or abnormal compact clusters of $CD34^+$ blasts may be present
- CD61 immunohistochemistry can highlight increased numbers or abnormal clusters of dysplastic megakaryocytes

**Cytogenetics**
- Essential in the diagnosis, subclassification, and prognostication of MDS
- In the 2008 WHO classification of MDS, characteristic MDS-related cytogenetic abnormalities can form the basis for a diagnosis of MDS in the setting of persistent unexplained cytopenias, even when there are insufficient morphologic features of myelodysplasia

**Differential Diagnosis**
- Cytopenia with hypercellular marrow: hemolytic anemia, immune-mediated or hypersplenism-related neutropenia or thrombocytopenia, viral infections (e.g., HIV, chronic parvovirus B19, cytomegalovirus, Epstein-Barr virus), hemophagocytosis, acute leukemia
- Cytopenia with normocellular or hypocellular marrow: inherited or acquired bone marrow failure syndromes, myelosuppressive infections, medication effect, toxic exposure, graft-versus-host disease affecting the marrow, T-cell large granular lymphocytic leukemia, hypocellular acute myeloblastic leukemia
- Dyserythropoiesis: severe hemolysis, marrow regeneration, megaloblastic anemia, effects of antimetabolite or antineoplastic therapy or other medications, congenital dyserythropoietic anemia
- Ring sideroblasts: alcohol, mitochondrial cytopathy, arsenic poisoning, zinc toxicity, pyridoxine deficiency, copper deficiency, anti-tuberculosis therapy
- Dysgranulopoiesis: G-CSF or GM-CSF, trimethoprim-sulfamethoxazole, valproate therapy, mycophenolate mofetil therapy
- Dysmegakaryopoiesis: sectioning artifact, HIV infection, valproate therapy
- Increased blood or marrow blasts: marrow regeneration, G-CSF or GM-CSF, leukemoid reaction, acute leukemia

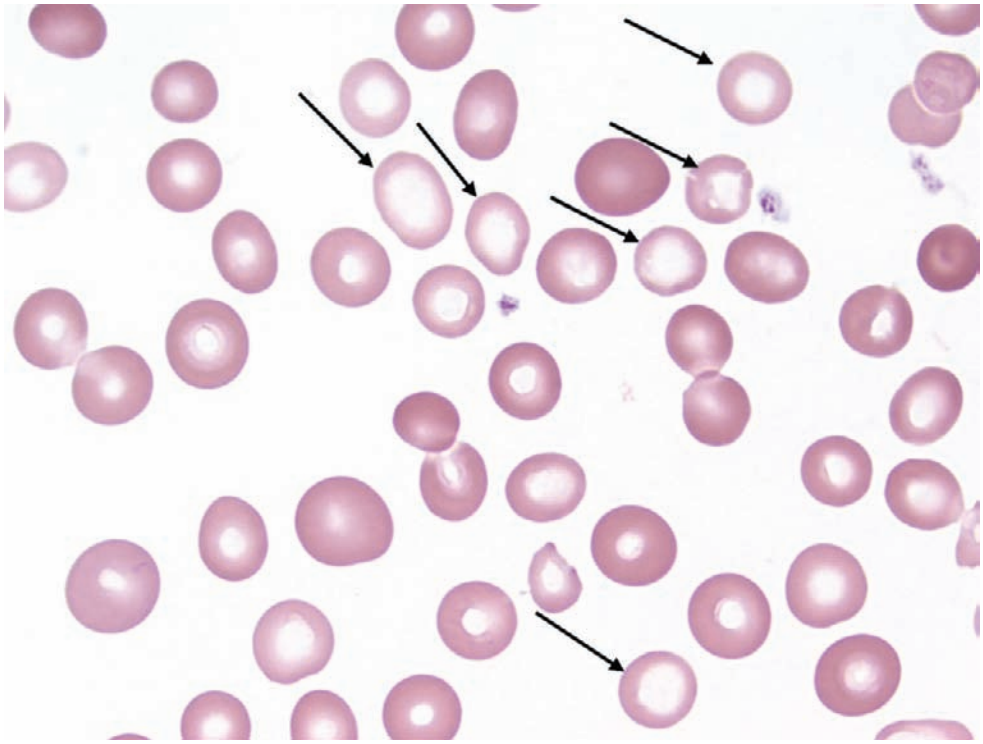

**FIGURE 18-2**

Dimorphic red blood cells in myelodysplastic syndrome. Peripheral blood smear from a patient with myelodysplastic syndrome, refractory anemia with ring sideroblasts, showing two populations of red blood cells—one normochromic and one hypochromic (*arrows*) (Wright-Giemsa stain).

dimorphic pattern with a mixture of normochromic and hypochromic erythrocytes (Figure 18-2). Atypically large and hypogranular platelets may be present. Neutrophil dysplasia may be observed in the cytoplasm as deficient secondary granulation, aberrantly fine and coalescent secondary granules, or pseudo Chédiak-Higashi granules. Dysplastic neutrophils may show nuclear hyposegmentation (pseudo Pelger-Huët anomalies), or less commonly, bizarre nuclear hypersegmentation (Figure 18-3). Most cases of MDS will retain a certain proportion of normal neutrophils with an adequate complement of secondary granulation; these should be contrasted with the hypogranular neutrophils to rule out the possibility of poor or pale staining as an explanation for the apparent abnormal granulation of neutrophils. Atypical hypogranulated eosinophils and basophils may also be seen in MDSs, although such findings are infrequent.

### Leukocyte Differential Count and the Enumeration of Circulating Blasts

The blood smear should be carefully examined for the presence of circulating blasts. If blasts are present in the peripheral blood, an accurate determination of their exact percentage is essential both for subclassification and for prognostication. In a study of approximately 1100 patients with low-grade MDS, Knipp and colleagues observed a difference in outcome with as few as 1% blasts in the peripheral blood. Compared to patients without PB blasts, those with 1% circulating blasts had both a shorter median survival (20 versus 47 months; $p < 0.00005$) and a higher cumulative risk of AML evolution. Based partly on such studies, in the 2008 World Health Organization (WHO) classification schema, patients with MDS are separated into different categories based on relatively small differences in the percentage of circulating blasts, less than 1%, 1%, 2% to 4%, or 5% or greater. Although a 100-cell leukocyte differential count had been used in the study by Knipp and colleagues as described previously, for adequate sampling to ensure an accurate determination of the circulating blast percentages, the International Working Group on Morphology of Myelodysplastic Syndrome recommends that a count of at least 200 cells be performed, with some investigators recommending a differential count of as many as 500 cells.

## Bone Marrow

The BM in MDS is often hypercellular. This paradox of marrow hypercellularity despite peripheral blood cytopenias is the direct result of the ineffective hematopoiesis that is characteristic of MDS. In some MDS cases, the BM may be normocellular or hypocellular, in which case the possibility of an evolving aplastic anemia or a

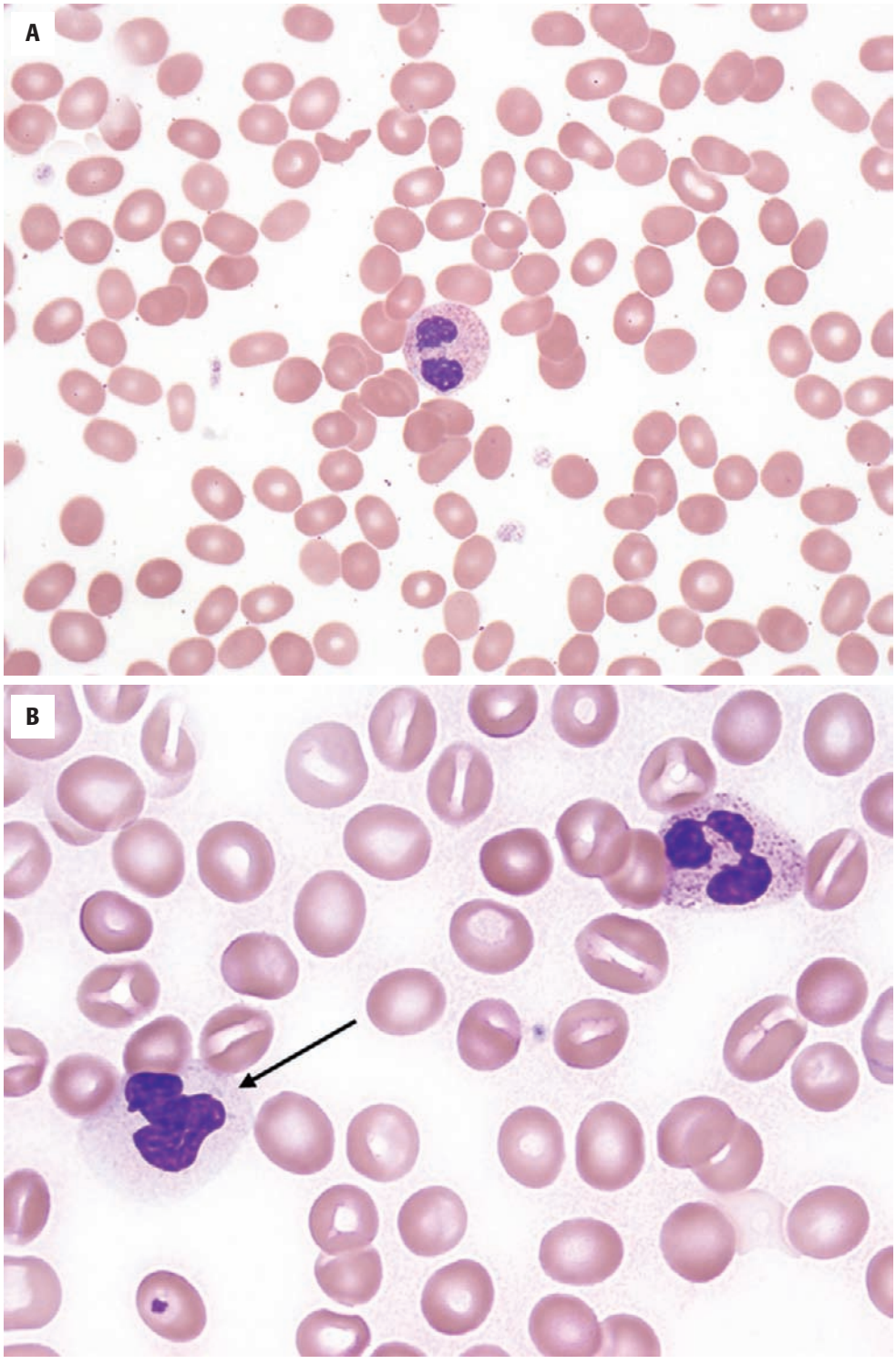


**FIGURE 18-3**

Examples of dysgranulopoiesis. **A,** Dysplastic neutrophil exhibiting pseudo Pelger-Huët anomaly with nuclear hyposegmentation. Note the thin, threadlike chromatin strand between the two nuclear lobes (Wright-Giemsa stain). **B,** Dysplastic neutrophil exhibiting cytoplasmic hypogranulation. In contrast to the normal amount of secondary granules present in the segmented neutrophil on the right side of the field, the band neutrophil *(arrow)* shows markedly decreased secondary cytoplasmic granules (Wright-Giemsa stain).

sampling artifact should also be considered, particularly if there are no definitive morphologic features of dysplasia.

#### Dyserythropoiesis

Dysplastic erythroid precursors may exhibit asynchronous nuclear-cytoplasmic development, nuclear abnormalities such as nuclear lobulation, irregular nuclear contours, karyorrhexis, atypical multinucleation, and internuclear chromatin bridging (Figure 18-4, A-C). Dyserythropoiesis may also manifest as ring sideroblasts, which are defined as erythroid precursors with five or more iron granules encircling at least one third of the cells' nuclear circumference (Figure 18-4, D). Dysplastic erythroblasts may show cytoplasmic vacuoles that stain positively for periodic acid-Schiff in a globular or coarsely punctate fashion (Figure 18-4, E).

#### Dysgranulopoiesis

Similar to the blood, dysplastic neutrophils and precursors with cytoplasmic hypogranulation or nuclear hyposegmentation can be seen in the BM aspirate smears. Dysplastic unilobated and nonlobated neutrophils can be distinguished from neutrophil myelocytes by the clumped, more condensed and more mature-appearing nuclear chromatin in the former.

#### Dysmegakaryopoiesis

Dysplastic megakaryocytes may be abnormally small with nonlobated or hypolobated nuclei, or they may be normal to large in size but with abnormally disconnected nuclear lobes (so-called osteoclast-like megakaryocytes; Figure 18-5, A-C). These features are best evaluated in the aspirate smears, because sectioning artifact may create the illusion of hypolobated or widely separated nuclear lobes in megakaryocytes. Clustering of small megakaryocytes is best visualized in the core biopsy sections and can be a helpful feature in support of a clonal process involving megakaryopoiesis, although the possibility of myeloproliferative neoplasms or marrow regeneration needs to be excluded.

#### Increased Blasts

Accurate enumeration of medullary blasts is essential in the diagnosis, classification, and prognostication of MDSs. A 500-cell differential count is recommended for the BM at the minimum. By morphologic evaluation, normal BM smears usually contain less than 3% blasts, although the cut-off level for MDS as first proposed by the French-American-British (FAB) group has remained at 5%. Substitution of a morphologic blast count with one obtained by flow cytometry immunophenotyping is not recommended, because dilution of the marrow aspirate by peripheral blood, specimen manipulation during processing for flow cytometry, and in some cases, the presence of blasts that lack CD34 expression may distort the marrow blast percentage as derived by flow cytometric immunophenotyping analysis.

#### Auer Rods

Based on the assumption that Auer rods indicate a more aggressive disease, their detection in MDS, regardless of the PB or BM blast percentages, constitutes one of the criteria in the 2008 WHO definition of the high-grade MDS category "refractory anemia with excess blasts-2" (RAEB-2). Rarely, Auer rods may be identified in cases with morphologic features that otherwise might be viewed as lower grade MDS. In a retrospective study involving three institutions, nine such cases of MDS were identified in which there were fewer than 5% marrow blasts but where Auer rods were present. These patients had a worse prognosis than would have been expected according to their calculated IPSS scores (from IPSS-0 to IPSS-1.0), with rapid progression to AML or shortened survival, or both.

## Ancillary Studies

### Cytochemistry

A periodic acid-Schiff stain of the marrow aspirate may highlight globular or coarsely punctate positive reactivity in erythroblasts with cytoplasmic vacuoles (see Figure 18-4, E). An iron stain of the marrow aspirate smear is necessary to look for ring sideroblasts (see Figure 18-4, D). Regarding the granulocytes, a combined butyrate esterase and chloracetate esterase stain can reveal an abnormally increased population of dual-esterase–positive cells in some cases of MDS, thus uncovering an additional feature of abnormal myeloid maturation, although this feature is not pathognomonic of MDS and can also be seen in myelomonocytic neoplasms and hematopoietic regeneration.

### Immunohistochemistry

A basic panel of antibodies that has been found to be useful in suspected cases of MDS includes the blast marker CD34 and a megakaryocytic marker such as CD31, CD42, or CD61. Immunohistochemical studies with CD34 stain the blast cells in most cases of MDS

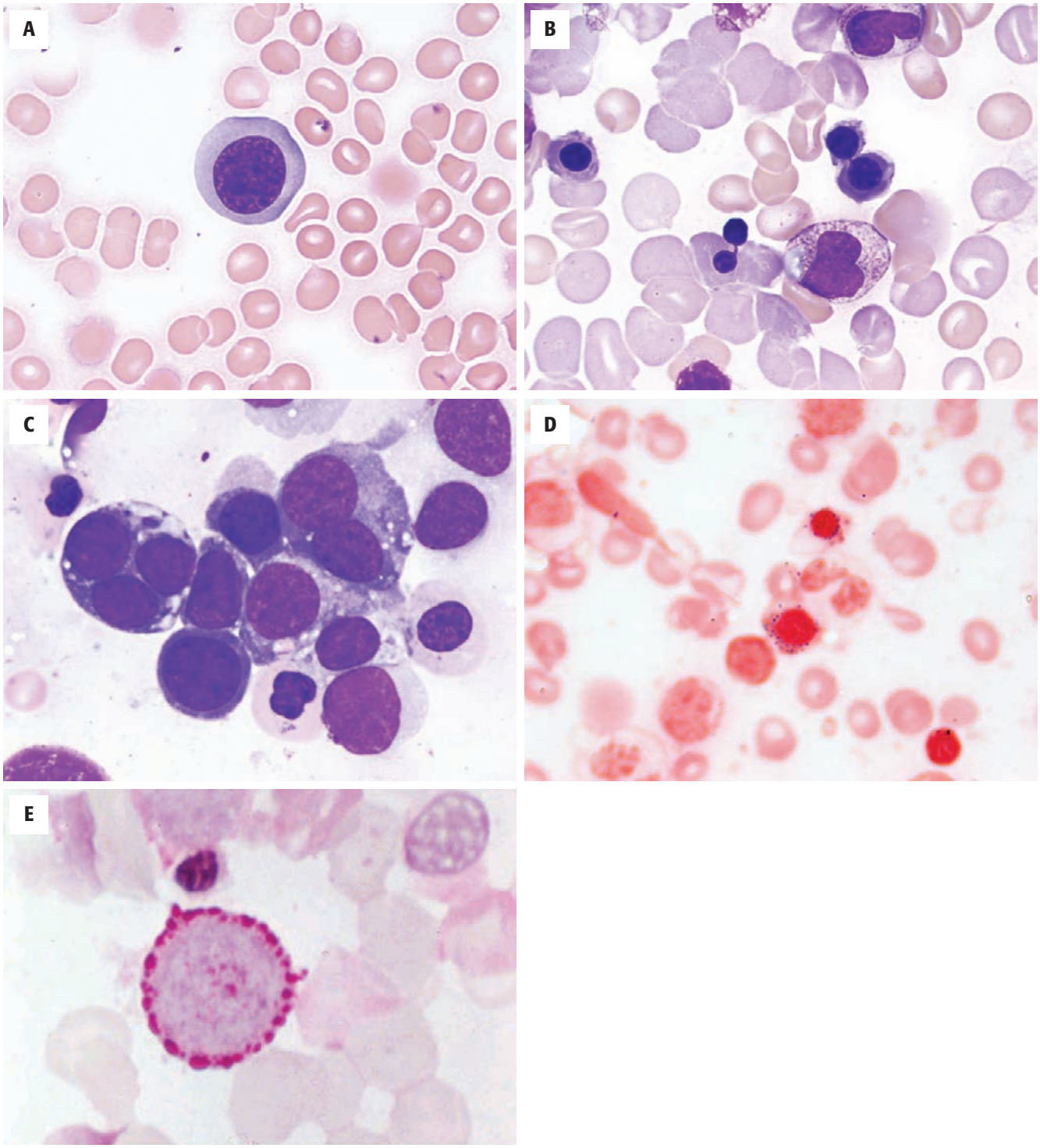


**FIGURE 18-4**

Examples of dyserythropoiesis in bone marrow aspirate smears. Dyserythropoiesis can manifest as megaloblastoid maturation **(A),** nuclear bridging **(B),** binucleation and multinucleation **(C),** ring sideroblasts **(D),** and cytoplasmic vacuoles **(E)** that stain periodic acid-Schiff–positive in a globular fashion. **A-C,** Wright-Giemsa stain; **D,** Dacie stain; **E,** periodic acid-Schiff stain.

and can be of great value in assessing the number and distribution of these cells. This is particularly important in MDS with fibrosis or hypocellular MDS. CD34 immunohistochemistry is also useful for assessing small compact blast cell aggregates that, defined as clusters of at least three cells in contradistinction to individually scattered $CD34^+$ cells, are highly suggestive of a neoplastic process. When detected in MDS, these are usually associated with the MDS subtypes with an excess of blasts. In the recent literature, the recommended term

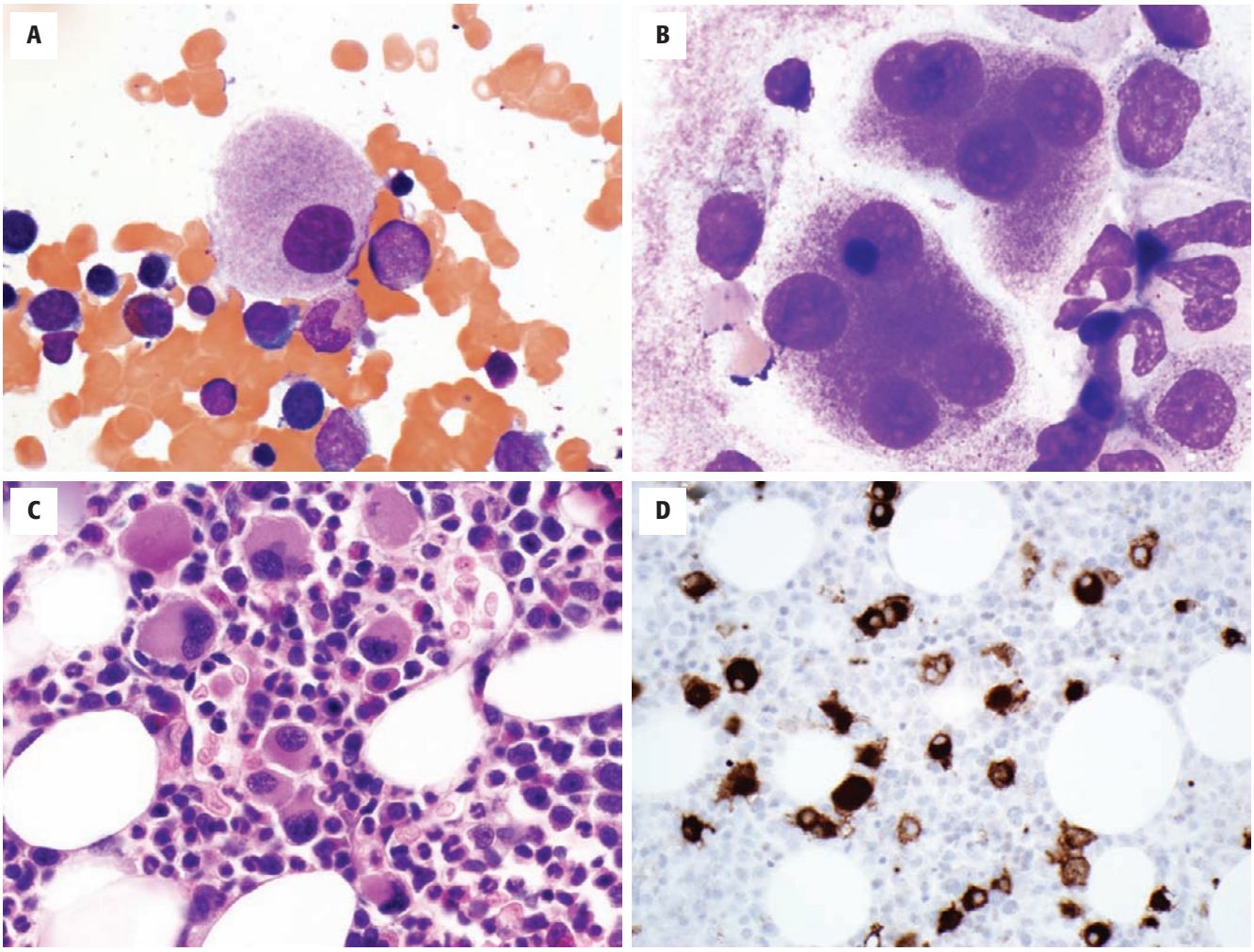


**FIGURE 18-5**

Examples of dysmegakaryopoiesis. **A,** Dysplastic small megakaryocyte with a nonlobated nucleus. The cell is mature, as evident by the abundant amount of platelet granules present in the cytoplasm (Wright-Giemsa stain). **B,** Dysplastic megakaryocytes with widely separate nuclear lobes (Wright-Giemsa stain). **C,** Clusters of dysplastic small and hypolobated megakaryocytes in this bone marrow section (hematoxylin and eosin). **D,** An immunohistochemical stain for CD61 highlights numerous small and nonlobated or hypolobated megakaryocytes.

for this finding is *multifocal accumulations of CD34⁺ progenitor cells,* which may be used in lieu of *abnormal localization of immature precursors*. In the few cases of MDS in which progenitor or blast cells are CD34⁻, CD117 can be used as an alternate marker, although caution should be used when interpreting this stain, because promyelocytes and potentially early erythroid precursors can stain positively for CD117. Megakaryocyte markers (CD31, CD42, or CD61) are used in the detection of both atypical accumulations (groups or clusters) and cytomorphologic atypia of megakaryocytes (Figure 18-5, *D*).

In rare cases in which there is mastocytosis associated with the MDS, staining for tryptase and CD117 may highlight clusters of spindle-shaped mast cells. Round tryptase-positive cells have also been described in some cases of MDS by some investigators; these could represent mast cells, basophils, or immature myeloid progenitors.

## Immunophenotype

The role of flow cytometric immunophenotyping in the diagnosis or management of patients with MDS remains somewhat unsettled. Studies from several groups have suggested that flow cytometric analysis can detect immunophenotypic dysplasia that corresponds to or possibly precedes the pathologic diagnosis of MDS. For example, according to such studies, dysgranulopoiesis can manifest immunophenotypically as absent CD10 or CD64 expression on granulocytes; expression of nonmyeloid antigens CD2, CD5, CD7, and CD19; diminished expression of CD11b, CD16, or both on myeloid cells or an abnormal CD16/CD13 pattern. Similarly, dyserythropoiesis can manifest immunophenotypically as abnormally low intensity of CD71 expression or as dyssynchronous expression of CD71 and glycophorin A on erythroid precursors.

Other investigators who have focused on the blast or progenitor compartment have reported that abnormalities involving expression of CD117, CD38, CD33, or of the lineage infidelity markers CD2, CD5, CD7, CD19, and CD56 might help to discriminate between MDS and a variety of cytopenias resulting from nonclonal disorders. A marked decrease in hematogones has also been described in patients with MDS compared to noncytopenic and non-MDS cytopenic control subjects. However, the specificity of this finding is not yet completely known, because similar decreases in hematogones have also been reported in pediatric patients with aplastic anemia.

From a prognostic standpoint, prior studies have shown that expression of myeloid cell immaturity, such as CD7 and CD117 on blasts, was associated with an unfavorable outcome as measured by survival, whereas expression of markers of myeloid cell maturation such as CD10 and CD15 was associated with a more favorable outcome. Other studies have reported that the presence of paroxysmal nocturnal hemoglobinuria (PNH)–type phenotype, defined as loss of CD55 or CD59 expression in at least 0.003% of cells in the blood of patients with MDS, was associated with generally a more indolent course and better hematologic responsiveness to immunosuppressive therapy. A recent study by Westers and colleagues of patients with MDS with IPSS low- and intermediate-1 risk MDS patients reported a lower response to erythropoietin stimulating agent, granulocyte-colony stimulating factor (G-CSF) therapy, or both, among those patients with immunophenotypically aberrant blasts compared to those with immunophenotypically normal blasts. In this study, immunophenotypic aberrancies on blasts consisted of expression of the lineage infidelity marker CD5, CD7, or CD56; a combination of CD5 and CD56 expression; expression of CD7 with loss of CD45; or loss of the myeloid antigen CD33.

However, despite this growing number of observations on immunophenotypic abnormalities in MDS since the first edition of this chapter, the specificity of such observations as described previously remains unclear, due largely to persistent uncertainties regarding the extent of the overlap between normal and dysplastic hematopoietic maturation, as well as uncertainties regarding changes in blast phenotypes during blast development, hematopoietic regeneration, and following growth factor stimulation. Thus, from the first European Leukemia Networking Conference on the role of flow cytometric immunophenotyping in MDSs, participants concluded that "further (prospective) validation of markers and immunophenotypic patterns . . . against control patient groups as well as further standardization in multicenter studies" would still be needed before definitive conclusions could be drawn on the role of this modality in the diagnosis and prognostication of MDS.

## Cytogenetics

Cytogenetic analysis of the BM is essential in the evaluation of patients with suspected MDS, both for diagnostic and prognostic purposes. The frequency of clonal cytogenetic abnormalities in MDSs ranges from 5% to 10% among patients with the lower-grade subtypes of MDS, where the blood and marrow blast counts are less than 5%, to as high as 70% among the higher-grade subtypes with excess blasts. Overall, the estimated frequency of chromosomal abnormalities among all patients with MDS is 50%. Therefore, whereas a normal cytogenetic result does not exclude the possibility of MDS, the presence of clonal cytogenetic abnormalities in patients with unexplained cytopenias and borderline or no appreciable morphologic abnormalities can be of great help in establishing the diagnosis of MDS. Table 18-1

**TABLE 18-1**

**Recurring Cytogenetic Abnormalities in Myelodysplastic Syndromes and Their Frequencies**

| Abnormality | Primary MDS (%) | Therapy-Related MDS (%) |
|---|---|---|
| ***Unbalanced*** | | |
| +8* | 10 | |
| –7 or del(7q) | 10 | 50 |
| –5 or del(5q) | 10 | 40 |
| del(20q)* | 5-8 | |
| –Y* | 5 | |
| i(17q) or t(17p) | 3-5 | |
| –13 or del(13q) | 3 | |
| del(11q) | 3 | |
| del(12p) or t(12p) | 3 | |
| del(9q) | 1-2 | |
| idic(X)(q13) | 1-2 | |
| ***Balanced*** | | |
| t(11;16)(q23;p13.3) | | 3 |
| t(3;21)(q26.2;q22.1) | | 2 |
| t(1;3)(p36.3;q21.2) | 1 | |
| t(2;11)(p21;q23) | 1 | |
| inv(3)(q21q26.2) | 1 | |
| t(6;9)(p23;q34) | 1 | |

From Swerdlow SH, Campo E, Harris NL, et al, editors: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue*, ed 4, Lyon, 2008, IARC.
*MDS,* myelodysplastic syndrome.
*In the 2008 World Health Organization classification, the presence of these abnormalities as the sole cytogenetic abnormality in the absence of morphologic dysplasia is not considered as definitive evidence for MDS. In contrast, the other abnormalities listed are considered to be "presumptive" evidence of MDS in the setting of persistent cytopenias even when there are no definitive morphologic features.

lists such specific cytogenetic abnormalities that, according to the 2008 WHO guidelines for classifying the MDSs, may be considered as "presumptive" evidence of MDS in the setting of unexplained persistent cytopenia, even in the absence of morphologic features of dysmyelopoiesis.

For the 50% patients with MDS without apparent karyotypic abnormalities, recent analyses by high-density single-nucleotide polymorphism arrays have uncovered molecular features of clonality. Using this approach in a carefully designed prospective study of 51 patients with MDS in which BM and/or sorted CD34-positive cell DNA were compared with the patients' own matched or paired buccal cell DNA, Heinrichs and colleagues reported clonal somatically acquired genomic abnormalities in approximately 40% of the patients studied. Among patients with a normal karyotype, 15% had clonal genomic abnormalities. Although not currently available as a clinical test, this technique may add to the list of analytical tools that can help in providing the requisite proof of clonality in some of the 50% of MDS patients with a normal karyotype or in those patients in whom there is a high index of suspicion for MDS.

When the diagnosis of MDS is clear, detection of certain types of cytogenetic anomalies can provide valuable prognostic information. For example, the presence of three or more chromosomal anomalies or of abnormalities involving chromosome 7 is considered to portend a poor prognosis. In contrast, a normal karyotype, an isolated del(5q), or an isolated del(20q) is generally associated with a good prognosis. Recently published data from a large MDS registry on 1931 primary MDS and 141 secondary MDS patients who had been treated with a variety of treatments confirm these earlier observations on the prognostic value of cytogenetics in MDS patients and, with follow-up between 90 and over 300 months, provide insights on some of the rarer cytogenetic abnormalities. For example, del(9q), del(15q), and t(15q), when occurring as noncomplex abnormalities, appear to confer a good prognosis, whereas other abnormalities such as rearrangements of 3q or +19 occurring as noncomplex abnormalities seem to be associated with an intermediate prognosis.

## Molecular Genetics

### For Proof of Clonality

Given the lack of demonstrable clonal cytogenetic abnormalities in approximately 50% of patients with MDS, Valent and colleagues have proposed the use of additional tests for clonality such as flow cytometry, human androgen receptor (HUMARA)-assay, gene chip profiling, or point mutation analysis (e.g., *RAS* mutations). Currently, these studies are not standardized, consistently available as a validated clinical test, or specific for MDS; they are not part of the 2008 WHO or NCCN guidelines in the diagnosis of MDS. Their role in the diagnosis of MDS will require further investigation.

### For Shedding Light on the Pathogenesis and Biology of MDSs

Since the first edition of this chapter, exciting advances have been made on the molecular genetic features of MDS. Previous reports have implicated disruptions of the *NRAS* oncogene, of the *TP53* and *IRF1* tumor-suppressor genes, and of the transcription factor genes *EVI1* and *MLL* in the pathogenesis of MDS. Recent additions to this growing list of single-gene mutations in patients with MDS have included those involved in cell signaling and differentiation, such as *CSF1R* and *RUNX1*, as well as those involved in cell cycle regulation, such as *TET2.* Somatic mutations of the histone methyltransferase gene *EZH2* have also been reported, and mutations involving the isocitrate dehydrogenase 1 (*IDH1*) gene were recently reported to carry a poor prognosis independently of other known prognostic factors such as karyotype, transfusion dependence, and IPSS score. The fact that each of these mutations appears to affect at most only 10% to 15% of patients with MDS studied suggests mutations that they might not be the primary pathogenetic driver, although this fact in itself would not necessarily preclude the potential diagnostic or prognostic value of these observations.

Analyses by gene expression profiling have enabled investigators to move beyond descriptions of single gene abnormalities and to evaluate instead the expression profiles of groups of genes, thus constructing possible pathways in the pathogenesis of MDS. For example, Pellagatti and colleagues report the interferon and thrombopoietin signaling pathways as the most frequently upregulated pathways in MDS patients, with involvement of *STAT1, IRF9, IFIT1,* and *IFITM1* genes and of *TPOR, STAT1,* and *SOS1* genes, respectively. Interestingly, in this same study, the immunodeficiency signaling, B-cell receptor signaling, IL4 signaling, and chemokine signaling pathways appear to be the most frequently deregulated pathways in patients with the lower-grade refractory anemia as compared to those patients with the higher-grade refractory anemia with excess blasts. This observation seems particularly intriguing in view of the known clinical responsiveness to immunosuppressive therapy among subsets of patients with low-grade MDS.

Although not specific to MDS, the concept of the epigenome and of epigenetic changes exerting another level of genetic controls provides a new and exciting intellectual framework for the purpose of therapeutic

development in MDSs. In this paradigm, expression or silencing of a gene does not require actual alterations at the level of the primary nucleotide sequence. Instead, it may occur by enzymatic reorganization of the three-dimensional structure of chromatin, thereby modulating the accessibility of that genetic region to transcriptions factors and other proteins involved in gene expression. Thus far, studies have suggested that such epigenetic modifications might include methylation of the cytosine residues in so-called CpG-rich islands, acetylation, phosphorylation, ubiquitination, or poly(ADP-ribosyl)ation. CpG islands are genomic regions located in or near promoters with a high content of CG dinucleotides. Cytosine methylation of these areas inhibits gene expression. Hypermethylation of the cell cycle regulator *p15* $^{INK4B}$ gene, the pro-apoptotic *DAP*-kinase gene, the ribosomal processing gene *RPS14*, and the *FHIT* gene, among others, has been identified in approximately 50% of patients with MDS, thus raising the attractive possibility of demethylation as a therapeutic strategy and possibly serving to explain the success of 5′ azacytidine in the treatment of patients with MDS (see Epigenetic Therapies later in this chapter). Histone acetylation at specific lysine residues on histones H3 and H4 has also been shown to represent another means of reorganizing the three-dimensional structure of chromatin that may allow for either repressive or permissive gene transcriptions.

Next generation sequencing is being applied to MDS and will likely impact our understanding of the molecular genetics of this group of diseased. For example, mutations of *SF3B1* have been found in the majority of cases of refractory anemia with ring sideroblasts. This provides a new avenue of research on the role of the spliceosome and abnormalities of RNA splicing in MDS.

In light of the growing number of molecular genetic advances in the few years between the first and current editions of this book, there is much to come in terms of understanding the pathogenesis and biology of MDSs. Currently, these molecular genetic modalities as described here are not yet available on a routine clinical basis to help in the diagnosis or prognostication of the individual patient being evaluated for possible MDS.

## DIAGNOSTIC CLASSIFICATION

### FRENCH-AMERICAN-BRITISH CLASSIFICATION

In 1975 the FAB cooperative group published the first major international effort to classify MDSs. This classification was based entirely on morphologic features identified in the peripheral blood and BM aspirate smears. In the initial proposal, two subtypes of "dysmyelopoietic syndromes" were defined: RAEB and chronic myelomonocytic leukemia (CMML). In 1982, refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), and RAEB in transformation (RAEB-T) were added. This subclassification considered the percentage of peripheral blood and marrow blasts, the percentage of ring sideroblasts, and the presence of a blood monocytosis. However, the number and extent of cytopenias and the extent of dysplasia were not explicitly addressed in the FAB classification system. Furthermore, among patients in those FAB categories of MDS without increased blasts (RA, RARS, and CMML), the wide range of survival (from less than 1 year to 5 to 6 years) caused some to question the clinical reliability of this classification system.

### 2001 WORLD HEALTH ORGANIZATION CLASSIFICATION

Some of the issues raised by the FAB classification were addressed in the WHO classification of MDSs published in 2001. In this next international effort at MDS subclassification, the importance of integrating morphology with other techniques such as cytogenetics and molecular genetics as well as with the clinical information was emphasized. Compared to the FAB classification, the 2001 WHO guidelines differed in several substantial ways. First, the proportion of blasts required for a diagnosis of AML was lowered from 30% to 20%, which effectively eliminated the FAB category of RAEB-T. Second, the high-grade MDS subcategory of RAEB was subdivided into RAEB-1 and RAEB-2. Third, to reflect the effects of multilineage dysplasia on overall survival and on the likelihood of transformation to acute leukemia, the low-grade FAB subtypes of RA and RARS were more strictly and narrowly defined, and a new category of refractory cytopenia with multilineage dysplasia (RCMD) with ring sideroblasts (RCMD-RS), or without, was added. Fourth, the 2001 WHO guidelines recognized the 5q- syndrome as a distinct and separate category of low-grade MDS, defined by the presence of del(5q) as the sole chromosomal abnormality with the appropriate associated morphologic and clinical features. Last, the subcategory of CMML was removed from the MDSs and assigned to the overlap group of "myelodysplastic/myeloproliferative neoplasms."

### 2008 WORLD HEALTH ORGANIZATION CLASSIFICATION

The 2008 WHO classification of MDSs included mostly refinements of the 2001 WHO guidelines, with the addition of a few rare MDS subtypes. An emphasis was placed on the exact percentages of circulating blasts, even when less than 5%, in the definition of the various MDS subtypes. Two new subcategories were added. The

**TABLE 18-2**

**Key Diagnostic Features of Myelodysplastic Syndrome Subtypes According to the 2008 World Health Organization**

| Name | Abbreviation | Peripheral Blood | Bone Marrow |
|---|---|---|---|
| Refractory cytopenias with unilineage dysplasia | RCUD | <1% blasts | <5% blasts; <15% ring sideroblasts |
| Refractory anemia | RA | Anemia | Unilineage erythroid dysplasia in ≥10% of cells |
| Refractory neutropenia | RN | Neutropenia | Unilineage granulocytic dysplasia in ≥10% of cells |
| Refractory thrombocytopenia | RT | Thrombocytopenia | Unilineage megakaryocytic dysplasia in ≥10% of cells |
| Refractory anemia with ring sideroblasts | RARS | Anemia, no blasts | Unilineage erythroid dysplasia, ≥15% ring sideroblasts, <5% blasts |
| Refractory cytopenia with multilineage dysplasia | RCMD | Cytopenias <1% blasts, no Auer rods | Dysplasia in ≥2 lineages, ± ring sideroblasts, <5% blasts, no Auer rods |
| Refractory anemia with excess blasts, type 1 | RAEB-1 | Cytopenias, <5% blasts, no Auer rods or 2%-4% blasts with <5% marrow blasts | Dysplasia in ≥1 lineages, 5%-9% blasts, no Auer rods |
| Refractory anemia with excess blasts, type 2 | RAEB-2 | Cytopenias, 5%-19% blasts, ± Auer rods | Dysplasia in ≥1 lineages, 10%-19% blasts, ± Auer rods |
| MDS associated with isolated del(5q) | Del(5q) | Anemia, normal or high platelet count, <1% blasts | Isolated del(5q), hypolobated megakaryocytes, <5% blasts |
| Childhood MDS, including refractory cytopenia of childhood (provisional) | RCC | Cytopenias | <5% blasts for RCC, usually hypocellular |
| MDS, unclassifiable | MDS-U | Cytopenias, ≤1% blasts | Does not fit other categories: Unilineage dysplasia but with pancytopenia; RCUD or RCMD picture, but with 1% circulating blasts; no definitive dysplasia, but persistent cytopenias with characteristic MDS-associated cytogenetic abnormalities |

From Swerdlow SH, Campo E, Harris NL, et al, editors: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue,* ed 4, Lyon, 2008, IARC.
±, with or without.

first, refractory cytopenia with unilineage dysplasia (RCUD), includes patients with RA and rare patients with refractory neutropenia (RN) or refractory thrombocytopenia (RT), thus accounting for all three individual cytopenias; it should be noted that although uncommon, patients in the last two categories would have been unclassifiable in the FAB classification and the previous 2001 WHO classification schemata. The second important category that was introduced was that of childhood MDS, including a provisional entity known as *refractory cytopenia of childhood* (RCC). The following section discusses in detail the diagnostic features of the different categories of MDSs recognized in the 2008 WHO classification. The key diagnostic criteria are presented in Table 18-2.

## REFRACTORY CYTOPENIAS WITH UNILINEAGE DYSPLASIA

The RCUD designation encompasses 10% to 20% of MDS cases and describes patients with refractory cytopenia associated with clear-cut dysplasia limited to one cell line. Refractory bicytopenia accompanied by unilineage dysplasia may be included in this category. Refractory pancytopenia that is associated with unilineage dysplasia should be designated as *MDS, unclassifiable*.

## REFRACTORY ANEMIA

Most patients with refractory anemia (RA) exhibit a normocytic and normochromic or macrocytic anemia. Blasts are less than 1% in the peripheral blood and less than 5% in the marrow. No Auer rods are present. Dyserythropoiesis is present in at least 10% of erythroid precursors in the marrow aspirate, but ring sideroblasts account for less than 15% of erythroid precursors. Less than 10% of the cells in the granulocytic or megakaryocytic lineage show dysplasia. In the absence of a cytogenetic abnormality characteristic for MDS, the diagnosis of refractory anemia is one of exclusion: it requires

cytopenia(s) of at least 6 months' duration and the exclusion of potential reactive causes (see Differential Diagnosis, Qualitative Defects, Dyserythropoiesis). In general, RA is viewed as a low-grade MDS with median survival in the range of 6 to 7 years and with less than 5% of cases progressing to AML.

## Refractory Neutropenia And Refractory Thrombocytopenia

Both RN and RT are rare and account for less than 1% to 2% of all MDS cases. Dysgranulopoiesis or dysmegakaryopoiesis should be present in at least 10% of granulocytic cells or megakaryocytes, respectively. For the diagnosis of RT, it is recommended that a minimum of 30 megakaryocytes be evaluated to ensure adequate sampling. Because of their uncommon occurrences, the diagnosis of RN or RT should be made with a great deal of caution. Non-neoplastic causes for isolated neutropenia or thrombocytopenia are much more common than clonal disease and should be excluded (see Differential Diagnosis, Quantitative Defects and Qualitative Defects, Dysgranulopoiesis and Dysmegakaryopoiesis).

## REFRACTORY ANEMIA WITH RING SIDEROBLASTS

Refractory anemia with ring sideroblasts is a low-grade MDS that is caused by abnormal mitochondrial iron metabolism; it is characterized by anemia, dyserythropoiesis, and 15% ring sideroblasts or greater. Ring sideroblasts are erythroblasts in which five or more iron granules encircle at least one third of the cells' nuclear circumference. There is no significant granulocytic or megakaryocytic dysplasia. The red blood cells often exhibit a dimorphic pattern with a mixture of normochromic and hypochromic cells, but a normochromic and macrocytic anemia may also be observed. There are no circulating blasts, and marrow blasts are less than 5%. It should be noted that ring sideroblasts can be found in any of the subsets of MDS, in AML, and in nonneoplastic diseases (see Differential Diagnosis, Qualitative Defects, Ring Sideroblasts).

RARS patients have a good prognosis, with a median survival of 7 to 9 years or longer and with a low rate of conversion to AML (less than 5%). When patients have both increased ring sideroblasts and a concurrent elevated platelet count ($\geq 450 \times 10^9$/L) with megakaryocytic features reminiscent of those seen in myeloproliferative neoplasms, consideration should be given to the provisional myelodysplastic/myeloproliferative neoplasm entity known as *refractory anemia with ring sideroblasts associated with marked thrombocytosis* (RARS-T). Whereas the *JAK2* V617F mutation is uncommon in MDS, it has been reported in 30% to 90% of patients with RARS-T. The recent discovery of frequent recurrent mutations in the spliceosome factor *SF3B1* in RARS and RARS-T (64% and 72% of cases, respectively) may open a new line of investigation and provide insight into the pathogenesis of these diseases.

## REFRACTORY CYTOPENIA WITH MULTILINEAGE DYSPLASIA

Accounting for approximately 30% of MDS cases, RCMD is characterized by cytopenia involving one or more lineages and dysplastic changes affecting at least 10% of cells in two or more of the hematopoietic lineages. There are less than 1% blasts in the blood, less than 5% blasts in the BM, and no Auer rods. Cases with greater than 15% ring sideroblasts can be designated as RCMD-RS, although this designation does not appear to confer any additional effect on prognosis. Cases meeting the criteria for RCMD but with persistently 1% circulating blasts should be considered as MDS, unclassifiable. Cases with multilineage dysplasia and less than 5% blasts in the BM but with 2% to 4% blasts in the blood should be classified as RAEB-1. With a reported median survival of approximately 30 months and with approximately 10% of patients progressing to AML, the diagnosis of RCMD carries a more unfavorable outcome compared with that of refractory cytopenia with unilineage dysplasia.

## REFRACTORY ANEMIA WITH EXCESS BLASTS

Subdivided into two subcategories, RAEB-1 and RAEB-2, RAEB as a group accounts for 40% of cases of MDS. Most cases of RAEB-1 have 5% to 9% blasts in the marrow, less than 5% blasts in the blood, and no Auer rods. Occasionally, according to the 2008 WHO classification, the diagnosis of RAEB-1 can be made with less than 5% medullary blasts if there are 2% to 4% circulating blasts. The category RAEB-2 describes cases in which there are 10% to 19% blasts in the marrow or 5% to 19% blasts in the blood, or both. The presence of Auer rods also qualifies a case as RAEB-2 when the blast percentages are less than 19%. Patients with RAEB have an unfavorable prognosis overall regardless of whether they may develop AML. The median survival for patients with RAEB-1 is approximately 18 months and 10 months for those with RAEB-2. Approximately 25% of patients with RAEB-1 and 33% of patients with RAEB-2 develop acute leukemia, with the highest rate (48%) observed among patients whose RAEB-2 designation was based on a peripheral blast count exceeding 5% or on the presence Auer rods.

## MYELODYSPLASTIC SYNDROME ASSOCIATED WITH AN ISOLATED DEL(5q) CHROMOSOMAL ABNORMALITY

This type of MDS is characterized by anemia with or without other cytopenias, less than 5% marrow blasts, less than 1% PB blasts, no Auer rods, and a sole cytogenetic abnormality of del(5q). Patients with MDS and with increased blasts or additional cytogenetic abnormalities should not be placed in this category. There are several regions on chromosome 5 that are deleted in patients with other types of MDS and AML, but an interstitial deletion affecting bands q31-q33 is characteristic of this MDS category. Mapping of the commonly deleted region suggests that haploinsufficiency for the ribosomal gene *RPS14* is central to the pathogenesis of this syndrome, with activation of TP53 and upregulation of the TP53 pathway resulting in defective erythropoiesis and anemia as observed both in the 5q– mouse model and in the human 5q– syndrome. Haploinsufficiency of the microRNA genes *miR-145* and *miR-146a* may be other cooperating events that account for abnormalities of the megakaryocytic lineage, including the thrombocytosis seen in some patients with the 5q- syndrome. Although there is a spectrum of clinical presentations associated with this MDS subtype, a typical profile that has been mentioned in the literature is that of an older woman with macrocytic anemia, normal or elevated platelet count, erythroid hypoplasia, megakaryocytes with nonlobated or hypolobated nuclei (Figure 18-6), and less than 5% blasts with no Auer rods. Before the use of the thalidomide derivative lenalidomide, MDS with isolated del(5q) was typically viewed as an indolent disease. In a retrospective single-institution study of 88 patients with MDS with isolated del(5q) as defined according to the 2008 WHO guidelines and covering a time span largely preceding the use of lenalidomide (1989–2009), Patnaik and colleagues reported a median overall survival of 5 years, with approximately 6% of patients showing disease progression. In this study, age greater than 70 years, transfusion needs at diagnosis, and dysgranulopoiesis detected at diagnosis were identified as predictive of a shortened survival. In the era of lenalidomide, whether or not these facts will hold will require future studies, although the assessment of this MDS subtype as indolent will likely remain (see Therapy).

## MYELODYSPLASTIC SYNDROME, UNCLASSIFIABLE

The MDS, unclassifiable (MDS-U), category is best viewed as one by default, when the features do not fit neatly under any of the well-defined MDS entities described previously. The category can be used in several situations:

1. Unequivocal unilineage marrow dysplasia in greater than 10% cells of the affected lineage, but with pancytopenia and thus failing the definitions of any of the RCUD

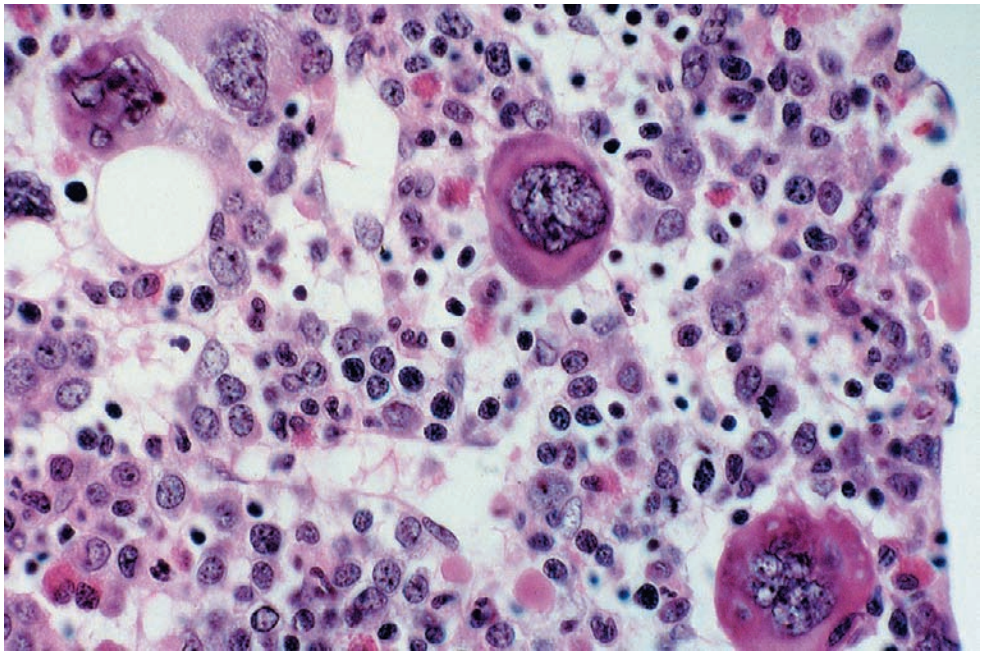

**FIGURE 18-6**

Myelodysplastic syndrome associated with an isolated del(5q) chromosomal abnormality. The dysplastic megakaryocytes in this bone marrow section are approximately normal in size, but the nuclei are hypolobated (hematoxylin and eosin).

2. Cases with features of RCUD or RCMD otherwise, but with 1% blasts in PB
3. MDS cases with less than 1% PB blasts, less than 5% BM blasts, dysplasia in less than 10% of the cells in one or more myeloid lineages, but with a cytogenetic abnormality otherwise considered as presumptive evidence of MDS (see Table 18-1)

Note that this last scenario signifies an important change brought forth by the 2008 WHO Classification of MDSs, in which a diagnosis of MDS can be rendered on the strength of cytogenetic results and the clinical history of refractory unexplained cytopenias, despite the lack of definitive morphologic findings. The incidence, rate of transformation to AML, and survival in patients with MDS-U are not known.

## CHILDHOOD MYELODYSPLASTIC SYNDROMES

MDSs represent only 4% of all pediatric hematologic cancers. In particular, de novo or primary MDS in children is exceedingly uncommon. Therefore the diagnosis of de novo MDS in a child should be made with considerable caution, because morphologic features mimicking or resembling dysplasia may be encountered in the BM of children with a wide variety of non-neoplastic conditions such as infections, metabolic disorders, vitamin deficiencies, and inherited or acquired BM failure syndromes.

Several differences exist between childhood and adult MDS. Unlike MDS in adults, thrombocytopenia and neutropenia rather than anemia and BM hypocellularity are more frequently observed in children with MDS; RARS and MDS associated with isolated del(5q) are exceedingly rare in children; the importance of multilineage versus unilineage dysplasia in childhood MDS is unknown; it is not known at present whether a blast threshold of 20% is superior to the FAB recommendation of 30% in distinguishing MDS from AML in this patient population; and it is also not known whether the RAEB-1 versus RAEB-2 distinction is of clinical value in this age group. Currently, without data indicating the contrary, the WHO recommends that the same definition for RAEB be used in this patient population as that described previously for adults with MDS, and that the same distinction between RAEB-1 and RAEB-2 be maintained at least for the purpose of future investigation.

## REFRACTORY CYTOPENIA OF CHILDHOOD

RCC, a provisional MDS entity, was introduced by the WHO in 2008 to include patients with persistent cytopenia, generally hypocellular BM, dysplasia in two different myeloid cell lineages or exceeding 10% in one single lineage, and fewer blasts than the thresholds established for RAEB (Figure 18-7). Morphologic features that are considered especially helpful in the diagnosis of RCC are pseudo Pelger-Huët neutrophils, hypogranular or agranular neutrophils, micromegakaryocytes, and islands of left-shifted erythroid precursors. More than half of patients with RCC have a normal karyotype; among the remaining patients, monosomy 7 is the most frequently detected clonal chromosomal abnormality. The diagnosis of RCC can be difficult to establish in the absence of a cytogenetic abnormality, because the hypocellular BM may preclude an adequate assessment for morphologic features of dysplasia, and it may also add to the differential diagnosis the additional consideration of an acquired or inherited BM failure syndrome. In difficult cases, repeated BM evaluations to include cytogenetic analysis may be necessary.

## OTHER CONSIDERATIONS IN THE DIFFERENTIAL DIAGNOSIS OF CHILDHOOD MYELODYSPLASTIC SYNDROMES

Acquired aplastic anemia (AA) is an autoimmune disorder characterized by increased apoptosis of hematopoietic stem cells and progenitor cells mediated by cytokines released by aberrantly activated oligoclonal T-cell populations. Approximately 10% to 20% of patients with acquired AA develop a clonal disease within the decade following their diagnosis; this is thought to occur as a result of the selective pressure applied on the stem cell pool by the cytotoxic T-cell population, which favors the evolution of somatically mutated stem cells. It is unclear whether the development of MDS is part of the extended natural history of AA, or whether it may be related to the therapies applied. Approximately 5% of patients with AA showed progression to clonal hematopoiesis before the widespread use of immunosuppressive therapy (IST), while up to 15% of long-term AA survivors developed secondary clonal disorders after treatment with IST. Some studies have shown a higher risk of transformation to MDS or AML with the addition of hematopoietic growth factors to IST regimens, and others have failed to show a statistically significant difference. In general, slight dyserythropoiesis is accepted in the BM of children with AA, but there should be no dysplasia in the granulocytic or megakaryocytic lineages. Some authors have suggested that erythroid islands with increased numbers of immature erythroid precursors or megaloblastic erythroid maturation were not seen in AA, but might be found in RCC. In the authors' personal experiences, these observations may not be absolute and are unlikely to be sufficiently specific in this differential diagnosis. Although earlier studies have described the presence of clonal or so-called transient cytogenetic abnormalities in some patients with AA, the detection of a cytogenetic

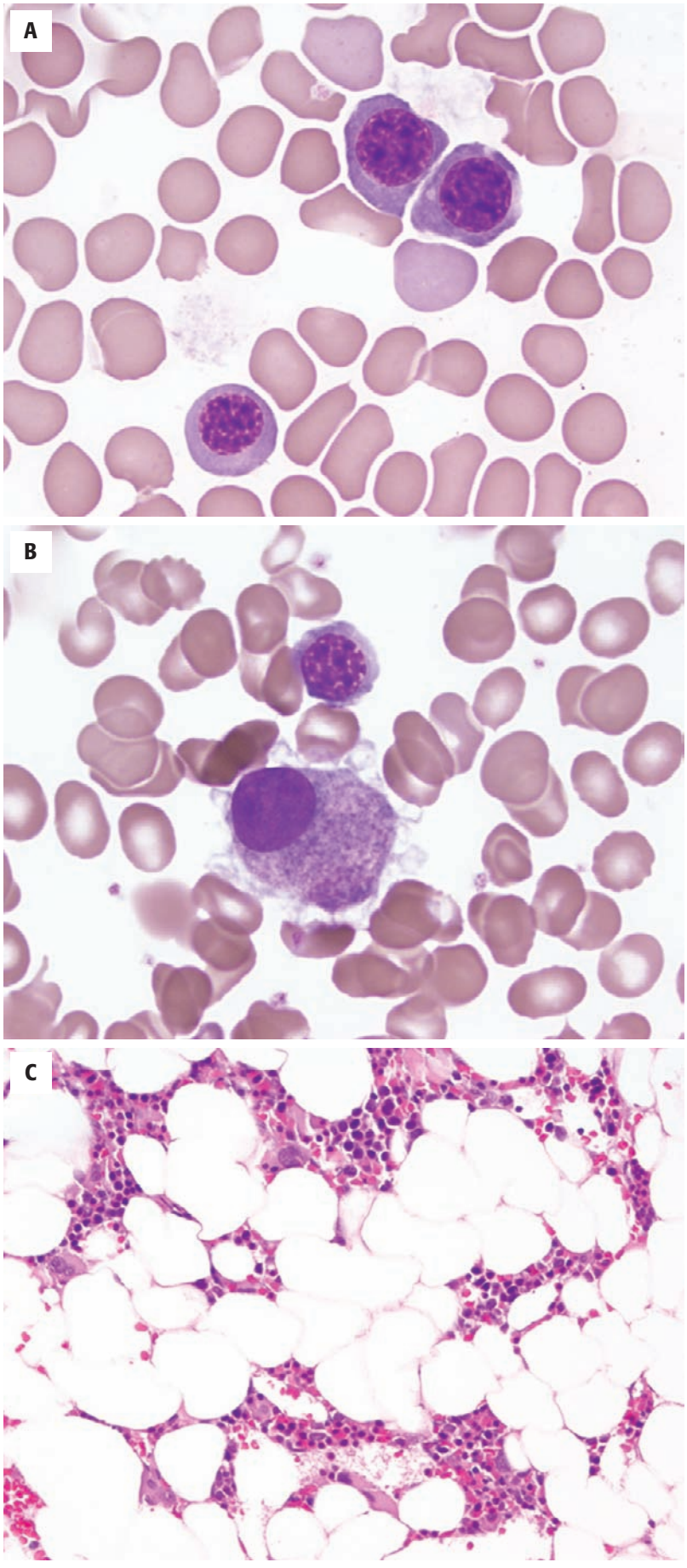


**FIGURE 18-7**

Refractory cytopenia of childhood. The bone marrow aspirate of a child with anemia and neutropenia showing megaloblastoid erythroid precursors **(A)** and a dysplastic megakaryocyte with a nonlobated nucleus (Wright-Giemsa stain, **B**). The marrow blast percentage was within normal limit, and no circulating blasts were identified. **C,** The bone marrow is hypocellular for age (hematoxylin and eosin). Chromosome analysis showed a derivative chromosome resulting in monosomy 7q and trisomy 1q.

abnormality in a patient with AA should be considered as strong evidence of potential evolution to MDS and should prompt close clinicopathologic and genetic monitoring. In children and adults with AA, the most commonly found cytogenetic abnormalities are aberrations of chromosome 7 and trisomy 8. Patients with AA who develop monosomy 7 or complex karyotype have an unfavorable prognosis with poor response to IST and a high rate of conversion to AML. In contrast, patients with trisomy 8 frequently show a good hematologic response to IST.

Fanconi anemia (FA) is an inherited BM failure syndrome associated with a high risk of progression to MDS and AML. In data from the International Fanconi Anemia Registry, MDS was reported in 7% of patients, with a cumulative incidence of 33% for hematologic malignancy (AML or MDS) by 40 years of age. MDS is a particularly challenging diagnosis in FA because of the frequent presence of dysplastic features, especially dyserythropoiesis,in the marrow of patients with FA. Dyserythropoiesis, most commonly characterized by irregular nuclear contours, budding nuclei, and karyorrhexis, is an almost universal finding in FA and may be marked (i.e., significantly higher than 10% of the cells) in some patients. Therefore, regardless of its severity, dyserythropoiesis as a sole abnormality should not be construed as evidence for MDS in these patients. A diagnosis of MDS should be considered in patients with FA when, in addition to dyserythropoiesis, dysplastic features are noted in one or both of the other two myeloid lineages, when there is an increase in BM blasts or when a clonal cytogenetic abnormality is detected. The most reliable morphologic features for MDS in patients with FA are increased blasts and dysgranulopoiesis, followed by dysmegakaryopoiesis and increased ring sideroblasts. The most frequently detected clonal chromosomal abnormalities in patients with FA who develop MDS include gains of material on chromosomes 1 and 3, loss of material on chromosome 7, and monosomy 7. When a clone is detected in FA in the absence of morphologic features of MDS, increased frequency of surveillance to include blood counts and BM evaluations should be considered, as indicated by the patient's clinical status, to monitor for potential progression to MDS or AML. Appropriate plans for stem cell transplantation should also be in place, because adverse changes may evolve rapidly.

## SPECIAL CONSIDERATIONS

### HYPOCELLULAR MYELODYSPLASTIC SYNDROME

Up to 20% of patients with MDS have a hypocellular BM, defined as less than 30% cellularity in patients younger than 60 years and less than 20% in those older than 60 years. Previous genotoxic exposure or therapy needs to be excluded because marrow hypocellularity can also be seen in therapy-related MDS. Hypocellular MDS (h-MDS) patients may have lower WBC counts and marrow blast percentages, but otherwise similar IPSS scores and frequencies of karyotypic abnormalities have been observed in this group compared with their normocellular or hypercellular MDS counterparts. Importantly, according to the few published reports on this entity, h-MDS patients appear to have a better overall survival that is independent of other risk factors. This survival benefit was demonstrated in all IPSS groups and across different cytogenetic risk groups. The lower prevalence of *RAS* and *AML1* mutations and SOCS1 hypermethylation in h-MDS—all of which are associated with poor prognosis—might serve to explain the better prognosis of this group of patients.

The distinction between h-MDS and aplastic anemia (AA) may be difficult or virtually impossible in some cases because of a number of overlapping features, such as megaloblastic features and macrocytosis, the presence of PNH-type cells, a good response to immunosuppressive therapy, and a favorable prognosis. Although features such as dysplastic granulocytes and megakaryocytes, an increased percentage of CD34+ cells in the marrow, increased reticulin fibrosis, and the identification of a clonal chromosomal abnormality may favor a diagnosis of h-MDS over AA, the possibility of a preexisting AA evolving or progressing to MDS should also be excluded. In addition to AA, the differential diagnosis of h-MDS should include other diseases such as hypocellular AML, hairy cell leukemia, and T-cell large granular lymphocyte leukemia. Even when a diagnosis of h-MDS is made, subclassification may prove difficult, especially in terms of separating between RA and RCMD or MDS-U and RCMD. Some investigators have proposed that for cases of h-MDS without increased blasts and without specific cytogenetic abnormalities, a diagnosis of hypocellular MDS may suffice.

### MYELODYSPLASTIC SYNDROME WITH FIBROSIS

Significant reticulin fibrosis has been reported in approximately 10% to 15% of patients with MDS. It is not clear whether MDS with fibrosis (MDS-f) should be considered as a distinct clinicobiologic entity, largely because criteria for its recognition have not been defined clearly in the past. Patients with MDS-f usually exhibit significant pancytopenia requiring red blood cell and platelet transfusions, multilineage dysplasia, and increased blasts. In this setting, most authors suggest that the diagnosis of MDS-f be considered if there is also a diffuse and dense increase in reticulin fibers with extensive intersections, which may be accompanied by the presence of bundles of collagen (i.e., reticulin

fibrosis of at least grade 2, in a 0 to 3 grading scale), in accordance with the European consensus guidelines. Recent studies have demonstrated that increased fibrosis in MDS is associated with lower overall survival and leukemia-free survival. The presence of grade 2 or 3 BM fibrosis represents an independent prognostic factor which determines a shift to a one-step higher risk group in the recently proposed WHO classification–based prognostic scoring system (WPSS).

One of the most important differential diagnostic considerations is acute panmyelosis with myelofibrosis, a rare subtype of acute myeloid leukemia that is characterized by the presence of 20% to 25% blasts and multilineage dysplasia, especially dysmegakaryopoiesis. Careful enumeration of blasts on touch imprints and by CD34 immunohistochemical studies on the marrow core biopsy sections is necessary in this differential diagnosis.

Other considerations in the broad differential diagnosis of MDS-f include some subtypes of AML, chronic myeloproliferative neoplasms, systemic mastocytosis, hairy cell leukemia, other non-Hodgkin lymphomas, metastatic tumors, and autoimmune diseases.

## MINIMAL DIAGNOSTIC CRITERIA FOR MYELODYSPLASTIC SYNDROME

What is required for the diagnosis of MDS? Given the broad differential diagnosis for anemia, neutropenia, and thrombocytopenia, the somewhat subjective nature of assessing morphologic features of dysmyelopoiesis, the variable degrees of dysmyelopoiesis from case to case, and the absence of cytogenetic proof of clonality in approximately half of MDS cases, this question of minimal diagnostic criteria for MDS arises frequently in clinical practice.

Following the Working Conference on MDS in 2006, Valent and colleagues put forth a proposal in which features already used in the various MDS classification schemes and in practice are organized into prerequisite criteria and MDS-related or decisive criteria, with the presence of both prerequisite criteria and one or more decisive criteria fulfilling the minimal requirements for a diagnosis of MDS. In this proposal, the two prerequisite criteria that must be fulfilled are (1) marked and constant (at least 6 months in duration) peripheral cytopenia and (2) the absence of all other hematopoietic and nonhematopoietic disorders as reasons for dysplasia or cytopenia. MDS-related or decisive criteria include the following: dysplasia in at least 10% of cells of one of the hematopoietic lineages; greater than 15% ring sideroblasts; 5% to 19% marrow myeloblasts; or an MDS-related karyotype.

In this proposal, the authors suggest the use of "co-criteria" if no decisive criteria are met. Currently, the co-criteria as listed in this proposal such as flow cytometric studies for immunophenotypic features of myelodysplasia or molecular genetic tests such as "HUMARA assay, gene chip profiling, or point mutation analysis" are not yet widely available as clinically validated tests, sufficiently standardized, or specific for MDS. Therefore a positive result by means of one of these modalities may not be sufficient for a definitive diagnosis of MDS in the absence of other definitive and clinically accepted features, and the current recommended standard of care is to continue with follow-up and repeated assessment as necessary.

## IDIOPATHIC CYTOPENIA OF UNCERTAIN SIGNIFICANCE

The term *idiopathic cytopenia of uncertain significance* has been proposed for patients whose negative or nondiagnostic blood and marrow pathologic findings do not meet the minimal criteria for MDS, but whose cytopenia of at least 6 months' duration cannot be explained by any other hematologic or nonhematologic disorders. Idiopathic cytopenia of uncertain significance is not a diagnosis, rather it is an acknowledgment of diagnostic uncertainty. The designation indicates the necessity of continued hematologic follow-up to document or exclude evolution to MDS. Recommended follow-up investigations include complete blood cell count with differential and serum chemistry at 1- to 6-month intervals, with a BM examination in patients with a high level of suspicion for MDS (e.g., patients with macrocytic anemia and transfusion dependency).

### Differential Diagnosis

The diagnosis of MDS and its subclassification carry a distinct prognosis and a finite probability of acute leukemic progression. Therefore it is imperative that all other diagnostic possibilities be thoroughly evaluated and excluded before embarking on the diagnosis of MDS. The following is a list of such alternate considerations.

#### Quantitative Defects

##### Cytopenia with Hypercellular Marrow

This scenario may be encountered in immune-mediated destruction of erythrocytes, neutrophils, or platelets, or it may be seen with sequestration owing to hypersplenism. However, without other confounding factors, immune-mediated destruction or hypersplenism typically do not result in a pancytopenia. Conversely, because MDS-RN or MDS-RT is rare, caution should be exercised when considering the diagnosis of

MDS based on an isolated neutropenia or thrombocytopenia, respectively. As rare as WHIM syndrome (an acronym for warts, hypogammaglobulinemia, infections, and myelokathexis) may be, it is perhaps no less likely than RCUD-RN as an explanation for an isolated neutropenia! A high-quality core biopsy specimen of adequate size is also necessary to rule out the possibility of a sampling bias, in which the hypercellular area may be only focal while the remainder of the marrow is in fact normocellular or hypocellular. Viral disorders, particularly HIV infection, can cause cytopenias with a hypercellular marrow. However, whereas the presence of cellular debris in association with an increased number of plasma cells and lymphocytes are frequently seen in the BM of HIV-positive patients, they are not usually present in that of patients with MDS. Cytopenias and pancytopenia have been reported in patients with chronic parvovirus B19, Epstein-Barr virus, and cytomegalovirus infections, especially among immunocompromised patients. Correlation with the clinical history and with serum serologies or viral DNA studies would be necessary, and a primary diagnosis of MDS should not be made in the face of active viral infections. Patients with hemophagocytosis may also exhibit pancytopenia and a hypercellular marrow, in which the presence of increased macrophages exhibiting varying degrees of active hemophagocytosis should discourage the diagnosis of MDS as a primary explanation for the cytopenias. Laboratory evaluation of serum ferritin and lactate dehydrogenase levels as well as coagulation profiles should be of help when considering this possibility of hemophagocytosis. Last, although acute leukemia can at times develop with cytopenia or pancytopenia despite a hypercellular marrow, the presence of 20% blasts or more in the blood or marrow should readily separate this consideration from that of MDS.

### Cytopenia with Normocellular or Hypocellular Marrow

Other causes of cytopenia with a normocellular or hypocellular marrow include aplastic anemia, paroxysmal nocturnal hemoglobinuria, myelosuppressive infections, medication effect, toxic exposure, graft-versus-host disease affecting the marrow, T-cell large granular lymphocytic leukemia, and acute leukemia. Examination of the BM typically reveals marrow hypocellularity in the first six conditions listed. Moreover, documentation of a normal hemogram within the preceding 3 to 6 months would suggest a more acute or subacute onset and would thus tend to exclude MDS. Instead, such a timeline should suggest exposure to medication, toxins, or infections as a cause for the cytopenia.

The clinical history should help to determine whether graft-versus-host disease is a likely explanation for the cytopenia. In T-cell large granular lymphocytic leukemia, laboratory evaluation typically reveals neutropenia with or without anemia, whereas thrombocytopenia as an isolated finding is uncommon. These patients often have increased circulating large granular lymphocytes, and flow cytometric immunophenotyping of the blood with immunohistochemical studies of the BM biopsy sections can help to detect phenotypically distinct T-cell populations. In the rare cases of hypocellular acute myeloblastic leukemia, identification of 20% or more myeloblasts in the marrow by morphology or CD34 immunohistochemistry, or both, should confirm the diagnosis of acute myeloblastic leukemia.

With the exception of acute leukemia, it would be unusual to find clear morphologic features of dysgranulopoiesis or dysmegakaryopoiesis in the two scenarios described, either in patients with cytopenia with a hypercellular marrow or cytopenia with a hypocellular or normocellular marrow. Although some cytologic atypia may be seen in granulocytes and megakaryocytes, definitive dysmyelopoietic features that are characteristic of and closely associated with MDS are rarely found in viral infections or at first presentation of a BM failure syndrome. Furthermore, certain medications may cause morphologic changes reminiscent of dysgranulopoiesis (see Differential Diagnosis and Qualitative Defects). Therefore a careful microscopic examination of well-prepared and well-stained blood and BM slides is essential to evaluate accurately for such features. In the absence of a distinctly increased blast population or of definitive dysplastic morphologic features, and in the absence of characteristic clonal cytogenetic abnormalities, continued monitoring of the patient's clinical and hematologic parameters with repeated BM examinations may be necessary.

## Qualitative Defects

### Dyserythropoiesis

Varying degrees of dyserythropoiesis may be seen in megaloblastic anemia, with antimetabolite therapy or with certain medications such as azathioprine and valproate, brisk hemolysis, marrow regeneration, congenital dyserythropoietic anemia, and mitochondrial cytopathy. Patients with megaloblastic anemia owing to severe vitamin $B_{12}$ or folate deficiency may develop pancytopenia with marked dyserythropoiesis. However, there should be no increase in blasts, and the morphologic features of megaloblastosis with giant neutrophilic bands and metamyelocytes as well as hypersegmented neutrophils are readily distinguishable from the cytoplasmic hypogranulation and nuclear hyposegmentation of MDSs. Laboratory evaluation for vitamin $B_{12}$ and folate levels and other related metabolites is crucial in confirming the diagnosis of a nutritional deficiency. A careful review of the patient's clinical and medication history with appropriate laboratory evaluation, including a work-up for hemolysis, is always useful to rule out the possibility of dyserythropoiesis caused by marrow regeneration, medication effect, or severe hemolysis. In congenital dyserythropoietic anemia, despite

considerable dyserythropoiesis, the granulocyte and megakaryocyte lineages are morphologically normal.

### Ring Sideroblasts

Ring sideroblasts can be seen with arsenic poisoning, antituberculosis therapy, pyridoxine deficiency, zinc toxicity, copper deficiency, and heavy alcohol use. A careful history of possible exposure is therefore essential to rule out these possibilities before embarking on a diagnosis of RARS. In the pediatric patient, the presence of ring sideroblasts in the BM should prompt an evaluation for a mitochondrial cytopathy such as Pearson syndrome, because RARS in this age group is extremely uncommon. (Pearson syndrome is a congenital multisystem disorder characterized by marked anemia, ring sideroblasts in the BM, neutropenia, thrombocytopenia, and pancreatic exocrine insufficiency.)

### Dysgranulopoiesis

The possibility of poor staining should first be excluded as an explanation for hypogranular-appearing neutrophils and precursors, and a diagnosis of MDS should never be based solely on the presence of pale neutrophils. G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy may result in hypogranular-appearing neutrophils, which may be a relative phenomenon because of the increased azurophilic granulation otherwise in other cells. G-CSF or GM-CSF therapy and trimethoprim-sulfamethoxazole therapy can sometimes result in pseudo Pelger-Huët neutrophils. Moreover, G-CSF or GM-CSF therapy can also result in left-shifted granulocytic maturation with circulating myeloblasts. Morphologic features of dysgranulopoiesis have also been described with valproate or mycophenolate mofetil therapy. With mycophenolate mofetil, dysgranulopoiesis is characterized by loss of normal nuclear chromatin condensation with neutrophil maturation, resulting in mature neutrophils showing more reticulated chromatin features that resemble those seen in mature monocytes; atypical nuclear hyperlobation may also be seen in some neutrophils. Nuclear fragments (akin to Howell-Jolly bodies in red blood cells) may also be seen in this setting as well as in HIV patients and in some patients undergoing chemotherapy. It is therefore advisable to repeat the blood and marrow examination after discontinuation of growth factor, trimethoprim-sulfamethoxazole, or valproate therapy before rendering the diagnosis of MDS.

### Dysmegakaryopoiesis

Sectioning artifact on trephine sections can render the appearance of a megakaryocyte as small, and the nucleus as hypolobated or even multinucleated. However, the more such abnormal-appearing megakaryocytes are seen in the BM trephine sections, the less likely is the possibility of artifact. In the authors' opinion, evaluation of the BM aspirate smears obviates this sectioning artifact. Other non-neoplastic causes of small and hypolobated megakaryocytes include HIV infection and certain medications, such as valproate. In addition to MDSs, clusters of megakaryocytes are also seen in myeloproliferative neoplasms and hematopoietic regeneration. With the recent introduction of thrombopoietin therapy, the effect of such agents on the morphologic features and distribution of megakaryocytes needs to be considered, and repeated marrow evaluation after discontinuation of such therapies is recommended before a diagnosis of MDS may be considered.

### Increased Blasts

Myeloblasts may be observed in the blood as part of a left-shifted granulocytic maturation resulting from G-CSF or GM-CSF therapy, a leukemoid reaction, or leukoerythroblastic reaction. Except for the relatively hypogranular or hyposegmented neutrophils that can be seen with G-CSF or GM-CSF therapy, dysmegakaryopoietic features should not be observed, the blood should exhibit a continuum of all intermediate stages of granulocytic precursors in addition to the blasts, the blast count rarely approaches 10% in the marrow, and there often is in parallel a notable left shift in granulocytic maturation with increased promyelocytes and myelocytes. If in doubt and if cytogenetic analysis reveals no characteristic abnormalities, one should exercise caution, and close clinical monitoring with follow-up hemograms and with a repeated BM examination may be necessary. When the percentages of circulating and medullary blasts approach or hover at approximately the 20% threshold, an accurate and well-sampled differential count of well-prepared and representative blood and marrow slides is essential to determine whether the blast proportions meet the definition of AML. If necessary, several fields, slides, or preparations may have to be examined to obtain an average. Hematogone hyperplasia can mimic increased blasts, although morphologically the more homogeneous and hyperchromatic nuclei of these BM B-lymphoid precursors differ from the more reticular chromatin structure of myeloid-lineage blasts, and flow cytometric immunophenotyping can be used to distinguish between the B-lineage of hematogones and the myeloid lineage of the increased blasts in MDSs. Difficulty can arise in differentiating blasts from promyelocytes, especially from dysplastic promyelocytes. The most helpful morphologic feature in this situation appears to be the presence of a Golgi zone, at least faintly visible, in promyelocytes.

## Prognostic Classification

Many individual prognostic factors have been studied in MDSs in an attempt to stratify patients into various risk groups and for the purpose of prescribing the

appropriate level of medical management and therapeutic intervention for each patient. In addition, various models have been developed for the purpose of predicting outcomes.

The IPSS was proposed in 1997 and based on a metaanalysis of a large group of patients with untreated primary MDS with a long follow-up period. At the time of the study, patients with 20% to 30% marrow blasts—now considered as having AML—were also included. In this scoring system, patients with MDSs were stratified into four different risk groups according to a combined score that considered the percentage of BM blasts, type of cytogenetic abnormalities, and number of cytopenias (Table 18-3). Patients in the low-risk group (IPSS-0) had a median survival of approximately 5 to 6 years with only approximately 20% progressing to AML when followed for as long as 19 years. In contrast, all patients in the high-risk group had developed AML by 3 years, and the median survival in this group of patients was less than 1 year.

Transfusion dependency has been demonstrated to represent an independent prognostic factor in patients with MDS. It can be considered as a reliable indicator of the severity of the disease, partly reflecting the presence of comorbidities, and is associated with a shorter survival and an increased risk of leukemic evolution. A prognostic model was recently developed that incorporated the 2001 WHO categories, cytogenetics, and transfusion dependency (see Table 18-3). This WPSS has been proposed as a dynamic prognostic model for predicting survival and leukemic evolution that could be applied to patients with MDS at any time during their clinical course. Compared with the four risk groups as defined by the IPSS, the WPSS was able to identify five risk groups of patients with different survival profiles, with the most relevant improvement observed among patients without excess blasts who might benefit from delayed treatment strategies. More recently, the WPSS was shown to be of value in stratifying the outcome of patients undergoing allogeneic stem cell transplantation.

The reader is referred to the primary literature for detailed discussions on the various advantages and disadvantages of the IPSS and WPSS models. It has been

**TABLE 18-3**

**Risk Classification of Patients with Myelodysplastic Syndromes**

**A. International Prognostic Scoring System***

| | Variable Scores | | | | |
|---|---|---|---|---|---|
| **Variable** | ***0.0*** | ***0.5*** | ***1.0*** | ***1.5*** | ***2.0*** |
| Bone marrow blasts (%) | <5 | 5-10 | — | 11-20 | 21-30 |
| Karyotype† | Good | Intermediate | Poor | — | — |
| Cytopenias‡ | 0 or 1 | 2 or 3 | — | — | — |

**B. WHO Classification-Based Prognostic Scoring System§**

| | Variable Scores | | | |
|---|---|---|---|---|
| **Variable** | ***0*** | ***1*** | ***2*** | ***3*** |
| 2001 WHO category | RA, RARS, 5q– | RCMD, RCMD-RS | RAEB-1 | RAEB-2 |
| Karyotype† | Good | Intermediate | Poor | — |
| Transfusion requirement¶ | No | Regular | — | — |

Data from Greenberg P, Cox C, LeBeau MM, et al: International scoring system for evaluating prognosis in myelodysplastic syndromes, *Blood* 89:2079–2088, 1997; Malcovati L, Germing U, Kuendgen A, et al: Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes, *J Clin Oncol* 25: 3503–3010, 2007.

*RA,* Refractory anemia; *RAEB,* refractory anemia with excess blasts; *RARS,* refractory anemia with ring sideroblasts; *RCMD-RS,* refractory cytopenia with multilineage dysplasia with ring sideroblasts.

*International Prognostic Scoring System Risk Groups: low (score 0); intermediate-1 (score 0.5-1.0); intermediate-2 (score 1.5-2.0); and high (score ≥2.5)

†Karyotype: *good* represents normal, -Y, del(5q), del(20q); *poor* represents complex (≥3 abnormalities) or chromosome 7 anomalies; *intermediate* represents other abnormalities.

‡*Cytopenias* were defined as a hemoglobin level of less than 10 g/dL, an absolute neutrophil count of less than $1.5 \times 10^9$/L, and a platelet count of less than $100 \times 10^9$/L.

§World Health Organization Classification–Based Prognostic Scoring System Risk Groups: very low (score 0); low (score 1); intermediate (score 2); high (score 3 to 4); and very high (score 5 to 6).

¶*Transfusion dependency* was defined as having at least one red blood cell transfusion every 8 weeks over a period of 4 months.

noted that the IPSS predates the new definition of AML at greater than 20% blasts, thereby limiting the applicability of the highest IPSS risk group. The IPSS might not apply to patients with secondary or therapy-related MDS, and it does not consider the adverse effect of certain other cytogenetic abnormalities. Regarding the WPSS, it is unclear whether severe anemia or the iron overload associated with the red blood cell transfusions is the independent factor responsible for the poorer overall survival in patients with MDS. Other investigators have indicated that the threshold for red blood cell transfusions varies by country and possibly according to regional medical practices, thereby introducing inconsistency to one of the cornerstone variables in the WPSS model.

In addition to the well-publicized IPSS and WPSS, other risk stratification models have evaluated the prognostic value of other variables, such as patient age, performance status, degree of anemia, BM fibrosis, immunophenotypes of myeloid progenitor cells, serum levels of lactate dehydrogenase, and new cytogenetic risk categories. This area of investigation is clearly active, and new information including an update of the IPSS should be forthcoming.

## Therapy

Previously, therapeutic options in MDSs have typically entailed either supportive care to alleviate symptoms related to the cytopenias or cytotoxic chemotherapy followed by hematopoietic stem cell transplantation with curative intent. Since the first edition of this text, several therapeutic options have become available that provide MDS patients and their health care providers with additional and alternative strategies at achieving disease stabilization. Decisions regarding the best treatment plan typically represent a balanced consideration of the patient's MDS subtype, the prognosis, the patient's symptomatology and comorbid clinical condition, and the goal of therapeutic intervention. A detailed discussion of such deliberations on the best appropriate treatment strategy is beyond the scope of this chapter; the reader is referred to the National Comprehensive Cancer Network website at www.nccn.org for the most recent and complete guidelines. The following discussion on current therapeutic options in MDS is brief by necessity.

## Hematopoietic Growth Factor Support

The goal of hematopoietic growth factor administration in MDS is to improve the symptoms related to the cytopenias. As such, this therapeutic approach is supportive and not curative. Approximately 20% of patients with MDS who were symptomatic from their anemia responded to erythropoietin therapy with or without concurrent G-CSF, particularly those with less than 500 mU/mL endogenous erythropoietin and without a del(5q) abnormality (please see the section on lenalidomide for use of this agent in the presence or absence of a del[5q] abnormality). The adverse effects of erythropoietin therapy reported in patients without MDS have not been found among patients with MDS, although the target hemoglobin level has nonetheless been lowered to 120 g/L. Although administration of G-CSF or GM-CSF to patients with neutropenia resulting from MDS has resulted in an improvement of the neutrophil count, currently such uses are not routine because no survival advantages have been shown in multicenter randomized phase III studies, and there was a suggestion that growth factor therapy might have accelerated progression to leukemia. Beyond platelet transfusion support for patients with thrombocytopenia, newer thrombopoietic agents such as romiplostim and the pegylated eltrombopag appear promising either alone or, as currently in phase II clinical trials, in combination with hypomethylating agents. Final conclusions on their role in the management of patients with MDS await further and definitive studies.

## Biologically Targeted Therapies: Immunomodulation

As discussed, increased proliferation and apoptosis of hematopoietic precursors have been shown in MDSs, and proinflammatory cytokines such as tumor necrosis factor α appear to play a role in mediating the latter. Therapeutic trials using agents such as the anti–TNF-α blockers etanercept in an attempt to modulate or block the inflammatory cytokines have been small in patient sample size. Without data from larger controlled trials, the role of such immunomodulatory agents as a mainstay in the treatment of MDS seems doubtful, at least in the foreseeable future. Based on reported improvements of cytopenia and survival for patients younger than 60 years and patients with lower-risk MDS, HLADR-15 histocompatibility type, and evidence of a PNH clone, immunosuppressive therapy with antithymocyte globulin or cyclosporin is the current standard of care for such subsets of patients.

## Epigenetic Therapies

### Demethylating Agents

Originally designed as a pyrimidine nucleoside analogue of cytidine to overcome cytarabine arabinoside resistance, 5′-azacitidine has since been shown to act as

a DNA methyltransferase inhibitor. In a series of phase I-II, II, and III multicenter randomized controlled trials sponsored by the Cancer and Leukemia Group B, patients with MDS and receiving 5′-azacitidine experienced statistically significant longer median time to leukemic progression, longer median survival and improved quality of life when compared to those patients in the arm receiving supportive care only. A recent re-analysis using the stricter response criteria of the International Working Group reaffirmed these superior results. A recent international multicenter controlled and open-label phase III trial of mostly patients with IPSS high or intermediate-2 risk MDS showed a statistically significant difference regarding both overall survival and time to acute leukemia progression between those receiving azacytidine and those in the conventional-care arm (24.5 months versus 15 months; $p < 0.0001$; and 17.8 months versus 11.5 months; $p < 0.0001$, respectively). In the current NCCN guidelines, azacytidine is part of the treatment for patients with either low- or high-risk (IPSS) MDS. In two nonrandomized studies, decitabine and azacytidine appeared comparable in terms of response rates and survival. As a result, the role of decitabine as an equal alternate demethylating agent in the treatment of MDS seems unclear. It remains to be determined whether decitabine has a role in specific situations, such as post-azacytidine failure or in combination with other agents.

#### Histone Deacetylase Inhibitors

Results of a few phase II studies using valproic acid as a single agent showed response rates ranging from 6% among patients with MDS with increased blasts to approximately 52% among patients with MDS without increased blasts. The value of combining histone deacetylase inhibitors with other agents such as all-*trans* retinoic acid, another hypomethylating agent, or other agents will require future studies.

#### Lenalidomide

For patients with low-risk MDS and with del(5q) as a sole cytogenetic abnormality, the use of lenalidomide as a single agent has resulted in high erythroid responses, with approximately two thirds of the patients showing an improvement of their hemoglobin levels for a median duration of 2.2 years. In addition, partial and complete cytogenetic responses have been reported in the range of 73% and 45%, respectively. Subsequent to the initial publication of these gratifying results and with further follow-up, it still remains unclear whether there is improved overall survival with long-term lenalidomide therapy; clinical and cytogenetic relapse have been reported in 50% of patients, possibly because of persistence of a del(5q) CD34+/CD90+/CD38(neg/low) stem-cell population that had evaded or resisted therapy. For these reasons, the long-term effects of lenalidomide on the management of patients with MDS will require further study. The use of this agent in patients without the del(5q) abnormality is currently not part of the NCCN guideline.

#### Cytotoxic Chemotherapy

For patients with MDSs with increased blasts approaching the level of acute leukemia, an induction chemotherapy regimen that is typically used in the setting of acute myeloid leukemia is an option. However, remissions tend to be short-lived and are achieved in only 30% to 50% of cases. Consolidation chemotherapy or hematopoietic stem cell transplantation, or both, often are a necessary next step.

#### Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation is the only curative therapy for patients with MDSs, but its use is limited by the typically older age of patients with MDSs who are often ineligible for many hematopoietic stem cell transplantation protocols. Recent advances in supportive care have enabled some transplantation programs to raise the age of eligibility to 65 years. The introduction of reduced-intensity and nonmyeloablative conditioning regimens in the last several years has provided another potential avenue to hematopoietic stem cell transplantation for older patients with high-risk MDS. Even with the eligibility age raised to 65 years, however, for a disease in which more than 80% of affected patients are older than 60 years, many patients with MDS remain ineligible for this curative therapy.

In addition to age and associated comorbidities, timing of transplantation in MDS is another issue for consideration. An analysis by Cutler and colleagues suggests that, whereas delayed BM transplantation is associated with maximal life expectancy for patients with MDS in the IPSS risk categories of low and intermediate-1, immediate transplantation should be considered for those in the intermediate-2 or high-risk categories.

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SECTION 4

# Disorders of Histiocytes, Mast Cells, Plasma Cells, Spleen, and Ancillary Techniques

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# 19

# Disorders of Histiocytes

■ Ronald Jaffe, MBBCh ■ Kudakwashe Chikwava, MBChB

## ■ DERIVATION OF HISTIOCYTES

The term *histiocyte* has come to encompass two cell lines, the monocyte-macrophages and the specialized antigen-presenting dendritic cells (DCs). It had been thought previously that myeloid-derived monocytes were the immediate precursors of macrophages and dendritic cells, but a marrow precursor of macrophages and dendritic cells that has no granulocyte potential has since been identified. The marrow macrophage dendritic cell precursor can give rise, under the influence of FLT3, to a common dendritic cell precursor that circulates as a preclassical DC or pre-plasmacytoid DC and that matures in tissues to classical or plasmacytoid dendritic cells. Under the influence of macrophage colony-stimulating factor (M-CSF), the macrophage dendritic cell precursor can produce monocytes that exit the blood under inflammatory stimuli and give rise to tissue macrophages (Figure 19-1).

A wide variety of tissue-specific macrophages are replenished from the marrow via precursors that include the monocytes; these include bone osteoclasts, brain microglia, liver Kupffer cells, renal mesangial cells, pulmonary alveolar macrophages, placental Hofbauer cells, serosal macrophages, and the interstitial histiocytes of the skin and connective tissues. The lymph nodes have their own resident macrophages that reside within the sinuses, the follicular germinal centers, and the nodal paracortex, and there is functional heterogeneity of the various macrophages. The spleen has follicular, sinus, and cordal macrophages. The macrophage is an incredibly active cell that is at the front line of the innate immune system. It has an extensive repertoire of pattern recognition motifs, and macrophages can phagocytose, digest, and destroy pathogens, become immune activated, and interact with DCs, B cells, and others to induce specific and acquired immunity, both cellular and humoral. The macrophages can modulate responses to intrinsic and extrinsic stimuli. Individuals, by virtue of their genetic heterogeneity, can vary in their ability to handle similar insults. Polymorphisms in interleukin (IL)-12R, for example, will determine a variety of granulomatous responses to mycobacterial infections. Macrophage production from myeloid marrow precursors is driven by IL-1, IL-3, M-CSF, and granulocyte-macrophage colony-stimulating factor (GM-CSF).

DCs, including the plasmacytoid DC (or plasmacytoid monocyte/interferon-producing cell), are also derived from the macrophage-DC precursor and circulate as a classical DC precursor following FLT3 stimulation. A preclassical DC and preplasmacytoid DC circulate and enter the tissues to form classical or plasmacytoid DCs. Like the tissue macrophage, there are various tissue DCs in the skin (Langerhans cell), the connective tissues (dermal and interstitial DCs), lymph nodes (interdigitating and follicular), spleen, and thymus. Only the brain appears to have no intrinsic DC pool. The germinal center follicular DCs are the only members of the DC family to have a nonmyeloid origin and are most likely derived from fibroblast-like mesenchymal cells in various tissues. DC replenishment can occur during periods of need from monocytes and is regulated, in part, by IL-4, GM-CSF, and tumor necrosis factor (TNF)α. There is plasticity in the system, and both macrophages and DCs can be driven in the direction of the other by various stimuli: cells with dendritic phenotype can be converted to macrophage phenotype by IL-10 or M-CSF. It is therefore not surprising that there are grey zones in which the distinction of macrophages from DCs is not absolute, and that histiocytic lesions commonly contain a mix of monocytes, macrophages, and DCs even if one cell type dominates. Macrophage fusion is a common phenomenon resulting in multinucleated giant cells. Under certain circumstances, such as incubation with IL-4, macrophages can assume an epithelioid appearance.

DCs exist in the periphery as sentinel cells, sampling the internal or external environment. When they encounter a danger signal through their cytokine or toll-like receptors, the cells become activated and proceed to mature, changing their phenotype from one adapted to antigen acquisition (pinocytic, phagocytic) to one more suited to antigen presentation, which occurs in the

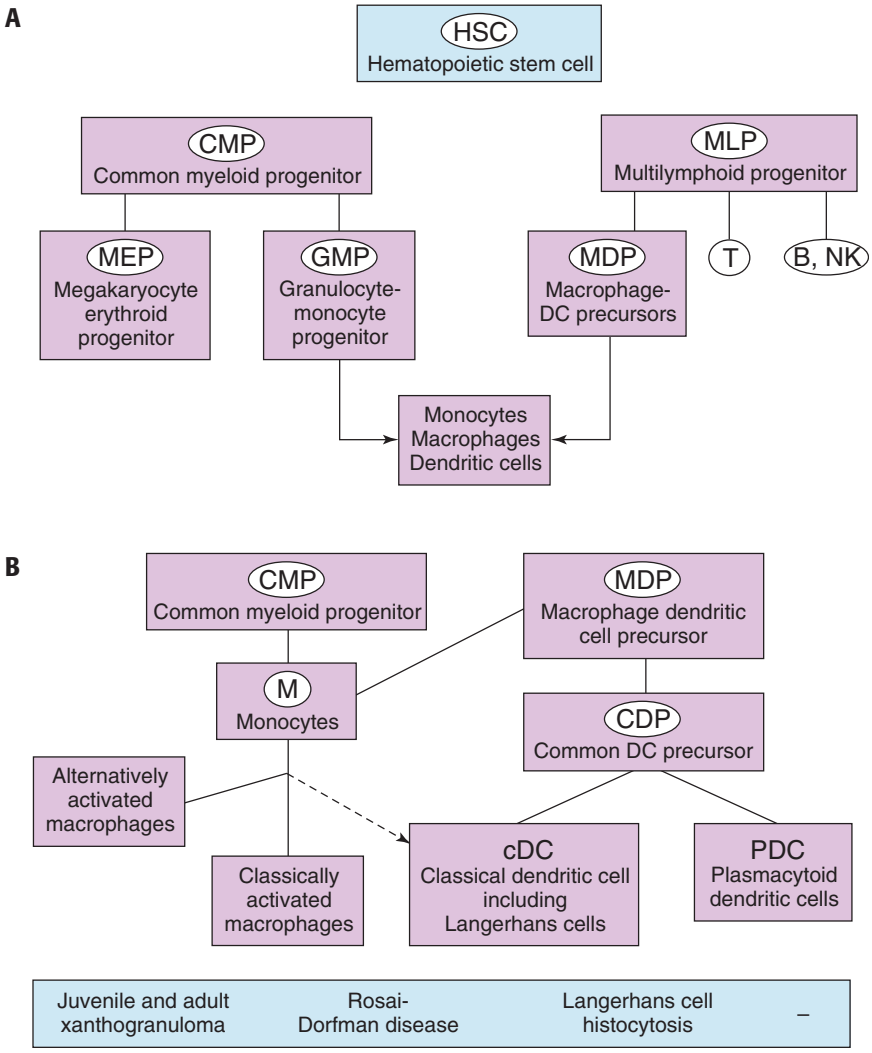


**FIGURE 19-1**

**A,** The multiple-lymphoid precursor derived from a hematopoietic stem cell gives rise to the macrophage-dendritic cell precursors. Under stable conditions, the macrophages and dendritic cells differentiate from this source. Under inflammatory conditions, the granulocyte-monocyte progenitor can augment the pool with inflammatory macrophages and dendritic cells. This model does not require transdifferentiation to explain phenotypic changes between T cells, B cells, or histiocytes. **B,** Precursors circulate as monocytes and the common dendritic cell precursor and enter the tissues as macrophages and dendritic cells. In this model, Langerhans cell histiocytosis would be derived from the common DC precursor, adult and juvenile xanthogranuloma family from monocyte-macrophages, and Rosai-Dorfman disease has features of both. (***A,*** *Data from Doulatov S, Notta F, Eppert K et al: Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development,* Nat Immunol *11:585–595, 2010;* ***B,*** *data from Geissmann F, Manz MG, Jung S, et al: Development of monocytes, macrophages, and dendritic cells,* Science *327:656–661, 2010.)*

central lymphoid tissues, lymph nodes, or spleen. DCs present antigen in a major histocompatibility complex–restricted manner to a variety of cell types, T lymphocytes, B cells, and natural killer (NK) or NKT cells to induce immune or tolerizing effects.

## IDENTIFICATION OF HISTIOCYTES

Histiocytes, DCs, and macrophages can be followed along their life cycle from precursors, through activation to mature functional cells by a wide panel of markers, surface and cytoplasmic, and by some of their histochemical characteristics. Table 19-1 lists some of these molecules, many of which are detected by flow cytometry, in situ by immunocytology, or on frozen sections. A significantly limited panel is available for the recognition of DCs and macrophages in fixed embedded tissues, and the panels can also be informative regarding maturation of the histiocytes. PU.1, an Ets family transcription factor, stains nuclei of both macrophages and DCs, but also myeloid cells and their leukemias. There are few histiocyte-related molecules that are informative on their own and are best used as panels of markers for tissue histiocytes. Table 19-2 shows antibodies that react almost exclusively with macrophages, those that are common to both macrophages and DCs (though the amount and pattern can differ), and the antibodies that are more exclusive to DCs. Some are informative regarding subsets, such as CD1a and Langerin for Langerhans cells and factor XIIIa for dermal and interstitial macrophages. CD123, the interleukin 3 receptor α, identifies the plasmacytoid DCs. Other molecules are more informative about DC maturation; CD83, DC-LAMP, and hi-fascin indicating an activated and mature phenotype. S100 has long used as a DC marker when present in high amounts, but it is variably expressed in subpopulations of activated macrophages and is thus of limited discretionary power.

**TABLE 19-1**
**Selected Antibodies Informative for Histiocytes**

| Cluster | Cell Function | Predominant Histiocyte |
|---|---|---|
| CD1a, b, c | T-cell response to nonpeptide lipids and glycolipids | DC |
| CD4 | MHC class II/HIV receptor | M, DC |
| CD11b | Complement C3b receptor | M |
| CD11c | CD11/CD18 receptor | M, DC |
| CD14 | Lipopolysaccharide receptor | M |
| CD25 | IL-2 receptor | M |
| CD31 | PECAM-1 | M |
| CD32 | Fc IgG receptor, low affinity | M |
| CD33 | Sialoadhesin | M |
| CD49 | Integrin receptors | M |
| CD64 | Fc IgG receptor, high affinity | M, DC |
| CD68 | Macrosialin | M, DC |
| CD83 | Ig superfamily | DC |
| CD86 | CD28/CD152 ligand | DC |
| CD91 | Low density lipoprotein related protein-1 | M |
| CD103 | Integrin α E | DC |
| CD116 | GM-CSF receptor α | M, DC |
| CD123 | IL-3 receptor α | PDC |
| CD163 | Hemoglobin/haptoglobin scavenger receptor | M |
| CD169 | Sialoadhesin | M |
| CD204 | Macrophage scavenger receptor | M |
| CD205 | DEC 205 | M, DC |
| CD206 | Macrophage-mannose receptor | M, DC |
| CD207 | Langerin | DC |
| CD208 | DC-LAMP | DC |
| CD209 | DC-SIGN | DC, M |
| CD254 | RANK-L | Tumor necrosis factor ligand M |
| CD283 | TLR3 | Toll-like receptor D |
| CD284 | TLR4 | Toll-like receptor M |
| CD303 | CLEC4 | C-type receptor PDC |

*DC,* Dendritic cell; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *Ig,* immunoglobulin; *IL,* interleukin; *M,* monocyte, macrophage; *MHC,* major histocompatibility complex; *PDC,* plasmacytoid dendritic cell.
NOTE: Many of the molecules are not unique to the histiocytes, but are expressed as indicated on monocytes, macrophages, or dendritic cells as well as other cells.

**TABLE 19-2**
**Antibodies and Enzymes Informative for Histiocytes in Tissues**

| Cell Type | Marker |
|---|---|
| Macrophages | CD14, CD163, acid phosphatase, nonspecific esterase |
| Macrophages and dendritic cells | CD68, HLA-DR, S100 |
| DCs | Immature Langerhans cells; CD1a, Langerin |
| Mature DCs | CD83, DC-LAMP, hi-fascin |
| Dermal macrophages | Factor XIIIa, CD163 |
| Plasmacytoid DCs | CD123 |
| Follicular DCs | CD21, CD35, clusterin |

*DC,* Dendritic cell.

## PHYSIOLOGIC EXCESS OF HISTIOCYTES

### MACROPHAGES

Macrophages derived from recruited monocytes will accumulate at sites of infection and inflammation wherever tissue damage and destruction occur. Scavenging, phagocytosis, and digestion of debris are major functions, although macrophages are also active participants in the subsequent repair and immune reactions. Lymph node reticular sinus histiocytes accumulate largely in response to local draining stimuli, inflammation, or tissue damage. Follicular tingible body macrophages are characterized by the presence of apoptotic debris within the cytoplasm and increase in the presence of apoptotic activity within the dark portion of the germinal center. Normal macrophages can be overloaded by increasing the amount of substrate they are expected to handle. Collections of xanthomatous (yellow) macrophages are also part of the repair and scavenging process following tissue injury, such as perforation of the appendix (Figure 19-2). Excessive turnover of cells with uptake of membrane-derived complex lipids leads to accumulation of ceroid–lipochrome-rich "sea-blue histiocytes," a term derived from their appearance on Wright or Wright-Giemsa type stains. Sea-blue histiocytes are an accompaniment of myeloproliferative discord in the bone marrow or high platelet phagocytosis in the spleen. Ceroid histiocytosis is also the explanation for most pigmented macrophages in the gastrointestinal mucosa and in chronic granulomatous disease. Intravenous alimentation, especially when lipid is added, can lead to the accumulation of ceroid-filled macrophages in the liver, spleen, and lungs. The

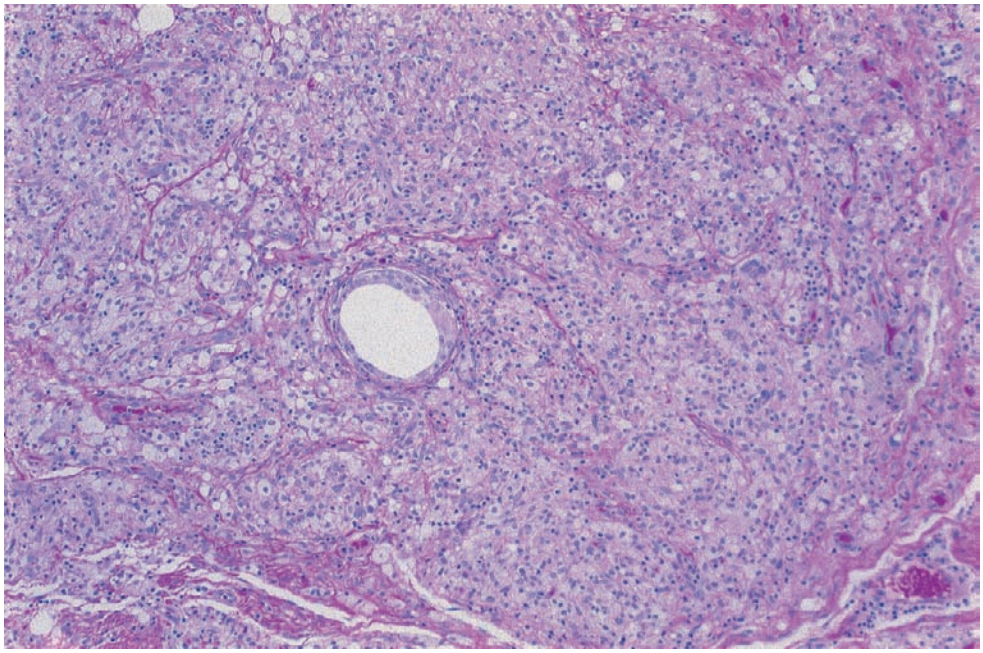

**FIGURE 19-2**

Xanthomatous inflammation. Aggregates and sheets of foamy macrophages surround a central cavity, all that remains of a ruptured thyroglossal duct cyst.

atheromatous plaque in large arteries has lipoproteins deposited in excess, and the accumulation of foamy lipid filled macrophages is characteristic.

## DENDRITIC CELLS

Germinal center responses require binding of antigen to follicular DCs that produce IL-6 leading to germinal center expansion, a process inhibited by corticosteroids. Follicular hyperplasia is common in childhood infections and autoimmune and rheumatic disorders. Cytologically atypical follicular DCs are seen in multicentric hyaline-vascular type Castleman disease, probably driven by human herpes virus 8–derived viral IL-6.

Accumulation of normal sentinel DCs can be seen along their migratory pathway when their numbers are increased in inflammatory processes. The best described is the paracortical DC hyperplasia known as *dermatopathic lymphadenopathy* but is not unique to that condition. Increased numbers of Langerhans cells from the periphery accumulate in the nodal paracortex in which they can form large, confluent, and nodular aggregates. Because these DCs are maturing or fully mature, they will have the phenotype of interdigitating DCs, $S100^+$, fascin-hi, $CD83^+$, DC-$LAMP^+$ with interspersed $CD1a^+$/$Langerin^+$ Langerhans cells (Figure 19-3). A diffuse paracortical DC hyperplasia in tonsils or lymph nodes can be an accompaniment of other infiltrating processes and can be misleading by diverting attention from the infiltrate, usually a leukemia or lymphoproliferative disorder.

# PATHOLOGIC EXCESS OF MACROPHAGES

## INFECTION AND FOREIGN MATERIALS

Macrophages can accumulate at sites of infection or tissue destruction as part of their physiologic role in repair. Osteomyelitis is an example in which the macrophages' presence in excess can sometimes mimic that of a neoplastic overgrowth such as Langerhans cell disease (Figure 19-4). Infection in the immune-suppressed individual can also be characterized by unconventional organisms and unusual responses. Atypical mycobacterial disease with mycobacteria avium intracellulare in particular has sheets of macrophages that harbor the organisms. *Mycobacterium tuberculosis* not only evades phagocytosis but inhibits apoptosis of infected macrophages, presumably by preventing mitochondrial damage and initiating plasma membrane repair. Malakoplakia with the typical Michaelis-Guttmann bodies is a disordered macrophage response to *Escherichia coli*. There are organisms that find safe haven in macrophages, shielded from other elements of the immune system, best characterized by mycobacterial spindle cell diseases including histoid leprosy and Whipple disease *(Tropheryma whippelii),* leishmania and rhinoscleroma (Mikulicz cells containing *Klebsiella rhinoscleromatis*).

Many organisms can induce epithelioid transformation of macrophages and a granulomatous response, some with a more acute inflammatory response, such as *Yersinia, Tularemia, Bartonella,* and *Brucella* spp. Other

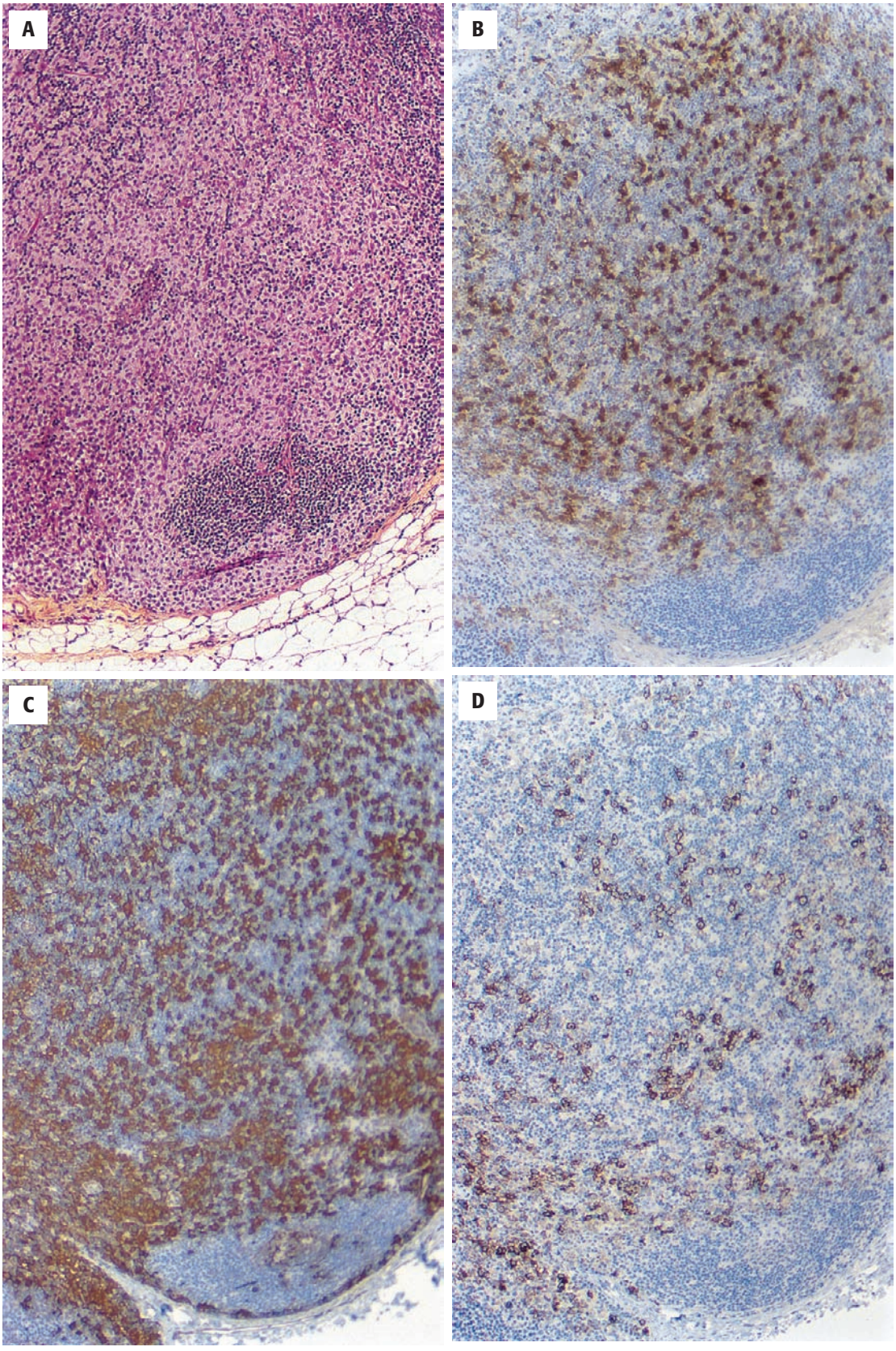


**FIGURE 19-3**

Aggregation (hyperplasia) of paracortical nodal dendritic cells. Dermatopathic lymphadenopathy, although the same pattern is seen in nodes draining nondermal sites. The paracortex is replaced by light-staining dendritic cells that have interdigitating DC phenotype **(A),** high S100 **(B),** high fascin expression **(C),** and numbers of CD1a-positive Langerhans cells **(D).** Contrast with LCH, which is a sinus disease.

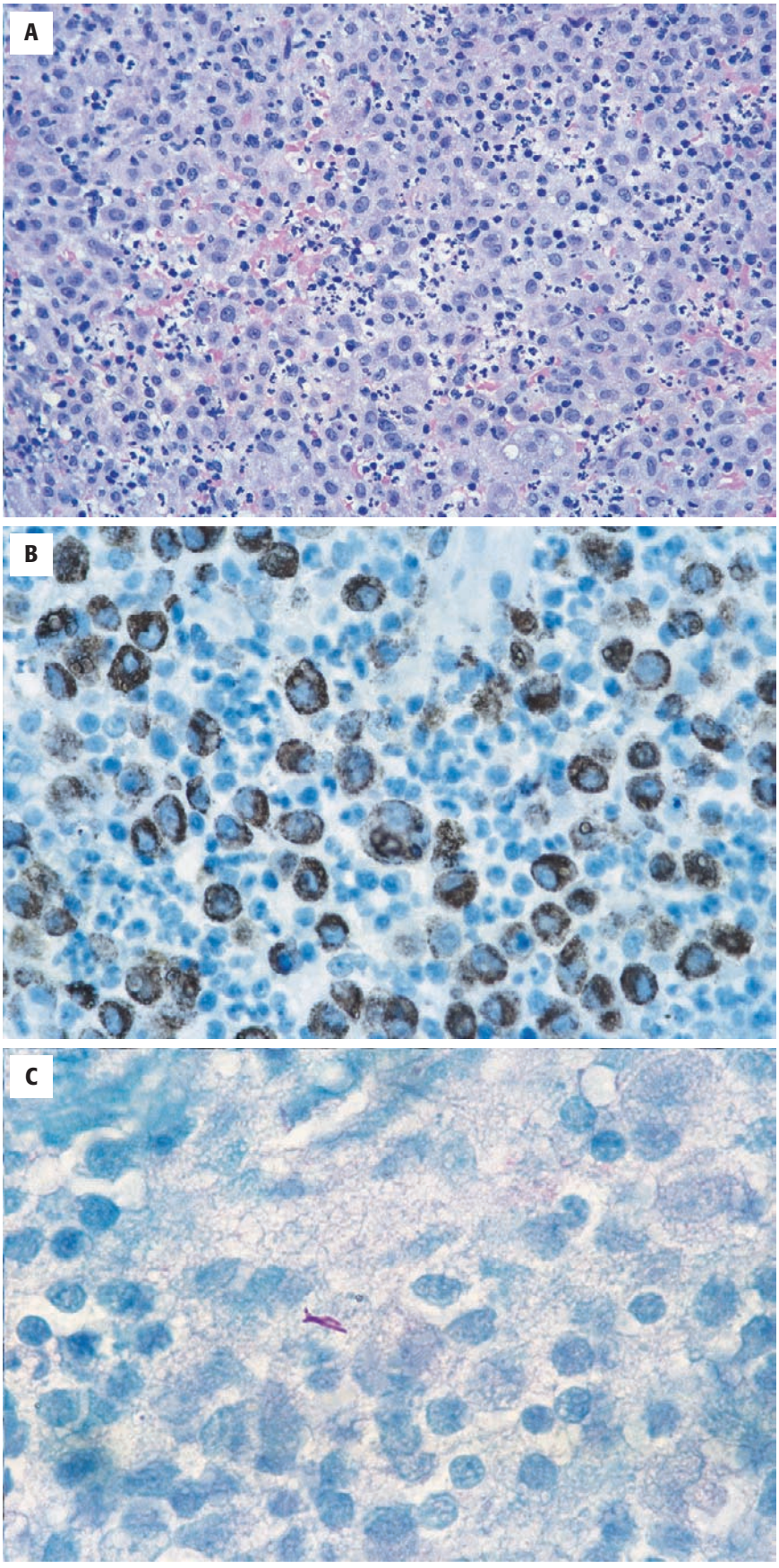


**FIGURE 19-4**

Bone with histiocyte-rich lesion. **A,** The histiocytes stain strongly for CD68 (PGM-1) and **(B)** were CD1a-negative. **C,** Acid-fast mycobacterial organisms are demonstrated.

organisms like *Mycobacterium, Pasteurella,* and *Burkholderia* spp. (the last causing glanders and melioidosis) have a more chronic course and innate epithelioid transformation, often with a giant cell component. Sarcoidosis is the prototype of the noninfectious epithelioid granuloma, but nonsarcoidal conditions such as common variable immune deficiency and Blau syndrome (*NOD2* mutations) can also manifest as granulomatous disease. Giant cell formation is also seen in a number of noninfectious circumstances with collections of epithelioid histiocytes at various tissue sites, sarcoidosis and Crohn disease are common examples. Beryllium exposure is less frequent. Foreign bodies of many kinds can lead to macrophage accumulation, often with a giant cell component, and some aggregates may be epithelioid. Talc, silicone, starch, and detritic granulomas from prostheses are examples of exogenous material, but crystal-storing histiocytes may accumulate endogenous crystals that are derived of immunoglobulin (Figure 19-5, A). Drugs such as paclitaxel (Taxol) can lead to accumulation of histiocytes with periodic acid-Schiff (PAS)-positive inclusions, whereas clofazimine produces histiocytes containing red crystals (see Figure 19-5, B, C). Some but not all foreign bodies are birefringent, and polarization is mandatory when confronted with a histiocytic aggregate of unknown type.

## XANTHOMAS AND XANTHOMATOUS INFLAMMATION

The xanthomatous (yellow) macrophage response in tissue repair is a feature when inflammatory cells and tissue necrosis are being scavenged. There are sites where the xanthomatous inflammation can be excessive and even simulate tumor in its extent. Xanthogranulomatous pyelonephritis is a chronic pyelonephritis of adults in which an exuberant xanthomatous process can replace much of the kidney and even extend into the retroperitoneum. It is rare in children. A similar process can affect the gallbladder. In both the gallbladder and kidney, bacteria can be isolated from most. There are instances of so-called necrobiotic xanthogranulomas in soft tissue, orbit, and rarely viscera, sometimes in association with paraproteinemia. These xanthogranulomas are not infectious in nature. A xanthoma is a mass or nodule composed almost entirely of lipid-rich foamy macrophages. Most are associated with hyperlipidemias, both primary inherited forms and secondary hyperlipidemic states such as diabetes mellitus, cholestatic liver disease or nephrotic syndrome. Xanthomas occur at various sites on the skin in crops as eruptive xanthoma, xanthelasma around the eyes, and tendinous xanthoma around the ankles, knees, hands, and elbows. The larger xanthomas may be associated with a center that contains fat necrosis, cholesterol crystals, and a surrounding foreign-body type giant cell reaction. Unlike the juvenile xanthogranuloma, the xanthoma cells express little factor XIIIa or fascin. Atherosclerotic lesions are characterized by xanthomatous macrophages.

## FUNCTIONAL DEFECTS OF HISTIOCYTES

Because of their specialized peculiar functions, macrophages are rich in lysosomes. In a number of inherited defects of the lysosomal apparatus, there is a deficiency of enzymes needed to degrade macromolecules or to transport the degraded substance out of the lysosome, leading to accumulation of naturally occurring metabolites with the lysosomes (lysosomal storage disorders). The classes of molecules involved include the mucopolysaccharidoses (MPS I-VII), glycoproteinoses, glycogenosis (type II), sphingolipidoses, lipidoses, the multiple enzyme deficiency disorders, and the lysosomal transport defects. Each of the defects included within these categories will have a peculiar tissue distribution depending on the substrate that fails to be catabolized or transported. The accumulation of the various substances will produce highly different phenotypic expression when viewed by light or electron microscopy, and both of these modalities have been combined with histochemistry to categorize the various disorders (Figure 19-6). On occasion, discovery of the storage cells in tissues might be the first clue to the presence of a metabolic disorder, and the physical characteristics of the storage material can give a lead to the biochemical or genetic testing needed for confirmation.

Considering the numerous functions that macrophages have in health and disease, it seems remarkable that so few defects have been documented that are restricted to these cells. Apart from the inherited lysosomal storage disorders, there are some examples that often share the deficiency with other cells. Autosomal recessive osteopetrosis affects osteoclasts from the same hematopoietic precursors as macrophages. When the responsible gene is *TCIRG1,* it affects the vacuolar proton pump of osteoclasts and gastric parietal cells. Fewer instances are caused by the *CLCN7* gene mutation that encodes a chloride channel. Hematopoietic stem cell transplant in the *TCIRG1* but not the *CLCN7* form can arrest the disease by providing functional monocytes and osteoclasts. The effect of these mutations on other macrophages, if any, is not known.

Leukocyte adhesion deficiency due to defects in ß-integrins hinders the ability of granulocytes and monocyte macrophages to accumulate at infection sites. Chronic granulomatous diseases involve the inability to provide an oxidative burst important in microbial killing. The responsible defects resulting in abnormal electron transfer from cytoplasmic nicotinamide adenine dinucleotide phosphate (NADPH) to molecular oxygen are common to neutrophils and

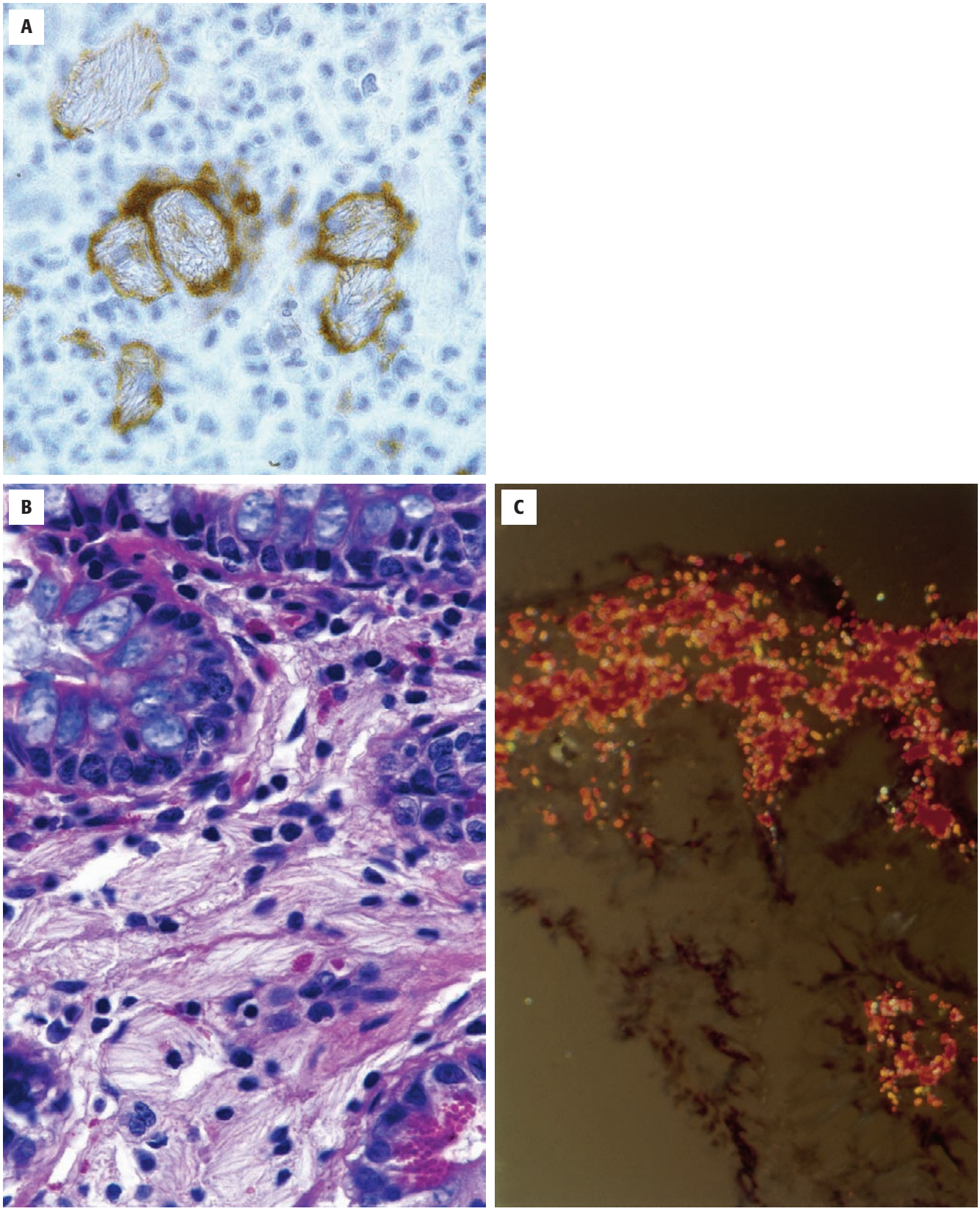


**FIGURE 19-5**

Crystals in macrophages. **A,** Bone marrow with refractile immunoglobulin crystals in macrophages stained for CD163, an example of crystal storing histiocytosis. **B,** Small bowel with intestinal macrophages that have a striated cytoplasmic appearance. **C,** Polarization of a frozen section of the bowel in **B** reveals the red crystals of clofazimine.

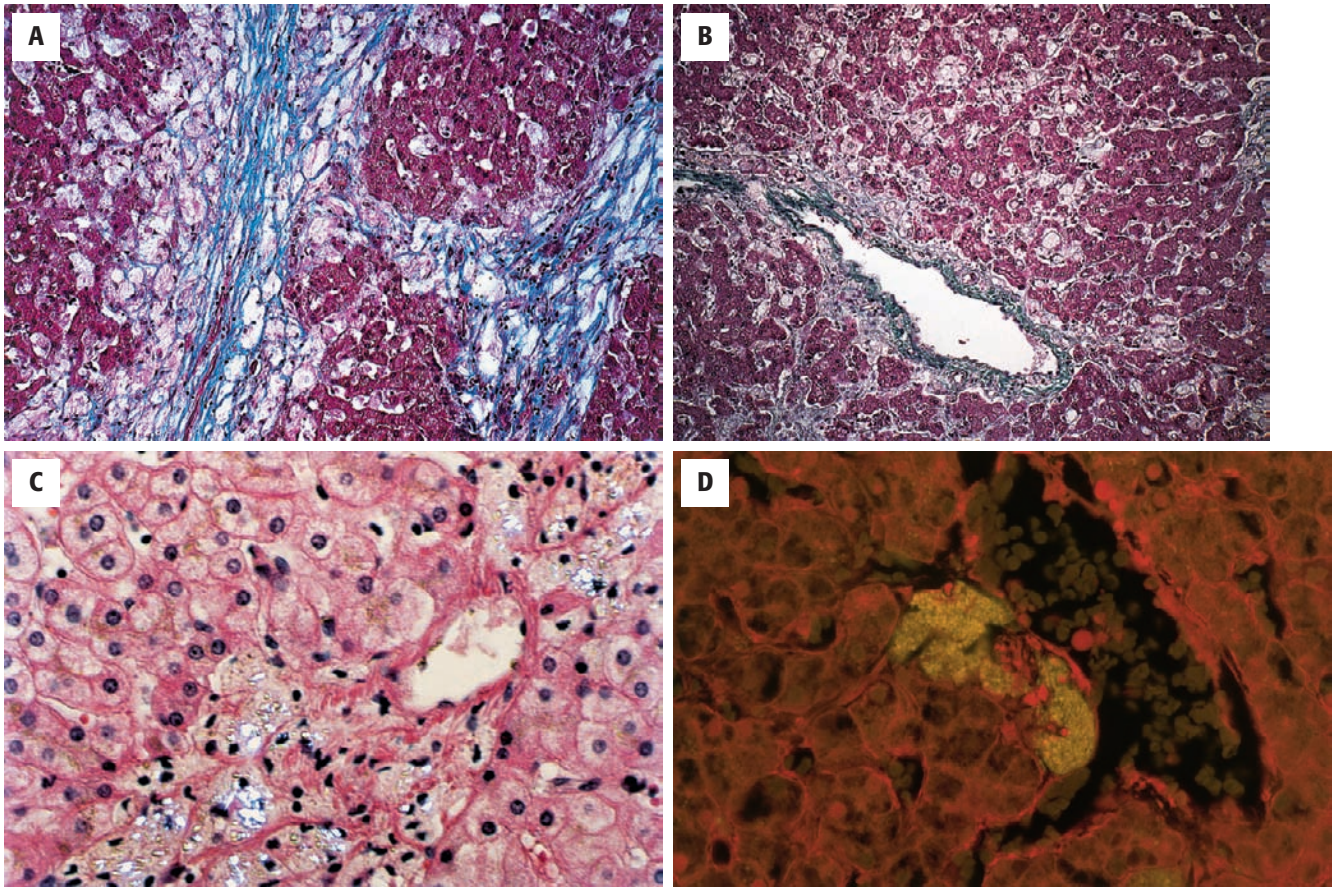


**FIGURE 19-6**

Liver with storage diseases that affect the sinusoidal macrophages. **A,** Gaucher disease in a Mallory trichrome stain has sheets of perifibrous macrophages with pale, striated cytoplasm. **B,** Niemann-Pick in a Masson trichrome has foamy pericentral macrophages. **C,** Cysteine crystals are seen in sinusoidal macrophages by polarization. **D,** Hermansky-Pudlak disease has ceroid-filled macrophages seen as yellow autofluorescence.

macrophage abnormalities of $gp91^{phox}$ and include a macrophage-specific component of NADPH oxidase.

Chronic granulomatous disease can develop for the first time as lymphadenopathy, and nodal involvement is seen in 50% of patients. Active chronic inflammation and granulomas occur, and occasionally pigmented macrophages might provide a clue to the diagnosis. The pigment is PAS positive and has the features of ceroid, the ultrastructural equivalent being lysosomal debris. The most frequent organisms in current experience are *Staphylococcus aureus,* followed by *Burkholderia cepacia, Serratia marcescens, Nocardia* species, *Aspergillus* species, *Salmonella* species, and bacille Calmette-Guérin.

Confirmation of the diagnosis of CGD is by direct measurement of superoxide production, ferricytochrome c reduction or dihydrorhodamine oxidation. Hermansky-Pudlak syndromes with oculocutaneous albinism and platelet storage disease are due to defects in intracellular protein trafficking that can affect lysosomes in some macrophages and melanocytes. Alveolar macrophages may be targeted in Hermansky-Pudlak syndrome type 1, in which progressive pulmonary fibrosis occurs.

The emerging field of toll-like receptors, their normal variation and defects is of importance because of their widespread expression on macrophages.

Kikuchi-Fujimoto disease (histiocytic necrotizing lymphadenitis) is a self-limiting condition characterized by proliferation and accumulation of histiocytes of unclear origin. The benign lymphadenopathy is associated with systemic symptoms and fever. Paracortical areas are filled with histiocytes, plasmacytoid DCs, $CD8^+$ T cells, and karyorrhectic debris. Later changes are more xanthomatous. The diagnosis is histopathologic by exclusion of other causes of inflammatory lymphadenopathy.

Tangier disease owing to high-density lipoprotein deficiency leads to the accumulation of cholesterol esters in lymphoid tissues, tonsils, lymph nodes, liver, and spleen. Functional effects on macrophage physiology have been documented for Gaucher disease. Hemophagocytic syndromes are also a serious consequence of the Griscelli syndrome, Chediak-Higashi syndrome, and Hermansky-Pudlak syndrome.

DC dysfunction has been identified in the X-linked Wiskott-Aldrich syndrome in which Wiskott-Aldrich

syndrome protein is functionally defective. Macrophages, DCs, and osteoclasts are affected and have disordered motility because of their inability to form podosomes. Common variable immune deficiency and CD40 defective hyperimmunoglobulin M disease may have diminished DC function, but it is not established that this is the primary defect. Because DCs can act as the portal for viral infection, they may be increased or selectively depleted in some viral infections, notably HIV.

## MACROPHAGE ACTIVATION SYNDROMES

Macrophage activation syndrome (MAS) in its most florid form is a severe, potentially fatal systemic inflammatory disorder characterized by uncontrolled activation and proliferation of macrophages and T cells associated with inflammatory cytokine upregulation.

Although macrophage functional patterns have been described as type 1 (Th1-driven) or type 2 (Th2-driven), a wide variety of microenvironmental factors result in a large number of different functional and phenotypic subtypes that are constantly being modulated. Macrophages can be immune activated by soluble factors such as cytokines, with interferon γ being the most classical example, but TNF, IL-1, IL-2, and macrophage migratory inhibitory factor (MIF) can also cause proinflammatory activation, as can lipopolysaccharide. These classically activated macrophages are referred to as *M1 macrophages*. Deactivation can be mediated by IL-4, IL-10, IL-13, and TGF-β. IL-4 and IL-13 induce an alternative antiinflammatory activation that enhances major histocompatibility complex class II expression and mannose receptor-mediated endocytosis. Activated macrophages participate in antimicrobial immunity by enhanced opsonization and phagocytosis and in cellular immunity by stimulating or inactivating lymphoid receptors largely through their active cytokine production and other proinflammatory mediators. Alternatively activated (i.e., M2) macrophages are heterogenous in terms of the cytokines that induce their differentiation; they play a role in tissue remodeling and repair, resistance to parasites, immunoregulation, and tumor promotion. Macrophages in MAS exhibit many characteristics of M2 macrophages, including the membrane and cytoplasmic expression of scavenger receptor CD163.

In response to a number of different stimuli, a systemic inflammatory or macrophage activation syndrome can develop. Viral infections are the best known, but bacterial infections, rheumatologic disorders, especially the systemic form of juvenile idiopathic arthritis, cancers, lymphoproliferative disorders, intravenous alimentation, and multiple organ failure are among other causes. MAS appears to be due to an unrestrained lymphocyte–NK cell–driven macrophage stimulation that leads to disseminated overactivity of the macrophages throughout the body, possibly mediated by TNF-α, and ineffective deactivation through depressed NK and cytotoxic CD8 T cells. An increase in the number and size of endogenous macrophages, with or without hemophagocytosis, is a feature of this condition that is best seen in the bone marrow, but also in spleen, liver, and lymph nodes (Figure 19-7). In its most severe expression, there are significant functional effects of the cytokine storm, with bone marrow depression, hepatomegaly with hepatocellular damage and raised hepatocellular enzymes, and effects on the clotting cascade. The condition abates when the inciting condition is treated or disappears, but in severe instances it can be fatal. Treatment with high doses of steroids, cyclosporine, or agents aimed at counteracting the effects of TNF-α, such as infliximab and etanercept, have been used with some success with treatment directed at the underlying inciting event. Macrophage activation syndrome is also referred to as *secondary hemophagocytic lymphohistiocytosis,* whereas the primary disorders are genetically programmed.

## CLASSIFICATION OF THE HISTIOCYTIC PROLIFERATIONS

Table 19-3 lists the classification of these disorders by the Histiocyte Society, and a more recent but limited classification by the World Health Organization.

## FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Familial hemophagocytic lymphohistiocytosis (FHL) refers to a group of recessively inherited disorders that have the clinical and pathologic manifestations of an acute and usually fatal from of uncontrolled macrophage activation syndrome. They are also referred to as *genetic* or *primary hemophagocytic lymphohistiocytosis* (HLH).

### Clinical Features

The clinical features are dominated by the effects of a hypercytokine state, and there is a wide range of clinical severity. There are diagnostic guidelines originally developed by the Histiocyte Society that include clinical, laboratory, and histopathologic findings because the constellation was important, but definitive molecular diagnosis is now available for some. A family history of parental consanguinity or prior sibling death may be elicited. Prolonged fever and cytopenias, most commonly anemia and thrombocytopenia within the first 2

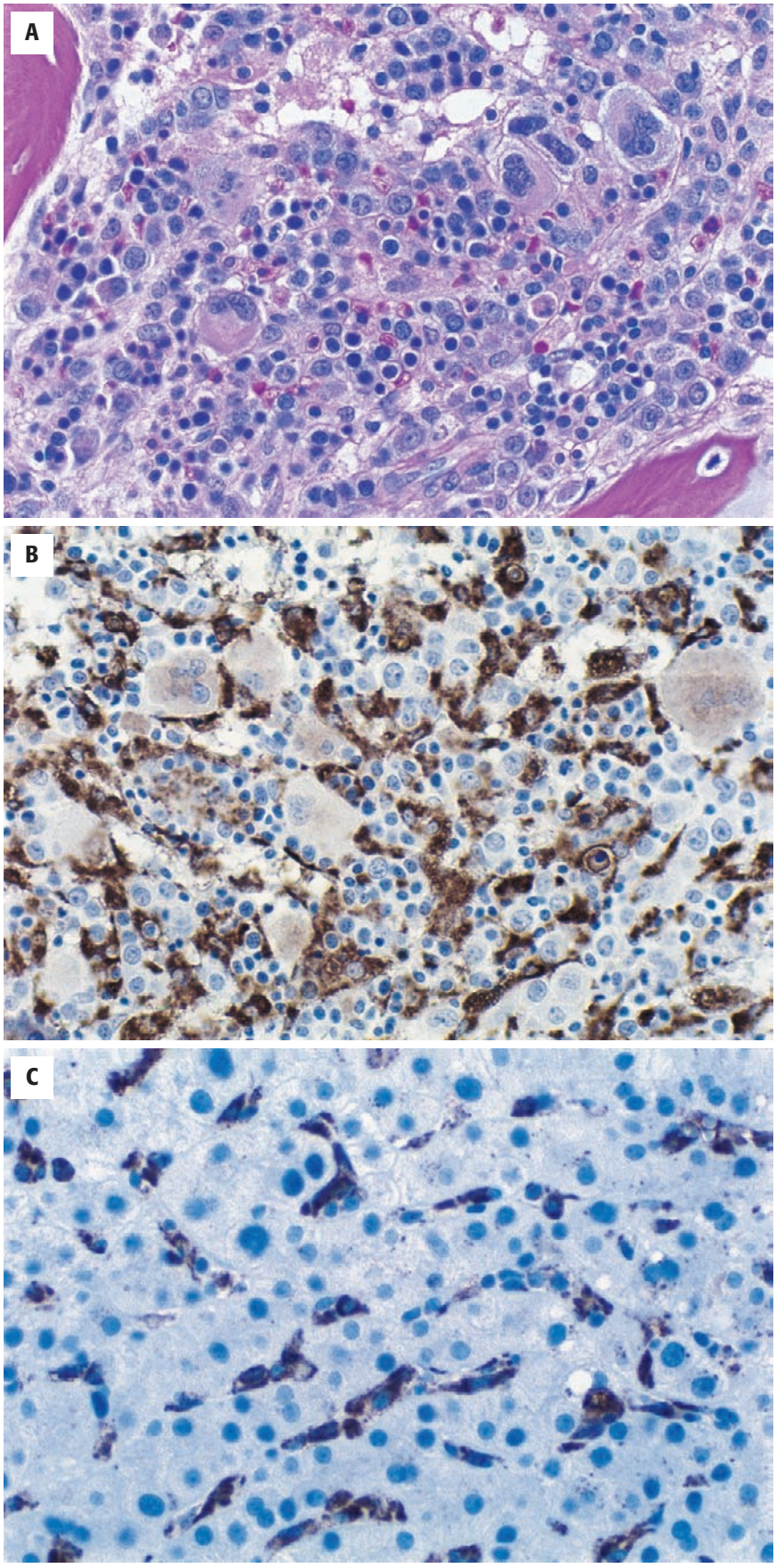


**FIGURE 19-7**

Macrophage activation syndrome: juvenile rheumatoid arthritis. **A,** A cellular marrow has mild maturational changes, but the histiocytosis is not obvious. **B,** Immunostain for CD68⁻ PGM-1, which does not cross-react with myeloid cells, reveals abundant and large macrophages, with some displaying hemophagocytosis. **C,** Liver enzymes were abnormal, but the only change was the prominence of the sinusoidal Kupffer cells with little hemophagocytosis.

## TABLE 19-3

### Classification of Histiocytic Disorders

| A Contemporary Classification of Histiocytic Disorders (Histiocyte Society, 1997*) | Combined Immunophenotypic and Morphologic Classification (World Health Organization, 2002†) |
|---|---|
| Disorders of varied biologic behavior<br>• Dendritic cell-related<br>  • Langerhans cell histiocytosis<br>  • Secondary dendritic cell processes<br>  • Solitary histiocytomas of various dendritic cell phenotypes<br>• Macrophage-related<br>  • Juvenile xanthogranuloma and related processes<br>  • Hemophagocytic syndromes<br>  • Primary hemophagocytic lymphohistiocytosis (familial and sporadic; commonly elicited by viral infections)<br>  • Secondary hemophagocytic syndromes<br>  • Infection-associated<br>  • Malignancy-associated<br>  • Other<br>  • Rosai-Dorfman disease (sinus histiocytosis with massive lymphadenopathy)<br>  • Solitary histiocytoma with macrophage phenotype<br>Malignant disorders<br>• Monocyte-related<br>  • Leukemias (FAB and revised FAB classifications)<br>  • Monocytic leukemia M5A and B<br>  • Acute myelomonocytic leukemia M4<br>  • Chronic myelomonocytic leukemia<br>  • Extramedullary monocytic tumor or sarcoma (monocytic counterpart of granulocytic sarcoma)<br>• Dendritic cell-related histiocytic sarcoma (localized or disseminated)<br>  • Specify phenotype (e.g., follicular dendritic cell, interdigitating dendritic cell)<br>• Macrophage-related histiocyte sarcoma (localized or disseminated)<br>• Juvenile and adult xanthogranulomas identified as being of macrophage, not dendritic cell type | Macrophage–histiocytic neoplasms<br>  • Histiocytic sarcoma<br>Dendritic cell neoplasms<br>  • Langerhans cell tumor<br>  • Langerhans cell sarcoma<br>  • Interdigitating cell tumor or sarcoma<br>  • Follicular dendritic cell tumor or sarcoma<br>Unclassifiable |

*Favara BE, Feller AC, Pauli M, et al: The WHO Committee on Histiocytic/Reticulum Cell Proliferations. Reclassification Working Group of the Histiocyte Society, *Med Pediatr Oncol* 29:157–166, 1997.
†Pileri SA, Grogan TM, Harris NL, et al: Tumours of histiocytes and accessory dendritic cells: an immunohistochemical approach to classification from the International Lymphoma Study Group based on 61 cases, *Histopathology* 41:1–29, 2002.
*FAB,* French-America-British.

### FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS—FACT SHEET

**Definition**

- A group of inherited conditions resulting in uncontrolled macrophage activation due to defective apoptosis, by T or NK cells

**Incidence and Location**

- 0.12/100,000 children per year (Sweden systemic)

**Morbidity and Mortality**

- Mortality is 100% untreated, 55% 3-year survival with bone marrow transplantation protocols HLH-94 and HLH-2004

**Gender, Race, and Age Distribution**

- Male to female ratio = 1, race distribution unknown
- 90% younger than 2 years
- Rare in older children or adolescents, but late occurrences seen

**Clinical Features**

- A molecular diagnosis consistent with HLH diagnosis
- Undetectable perforin in 40% (flow cytometry)
- In the absence of genetic information, diagnosis requires five of the following eight features:
  - Fluctuating fever
  - Hepatosplenomegaly
  - Bicytopenia, especially thrombocytopenia
  - Hypertriglyceridemia >2.0 mmol/L or >3 SD fasting values, or hypofibrogenemia <1.5 g/L or <3 SD
  - Hemophagocytic histiocytosis (bone marrow, liver, spleen, cerebrospinal fluid)
  - Low or absent NK-cell function
  - Hyperferritinemia >500 μg/L
  - Increased soluble CD25 (>2400 U/mL)

years of life may be associated with neurologic and meningeal signs and symptoms, hepatomegaly with evidence of hepatocellular dysfunction, splenomegaly, and sometimes a skin rash. Laboratory features include hypofibrinogenemia alone or with hypertriglyceridemia, hyperferritinemia, and high levels of circulating soluble CD25 (IL-2 receptor). NK cell function is low or absent.

## Pathologic Features

### Gross Findings

Gross findings are nonspecific and are limited to hepatomegaly and splenomegaly.

### Microscopic Findings

An excess of activated macrophages is the hallmark, usually with prominent hemophagocytosis. These cells can be found in the bone marrow, spleen, and lymph nodes. In the liver, the infiltrate is largely portal with the accompaniment of T lymphocytes (i.e., lymphohistiocytosis). In addition to this chronic hepatitis-like appearance, three other histopathologic patterns have been described. These are a leukemia-like pattern, a histiocyte storage–like pattern, and a neonatal giant cell hepatitis-like pattern. The hemophagocytic cells may be found in the central nervous system (CNS), especially the spinal fluid (Figure 19-8). The macrophage activation and hemophagocytosis can be cyclical, and intervening biopsy specimens (e.g., marrow) may be negative at first examination or during troughs of activity.

**HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Hemophagocytic histiocytosis in bone marrow, spleen, lymph nodes, liver, cerebrospinal fluid
- Liver with portal lymphohistiocytosis and Kupffer cell activation with hemophagocytosis
- Bone marrow possibly cellular and active in the face of cytopenias early on, with progressive depletion of hematopoiesis and increasing content of large phagocytic histiocytes

**Fine-Needle Aspiration Biopsy Findings**
- Histiocytosis with hemophagocytosis on bone marrow or spleen

**Immunohistochemical Features**
- Increased numbers of marrow histiocytes CD68 (PGM-1)
- Increased macrophages in spleen, CD68, CD163

**Differential Diagnosis**
- Secondary macrophage activation with viral infection especially EBV, rheumatologic conditions, T-cell malignancies
- X-linked lymphoproliferative syndrome, EBV
- Griscelli syndrome
- Chediak-Higashi syndrome

## Ancillary Studies

### Molecular and Cytogenic Features

FHL1 (Online Mendelian Inheritance in Man [OMIM] no. 267700) is mapped to 9q21, but the genetic defect is not yet determined. Approximately 30% to 50% of patients have mutations in the perforin gene FHL2 (FHL2, OMIM no. 603553), most of which lead to reduced or absent perforin expression on peripheral blood cells, a feature that can be documented by flow cytometry. A smaller number have FHL3, the form characterized by mutations in the *Munc* 13-4 gene (FHL, OMIM no. 608898) important for perforin transport as well as docking and fusion of cytotoxic granules with the cytoplasmic membrane, but perforin content is normal. FHL4, (OMIM no. 603552) is caused by mutations in the syntaxin-11 gene and FHL5 (OMIM no. 613101) by mutations in the syntaxin-binding protein-2 gene.

There are also inherited defects in other genes that predispose to hemophagocytic syndromes, most notably Griscelli syndrome (RAB27A one of the MUNC 13-4 effector molecules), Chediak-Higashi syndrome (LYST1), the Hermansky-Pudlak-2 syndrome (AP3B1) and X-linked lymphoproliferative syndrome, SAP/SH2D1A, and especially the *BIRC4* genes.

### Immunohistochemistry

The demonstration of an excess of activated macrophages is key to the recognition of the condition, but a definitive diagnosis may require genetic documentation when possible. Marrow macrophages in FHL are demonstrated using CD163 or anti-CD68 PGM-1 antibody that has little cross-reaction against hematopoietic precursors, and hemophagocytosis may be evident. At all other sites, CD68 (KP-1 or PGM-1) and CD163 will demonstrate the macrophages effectively, including in the portal areas of the liver. In patients who have a perforin mutation, the CD3/CD8 cells do not stain for perforin by immunohistochemistry (Figure 19-9).

### Fine-Needle Aspiration Biopsy

Marrow is the most commonly examined aspirate, although splenic puncture is more commonly done in Europe. In both instances, the presence of large numbers of big, cytoplasm-rich but bland macrophages that have hemophagocytosis, especially erythrophagocytosis, is typical during active episodes.

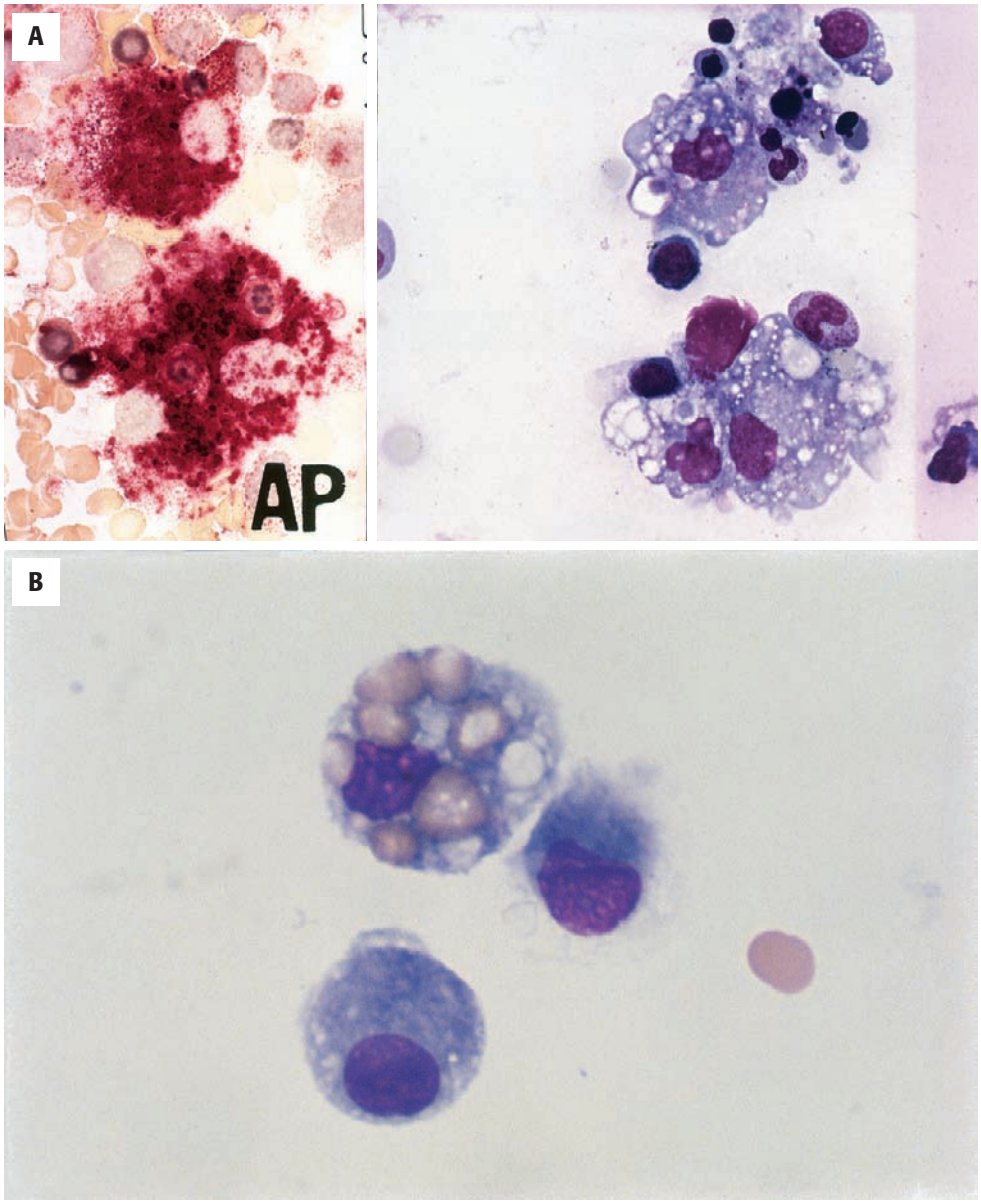


**FIGURE 19-8**

Hemophagocytic lymphohistiocytosis. The bone marrow in active phases will have large, actively phagocytic macrophages that may contain other formed blood elements. **A,** Acid phosphatase (AP) is demonstrable in the cytoplasm. **B,** Similar cells that have intracytoplasmic erythrocytes are present in spinal fluid.

## Differential Diagnosis

The most important differential diagnosis is from the secondary macrophage activation syndromes that are treated by targeting the inciting condition: viral infection, rheumatologic condition, T-cell malignancy, and hyperalimentation (fat overload syndrome). Molecular diagnosis, when available, is definitive. The differential diagnosis can be confounded not only because the clinicopathologic features in individual instances are so variable, but also because infection can serve as the trigger for a crisis in the primary and familial hemophagocytic lymphohistiocytoses mimicking a secondary infection–associated macrophage activation syndrome. It should also be noted that the most common cause of erythrophagocytosis is not hemophagocytic syndrome, but a minor transfusion reaction.

## Prognosis and Therapy

Primary familial hemophagocytic lymphohistiocytosis is rapidly fatal with a mean survival of 2 months unless treated effectively. The HLH94 protocol (now HLH-2004) stabilizes patients using immune suppression

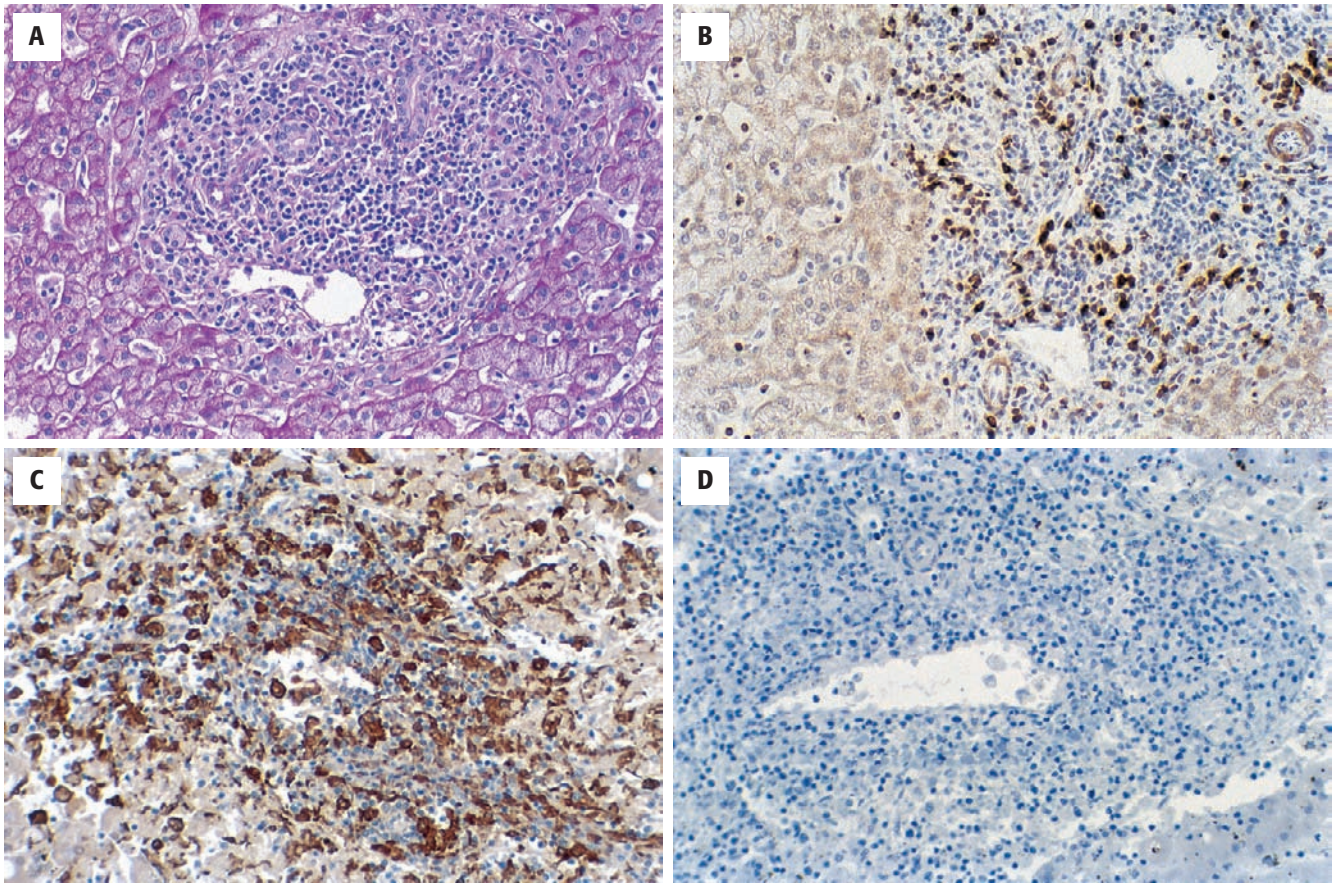


**FIGURE 19-9**

Liver in hemophagocytic lymphohistiocytosis. **A,** The infiltrate is mostly portal and consists of a mix of macrophages and lymphocytes. **B,** In more than half of the familial cases, CD3/CD8 will be demonstrable on the lymphocytes, and perforin is present. **C,** In instances of FHL2, the vast numbers of portal macrophages are seen with CD163, but **(D)** perforin is not detectable on the lymphocytes.

and chemotherapy and follows that with bone marrow transplantation, with a survival rate of approximately 50%.

## CYTOPHAGIC HISTIOCYTIC PANNICULITIS

Cytophagic histiocytic panniculitis appears to be a localized expression of hemophagocytic lymphohistiocytosis. Some cases of cytophagic histiocytic panniculitis are associated with instances of familial hemophagocytic syndrome, but the local effect may predominate. Other instances appear to be examples of infection-associated macrophage activation, most commonly harboring Epstein-Barr virus (EBV) in the panniculitis. Both familial hemophagocytic syndrome and infection associated macrophage activation must be separated from subcutaneous panniculitis-like T cell lymphoma in which the lymphoma drives the histiocytic component. Most are examples of the systemic SPTL-αβ with αβ rearranged T cells that are $CD3^{+}CD8^{+}$ cytotoxic cells. There is loss of CD2, CD5, and CD7 in up to 50% of cases.

## ROSAI-DORFMAN DISEASE

Also known as *sinus histiocytosis with massive lymphadenopathy* after its most common presentation, Rosai-Dorfman disease is a benign condition of a unique histiocyte and has a variable clinical course.

### Clinical Features

The usual presentation is painless, large and firm, usually bilateral cervical nodes associated with fever, leukocytosis, anemia, raised sedimentation rate, and a polyclonal hypergammaglobulinemia. The disease can also occur in nonnodal sites. In 40% of cases with or without cervical node involvement, skin and subcutaneous tissue, the orbit, bone, and CNS coverings are the more frequent sites, although involvement of any organ (except bone marrow and spleen) has been described. Extranodal involvement presents most commonly as a mass, and the diagnosis comes as a surprise. Multifocal

**ROSAI-DORFMAN DISEASE—FACT SHEET**

**Definition**
- A benign histiocyte infiltrate most common in cervical lymph nodes causing striking enlargement, also occurring in extranodal sites
- Lesion identified at all sites by the unique Rosai-Dorfman histiocyte that has cytoplasmic emperipolesis

**Incidence and Location**
- Sporadic with rare clusters of cases
- Cervical lymph nodes
- Waldeyer's ring and upper respiratory tract
- Skin and subcutaneous, trunk and proximal limbs, orbit, bone, meninges, breast

**Morbidity and Mortality**
- Cosmetic problems owing to massive lymphadenopathy
- Regress without residua in most instances
- May cause serious disease at vulnerable sites such as brain

**Gender, Race, and Age Distribution**
- Adolescents and young adults (mean, 20 years)
- Blacks affected more commonly; males more than females for cervical lymphadenopathy
- Equal sex ratio for extranodal disease

**Clinical Features**
- Massive painless bilateral cervical lymphadenopathy with hyperglobulinemia and occasional autoimmune antibodies, uncommon systemic symptoms
- Space occupying lesions at other sites, with or without cervical node involvement

**Radiologic Features**
- Space occupying lesions without distinguishing features

**Prognosis and Treatment**
- Indolent course with slow spontaneous regression of the infiltrate and associated autoimmune phenomena, when present
- Corticosteroids and surgery for progressive disease, rarely chemotherapy for refractory or life-threatening lesions

**ROSAI-DORFMAN DISEASE—PATHOLOGIC FEATURES**

**Gross Findings**
- Massive cervical node involvement with capsular thickening, matting of adjacent nodes, and yellow color

**Microscopic Findings**
- Lymph nodes and extranodal sites have zones of very large pale cells alternating with lymphocyte- and plasma cell–rich areas
- Exceptionally large cells greater than 75 μm with pale, water-clear cytoplasm and large round hypochromatic nuclei containing a single nucleolus
- Cytoplasmic emperipolesis of intact cells mostly lymphocytes, less commonly neutrophils, eosinophils
- Interspersed plasma cells prominent in most
- Neutrophil pseudoabscesses
- Xanthoma cells and spindle cells with collagen deposition in regressing extranodal lesions

**Ultrastructural Features**
- Emperipolesis, intact inflammatory cells within cytoplasm

**Fine-Needle Aspiration Biopsy Findings**
- There is a population of exceptionally large histiocytes that have abundant pale cytoplasm with emperipolesis. The features are distinctive and immunocytologic confirmation is not required. S100 staining requires prior fixation because of high solubility

**Immunohistochemical Features**
- Large histiocytes have CD14, CD163, CD68, S100, fascin

**Differential Diagnosis**
- Langerhans cell histiocytosis is $CD1a^+$/$Langerin^+$. Granulomatous disease and draining histiocytes near prosthesis lack S100/fascin
- Foci of Rosai-Dorfman reaction can be seen in lymph nodes that harbor Hodgkin disease, acquired lymphoproliferative syndrome, or LCH without other manifestations of Rosai-Dorfman disease

lesions, nodal and extranodal, can simulate malignancy. Most cases occur in adolescents or young adult males with only rare intrafamily clusters.

## Radiologic Features

Imaging manifestations are nonspecific, and fludeoxyglucose positron emission tomography (FDG-PET) shows increased metabolism. These features do not distinguish Rosai-Dorfman disease from malignancy or other causes of lymphadenopathy. However, concomitant cervical lymphadenopathy with orbital or paranasal sinus mass should suggest further testing. Decreased T2-weighted signal intensity in dural-based lesions and lack of arteriovenous shunting on angiography helps to rule out meningioma.

## Pathologic Features

### Gross Findings

The nodes are often strikingly enlarged and firm and have thick fibrous capsules matting them together. The cut surface is commonly yellow from lipid.

### Microscopic Findings

In the lymph nodes, the lesion involves the sinuses that are markedly distended. Often the distention and distortion are so extensive that the sinus pattern is not recognizable. In soft tissues, the lesions are commonly

confluent with aggregates of lesional cells and intervening inflammatory cells that produce an alternating dark and light effect or simulate lymph node sinuses (i.e., sinusoidal pattern). The lesional Rosai-Dorfman cells are unique. The cells are large, often 75 μm or more, with a central large hypochromatic nucleus set in an abundant pale water-clear cytoplasm. Nucleoli are single, moderately large, and distinct. Mature lymphocytes, many plasma cells, and neutrophils are often present between the histiocytes and within the cytoplasm. The presence of intact cells within the cytoplasm, often as many as 5 to 10, is the emperipolesis that is characteristic of this condition (Figure 19-10). Neutrophil-rich suppuration may confound the picture. Late or involuting lesions have fewer Rosai-Dorfman cells, more xanthoma cells, and spindled fibroblasts.

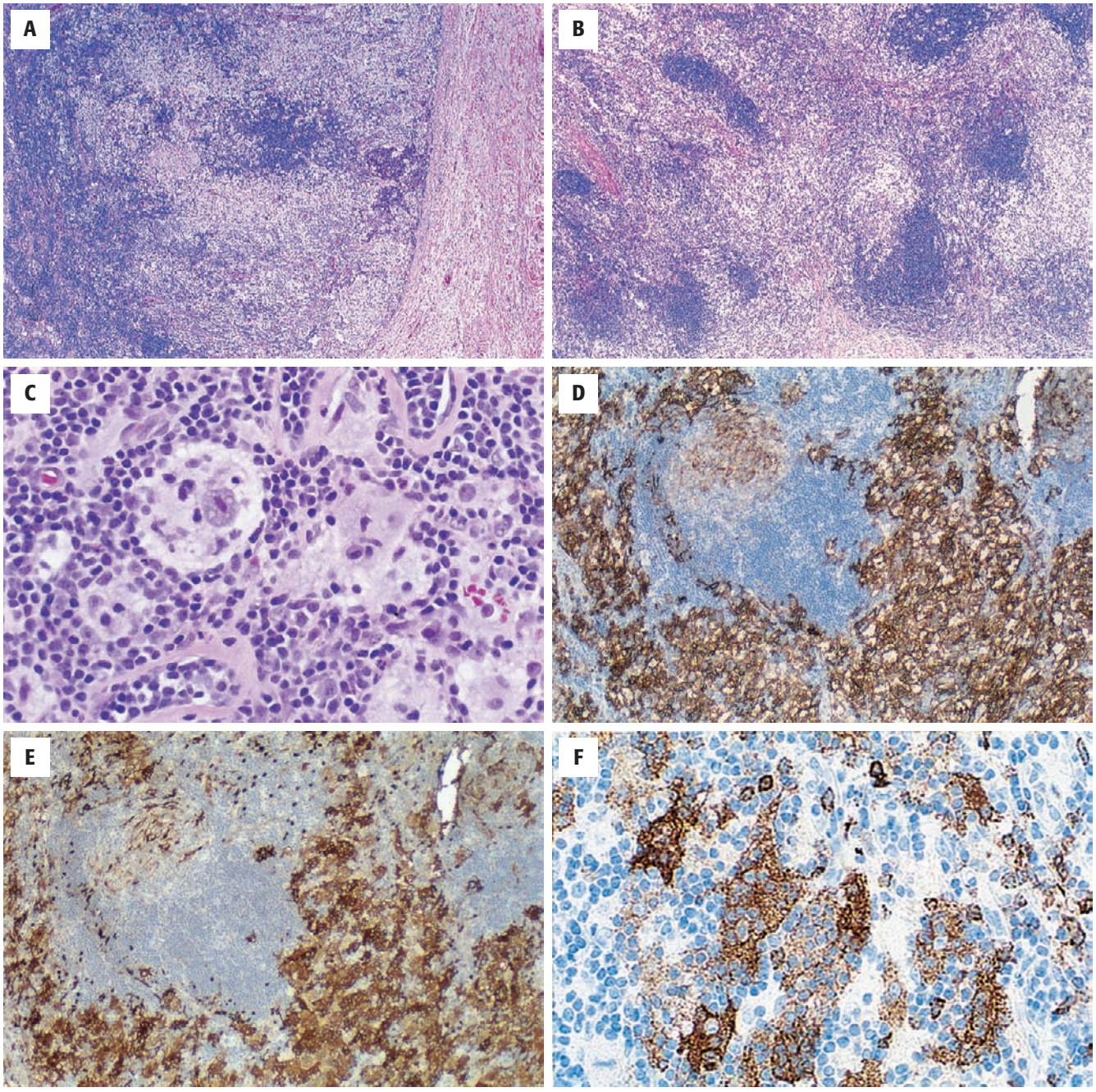


**FIGURE 19-10**

Rosai-Dorfman disease. **A** and **B,** The thick capsule and accumulation of pale histiocytes is demonstrated though their presence in the sinuses is not obvious. **C,** The large cells that have clear cytoplasm that includes whole cells (emperipolesis) and the nuclei are eccentric and large and have a prominent nucleolus in a hypochromatic background. The cells have sharp membrane staining for CD14 **(D)** and cytoplasmic and nuclear staining for S100 **(E),** and large cells with their emperipolesis are well shown with immunostain for CD163 **(F).**

## Ancillary Studies

### Electron Microscopy

Intact lymphocytes and phagolysosome structures are seen in the macrophages, but no Birbeck granules are present.

### Molecular and Cytogenic Features

There are no known molecular or cytogenetic features, and the large Rosai-Dorfman cells are not clonal.

### Immunohistochemistry

The cells mark as macrophages–DC hybrids. They express strong macrophage markers, variable surface CD14, and membrane CD163, and they have fine granular cytoplasmic CD68. In addition, and most informatively, there is the strong expression of S100 and fascin in their cytoplasm, outlining the emperipolesis (see Figure 19-10).

### Fine-Needle Aspiration Biopsy

The exceptionally large Rosai-Dorfman cells that have a central round nucleus and abundant cytoplasm that contains emperipolesis of lymphocytes, plasma cells, or neutrophils are virtually diagnostic. Confirmation of the phenotype completes the diagnosis.

## Differential Diagnosis

Reactive and draining nodes with sinus histiocytosis do not have classical Rosai-Dorfman features; the cells are much smaller. Langerhans cell histiocytosis (LCH) involves the sinuses of lymph nodes, but the LCH cells are also smaller with nuclei that are grooved or complexly folded, unlike the large Rosai-Dorfman nucleus. Nodes draining prosthetic implants can more closely simulate Rosai-Dorfman disease, usually in older patients. Rarely, intermixed foci of Rosai-Dorfman reaction can be seen in lymph nodes that harbor other conditions such as acquired lymphoproliferative syndrome, Hodgkin disease, or Langerhans cell histiocytosis. Late lesions that have only few Rosai-Dorfman cells can simulate inflammatory pseudotumors.

## Prognosis and Therapy

Some large nodes regress rapidly and spontaneously without treatment, whereas most regress and recur over a period of years. Soft-tissue lesions can be indolent and respond only poorly to corticosteroids, but chemotherapy has been effective. Mortality has been due to disease at vulnerable sites, such as the CNS or upper respiratory system, or associated immunologic disorders, lymphoma, leukemia, or exceptional instances of solid tumor.

# Reticulohistiocytoma and Multicentric Reticulohistiocytosis

Two forms of reticulohistiocytoma are recognized: solitary reticulohistiocytoma (a cutaneous lesion also called *epithelioid histiocytoma*) and multicentric reticulohistiocytosis (MRH; a cutaneous and arthropathic condition). Both are characterized by infiltration by the same type of histiocyte. Visceral symptoms and muscular, cardiopulmonary, ocular, thyroid, and submandibular involvement have been reported occasionally. Lymph node involvement is rare. The solitary lesion is seen in neonates and young adult males, whereas MRH is more common in older females. MRH is associated with hyperlipidemia, underlying malignancy, or an autoimmune disorder in half the patients.

## Pathologic Findings

The lesional histiocyte is large (50 to 100 μm) with one to three eccentric, oval, or grooved nuclei and abundant, deeply eosinophilic glassy cytoplasm that stains lightly with PAS and resists diastase. Early lesions may contain many macrophages and lymphocytes mimicking other histiocytoses. Phagocytosis of connective tissue or cellular components may be seen. The cells stain for CD163, CD14, and CD68, but not S100 or CD1a (Figure 19-11). Fascin stains the lesional cells lightly and variably. Light staining for factor XIIIa and muscle-specific actin are said to distinguish the solitary lesion from that of MRH. Electron microscopy shows large mononucleated or multinucleated cells with numerous peripheral microvilli. Birbeck granules are absent. The differential diagnosis includes Rosai-Dorfman disease, but the cytoplasm of the MRH cells neither is pale nor contains emperipolesis. The cells are larger than those of the juvenile xanthogranuloma (JXG) family and have been confused for melanoma cells, but S100 stain is negative. The nonneural (S100-negative) granular cell lesion can look similar.

## Prognosis and Therapy

A congenital skin form is self limiting. Most children have benign epithelioid histiocytomas of the skin,

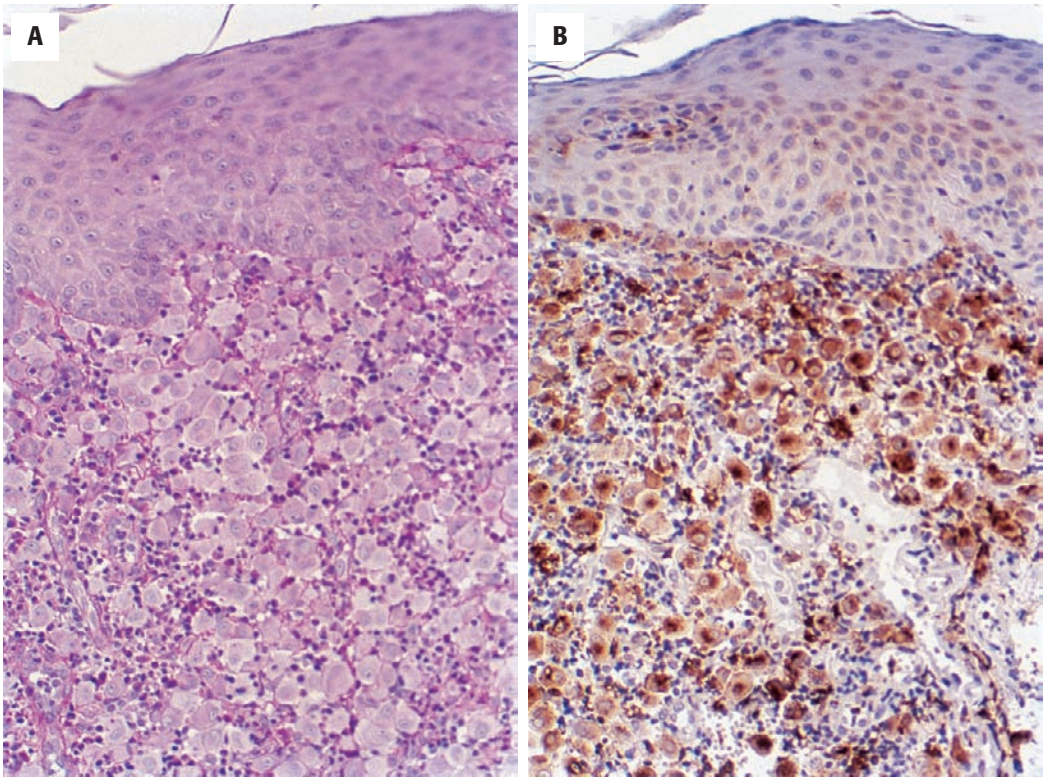


**FIGURE 19-11**

Reticulohistiocytoma. **A,** The skin lesion contains large cells that have abundant glassy cytoplasm, lightly PAS-positive in contrast to the intervening inflammatory cells. **B,** CD68 (KP-1) stains the cytoplasm with a paranuclear accentuation.

whereas 50% of adults progress to a deforming polyosteoarthropathy.

## LANGERHANS CELL HISTIOCYTOSIS

LCH is a disorder characterized by clonal proliferation of abnormal histiocytic cells that have the characteristics of Langerhans cells.

### Clinical Features

LCH affects children primarily, but there is an increasing incidence in adults. In children, the disease can be limited to a single site unifocal (e.g., bone, soft tissue, skin lesions), multiple foci in a single system (multifocal bone or multiple lymph node lesions), or multisystem disease that involves two or more organ systems. Involvement of some organs confers high risk; these are the liver, lungs, bone marrow, spleen, and penetrating skull lesions. Mortality in these high-risk children with multisystem disease, often younger than 2 years, remains 30% to 50%. In adults, the disease affects the lungs of smokers, although bone and skin lesions occur. Common but nonspecific B-type symptoms can include fever, weight loss, and fatigue. The specific clinical manifestations depend on the site of involvement. Bone involvement, monoostotic or polyostotic, is the most frequent presentation and is commonly associated with pain, pathologic fractures, vertebral collapse, and local soft-tissue extension. Skin involvement often involves flexures and the scalp with a seborrheic rash that may be petechial. Newborns have more papular lesions that commonly regress. The liver can be involved by LCH infiltration of the major bile ducts that causes a sclerosing cholangitis, biliary strictures, and ends in a biliary cirrhosis. The lungs are more commonly involved as part of multifocal disease in children, but may often be the only site in smoking adults. Interestingly, some children who have recovered from early childhood disease have developed pulmonary LCH when they began smoking as adolescents. Pulmonary involvement leads to pneumothorax, interstitial and peribronchial fibrosis, and respiratory compromise. Central nervous system involvement by active LCH involves the hypothalamic-posterior pituitary axis, causing irreversible diabetes insipidus, or growth retardation, and it can involve the choroid plexus and meninges. Late CNS involvement, years later, appears to be indirect without active LCH but with bilateral, usually cerebellar demyelinating foci resulting in progressive ataxia, dysarthria,

**LANGERHANS CELL HISTIOCYTOSIS—FACT SHEET**

**Definition**
- LCH is a group of poorly characterized disorders, mostly clonal, characterized by the accumulation of bone marrow-derived Langerhans cell histiocytes (LCH cells)

**Incidence and Location**
- 0.54 to 0.9 per 100,000 children (Denmark, Sweden) and unknown in adults; probably underestimates the true incidence since many are not reported
- Rare familial cases
- Bone and soft tissue most common, skin, lung in smoking adults, lymph node, liver, bone marrow

**Morbidity and Mortality**
- Highest mortality in those younger than 2 years (30% to 50%) who have "risk organ" involvement (e.g., liver, lungs, marrow, spleen)
- Morbidity high for multiple bone lesions and adult multisystem or lung disease (91% 5-year survival)
- Unifocal bone disease, eosinophilic granuloma, usually self-limiting

**Gender, Race, and Age Distribution**
- Male predominance 2:1 in children, 1.4:1.2 in adults
- Age range from neonates to adults
- Neonatal skin-only papular disease often self-limited (Hashimoto-Pritzker disease)
- Systemic disease, younger than 2 years (Letterer-Siwe disease)
- Multiple bone, soft tissue disease, 2 to 10 years (Hand-Schüller-Christian disease)
- Solitary bone, soft tissue disease, 5 to 15 years (eosinophilic granuloma)
- Adult disease, mean age 33 years (SD = 15 years), 30% single system, 70% multisystem

**Clinical Features**
- Symptoms vary according to site and organs involved
- Skeletal pain, soft tissue mass in bone lesions
- Extraosseous manifestations: diabetes insipidus, seborrhea-like skin rash, pulmonary abnormalities, lymphadenopathy, hepatosplenomegaly, and pancytopenia

**Radiologic Features**
- Osteolytic intramedullary bone lesions, poorly demarcated in early and active lesions
- Osteolytic intramedullary bone lesions, well demarcated by sclerotic rim in older and regressing lesions
- Peribronchial and interstitial lung changes progressing to honeycombing in some
- Loss of normal pituitary signal and thickening of the pituitary stalk

**Prognosis and Treatment**
- Localized skin and bone lesions can be observed or resected, treated intralesionally or topically with steroids or given low dose radiation to bone
- Multisystem LCH given systemic chemotherapy according to LCH III protocol
- Recurrent or refractory disease; 2 chlorodeoxyadenosine (2CDA) or bone marrow transplantation
- Adult lung disease
- Cessation of smoking, corticosteroids

nystagmus, hyperreflexia, dysdiadochokinesia, dysphagia, and blurred vision. Hematopoietic involvement is seen with bone marrow and spleen infiltration, but the cytopenias that are common are functional (possibly cytokine mediated) because the hematopoietic marrow is preserved. Lymph nodes and the gastrointestinal tract can be involved, resulting in lymphadenopathy and sometimes life-threatening malabsorption, diarrhea, and protein-losing enteropathy. The kidney and gonads are almost never affected. Because of the widespread and protean possibilities, LCH when first diagnosed at any site is evaluated by staging, a clinical survey that maps the extent of involvement.

## Radiologic Features

### Bone and Adjacent Soft Tissue

In the early stages, expanding new bone lesions may have a rapidly growing, lytic appearance with poorly defined margins that mimic malignant disease. The presence of pathologic fractures can confound the picture. Older and involuting lesions have an osteolytic center and sclerotic, sharply defined borders, and the differential diagnosis includes low-grade lesions such as chronic osteomyelitis. Cranial bone involvement with adjacent soft tissue disease is characteristic (Figure 19-12). Computed tomography or magnetic resonance imaging (MRI) may help to ascertain the extent and character of the bone lesion before biopsy. Early lung involvement is best demonstrated by high-resolution computed tomography that reveals a delicate interstitial and cystic change that is bronchocentric and spares the intervening lung. Late disease is nodular, fibrotic, scarring, and honeycombing on imaging studies. Pleural bullae are responsible for the bouts of pneumothorax. Imaging of the liver may demonstrate the features of a sclerosing cholangitis. Central nervous system disease in the early infiltrative phase is characterized by posterior pituitary stalk involvement, and there can be space-occupying choroid or meningeal masses. Late disease is seen best by MRI that reveals the symmetrical neurodegenerative foci of the cerebellum and basal ganglia. An age-appropriate survey of the rest of the skeleton to look for other lesions is often performed once a diagnosis is established. More sensitive modalities such as whole-body FDG-PET scans can localize new lesions and are more informative of disease activity.

## Pathologic Features

### Gross Findings

There are virtually no early or late changes in any of the organs that will be suggestive of LCH.

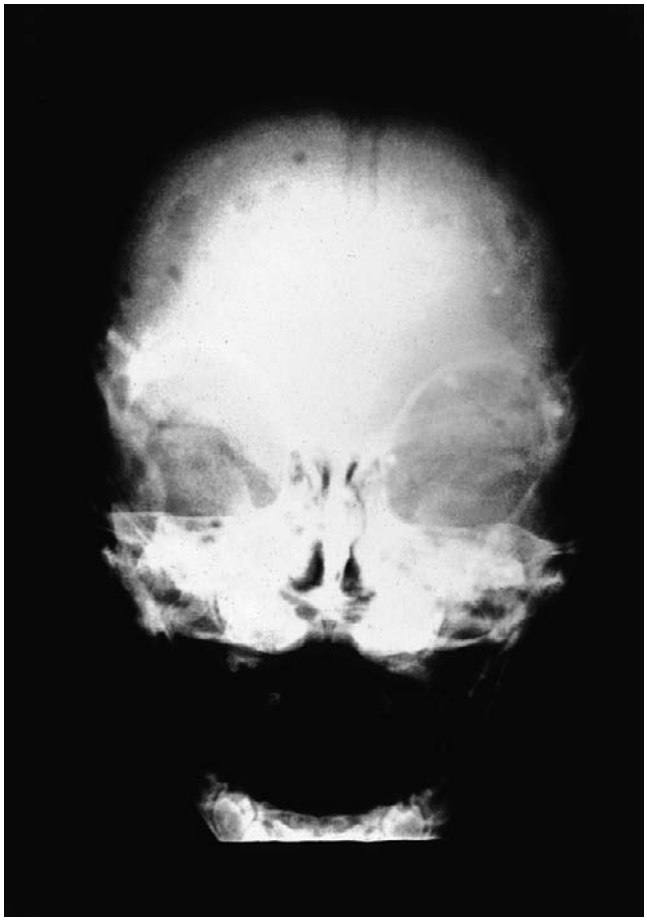

**FIGURE 19-12**

Langerhans cell histiocytosis. A radiograph of the head in this 4-month-old child who had widespread active diseases shows the presence of bilateral punched-out parietal lesions in the calvarium. The sclerotic rims, not seen here, are more characteristic of chronic or long-standing disease.

**LANGERHANS CELL HISTIOCYTOSIS—PATHOLOGIC FEATURES**

**Gross Findings**

- Bone lesions may be sharply demarcated or grey to yellow depending on lipid content

**Microscopic Findings**

- Cells are oval and measure 15 to 25 μm, usually nested in sheets or clusters
- Nuclei are oval or grooved or have complex foldings.
- Nucleoli are inconspicuous
- Cytoplasm is pale
- Binucleated and multinucleated forms are common with osteoclast-like cells in bone and contiguous soft-tissue lesions.
- Mitoses are variable, never atypical
- Eosinophils are variable and may be absent to overwhelming, with microabscesses filled with Charcot-Leyden crystals
- Plasma cells are rare within in the histiocytic aggregates

**Ultrastructural Features**

- There are classic dendritic cells and intervening macrophages. The LCH cells have sparse organelles
- Birbeck granules are required for ultrastructural confirmation

**Fine-Needle Aspiration Biopsy Findings**

- Bone, lymph node, thyroid, and hypercellular lesions with histiocytes having moderately abundant pale cytoplasm and a grooved or folded nucleus
- Eosinophils possibly not obvious

**Immunohistochemical Features**

- Oval histiocytes with CD1a/Langerin and high sensitivity and specificity
- S100 nuclear/cytoplasmic, sensitive not specific; vimentin
- CD68 and HLA-DR-low, paranuclear

**Differential Diagnosis**

- Bone osteomyelitis, especially chronic recurrent culture-negative
- Soft-tissue, inflammatory, and granulomatous processes
- Hodgkin lymphoma with eosinophils
- Foci of $CD1a^+$ DC hyperplasia can occur in lymph nodes containing lymphoma, simulating LCH, but extranodal disease never seen in these instances

### Microscopic Findings

Diagnosis of LCH is made by identifying the lesional cells as LCH cells with Langerhans cell phenotype. The cells are moderately large (20 to 25 μm), oval, and not dendritic in shape, with a grooved (coffee bean) or complex folded nuclear profile. Nuclei are commonly single or two to three per cell, and there are osteoclast-type multinucleated cells in many sites that may harbor the folded LCH nucleus (Figure 19-13). Cytoplasm is abundant and pale and may have few fine granules. The gold standard for the diagnosis remains the phenotypic confirmation of cells that look cytologically appropriate for LCH. In some sites, most notably bone, eosinophils can be interspersed often in large numbers, albeit unevenly. Although the lesions were formerly called *eosinophilic granulomas,* eosinophils are not required for the diagnosis, which rests on identifying the LCH cell. In bone and soft tissue, early lesions contain sheets of LCH cells with inflammatory cells, mostly T cells largely restricted to the periphery. Phagocytic macrophages and osteoclast-type giant cells can be present or even dominate the picture. The LCH cells disappear as lesions regress, and it is possible to biopsy a late, healing lesion from which the LCH cells have disappeared and therefore be unable to confirm the diagnosis. In the skin, the process is usually epidermotropic, hugging the epidermis and filling the papillary dermis. The cells are characteristically large and oval. Lymph node involvement is strictly sinus in distribution at first, spilling over into the paracortex and thus following the normal migratory pathway for sentinel DCs reaching the lymph node (Figure 19-14). Liver involvement is almost uniquely biliary, with LCH cells in the epithelium of large-caliber bile ducts where they cause a sclerosing cholangitis (Figure 19-15). The cells can migrate peripherally within the biliary tree, usually between the basement

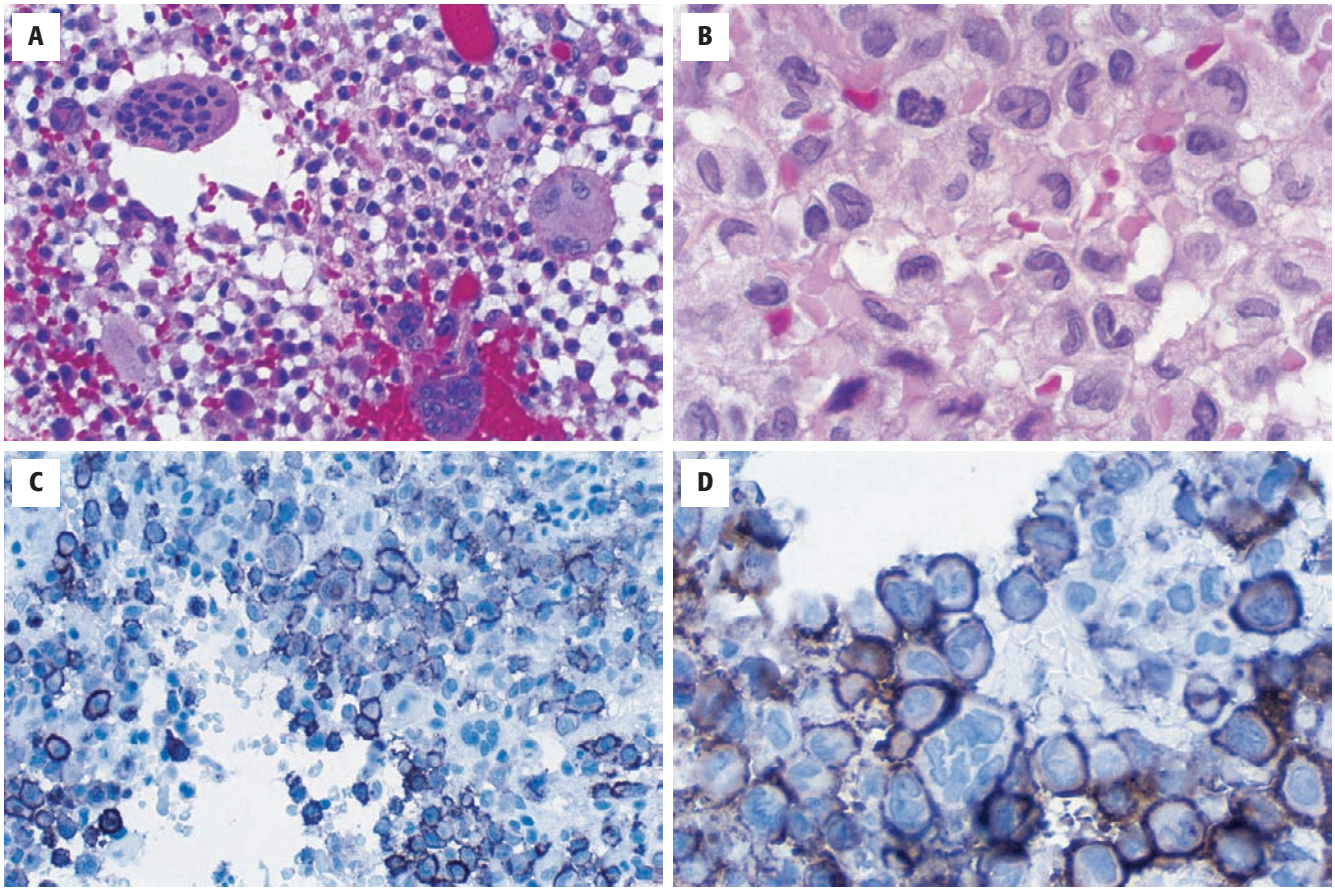


**FIGURE 19-13**

Langerhans cell histiocytosis (LCH): bone. This lesion has few eosinophils, but osteoclast-type giant cells are present. **A,** The LCH cells predominate. **B** and **C,** Strong membrane staining for CD1a is demonstrable. **D,** Mononuclear and multinucleated LCH cells have CD1a staining, and the angular nuclear profile is typical. Langerin stains the LCH cells.

membrane and the biliary epithelial cells. When there is extensive involvement, parenchymal nodules can occur. Lung involvement is peribronchial with extension of LCH cells into peribronchial alveolar walls. Bone marrow involvement is often subtle; first because there may only be few LCH cells in small clusters, and second because there may be a reactive macrophage histiocytosis that obscures the picture. Posterior pituitary, leptomeningeal and choroid involvement has the characteristic large, oval LCH cells. Three types of lesions are described in CNS LCH: (1) circumscribed granulomatous lesions including variable numbers of $CD1a^+$ cells and numerous $CD8^+$ T cells most commonly in circumventricular sites and the pituitary; (2) $CD1a^+$ cell-rich and CD8 T lymphocyte-rich granulomas in infundibular sites extending to the hypothalamus and adjacent CNS parenchyma; and (3) $CD1a^+$ cell poor, $CD8^+$ T cell–rich lesions most often in the cerebellum. The latter two are associated with neuronal and axonal loss. The splenic involvement can also be difficult to define except in those patients who form LCH nodules in the spleen. Gastrointestinal involvement fills the lamina propria and can be subtle until one recognizes that the usual cell mix is replaced by a single population of LCH cells. Thyroid and thymus can be involved with sheets of oval LCH cells that have the diagnostic phenotype.

## Ancillary Studies

### Molecular and Cytogenic Features

A BRAF V600 mutation has been described in 57% of patients in one study. Loss of heterozygosity of tumor suppressor genes has been documented in adults with pulmonary LCH, specifically for putative genes on 9p and 22q, as well as 1p and chromosome 7 in bone lesions from children. A distinct profile of gene expression compared to epidermal Langerhans cells has been described that includes overexpression of genes associated with immature myeloid DCs that are suggested to provide the LCH precursors. A study using loss of heterozygosity has showed evidence of more mutational events at gene loci with tumor suppressor genes in extensive and higher-risk forms of LCH. A subsequent study using

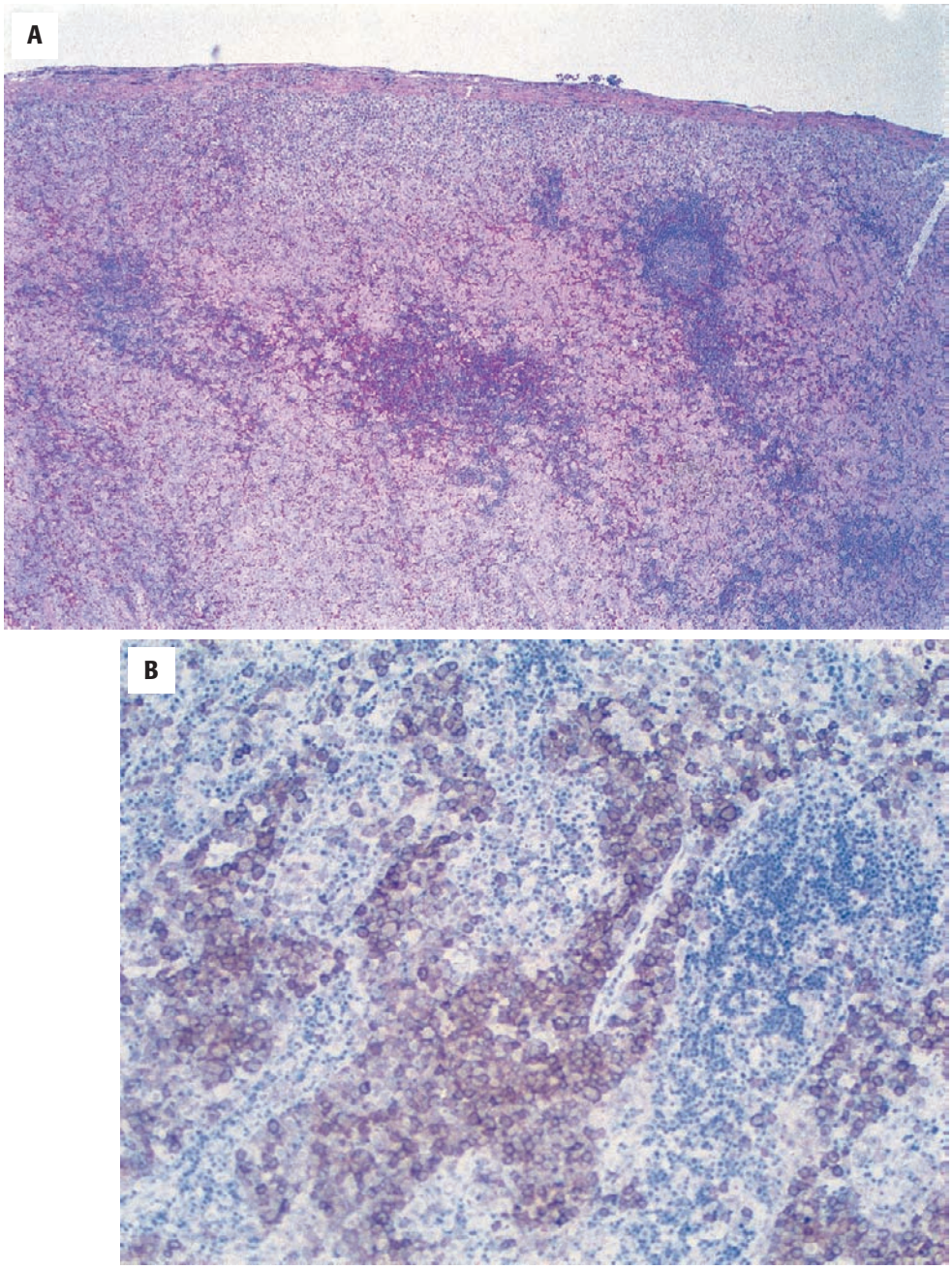


**FIGURE 19-14**

Langerhans cell histiocytosis (LCH): lymph node. **A,** The lymph node is replaced by sheets of LCH cells, and no particular distribution can be discerned. Few residual follicles remain. **B,** CD1a immunostain reveals that in the lymph node, sinus LCH has strong CD1a presence, whereas the paracortical cells have little to no CD1a expression but enhanced HLA-DR surface pattern, suggesting limited maturation.

conventional cytogenetics, array-based comparative genomic hybridization, and single nucleotide polymorphisms on a $CD1a^+$ sorted population failed to show any abnormality.

### Ultrastructure Features

The Birbeck granule was originally identified in Langerhans cells of the skin then in adult pulmonary LCH and later in cutaneous and bone lesions of childhood. The structure forms where the C-type lectin Langerin (CD207) accumulates and the Birbeck granule appears to be related to endosomal trafficking. The Birbeck granule is a rod-shaped bilaminar disc with an internal zipperlike pattern of striations, often with a bulbus dilatation at one end, like a tennis racquet. The granule is found wherever Langerhans cells are seen in the body, but are not unique to Langerhans cells and

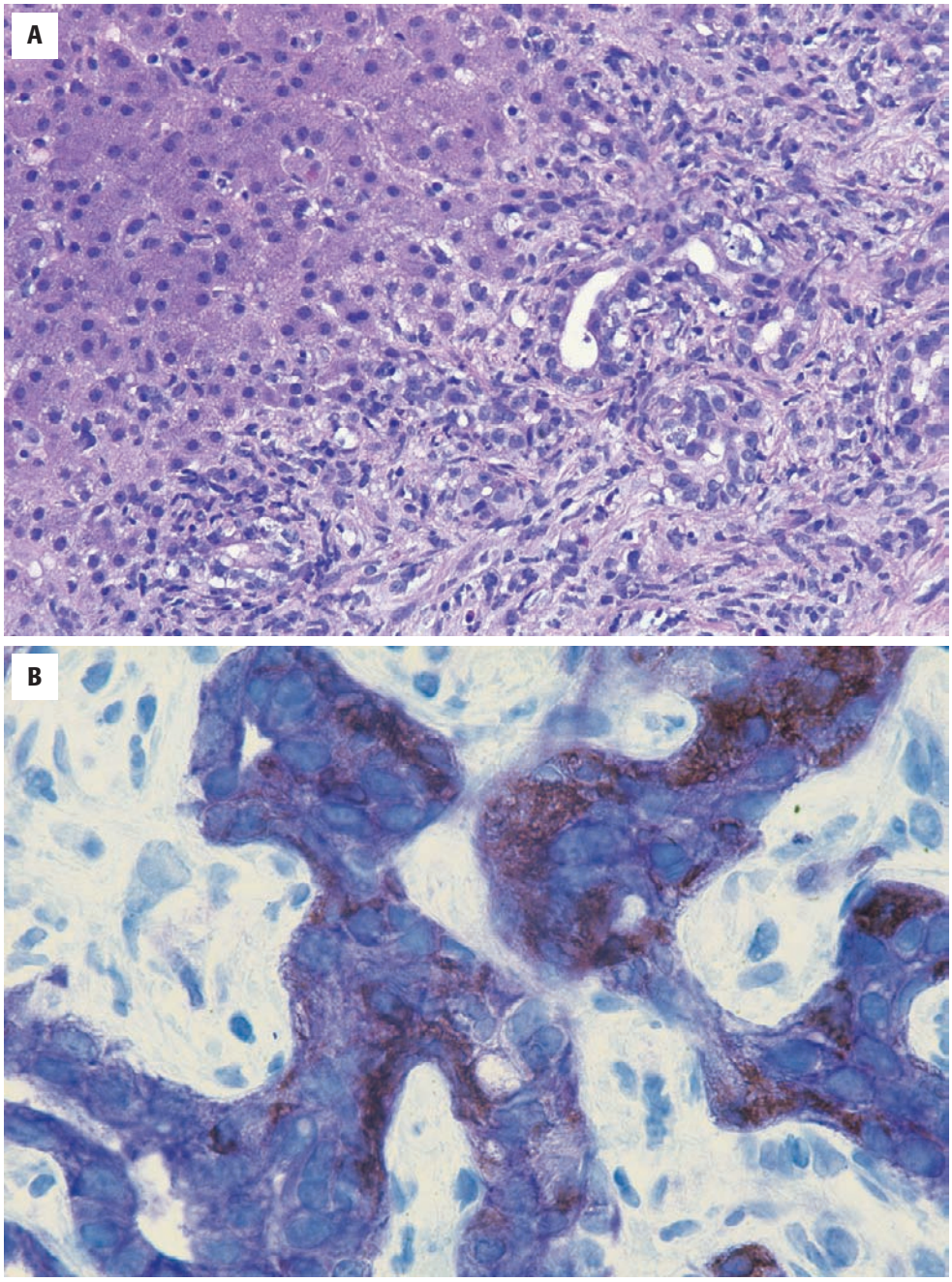


**FIGURE 19-15**

Langerhans cell histiocytosis (LCH): liver. **A,** The liver has biliary obstructive features with prominent bile duct dilatation and ductular proliferation, but no infiltrate is detected. **B,** Double stain for epithelial cells (cytokeratins AE1/AE3, blue) and CD1a, brown, show the subtle presence of LCH cells percolating between the epithelial cells and the basement membrane.

have been described in other cells (Figure 19-16). LCH cells have few cytoplasmic organelles and filopodia at the cell surface.

#### Immunohistochemistry

The cells of Langerhans cell histiocytosis have a stable phenotype: vimentin$^+$, S100$^+$, CD1a$^+$, Langerin$^+$. They have small, paranuclear intracytoplasmic accumulations of CD68 and human leukocyte antigen (HLA)-DR. In sites such as the paracortex of lymph nodes, the LCH cells can show some evidence of limited maturation in which CD1a is focally lost and membrane HLA-DR expression is increased.

#### Fine-Needle Aspiration

Lesions that are aspirated are commonly mixed in nature, and the LCH cell must be identified. Presumptive diagnosis rests on the cytologic features of large,

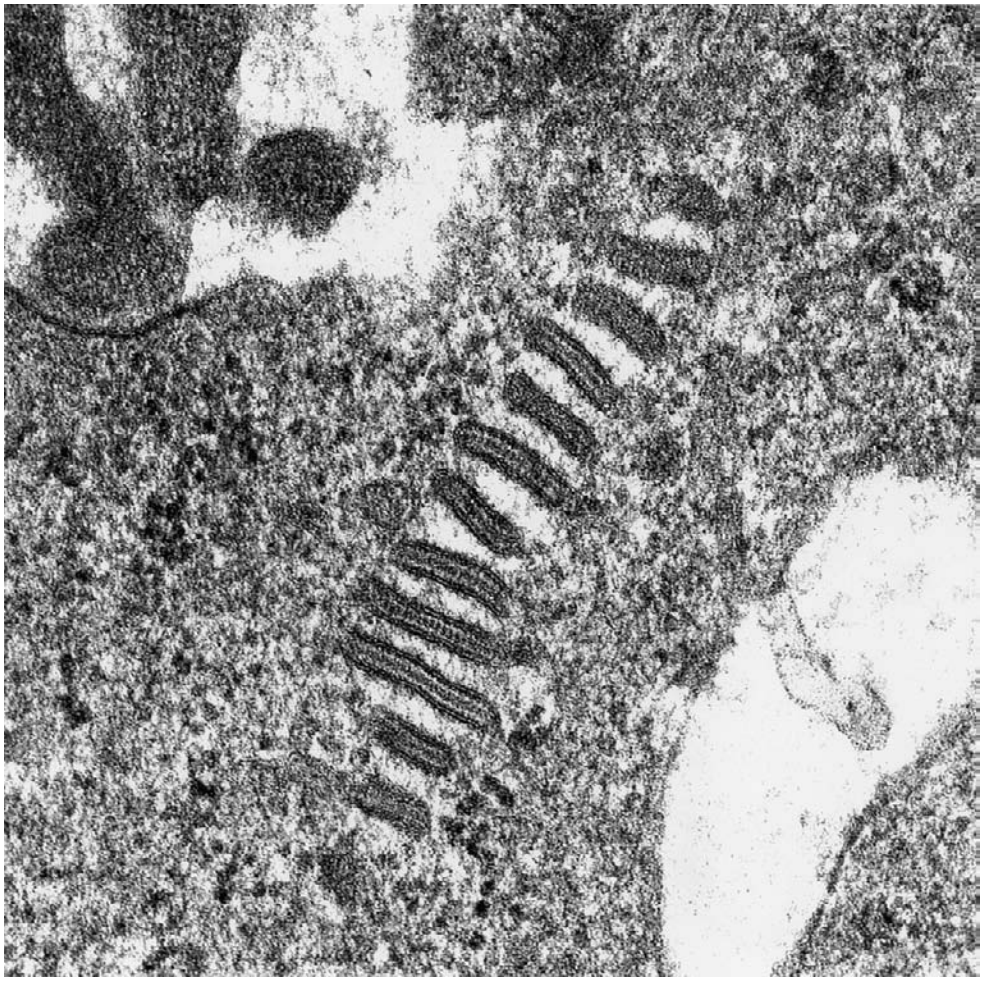

**FIGURE 19-16**

Langerhans cell histiocytosis (LCH): Birbeck granules. Electron microscopic examination of an LCH lesion reveals an unusual palisade of Birbeck granules with the laminar membranes and the central zipper (original magnification, ×135,000).

bland, oval histiocytes that are nonphagocytic and have pale cytoplasm and folded nuclei. Immunocytology for CD1a or Langerin is definitive (Figure 19-17). S100 is soluble and does not survive without fixation. Pulmonary involvement can be inferred when the imaging is compatible and more than 12% of the larger bronchoalveolar cells are $CD1a^+$ or display Langerin.

## Differential Diagnosis

The differential diagnosis depends on the site of involvement. Skin lesions of mastocytosis (urticaria pigmentosa), juvenile xanthogranuloma, and nevi can simulate LCH, the last also being $S100^+$. An increase in perivascular $CD1a^+$ Langerhans cells that are spindled or dendritic in shape is a feature of many chronic dermatoses, most notably chronic scabies.

Bone lesions are most likely to be confused with osteomyelitis, especially chronic recurrent multifocal culture-negative disease. LCH has few plasma cells in between the lesional LCH cells, but may have more in the surrounding inflammatory reaction. Hodgkin lymphoma can contain eosinophils and scattered $CD1a^+$ cells, and Rosai-Dorfman disease can also produce bone lesions. In late and healing lesions where LCH cells are few, fibrotic or fibrohistiocytic lesions enter the differential diagnosis.

Lung lesions that are fibrotic and that have few LCH cells resemble fibrosing interstitial diseases with honeycombing in the most severe instances. Alveolar macrophages can simulate LCH cells but are not $CD1a^+$. Spontaneous pneumothorax can be associated with an eosinophilic pleural reaction.

Bone marrow involvement by LCH is obscured by the common finding of a marrow macrophage histiocytosis. Only $CD1a^+$/$Langerin^+$ large cells can be considered informative because they are rare in the normal marrow, but S100 can be confounding by staining stromal cells, fat cells, some T cells, and activated macrophages.

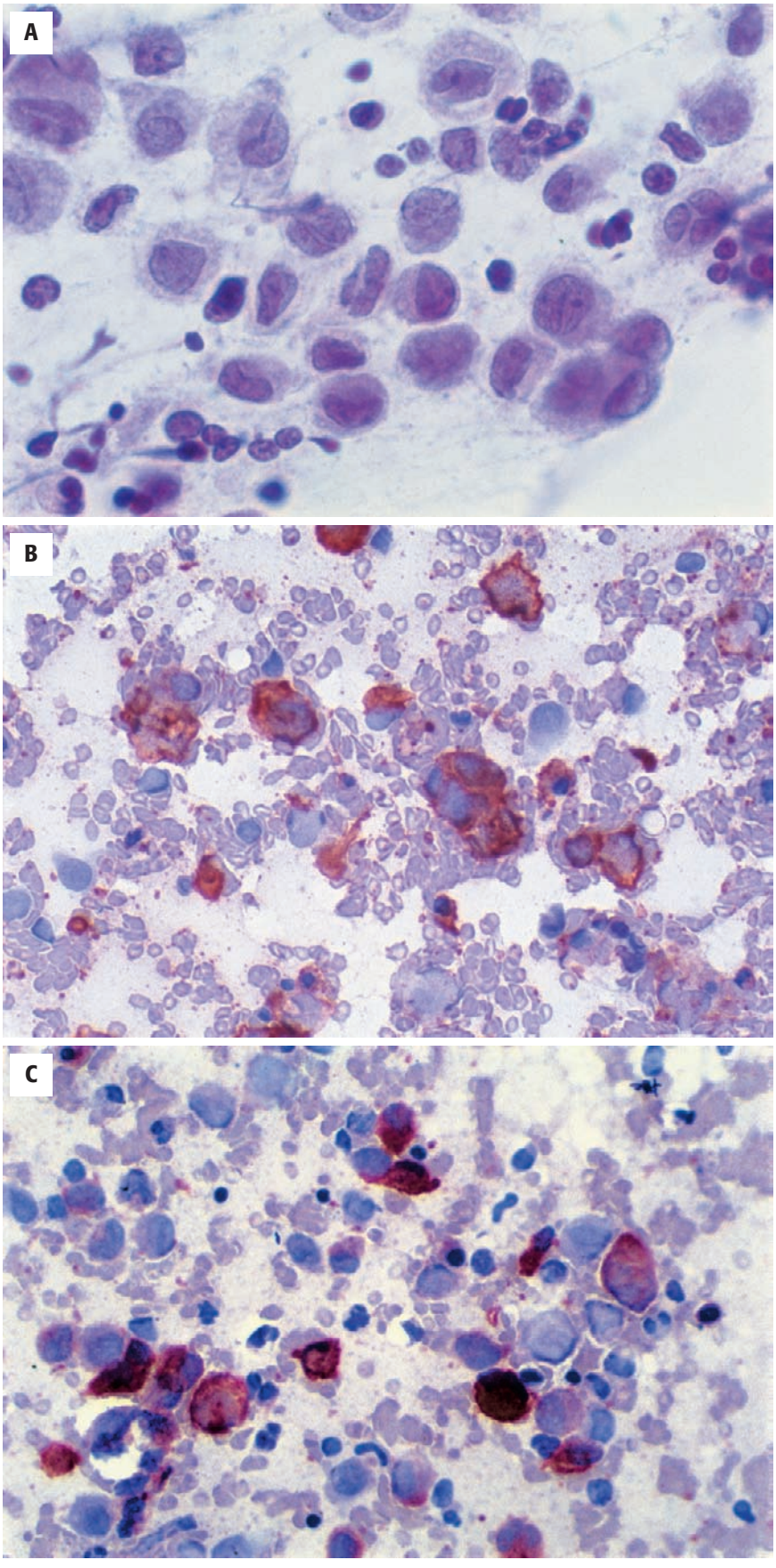


**FIGURE 19-17**

Langerhans cell histiocytosis: aspirate. Aspiration of a temporal soft-tissue mass has a mixed cell population. **A,** The angular nuclear profiles are shown and with the complex grooving pattern. Immunostains for CD1a **(B)** and Langerin **(C)** confirm the diagnosis.

Liver involvement is simulated by macrophage activation in children with widespread disease who may have hepatomegaly and hypoalbuminemia. An identifiable peribiliary infiltrate, $S100^+/CD1a^+/Langerin^+$ is diagnostic, but biliary obstructive changes on a biopsy specimen are presumptive evidence of LCH involvement downstream. Juvenile xanthogranuloma lesions also have a portal distribution but have a different phenotype and spare the bile ducts.

Pituitary lesions must be distinguished from germ cell tumors. The late demyelinating CNS lesions do not have a LCH cell presence. Choroid plexus and dural lesions of LCH acquire large numbers of xanthoma cells during the regressing and healing phases and simulate reactive histiocytosis or even juvenile xanthogranuloma.

### Prognosis and Therapy

The prognosis depends on the site and extent of disease, with the high-risk sites having a poorer outcome. Diabetes insipidus is often permanent. The greater the number of organ systems involved in small children, the worse the outcome with highest mortality, with 30% to 50% reserved for those with high-risk organ involvement (e.g., marrow, liver, lungs, spleen) and high risk of long-term CNS effects if there is cranial bone involvement. Poor initial response to chemotherapy in the systemic form also portends a poor outcome. Adults with smoking-related lung disease have variable outcomes; some regress on cessation and with steroids, but others require chemotherapy or progress. Mortality is 25%. Monoostotic bone lesions often regress no matter what modality is used, but can leave impairment at the site (e.g., vertebral collapse). Indomethacin or intralesional steroids are used with polyostotic bone lesions, low-dose radiation or chemotherapy with vinblastine–prednisone is used. In multisystem disease, the LCH-III protocol used corticosteroids and vinblastine, and etoposide was added for nonresponders. Immune suppression with cyclosporine, cladribine, or bone marrow–cytosine-arabinoside hematopoietic stem cell transplantation, as well as lung or liver transplantation in some instances, has been used in refractory disease.

Anecdotal reports describe treatment of low-risk cases with cyclosporin-A, thalidomide, interferon α, or etanercept. Experimental testing using anti-CD1a for diagnostic immunolocalization and treatment has shown promising results. Transition from bona fide LCH to later Langerhans cell sarcoma (dendritic sarcoma, Langerhans' phenotype) is exceptionally rare, and there is reason to believe that the LCH in these instances was cytogenetically different to begin with (see Langerhans Cell Sarcoma).

## Non–Langerhans Cell Histiocytosis

## Juvenile Xanthogranuloma Family

### Clinical Features

The juvenile xanthogranuloma family of lesions has many parallels to LCH. There are small solitary lesions, mostly cutaneous, but also larger and deep lesions that, like the skin lesions, are amenable to slow regression. A small subpopulation of young children, mostly younger than 1 year, have widespread systemic JXG with involvement of the skin, subcutaneous tissues, liver, spleen, lung, bone, meninges, and eye (iris) and less commonly the brain. Rare overlap with LCH occurs in the same patient, concurrent or asynchronous, but JXG does not involve the lymph nodes.

Localized variants in children include deep JXG that can involve muscle or retroperitoneum and benign cephalic histiocytosis in which clusters of lesions occur on the head, face, or scalp. Xanthoma disseminatum, which is more common in young adults, involves a myriad of lesions that involve skin and mucosa with special predilection for the upper aerodigestive tract. Like LCH, pituitary-hypothalamic involvement with diabetes insipidus is described. There is a higher incidence of JXG in patients with neurofibromatosis 1.

### Radiologic Features

Most forms of juvenile xanthogranuloma family have no unique imaging features beyond documenting a space-occupying lesion at a particular site.

### Pathologic Features

#### Gross Findings

There are no gross characteristics other than the appearance of the skin lesions. The nodules (1 to 10 mm in diameter) are described as being yellow to red-brown with overlying telangiectasia, solitary or multiple. Xanthoma disseminatum has myriads of skin and mucosal lesions that are raised and pink in early stages.

#### Microscopic Findings

All forms of the juvenile xanthogranuloma family (much like LCH) are characterized by the presence of

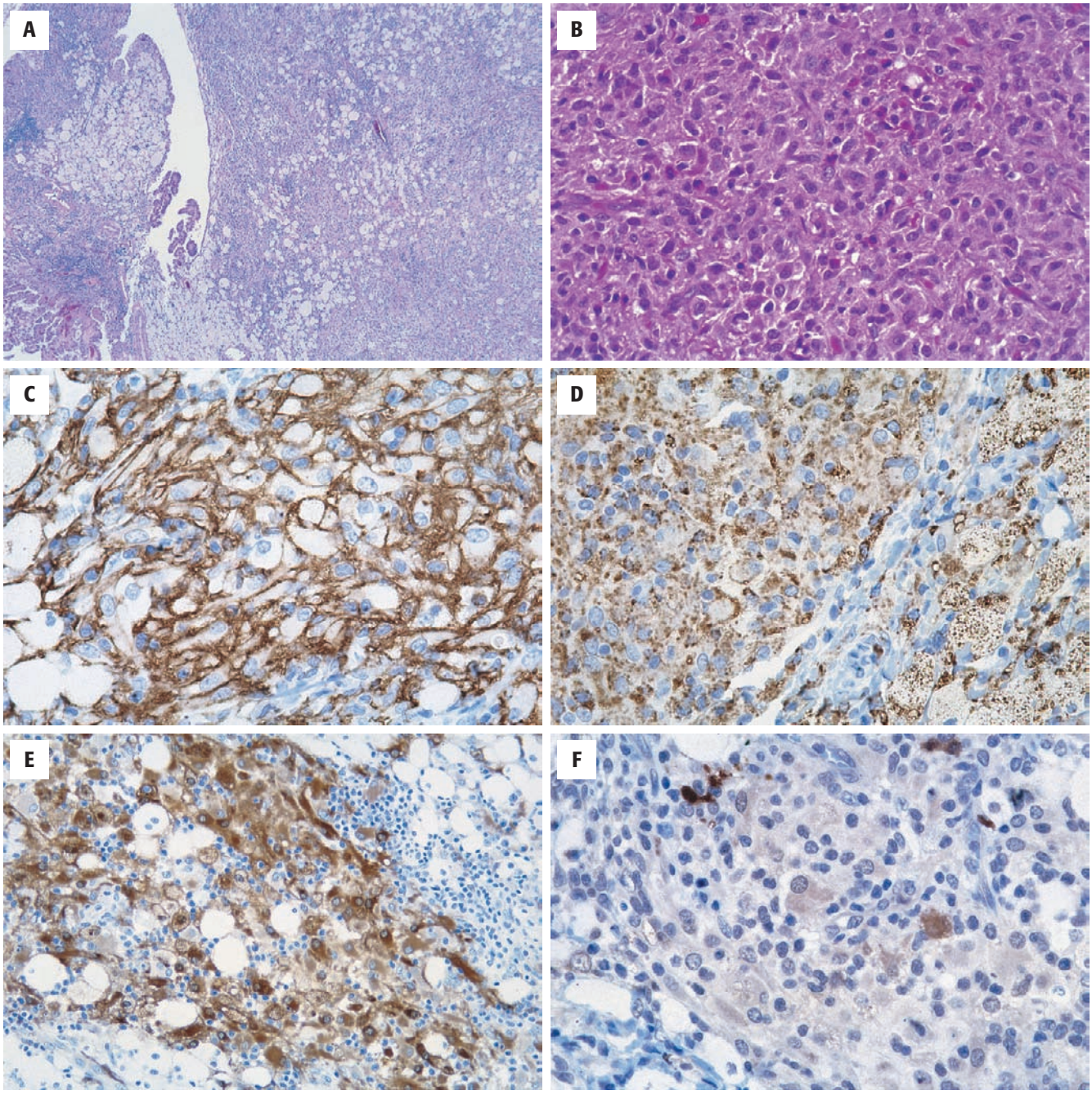


**FIGURE 19-18**

Juvenile xanthogranuloma family. Lesions occur most commonly on the skin, but mucosal sites, especially the upper aerodigestive tract, are vulnerable. **A,** Laryngeal lesion. **B,** The cells have a bland cytoplasm-rich appearance similar to Langerhans cell disease. **C,** The cells have macrophage markers CD14 and CD68 **(D). E,** Cytoplasmic Factor XIIIa is present, but S100 is generally low to absent **(F).**

a dominant histiocyte with classical appearance and phenotype. Early lesions have small histiocytes that have folded bland nuclei and a moderate amount of faintly vacuolar cytoplasm (Figure 19-18). Touton giant cells with a wreath of nuclei around a central eosinophilic core and a xanthomatous periphery are seen in approximately 85% but not required for diagnosis. As the lesions age, the presence of xanthomatous cells becomes more prominent, and spindle cells also increase in number. Lymphocytes (T cells, CD3, CD43) are interspersed, and there may be eosinophils that are occasionally prominent.

## Ancillary Studies

### Molecular and Cytogenetic Features

No consistent abnormalities have been found, but some lesions have demonstrated clonality.

#### Ultrastructural Features

The cells are bland histiocytes that lack the Birbeck granule and usually have intracytoplasmic lipid droplets. A number of nonspecific intracytoplasmic findings such as dense bodies, wormlike bodies, and popcorn bodies are described.

#### Immunohistochemistry

The phenotype of the histiocyte at the core of the entire range of lesions is highly characteristic. The lesions have the macrophage markers CD14, strong membrane staining, and CD68 (KP-1, PGM-1), coarsely granular cytoplasmic staining, nonphagocytic, and membrane CD163. The cells stain variably for factor XIIIa and fascin, often most strongly at the perimeter of lesions. CD1a and Langerin are absent, and S100 is either lacking or present in low-variable amounts in less than 30% of cases (see Figure 19-18). Vimentin is also documented, but LCA is absent. An $ALK^+$ variant has been described.

#### Fine-Needle Aspiration Biopsy

The lesions are represented by bland histiocytes rich in lipid droplets, and only the phenotype as described previously is definitive when combined with the relevant clinical information.

### Differential Diagnosis

The major differential diagnosis is from Langerhans' cell histiocytosis. CD1a, Langerin, and S100 should discriminate between the two. Eruptive xanthomas, associated with hyperlipidemias, require documentation of the serum lipids. Older lesions that contain more spindle cells, especially the progressive nodular histiocytosis variant, simulate the fibrous histiocytomas, but the JXG-family phenotype is distinctive. The dermal fibrous histiocytomas may have large numbers of factor XIIIa cells interspersed. Rosai-Dorfman cells are much larger with paler cytoplasm and $S100^+$; reticulohistiocytoma cells are larger, more eosinophilic, and glassy. Nevus cells can confound, but the S100 stain will usually discriminate by being uniformly strong.

### Prognosis and Therapy

The prognosis and therapy vary for the different clinical types at their characteristic ages. Solitary lesions in the young usually regress slowly. Systemic JXG and xanthoma disseminatum may involute, but can involve vulnerable sites such as the brain or larynx and may need low-dose chemotherapy. Rarely, death in the systemic form has been associated with fulminant hepatic failure (possibly cytokine mediated) or brain involvement.

## ADULT XANTHOGRANULOMA FAMILY

### Clinical Features

Like the juvenile xanthogranuloma family, adult xanthogranuloma can vary from single lesions to multiple lesions, xanthoma disseminatum, and systemic-form Erdheim-Chester disease. Localized lesions are common around the orbit, where a number of clinical variants are recognized: adult onset xanthogranuloma, necrobiotic xanthogranuloma, adult-onset asthma, periocular xanthogranuloma, and the ocular involvement of systemic Erdheim-Chester disease. Erdheim-Chester disease has a predilection for the hypothalamic-pituitary axis with the development of diabetes insipidus, much like Langerhans' cell histiocytosis, and in a number of instances it has been associated with LCH at various sites. Erdheim-Chester disease is characterized by symmetrical bony sclerosis and systemic involvement mostly of the lung, retroperitoneum, kidney, and heart. The biology is aggressive with 50% mortality.

### Radiologic Features

Solitary and localized forms have no distinctive imaging features, but Erdheim-Chester disease is in part a radiologic diagnosis characterized by polyostotic sclerosis. This disease involves the bones around the knee, less frequently the elbow and the diaphyses, and metaphyses have a coarsely increased trabecular pattern of medullary sclerosis and cortical thickening on radiography (Paget's disease–like pattern). Computed tomography demonstrates increased density, and MRI highlights low-signal marrow replacement on fat-suppressed T1-weighted images, mixed signals on T2, and some enhancement after gadolinium contrast. FDG-PET has been used for identification of new lesions and monitoring response to therapy. Bone scintigraphy is said to show pathognomonic bilateral and symmetric increased uptake affecting both diaphysis and metaphysis of the femur and tibias.

### Pathologic Features

#### Gross Findings

There are no gross characteristics other than the appearance of the skin lesions as described for the juvenile xanthogranuloma family.

#### Microscopy Findings

The histopathology and phenotype are identical to those of juvenile xanthogranuloma. The dominant cell is oval in shape or slightly spindled and has an oval nucleus without the complexity of the LCH nucleus. The phenotype is that of a macrophage, $CD14^+CD68^+CD163^+F13a^+$ and $fascin^+$ with little or no S100, CD1a, or Langerin. In the orbit, the adult xanthogranuloma and asthma-associated xanthogranulomas are identical. The necrobiotic xanthogranulomas have areas of central tissue necrosis, and the phenotype has proved to be more variable that may reflect evolution over time. Erdheim-Chester disease shows the classical juvenile xanthogranuloma phenotype.

### Differential Diagnosis

Distinction from hyperlipidemic eruptive xanthomas is required. Erdheim-Chester disease, by virtue of its wide anatomical distribution, will have different regional patterns for example in bone and retroperitoneum. In contrast to the lgG4-sclerosing lesions, lgG4 content is not increased in the lesions. The combination of histopathologic features and phenotype must be taken in concert with the clinical and imaging findings for definitive diagnosis.

### Prognosis and Therapy

Xanthoma disseminatum and progressive nodular histiocytosis can be locally aggressive and destructive with some capacity to involute. The Erdheim-Chester systemic variant has remissions and progression with greater than 50% mortality and considerable morbidity. Chemotherapy, radiation, and immunotherapy using interferon α have been used with some success.

## OTHER NON–LANGERHANS CELL HISTIOCYTOSES

On rare occasion, a solitary or systemic histiocytosis cannot be characterized as Langerhans' cell or juvenile xanthogranuloma in type, because they fail to fulfill the phenotypic requirements. The terms *indeterminate cell lesion* or *histiocytosis* have been used in this situation, where the indeterminate cell is defined as a Langerhans-like DC that is $CD1a^+$ but lacks a Birbeck granule on ultrastructural examination. That definition is unsatisfying for a number of reasons. Langerhans cell histiocytosis can have sparse Birbeck granules, and current practice does not require electron microscopy for complete diagnosis. Recently it has been shown that absence of Langerin expression on these lesions is a surrogate for the lack of Birbeck granules. Immunohistochemistry shows proliferating cells that are usually positive for CD14, CD1a, CD68, and factor XIIIa. The patients are more commonly adults who have solitary or multiple skin lesions, but a more disseminated and visceral presentation in children and adults is described. The histopathology is that of a bland histiocytic lesion similar to Langerhans cell disease, although nuclear grooving and complexity may be less, and eosinophils are generally sparse. A malignant sarcomatous counterpart, indeterminate cell sarcoma, is described in addition to an association with myeloid leukemia.

There are rare Langerhans cell–like lesions—solitary, multiple, and systemic—that closely resemble LCH but lack CD1a expression. These DC histiocytomas and histiocytoses have been seen in children and young adults and appear to be composed of Langerhans-type cells that are more mature than LCH cells and have lost CD1a expression, whereas HLA-DR expression on the surface is upregulated and S100/fascin expression is high and more like that of the interdigitating DCs, although they are not spindled. In support of the relationship to LCH, one case occurred at the site of a previous classical LCH. A collection of some of these cases has shown that the clinical spectrum is similar to LCH, but that the CNS and its coverings are more likely to be involved and local recurrence is more common than is seen with LCH. LCH-type treatment has been effective. A sarcomatous counterpart exists: DC sarcoma, not otherwise specified.

## COMBINED LANGERHANS AND NON-LANGERHANS' HISTIOCYTOSIS

### LANGERHANS CELL HISTIOCYTOSIS AND JUVENILE XANTHOGRANULOMA

Rare instances are described in which the two patterns are discernible in the same lesion, but more commonly clinical LCH is followed by lesions that are clinically and morphologically JXG in type. This asynchronous event needs to be distinguished from the xanthomatous (JXG-like) histiocytic reaction that accompanies regressing or treated LCH most conspicuously in the meninges or orbit. There are a number of examples of Erdheim-Chester disease that have had focal LCH lesions as well.

### LANGERHANS CELL HISTIOCYTOSIS AND ROSAI-DORFMAN DISEASE

Coexistence of LCH and Rosai-Dorfman disease can occur at different sites synchronously or asynchro-

nously and even at the same site as distinctly identifiable areas of growth.

## HISTIOCYTOSIS FOLLOWING ACUTE LYMPHOBLASTIC LEUKEMIA OR FOLLICULAR LYMPHOMAS

A variety of histiocytic lesions—focal, systemic, and malignant—has been described following prior acute lymphoblastic leukemias or follicular lymphomas. The histiocytic lesions may share the cytogenetic or molecular signature of the prior leukemia or lymphoma. Although the histiocytic lesions may fit the morphologic categories of Langerhans cell disease, juvenile or adult xanthogranuloma, and Rosai-Dorfman disease, their biologic behavior is commonly more aggressive. Instances of histiocytic sarcoma are high grade.

## HISTIOCYTIC MALIGNANCIES

### HISTIOCYTIC SARCOMA

Histiocytic sarcoma is a large-cell hematopoietic malignancy that develops as an epithelioid lymphoma in nodal or extranodal sites, and the diagnosis is confirmed by demonstrating an immunophenotype of macrophages, DCs, or both. Some have occurred after acute lymphoblastic leukemias, lymphomas, or mediastinal germ cell tumors.

#### HISTIOCYTIC SARCOMAS—FACT SHEET

**Definition**

- Histiocytic sarcomas are a collection of malignancies that are cytologically malignant and are categorized by their phenotypic affiliation to the various histiocytes, macrophages, and dendritic cells

**Incidence and Location**

- Incidence is rare, with mostly single case reports
- Some follow a prior leukemia and may contain the same molecular signal
- Some are lymphoma-like and nodal; others are soft-tissue lesions

**Gender, Race, and Age Distribution**

- Mostly adult though childhood examples are described

**Clinical Features**

- Symptoms vary according to site of presentation
- Nodal disease and systemic involvement in some is accompanied by B-type systemic effects

**Radiologic Features**

- No specific findings other than nodal or extranodal space-occupying lesions

**Prognosis and Treatment**

- Localized lesions in general may have low-grade behavior after resection
- Recurrences, multiple lesions, and systemic disease are relatively refractory to current chemotherapy, with high mortality

#### Clinical Features

Adults are involved; mean ages are 44 to 55 years, and males and females are affected equally. Presentation is commonly as a mass, with the involved sites being lymph node, soft tissue, or gastrointestinal tract, and obstructive features may dominate gastrointestinal presentations. Rarely, patients who have a more systemic form have been diagnosed with malignant histiocytosis, which is a historically fraught term.

#### Pathologic Features

##### Microscopic Findings

The microscopic appearance is dominated by large (greater than 20 μm) epithelioid cells that have abundant eosinophilic cytoplasm. The abundance of the cytoplasm is a clue to the diagnosis, because phagocytic activity is hardly ever seen, although cytoplasmic vacuolation may be present. Nuclei are oval and variable in size with a large amphophilic nucleolus (Figure 19-19). Binucleation is common, and anaplastic pleomorphism with giant cells is seen focally in some. The proliferating cells may be diffuse or sinusoidal in their distribution in the lymph nodes, liver, or spleen. Lymphocytes, less commonly eosinophils, may be admixed.

#### Ancillary Studies

##### Molecular and Cytogenetic Features

The cases that have occurred following a prior leukemia generally have the same clonal markers as the prior leukemia.

##### Electron Microscopy

No cell processes or tight junctions are present, but there is a prominent Golgi and secondary lysosomes. Birbeck granules are absent.

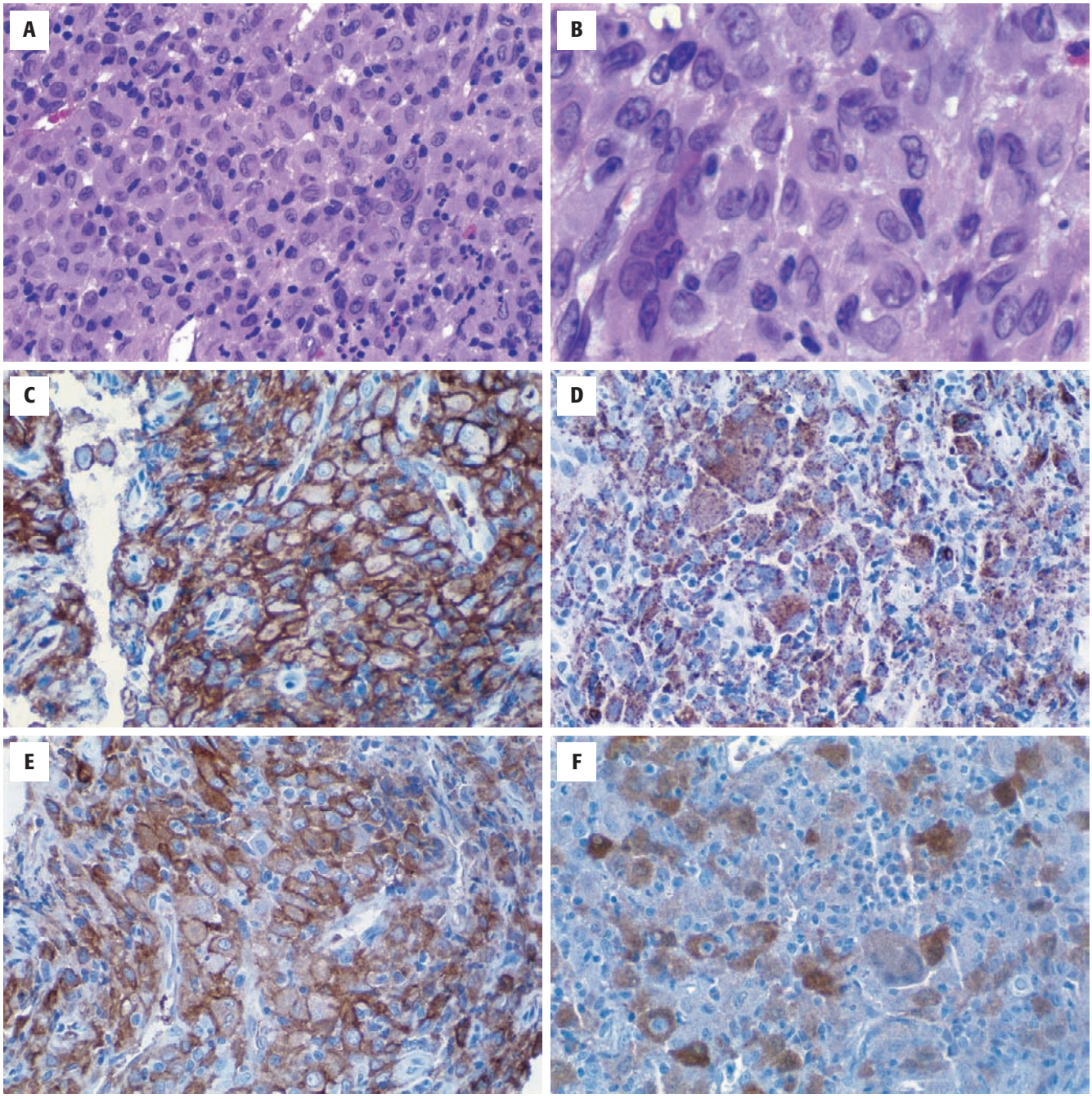


**FIGURE 19-19**

Histiocytic sarcoma. **A** and **B,** The cell type can vary from epithelioid to cells with more complex nuclei. The phenotype is macrophage-like with CD14 **(C),** CD68 **(D),** and CD163 **(E). F,** S100 expression is variable but low. This lesion followed a B-cell acute lymphoblastic leukemia by 10 years.

#### Immunohistochemistry

The immunophenotypic pattern is variable: the cells generally stain for LCA and CD45RO. Some have a more macrophage-like pattern with $CD14^+CD68^+CD163^+$ with variable lysozyme expression. Typical cases are CD1a/Langerin negative. Others are more dendritic with S100, fascin, or even Langerin in a few. B- and T-cell specific markers are absent, and CD30 is not expressed.

## Differential Diagnosis

The differential is wide—generally that of other large cell lymphomas with epithelioid features, T cell, B cell, or even granulocytic sarcomas, Hodgkin lymphoma, metastatic carcinomas, epithelioid sarcoma, and melanoma. Monocytic and monoblastic leukemias must be excluded by bone marrow examination.

**TABLE 19-4**
**Immunohistochemical Features**

| | LCA | CD14 | CD68 | CD163 | Lysoz | S100 | CD1a | Langerin | Fascin | CD21 | CD35 | Clusterin | FXIIIa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HS | + | ++ | ++ | ++ | ++ | +/– | – | – | – | – | – | N/D | – |
| LCS | – | – | +/– | – | – | + | ++ | + | – | – | – | – | – |
| IDC | – | – | +/– | – | – | ++ | – | – | ++ | – | – | +/– | – |
| FCS | – | – | +/– | – | – | – | – | – | +/– | ++ | ++ | ++ | – |
| JXGS | – | ++ | ++ | ++ | – | – | – | – | + | – | – | – | + |

*FCS,* Follicular cell tumors; *HS,* histiocytic sarcoma; *IDC,* interdigitating cell sarcoma; *JXGS,* histiocytic sarcoma, juvenile xanthogranuloma phenotype; *LCS,* Langerhans cell sarcoma.

**HISTIOCYTIC AND DENDRITIC CELL SARCOMAS—PATHOLOGIC FEATURES**

**Gross Findings**
- Nodal or soft-tissue lesions are firm
- The follicular dendritic cell lesions are exceptionally circumscribed with pushing borders

**Microscopic Findings**
- A variety of appearances, generally stubby oval to spindled cells, some epithelioid
- Histiocytic sarcoma, predominantly epithelioid
- Interspersed lymphocytes, T cells predominant in interdigitating cell lesions, B cells in follicular dendritic cell lesions

**Ultrastructural Features**
- Interdigitating and follicular DC lesions have processes, more profound interdigitation in the former
- True desmosomes in follicular DC tumors
- No Birbeck granules

**Fine-Needle Aspiration Biopsy Findings**
- Epithelioid or spindle cell lesions that have interspersed lymphocytes
- Lesional cells have characteristic phenotype

**Immunohistochemical Features**
- See Table 19-4

**Differential Diagnosis**
- The histiocytic dendritic cell lesions must be distinguished from each other (see Table 19-4)
- Histiocytic sarcoma has the differential diagnosis of epithelioid cell tumors and large cell lymphomas, Hodgkin lymphoma, metastatic carcinoma, epithelioid sarcoma, and melanoma. Extramyeloid granulocytic processes, especially monocytic leukemias, may have similar features
- The spindle cell dendritic processes LCS, interdigitating dendritic cell sarcoma (IDS), and follicular dendritic cell sarcoma (FCS) must be distinguished from other spindle cell lesions, myoid, gastrointestinal stromal tumors, myofibroblastic, and angiomatoid fibrous histiocytoma

### Prognosis and Therapy

For patients with localized disease, the outcome following removal and therapy may be good. The majority of patients have high-stage disease at presentation, and the biologic behavior is that of a high-grade sarcoma with aggressive clinical course, poor response to therapy, and high mortality (60% to 80%) from progressive disease.

## LANGERHANS CELL SARCOMA

Langerhans cell sarcoma is a high-grade malignant proliferation of dendritic histiocytes that have an immature Langerhans cell phenotype. Although one case evolved from a prior Langerhans cell histiocytosis, almost all arise de novo in adults. The tumor develops as soft-tissue masses, nodal tumors, or as multiorgan involvement with spleen, liver, lung, and bone marrow disease. Most cases are multifocal high-stage disease, fewer are primarily nodal. The mean age is 46 years (range, 8 to 81 years) with an equal sex ratio.

### Pathologic Features

When lymph nodes are involved, the pattern is that of sinus spread but may be obscured. The constituent cells

are large and oval to spindled, with malignant to anaplastic cytologic features, variation in nuclear size and shape, and atypical mitoses. The nuclei may be grooved or folded, like the typical complex foldings of the LCH cells. Mitoses including atypical forms are common. The cytoplasm is generous and eosinophilic. Interspersed eosinophils are sparse. Immunohistochemistry reveals the same phenotype as the LCH cell (i.e., $S100^{+}CD1a^{+}Langerin^{+}$), although expression may be more variable (Figure 19-20). Ultrastructural confirmation of a Birbeck granule is usual. CD68 is present in small amounts in a paranuclear distribution. Lysozyme is not present, and follicular DC markers, CD21, and CD35 are absent. CD56 expression is said to identify a more refractory clinical subtype. Rarely CD30 may be expressed.

## Differential Diagnosis

Distinction from LCH is based on the cytologic pleomorphism and, in most instances, the anaplasia and atypical mitoses. The differential diagnosis includes LCH and other $CD1a^{+}$ infiltrates, such as some acute myeloid leukemias, and rarely $CD1a^{+}$ T lymphoblastic leukemias and lymphomas. Indeterminate cell histiocytosis (and sarcoma) refers to a related condition that is $CD1a^{+}$ but lacks the Birbeck granule or Langerin expression. Because Langerhans cell sarcoma (LCS) is variable in its phenotypic expression, there may not be a clear dividing line between indeterminate cell histiocytosis (and sarcoma) and LCS. The distinctive phenotype distinguishes LCS from other spindle cell sarcomas, most notably follicular DC and interdigitating DC tumors.

## Prognosis and Therapy

LCS is aggressive, with greater than 50% mortality from progressive disease.

# INTERDIGITATING DENDRITIC CELL SARCOMA

Interdigitating cell sarcoma is a malignant proliferation of cells that have a mature interdigitating DC phenotype.

## Clinical Features

The tumors develop as masses, most commonly involving lymph nodes, but extranodal sites such as the nasopharynx, intestine, retroperitoneum, mesentery, skin, and testis have been described. While commonly asymptomatic, B symptoms and signs including diffuse lymphadenopathy occur in patients with high-stage disease. The median age is 58 years (range, 2 to 88 years) with sex parity.

## Pathologic Features

### Gross Findings

The tumors have a lobular pattern and are firm as befitting spindle cell lesions with gross necrosis being unusual.

### Microscopic Findings

When lymph nodes are partially involved, the lesions may be seen to be paracortical, a helpful distinction from the follicular lesions. Like the other DC sarcomas, the interdigitating cell sarcoma is cytologically malignant, but not anaplastic, and has the general appearance of a spindle-cell sarcoma of medium sized cells (greater than 20 μm) that forms fascicles and whorls. Round and oval cell areas may be present and can predominate (Figure 19-21). Nuclei are oval to elongated and chromatic with finely aggregated chromatin and a distinct small nucleolus. Rarely, the nuclei can be folded and simulate Langerhans' cell nuclei. Small T cells commonly abound between the spindle cells.

## Ancillary Studies

### Ultrastructural Features

Spindled lesions have long, interdigitating cytoplasmic extensions, but the features of Langerhans cells, the Birbeck granule, and follicular DCs (true desmosomes) are not demonstrable.

### Immunohistochemistry

The immunohistologic profile is that of a mature DC, although there are gaps in their characterization. CD45 is negative (or weak), although CD45RB is expressed. The spindle cells express vimentin, high S100, high HLA class II, and high fascin (see Figure 19-21). CD30 and mature DC markers are absent. Small amounts of CD68 have been reported in some, but other macrophage markers (CD163) are absent, and neither Langerhans cell (CD1a, Langerin) nor follicular DC markers (CD21, CD35) are described. Clusterin is identified only at low levels. Proliferative activity (assessed using the

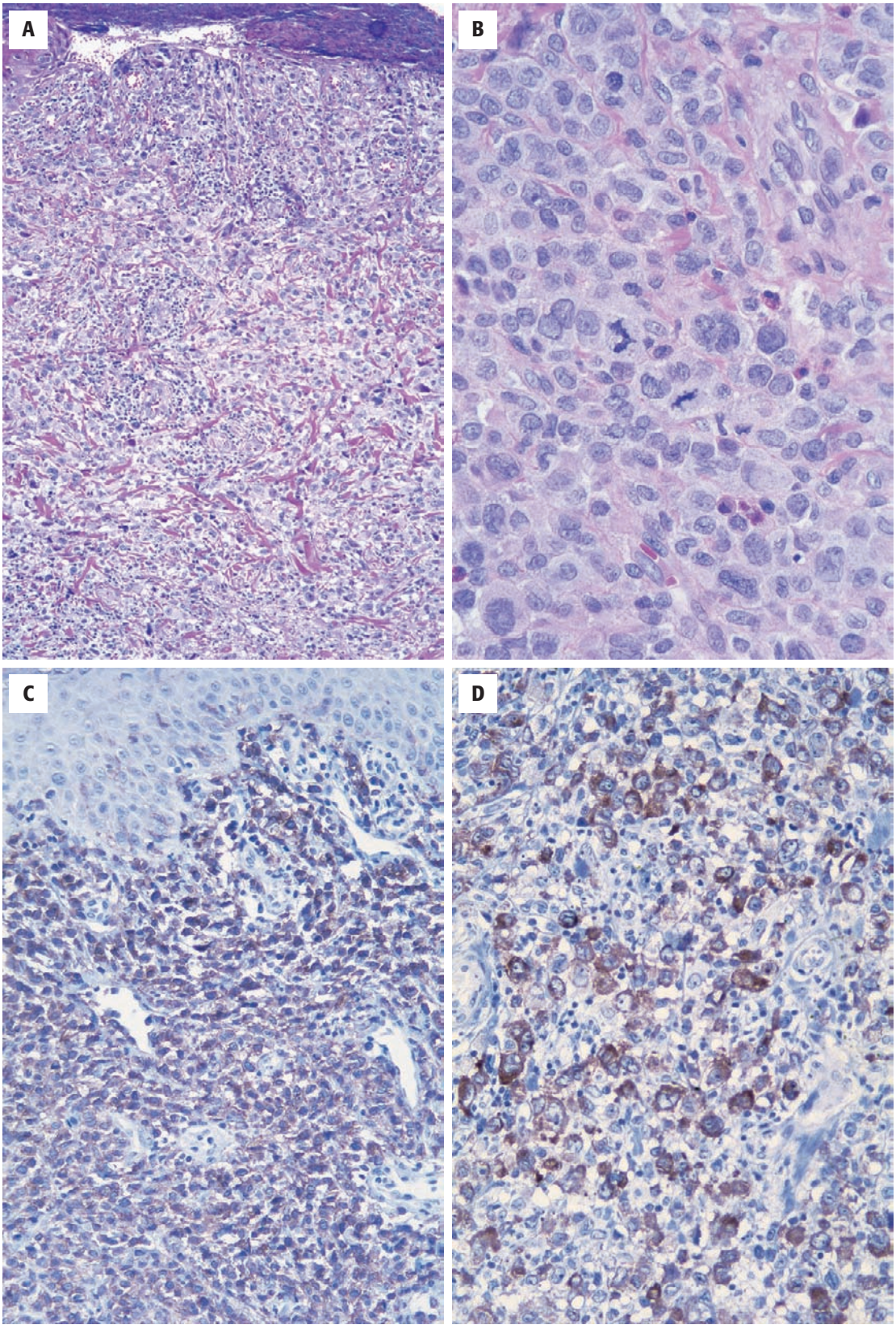


**FIGURE 19-20**

Langerhans cell sarcoma. **A,** The lesions have more nuclear pleomorphism than would be expected from LCH. **B,** Atypical mitosis is present. The Langerhans phenotype requires CD1a **(C)** and Langerin expression **(D).**

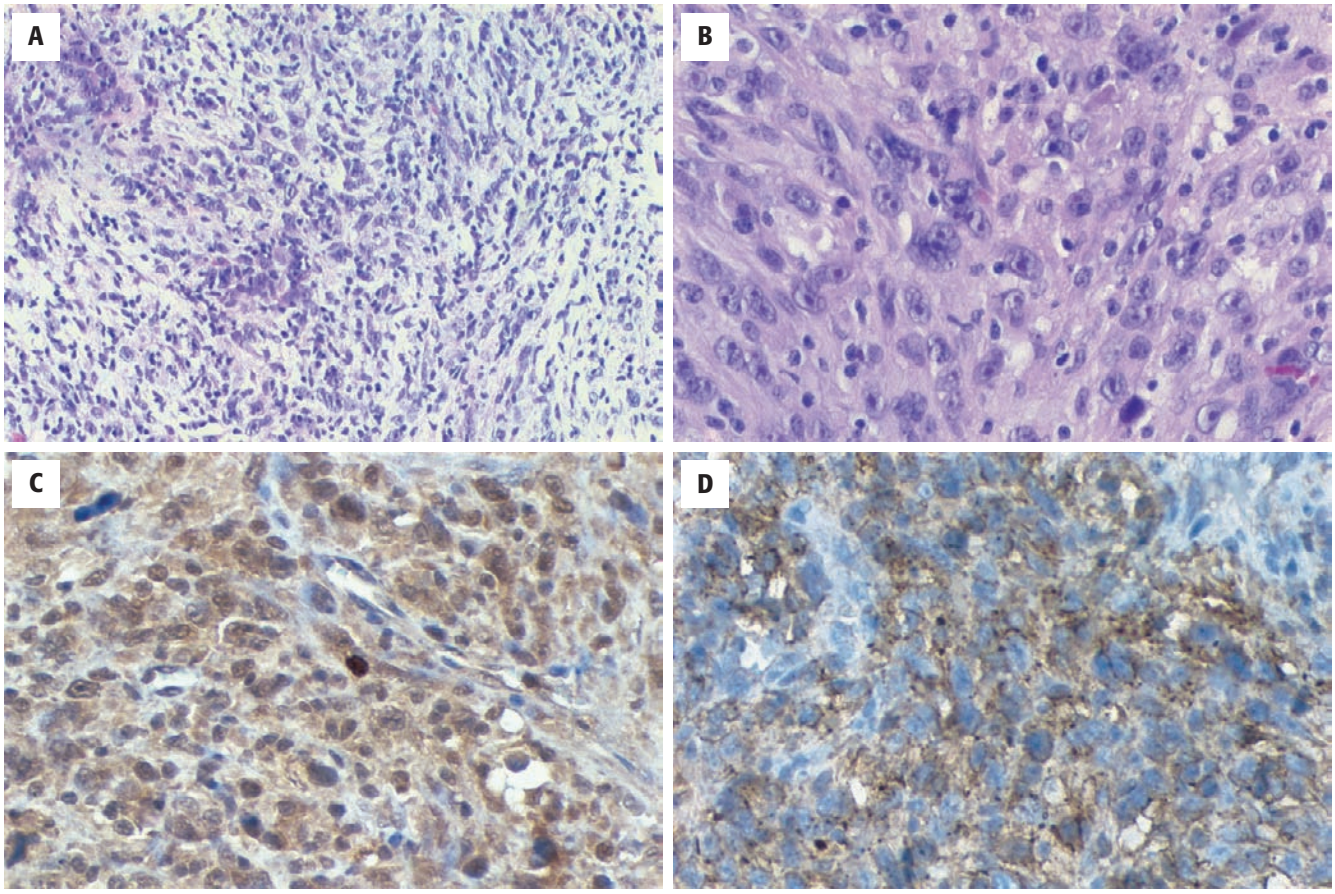


**FIGURE 19-21**

Interdigitating cell sarcoma. **A** and **B,** The lesions are generally spindled with high-grade nuclear features. The phenotype is that of mature dendritic cells with high S100 expression **(C)** and human leukocyte antigen HLA class II **(D).**

MIB-1 antibody) is generally modest (approximately 10%), and p53 expression has been mentioned as a correlate of poor outcome.

### Differential Diagnosis

The differential diagnosis is wide and covers the gamut of spindle cell sarcomas, the other DC sarcomas, and the fibrohistiocytic lesions. Muscle tumors have a spectrum of myoid markers. Inflammatory pseudotumors are recognized by their myofibroblastic qualities and occasional ALK expression. Melanomas (also $S100^{+}$) have HMB45 and tyrosinase. The nodal site, paracortical involvement, and phenotype are distinctive.

### Prognosis and Therapy

The interdigitating cell sarcoma appears to be aggressive, with remissions and local recurrences, but with a 50% mortality rate. Localized disease that is resected, however, may have a much better outcome.

## FOLLICULAR DENDRITIC CELL TUMORS AND SARCOMAS

### Clinical Features

Follicular DC tumors, including sarcoma, are spindle cell lesions that develop as a large, slow-growing mass in nodal or extranodal sites. Adults are primarily affected with no sex predilection, except for the inflammatory pseudotumor variant that has a marked female preponderance. There are associations with hyaline-vascular Castleman follicular hyperplasia and, rarely, with EBV. Most tumors arise in cervical lymph nodes, and mediastinal and retroperitoneal involvement is also noted. Extranodal sites include skin and soft tissue, tonsil, gastrointestinal tract, liver, and spleen. The lung is the most usual metastatic site; lymph nodes and liver are involved less commonly. Systemic symptoms are more common in the inflammatory pseudotumor variant.

## Pathologic Features

### Gross Findings

Tumors are solid and firm, with smaller lesions being seen in lymph nodes and larger lesions in visceral organs or soft tissue. The borders are smooth, well demarcated, and push into the surroundings.

### Microscopic Findings

In lymph nodes the growth pattern can be serpiginous, leaving a residual node between the nodules. The cells are spindle shaped, and the lesions are commonly highly cellular with a tight, whorling, meningioma-like pattern. The cell shape varies from elongated spindle cells to plump or ovoid, with moderate amounts of brightly eosinophilic cytoplasm and indistinct cell borders. Nuclei are usually single with occasional binucleated or multinucleated cells. The nuclear outline is oval, and the degree of pleomorphism can vary, although uniformly oval nuclei are the rule. The nuclear membrane is sharp, and the nuclei are chromatic with fine to coarsely granular chromatin and small distinct nucleoli (Figure 19-22). High-grade lesions have prominent nuclear size variability with frequent and atypical mitoses greater than 30 per 10 high-power fields. These lesions also have necrosis. Lymphocytes are mostly B cells interspersed, sometimes in broad sheets and sometimes aggregated around blood vessels. The inflammatory pseudotumor-like variant of follicular DC sarcomas involves the liver and spleen predominantly. It has spindle cells dispersed in a background of lymphocytes and plasma cells with foci of necrosis and hemorrhage.

## Ancillary Studies

### Ultrastructural Features

The spindle cells have cytoplasmic projections that interdigitate and display typical desmosomes. Organelles, including lysosomes, are sparse and no Birbeck granules are present.

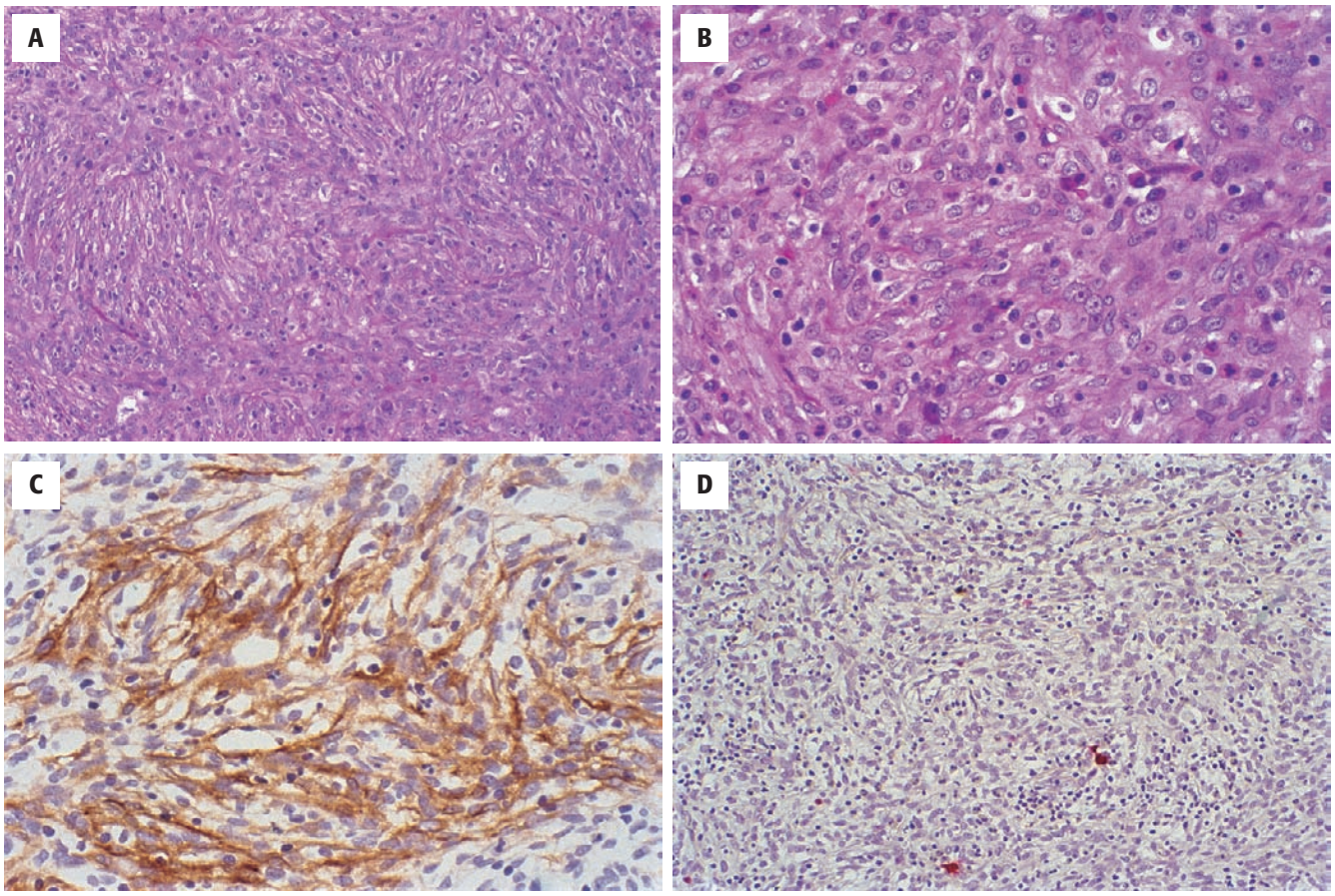


**FIGURE 19-22**

Follicular dendritic cell tumor. The whorls of short spindled cells **(A)** or more epithelioid plump cells **(B)** are both consistent with the follicular dendritic cell lesions. **C,** The follicular dendritic cell markers, such as CD21 and CD35, or the ultrastructural demonstration of desmosomes are required (not shown). **D,** S100 is generally absent. *(Courtesy of Dr. Steven Swerdlow, University of Pittsburgh Medical Center.)*

### Immunohistochemistry

The tumors have a unique and consistent phenotypic profile that includes the follicular DC markers CD21 and CD35. CD23, CD68, fascin, and clusterin are also expressed, but CD1a, S100, actin, desmin, and cytokeratin are not (see Figure 19-22). EMA has been reported to be present in a variable amount in most cases. The inflammatory variant of follicular DC tumor has nuclear EBV RNA and Southern blot studies reveal the presence of virus in monoclonal episomal form.

### Fine-Needle Aspiration Biopsy

Spindle to oval cell lesions have interspersed lymphoid cells. The phenotypic confirmation is required.

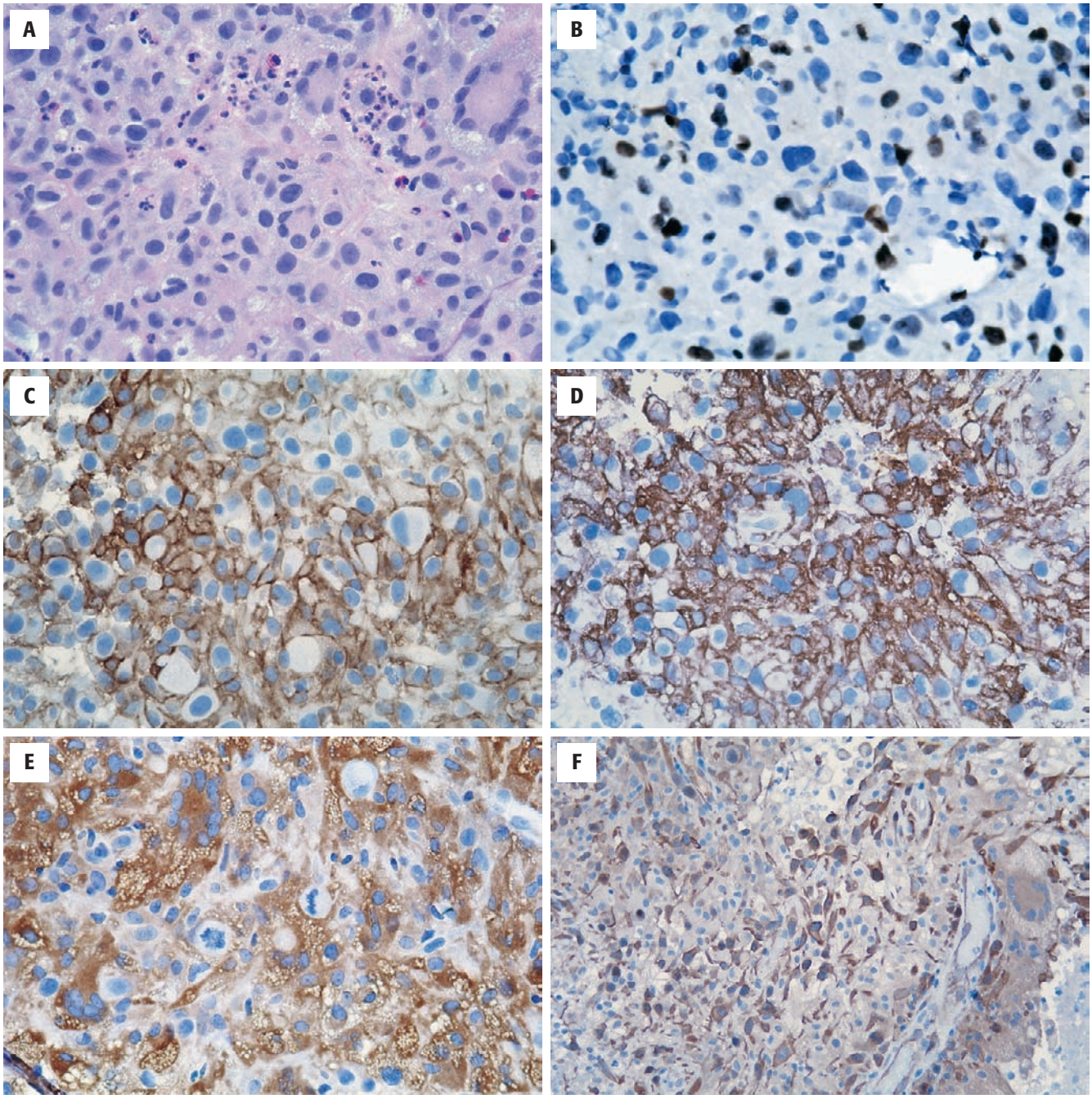


**FIGURE 19-23**

Histiocytic sarcoma, juvenile xanthogranuloma phenotype. Adult female with brain lesions. **A,** The histologic appearance is that of juvenile xanthogranuloma with a Touton cell, but there is pleomorphism and tumor necrosis. **B,** Cell cycle is vigorous, Ki-67 immunostain. The phenotype is that of the juvenile xanthogranuloma family, CD14 **(C),** CD163 **(D),** factor XIIIa **(E),** fascin **(F),** CD68 (not shown), and no S100 (not shown).

### Differential Diagnosis

The differential diagnosis includes the spindle cell lesions, most appropriate to the site of the presentation. In the lymph node, distinction from other DC lesions such as the interdigitating cell sarcoma, nodular sclerosing Hodgkin lymphoma, and fibroblastic reticular cell tumor is required. In soft tissues, liver, and spleen, the inflammatory myofibroblastic tumors, angiomatoid fibrous histiocytoma, and meningioma must be excluded. In the gastrointestinal tract, gastrointestinal stromal tumors and smooth muscle tumors are similar, as are the spindle-cell posttransplant lesions and the mycobacterial spindle cell pseudotumor.

### Prognosis and Therapy

The biologic behavior is generally indolent and low grade, with localized lesions being treated by excision, but late local recurrence is seen in 50%. Eventually, distant metastases can occur in 25% of patients, and less than 20% eventually die of their disease. Large lesions and those that are organ-based and that have cytologic anaplasia and necrosis may have a more fulminant course.

## HISTIOCYTIC SARCOMA WITH JUVENILE XANTHOGRANULOMA PHENOTYPE

A histiocytic lesion with sarcomatous cytologic features and a rapidly malignant clinical course has been seen. Generalizations about the clinical presentation are not warranted because of the rarity; however, the constituent atypical histiocytes have the JXG family appearance and phenotype of CD14, CD163, CD68, FXIIIa, and fascin, with negative S100 (Figure 19-23).

## DENDRITIC CELL SARCOMA, NOT OTHERWISE SPECIFIED

The designation *dendritic cell sarcoma, not otherwise specified,* is reserved for the rare lesions that do not fit easily into one of the defined DC categories—Langerhans cell, interdigitating cell or follicular cell—as well as the juvenile xanthogranuloma phenotype. Because there are occasional lesions with hybrid features that cross rigid and rather arbitrary boundaries, they too could occupy this category.

## FIBROBLASTIC RETICULAR CELL SARCOMA

The provenance of this lesion is undetermined but included here because of the histopathologic resemblance to the nodal DC lesions. The lesion has been described in adolescents and adults, and the key to their phenotypic differentiation from the DC lesions is their consistent lack of the Langerhans, interdigitating, and follicular phenotypes, but they strongly express cytokeratins and variably, actins, desmin, and CD68.

### SUGGESTED READING

The complete reference list is available online at www.expertconsult.com.

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20

# Mastocytosis

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## INTRODUCTION

Mast cells (MCs), initially recognized in 1878 by Paul Ehrlich, are unique inflammation mediator cells because they contain distinctive metachromatic and electron-dense cytoplasmic granules. These cells derive from bone marrow pluripotent hematopoietic progenitors and then transmigrate and subsequently mature in various organs, under the influence of a complex network of cytokines and growth factors. The central piece of this network is activation of the tyrosine kinase receptor KIT by its ligand, stem cell factor (SCF).

MC progenitors undergo differentiation and sequentially acquire distinct morphologic, cytochemical and immunophenotypic properties, and metachromatic granules, which can be categorized as four major stages: a nongranulated (tryptase-positive) blast, a metachromatically granulated blast (metachromatic blast), a promastocyte (atypical MC type II, or MCs with bilobed or multilobed nuclei), and a mature MC (typical mononuclear MC). Phenotypically, the precommitted MC progenitors express surface CD13, CD34, CD38, and CD117. Once committed, MCs downregulate CD34 and several other receptors, but maintain strong surface CD117 expression. Under normal physiologic conditions, MCs and their precursors do not express surface CD2, CD25, or CD35. MC granules contain numerous mediators, such as tumor necrosis factor α, histamine, acid hydrolases, cathepsin G, and carboxypeptidase. On activation by numerous stimuli in either an immunoglobulin E receptor–dependent or an independent manner, MCs instantaneously release these mediators and generate lipid-derived substances that induce abrupt allergic responses.

Mastocytosis, also known as *mast cell disease,* denotes a heterogeneous group of disorders characterized by abnormal growth and accumulation of morphologically and immunophenotypically aberrant MCs in skin (cutaneous mastocytosis [CM]) or in one or more extracutaneous systems (systemic mastocytosis [SM]). Childhood CM frequently regresses spontaneously, whereas virtually all adult-onset CM persists and may progress to SM. Most mastocytoses, especially SM, are clonal disorders. The aberrant MC proliferation and release of MC granular contents account for the unique clinicopathologic features of mastocytosis. Over the past several years, various proposals have been made to classify mastocytosis. This chapter focuses on the 2008 World Health Organization (WHO) classification system and other recent pertinent publications.

### Clinical Features

CM is more frequent in children than SM, and 80% of children with CM have disease manifestation by 6 months of age. Conversely, virtually all cases of SM occur in adults after age 30 years, and its occurrence peaks between the ages of 50 and 80 years. Depending on the pattern of referral, CM presenting as urticaria pigmentosa (UP) is associated with 50% to 100% of patients with SM. No significant sex predilection has been reported for either CM or SM.

Clinical manifestations of mastocytosis are organized into four major categories: constitutional symptoms (e.g., fatigue, weight loss, fever, sweats), cutaneous manifestations (e.g., pruritus, urticaria, dermatographism), mediator release syndromes (e.g., abdominal pain, diarrhea, nausea and vomiting, hypertension, headache, flushing, syncope, hypotension, tachycardia, respiratory symptoms), and organ dysfunction from tissue infiltration by neoplastic MCs.

Cutaneous manifestations are the most common symptoms in mastocytosis. Structural lesions are the most common form, represented as red-brown macules, papules, and plaques of UP/maculopapular CM (UP/MPCM; Figure 20-1). The lesions of UP/MPCM tend to cluster on the upper arms, shoulders, trunk, and thighs while sparing the palms, soles, face, and scalp. Telangiectases, petechiae, or ecchymoses may occur in the lesions or in adjacent skin. Other cutaneous manifestations, including mastocytoma and diffuse CM, are typically seen in children. Lesions of mastocytoma (solitary or numerous) are red-brown nodules or plaques and are up to several centimeters in diameter. Diffuse CM may appear as grossly normal skin or as red-brown,

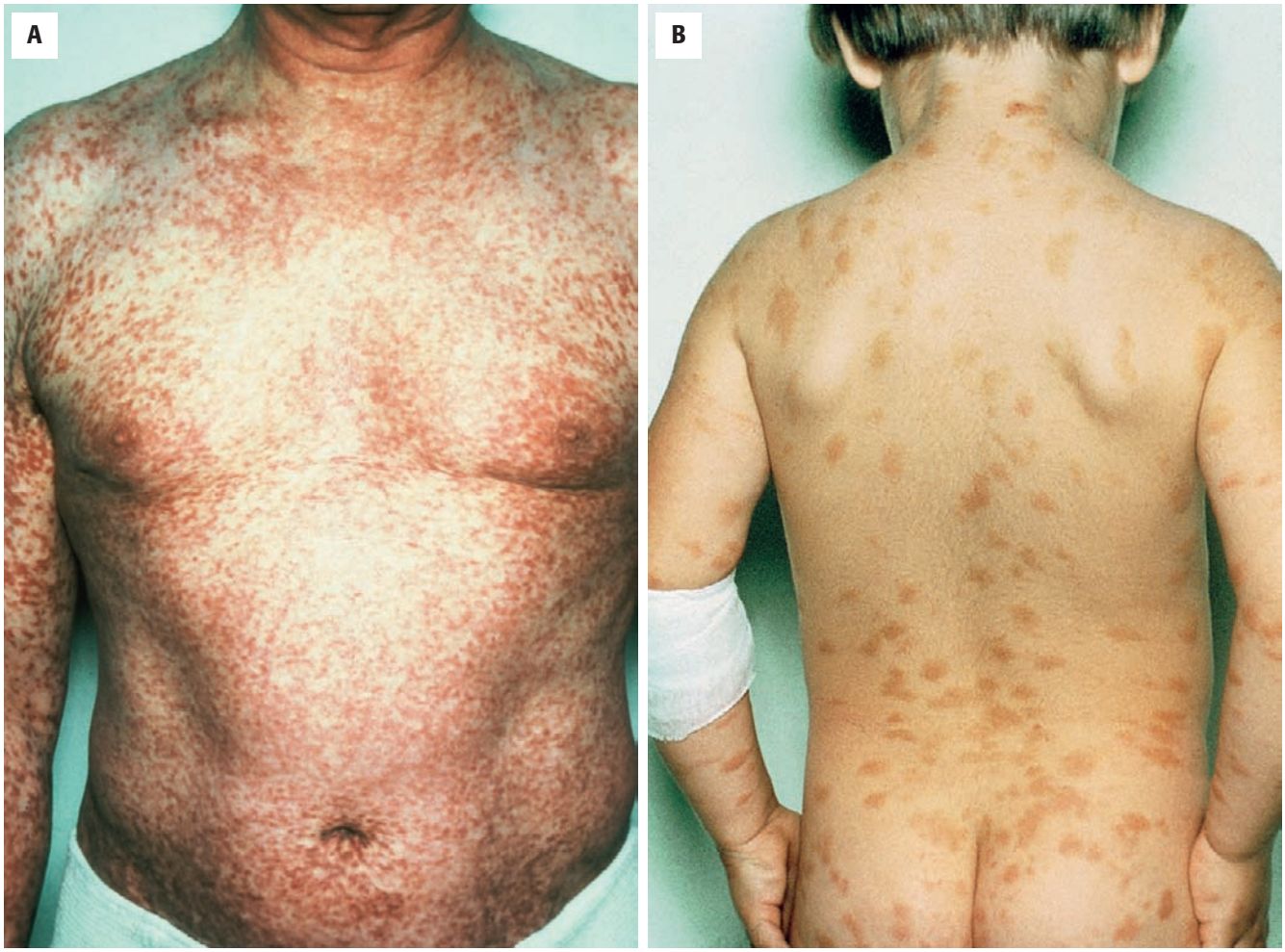



**FIGURE 20-1**

Cutaneous mastocytosis. **A,** The most common cutaneous lesions in adults with mastocytosis (i.e., urticaria pigmentosa) are red-brown macules in large numbers with highest density on the trunk. **B,** In children, the lesions tend to be papular and less in number.

thickened, edematous skin with an orange-peel texture. The cutaneous lesions of all forms may induce urticaria when stroked (Darier sign). Blistering or bullous formation, an exaggerated Darier sign, is frequently observed in children.

Mediator release symptoms (MRSs) caused by MC degranulation and the release of biologic amines (e.g., histamine), proteolytic enzymes (e.g., tryptase), proteoglycans (e.g., heparin), prostaglandin $D_2$, and leukotriene $C_4$ include recurrent syncope, hypotensive shock, severe bone pain, headache, lightheadedness, anaphylaxis, flushing, pruritus, urticaria, and gastrointestinal (GI) symptoms. Identification of the characteristic skin lesions, as well as associated MRSs, is the key to early recognition of mastocytosis. Importantly, an initial manifestation of SM can masquerade as GI, cardiovascular, respiratory, skeletal, or neuropsychiatric symptoms with or without skin involvement.

GI symptoms caused by either MC mediators or direct infiltration are frequent manifestations in mastocytosis. The common symptoms are abdominal pain, diarrhea, nausea, and vomiting. Abdominal pain can manifest as either typical dyspeptic pain or nondyspeptic discomfort. The former actually may be associated with peptic disease. Nondyspeptic pain occurs often in association either with consumption of alcohol or certain foods or with stress. Involvement of the small intestine beyond the duodenum can cause lower abdominal pain, diarrhea, and steatorrhea secondary to malabsorption.

Cardiovascular symptoms include syncope and episodic vascular collapse. Episode severity and frequency vary greatly, and fatalities can occur in patients with severe and prolonged hypotension. The symptoms sometimes are preceded by lightheadedness or palpitations, or they are precipitated by exposure to specific medications, sudden temperature alterations, friction (rubbing of skin), alcohol consumption, or stress.

Respiratory manifestations caused by MC mediators include asthma phenotype (wheezing and dyspnea) and rhinitis. Occasionally, the symptoms are part of anaphylactic shock and have significant effects on patient's

morbidity and mortality. Skeletal symptoms are related to bone involvement and include bone pain that usually affects the long bones, fractures, and severe osteoporosis with deforming kyphoscoliosis. Neuropsychiatric symptoms include various types of headache, including a typical mild frontal, dull, nonpounding type; a vascular variant with migraine characteristics; and headache associated with rhinorrhea, pruritus, and lacrimation suggestive of a histaminergic variety. Other neuropsychiatric symptoms include decreased attention span, difficulty in concentration, forgetfulness, irritability, depression, poor motivation, confusion, anger, anxiety, lethargy, and somnolence.

## Radiologic Features

Patients with SM may have distinct skeletal or abdominal radiologic abnormalities. Diffuse or, less frequently, circumscribe bone lesions including osteoporosis, osteosclerosis, or both, occur in approximately 70% of cases (Figure 20-2). Scintigraphic bone scans are generally more sensitive than routine radiographs in detecting SM-related bone abnormalities. The diffuse lesions often are poorly demarcated with various degrees of

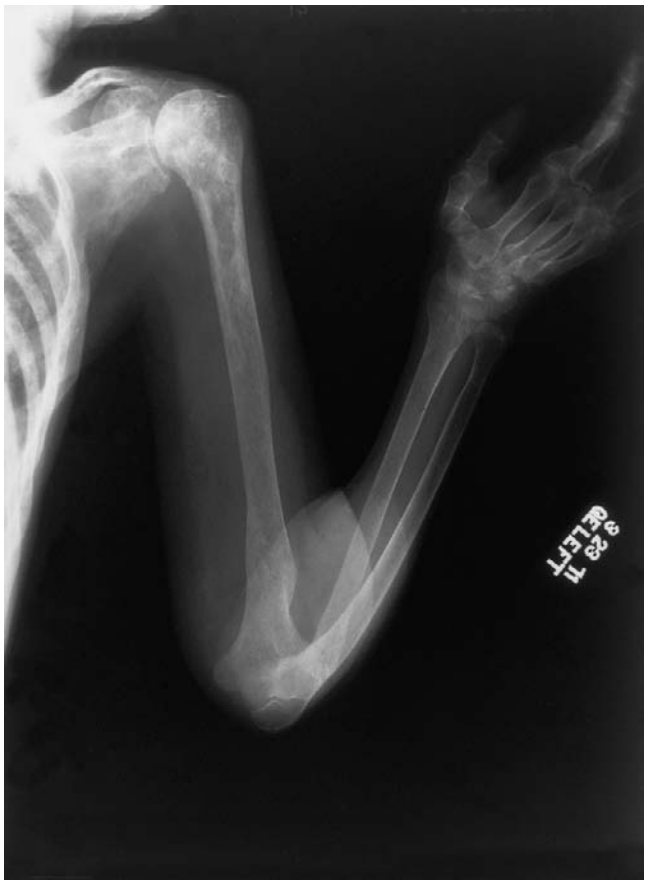



**FIGURE 20-2**

Systemic mastocytosis. Radiologic features of the shoulder and upper extremity show mixed osteoblastic and osteolytic lesions.

### MASTOCYTOSIS—FACT SHEET

**Definition**

- The abnormal growth and accumulation of mast cells in skin and any extracutaneous organ

**Incidence and Location**

- Rare, estimated annual incidence, 6.6 per 1 million population
- Predominantly involves the skin, bone marrow, liver, spleen, lymph nodes, and gastrointestinal tract

**Sex, Race, and Age Distribution**

- No sex predilection in either cutaneous or systemic mastocytosis
- More frequently reported in white patients
- CM is most common in children, with 80% showing lesions by 6 months of age
- SM is most common in adults older than 30 years

**Clinical Features**

- Skin: pruritus, urticaria, dermatographism, pigmentation
- Constitutional: fatigue, weight loss, fever, sweats
- Skeletal: bone pain, arthralgia, fractures
- Mediator release symptoms:
  - Abdominal pain, diarrhea, nausea, and vomiting
  - Headache, hypertension, flushing
  - Syncope, hypotension, tachycardia

**Radiologic Features**

- Bone: osteoporotic and osteosclerotic changes
- Gastrointestinal: peptic ulcer, abnormal mucosal patterns, motility disturbances
- Hepatosplenomegaly
- Possible retroperitoneal lymphadenopathy

**Morbidity and Mortality Rates**

- All mastocytosis types may have disabling symptoms related to mast cell mediator release
- Systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease: clinical course determined by the associated hematologic disorder
- Aggressive systemic mastocytosis: progressive course with organ dysfunction
- Mast cell leukemia: most aggressive, with survival of weeks or months

**Prognosis and Therapy**

- Pediatric cutaneous mastocytosis mostly regresses spontaneously
- Prognosis of systemic mastocytosis is directly correlated with 2008 WHO clinicopathologic classification
- Mediator release symptoms in cutaneous mastocytosis and indolent systemic mastocytosis can be alleviated by mediator-targeting drugs
- Aggressive systemic mastocytosis requires cytoreductive therapy.
- FIP1L1-PDGFRA–positive systemic mastocytosis with eosinophilia can be treated effectively with low-dose imatinib mesylate therapy
- Molecular-targeted therapies are being investigated

osteoblastic and osteolytic changes. Widely spread diffuse bone lesions usually accompany an SM progression. The circumscribed lesions radiologically may resemble metastatic carcinoma, multiple myeloma, or Paget disease.

The frequency of abnormal radiographic findings in the GI tract differs widely (from 10% to 38%) in the various reported series of SM. The most frequent radiographic findings in the GI tract are due to peptic ulcer disease, followed by abnormal mucosal patterns and motility disturbances. The mucosal abnormalities reported in patients with SM include gastric rugal hypertrophy, coarsening or loss of mucosal folds, patchy mucosal lesions, diffuse mucosal thickening, nodular lesions, and single or multiple polyps. Motility disturbances are usually found in patients who have diarrhea; however, both rapid and delayed GI transit times have been reported. Liver and spleen scans in patients with SM usually show hepatosplenomegaly with an abnormal uptake pattern consistent with diffuse parenchymal disease.

## Pathologic Features

### Morphology of Normal MCs and Precursors

Before attaining its mature morphologic features characterized by a mononuclear form with abundant metachromatic cytoplasmic granules, an MC probably differentiates from an agranular blast stage, a metachromatic blast stage with a few metachromatic granules, and an immature promastocyte stage with bilobed or multilobed nuclei and typical metachromatic cytoplasmic granules. Importantly, the sequence of nuclear changes from immature bilobed or multilobed nuclei to the mature mononuclear form differs from that of granulocytes, and it is difficult to morphologically distinguish MCs from basophils at the stage of metachromatic blasts.

### WHO Classification of Mastocytosis

Mastocytosis is broadly classified into two categories: CM and SM. The diagnosis of CM requires a typical clinical presentation and histologic evidence of skin infiltration by morphologically abnormal MCs. SM is defined by involvement of at least one extracutaneous organ with or without evident CM. It is further classified into six subcategories (Table 20-1): indolent SM (ISM), aggressive SM (ASM), SM with associated clonal hematologic non-MC lineage disease (SM-AHNMD), MC leukemia (MCL), MC sarcoma (MCS), and extracutaneous mastocytoma.

Both the previous (2001) and current (2008) WHO classification schemes use one major criterion and four minor criteria to distinguish the SM from a reactive MC hyperplasia (Table 20-2). The major criterion is the evident, multifocal, dense infiltrates of MCs (15 MCs or more in aggregates) in sections of bone marrow or other extracutaneous organs. The four minor criteria are intended to establish an aberrant clonal MC process: (1) more than 25% of MCs in biopsy sections of bone marrow or other extracutaneous organs or on bone marrow aspirate smear are spindle shaped or have other atypical morphologic features, such as hypogranular cytoplasm or multilobed nuclei; (2) detection of an activating point mutation at codon 816 of *KIT* in bone marrow, blood, or other extracutaneous organ; (3) aberrant surface MC expression of CD2 or CD25, or both; and (4) serum total tryptase level persistently greater than 20 ng/mL. It is important to remember that the fourth minor criterion is only valid when there is no evidence of non-MC clonal myeloid disorder. The diagnosis of SM requires either one major criterion and one minor criterion, or three minor criteria.

### Cutaneous Mastocytosis

CM includes three distinct clinicohistopathologic entities: UP/MPCM (see Figure 20-1), diffuse CM, and solitary mastocytosis of the skin. The histopathologic feature of all CM lesions is an infiltrate by MCs (Figure 20-3). Only partial correlation exists between the histologic patterns and the clinical appearance of lesions. The diagnosis of CM requires the demonstration of typical clinical findings and histologic evidence of cutaneous infiltration by MCs.

#### Urticaria Pigmentosa/Maculopapular Cutaneous Mastocytosis

UP is the most frequent form of CM (see Figure 20-1, A). In children, the lesions tend to be papular (see Figure 20-1, B) and are characterized by aggregates of elongated MCs, which typically fill the papillary dermis and extend as aggregates and sheets into the reticular dermis, often following the vasculature (see Figure 20-3). In adults, the lesions tend to be macular in large numbers, more darkly pigmented, and sometimes associated with telangiectasia (see Figure 20-1, A). A rare form of telangiectasia macularis eruptiva perstans is characterized by a small number of larger lesions (2 to 6 mm), which are lightly pigmented, telangiectatic macules with minimal or no increase in the number of MCs. Adult UP tends to have fewer MCs than the lesions in children.

#### Diffuse Cutaneous Mastocytosis

This lesion is less frequent than UP/MPCM and occurs in children exclusively. Patients usually exhibit erythroderma involving almost the entire skin. The skin may be simply red or may have a red-to-yellow color. Diffuse edema and thickening with a doughy consistency may be observed. Histologically, a skin biopsy specimen usually shows a bandlike infiltrate of MCs in the papillary and upper reticular dermis. Occasionally, MCs extend into the deep dermis in heavily infiltrated skin lesions.

## TABLE 20-1

### 2008 World Health Organization Classification and Related Clinical Findings of Mastocytosis

***Classification***

- Cutaneous mastocytosis: absent systemic mastocytosis
- Systemic mastocytosis
  - Indolent SM: skin lesions frequently present; no C findings or SM-AHNMD; low mast cell burden (≤30% bone marrow infiltration by MCs).
    - Isolated bone marrow mastocytosis: no skin lesions
    - Smoldering systemic mastocytosis: no C findings, but at least two B findings
- Systematic mastocytosis with associated clonal hematological non–mast cell lineage disease: clonal non–mast cell lineage disorders including MDS, MPN, AML, lymphoma, or other hematologic neoplasm that meets the classification
- ASM: no evidence of MCL, at least one C finding, usually fewer skin lesions
  - Lymphadenopathic mastocytosis with eosinophilia: progressive lymphadenopathy with peripheral eosinophilia, frequent bone involvement, and hepatosplenomegaly; usually no skin lesions; no rearrangement of *PDGFRA*
- Mast cell leukemia: bone marrow aspirate smears show 20% MCs or greater, usually no skin lesions
  - Typical MCL: MCs ≥10% of peripheral blood leukocytes
  - Aleukemic MCL: MCs <10% of peripheral blood leukocytes
- Mast cell sarcoma: unifocal tumor with anaplastic mast cells in destructive growth pattern; no evidence of SM
- Extracutaneous mastocytoma: unifocal mast cell tumor with low grade cytology and nondestructive growth pattern; no evidence of CM or SM

***Related Clinical Findings***

- B findings
  - Bone marrow biopsy specimen showing >30% infiltration by mast cells or serum total tryptase level >200 ng/mL, or both
  - Normal or borderline normal blood counts with features of myelodysplasia or myeloproliferation in non–mast cell lineages that are insufficient for a definitive diagnosis of AHNMD
  - Hepatomegaly without impairment of liver function, splenomegaly without hypersplenism, lymphadenopathy
- C findings
  - Cytopenia but no overt AHNMD (ANC <1.0 × $10^9$/L; hemoglobin <10 g/dL; or platelets <100 × $10^9$/L)
  - Palpable hepatomegaly with impairment of liver function, ascites, or portal hypertension
  - Skeletal involvement with large osteolytic lesions or pathologic fractures
  - Palpable splenomegaly with hypersplenism
  - Malabsorption with weight loss owing to gastrointestinal mast cell infiltration

From Horny HP, Akin C, Metcalfe DD, et al: Mastocytosis. In Swerdlow SH, Campo E, Harris NL, et al, editors: *WHO of Tumours of Haematopoietic and Lymphoid Tissue,* ed 4, Lyon, 2008, IARC, pp 54–63.
*AHNMD,* Associated clonal hematologic non–mast cell lineage disease; *ANC,* absoulute neutrophil count; *ASM,* aggressive systemic mastocytosis; *CM,* cutaneous mastocytosis; *MPN,* myeloproliferative neoplasm; *MC,* mast cell; *MCL,* mast cell leukemia; *MDS,* myelodysplastic syndrome; *MPN,* myeloproliferative neoplasm; *SM,* systemic mastocytosis.

## TABLE 20-2

### Criteria for Cutaneous Mastocytosis and Systemic Mastocytosis

***Cutaneous Mastocytosis (CM)***

Skin lesions show the typical clinical findings of urticaria pigmentosa/maculopapular CM, diffuse cutaneous mastocytosis, or solitary mastocytoma and typical histologic infiltrates of mast cells in a multifocal or diffuse pattern in an adequate skin biopsy specimen. In addition, a diagnostic prerequisite for the diagnosis of CM is the absence of features or criteria sufficient to establish a diagnosis of SM.

***Systemic Mastocytosis (SM)***

The diagnosis of SM can be made when one major criterion and one minor criterion are present or when three minor criteria are fulfilled.

***Major Criterion***

Multifocal, dense infiltrates of mast cells (≥15 mast cells in aggregates) in bone marrow or other extracutaneous organs

***Minor Criteria***

1. In biopsy sections of bone marrow or other extracutaneous organs, greater than 25% mast cells in the infiltrate are spindle shaped or have atypical morphologic characteristics. Alternatively, of all mast cells in a bone marrow aspirate smear, greater than 25% are immature or atypical.
2. An activating point mutation at codon 816 of *KIT* in bone marrow, blood, or other extracutaneous organ is detected.
3. Mast cells in bone marrow, peripheral blood, or other extracutaneous organ(s) express CD2 or CD25, or both, in addition to normal mast cell markers.
4. Serum total tryptase level are persistently greater than 20 ng/mL, unless an associated clonal myeloid disorder is present, in which case this parameter is not valid.

From Horny HP, Akin C, Metcalfe DD, et al: Mastocytosis. In Swerdlow SH, Campo E, Harris NL, et al, editors: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue,* ed 4, Lyon, 2008, IARC, pp 54–63.

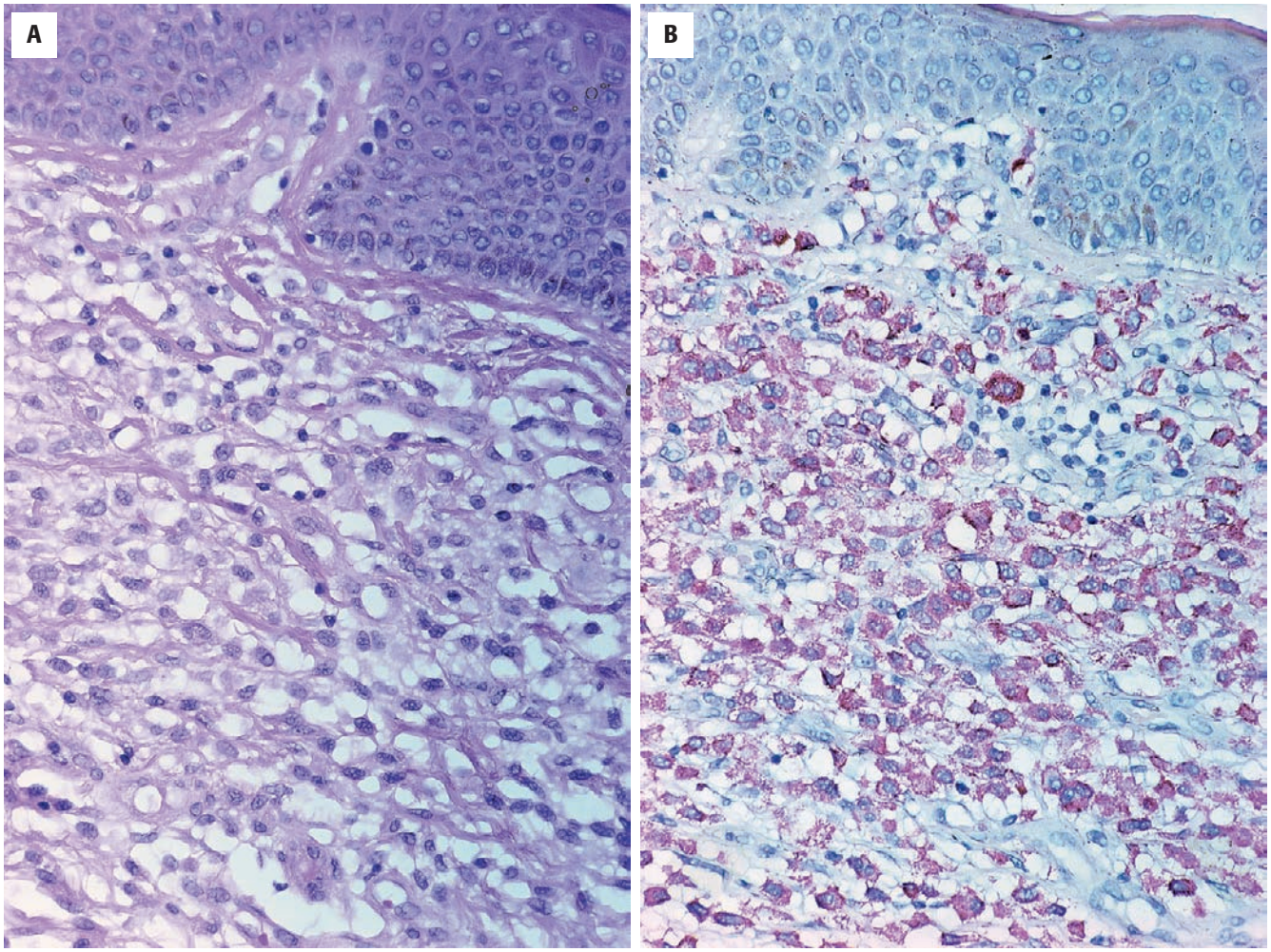



**FIGURE 20-3**

Histologic and histochemical features of cutaneous mastocytosis. **A,** Section of skin biopsy specimen shows sheets and aggregates of elongated mast cells involving the upper dermis and papillary dermis (H&E-stained, original magnification ×240). **B,** Histochemical stain of chloracetate esterase shows positive staining in all atypical mast cells (original magnification ×240).

### Solitary Mastocytosis of the Skin

Solitary CM, a rare variant of CM that usually occurs in infants, develops as nodules or plaques larger than 1 cm in diameter. Usually, the nodules or plaques are located on the extremities, but they can also occur on the face, scalp, or trunk, or in any combination of these locations. Histologically, sheets of MCs with abundant cytoplasm fill the papillary and reticular dermis and may extend into the deep dermis and subcutaneous tissues.

## Systemic Mastocytosis: Bone Marrow Aspiration and Biopsy

### Cytologic Characteristics of MCs

Morphologic examination of bone marrow aspirate and biopsy specimen is a critical step in the workup of SM. It is important to note that MC lesions commonly consist of patchy clusters of spindle-shaped MCs with associated subtle reticulin fibrosis. Therefore, when evaluating a bone marrow aspirate smear, it is essential to pay close attention to the thick, inadequately spread marrow particles and the poorly stained areas where the abnormal MC clusters commonly appear. Careful examination of adequately spread and stained areas surrounding these thick marrow particles can identify individual atypical MCs commonly intermixed with eosinophils.

In contrast to normal MCs with abundant cytoplasmic granules and centrally located round nuclei (Figure 20-4, A, B), MCs in SM are cytologically atypical, which can be categorized into two types. Type I atypical MCs have excessive cytoplasmic extensions, elongation of nuclei, and hypogranular cytoplasm (see Figure 20-4, C, D). These atypical features are seen in most SM cases (greater than 90%); however, the variation is wide in the degree of cytoplasmic extensions, nuclear elongation, and cytoplasmic hypogranulation among cases or even within the same case (Figure 20-5, A-C). In rare

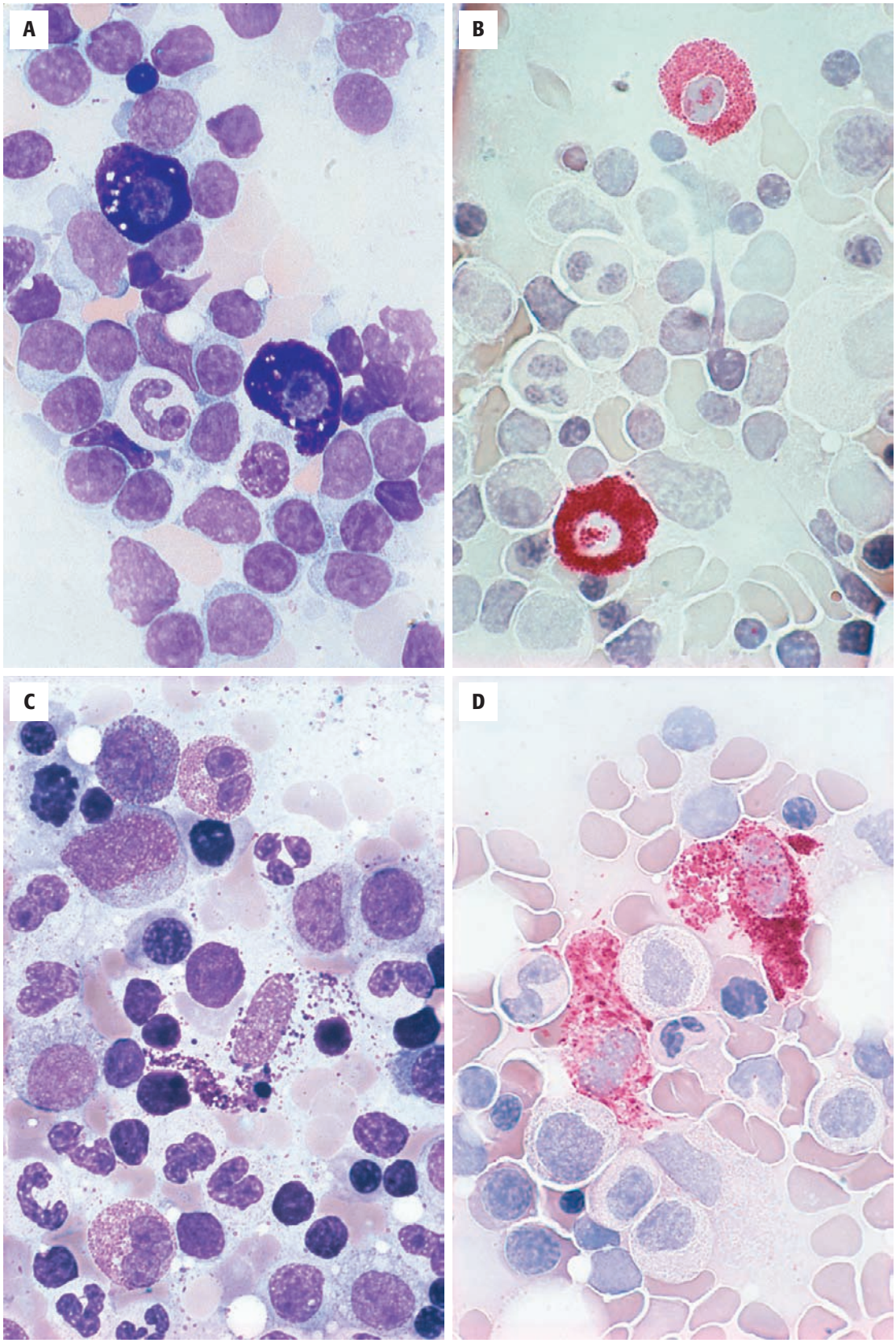



**FIGURE 20-4**

Microscopic features and cytochemical characteristics of normal and atypical mast cells. **A,** Normal or reactive mast cells are characterized by a centrally located round nucleus and abundant basophilic granules packed in the well-defined cytoplasm (Wright-Giemsa stain, original magnification ×1000). **B,** Bone marrow smear showing strong aminocaproate esterase staining in normal mast cells and negative staining in all other hematopoietic cells (original magnification ×1000). **C,** Bone marrow aspirate smear from a patient with systemic mastocytosis (SM) showing the cytologic characteristics of atypical mast cells in SM, including excessive cytoplasmic extensions, elongation of nucleus, and hypogranular cytoplasm (Wright-Giemsa stain, original magnification ×1000). **D,** Atypical mast cells in SM also show positive aminocaproate esterase staining (original magnification ×1000).

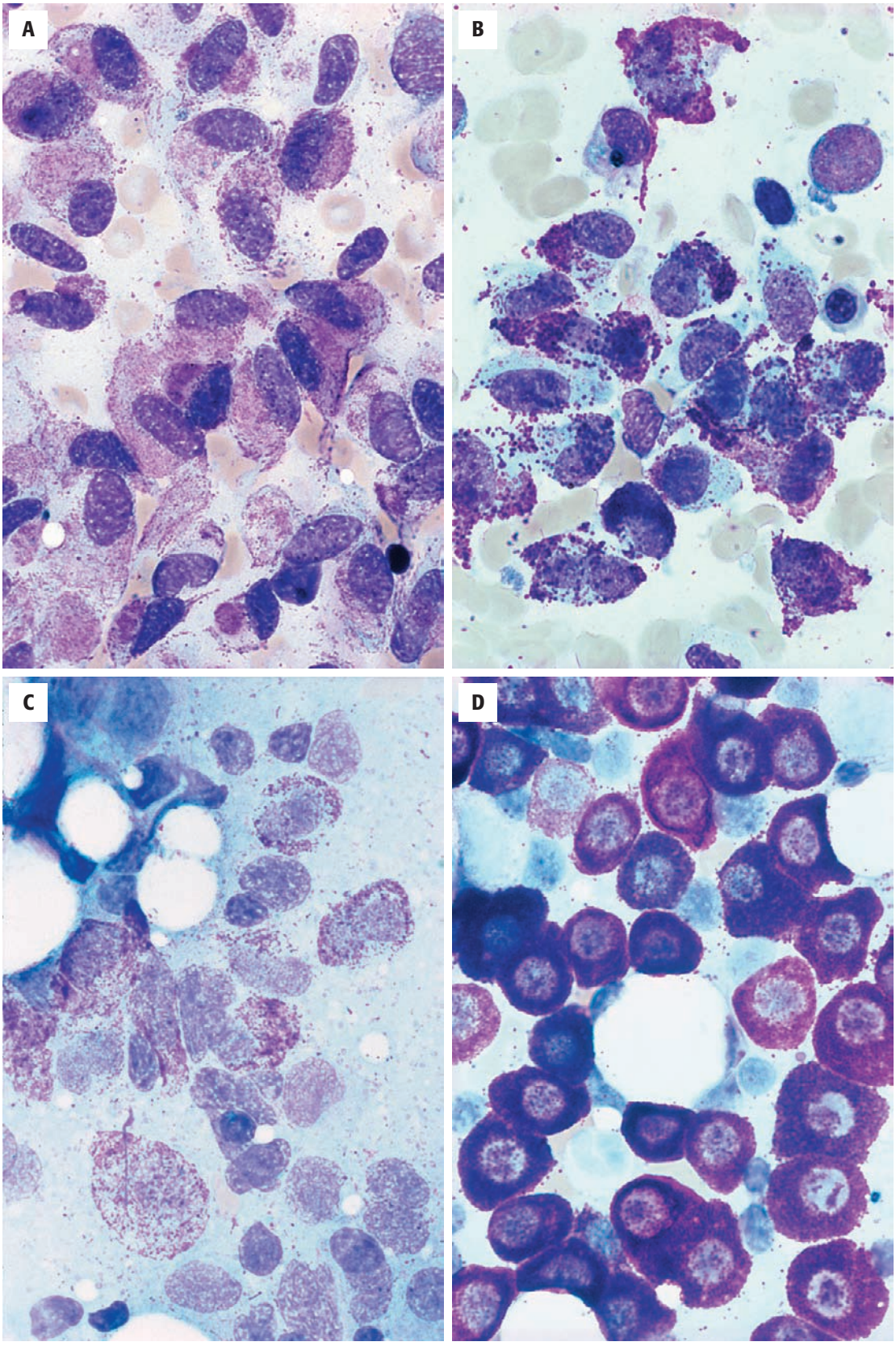



**FIGURE 20-5**

Cytologic variation in systemic mastocytosis. **A-C,** Wide variations exist in the degree of cytoplasmic extension, nuclear elongation, and cytoplasmic hypogranulation (Wright-Giemsa stain, original magnification ×1000). **D,** In rare cases, individual mast cells are cytologically indistinguishable from normal mast cells, but have excessive cell numbers in sheets (Wright-Giemsa stain, original magnification ×1000).

**TABLE 20-3**

**Cytochemical and Immunohistochemical Characteristics of Hematopoietic Cells with Prominent Cytoplasmic Granules and/or Positive for Tryptase**

| Stain | Mast Cells | Basophils | Tryptase-Positive Myeloblasts |
|---|---|---|---|
| ***Cytochemical*** | | | |
| Peroxidase | – | – | ++ |
| Toluidine blue | +++ | ++ | ± |
| Chloracetate esterase | +++ | – | + |
| Aminocaproate esterase | ++ | – | ± |
| ***Immunohistochemical*** | | | |
| Tryptase | +++ | ± | + |
| CD117 (c-kit) | +++ | – | ± |
| CD34 | – | – | ± |
| CD25 | ++/–* | – | ± |

*+++*, Strongly positive; *++*, moderately positive; *+*, weakly positive; *±*, either positive or negative; *–*, negative.
*Positive in abnormal clonal mast cells, but negative in normal or reactive mast cells and clonal mast cells of well-differentiated systemic mastocytosis.

cases, the cytologic features of individual bone marrow MCs may be indistinguishable from normal mature MCs, but show an excessive number of MCs that are often present in sheets (see Figure 20-5, D). Such cases recently have been described as a potential new variant, well-differentiated SM. Type II atypical MCs are immature forms of MCs, including immature promastocytes exhibiting bilobed or multilobed nuclei or metachromatic blasts (Figure 20-6). These MCs are more common in aggressive forms of mastocytosis, especially MCL. Both normal appearing MCs in well-differentiated SM and metachromatic blasts need to be distinguished from other tryptase-positive round cell infiltrates, including tryptase-positive blasts in acute myeloid leukemia or neoplastic basophils in acute basophilic leukemia and chronic myelogenous leukemia, through cytochemical or immunophenotyping studies (Table 20-3).

Apart from MCs, all other lineages of the bone marrow should be examined to identify concomitant myelodysplasia, myeloproliferation, or lymphoproliferative or plasma cell proliferative disorders. In such cases, the diagnosis of SM-AHNMD can be established when diagnostic criteria of both SM and the non-MC clonal hematologic malignancy are fulfilled.

### Histology of Pathologic Lesions

In most cases of SM, multifocal, sharply demarcated aggregates of MCs are found in paratrabecular or perivascular locations in bone marrow biopsy specimens (Figure 20-7, A, B). In specimens with lymphoid aggregates, irregular clusters of MCs can develop at the periphery of the lymphoid aggregates (see Figure 20-7, C). The focal lesions are composed of varying proportions of MCs, lymphocytes, eosinophils, and fibroblasts. Marked fibrosis and thickening of the adjacent bony trabeculae are frequent (see Figure 20-7, A). In sections stained with hematoxylin and eosin (H&E), atypical MCs with elongated nuclei and clear cytoplasm may resemble fibroblasts or histiocytes. Therefore the focal lesions of mastocytosis were historically described as *fibrohistiocytic lesions with eosinophilia*. The degree of eosinophilia can vary from a few eosinophils at the margins of the lesion to extremely intense eosinophilia overshadowing the actual MC infiltrates (Figure 20-8). In addition, the degree of bone marrow involvement by mastocytosis can vary from minimal involvement showing small clusters of MCs around the lymphoid aggregates to diffuse replacement of the bone marrow in advanced-stage SM or MCL (Figures 20-9, C, and 20-10, A).

## Systemic Mastocytosis Variants (WHO Classification)

Six major and three provisional SM variants are recognized by the 2008 WHO classification scheme (see Table 20-1).

### Indolent Systemic Mastocytosis

Typical ISM is characterized by modest (less than 30%) bone marrow involvement with multifocal dense infiltrates of MCs in a background of normocellular marrow with normal hematopoiesis, an indolent clinical course, and the presence of maculopapular skin lesion in most cases (see Figure 20-9, A). However, isolated bone marrow mastocytosis with associated MRSs also exist, and these patients generally have clinical courses similar to those of patients with usual ISM. Isolated bone marrow mastocytosis, likely a variant of ISM, is currently recognized by the 2008 WHO classification scheme as a provisional entity. Some cases of ISM may progress. A combination of at least two of these so called B findings (see Table 20-1) is indicative of disease progression, and such cases can be classified as *smoldering SM*.

### Systemic Mastocytosis with Associated Clonal Hematologic Non–Mast Cell Lineage Disease

In SM-AHNMD, criteria for SM are met. In addition, features are present that allow for diagnosis of a second disorder that satisfies WHO guidelines for myelodysplastic syndrome, myeloproliferative neoplasm, myelodysplastic/myeloproliferative neoplasm, acute myeloid leukemia, lymphoma, or myeloma (see Figure 20-9, B). The most frequent AHNMD is myeloproliferative neoplasm (40% to 50%), followed in frequency by chronic myelomonocytic leukemia (20% to 30%), myelodysplastic syndrome (10% to 20%), and acute myeloid leukemia

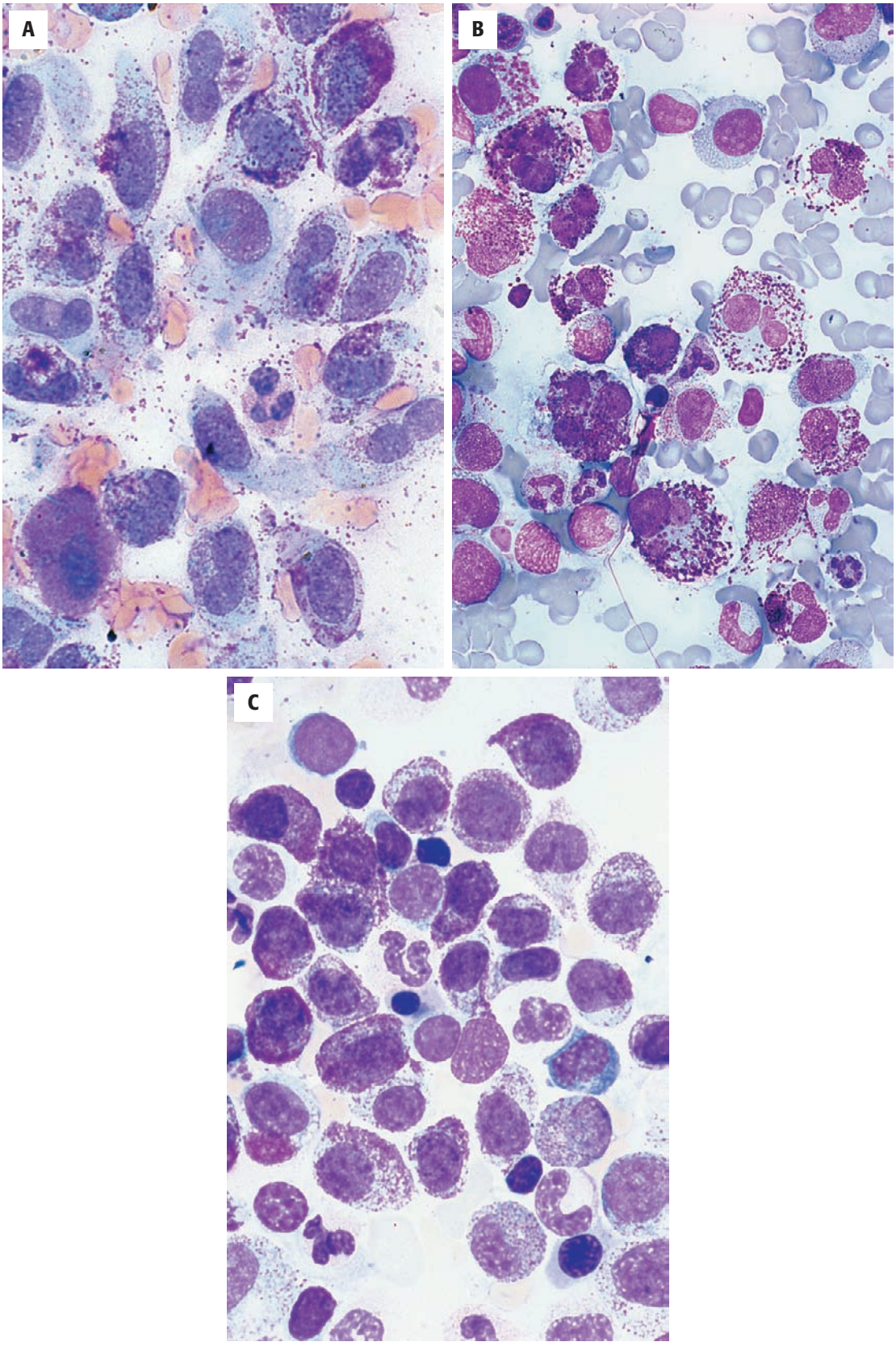



**FIGURE 20-6**

Cytologic features of aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL). In ASM **(A, B)** and MCL **(C),** more immature forms of atypical mast cells, including immature promastocytes exhibiting metachromatic blasts **(A, C)** or bilobed or multilobed nuclei **(B),** may be seen in bone marrow aspirate smears (Wright-Giemsa stain, original magnification ×1000).

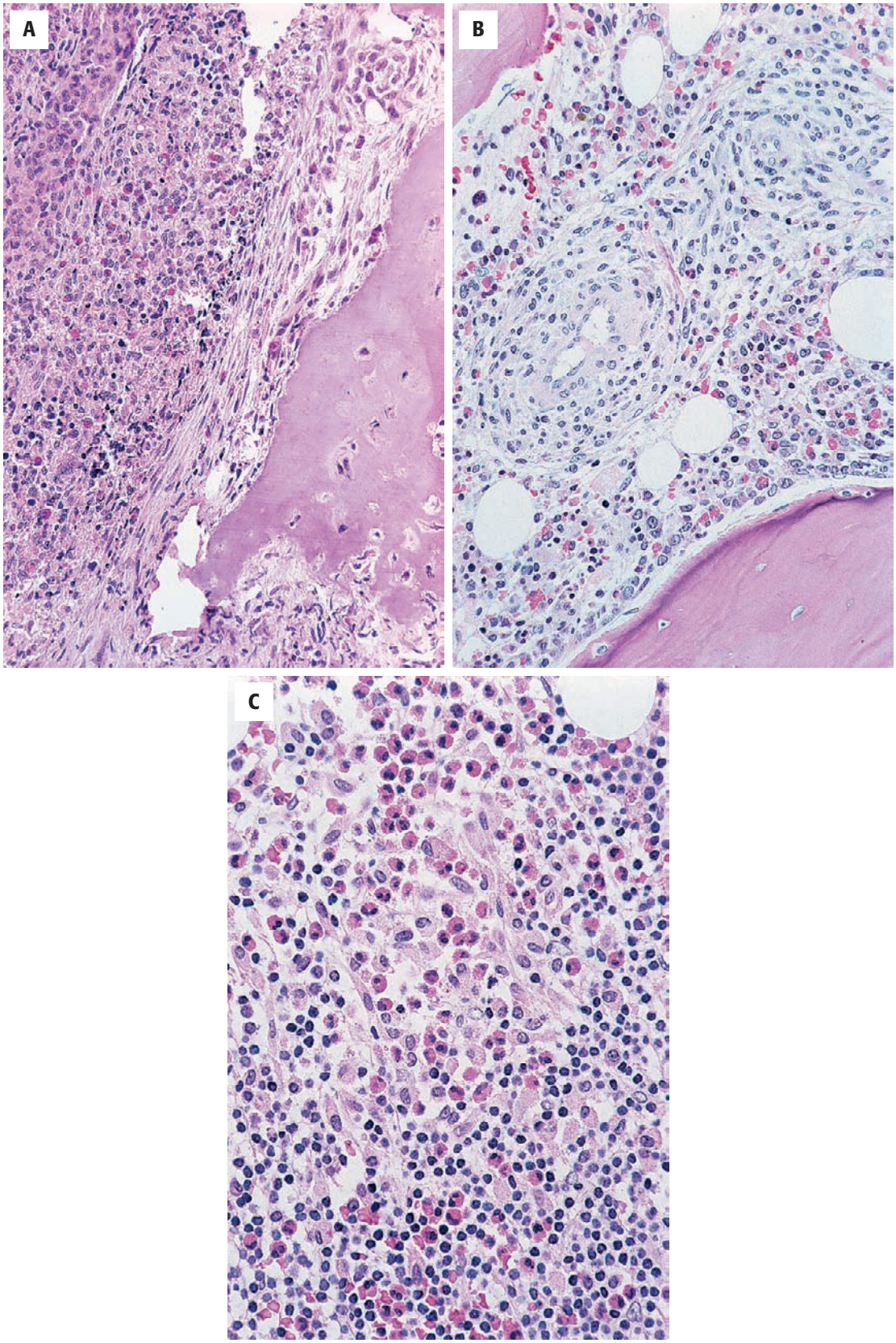



**FIGURE 20-7**

Histologic features of bone marrow biopsy in systemic mastocytosis. Osteoblastic changes and thickening of bony trabecular **(A)** may be associated with paratrabecular mast cell infiltrates (H&E stain, original magnification ×240). Multifocal aggregates of atypical mast cells are found in paratrabecular **(A)** or perivascular **(B)** locations (H&E stain, original magnification ×240). **C,** In specimens with lymphoid aggregates, irregular clusters of mast cells are seen at the periphery of the lymphoid aggregate (H&E stain, original magnification ×400). The mast cells have abundant clear cytoplasm, a distinct cytoplasmic border, and oval or reniform nuclei with a delicate chromatin network. Eosinophils are frequently within and particularly at the margin of mast cell lesions.

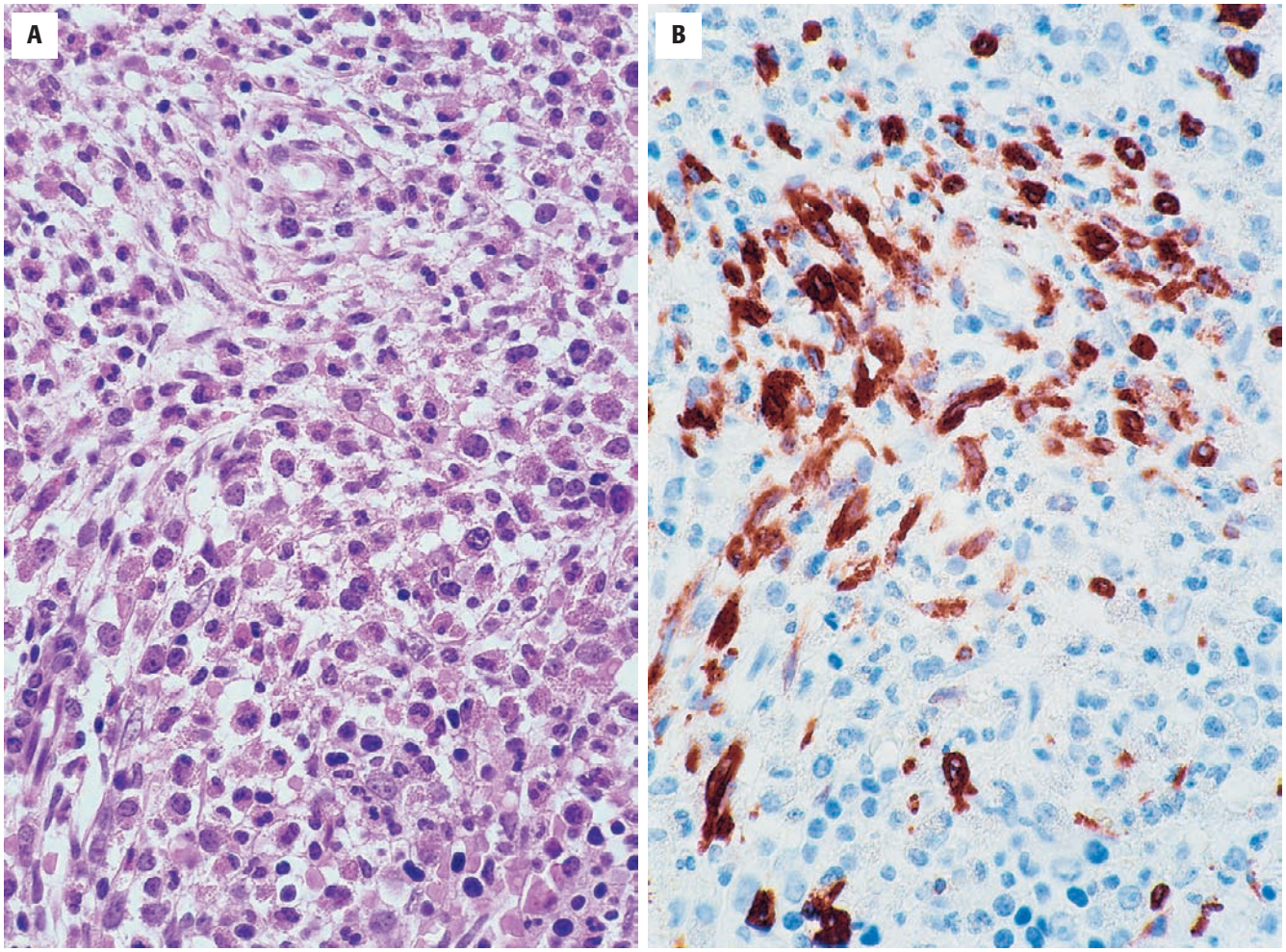



**FIGURE 20-8**

Histologic and immunohistochemical features of systemic mastocytosis with eosinophilia. In some cases, extremely intense eosinophilia can overshadow the actual mast cell infiltrates **(A)** and require mast cell–specific tryptase immunostain to highlight the atypical mast cell infiltrates, usually in loose aggregates (**B,** original magnification ×400).

(1% to 5%). The presence of *KIT* mutations in both neoplastic MCs and non-MC neoplastic myeloid cells supports the assumption that a common neoplastic precursor gives rise to both MC and non-MC components of SM-AHNMD. Such findings have not been reported in SM-lymphoma or SM-myeloma, suggesting that lymphoma or myeloma may be merely a collision lesion. Interestingly, a recent study showed that as much as 30% to 40% of SM-AHNMD cases had associated eosinophilia, which should be distinguished from platelet-derived growth factor receptor α (*PDGFRA*) translocation–associated myeloid neoplasms through molecular studies.

### Aggressive Systemic Mastocytosis

ASM is characterized by a progressive infiltration of various organs by neoplastic MCs with consequent impairment of organ function. To have a diagnosis of ASM, the patient should have at least one C finding (see Table 20-1). Histologic evaluation of the bone marrow in ASM usually shows a variable degree of mixed dense, focal, and diffuse MC infiltration (see Figure 20-9, C, D).

### Mast Cell Leukemia

MCL is rare and is characterized by leukemic bone marrow infiltration by immature neoplastic MCs. It has a rapidly progressive course similar to other types of acute leukemia. The diagnosis requires that the cytologically atypical MCs are more than 20% of bone marrow nucleated cells on bone marrow aspirate smear or more than 10% of leukocytes in peripheral blood. The bone marrow typically shows extensive replacement of normal bone marrow by immature or cytologically atypical MCs. The pattern may be diffuse, interstitial, or a combination of the two. In sections stained with H&E, bone marrow is usually infiltrated by sheets of atypical MCs with abundant clear-to-granular cytoplasm and round-to-oval nuclei (see Figure 20-10, A, B). The pattern of infiltration and the morphologic features of

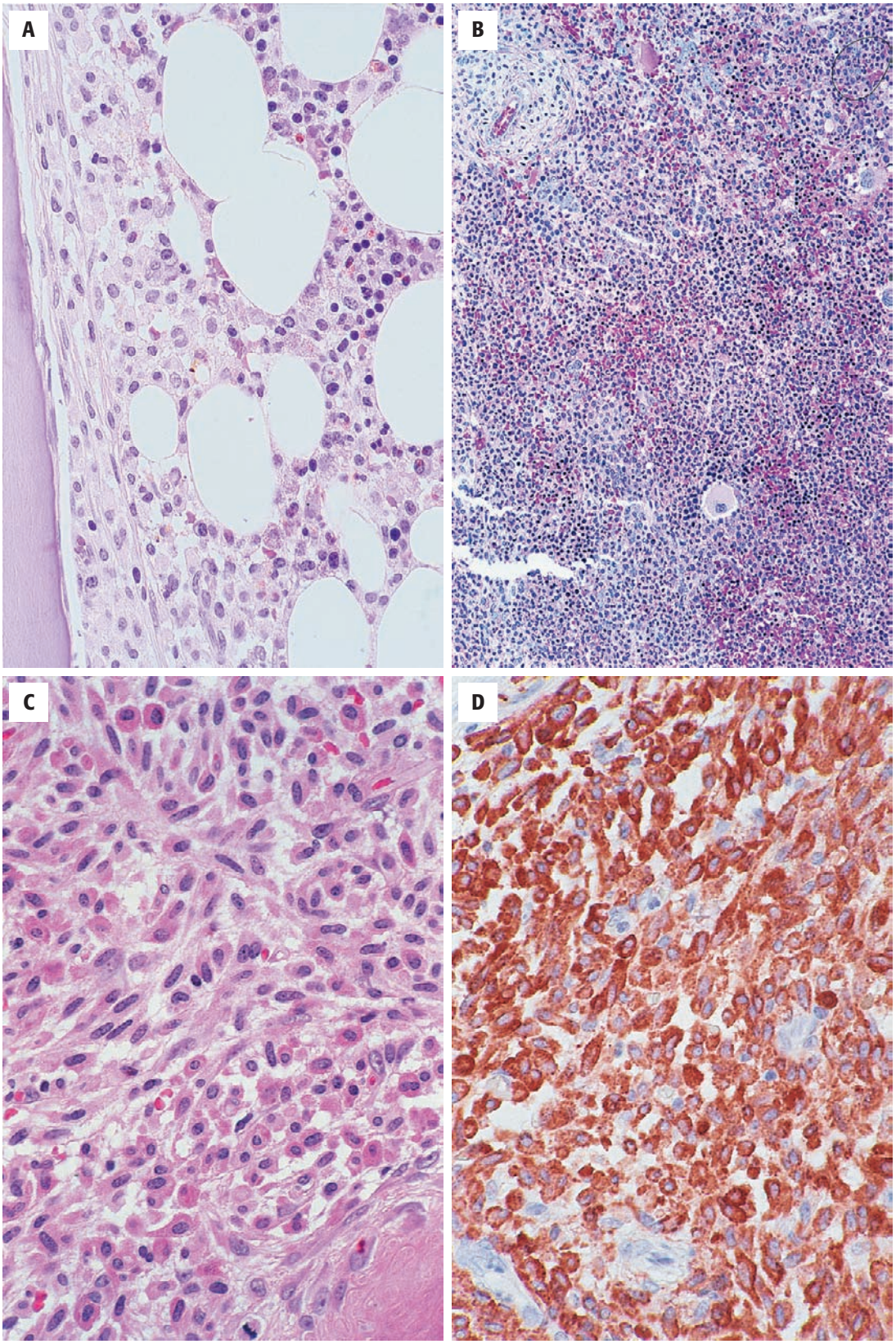



**FIGURE 20-9**

Histologic and immunohistochemical features of subtypes of systemic mastocytosis. **A,** Indolent systemic mastocytosis showing paratrabecular atypical mast cell infiltrates in a background of normocellular marrow with normal hematopoiesis (H&E stain, original magnification ×400). **B,** Systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease showing a perivascular atypical mast cell aggregate *(left upper corner of field)* in a background of markedly hypercellular marrow owing to associated hematologic disease (H&E stain, original magnification ×96). **C,** Aggressive systemic mastocytosis showing extensive bone marrow involvement by spindle-shaped atypical mast cells with elongated nuclei and few scattered eosinophils (H&E stain, original magnification ×400). **D,** Immunohistochemical tryptase staining showing strong staining of all atypical mast cells (original magnification ×400).

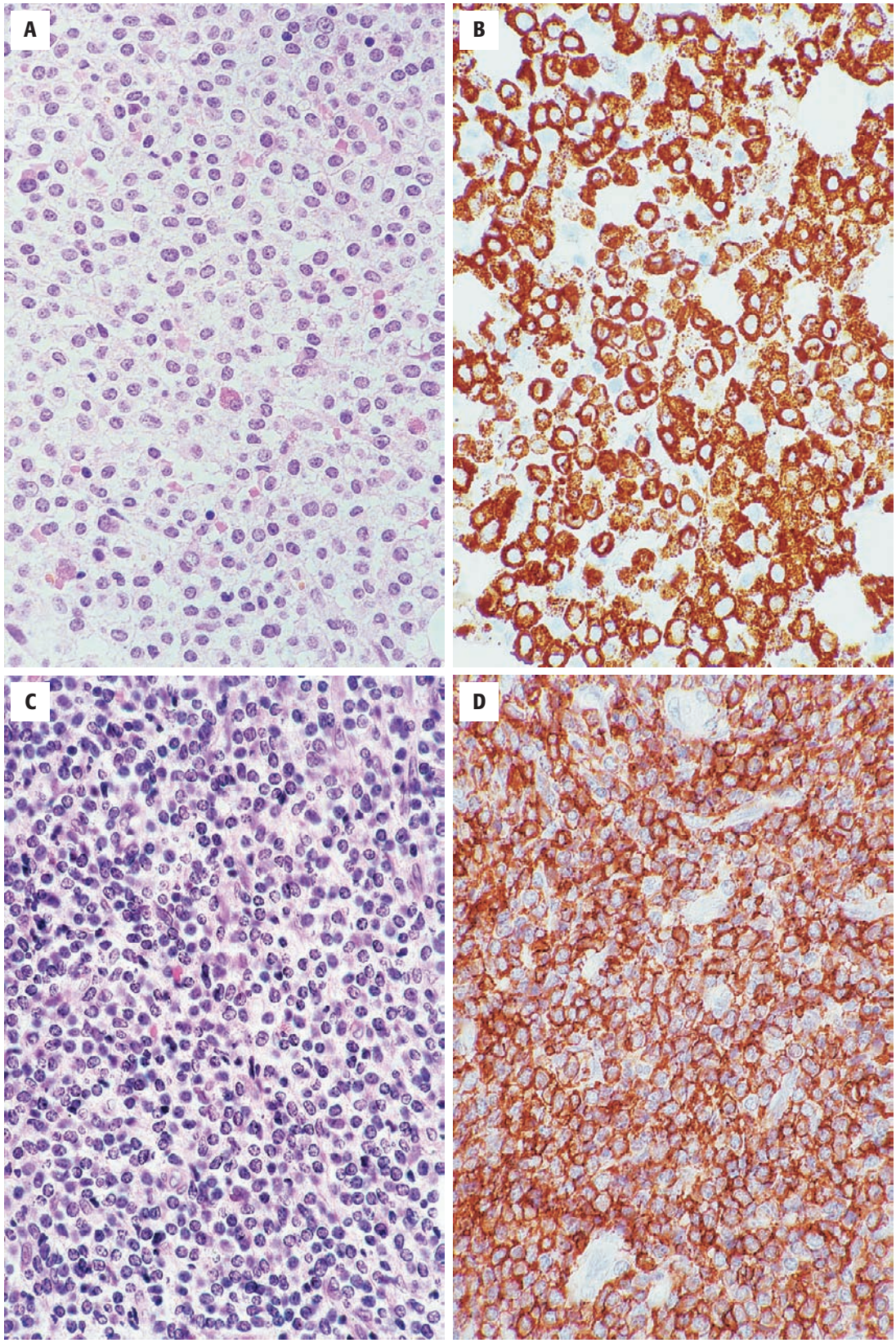



**FIGURE 20-10**

Histologic and immunohistochemical features of mast cell leukemia (MCL) and mast cell sarcoma (MCS). **A,** MCL showing extensive interstitial infiltration of bone marrow by immature mast cells with morphologic features of immature cells with abundant clear cytoplasm and round to oval nuclei (H&E, original magnification ×400). **B,** Strong tryptase immunostaining of leukemic cells confirmed the mast cell lineage (original magnification ×400). **C,** MCS that clinically presented as a local destructive tumor (H&E, original magnification ×400). The neoplastic cells are immature with a high nuclear-to cytoplasm ratio, fine nuclear chromatin, and clear cytoplasm. The cells are negative for most immunohistochemical markers for hematolymphoid cells, except CD43 (**D,** original magnification ×400) and tryptase.

the cells may resemble those of hairy cell leukemia. Type II immature atypical MCs are frequent. MCL is further subclassified as aleukemic MCL when the circulation of MCs in peripheral blood is less than 10%.

### Mast Cell Sarcoma

MCS is defined by a local destructive growth of cytologically anaplastic MCs without systemic involvement at presentation. The neoplastic cells are usually immature with a high nuclear to cytoplasmic ratio, prominent nucleoli, and a hypogranulated cytoplasm (Figure 20-10, C, D).

### Extracutaneous Mastocytoma

Mastocytoma is a localized unifocal growth of tissue MCs without systemic involvement. In contrast to mastocytoma of skin, extracutaneous mastocytomas, primarily reported in the lung, are exceedingly rare. In contrast to MCS, mastocytomas do not show a destructive growth pattern, and MCs in mastocytoma show a low-grade cytology.

### Three Potential Variants of SM that Are Not Yet Fully Recognized by WHO Classification

- **Well-differentiated SM.** Well-differentiated SM manifests as an exclusively compact, multifocal infiltration by round and $CD25^-$ MCs. The clonal MCs carry *KIT F522C* or other mutations outside *KIT* codon 816. Among the one major and four minor criteria of SM, only serum tryptase level is reportedly abnormally elevated in this variant.
- **Occult mastocytosis.** The term *occult systemic mastocytosis* is used for rare cases of SM that initially are obscured by a malignant hematologic disorder in SM-AHNMD. Usually, after chemotherapy and disappearance of the associated neoplasm, typical compact MC infiltrates are revealed.
- **Monoclonal MCs with undetermined significance/monoclonal MC activation syndrome.** Monoclonal MCs with undetermined significance describes cases that have evidence of an immunophenotypic or genetic clonal MC population but do not meet the diagnostic criteria of SM. Some patients may have MC MRSs. Although both monoclonal MCs with undetermined significance and monoclonal MC activation syndrome may be possible early lesions of SM, they require further clinical, pathologic, and genetic characterization.

In light of the somewhat complicated clinicopathologic diagnostic criteria and existence of various SM subtypes, a systemic approach (Figure 20-11) will help to facilitate the effective workup for accurately diagnosing SM.

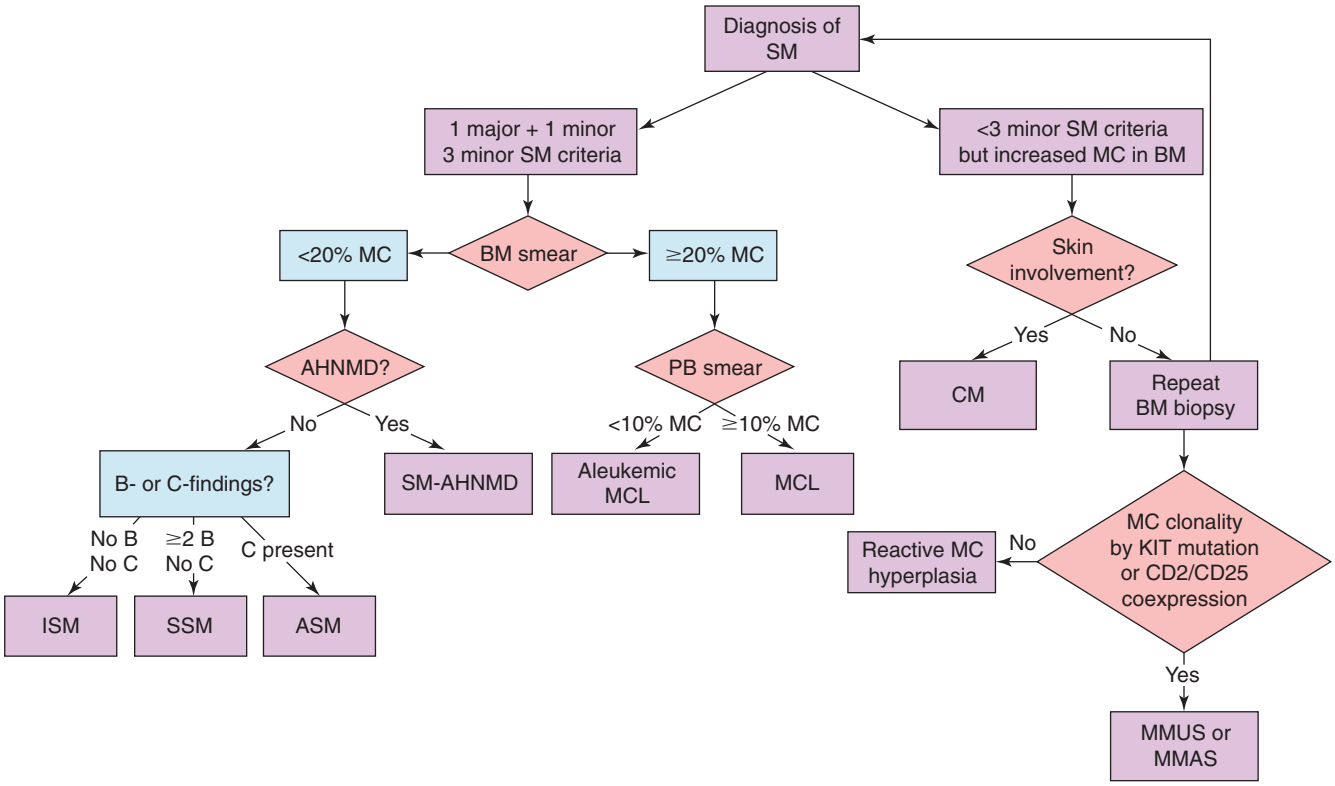



**FIGURE 20-11**

Systemic approach to workup and diagnosis of a mast cell disorder. *ASM,* Aggressive systemic mastocytosis; *CM,* cutaneous mastocytosis; *ISM,* indolent systemic mastocytosis; *MC,* mast cell; *MCL,* mast cell leukemia; *MMUS,* monoclonal mast cells with undetermined significance; *MMAS,* monoclonal mast cells with activation syndrome; *SM,* systemic mastocytosis; *SM-AHNMD,* systemic mastocytosis–associated clonal hematologic non–mast cell lineage disease; *SSM,* smoldering systemic mastocytosis.

### Microscopic Findings in Systemic Mastocytosis: Liver Biopsy

Most liver biopsy specimens from patients with SM show some degree of fibrosis and chronic inflammatory cellular infiltration with plasma cells, lymphocytes, eosinophils, and mononuclear fibroblast like cells (including MCs) in the portal area (Figure 20-12). The predominant infiltrate appears to be mononuclear cells. However, differentiating MCs from other mononuclear cells is usually difficult on H&E–stained sections. The presence of eosinophils, fibrosis, and oval or elongated nuclei of the mononuclear cells in portal areas may suggest MCs and require cytochemical or immunohistochemical stains for confirmation. In cases of MCL, both portal and sinusoidal MC infiltrates may be present.

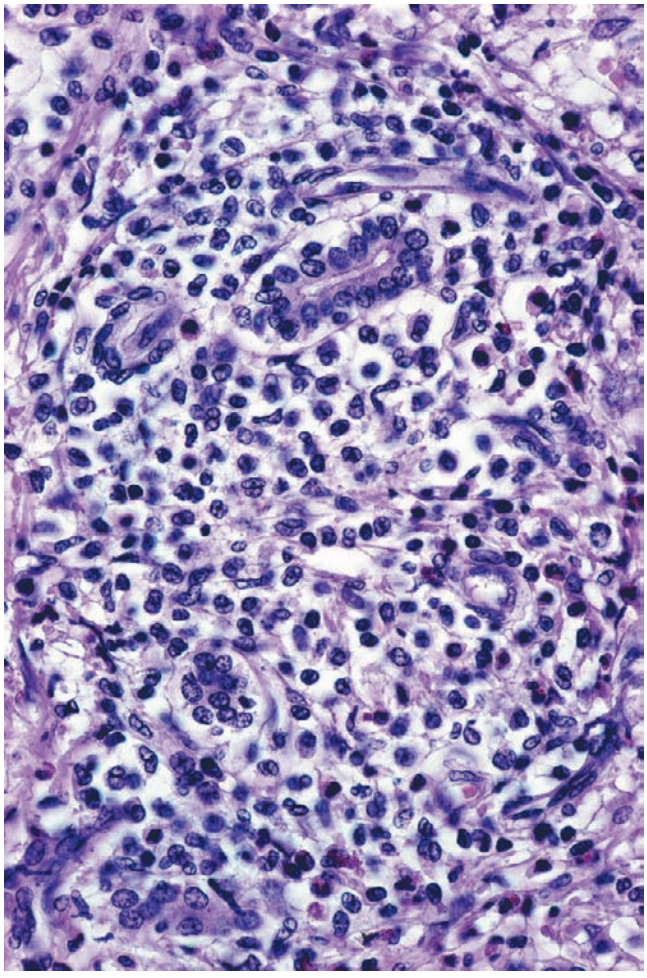


**FIGURE 20-12**

Histologic features of the liver in systemic mastocytosis. Sections of liver biopsy show intense portal infiltration by atypical mast cells intermixed with a few eosinophils (H&E stain, original magnification ×400).

### Microscopic Findings in Systemic Mastocytosis: Lymph Node

Central lymph nodes (abdominal or thoracic) frequently show definitive involvement by SM. The abnormal MC infiltrates may be observed in any compartment of the lymph node. Common features of lymph node involvement are mixed MCs and eosinophil infiltration in the perifollicular and paracortical areas with associated increased vascularity or fibrosis, or both (Figure 20-13, A, B). Occasionally, dense eosinophils (eosinophilic abscess) may mask the MCs. In rare cases, MC aggregates partially or completely replace lymphoid follicles, showing features that resemble follicular lymphoma (see Figure 20-13, C). Intense MC infiltration in the medullary cords of the lymph node may occur in rare cases. Selective intense sinusoidal MC infiltration with dilatation of medullary sinuses resembling sinus histiocytosis may be seen in cases of MCL (see Figure 20-13, D).

### Microscopic Findings in Systemic Mastocytosis: Spleen

MC infiltrates may involve all compartments of the spleen, including the fibrous trabeculae and the white and red pulp. The most frequent location of MC infiltrates is adjacent to and within the fibrous trabeculae (Figure 20-14, A). The MCs may be found in small clusters, aggregates, sheets, or perivascular distribution. Eosinophilia, fibrosis, and plasmacytosis are frequently observed in the areas of MC infiltration. White pulp infiltrates are often manifest as small clusters of MCs in the marginal zone of the lymphoid follicles (see Figure 20-14, B). Diffuse red pulp MC infiltration is seen only in spleens of patients with MCL (see Figure 20-14, C). In addition, extramedullary hematopoiesis, focal sinusoidal dilatation, and increased numbers of myeloid elements may occur in the red pulp.

### Microscopic Findings in Systemic Mastocytosis: Gastrointestinal Tract

Endoscopic mucosal biopsy specimens from the upper or lower GI tract of patients with mastocytosis usually show increased numbers of plasma cells and eosinophils and, in some cases, increased numbers of MCs in the lamina propria and submucosa (Figure 20-15). Extensive submucosal infiltration by MCs has been documented in a cholecystectomy specimen showing contracted gallbladder with thickened walls.

## Ancillary Studies

Over the years, numerous cell type–specific cytochemical and immunochemical markers have been developed

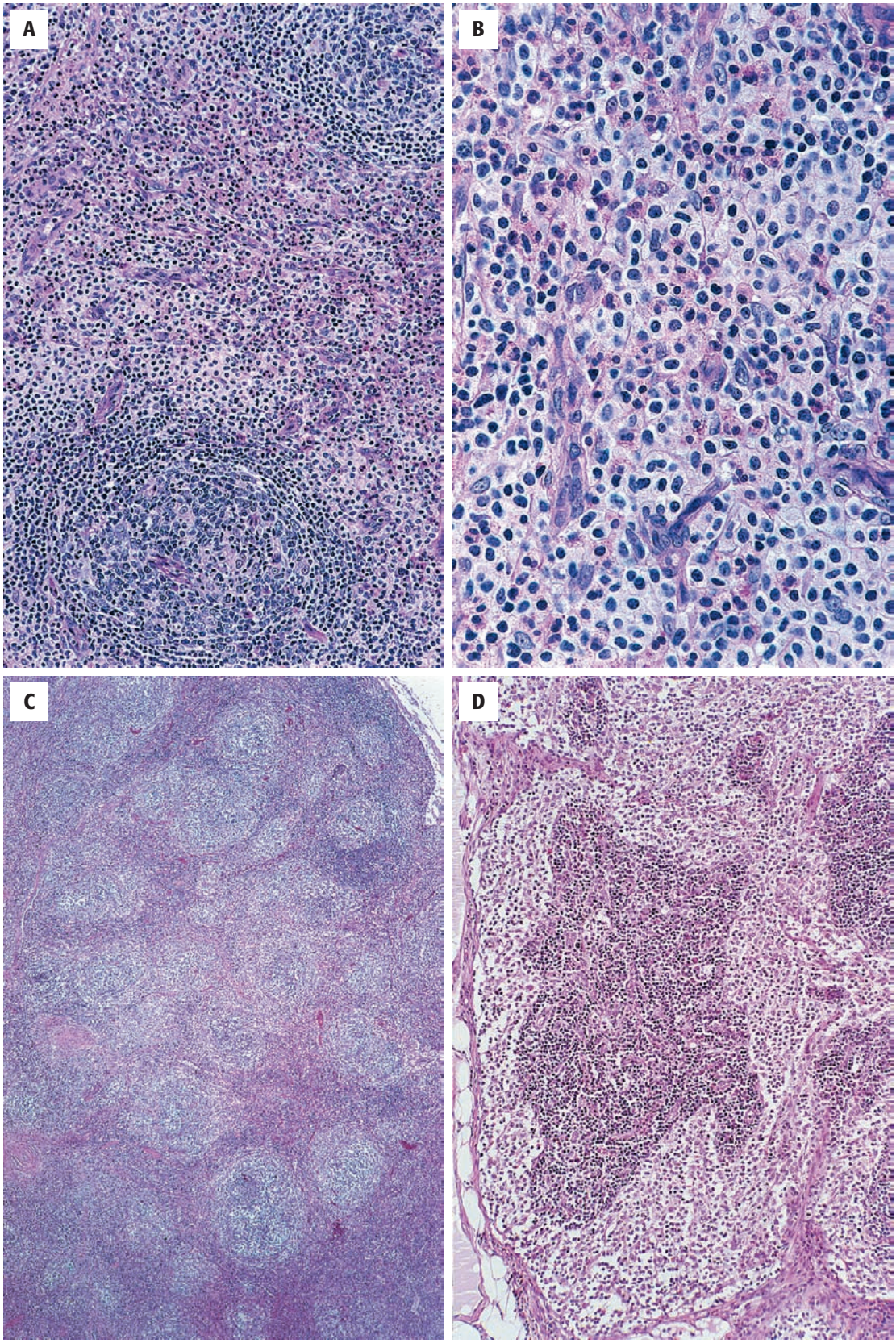



**FIGURE 20-13**

Histologic features of the lymph node in systemic mastocytosis. **A,** Lymph node section shows mixed cellular infiltration in the perifollicular and paracortical areas, associated with increased vascularity (H&E stain, original magnification ×250). Scattered aggregates of mast cells are present predominantly in the perifollicular regions. **B,** Tight mast cell aggregate surrounded by many eosinophils and vessels (H&E stain, original magnification ×400). The mast cells have abundant clear cytoplasm, a distinct cytoplasmic border, and oval or reniform nuclei with a delicate chromatin pattern. **C,** Lymph node section shows mast cell replacement of germinal centers, giving an appearance resembling a follicular lymphoma (H&E stain, original magnification ×96). **D,** A lymph node section from a patient with mast cell leukemia shows marked sinusoidal infiltration by atypical mast cells with abundant clear cytoplasm, giving an appearance resembling a sinus histiocytosis (H&E stain, original magnification ×160).

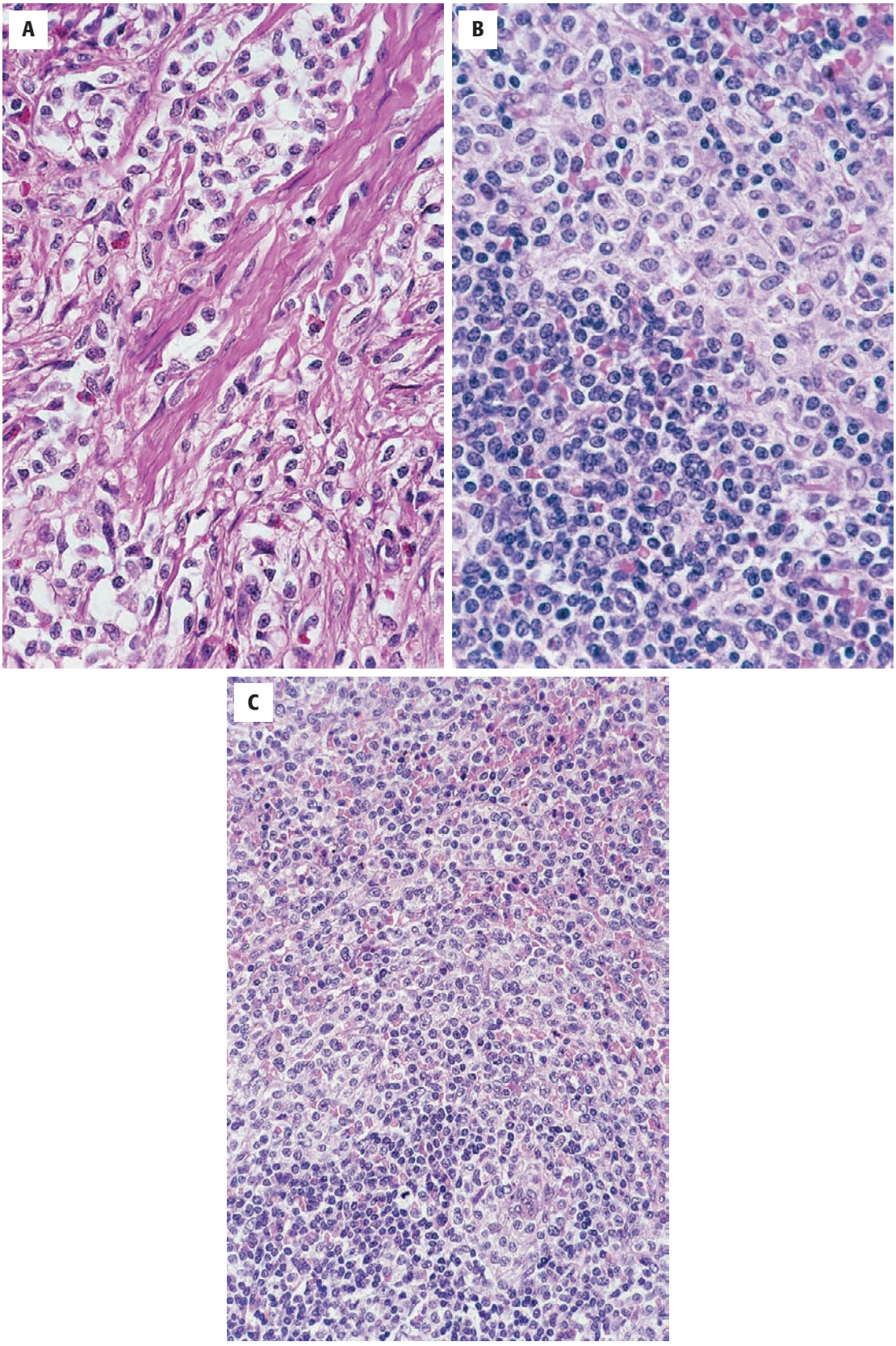



**FIGURE 20-14**

Histologic features of the spleen in systemic mastocytosis. **A,** High-power view of splenic section shows fibrosis and thickening of splenic trabeculae associated with mast cell infiltrates (H&E, original magnification ×400). **B,** Splenic white pulp shows clusters of mast cells with abundant clear cytoplasm located at the edge of the marginal zone of this splenic lymphoid follicle (H&E, original magnification ×400). **C,** Splenic section from a case with mast cell leukemia shows diffuse atypical mast cell infiltration in the red pulp and a small perivascular cluster of immature mast cells in the white pulp (H&E, original magnification ×240).

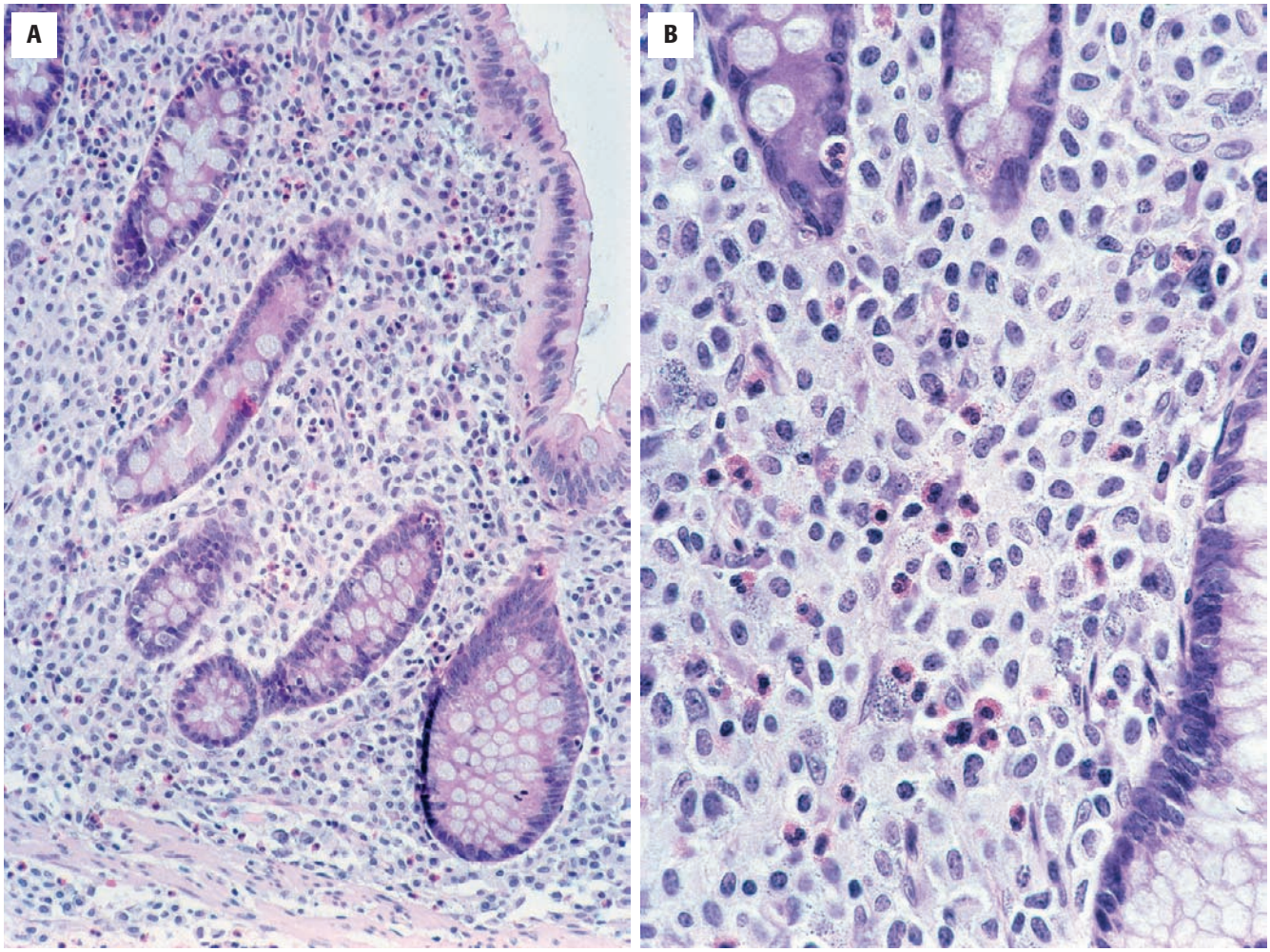



**FIGURE 20-15**

Histologic features of the gastrointestinal tract in systemic mastocytosis. **A,** Colonic section shows extensive mast cell infiltration in the lamina propria and in the submucosa (H&E stain, original magnification ×160). **B,** Colonic section shows extensive mast cell infiltration intermixed with a few scattered eosinophils in the lamina propria (H&E stain, original magnification ×400).

for the identification of normal and abnormal MCs. Subsequently, the diagnostic accuracy of mastocytosis has been vastly improved.

#### Cytochemical and Histochemical Stains

Cytochemical and histochemical stains of bone marrow aspirate smears or biopsy specimens are easy and helpful tools for the diagnosis of mastocytosis (see Table 20-3). Wright-Giemsa stain can highlight the basophilic granules of MCs (see Figure 20-4, A, C) and the accompanying eosinophils. MC granules can be visualized readily with toluidine blue stain because of their metachromatic properties. MCs contain granules rich in aminocaproate esterase (see Figure 20-4, B and D) and chloracetate esterase (see Figure 20-3, B), which can be demonstrated easily in smears or paraffin sections. Aminocaproate esterase–positive granules are found only in MCs, whereas chloracetate esterase-positive granules are also present in neutrophils. Of note, decalcification and conventional tissue processes can diminish or totally abolish the metachromatic granules of MCs. They can be preserved better in plastic embedding without decalcification.

#### Immunohistochemical Stains

Tryptase, KIT (CD117), and CD25 immunohistochemical (IHC) stains are recommended for the initial workup for SM (see Table 20-3); however, tryptase is not specific for MCs. Abnormal basophils in chronic myelogenous leukemia and acute basophilic leukemia or myeloblasts in rare acute myeloid leukemia may show positive IHC staining for tryptase. MCs can be distinguished from basophils by their distinctive strong CD117 expression. Although myeloblasts also may express CD117, their expression is much weaker than that of MCs. The aberrant CD2 or CD25 expression, or both, by neoplastic MCs may be used to differentiate them from normal bone marrow MCs. The CD25 expression

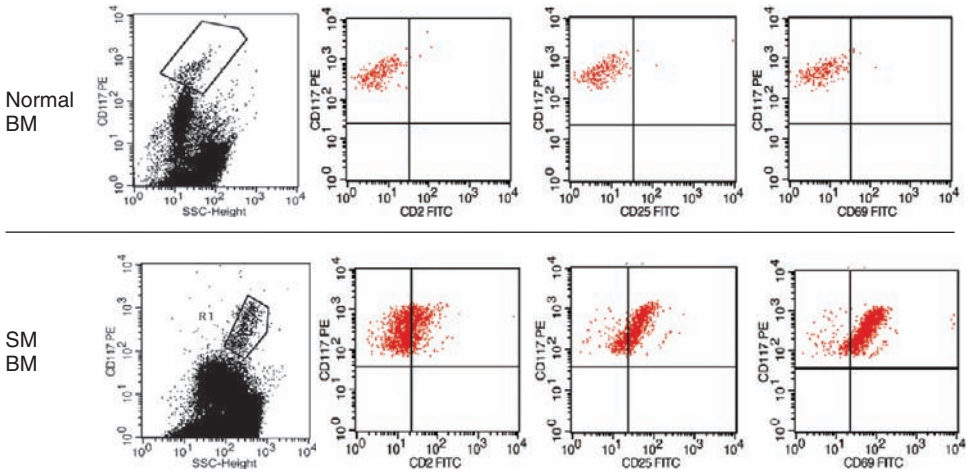


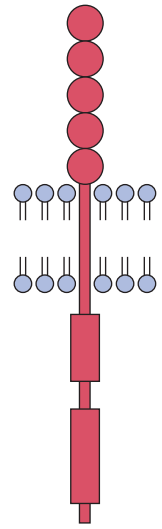

**FIGURE 20-16**

Flow cytometric immunophenotyping of mast cells in normal bone marrow and in bone marrow with systemic mastocytosis. Mast cell population is first gated based on the bright CD117 expression and distinct side scatter characteristics. Surface expressions of CD2, CD25, and CD69 are then examined. Neoplastic mast cells aberrantly express CD2, CD25, and brighter CD69.

is found in more than 80% of SM cases. Conversely, the CD2 expression is usually weaker and less frequent than CD25. The interpretation of CD2 immunohistochemical stain sometimes may be hindered by background T cells. Nuclear phosphorylated STAT5 has been shown to be present in MCs in patients with SM, likely reflecting the abnormal KIT tyrosine kinase activity central to the pathogenesis of SM.

### Flow Cytometric Immunophenotyping

Flow cytometry has the advantage of simultaneously analyzing multiple MC surface markers using various fluorescent-conjugated monoclonal antibodies. Although bone marrow MCs are rare (normal range, 0.002% to 0.008%), they can be distinguished immunophenotypically from other myeloid precursors by their strong CD117 and negative CD34 surface expressions. Bone marrow MCs also invariably express CD9, CD11c, CD29, CD33, CD43, CD44, CD45, CD49d, CD49e, CD51, CD54, and CD71 and the high-affinity immunoglobulin E receptor. Both CD2 and CD25 have been shown to be constantly absent in normal and reactive MCs. Conversely, neoplastic MCs from SM show unique immunophenotypic characteristics, including aberrant surface expression of CD2 and CD25 antigens, together with abnormally high levels of the activation-related antigens CD35, CD63, and CD69 (Figure 20-16). Therefore a minimal standard flow cytometry panel should include CD2, CD25, and CD117. The MC population can be first gated by its bright CD117 and typical forward–side light scatter characteristics. CD2 and CD25 full or partial expression by MCs is considered evidence of an aberrant MC clone (see Figure 20-16). CD2 expression usually is weaker and less frequent than CD25 expression; consequently, a more sensitive fluorochrome, such as phycoerythrin, is preferred for CD2.



| Domains | Amino Acid Residues |
|---|---|
| N-terminal | 1-22 |
| Extracellular | 23-520 |
| Transmembrane | 521-543 |
| Juxtamembrane | 544-581 |
| Tyrosine kinase 1 | 582-684 |
| Tyrosine kinase 2 | 762-937 |
| C-terminal | 938-976 |

**FIGURE 20-17**

KIT protein domains and corresponding amino acid sequences.

### Molecular and Cytogenetic Features

KIT and PDGFRA/B belong to the class III receptor tyrosine kinases characterized by 5 immunoglobulin-like domains in the extracellular ligand-binding region, a sole transmembrane domain, a juxtamembrane domain, two intracellular kinase domains divided by a kinase insert (KI) domain, and a c-terminal domain (Figure 20-17). The *KIT* and *PDGFRA* genes are located on chromosome 4q11-q13; the *PDGFRB* gene is located on chromosome 5q31-q33.

### Activating Mutation of *KIT* in Mastocytosis

KIT is expressed in MCs, myeloblasts, granulocytic and erythroid precursors, GI stromal cells, melanocytes, and germ cells. Its ligand SCF is expressed by fibroblasts, endothelial cells, bone marrow stromal cells, and cells of the reproductive system. Binding of SCF to the surface domain of KIT induces receptor dimerization, trans-phosphorylation, and tyrosine kinase activation and subsequently leads to MC differentiation, proliferation, and migration.

The identification of SCF as the essential growth factor for MCs led to the discovery of the pathogenetic role of KIT in mastocytosis. In HMC-1, a human MCL cell line, two *KIT* mutations have been identified—one at codon 560 (juxtamembrane domain), causing substitution of glycine for valine, and another at codon 816 in the enzymatic pocket/activation loop domain, causing substitution of valine for asparagine (Table 20-4). The enzymatic pocket-type mutations at codon 816 induce ligand-independent phosphorylation of KIT and are termed *activating mutations,* recognized by the 2008 WHO classification scheme as a minor criterion. In contrast, juxtamembrane domain mutations cause disruptions of the regulation of phosphorylation and kinase activity of KIT and are termed *regulatory mutations*.

Soon after these discoveries, various mutations of *KIT* were identified in mastocytosis. Among the mutations found, the most common is the mutation of asparagine to valine at codon 816 (*KIT* D816V) in more than 90% of SM cases. This mutation is found in most patients with ISM and in a subset of patients with ASM, SM-AHNMD, or MCL, but it is less common in typical pediatric cases of CM. In general, the KIT mutants at the activating loop are resistant to imatinib mesylate (a tyrosine kinase inhibitor; see Table 20-4). Interestingly, the F522C or I817V mutation–related SMs usually have round neoplastic MCs and extensive bone marrow involvement. Because of their morphologic resemblance to normal MCs, they are currently considered as a variant of SM, well-differentiated SM.

In suspected SM, bone marrow aspirate or cells detached from marrow smears, or paraffin-embedded biopsy material can be used to test for *KIT* mutations. Although peripheral blood can be used for *KIT* mutation analysis, its sensitivity is less than that of bone marrow aspirate, especially in ISM. Commonly used molecular

**TABLE 20-4**

**Summary of *KIT* Mutations in Mastocytosis and Non–Mast Cell Neoplasms**

| Function Domain | Exon | *KIT* Mutation | Nature of Mutation | Mastocytosis Variant (Frequency %) | Non–Mast Cell Myeloid Neoplasms | Non-Hematologic Neoplasms (Frequency %) | Imatinib Mesylate |
|---|---|---|---|---|---|---|---|
| Extracellular | 8 | del D419 | Unknown | Familial SM (<5) | AML with inv(16) | Familial GIST (<5) | Sensitive |
| | 9 | K509I | Unknown Familial SM (<5) | | NA | | Sensitive |
| Transmembrane | 10 | F522C | Regulatory | WDSM (<5) | NA | NA | NA |
| | 10 | A533D | Regulatory | Familial CM (<5) | NA | NA | Resistant |
| Juxtamembrane | 11 | V559I | Regulatory | SM (<5) | NA | NA | Resistant |
| | 11 | V560G | Regulatory | SM (<5) | NA | NA | NA |
| Activation loop | 17 | R815K | Unknown | Pediatric CM (<5) | NA | NA | NA |
| | 17 | D816V | Activating | SM (>90) | AML with inv(16) or t(8;21) | Germ cell tumor (3-10) | Resistant |
| | 17 | D816Y | Activating | SM (<5) | AML | Germ cell tumor (<5) | Resistant |
| | 17 | D816H | Unknown | SM-AML (<5) | AML | Germ cell tumor (<5) | Resistant |
| | 17 | D816F | Activating | SM (<5) | NA | NA | Resistant |
| | 17 | I817V | Unknown | WDSM (<5) | NA | NA | NA |
| | 17 | InsV815-I816 | Unknown | SM (<5) | NA | NA | NA |
| | 17 | D820G | Unknown | SM (<5) | NA | NA | NA |
| | 17 | E839K | Inactivating | CM (<5) | NA | NA | NA |

*AML,* Acute myeloid leukemia; *CM,* cutaneous mastocytosis; *GIST,* gastrointestinal stromal tumor; *NA,* not applicable; *SM,* systemic mastocytosis; *WDSM,* well-differentiated systemic mastocytosis.

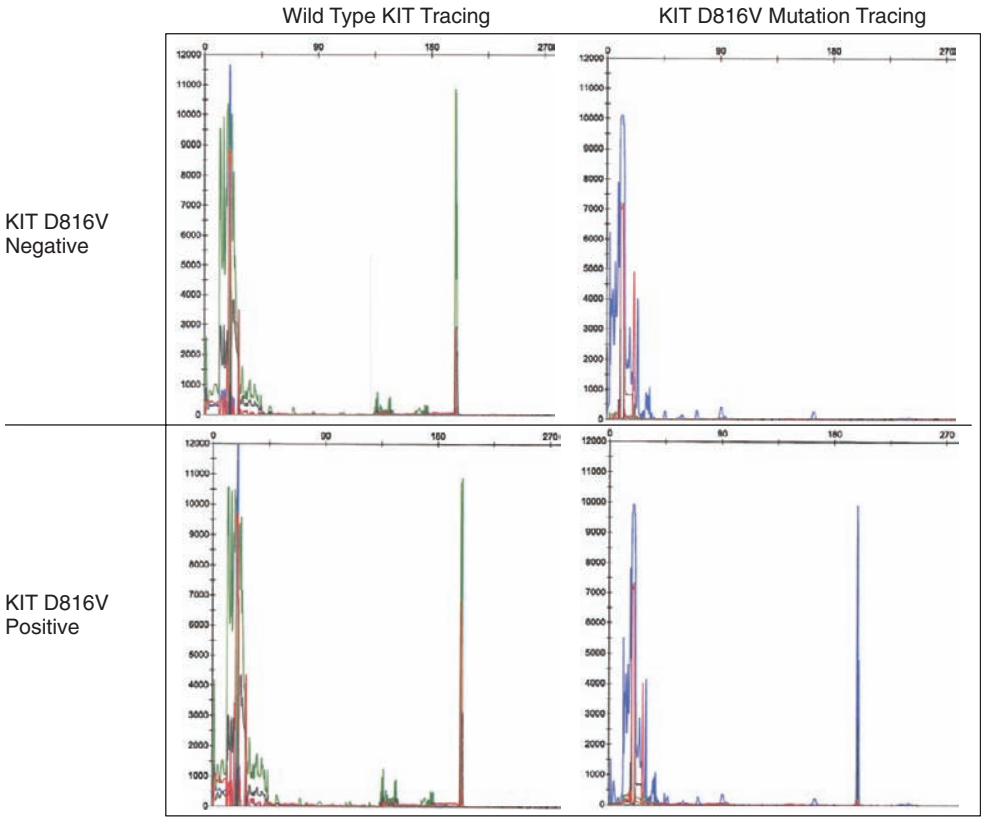



**FIGURE 20-18**

*KIT D816V* mutation analysis with allele-specific oligonucleotide polymerase chain reaction with fragment analysis (on an ABI 3130xl genetic analyzer). The *left panels* (green) are the tracings of wild type *KIT*. The *right panels* (blue) are tracings of *KIT D816V* mutation. The *upper panels* are tracings of a normal negative control bone marrow aspirate sample. The *lower panels* are tracings of a bone marrow aspirate specimen of a patient with systemic mastocytosis.

techniques to detect the most frequent D816V mutations include real-time polymerase chain reaction (PCR) with restriction fragment length polymorphism, peptide nucleic acid–mediated PCR, and allele-specific PCR (Figure 20-18). Negative results on D816V mutation analysis can be caused by sampling issues resulting from patchy bone marrow involvement or SM with different *KIT* mutations, especially in more aggressive forms. For indolent SM, *KIT* sequencing may be indicated to identify rare variants that could potentially be sensitive to imatinib mesylate. Importantly, *KIT* mutations are not specific for mastocytosis because they can be present in other hematologic and nonhematologic malignancies, such as GI stromal tumors, melanomas, and germ cell tumors (see Table 20-4).

### FIP1L1-PDGFRA Fusion Tyrosine Kinase in Hypereosinophilic Syndrome and Mastocytosis

Platelet-derived growth factor (PDGF) was originally purified from platelets. Later, it was found to be produced by various cells, including endothelial cells, vascular smooth muscle cells, activated monocytes and macrophages, osteoclasts, erythroblasts, and plasma cells, and to exert its effects on mesenchymal cells, such as fibroblasts. Two different PDGF chains (A and B) form three different PDGF dimeric isoforms—the AA and BB homodimers and the AB heterodimer. Similarly, two different PDGF receptors exist: the α receptor (PDGFRA) binds all the PDGF isoforms, and the β receptor (PDGFRB) binds only PDGF-BB. The binding of the ligand induces dimerization of α or β subunits, or both, leading to three possible receptor dimers. Dimerization is a prerequisite for receptor autophosphorylation and kinase activation, resulting in the signal being transduced to the interior of the target cell by a series of tightly regulated molecular interactions.

The recent identification of FIP1-like-1–PDGFRA *(FIP1L1-PDGFRA)* fusion in cases of hypereosinophilic syndrome, chronic eosinophilic leukemia, and SM adds to the growing list of activated fusion tyrosine kinases linked to the pathogenesis of chronic myeloproliferative disorders. The unifying presence of the *FIP1L1-PDGFRA* fusion has resulted in these cases being defined as myeloid and lymphoid neoplasms with *FIP1L1-PDGFRA* in the 2008 WHO classification. This fusion results in a gain-of-function fusion protein deriving from a cryptic interstitial deletion of genes rather than a reciprocal chromosomal translocation. This interstitial chromosomal deletion involves an

approximately 800-Kb DNA segment, including the cystein-rich hydrophobic domain 2 locus at 4q12. Demonstration of the loss of one of the two cystein-rich hydrophobic domain two alleles with interphase fluorescence in situ hybridization can be used as a surrogate for the *FIP1L1-PDGFRA* fusion.

Besides eosinophilia, MC proliferation is a distinct feature of *FIP1L1-PDGFRA*–associated myeloproliferative neoplasm. The MCs may be loosely distributed or may be in loose, noncohesive clusters. Occasional clusters of spindle-shaped and CD25-expression atypical MCs develop with features that are indistinguishable from SM. Patients with *FIP1L1-PDGFRA*-positive eosinophilic disorder are responsive to low-dose imatinib mesylate treatment. Recently, various *PDGFRA* translocations (e.g., *KIF5B-PDGFRA, CDK5RAP2-PDGFRA, ETV6-PDGFRA*, and *BCR-PDGFRA*) have been reported.

## Differential Diagnosis

The pathologic lesions of SM are most distinctive in the bone marrow and typically consist of clusters or aggregates of MCs (mononuclear cells with abundant cytoplasm) on sections stained with H&E. These lesions usually are associated with various degrees of eosinophilic infiltrates. In some cases, especially those with *FIP1L1-PDGFRA* fusion, the intense eosinophilia can overshadow the MC infiltrates, making it difficult to distinguish the process from hypereosinophilic syndrome. In this situation, the tryptase immunostain is helpful in highlighting the atypical MC infiltrates that frequently are distributed in loose aggregates rather than tight clusters (see Figure 20-8, B).

Monocytic nodules, also known as *plasmacytoid dendritic cell nodules,* in myeloproliferative/myelodysplastic syndrome may appear as aggregates of mononuclear cells, resembling MC clusters. However, such lesions usually do not have eosinophilic infiltrates and should not immunostain positively for tryptase.

In cases of MCL, the heterogeneous pattern seen in cytoplasmic granulation, nuclear morphologic features, and interstitial infiltration of the bone marrow might sometimes resemble that of other hematologic malignancy, such as acute myeloid leukemia, acute basophilic leukemia, and hairy cell leukemia. Cytochemical and IHC studies (see Table 20-3) are helpful in the differential diagnosis of these entities. Because the pathologic manifestations of SM in the spleen and lymph node include a broad spectrum of histologic findings, many entities must be considered in the morphologic differential diagnosis.

In the spleen, SM can be confused with a myeloproliferative disorder or hairy cell leukemia, especially when diffuse red pulp infiltration occurs in the spleen of patients with MCL. Furthermore, MCs also possess tartrate-resistant acid phosphatase, a diagnostic tool for hairy cell leukemia. Differential diagnosis of SM requires a constellation of MC-specific IHC studies (see Table 20-3).

SM infiltrates in lymph nodes can mimic many malignant conditions. They may resemble T-cell lymphoma because of the paracortical distribution, the clear cytoplasm of the MCs in some cases, and the associated vascular proliferation and eosinophilia (see Figure 20-13, A, B). However, MCs in SM infiltrates are relatively monomorphous, in contrast to neoplastic T cells, which often have a heterogeneous cellular composition with variable proportions of small, intermediate, and large lymphoid cells. When MC infiltrates replace the lymphoid follicles, the pattern can give a striking resemblance to a follicular hyperplasia or lymphoma (see Figure 20-13, C). However, careful examination of the cytologic features of the cells in the follicles shows that the MC cytoplasm is more abundant, is granular, and stains metachromatically. The presence of associated eosinophilia and extramedullary hematopoiesis can be helpful clues that the process is not a follicular hyperplasia or lymphoma. The marked sinusoidal infiltration by MCL in a lymph node needs to be distinguished from sinus histiocytosis (see Figure 20-13, D). This distinction may be difficult morphologically because the hypogranular MCs closely resemble histiocytes. However, histochemical staining for chloracetate esterase or IHC staining for CD117, tryptase, or CD25 can result in the characteristic staining of the MCs in the sinuses. MCs also can resemble Langerhans histiocytes because of their eosinophilic cytoplasm, occasional folded nuclei, and frequent association with eosinophils. However, Langerhans cells have a more characteristic nuclear groove and can be distinguished from MCs by S100 and CD1a immunostains.

## Prognosis and Therapy

CM in children usually regresses spontaneously before or during puberty. In adults, CM is often associated with SM, which in adults is markedly heterogeneous in its clinical display, bone marrow histologic appearance, underlying molecular lesion, and treatment response. Patients with ISM usually have a normal life expectancy. Infrequently, ISM progresses into a more aggressive disease. In aggressive SM without associated hematologic disorder, increased bone marrow MC and eosinophil content, an elevated serum alkaline phosphatase level, anemia, and hepatosplenomegaly portend a poor prognosis. MCL and MCS are clinically aggressive disorders with no effective therapy. Patients with MCL or MCS usually have an overall survival of weeks

to months. By comparison, the clinical course of SM-AHNMD is usually dictated by the associated hematologic disorder.

In general, treatment of mastocytosis is indicated when patients have MC MRSs, UP, or organ dysfunction from aggressive SM. MRSs and UP can accompany both indolent and aggressive SM but occur less frequently in SM-AHNMD. Cytoreductive therapy is usually reserved for aggressive SM and not for indolent SM, MRSs, or UP.

Noncytoreductive drugs for MRSs include oral histamine$_1$ and histamine$_2$ antagonists, as well as cromolyn sodium. In addition, patients with a propensity to vasodilatory shock should wear a medical alert bracelet and carry an epinephrine autoinjector for self-administration of subcutaneous epinephrine. For UP, both intralesional and topical corticosteroids produce a substantial response. Oral-based psoralen ultraviolet A photochemotherapy is also effective in UP.

Based on recent publication, categorizing aggressive SM as either associated or not associated with blood eosinophilia is therapeutically relevant. Patients who have SM with associated eosinophilia may carry *FIP1L1-PDGFRA* translocation. These patients usually have a durable response to low-dose (100 mg/day) imatinib mesylate therapy. In contrast, none of the patients with *FIP1L1-PDGFRA*–negative SM associated with eosinophilia responded to treatment with imatinib mesylate, even with an increased dose of 400 mg/day.

For the other SM patients who do not have *FIP1L1-PDGFRA*, interferon α continues to be the initial drug of choice. Cladribine (2-chlorodeoxyadenosine), a purine nucleoside analogue, is an alternative option for interferon-resistant disease. Currently, molecularly targeted therapies are being explored, including dasatinib (a dual SRC/ABL inhibitor), PKC412 (a multitargeted kinase inhibitor), AP23464 (an adenosine triphosphate–based inhibitor), MLN518 (a quinazoline-based inhibitor), indolinone compounds, OSI-930 (a thiophene-based inhibitor), and nilotinib (an aminopyrimidine-based inhibitor). These new targeted therapeutic compounds may have future roles in the treatment of mastocytosis.

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The complete reference list is available online at www.expertconsult.com.

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21

# Plasma Cell Neoplasms and Related Disorders

Pei Lin, MD

## INTRODUCTION

Monoclonal expansion of plasma cells and their precursors gives rise to a spectrum of plasma cell neoplasms. These include monoclonal gammopathy of undetermined significance (MGUS; 67%), plasma cell myeloma (multiple myeloma; 14%), solitary plasmacytoma of bone or extraosseous tissue (3%), primary amyloidosis (AA; 9%), and immunoglobulin (Ig) chain deposition diseases (less than 1%). Plasma cell myeloma is the prototype of plasma cell neoplasm and occurs as a generalized disease involving primarily bone marrow; it is associated with serum monoclonal protein and lytic bone lesions. In this chapter, myeloma and its variants are separately described from other types of plasma cell neoplasms.

## PLASMA CELL MYELOMA

### Clinical Features

Plasma cell myeloma is a disease of older people and is rare in people younger than 40 years (less than 2% are younger than 40 years). The median age is approximately 69 years. The disease also has a predilection for blacks compared with whites (2:1). The incidence is approximately 5 males and 3 females per 100,000 among whites, and 10 males and 7 females per 100,000 among blacks. The incidence is approximately 2 per 100,000 in Asians.

Presenting symptoms are variable and depend on the extent of tumor burden and activity. Most patients have bone pain resulting from tumor infiltration and pathologic fractures. Fatigue and weakness secondary to anemia, as well as renal failure and recurrent infection (more than two episodes in 12 months) are also common. Less frequent symptoms include hyperviscosity syndrome, hypercalcemia, and spinal cord

**PLASMA CELL MYELOMA—FACT SHEET**

**Definition**
- Monoclonal proliferation of plasma cells or their precursors leading to tumor formation, excess immunoglobulin monoclonal protein, and osteolytic bone disease, symptomatic or asymptomatic

**Incidence and Location**
- One percent of all malignancy and 10% of hematologic tumors
- Nearly all are preceded by a phase of MGUS
- 4.5 per 100,000 per year in the United States
- More than 20,000 new cases are diagnosed each year
- Predominantly involves bone marrow, but may occur at extramedullary sites

**Morbidity and Mortality**
- Plasma cell myeloma is incurable
- Median survival averages 3 years using standard therapy, 5 years with dose-intensive therapy and stem cell transplantation, and 7 years with immunomodulatory drugs and proteasome inhibitor
- Rare patients with low tumor burden survive 10 to 15 years

**Gender, Race, and Age Distribution**
- Male predominance (male:female = 2:1)
- Incidence is higher in blacks (2:1)
- Mostly elderly; median age at onset 65 years, but 2% to 4% occurs in people younger than 40 years

**Clinical Features**
- Bone pain, anemia, recurrent infection, hypercalcemia, hyperviscosity, and renal failure; spinal cord compression (10%); peripheral neuropathies caused by amyloidosis
- Asymptomatic myeloma diagnosed incidentally, but with high risk for progression to symptomatic myeloma

**Radiologic Features**
- Punched-out osteolytic lesions in vertebral bodies, skull, ribs, humerus, and femur

**Prognosis and Therapy**
- Poor prognostic indicators include high serum β2M, low serum albumin level, high PCLI or Ki-67, hypodiploidy, t(4;14), t(14;16), deletion of chromosome 13 and del17/p53
- Conventional chemotherapy agents or novel agents with or without stem cell transplantation

compression as a result of vertebral body fracture or epidural tumor mass.

The diagnosis of myeloma is based on clinical and laboratory findings. Diagnosis generally requires 10% or more monoclonal plasma cells in the bone marrow or a plasmacytoma proved by biopsy. However, patients who meet the criteria may be asymptomatic and in stable condition for years without therapy. Conversely, a small subset of patients who have symptomatic and progressive disease show 10% or less of monoclonal plasma cells in the bone marrow. Based on the proposals of International Myeloma Working Group, the current World Health Organization Classification describes two types of myeloma: asymptomatic or smoldering myeloma (SMM) and symptomatic or active myeloma. In the presence of myeloma-related end organ damage and tissue impairment—defined as hypercalcemia, renal insufficiency, anemia, or bone lesions—the diagnosis of symptomatic myeloma can be established without a minimal level of serum M-protein or bone marrow plasmacytosis. Diagnosis of smoldering myeloma still requires a minimum of 10% of monoclonal plasma cells or 30 g/L of M-protein in the serum, or both, to distinguish it from MGUS. Smoldering myeloma is usually identified incidentally during routine physical examination or laboratory testing and is monitored closed for progression to symptomatic myeloma (Table 21-1).

Solitary plasmacytomas of the bone are tumors of neoplastic plasma cells localized to a single bone and represent 3% of plasma cell neoplasms. The tumor primarily affects the axial skeleton, and the thoracic vertebrae are involved most frequently. Most patients have localized bone pain, and some may have neurologic symptoms caused by spinal cord or root compression. The patients are usually free of anemia, hypercalcemia, and renal insufficiency. Serum or urine monoclonal protein is detectable in 60% of patients, usually at low levels. Most patients (70%) eventually develop systemic disease at a median of 2 to 4 years.

Solitary extramedullary plasmacytomas tend to be localized to the head and neck regions, where 80% of cases occur, although they can occur in many other parts of the body such as the gastrointestinal tract, central nervous system, and skin. The majority of the tumors do not produce detectable serum paraprotein (less than 25%) and the tumors rarely spread (less than 30%).

It is possible that some of these tumors represent extranodal marginal zone lymphoma with marked plasmacytic differentiation. The 10-year survival rate is 70% compared with 40% in solitary plasmacytoma of the bone. Approximately 15% of patients may subsequently develop myeloma.

The diagnosis of solitary plasmacytoma of the bone or soft tissue is established only after extensive radiologic imaging, especially magnetic resonance imaging, to exclude occult systemic disease. Risk of local recurrence and dissemination is high in patients with solitary plasmacytoma of the bone with persistent serum monoclonal proteins after local radiation therapy.

Plasma cell leukemia (PCL), either primary or secondary, is defined as an absolute plasma cell count of more than $2 \times 10^9$/L or plasma cells constituting more than 20% of the total leukocyte count in the peripheral blood. Primary PCL represents initial presentation of disease and constitutes less than 5% of newly diagnosed cases of myeloma. The patients are younger than myeloma patients, with a median age of 55 years at diagnosis. Secondary PCL is essentially peripheralized myeloma at the terminal stage of disease as a result of excessive tumor growth, representing 1% of myeloma cases. Primary PCL is more common than secondary PCL, constituting 60% of leukemic cases, and it is an aggressive disease frequently associated with high tumor burden, extramedullary dissemination, and adverse prognosis with a median survival of 7 to 11 months. Hepatosplenomegaly is more common (50%) and lytic

**TABLE 21-1**

**World Health Organization Diagnostic Criteria of Myeloma and MGUS**

***Symptomatic Plasma Cell Myeloma***

M-protein in serum or urine, or both
Bone marrow (clonal) plasma cells or plasmacytoma
Related organ or tissue impairment (end organ damage, including bone lesions)
Myeloma-related organ or tissue impairment (end organ damage):
- Serum calcium >0.25 mmol/L (1 mg/dL) above the upper limit of normal or >2.75 mmol/L (11 mg/dL)
- Renal insufficiency: creatinine >173 mmol/L (1.96 mg/dL)
- Anemia: hemoglobin 2 g/dL less than the lower limit of normal or hemoglobin <10 g/dL
- Bone lesions: lytic lesions or osteopenia with compression fractures
- Other: symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections (>2 episodes in 12 months)

***Asymptomatic (Smoldering) Myeloma***

M-protein in serum >30 g/L
Bone marrow clonal plasma cells >10%
No related organ or tissue impairment (including bone lesions) or symptoms

***Monoclonal Gammopathy of Undetermined Significance***

M-protein in serum <30 g/L
Bone marrow clonal plasma cells <10% and low level of plasma cell infiltration in a trephine biopsy
No evidence of other B-cell proliferative disorders
No related organ or tissue impairment (including bone lesions)

***Light Chain Monoclonal Gammopathy of Undetermined Significance****

Abnormal FLC ratio (<0.26 or >1.65)
Increased level of the appropriate involved light chain (increased κ FLC in patients with >1.65 and increased λ FLC in patients in ratio <0.26)
No immunoglobulin heavy chain expression on immunofixation

*Although not described in the World Health Organization classification, these criteria have been proposed for the diagnosis of light chain monoclonal gammopathy of undetermined significance.

bone lesions are less common (18%) in primary PCL, compared to secondary PCL (17% and 70%, respectively). Hypercalcemia, cytopenia, and renal insufficiency are more common in both primary and secondary PCL compared with myeloma.

Osteosclerotic myeloma is a rare form of myeloma (less than 1%) usually seen in POEMS (polyneuropathy, organomegaly, endocrinopathy, M component, and skin changes) syndrome. The bone changes are characterized by osteosclerosis rather than lytic lesions. The monoclonal protein is usually of IgA λ type and generally less than 3 g/dL. Lymph nodes may show Castleman disease.

## Radiologic Features

### X-Ray

A skeletal survey is essential in patients suspected of myeloma. The classic radiographic image of myeloma is that of multiple, lytic, well-circumscribed lesions within the skull, spine, and pelvis (Figure 21-1). In solitary plasmacytoma of the bone, the lesions appear to be a localized expansion of a single bone and occasionally are associated with a soft-tissue mass. In POEMS syndrome, sclerotic lesions may be observed on radiographs.

### Computed Tomography

Computed tomography (CT) is usually not required because the standard skeletal surveys usually detect most of the lesions. However, CT may be useful in patients with suspected myeloma and in whom the skeletal survey is negative. CT-guided percutaneous biopsy is useful in patients with suspected osseous or extramedullary plasmacytomas.

### Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is superior to CT in detecting soft-tissue lesions; therefore it is particularly useful in assessing spinal cord compression and extramedullary lesions to determine the extent of involvement. It is critical that thorough staging with magnetic resonance imaging be performed in all patients with solitary plasmacytoma of bone to rule out multifocal disease. Magnetic resonance imaging of the spine and pelvis are recommended to detect occult lesions or identify patients with asymptomatic myeloma for evidence of early progression to symptomatic myeloma. The typical appearance of a myeloma infiltrate is a low signal intensity on T1-weighted images, which becomes high

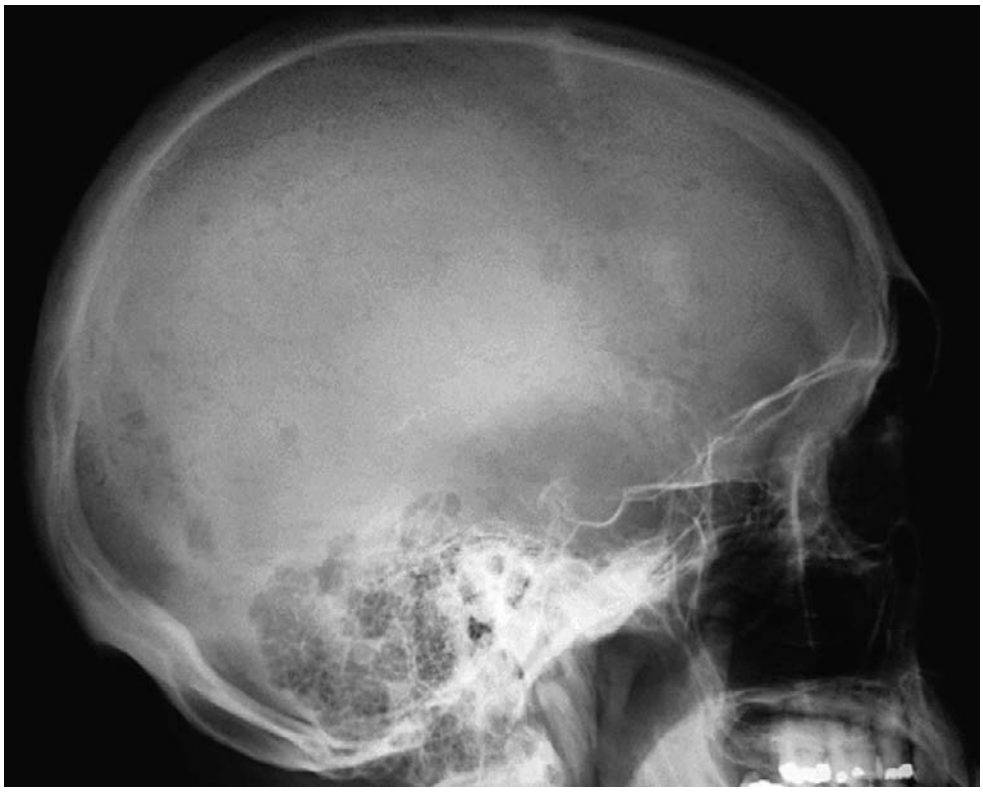

**FIGURE 21-1**
Radiographic image of the skull. Punched-out osteolytic lesions characteristic of multiple myeloma are shown.

on T2-weighted sequences. Myeloma lesions also tend to enhance with gadolinium administration. Whole-body fluorodeoxyglucose/positron emission tomography (PET) imaging is also not required, but it can be used to detect occult lesions to exclude MGUS.

## Pathologic Features

Plasma cell enumeration and cytologic evaluation remain essential for classification and prognostication. Bone marrow aspirate tends to underestimate the extent of disease, especially in cases associated with marked fibrosis. A bone marrow biopsy should be performed, at least in the initial evaluation, to assess the extent and pattern of infiltration as well as evidence of myeloma-associated bone changes, amyloid deposition, and fibrosis. Symptomatic myeloma usually has greater than 30% plasma cells, whereas SMM and MGUS usually have low numbers of plasma cells that tend to be dispersed in the interstitium or form small clusters.

Most patients usually exhibit systemic disease, and a biopsy of the iliac crest yields diagnostic material. In a small number of patients, the infiltrate may be patchy, and a negative biopsy result does not exclude the possibility of myeloma. In these patients, a repeated biopsy of different sites is indicated.

**PLASMA CELL MYELOMA—PATHOLOGIC FEATURES**

**Gross Findings**
- Fish flesh, hemorrhagic tumor

**Microscopic Findings**
- Atypical or immature plasma cells proliferate in large clusters in the bone marrow forming space-occupying lesions associated with increased osteoclastic activity and intramedullary fibrosis; may be associated with amyloid deposition

**Ultrastructural Findings**
- Prominent Golgi region and dilated rough endoplasmic reticulum
- Cytoplasmic crystalline inclusions

**Fine-Needle Aspiration Biopsy Findings**
- Atypical or immature plasma cells

**Immunohistochemical Findings**
- $CD138^+$, $CD38^+$, κ or λ immunoglobulin light chain; $CD56^+$ (55% to 60%) or CD79a (50% to 70%); CD117+ (20% to 30%) or $CD20^{-/+}$ (20%), CD45dim/$^-$, $PAX5/BSAP^-$

**Differential Diagnosis**
- Reactive plasmacytosis
- Acute monoblastic leukemia or idiopathic myelofibrosis
- Non-Hodgkin lymphoma or leukemia with plasmablastic/plasmacytic/plasmacytoid differentiation
- Epithelial tumors

## Gross Findings

The involved bone shows fish-flesh mass with hemorrhage or necrosis.

## Microscopic Findings

### Bone Marrow

Plasma cell enumeration is performed using a differential count of 200 to 500 cells. Normal plasma cells constitute 1% to 4% of nucleated cells in the marrow and are mature Marschalko type with abundant basophilic cytoplasm, perinuclear hof, and a "spoke-wheel" nuclear pattern.

The neoplastic plasma cells often show a variable degree of immaturity or atypia. Asynchronous maturation of the nuclear and cytoplasm, dispersed chromatin, a high nuclear and cytoplasmic ratio, and prominent nucleoli are features of immaturity. Atypia usually manifests as bizarre nuclear forms and shapes and marked variation in nuclear size. These features are strong evidence of malignancy even if immunoglobulin light chain analysis fails to demonstrate monoclonality as in cases of nonsecretory myeloma. Binucleated or occasionally trinucleated forms may be observed in reactive conditions and are not necessarily indications of malignancy.

A variety of cytoplasmic inclusions may form because of the accumulation of cytoplasmic immunoglobulin, sometimes forming crystalline rods. Russell bodies are the most common cytoplasmic inclusions. Cells containing inclusions are described as grape cells, flaming cells, Mott cells, Gaucher-like cells, and thesaurocytes. Dutcher bodies are nuclear inclusions. In rare cases of monoclonal gammopathies (including multiple myeloma, MGUS, and lymphoplasmacytic lymphoma) crystalline material can be found also in histiocytes—so-called crystal storing histiocytosis. The crystalline material has been shown to occur overwhelmingly in plasma cell neoplasms expressing immunoglobulin κ light chain.

Two grading systems have been proposed to describe the spectrum of plasma cell morphology in myeloma: the Greipp system and the Bartl system. In the Greipp system (Table 21-2), the plasma cells are divided into four subgroups: mature, intermediate, immature, and plasmablastic. In the Bartl system, six subtypes are described, as shown in Table 21-3. Examples of different Bartl grade tumors are illustrated in Figure 21-2. The Bartl grading system also described six patterns of

**TABLE 21-2**

**Greipp Plasma Cell Grading System**

***Mature Type***

Dense chromatin clumping
Nucleus <8 μm
Nucleolus <1μm
Cytoplasm well developed
Nucleus eccentrically placed with a prominent hof

***Intermediate Type***

Not meeting criteria of other types

***Immature***

Diffuse chromatin pattern
Nucleus >10 μm or nucleolus >2 μm, and abundant cytoplasm
Nucleus eccentrically placed with a hof

***Plasmablastic***

Very high nuclear/cytoplasmic ratio; scanty cytoplasm; and a central, immature, large nucleus with a reticular chromatin pattern, prominent nucleolus, little or no hof, or with marked pleomorphism

**TABLE 21-3**

**Bartl Grading System**

***Low Grade***

Marschalko: eccentric cartwheel nuclei, perinuclear hof, basophilic cytoplasm, few nucleoli or mitotic figures, primarily interstitial
Small cell: smaller than Marschalko; round, lymphocytic nucleus; narrow rim of basophilic cytoplasm; associated with plasma cell leukemia

***Intermediate Grade***

Cleaved: notched, cleaved nuclei of variable size; high nuclear to cytoplasmic ratio; small hof often present; often associated with packed marrow with fibrosis, associated with plasma cell leukemia
Polymorphous: cellular and nuclear polymorphism; 25% may show prominent central nucleoli
Asynchronous: marked nuclear and cytoplasmic maturation asynchrony; >50% cells have large eccentric nuclei, prominent nucleoli abundant basophilic cytoplasm, pronounced nuclear hof

***High Grade***

Blastic: large nuclei, prominent centrally located nucleoli, and moderate rim of basophilic cytoplasm, faint perinuclear hof

infiltration as interstitial, interstitial with paratrabecular sheets, interstitial/nodular, nodular, packed, and sarcomatous. The Greipp system was developed for use in bone marrow aspirate smear samples, whereas the Bartl system was developed for use in tissue section. Both have shown poor outcome in patients with plasmablastic or high grade cytologic features.

In addition to morphologic assessment of the plasma cells, vasculature and bone changes are also essential parts of the overall evaluation. Amyloid deposition and increased osteoclastic activities may accompany the plasma cell infiltrate. Increased bone resorption and bone remodeling are features distinguishing symptomatic myeloma from MGUS.

#### Peripheral Blood Smear

Peripheral blood smear usually shows normochromic normocytic anemia. Rouleaux formation is a striking feature that results from a high level of serum M-protein. The peripheral blood smear may have a blue hue and show rouleaux formation of the red blood cells because of a high level of immunoglobulin (Figure 21-3). Normochromic normocytic anemia is a frequent finding. Leukopenia or thrombocytopenia is usually present at the advanced stage of disease when the tumor burdens are heavy. The circulating plasma cells may resemble plasmacytoid lymphocytes or myeloblasts. Primary PCL is often associated with small cell morphology resembling low-grade B-cell lymphoma or leukemia with plasmacytoid differentiation.

## Ultrastructural Findings

Electron microscopy is usually not required for the diagnosis of myeloma. The typical features of plasma cells are a prominent Golgi region and dilated rough endoplasmic reticulum. Cytoplasmic crystalline inclusions may also be present.

## Ancillary Studies

#### Laboratory Findings

Laboratory evaluations are essential for diagnosis, follow-up, and prognosis. Serum protein electrophoresis (SPEP), urine protein electrophoresis of an aliquot from a 24-h urine collection, and immunofixation electrophoresis (IFE) are used for detection and quantification of monoclonal protein (M protein). Other tests such as $\beta_2$-microglobulin (β2M), C-reactive protein, lactate dehydrogenase, interleukin (IL) 6, plasma cell labeling index, and bone markers serve as biomarkers for tumor load or tumor activity. Serum chemistry panels include calcium and creatinine to detect hypercalcemia and renal failure.

Most patients have monoclonal serum protein (M component). IgG is most common (55%), followed by IgA (20%), light chain only (20%), IgD (1.5%), IgM (0.5%), and IgE (0.5%). Nonsecretory myelomas account for 2% of cases. Urine monoclonal immunoglobulin light chain protein, also termed the *Bence-Jones protein,* can be detected in 80% of patients.

M protein (or M component) appears as a discrete band on SPEP or as a narrow spike or peak usually in

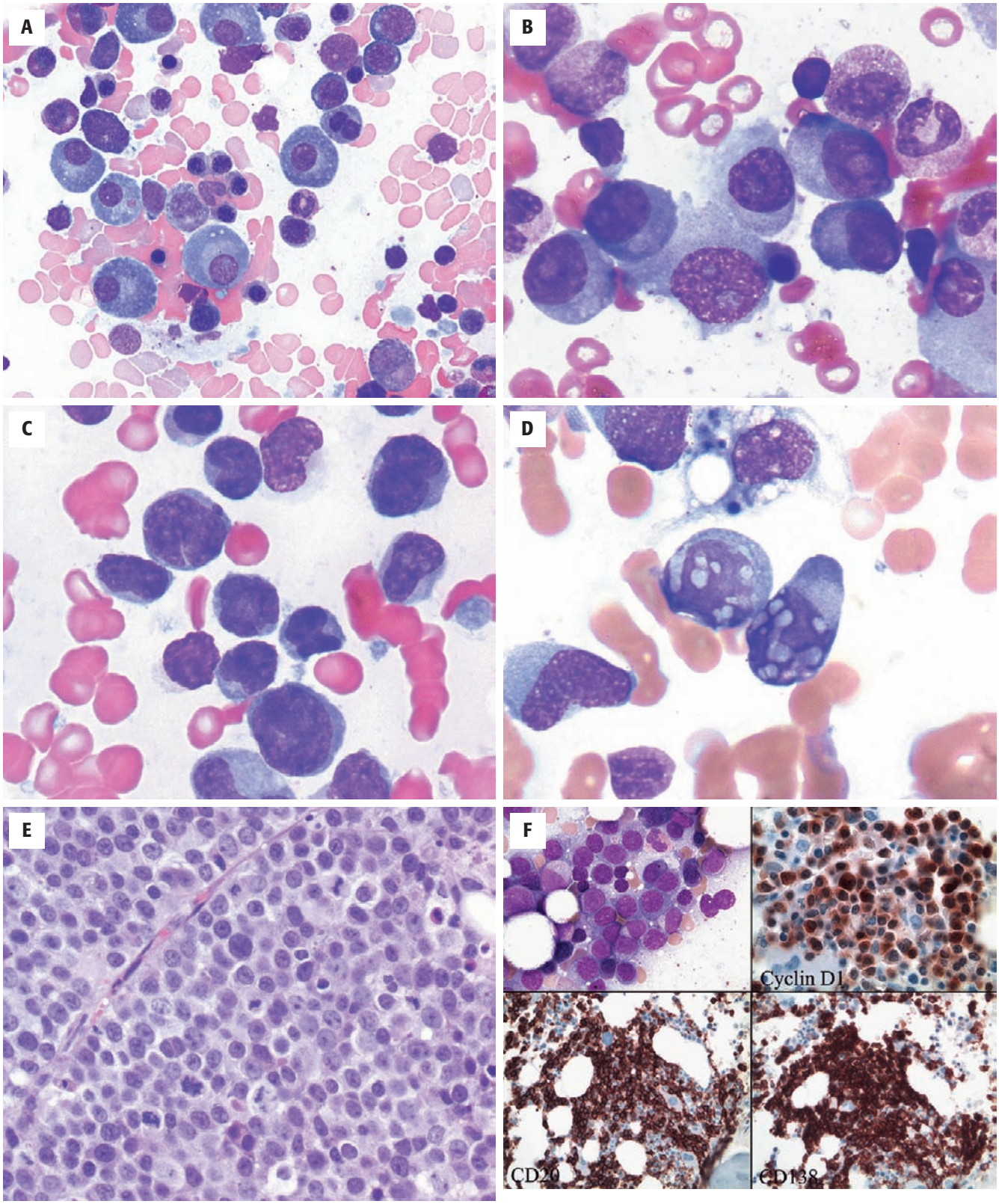


**FIGURE 21-2**

Multiple myeloma. **A,** Myeloma cells of low-grade cytology. Some plasma cells have cytoplasmic immunoglobulin inclusions (Wright-Giemsa stain, original magnification ×500). **B,** Myeloma cells of intermediate-grade cytology. The nucleoli are prominent and cytoplasmic-nuclear maturation is asynchronous (Wright-Giemsa stain, original magnification ×1000). **C,** Myeloma cells of intermediate-grade cytology. The cell size and nucleus are polymorphic (Wright-Giemsa stain, original magnification ×1000). **D,** Plasma cells of intermediate grade with cytoplasmic immunoglobulin inclusions (Wright-Giemsa stain, original magnification x1000). **E,** Bone marrow biopsy of plasmablastic myeloma (hematoxylin and eosin stain, original magnification ×400). Note the increased mitotic figures. **F,** Small-cell variant of plasma cell myeloma resembling lymphoplasmacytic lymphoma (Wright-Giemsa stain, original magnification ×1000). The neoplastic cells are positive for CD20, CD138, and cyclin D1 (nuclear and cytoplasmic staining; immunohistochemistry).

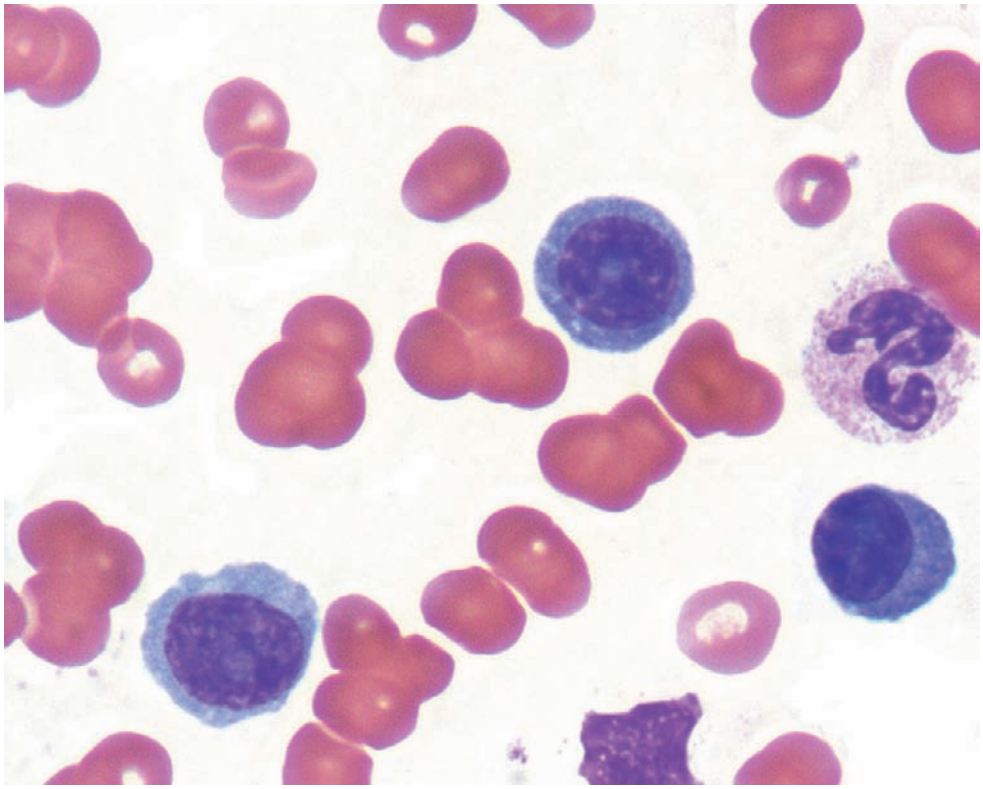

**FIGURE 21-3**

Peripheral blood smear of a patient with plasma cell leukemia. The circulating plasma cells show small, mature morphology. The background red blood cells show rouleaux formation resulting from a high level of M protein (Wright-Giemsa stain, original magnification ×1000).

the β or γ region of the densitometer tracing, but may be anywhere between the $\alpha_1$ and post-γ regions (Figure 21-4). In light chain–only myeloma, a distinct band or spike may not appear on SPEP, and hypogammaglobulinemia may be the only finding. In IgD or IgE myeloma, the routine SPEP, which uses antibodies against IgG, IgA, and IgM, will not detect an abnormal band. Using antibodies against IgD and IgE will confirm the diagnosis. IFE determines the type of M protein and detects M proteins beyond the sensitivity level of SPEP (less than 5.0 g/L).

M protein is used to confirm the diagnosis and for follow-up to assess the response to treatment. Serial quantification of M protein by densitometer tracing allows easy assessment of tumor load reduction. Clinically, partial remissions are defined as more than 75% of reduction of serum M protein or more than 95% reduction of urine M protein, and the complete remission is defined as absence of M protein.

In patients treated with myeloablative chemotherapy followed by bone marrow transplantation, reconstitution of humoral immunity can create a transient oligoclonal or monoclonal pattern that should be distinguished from the original M protein band. Comparing the IFE pattern before and after therapy may help to determine whether residual disease is present. Sensitivity of IFE is considered to be comparable with monoclonal immunoglobulin gene rearrangement assessed by polymerase chain reaction assay.

Total serum IgG, IgA, and IgM are measured by nephelometry, a tool more useful when the M protein is small. Suppression of uninvolved immunoglobulin is generally an indication of overt disease, but it can be seen in MGUS and thus is not a reliable discriminator of the two conditions. Discordant readings between nephelometry and densitometer tracing, with a lower reading by the latter, are usually the result of high levels of γ-globulin.

Serum free light chains (FLCs) are measured by nephelometric methods, and the ratio of κ and λ light chains is calculated. FLC ratio is most useful in cases with minimal serum M protein and nonsecretory or light chain–only myeloma and in monitoring patients in clinical remission when the M protein levels are low. FLC ratio is increasingly replacing urine protein electrophoresis for detection of the Bence-Jones protein. Abnormal FLC ratio at initial diagnosis also predicts risk for progression from asymptomatic to symptomatic myeloma. It is also useful in diagnosis of MGUS, amyloidosis (AL), and light chain deposition disease (LCDD) when M component is low.

β2M, reflecting cell turnover and renal function, is used as a prognostic marker. C-reactive protein is an

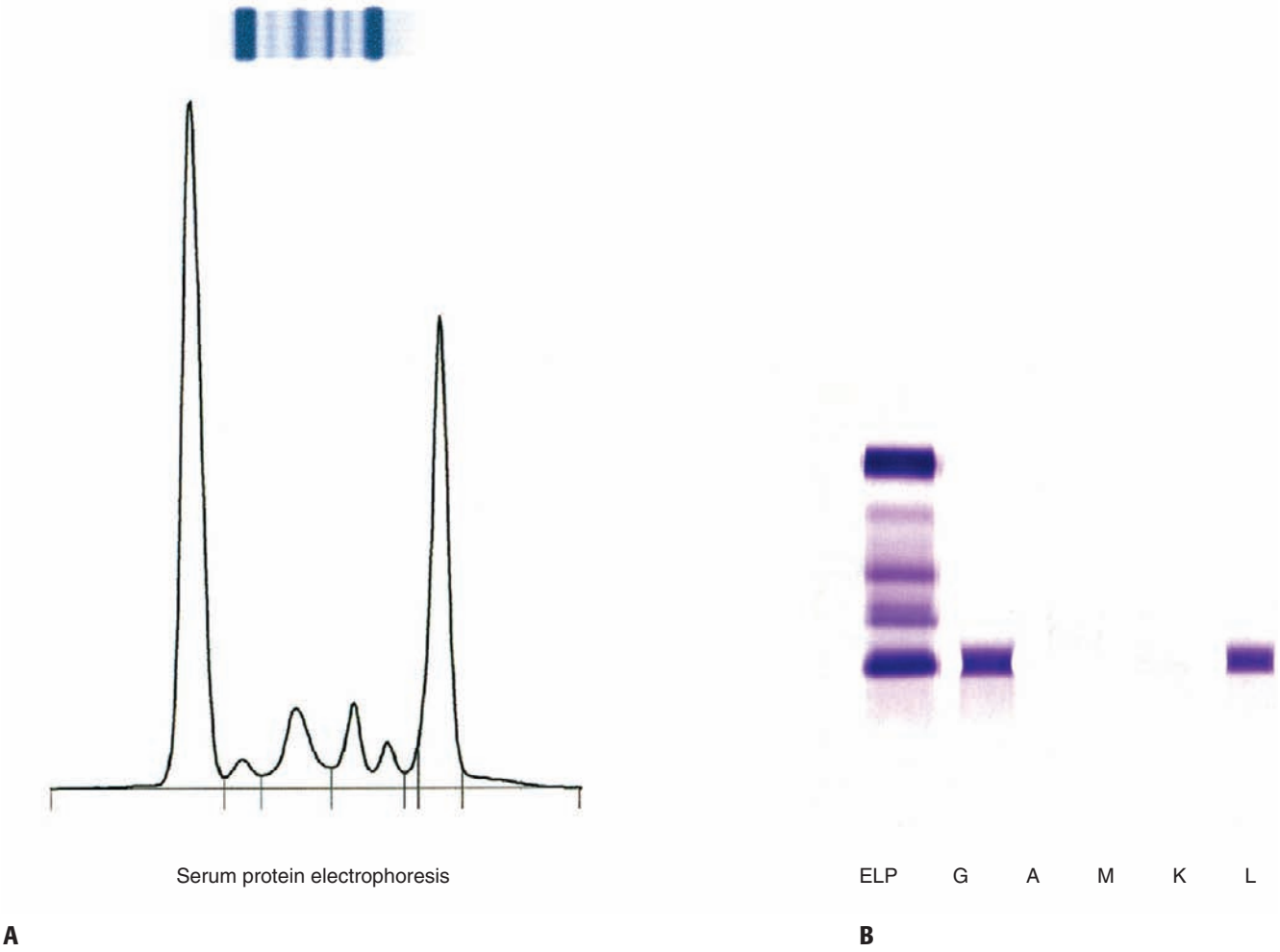


**FIGURE 21-4**

Electrophoresis. **A,** Serum protein electrophoresis demonstrates an M-protein peak. **B,** Immunofixation electrophoresis confirms it to be monoclonal IgG λ type. *ELP,* Electrophoresis; *G,* IgG; *A,* IgA; *M,* IgM; *K,* kappa; *L,* lambda antisera used for immunofixation.

acute phase reactant that is produced by the liver under the control of cytokines such as IL-6. Its level correlates with disease activity in myeloma. Lactate dehydrogenase is usually elevated in patients with extensive lymphadenopathy and indicates a poor prognosis.

In the International Staging System β2M and albumin (ALB) levels are used to define three stages of myeloma:

Stage 1: β2M < 3.5, ALB ≥ 3.5
Stage 2: β2M < 3.5, ALB < 3.5, or β2M = 3.5 to 5.5
Stage 3: β2M ≥ 5.5

β2M levels are measured in milligrams per deciliter; ALB levels are measured in grams per deciliter.

The plasma cell labeling index measures synthesis of DNA by using a monoclonal antibody (i.e., BU-1). The antibody BU-1 reacts with the bromodeoxyuridine that is incorporated into the DNA synthesis by the cells in S phase. Patients with symptomatic myeloma usually have a plasma cell labeling index of more than 1% and more than 5% in the advanced stage of disease.

Biochemical markers of bone resorption provide more sensitive information about bone turnover. These markers include amino- and carboxy-terminal cross-linking telopeptide of type I collagen (NTX and CTX, respectively) or cross-linked carboxyterminal telopeptide of type I collagen (ICTP). They can be used to monitor disease and treatment response.

### Fine-Needle Aspiration

CT-guided percutaneous biopsy or fine-needle aspiration is useful in obtaining tissues from deep-seated plasmacytomas. Other situations in which fine-needle aspiration may be used include abdominal fat pad aspiration and diagnosis of amyloid deposition.

### Cytochemistry

The plasma cells are reactive with acid phosphatase and nonspecific esterase. These stains are rarely used clinically for the purpose of identifying plasma cells.

### Flow Cytometry Immunophenotyping

Although in many instances flow cytometry immunophenotyping is not required for the diagnosis, it has been used increasingly for several reasons. In morphologically challenging tumors or nonsecretory myeloma, identification of monoclonal plasma cells or aberrant marker expression on plasma cells facilitates the diagnosis. The aberrant markers can also be used to detect minimal residual disease or as potential targets of therapy.

CD138 is the best marker for identifying benign and malignant plasma cells. CD38 is less specific than CD138, because it is also expressed in T cells and some B cells. CD38 is bright in benign plasma cells and slightly dimmer in malignant plasma cells. Benign plasma cells are typically positive for CD19, CD27, and CD45 and negative for CD117 and CD56. Malignant plasma cells are usually positive for CD27dim, CD28, CD56, and negative for CD19, CD20, and CD45, or dimly positive for CD45. However, CD45 and CD20 can be expressed at moderate or higher intensity in 10% and 20% of cases, respectively. CD56 is negative in about 40% to 45% of cases. CD117 is aberrantly expressed in 30% of cases and is a useful marker for malignancy. Myeloid markers CD13 and CD33 or CD10 can also be identified in a small subset of cases. PCL tends to be positive for CD20 (50%) but negative for CD117 and CD56 compared to myeloma. Approximately 90% of secondary PCL cases express CD28 as compared with 30% in primary PCL. The plasma cells express cytoplasmic but not surface Ig light chain.

Plasma cell gating is best achieved by using a combination of CD138, CD38, CD45, and light-scatter pattern. The detection sensitivity is 1 in $10^4$ to $10^5$ or higher in cases with aberrant marker expression or aneuploidy. The tumor ploidy can be measured by the DNA index. A DNA index of less than 0.95, 0.95 to 1.05, greater than 1.05, and 1.75 to 2.00 defines hypodiploid, pseudodiploid, hyperdiploid, and near tetraploid myeloma, respectively. Assays designed to detect minimal residual disease are usually based on the differential expression patterns of CD19, CD45, CD56, and CD117 between benign and malignant plasma cells. A simple modified assay using CD138, cytoplasmic immunoglobulin light chain, and a nuclear staining dye to measure DNA ploidy in the monoclonal plasma cells can also be used effectively in the clinical setting. Patients who have evidence of minimal residual disease (defined as greater than 0.01% with at least 100 aberrant plasma cells identified) detected by flow cytometry at day 100 after autologous stem cell transplantation have worse survival than those without.

### Immunohistochemistry

As mature plasma cells are difficult to distinguish from lymphoid and erythroid cells on regular hematoxylin and eosin sections, immunostains for plasma cell markers should be performed on the biopsy specimen, at least in the initial evaluation, to assess the extent and pattern of involvement.

The most useful antibody for identifying plasma cells in frozen or paraffin-embedded tissue is syndecan-1 (CD138; see Figure 21-2, F). When combined with κ and λ, syndecan-1 provides a more accurate assessment of tumor load. This is particularly important in cases of SMM, which require a minimal number of 10% monoclonal plasma cells. A caveat is that immunoblastic–plasmablastic large B-cell lymphoma and plasmablastic lymphoma are also positive for CD138. A positive result may represent bone marrow involvement by lymphoma rather than myeloma.

Ki-67 (MIB-1) is used more frequently than plasma cell labeling index as a surrogate marker for proliferation. Ki-67 of more than 10% is considered an indication of intermediate or high proliferation activity. Dual staining of Ki-67 with κ and λ or CD138 allows distinction between proliferating plasma cells and other marrow elements, especially the rapidly dividing erythroid precursors. Most cases also express CD79a and MUM-1. CD20 is expressed in one fourth of cases and does not reliably distinguish myeloma from other mature B-cell neoplasms. PAX5 is usually negative or weakly positive with occasional exceptions. In 40% to 45% of cases, cyclin D1 is expressed, typically in a nuclear and cytoplasmic pattern.

### Cytogenetics

Conventional cytogenetic analysis detects structural or numerical chromosomal abnormalities, usually complex, at diagnosis in only approximately 30% of cases. In most instances, the low proliferating tumor cells yield no informative result. However, genetic aberrations can be demonstrated in nearly all myelomas by interphase fluorescence in situ hybridization (FISH) analysis. Plasma cell enrichment by CD138 magnetic beads may enhance the yield of interphase FISH when the plasma cells are at a low level.

Translocations involving the switch region of Ig heavy-chain locus on chromosome 14q32 are the most common recurrent cytogenetic abnormalities, occurring in approximately 50% of cases. More than 50 partner genes have been described; the five most common ones representing 40% of the cases with *IGH@* translocation are located at 11q, 4p, 16q, 6p, and 20q. The genes and their functions are mostly related to expression of cyclin D family protein and cell cycle regulation (Table 21-4). These five *IGH@* translocations are also detectable in MGUS and are considered primary and possibly initiating events.

The breakpoint in t(11;14) involves part of *CCND1* gene that is located more centromeric than that in mantle cell lymphoma. The t(11;14) is more common in primary PCL (71%) compared with secondary PCL

**TABLE 21-4**

**Five Most Common Partner Genes Involved in Translocations of Immunoglobulin Heavy Chain Gene**

| Chromosome | Gene | Percentage |
|---|---|---|
| 11q13 | *Cyclin D1* | 15 |
| 4p16.3 | *FGFR3 & MMSET* | 15 |
| 16q23 | *C-maf* | 5 |
| 6p21 | *Cyclin D3* | 3 |
| 20q11 | *mafB* | 2 |

(23%). The t(4;14) interrupts both fibroblast growth factor (FGF) receptor 3 gene *(FGFR3)* and multiple myeloma SET domain *(MMSET)* genes, and is best identified by FISH because the breakpoint involves the telomeric part of the chromosome 4.

Besides structural aberrations, numerical aberrations are also common. Trisomy most frequently involves the odd numbered chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, whereas monosomy and partial deletions affect mainly chromosomes 6, 13, 16, and 22. Gains of 1q are the most common structure aberrations in myeloma, detectable in up to 40% of those with an abnormal karyotype. Monosomy 13 or interstitial deletion of 13 (13q14) is detectable in 15% to 20% of newly diagnosed myeloma by conventional cytogenetics and 50% of cases by interphase FISH. Del13 can also be found in MGUS.

Based on the number of chromosomes, myeloma cases can be broadly divided into two groups: the hyperdiploid (48 to 75 chromosomes) and non-hyperdiploid myeloma (less than 48 or greater than 75 chromosomes). Hyperdiploid represents approximately half of cases and frequently carries trisomies. The five recurrent translocations described previously and del13 are by far more common in non-hyperdiploid than hyperdiploid, suggesting that different molecular genetic pathways are involved in pathogenesis of the two subgroups.

Other genetic aberrations described in myeloma include *MYC* rearrangement, usually involving non-*IGH@* partners, occurring in 10% to 15% of cases; amplification of 1q21 (resulting in putative cyclin dependent kinase subunit 1 (*CKS1*B) gene amplification) in 30% to 40%; *N* or *K-RAS* mutations in 15% and 15% of cases each; *p16* methylation in 20% to 30%, deletion or methylation of *p18* in 20% to 30% and *TP53* mutations or deletions in 10% of cases. Mutations in NFκB pathway components such as *TRAF3, cIAP1/2, CYLD,* and *NIK* are also identified in myeloma. Unlike the five primary translocations and 13q deletions, *MYC* rearrangement or *RAS* mutations are rare in MGUS. In addition, many of these genetic abnormalities have prognostic importance and are mentioned under Prognosis and Therapy.

### Gene Expression Profiling, Single Nucleotide Polymorphisms, and MicroRNA Microarrays

The advent of new genomic technology allows global investigation of genes altered in myeloma and has resulted in new ways of classifying myeloma. The translocation/cyclin D classification scheme proposes eight molecular subgroups—6q21, 11q13, D1, D1+D2, D2, non, 4p16, and maf—based on the predominant activation pattern of cyclin D protein and the pattern of *IGH@* translocations identified by DNA microarray. The 70-gene model proposed by the Arkansas group emphases the potential of expression arrays as a guide to clinical practice. Other technologies, such as complimentary genomic hybridization–based mapping array, also allow investigation of key candidate genes in the context of complex chromosomal gain and loss. Single nucleotide polymorphism array has found additional prognostic markers, including del(12p13.31) as an independent adverse marker and amplification of (5q31.1) as a favorable marker. Similarly, microRNA-based array and proteomics have also shed light on mechanisms controlling myeloma growth and disease progression. MicroRNA may also serve as biomarkers for diagnosis, prognosis, and monitoring of residual disease. These techniques are largely still in the realm of clinical research, but they will likely become incorporated in some form in the near future.

## Differential Diagnosis

A variety of reactive conditions are associated with plasmacytosis and sometimes up to 30% of marrow cells. Autoimmune disorders, hepatitis C, HIV infections, and Hodgkin lymphoma are a few examples. Nuclear immaturity and marked atypia are not features of reactive plasmacytosis and are strong indications of malignancy. Reactive plasmacytosis is also frequently associated with increased eosinophils, mast cells, and megakaryocytes. Benign plasma cells usually occur in small clusters of five or six cells in perivascular areas, while the myeloma cells proliferate in sheets and infiltrate and replace normal hematopoietic elements with increased bone resorption. Demonstration of monoclonality by immunoglobulin light chain analysis confirms a neoplastic process. Identification of aberrant marker expression such as CD56 or CD117 provides additional supporting evidence of malignancy.

Large immature myeloma cells may resemble monoblasts, react with nonspecific esterase, and express myeloid-associated markers such as CD33, causing confusion with acute monoblastic leukemia. CD138 is an extremely helpful marker for the distinction of the two entities. The monoblasts are negative for CD138.

The polymorphous subtype of myeloma is frequently associated with fibrosis. The tumor cells show marked nuclear irregularity and can be confused with idiopathic chronic myelofibrosis. Demonstration of CD138 expression by the infiltrating cells confirms a plasmacytic origin.

Monoclonal protein can be detected in a variety of non-Hodgkin lymphomas or chronic lymphocytic leukemia. Because a small amount of monotypic B cells of identical idiotype can be detected in myeloma patients, the differential diagnosis of lymphoma with marked plasmacytic differentiation versus myeloma arises. In the bone marrow, chronic lymphocytic leukemia with plasmacytoid differentiation or lymphoplasmacytic lymphoma–Waldenström macroglobulinemia is the most commonly entertained differential diagnosis. The neoplastic plasma cells in myeloma are usually $CD138^{+}CD19^{-}CD45^{-}$, whereas plasma cells in LPL tend to coexpress CD19, CD45, and CD138. Of note, myeloma cases with *IGH@-CCND1* translocation are often $CD20^{+}$ with lymphoplasmacytic morphology. Therefore cases of suspected LPL in bone marrow should be examined for this translocation or cyclin D1 overexpression by immunohistochemistry to exclude plasma cell myeloma. Furthermore, in lymphoplasmacytic lymphoma samples obtained after chemotherapy, the plasma cells persist and become the predominant component while the lymphoid cells are eliminated. In this setting, detection of IgM paraprotein and CD45+CD19+ plasma cells strongly favors a diagnosis of lymphoplasmacytic lymphoma–Waldenström macroglobulinemia.

When evaluating small extramedullary tissue biopsy specimens, such as gastrointestinal or ocular tissue, immunohistochemistry is often the only available tool for immunophenotyping, and the differential diagnosis often includes marginal zone B-cell lymphoma with marked plasmacytic differentiation. Expression of CD79a by the neoplastic cells is not a reliable marker for the distinction of the two entities. Pax5/BSAP is usually absent in myeloma and can be useful in these settings. In the rare cases of myeloma expressing CD20 and Pax5/BSAP, evidence of CD138 and immunoglobulin heavy chain IgG or IgA type expression may be helpful.

The distinction of a high-grade extramedullary plasmacytic neoplasm from immunoblastic–plasmablastic lymphoma can be extremely difficult. The immunophenotypes may be indistinguishable. Epstein-Barr virus–encoded RNA in situ hybridization is usually positive in plasmablastic lymphoma and negative in myeloma. Correlation with other clinical features such as serum M protein and radiographic imaging may be helpful in difficult cases.

Plasmacytosis in MGUS usually is less than 10% of total cellularity and lacks cytologic atypia. Immunoglobulin κ-to-λ ratio may fall within the normal range when analyzed by immunohistochemistry, because the polytypic plasma cells are frequently intermixed. Flow cytometry allows better discrimination of monotypic plasma cells from polytypic plasma cells in this setting.

Localized plasmacytoma of bone needs to be distinguished from metastatic tumor. In this situation, CD138 is not useful because epithelial tumors also express this marker. Demonstration of immunoglobulin light chains expression and restriction is critical.

## Prognosis and Therapy

The median survival of myeloma patients averages approximately 3 years with standard therapy (melphalan and prednisone), and 5 years with dose-intensive therapy and autologous stem cell transplantation. Novel agents such as immunomodulatory drugs, thalidomide, lenalidomide, the proteasome inhibitor bortezomib, as well as liposomal doxorubicin have significantly improved the survival to 7 years. New generation of immunomodulatory and proteasome inhibitors (pomalidomide and carfilzomib) further enhances therapeutic efficacy especially in refractory patients. However, myeloma remains an incurable disease and survival is highly variable among different patients. Risk stratification is the key to improve survival. Besides age and performance status, disease stage is one of the most useful prognostic indicators. The simplified International Staging System described above, which is based on serum levels of β2M and ALB , has largely replaced the Durie-Salmon Staging System (Table 21-5) to measure tumor burden and activity. The median survival for International Staging System stage I, II, and III diseases is approximately 62, 44, and 29 months, respectively, before the era of novel therapy.

**TABLE 21-5**

**Durie-Salmon Staging System**

| |
|---|
| ***Stage 1 (All Criteria Must Be Met)*** |
| Hemoglobin >100 g/L |
| Normal serum calcium |
| Normal bone radiograph or single bone plasmacytoma |
| Low M protein levels: IgG < 50 g/L, IgA < 30 g/L, urine light chain < 4 g per 24 hours |
| ***Stage 2*** |
| Neither Stage 1 or 3 |
| ***Stage 3 (One or More Criteria Must Be Met)*** |
| Hemoglobin <85 g/L |
| Serum calcium >12 mg/dL |
| Multiple lytic bone lesions |
| High M protein levels: IgG > 70 g/L, IgA > 50 g/L, urine light chain > 12 g per 24 hours |

Subgroups: A, serum creatinine <2 mg/dL; B, serum creatinine >2 mg/dL.

Molecular genetics and cytogenetics add additional value to risk stratification as they explore the tumor biology. An abnormal karyotype identified by conventional cytogenetics is indicative of highly proliferative tumors and inferior survival. Furthermore, deletion of chromosome 13 or 13q14, hypodiploidy, and monosomy 17/*TP53* are associated with a poor prognosis. Detection of t(4;14), t(14;16), or t(14;20) by FISH also predicts an inferior survival, whereas deletion of 13 detected by FISH in the absence of an abnormal karyotype is not predictive of an inferior survival. Data regarding the values of gain of 1q and deletion of 1p are controversial. The definition of high risk factors may evolve with novel anti-myeloma therapy.

Laboratory parameters that correlate with poor prognosis include plasma cell labeling index (more than 2% to 3%), Ki-67 labeling index, elevated serum β2M (more than 2.5 mg/dL), C-reactive protein, high lactate dehydrogenase, and hypoalbuminemia. C-reactive protein is a surrogate marker for IL-6, a growth factor for myeloma cells.

Although asymptomatic, the risk for SMM to evolve to symptomatic myeloma is 10% per year for the first 5 years and 3% per year for the next 5 years and 1% to 2% per year for the next 10 years. The median time to progression is approximately 5 years. The current model for risk stratification of SMM is based on three parameters: abnormal FLC ratio (≤0.125 or ≥8), bone marrow plasma cells 10% or greater and serum M protein 30g/L or greater. Patients with one to three risk factors had 5-year progression rates of 25%, 51%, and 76%, respectively; the corresponding median times to progression are 10, 5.1, and 1.9 years, respectively. The Spanish group proposes that a ratio of 95% or greater of aberrant plasma cells to normal plasma cell identified by flow cytometry together with suppression of uninvolved γ-globulin could stratify SMM patients into three prognostic groups, with a progression risk of 5 years at 72%, 46%, and 4% if the patient has two, one, or none of these risk factors.

Development of new antimyeloma drugs aims at targeting interaction of myeloma cells and their bone marrow microenvironment. Adhesion molecules on extracellular matrix proteins and bone-marrow stromal cells regulate the homing of myeloma cells. The adherence of myeloma cells to stromal cells leads to the overproduction of several cytokines and growth factors such as IL-6, insulin-like growth factor 1, tumor necrosis factor α, stromal cell–derived factor-1 α, and vascular endothelial growth factor through autocrine and paracrine loops. These factors then activate multiple signaling pathways including JAK/STAT, PI3k/Akt/NF-κB, and Wnt, which in turn promote angiogenesis, survival, and drug resistance of myeloma cells. Conversely, myeloma bone lesions develop as a result of increased cytokines, such as macrophage inflammatory protein 1α, released by myeloma cells, causing overexpression of the receptor activator of nuclear factor-κB ligand (RANKL) by osteoblasts and reduction of its decoy receptor osteoprotegerin. The imbalance of the two molecules leads to increased bone resorption and decreased bone formation, which then releases a range of cytokines further promoting myeloma growth. Drugs such as bisphosphonates (pamidronate and zoledronic acid), which regulate bone metabolism, are effective in anti-myeloma and improving survival. Anti–IL-6 antibody, RANKL antagonist, kinase inhibitors, antibodies targeting plasma cells (anti-CS1), and histone deacetylase inhibitors are in clinical trials. In keeping with advances in patient outcomes, these new therapeutic strategies may eventually render myeloma a controllable chronic disease if not a curable disease.

## MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

MGUS is an asymptomatic disorder in which a monoclonal immunoglobulin is secreted by low proliferating plasma cells in the bone marrow. The M protein is often detected incidentally during routine laboratory evaluation. There is no evidence of myeloma, primary amyloidosis, or non-Hodgkin lymphomas at the time of diagnosis. MGUS, however, can evolve to any of these conditions eventually. The current criteria for diagnosis of MGUS are as follows: serum M protein less than 30 g/L, bone marrow plasma cells less than 10%, and absence of end organ damage as a result of plasma cell proliferation.

### Clinical Features

The incidence of MGUS increases with age, occurring in 3% of the population older than 50 years, 5% older than age 70 years, and 10% older than age 80 years. Relatives of patients with MGUS have increased risk for MGUS and myeloma, suggesting genetic susceptibility.

It is important to distinguish whether MGUS is a result of lymphoid neoplasm or plasma cell neoplasm because they have different clinical implications. Currently, three major types of MGUS are recognized: IgG/IgA-MGUS, IgM-MGUS, and light chain–MGUS. The IgG type is most common (70%), followed by IgM (15%) and IgA (12%). IgM-MGUS is related to lymphoproliferative disorders and may evolve to lymphoplasmacytic lymphoma–Waldenström macroglobulinemia, other non-Hodgkin lymphoma, amyloidosis, or chronic lymphocytic leukemia rather than myeloma. The light chain type (3%), also known as *Bence-Jones proteinuria of undetermined significance,* is defined by an abnormal FLC ratio of less than 0.26 or greater than 1.65 without

**MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE—FACT SHEET**

**Definition**
- Asymptomatic plasma cell neoplasms associated with low levels of serum IgM protein and low proliferating cells
- Three types: IgG/A, IgM, and light chain

**Incidence and Location**
- One percent and 3% of individuals older than 50 and 70 years, respectively
- Involving bone marrow

**Morbidity and Mortality**
- One percent annual risk and 25% cumulative probability of evolving into overt myeloma, plasmacytoma, lymphoplasmacytic lymphoma–Waldenström macroglobulinemia, or amyloidosis

**Gender, Race, and Age Distribution**
- Elderly, male predominance
- Incidence is higher in blacks (2:1)

**Clinical Features**
- Asymptomatic

**Radiologic Features**
- No evidence of lytic bone lesion

**Prognosis and Therapy**
- The condition is stable unless it progresses to overt myeloma or other related diseases. Progression may be gradual or sudden. Increasing M protein levels, plasmacytosis, and abnormal FLC ratio pose at higher risk
- It is managed by observation only during the stable phase

**MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE—PATHOLOGIC FEATURES**

**Microscopic Findings**
- Interstitial mild plasmacytosis (<10%) of mature forms devoid of marked atypia
- No associated bone changes or fibrosis

**Immunohistochemical Findings**
- May not show distinct immunoglobulin light chain restriction owing to coexistence of polyclonal plasma cells

**Differential Diagnosis**
- Asymptomatic plasma cell myeloma (≥10% monoclonal plasma cells and/or ≥30g/L M protein)

evidence of Ig heavy chain expression by IFE. It may evolve to light chain–only myeloma. An intermediate stage, so-called idiopathic Bence Jones proteinuria that is similar to smoldering myeloma, may be observed in some patients before the onset of symptomatic myeloma.

The κ-to-λ ratio is approximately 2:1. Bence Jones protein is usually absent or present in small amounts in approximately two thirds of patients; it does not indicate progression to myeloma. Although asymptomatic, patients with MGUS are at a higher risk for bone fractures and deep vein thrombosis than their healthy counterparts.

## Pathologic Features

Bone marrow biopsy and aspirate show a mild increase in plasma cells, usually less than 10% (median, 3%) that are scattered throughout the interstitium. Large aggregates of plasma cells are not seen, and bone trabeculae are unremarkable. Lack of marked cytologic atypia and an infiltrative pattern help to distinguish MGUS from myeloma.

## Ancillary Studies

The size of the M protein is usually less than 15 g/L in 80%. Flow cytometry identifies both immunophenotypically aberrant and normal plasma cells.

By FISH analysis, aneuploidy is a consistent finding in MGUS. Monosomy 13 and 14q32 translocations are detectable in 21% to 45% and 50%, respectively, with the former being usually detected in a subset of clonal population. It is not useful for prediction of progression.

## Differential Diagnosis

There are no known molecular or cytogenetic markers that reliably distinguish MGUS from myeloma. Microarray analysis reveals significant overlap between gene expression patterns of MGUS and myeloma. The progress of MGUS to myeloma is likely a result of stepwise accumulation of genetic changes. Activating *K-RAS* mutations are not observed in MGUS patients but are detectable in 15% of myeloma patients. *MYC* rearrangement is observed in 3% to 4% of MGUS/SMM cases and 15% of myelomas. These genetic changes are likely involved in disease progression.

Morphologically, plasma cells in MGUS generally lack marked nuclear atypia and immaturity seen in myeloma. The plasma cells are usually singly dispersed in the interstitium.

The IgM type of MGUS is distinguished from the so-called IgM-related disorders by the absence of symptoms attributable to the M protein. In IgM-related

disorders, the patients may have minimal serum M protein, but there is no evidence of lymphoma. The patients, however, may suffer from more debilitating complications, such as autoimmune hemolytic anemia, cryoglobulinemia, and peripheral neuropathy owing to IgM binding to the myelin-associated glycoprotein and cryoglobulinemia.

### Prognosis and Therapy

The incidence of progression of MGUS to myeloma or related disorders is approximately 1% each year. Cumulative incidence of progression is approximately 10% at 5 years, 15% at 10 years, and 20% at 15 years. The interval from recognition of MGUS to diagnosis of myeloma ranged from 1 to 32 years (median, 10.4 years).

Nearly all myelomas are preceded by a period of MGUS, which can progress suddenly or gradually through a smoldering phase to symptomatic myeloma. It is therefore critical to identify patients at risk for progression for better management. Because MGUS and myeloma share common molecular genetic features, the current models of risk stratification are based on clinical or laboratory features. Progressive increase in the size of M-protein predicts a higher risk. In the model proposed by the Mayo Clinic group, patients with low-risk MGUS have less than 15 g/L M-protein, IgG type, and a normal FLC ratio. Patients with intermediate or high risk have a greater than 15 g/L M-protein IgA-type and an abnormal FLC ratio (<0.26 or >1.65). In the model proposed by Perez-Persona, a ratio of aberrant plasma cells to normal plasma cell greater than or equal to 95% and DNA aneuploidy are risk factors for progression of MGUS.

Besides myeloma, other diseases that can evolve in patients with MGUS include primary amyloidosis or plasmacytoma. Patients with MGUS are usually observed without chemotherapy and monitored regularly for progression, with frequency dictated by risk as determined by models just described. Overtreating these patients is harmful because of the risk of myelodysplastic syndrome.

## PRIMARY AMYLOIDOSIS

Amyloidosis is a syndrome caused by accumulation of insoluble proteins with a β-pleated sheet configuration. Amyloidosis can be primary, secondary, or familial according to the cause. The four major types of amyloid fiber are AL, AA, AF, and Aβ2m. Serum amyloid P component is present in all four types of amyloidosis regardless of underlying cause. Rare cases of amyloidosis are hereditary, because of mutations of transthyretin or fibrinogen α-chain.

Primary AL is associated with plasma cell neoplasm, whereas secondary AA is associated with chronic inflammation such as in rheumatoid arthritis. AF is found in familial amyloidosis, and Aβ2m is related to hemodialysis. The AL contains a part of the variable region of the light chain, most often λ (75%) and sometimes κ (25%). AA contains serum amyloid A (prealbumin), a protein derived from an acute phase reactant. This section focuses on primary amyloidosis.

### Clinical Features

The incidence of AL is 8 per 1 million person-years, with a median age of 63 years at onset. There is a male predominance. The symptoms are vague and nonspecific in the early stage. The most common complaints are fatigue and malaise. Amyloid infiltrates cause organ damage and organ dysfunction. Thus, the clinical presentations are related to the dominant organ involved

**PRIMARY AMYLOIDOSIS—FACT SHEET**

**Definition**
- Plasma cell neoplasms associated with abnormal light deposition in the tissue as amyloid fibers causing organ damage and dysfunction

**Incidence and Location**
- Eight per 1 million person-years; approximately 2000 new cases each year
- Predominantly involves heart, kidney, liver, and nervous system

**Morbidity and Mortality**
- Median survival is 1 to 2 years; major vital organ failure

**Gender and Age Distribution**
- Male predominance; median age is 63 years at onset
- Sixty percent of patients aged 50 to 70 years at diagnosis; 10% younger than 50 years

**Clinical Features**
- Symptoms are related to the dominant organ involved and the extent of organ systems involved
- Restrictive cardiomyopathy, nephrotic syndrome, hepatic failure, gastrointestinal symptoms, and neurologic dysfunctions

**Radiologic Features**
- Echocardiography demonstrates concentric thickening of the ventricular wall and the septum

**Prognosis and Therapy**
- Median survival is 1 to 2 years; cardiac involvement predicts poor prognosis
- Chemotherapy with or without stem cell transplantation to eliminate neoplastic clone

and the extent of organ systems involved. Renal involvement results in nephrotic syndrome or renal failure. Cardiac involvement with amyloid deposition in the extracellular space and the conduction system results in restrictive cardiomyopathy and arrhythmia. Gastrointestinal tract infiltration can cause macroglossia, hemorrhage, malabsorption, diarrhea, and obstruction. Vascular damage results in facial and periorbital purpura (the raccoon sign). Soft-tissue accumulation can lead to carpal tunnel syndrome and to "shoulder pads" sign. Deposits in nerves result in sensorimotor peripheral neuropathy. Amyloid deposition can result in mass formation—so-called amyloidoma. Coagulopathy caused by binding of the amyloid fibers with factor X can cause severe bleeding complications. The major causes of death are congestive heart failure and fatal arrhythmia. Misdiagnosis as temporal arteritis or Sjögren syndrome can delay the correct diagnosis and therapy.

## Pathologic Features

Grossly, the involved organs appear to be waxy and stiff. On hematoxylin and eosin–stained sections, the amyloid fibers have an amorphous, eosinophilic appearance often associated with cracking artifact and multinucleated giant cell reactions (Figure 21-5). The amyloid fibers appear apple-green birefringent when stained with Congo red and viewed under polarized light.

Primary amyloidosis is essentially MGUS that is associated with amyloid deposition in tissue that causes damage. Of note, the organ dysfunction caused by amyloid deposition should not be taken as evidence of end organ damage for a formal World Health Organization diagnosis of multiple myeloma. The number of plasma cells is in the range of MGUS that is best appreciated by immunohistochemistry or flow cytometry immunophenotyping. A low level of serum M protein can be detected in 80% of patients. Evaluation of bone marrow suspected of plasma cell neoplasms should always include vasculature and periosteum, where the amyloid deposits are likely to occur. Other locations are bone marrow medullary cavities.

Abdominal subcutaneous fat pad aspiration is the diagnostic test used most often. In patients who have a serum M component and are suggested to have primary amyloidosis, combined fat pad and bone marrow biopsy with Congo red stains yields a diagnosis in 90% of cases, whereas each method alone may detect up to 80% and 50% of cases, respectively. Direct biopsy of the involved organ is rarely required and carries the risk of bleeding. Primary amyloidosis is most commonly associated with two particular immunoglobulin λ light chain variable regions, the 3r (λIII) and the 6a (λVI).

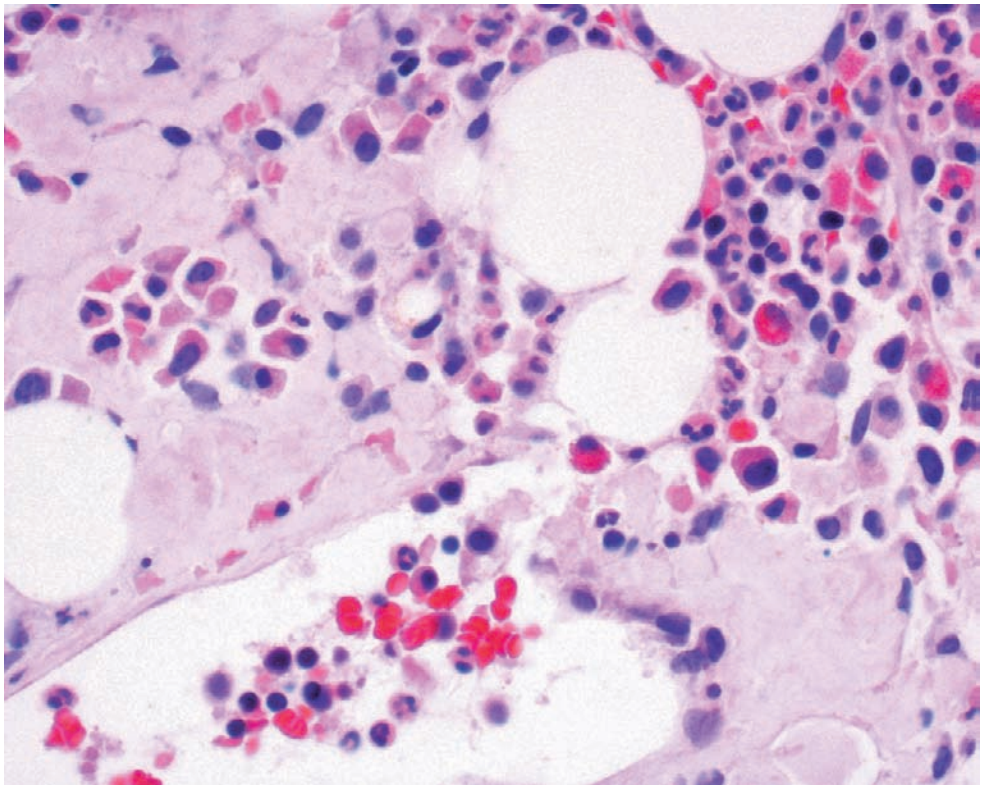

**FIGURE 21-5**
Amyloid deposits in the bone marrow medullary space (original magnification ×400).

**PRIMARY AMYLOIDOSIS—PATHOLOGIC FEATURES**

**Gross Findings**
- Involved tissue may have a waxy, stiff quality

**Microscopic Findings**
- Eosinophilic, amorphous deposits in the medullary space, vessel walls, or the soft tissue (especially the periosteum in bone marrow trephine biopsy) that are stained red by Congo red stain and apple-green birefringent in polarized light

**Ultrastructural Findings**
- Nonbranching linear fibrils 7 to 10 nm in diameter and of variable length

**Fine-Needle Aspiration Biopsy Findings**
- Amorphous waxy, basophilic material on Diff-Quick stain or cyanophilic clumps of material with fissures on Papanicolaou stain

**Immunohistochemical Findings**
- Positive for κ or λ light chain and amyloid P component

**Laboratory Findings**
- Low level of M protein by IFE or abnormal FLC ratio
- Measurement of troponin, brain natriuretic peptide

**Differential Diagnosis**
- LCDDs or HCDDs

## Ancillary Studies

Immunohistochemistry or flow cytometry immunophenotyping using a panel of antibodies including CD138, in conjunction with immunoglobulin light chains, will highlight plasma cells and determine monoclonality. The AL amyloid can be detected using antibody specific for AL or amyloid P component. By electronic microscopy, amyloid fibers appear as a mass of nonbranching linear fibrils measuring 7 to 10 nm in diameter and of variable length. In one third of patients with the AL, no distinct spike is visible by SPEP. IFE is more sensitive in patients with a low level of M protein and should be performed to confirm the presence of M protein. Serum FLC assays can determine the type of the M protein and confirm the diagnosis; they are also useful for monitoring response to therapy.

## Differential Diagnosis

As AL is treated with chemotherapy and autologous transplantation, it is critical that other forms of amyloidosis be excluded. This can be challenging when non-AL coexists with MGUS with minimal plasmacytosis in the bone marrow, mimicking AL. A useful hint to the presence of AL is amyloid deposition in the bone marrow. Congo red stain should be performed routinely in the bone marrow biopsy of all patients with suspected AL. Another feature of AL is its heterogeneous clinical presentation with multiorgan involvement. Molecular studies with polymerase chain reaction amplification and DNA sequence analysis will detect patients with mutant transthyretin or fibrinogen A α-chain variants. Mass spectrometry allows specific typing of amyloid fibers and is considered the gold standard.

The diagnosis of primary amyloidosis requires direct evidence of amyloid-induced organ damage as a result of monoclonal plasma cell proliferation. All the following criteria need to be fulfilled: presence of amyloid-related systemic syndrome (e.g., renal, liver, heart, gastrointestinal tract, or peripheral nerve involvement); positive amyloid staining with Congo red or EM in any tissue, confirming that the amyloid is composed of immunoglobulin chain by immunostaining, mass spectrometry or other techniques; and detection of monoclonal plasma cell proliferation such as serum or urine M protein, abnormal FLC ratio, or monotypic plasma cells in bone marrow. The distinction between AL and light or heavy chain deposition diseases is discussed in the following section.

## Prognosis and Therapy

The median survival of patients with AL is 1 to 2 years. The patients usually succumb to progressive organ failure. The prognosis largely depends on the dominant organ involvement. Cardiac involvement predicts a poor prognosis. Using N-terminal pro-brain natriuretic peptide and serum troponin T values as surrogate markers of cardiac involvement at a cutoff value of 332 pg/mL and 0.035 μg/L, respectively, patients can be divided into three risk groups with median survivals of 26.4 (both markers below cut-off), 10.5 (one above cutoff), and 3.5 (both markers above the cutoff) months, respectively. Stem cell transplant is a preferred therapy, but only 20% of patients are eligible. Nontransplantable patients are treated with various agents to eliminate the neoplastic cells.

# MONOCLONAL LIGHT AND HEAVY CHAIN DEPOSITION DISEASES

Monoclonal LCDD and heavy chain deposition disease (HCDD) are caused by deposition of abnormal heavy or light chains secreted by neoplastic plasma cells. In LCDD, HCDD, or combined LCDD and HCDD, the abnormal proteins do not have a sheet structure or contain P component but are deposited in tissue, as in

amyloidosis, causing organ damage and dysfunctions. LCDD and HCDD are frequently underdiagnosed. In adult patients with unexplained nephrotic syndrome, the index of suspicion should be high for amyloidosis or LCDD.

The κ light chain, especially products of $V_{\kappa I}$ and $V_{\kappa IV}$, is more often involved in LCDD than is the λ light chain. Multiple mutations of the variable region alter amino acid sequence and enhance their tissue affinity. Free light chains filtered through the glomeruli tend to deposit in the kidney when the amount exceeds the degradation capacity of the renal tubular epithelia. Free heavy chains usually do not circulate in blood. Deposition of heavy chains usually results from structural deletion of the CH1 constant domain of the heavy chain, which then prevents its association with heavy chain binding protein and development of HCDD.

## Clinical Features

Combined LCDD and HCDD and isolated HCDD are rare. The patients with LCDD are usually younger (30 to 50 years old) and predominantly female compared with patients with amyloidosis. The patients usually have acute renal failure with profound proteinuria or hematuria. Less commonly, the patients have cardiomyopathy or hepatic or pulmonary insufficiency.

**LIGHT OR HEAVY CHAIN DEPOSITION DISEASES—FACT SHEET**

**Definition**
- Abnormal immunoglobulin secreted by neoplastic plasma cells that are not amyloid material deposited in tissue

**Incidence and Location**
- Rare; tends to be underdiagnosed, involving major organs as in amyloidosis, with the kidney being the most common

**Morbidity and Mortality**
- Median survival is 1 to 2 years; most often the patients die of organ failure

**Gender and Age Distribution**
- 30 to 50 years; predominantly female

**Clinical Features**
- Nondiabetic nephrotic syndrome most common presentation

**Radiologic Features**
- Nonspecific

**Prognosis and Therapy**
- Poor; treated with chemotherapy to eliminate monoclonal plasma cells

**LIGHT OR HEAVY CHAIN DEPOSITION DISEASES—PATHOLOGIC FEATURES**

**Gross Findings**
- Waxy, stiff

**Microscopic Findings**
- Eosinophilic, amorphous material that does not stain for Congo red; linear ribbon-like deposition along the basement membrane of the glomeruli and the vasculatures

**Ultrastructural Findings**
- Discrete, electron-dense, punctate deposits

**Fine-Needle Aspiration Biopsy Findings**
- Amorphous material similar to amyloid, but negative on Congo red stain

**Immunofluorescence Findings**
- The deposits are positive for κ or λ light chain or heavy chain by immunofluorescence stain

**Differential Diagnosis**
- Amyloidosis, diabetic nephropathy

## Pathologic Features

Monoclonal immunoglobulin deposits in tissue as amorphous eosinophilic material resembling amyloid, but does not stain for Congo red. The diagnosis of LCDD is usually established by renal biopsy with appropriate ancillary studies. In the kidney, LCDD is characterized by nodular glomerulosclerosis, resembling diabetic nephropathy. Deposition in the renal vasculature results in proliferative vasculopathy. The renal tubules show interstitial disease.

As in AL, most cases (50% to 60%) of LCDD have minimal plasmacytosis in the bone marrow. Immunohistochemistry is usually required to demonstrate monoclonality of the plasma cells. The bone marrow is involved with overt myeloma only in 10% of patients.

## Ancillary Studies

By immunofluorescence, abnormal immunoglobulin appears along the renal glomerular and tubular basement membrane as a linear ribbonlike deposition. By electron microscopy, the abnormal immunoglobulin typically appears as discrete, electron-dense, punctate deposits. These deposits are usually demonstrated in the kidney biopsy specimen. Similar changes can also be observed in the dermal–epidermal junction of the skin biopsy specimens.

### Serum Protein Electrophoresis and Immunofixation

Serum M protein is usually minimal in most patients, but helps to establish the diagnosis of plasma cell neoplasms. Serum FLC ratio is useful in diagnosing LCDD.

### Differential Diagnosis

The differential diagnosis is amyloidosis. The abnormal immunoglobulin in LCDD or HCDD is Congo red negative.

### Prognosis and Therapy

The overall prognosis is poor with an overall survival of 1 to 2 years, regardless of plasma cell tumor load.

## HEAVY CHAIN DISEASE

Heavy chain disease (HCD) is a group of rare B-cell neoplasms that exhibit marked lymphoplasmacytic differentiation and are associated with an excessive amount of a monoclonal heavy chain devoid of light chain. The clinical presentations of HCD are variable and depend on the type of monoclonal immunoglobulin heavy chain produced. The three major types of HCD, in decreasing order of frequency, are α-, γ-, and μ-chain diseases. The α-chain disease is associated with extranodal marginal zone B-cell lymphoma. The γ-chain disease is usually classified as lymphoplasmacytic lymphoma. The μ-chain disease is considered a variant of chronic lymphocytic leukemia.

**HEAVY CHAIN DISEASE—FACT SHEET**

**Definition**
- B-cell neoplasms associated with secretion of monoclonal immunoglobulin heavy chain devoid of light chain; αHCD, γHCD, and μHCD are the three most common types

**Incidence and Location**
- Incidence is rare
- Sites of involvement are variable depending on the type of disease
- αHCD involves small intestine or mesenteric lymph nodes; γHCD and μHCD involve bone marrow or other extramedullary sites

**Morbidity and Mortality**
- Markedly variable ranging from months to years

**Gender and Age Distribution**
- Young adults in αHCD, elderly males in γHCD and μHCD

**Clinical Features**
- Gastrointestinal symptoms in αHCD, chronic lymphocytic leukemia, or Waldenström macroglobulinemia–like presentations in γHCD and μHCD with hepatosplenomegaly and peripheral lymphocytosis
- Autoimmune disorders common in γHCD

**Radiologic Features**
- Nonspecific

**Prognosis and Therapy**
- Prognosis is variable
- Early stages of αHCD are managed with oral antibiotics; early stages of γHCD and μHCD are observed only; advanced stages of all three types of HCD disease require chemotherapy

### α-CHAIN DISEASE

#### Clinical Features

αHCD, also known as *Mediterranean lymphoma,* typically affects young adults in the second and third decades of life from the Mediterranean area or Middle East. Environmental factors such as poor hygiene and malnutrition with repeated acute infectious diarrhea and chronic parasitic infestations have been implicated. Presenting symptoms are most often gastrointestinal, such as abdominal pain, diarrhea, and malabsorption. The disease appears to evolve from chronic infections that may initially respond to antibiotic treatment. Recent studies suggest that *Campylobacter jejuni* may be an important pathogenic micro-organism.

#### Pathologic Features

The neoplastic infiltrate primarily involves the small intestine and may spread to the mesenteric lymph nodes and show features of marginal zone B-cell lymphoma. In the early stage of the disease, the lamina propria is infiltrated by small mature lymphocytes, plasmacytoid lymphocytes, and plasma cells producing only α-chain protein. Lymphoepithelial lesions may be present with associated villous atrophy. As the disease progresses, the infiltrate extends to the submucosa, and large immunoblasts are increased. In the advanced stage the infiltrate becomes transmural, and the tumor may transform to immunoblastic large B-cell lymphoma.

**HEAVY CHAIN DISEASE—PATHOLOGIC FEATURES**

**Gross Findings**
- Mass formation; ulcer in αHCD

**Microscopic Findings**
- Marginal zone B-cell lymphoma in αHCD with infiltrate of mature lymphoplasmacytic cells associated with villous atrophy
- Lymphoplasmacytic lymphoma in γHCD and μHCD associated with lymphocytosis

**Ultrastructural Findings**
- Nonspecific

**Fine-Needle Aspiration Biopsy Findings**
- Lymphoplasmacytic infiltrate

**Immunohistochemical Findings**
- The lymphoid cells are positive for pan B–cell markers

**Differential Diagnosis**
- Chronic lymphocytic leukemia

### Ancillary Studies

SPEP is usually normal or shows only hypogammaglobulinemia without a distinct spike. Sometimes the abnormal protein appears as a broad band extending from the α2-β2 region. IFE with antibody specific for α is needed to confirm the diagnosis.

### Prognosis and Therapy

Survival varies from a few months to several years, depending on the stage of the disease.

## γ-CHAIN DISEASE

### Clinical Features

γHCD, also known as *Franklin disease,* is a heterogeneous disease with variable clinical presentation. It affects mostly elderly people in their 60s. "B type" of symptoms, anemia, recurrent infection, lymphadenopathy, and hepatosplenomegaly are common presenting symptoms. There are usually no lytic bone lesions. Autoimmune diseases may occur in as many as one third of patients manifesting as autoimmune hemolytic anemia, Sjögren syndrome, rheumatoid arthritis, systemic lupus erythematous, or thyroiditis. The abnormal protein consists primarily of the Fc region of the heavy chain with a normal carboxy terminal end followed by an internal deletion of the V and the entire CH1 domain.

### Pathologic Features

The lymph nodes, bone marrow, spleen, and liver are frequently involved. The neoplastic infiltrate in some cases is composed predominantly of lymphoplasmacytoid cells falling into the category of lymphoplasmacytic lymphoma. Other cases may resemble processes such as splenic marginal zone or splenic diffuse red pulp small B-cell lymphoma.

### Ancillary Studies

SPEP usually detects no abnormal protein. IFE confirms monoclonal γ-heavy chain without light chain production. The low molecular weight of the γ-heavy chain allows the abnormal protein to be detected in the serum and urine.

### Prognosis and Therapy

Survival varies greatly from months to years depending on the clinical features. Those with an indolent disease are often managed with observation only, whereas those with aggressive disease generally require chemotherapy with agents such as fludarabine, cyclophosphamide, or doxorubicin.

## μ-CHAIN DISEASE

### Clinical Features

μHCD is a rare and indolent disease that clinically resembles chronic lymphocytic leukemia or Waldenström macroglobulinemia. The patients usually exhibit hepatosplenomegaly without lymphadenopathy.

### Pathologic Features

The peripheral blood shows lymphocytosis. The bone marrow is infiltrated by small lymphocytes, plasmacytoid lymphocytes, and mature plasma cells with cytoplasmic vacuoles, as in lymphoplasmacytic lymphoma–Waldenström macroglobulinemia.

## Ancillary Studies

IFE detects excess monoclonal μ-chains in the serum. In contrast to αHCD or γHCD, Bence-Jones protein of monoclonal κ light chain type can be detected in the urine of two thirds of patients with μHCD and may be associated with cast nephropathy.

## Differential Diagnosis

γHCD with marked plasmacytic differentiation needs to be distinguished from myeloma. Those with more peripheralized disease need to be distinguished from chronic lymphocytic leukemia by immunophenotyping.

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The complete reference list is available online at www.expertconsult.com.

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# 22

# Disorders of the Spleen

■ Robert S. Ohgami, MD, PhD ■ James M. Ziai, MD ■ Daniel A. Arber, MD

## ■ INTRODUCTION

The spleen is a secondary lymphoid organ that functions in the development of the immune response and in blood filtration. In the spleen, blood-borne antigens are presented to lymphocytes and macrophages to facilitate antibody development and cytotoxic immune responses. In addition, senescent red blood cells and blood-borne foreign substances are introduced to and removed by macrophages. Finally, the spleen also serves as a reservoir for platelet and granulocytic storage, dynamically adjusting cellular contents in the peripheral blood during times of stress.

## ■ NORMAL SPLEEN ANATOMY AND HISTOLOGY

The average adult spleen measures 12 × 7 × 3.5 cm, weighs 150 g, and contains a blood volume of 300 mL. The medial portion is concave and divided into the gastric (anterior) surface, which abuts the hilum and tail of the pancreas, and renal (posterior) surface, which abuts the upper pole of the left kidney and adrenal gland. The main blood supply to the spleen is the splenic artery. At the hilum, the splenic artery branches into the superior polar, superior middle, inferior middle, and inferior polar splenic arteries. Venous drainage is primarily through the splenic vein, which arises from the hilum and courses along the superior aspect of the pancreas to join the superior mesenteric vein forming the portal vein. The left gastroepiploic veins also provide ancillary drainage for the spleen.

The spleen is divided into two visually distinct components: red pulp and white pulp. The red pulp is a three-dimensional network of cords and sinuses that compose approximately three fourths of the splenic volume. The cords are reticular fibers, a matrix of collagenous connective tissue, with admixed myofibroblasts and fibroblasts; scattered lymphocytes, plasma cells, and macrophages are also seen. Between these cords lie the splenic sinuses, which are filled with peripheral blood cells and macrophages; these sinuses are lined by plasma cells and plasmablastic cells that migrate from follicles. In contrast, the white pulp constitutes the remaining one fourth of the spleen and consists of B- and T-lymphocytes that are found either around vessels or as independent follicles. Lymphocytes around vessels form the periarteriolar lymphoid sheath and are predominantly T cells, whereas lymphoid follicles are composed predominantly of B cells with some admixed T cells. Generally, follicles are found adjacent to the periarteriolar lymphoid sheath, but can also be seen in isolation within the red pulp. Germinal centers are not a usual component of the white pulp in adulthood (Figure 22-1). Finally, the marginal zone constitutes the area between the white and red pulps and contains both lymphocytes and macrophages; it is here that the presentation of peripheral blood elements to lymphocytes and macrophages occurs.

## ■ TRAUMA AND CONGENITAL ABNORMALITIES

Both trauma and congenital abnormalities of the spleen result in distinct clinical and pathologic findings, which warrant discussion here.

### SPLENIC RUPTURE

Splenic rupture can be classified as spontaneous or delayed. Spontaneous splenic rupture is more common in males and typically occurs in relation to infarction, thrombocytopenia, tumors, lymphoma, or leukemia. *Delayed splenic rupture* is defined as development of splenic hemorrhage more than 7 days after an injury and is thought to be the result of an evolving initial splenic contusion. Symptoms of acute or delayed splenic rupture include abdominal pain with referred shoulder pain, abdominal distention, and signs of bleeding or shock. Ruptured spleens, even those removed acutely, characteristically show reactive white pulp changes in addition to subcapsular hemorrhage. Emergent splenectomy is the only definitive therapy.

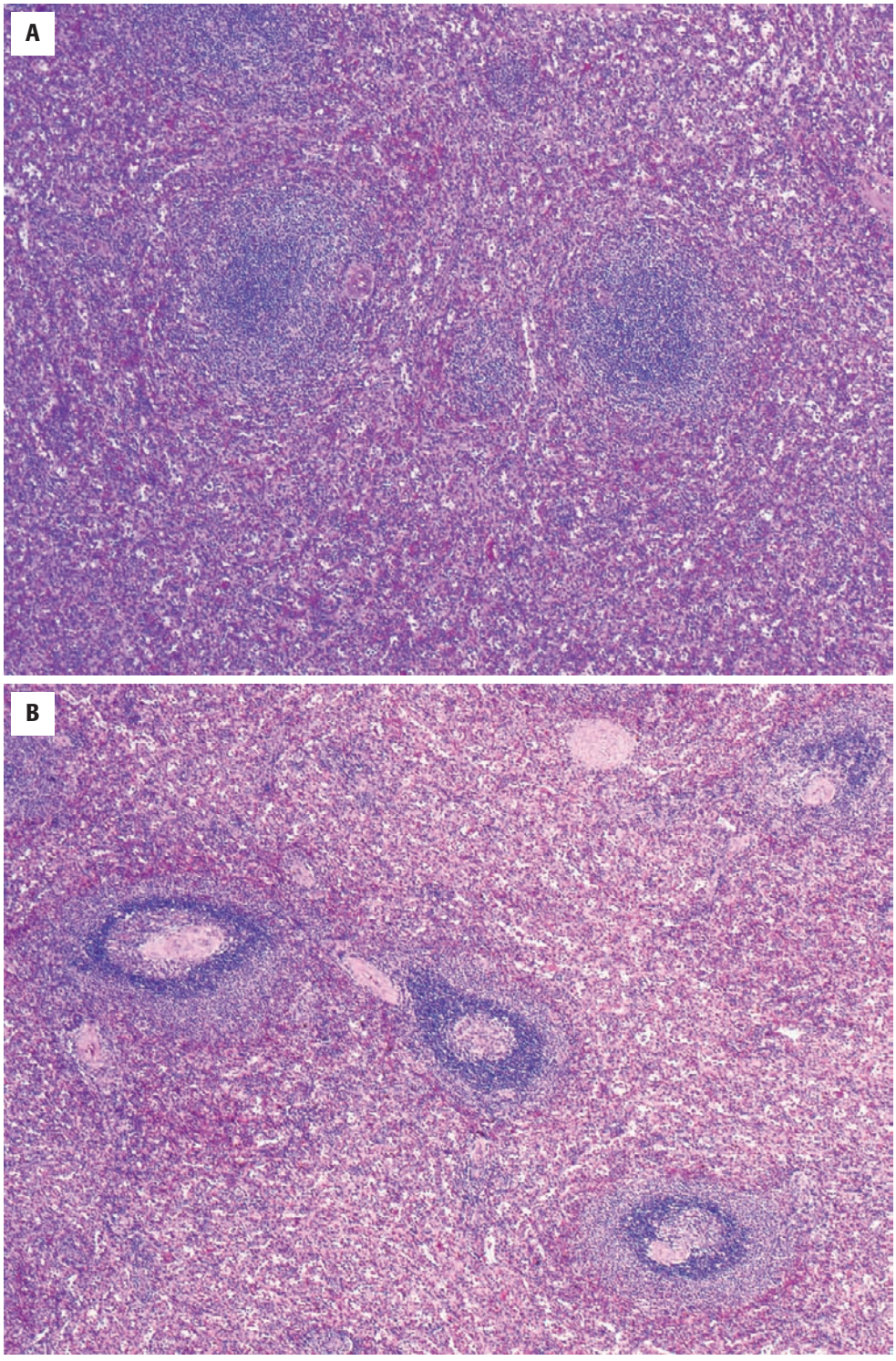


**FIGURE 22-1**

Normal and reactive spleen. **A,** The normal adult spleen shows aggregates of white pulp small lymphocytes without distinct germinal center formation. The red pulp containing the splenic cords and sinuses resides between the lymphoid component. **B,** In an adult with autoimmune or infectious conditions, including immune thrombocytopenic purpura, the splenic white pulp is evenly distributed but expanded with distinct germinal centers, mantle zones of dark staining cells, and an outer marginal zone of medium sized cells with more abundant, pale staining cytoplasm. These distinct zones are also seen in normal spleens of children.

## SPLENOSIS

One consequence of splenic rupture is splenosis, which is dissemination of splenic tissue to a separate anatomic site. This phenomenon is also a common sequela of trauma or surgery. It occurs in up to 75% of trauma patients undergoing splenectomy and is thought to result from seeding or hematogenous spread of tissue, or both. Splenosis usually occurs in the abdominal and pelvic cavities, but intrathoracic, subcutaneous, intrahepatic, and intracranial cases have been described. The heterotopic splenic tissue generally demonstrates abnormal histology; it lacks trabecular structures and has poorly formed white pulp, although the red pulp is normal. Functionally these splenosis nodules are capable of clearing senescent erythrocytes, but do not offer protection against encapsulated bacteria. Splenosis nodules are often detected on CT, raising concern for malignancy; however, they are benign and removal is required only in symptomatic cases.

## ACCESSORY SPLEEN

A relatively common congenital variation is that of an accessory spleen, which is seen in up to 20% of otherwise normal individuals and is found in greater frequency (up to 30%) in patients with hematologic disorders. It is thought to result from inadequate fusion of primary lobules during the second or third trimester of fetal development. Most patients with an accessory spleen have only one, although multiples are not uncommon. In the majority of cases, an accessory spleen is found near the hilum or in the supporting ligaments or greater omentum. Other notable locations are (in order of frequency) the gastrocolic ligament, pancreatic tail, greater omentum, greater curvature of the stomach, the splenocolic ligament, the small and large bowel mesentery, the left broad ligament in women, and the left spermatic cord in men. Accessory spleens are histologically and functionally identical to the native spleen. As such, if a patient fails therapeutic splenectomy, the possibility of an accessory spleen should be considered.

## ASPLENIA

Asplenia is congenital or acquired. Congenital asplenia is rare and inherited in a predominantly autosomal dominant pattern. It often develops as pneumococcal sepsis in an infant or newborn. Although it can occur in isolation, it is also associated with heterotaxy syndromes. The most common of these syndromes is the Ivemark syndrome, in which right-sided organs are duplicated and organs that are normally present on the left side are absent. Other associated conditions include Pearson syndrome, Stormorken syndrome, Smith-Fineman-Myers syndrome, ATR-X syndrome, Fanconi anemia, and autoimmune polyendocrine syndrome type 1. However, the most common cause of asplenia is secondary to trauma, infarction, or surgery. Regardless of etiology, the most important consequence of asplenia is increased susceptibility to infection by encapsulated organisms, most commonly *Streptococcus pneumoniae. Neisseria meningitidis, Escherichia coli, Haemophilus influenzae, Staphylococcus* species, and *Streptococcus* bacteremia, which occur at rates ranging from 7% to 12% in asplenic patients. Interestingly, this increased susceptibility is due to defective immunoglobulin (Ig) M and opsonin production.

Asplenia is evident in the peripheral blood smear, which demonstrates anisopoikilocytosis of red blood cells including acanthocytes, echinocytes, and target cells as well as red blood cell inclusions such as Pappenheimer and Howell-Jolly bodies.

## SPLENIC-GONADAL FUSION

Another congenital anomaly is that of splenic-gonadal fusion, which results from fusion of the splenic anlage with the gonadal mesoderm of the left urogenital fold (Figure 22-2). Typically a disorder of young males (male-to-female ratio, 20:1), it often manifests as a scrotal mass identified during physical examination for cryptorchidism or during inguinal hernia repair. The condition exists in two forms. The first more common form is characterized by a continuous cord of splenic or fibrous tissue between the normal spleen and ectopic mass. The second, discontinuous type, shows no connection between spleen and mass. Associated cryptorchidism, micrognathia, and limb defects are commonly associated with continuous splenic-gonadal fusion, but are rarely seen with the discontinuous form. Less commonly, cardiac defects, spina bifida, cleft palate, and imperforate anus can be seen. Typically, the ectopic splenic tissue is confined to the tunica vaginalis. It is well demarcated from the gonad and has a fibrous capsule and a burgundy red-to-purple cut surface. Histologically it is identical to the native spleen, although fibrosis, thrombosis, calcification, fat degeneration, or hemosiderin deposits can be observed.

## SPLENIC CYST

One of the most common benign tumors of the spleen is the splenic cyst. These tumors have a male predominance and typically appear in the third decade of life. They are designated as primary (true) or secondary (false). Primary cysts represent approximately 20% of all splenic cysts. They are unilocular and have a firm, fibrous, trabecular wall that is lined by mesothelial cells or squamous epithelium (Figure 22-3). Notably, the

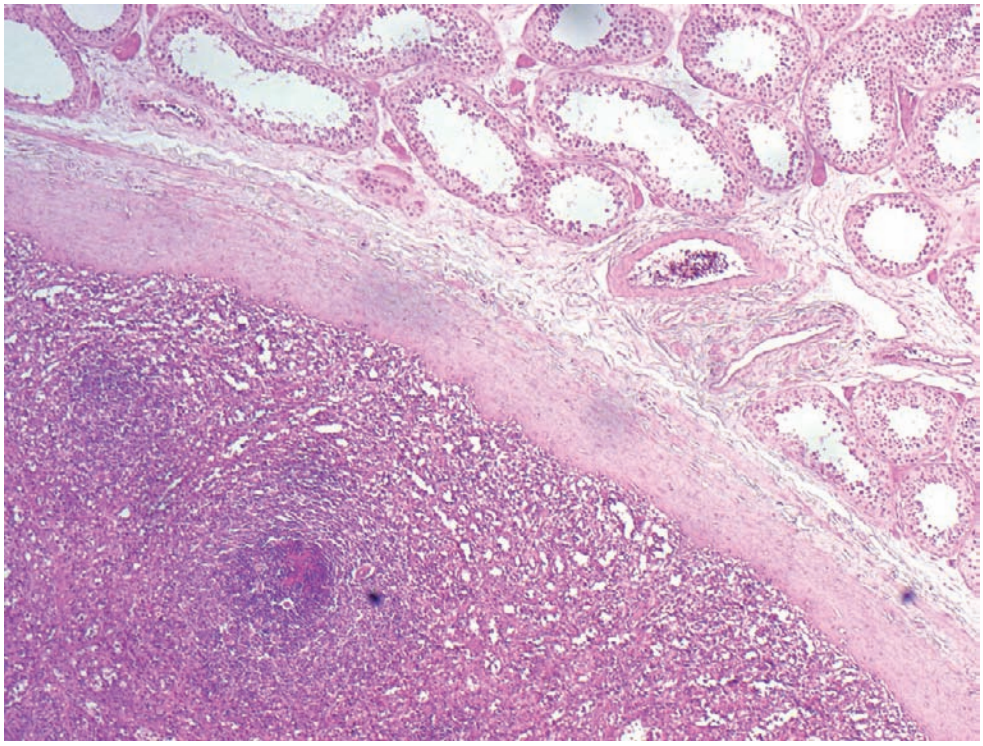

**FIGURE 22-2**

Splenic-gonadal fusion. Seminiferous tubules *(top right),* usually without spermatogenesis, are present fused with normal splenic tissue.

**SPLENIC CYST—FACT SHEET**

**Clinical Features**

- Primary cyst: male predominance; third decade of life
- Secondary cyst: often associated with history of trauma

**Gross Features**

- Primary cyst: unilocular with a firm fibrous trabeculated wall
- Secondary cyst: unilocular with a nontrabeculated thin wall

**Morphology**

- Primary cyst: wall lined by mesothelial cells or squamous epithelium
- Secondary cyst: wall with absence of epithelial lining

**Prognosis and Therapy**

- Treatment is a simple splenectomy
- Incomplete resection in primary cysts leads to recurrence

epithelial lining of primary cysts may be patchy, with denuded areas present that may simulate a secondary cyst. Primary cysts can be further subdivided into parasitic and nonparasitic types. Parasitic cysts, though uncommon, are typically attributable to *Echinococcus* species and are readily identified by the presence of parasite scolices in the cyst contents. Nonparasitic primary cysts appear to arise from congenital inclusions of capsular mesothelium. Interestingly, patients with primary cysts may have elevations of CA19-9 and carcinoembryonic antigen. Treatment in symptomatic cases requires a complete splenectomy because incomplete resection often leads to recurrence.

Secondary cysts represent approximately 80% of splenic cysts and are often associated with a history of abdominal trauma. These cysts are unilocular and thin walled and differ from primary cysts by the complete absence of an epithelial lining; therefore they are unlikely to recur even if only partially resected.

## INFLAMMATION OF THE SPLEEN

Inflammation of the spleen commonly results in splenomegaly. Generally it is a response to local or systemic infectious or noninfectious diseases.

### FOLLICULAR HYPERPLASIA

Germinal center formation in the white pulp is a normal finding in the childhood spleen, but its presence is abnormal in adults (see Figure 22-1, B). Reactive follicular hyperplasia can be seen as a result of a systemic infection as in bacterial sepsis, measles, typhoid fever, and AIDS. In addition, autoimmune conditions such as

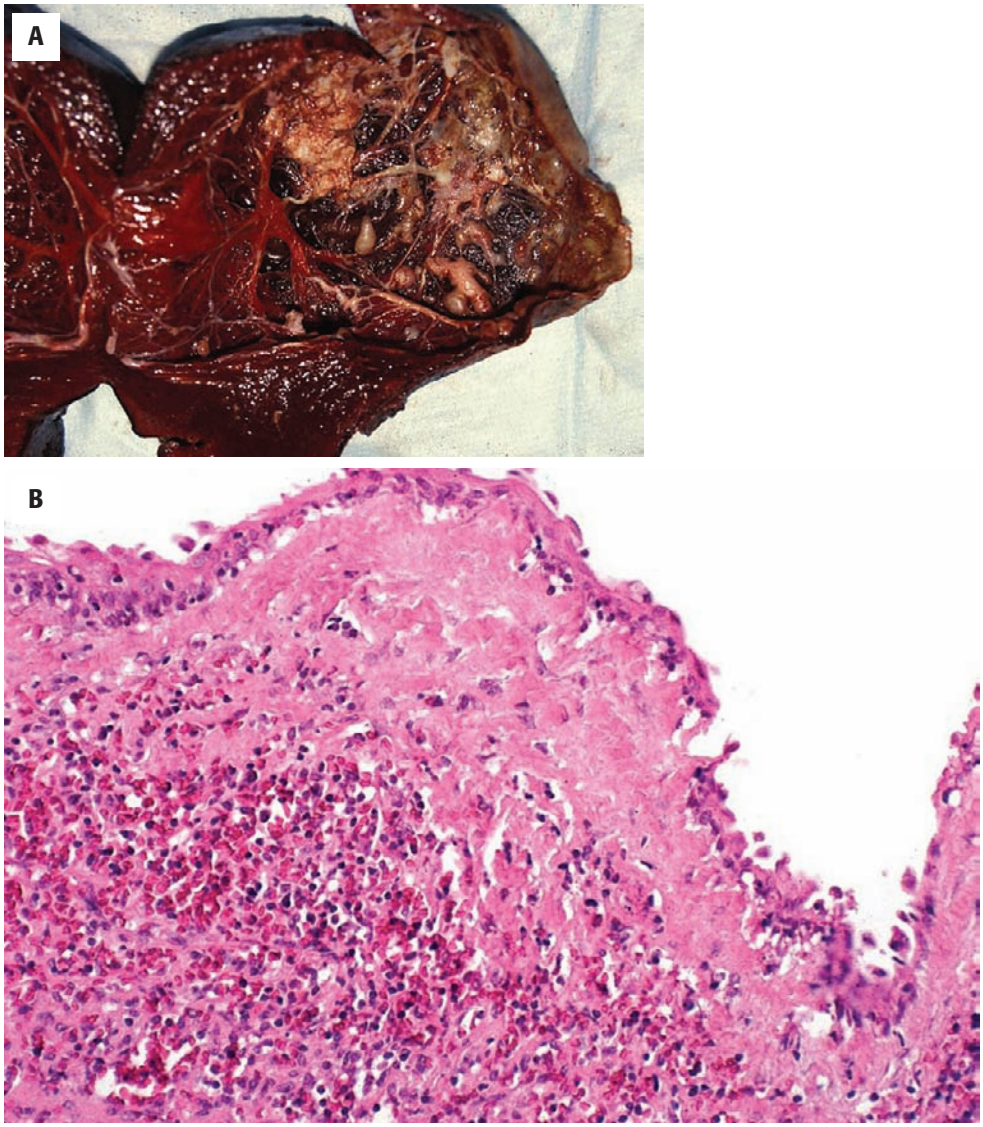


**FIGURE 22-3**

Primary splenic cyst. **A,** Grossly, the cyst is trabeculated without serious or slightly bloody fluid contents. **B,** The cyst wall shows an epithelial lining, which should at least be focally present in all primary cysts. The lining may be mesothelial, squamous, or a mixture of cell types.

rheumatoid arthritis and idiopathic thrombocytopenic purpura (ITP) can lead to follicular hyperplasia. The histologic findings are those of a balanced and proportional expansion of all lymphoid compartments: follicles, mantle zone, marginal zone, and peripheral arteriolar sheaths. Disproportionate expansion of B cells in the mantle or marginal zones should be cautiously evaluated, because these can be subtle indications of involvement by lymphoma.

## SPLENIC ABSCESS

Splenic abscesses are rare, with a predicted frequency of less than 0.7% based on autopsy studies, and may be single or multiple. The most common etiology is hematogenous seeding of bacteria from a secondary site (e.g., heart, lung, urinary tract). Other well-known predisposing conditions are splenic trauma and hemoglobinopathies. Most abscesses are well circumscribed with a thick nonepithelialized fibrous wall and central accumulation of necrotic tissue associated with acute inflammatory cells. Preserved splenic tissue surrounding the abscess may show white pulp hyperplasia. All abscesses should be cultured for bacterial and fungal organisms; however most cases are associated with Gram-negative bacilli, *Staphylococcus aureus,* or *Streptococcus* species, although *Salmonella* species is commonly seen in sickle cell patients. Fungal infection, though rare, can be seen. *Candida, Aspergillus,* and *Cryptococcus* species are the most common culprits.

## INFECTIOUS MONONUCLEOSIS IN THE SPLEEN

Nearly half of all patients with infectious mononucleosis demonstrate splenomegaly. Although most patients show no significant complications, a well-known adverse outcome from Epstein-Barr virus (EBV) infection of the spleen is splenic rupture secondary to trauma; however, most cases of rupture are minor in severity. The morphologic findings of infectious mononucleosis are varied, but the spleen can enlarge because of expansion of the red pulp and white pulp with a spectrum of immunoblasts, reactive lymphocytes, and plasma cells. In dramatic cases, Reed-Sternberg–like cells may be seen. In such cases, evaluation of these cells with immunohistochemical stains for CD30 and CD15 must be made with caution, because CD30 will be positive in the reactive immunoblasts, but these cells should generally not express CD15. In addition, the histologic picture can mimic that of other non-Hodgkin lymphomas. In these cases, careful evaluation of the splenic architecture should provide necessary information, because lymphomas will unequally expand the spleen, unlike infectious mononucleosis, in which the underlying balance of white and red pulp elements will be retained.

## GRANULOMAS

Infection with mycobacteria, fungus, *Brucella* species, or EBV can result in extensive necrotizing granulomas that typically involve the white pulp and are generally well circumscribed. However, granulomas can also be seen as malignancies such as hairy cell leukemia and both Hodgkin and non-Hodgkin lymphomas. In addition, sarcoidosis, uremia, and selective IgA deficiency can result in splenic granulomas. Finally, lipogranulomas—collections of vacuolated histocytes surrounded by plasma cells and lymphocytes—are a relatively common benign finding in North America, but not so in other parts of the world. Such geographic stratification together with other correlative data have led to the hypothesis that lipogranulomas are caused by differences in dietary intake or packaging of foods, ultimately resulting in absorption of mineral oils through the intestine and distribution throughout the body and to the spleen.

## METABOLIC DISEASES WITH SPLENIC MANIFESTATION

The most common congenital metabolic diseases with significant pathologic manifestations within the spleen are the lysosomal storage diseases, which demonstrate morphologic findings similar to those seen in the marrow (see Chapter 6). In general, these congenital enzyme deficiencies develop early in infancy or childhood and result in splenomegaly.

## GAUCHER DISEASE

The most common of the lysosomal storage diseases is Gaucher disease, which is an autosomal recessively inherited deficiency of glucocerebrosidase (β-glucosidase). The spleen is typically diffusely enlarged, and the cut surface is pale and filled with large collections of Gaucher cells, histiocytes with eccentric nuclei, and abundant tissue paper–like blue-gray foamy cytoplasm (Figure 22-4, A). These cells are best appreciated on smear or touch preparations. The major differential diagnosis is that of other lysosomal storage diseases and in particular, Niemann-Pick disease; however, one should also consider hematologic malignancies, many of which demonstrate Gaucher-like cells. Prognosis is variable and depends on the subtype of Gaucher disease. Patients can undergo recombinant enzyme replacement therapy to prevent complications.

## NIEMANN-PICK DISEASE

Niemann-Pick disease is rare, occurring in an estimated 1/120,000 live births and is inherited in an autosomal recessive pattern. It results from a deficiency of either acid sphingomyelinase (types A and B) or Niemann-Pick C proteins (types C and D). Although Niemann-Pick disease can be typed according to enzyme deficiency, the disease is also separated into five categories based on clinical features (type IA, IS, IC, IIS, and IIC). In the case of type IA and IS, the spleen is characteristically massively enlarged, often 10 times normal size. Cut cross-sections demonstrate homogeneously pale tissue. Microscopic examination reveals diffuse expansion of the red pulp by sphingomyelin-laden foamy macrophages with characteristic small, uniform, mulberry-like cytoplasmic globules.

## OTHER METABOLIC DISEASES INVOLVING THE SPLEEN

The mucopolysaccharidoses (MPS) are progressive diseases resulting from the inability to effectively process glycosaminoglycan. These are inherited autosomal recessively with the exception of MPS 2, or Hunter syndrome, which is X-linked recessive. Hepatosplenomegaly is a common presentation. Microscopic examination of the spleen will demonstrate plump vacuolated histiocytes. A diagnosis can generally be made by urine analysis, which reveals increased concentration of glycosaminoglycan fragments. Most patients can be screened initially with an enzyme assay and by

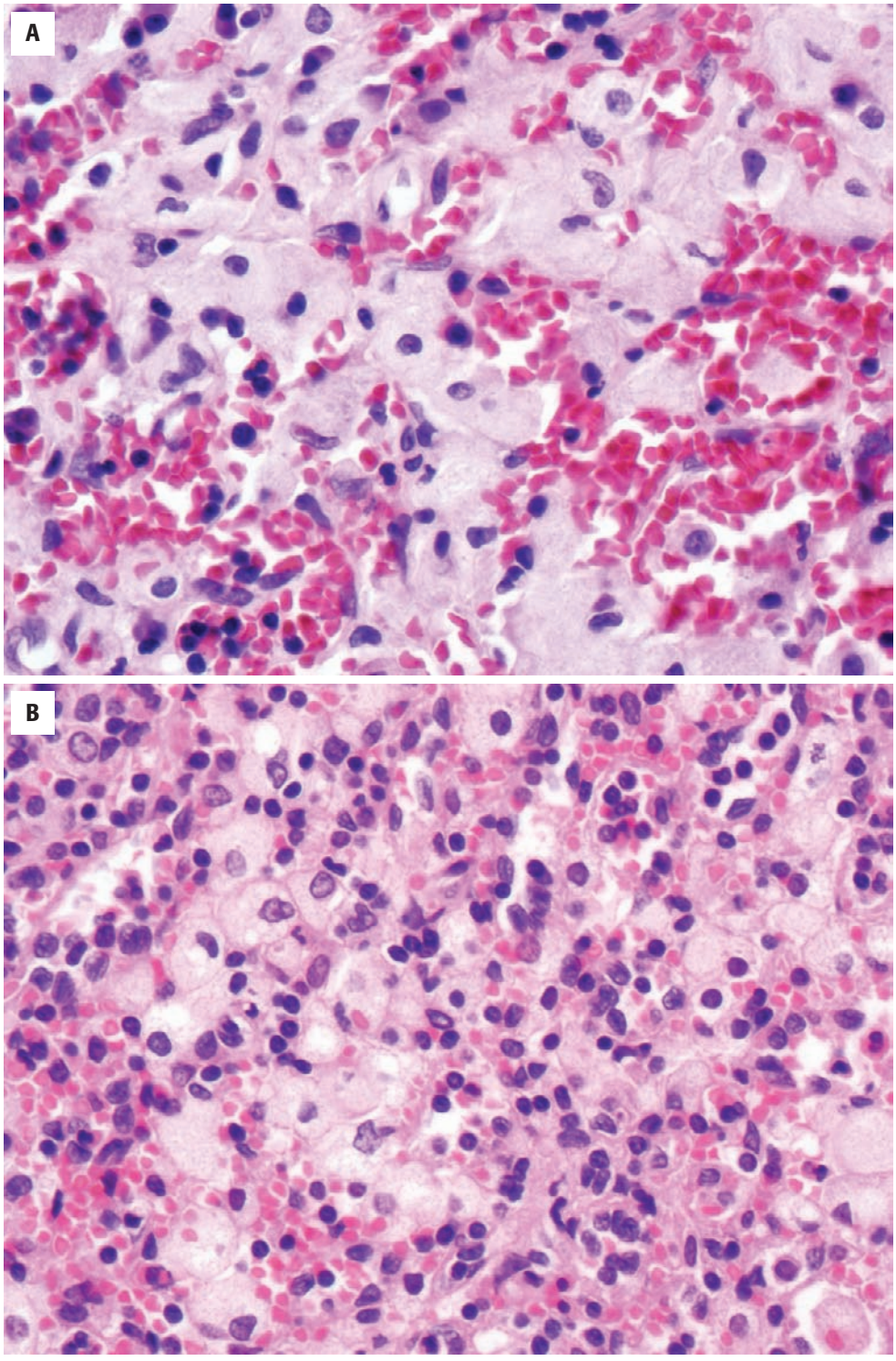


**FIGURE 22-4**

Storage diseases in the spleen. **A,** Gaucher disease frequently involves the spleen with extensive red pulp replacement by large histiocytes with so-called tissue paper cytoplasm. **B,** The less specific ceroid histiocytes also involve the red pulp with less distinctive features.

sequencing known mutations. Tangier disease, which is due to defective cholesterol transport, presents in childhood with hepatosplenomegaly and premature coronary atherosclerosis. Tissues demonstrate accumulation of foamy cholesterol-laden macrophages, particularly in the spleen. In Tay-Sachs disease, hexosaminidase A deficiency, there is accumulation of GM2 ganglioside in the heart, liver, and spleen leading to plump vacuolated histocytes. Involvement of the central nervous system with vacuolated neurons is also predominant. In addition,

nondescript foamy histiocytes are seen in many other metabolic diseases, including Fabry disease, Wolman disease, and von Gierke disease. Finally, sea blue histiocytes may be found nonspecifically in many lysosomal storage disorders and in Hermansky-Pudlak syndrome, chronic myelogenous leukemia, red blood cell disorders, and autoimmune disorders. These histiocytes are associated with massively increased cell destruction, and in these cases the macrophage cytoplasm is filled with insoluble lipid pigment, called *ceroid* (see Figure 22-4, B). Compared with Gaucher cells, these cells are stained more intensely blue with Wright-Giemsa, and the inclusions are globular rather than fibrillary.

## HYPERSPLENISM-RELATED DISORDERS

### IDIOPATHIC THROMBOCYTOPENIC PURPURA

Therapeutic splenectomy is occasionally performed on patients refractory to medical therapy in order to raise platelet counts. The spleen in ITP is generally unremarkable in gross appearance. Characteristically the white pulp demonstrates attenuated mantle zones with hyperplastic marginal zones containing increased immunoblasts and plasma cells. In cases of ITP, the presence of splenic congestion or white pulp expansion should raise suspicion for infarction or lymphoma, respectively.

### THROMBOTIC THROMBOCYTOPENIC PURPURA

Thrombotic thrombocytopenic purpura (TTP) is a rare adult disorder with an incidence of approximately 4 per 100,000 (see Chapter 2). Treatment involves fresh frozen plasma, and splenectomy is uncommonly performed for refractory or relapsing patients. Grossly, the spleens of patients with TTP are not typically splenomegalic (mean weight, 214 g). The histologic features are somewhat varied, but common characteristics are subendothelial deposits, hemophagocytosis, arteriolar thrombi, B-cell hyperplasia, and periarteriolar concentric fibrosis. Hemosiderosis also occurs and can be highlighted with a Prussian blue stain. Less typical findings include extramedullary hematopoiesis, endothelial cell proliferation, infarcts, and blood lakes.

### CONGESTIVE SPLENOMEGALY

Portal hypertension ultimately results in congestive splenomegaly. Most cases are the result of splenic, hepatic, or portal vein thrombosis, congestive heart failure, or cirrhosis. Rarely, cases of portal hypertension-associated splenomegaly have no discernible cause and are termed *idiopathic portal hypertension*. Grossly, the spleen appears large, firm, and dark. On histologic analysis, the white pulp is decreased and there is marked sinusoidal and venous dilatation with numerous hemosiderin-laden macrophages and red pulp fibrosis. Focal hemorrhage is often seen, sometimes with development of sclerotic nodules. Resolution of splenomegaly is achieved by treating the underlying condition.

### OTHER HYPERSPLENISM-RELATED DISORDERS

In hereditary spherocytosis, splenomegaly is common and is the result of massive congestion of splenic cords by spherocytes. Hyperplastic follicles also may be seen. Splenectomy, which is typically reserved for patients with severe disease, corrects the anemia.

Similar to hereditary spherocytosis, acquired immune-mediated hemolytic anemia results in congestion of the splenic cords or sinuses, or both, by numerous erythrocytes, which gives the spleen an overall dark, red, and firm gross appearance. Acquired hemolytic anemias have varied etiologies and may be the result of toxins (bacterial hemolysins), plasma lipid abnormalities, parasites, and immune reaction. The immune-mediated hemolytic anemias are usually Coombs-positive (either autoimmune or alloimmune in the setting of prior transfusion). The lining cells of sinuses are often prominent, and foci of extramedullary hematopoiesis may be observed; splenic infarcts may be found in about one quarter of cases. Splenectomy is typically reserved for cases of autoimmune hemolytic anemia refractory to medical management and achieves remission in approximately 50% of cases.

In sickle cell disease, the two major sequelae in the spleen are autoinfarction and splenic sequestration. Typically, sickle cell patients with sequestration are young, and grossly their spleens are enlarged and show hemorrhagic infarcts. On histologic examination, the spleen shows hemorrhagic necrosis and sickle cells within the splenic cords. Spleens from older patients with sickle cell disease are small and fibrotic, and on microscopy they show white pulp atrophy with infarcts encrusted with iron and calcium (Gamna-Gandy bodies).

## SPLENIC LYMPHOMAS–LEUKEMIAS

Six splenic lymphomas–leukemias that preferentially localize to the spleen are splenic marginal zone lymphoma, hairy cell leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant of the spleen, hepatosplenic T-cell lymphoma, and micronodular T-cell–histiocyte-rich large B-cell lymphoma

(MTLBL) of the spleen. Interestingly these lymphomas and leukemias are generally not confined to the spleen and involve the marrow and peripheral blood. With the exception of the provisional World Health Organization (WHO) category of splenic diffuse red pulp small B-cell lymphoma and MTLBL, these are discussed in the chapters on small B-cell lymphomas, B-cell leukemias of mature lymphocytes, and peripheral T-cell lymphomas.

## SPLENIC DIFFUSE RED PULP SMALL B-CELL LYMPHOMA

Splenic diffuse red pulp small B-cell lymphoma (SRPL) is rare and represents less than 1% of non-Hodgkin lymphomas. Currently they fall under the ill-defined WHO designation of B-cell lymphoma–leukemia, unclassifiable. The two best-known provisional entities in this group of lymphoma–leukemias are SRPL and HCLv. The latter has been discussed in Chapter 12. Nearly all patients with SRPL will have disseminated disease, including bone marrow and rarely cutaneous involvement. Patients are more frequently male (male-to-female ratio, approximately 2:1) and generally are elderly (median age greater than 65 years). Although splenomegaly is common, B-type symptoms are rare. Interestingly, a lymphocytosis is often present, occasionally in association with thrombocytopenia, and laboratory values may demonstrate a high lactate dehydrogenase and β2-microglobulin levels.

Grossly, cases of SRPL demonstrate a homogeneous increase in the size of the spleen, which on cut section appears red-brown and lacks any distinct nodularity. The microscopic features are those of expansion of the red pulp by monotonous populations of small to medium-sized lymphocytes with round to oval nuclei, single small nucleoli, and pale cytoplasm (Figure 22-5). In contrast, the white pulp may appear atrophic. In the peripheral blood, these tumor cells typically are greater than 25% of lymphocytes and appear small to medium in size with clumped nuclear chromatin, round nuclei, and broad or fine villous cytoplasmic projections. Occasionally cells with prominent central nucleoli may be seen in the blood and raise the differential diagnosis of prolymphocytic leukemia and hairy cell leukemia-variant; however villous projections are not a feature of prolymphocytic leukemia. As stated previously, bone marrow involvement is characteristic with lymphoma cells interspersed throughout sinusoids. Neoplastic lymphoid aggregates are not typically seen.

Immunohistochemical studies show that the neoplastic cells express CD20, CD79a, CD19, CD22, and BCL2. In addition, they are generally also positive for FMC7, DBA.44(CD76), and IgG; a subset of cases also are positive for CD11c. In contrast, MUM1, annexin A1, CD25, IgD, CD5, CD10, CD103, CD123, CD23, and BCL6 are absent. Interestingly, weak expression of CD5, CD43, CD103, CD123, or CD23 may be seen by flow cytometry.

Detailed molecular analyses have shown that these lymphomas have clonal *IGH@* rearrangements with few somatic hypermutations and *TP53* alterations are frequently seen resulting in increased p53 expression.

Treatment is varied; however, as the disease is typically indolent, no treatment at all may be required, although a subset of patients may benefit from splenectomy with or without chemotherapy.

The differential diagnosis includes hairy cell leukemia (HCL), splenic marginal zone lymphoma (SMZL), and HCL variant (HCLv). The blood smear morphology and lack of CD103 and CD25 expression by SRPL allows distinction from HCL, while the red pulp pattern of SRPL distinguishes it from SMZL. Expression of CD103 by HCLv and its usual absence in SRPL can help to separate these two entities from each other, but overlap may exist between rare cases of HCLv and SRPL. Availability of blood smear, bone marrow, and spleen for morphologic evaluation and full phenotypic characterization are necessary for the best subclassification. Without splenic histopathology, indications of SRPL include appropriate villous lymphocyte blood smear morphology and typical immunophenotype with purely sinusoidal bone marrow involvement.

**SPLENIC DIFFUSE RED PULP SMALL B-CELL LYMPHOMA—FACT SHEET**

**Clinical Features**
- Elderly (>65 years old) with male predominance
- Lymphocytosis occasionally with thrombocytopenia
- Splenomegaly is common

**Morphology**
- Monotonous population of small to medium-sized lymphocytes with round to oval nuclei, single small nucleoli, and pale cytoplasm
- Red pulp expansion by lymphoma cells

**Immunophenotype**
- $CD20^+$, $CD19^+$, $CD79a^+$, $CD22^+$, $DBA.44^+$, $IgG^+$, $FMC7^+$, $CD11c^{+/-}$, $IgD^-$, annexin $A1^-$, $CD25^-$, $CD103^-$, $CD123^-$, $CD10^-$, $BCL6^-$, $CD23^-$, $CD38^-$, $BCL1^-$

**Prognosis and Therapy**
- Indolent incurable disease that may require no treatment
- Splenectomy with or without chemotherapy

**Differential Diagnosis**
- Hairy cell leukemia and hairy cell leukemia variant
- Prolymphocytic leukemia
- Chronic lymphocytic leukemia or small lymphocytic lymphoma
- Splenic marginal zone lymphoma
- Lymphoplasmacytic lymphoma

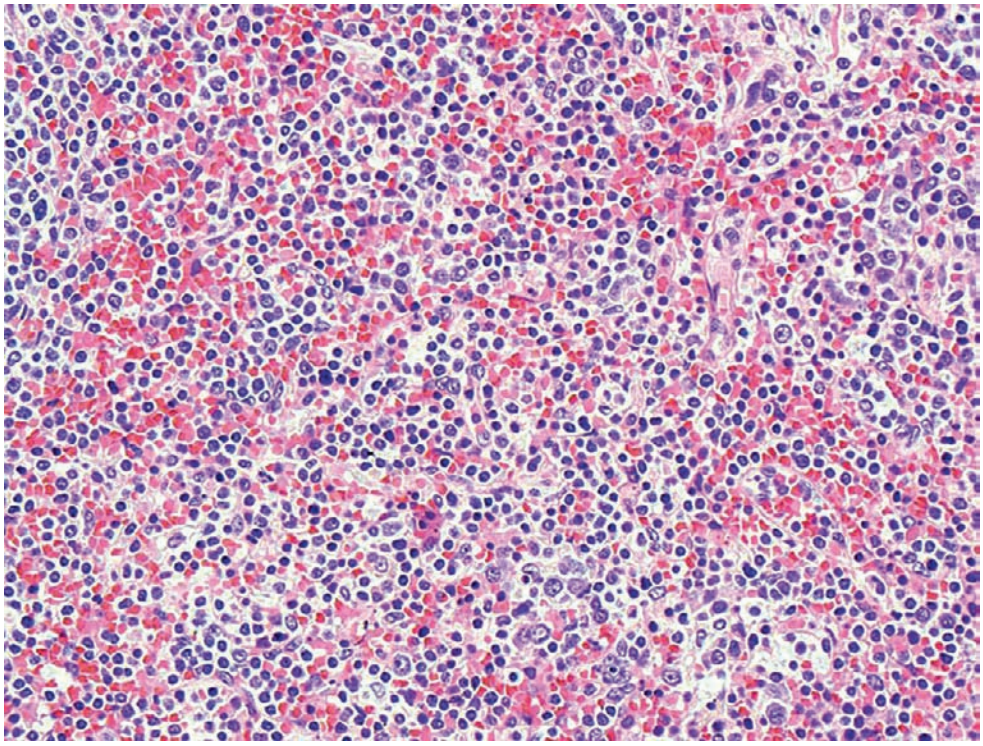

**FIGURE 22-5**

Splenic diffuse red pulp small B-cell lymphoma. The red pulp is infiltrated by small, mostly round B lymphocytes. The morphologic and immunophenotypic features (see text) are similar to the cells of splenic marginal zone lymphoma, but the pattern of infiltration is more similar to hairy cell leukemia.

## MICRONODULAR T-CELL/HISTIOCYTE-RICH LARGE B-CELL LYMPHOMA OF THE SPLEEN

Large B-cell lymphoma can occasionally develop as a primary single mass or multiple masses of the spleen. One rare variant of diffuse large B-cell lymphoma, MTLBL, has been described and constitutes a specific subset with extremely poor prognosis. Patients with MTLBL are more frequently male (male-to-female ratio, 2:1) and exhibit splenomegaly, anemia, and B type symptoms. Interestingly, on gross appearance, the spleen, though enlarged, does not show a discrete mass, and only on histologic analysis is clearly diffusely expanded by small discrete micronodules throughout white pulp. Classically the red pulp is spared. Numerous small T cells and histiocytes are seen with scattered rare (less than 10%) large atypical B cells within nodules and few, if any, small mature B cells. The large neoplastic B cells can have a somewhat pleomorphic appearance as atypical centroblasts, lymphocytic and/or histiocytic Reed-Sternberg variants (L&H cells), or Reed-Sternberg cells, thus complicating the diagnosis. Immunohistochemical studies demonstrate these B cells to be positive for CD20, CD79a, BCL6, and OCT2 with variable staining for epithelial membrane antigen (EMA) and CD30; these cells are negative for CD15. Importantly, as in T-cell/histiocyte-rich large B-cell lymphoma in nodal sites, follicular dendritic cell networks as demonstrated by CD21, CD23, or CD35 should be absent. Most cases show involvement of the perisplenic lymph nodes and bone marrow by rare atypical large B cells though diffuse sheets of large B cells can also be seen. MTLBL may be misdiagnosed as a reactive inflammatory lesion, or other non-Hodgkin or Hodgkin lymphoma if immunohistochemical studies are not performed.

## LEUKEMIAS SECONDARILY INVOLVING THE SPLEEN

Both myeloid and lymphoid leukemias commonly involve the spleen and result in generalized enlargement and expansion of the red pulp in both the splenic cords and sinuses.

## CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA

Involvement of the spleen by chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) with resultant splenomegaly is rarely a presenting complaint; however, it is often a deciding factor in initiating treatment because it heralds development of complications such as ITP, autoimmune hemolytic anemia and hypersplenism. The spleen demonstrates a miliary gross pattern that is similar to many other small B-cell

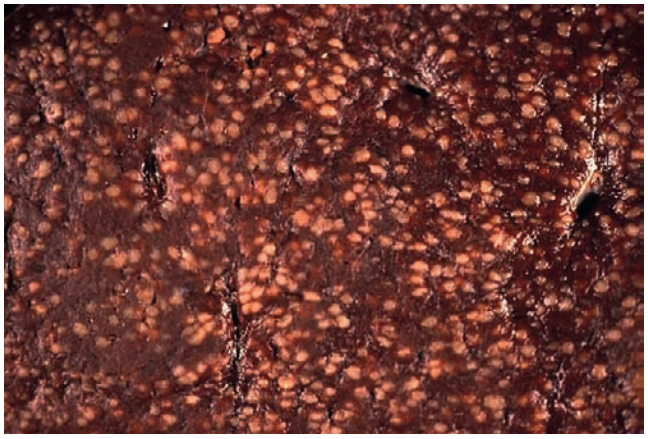

**FIGURE 22-6**

Miliary gross pattern of splenic involvement. This pattern usually represents an expansion of the white pulp and is common with splenic involvement by lymphomas of small B lymphocytes, including splenic marginal zone lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, and follicular lymphoma. In this case, the spleen is involved by chronic lymphocytic leukemia.

lymphomas (Figure 22-6). The cells of CLL/SLL predominantly involve the white pulp; they also infiltrate the red pulp. In cases with significant numbers of prolymphocytes, which can be easily distinguished by their large nucleoli, splenomegaly is often massive. The differential diagnosis includes other small cell lymphomas, including lymphoplasmacytic lymphoma, mantle cell lymphoma, and follicular lymphoma. With the exception of a subset of cases of lymphoplasmacytic lymphoma, these disorders all infiltrate and expand the splenic white pulp. As such, the diagnosis rests largely on immunophenotypic and molecular studies, with CLL showing dim CD20 and dim surface light chain expression with aberrant CD5 expression on $CD23^+$ B cells. Patients with CLL and splenic involvement are typically advanced stage and have shorter survival times. Interestingly, studies have shown improved survival in patients with hemoglobin less than 10 g/dL or platelet count less than $50 \times 10^9/\mu L$ who received splenectomy in addition to standard chemotherapy.

## ACUTE MYELOID LEUKEMIA AND ACUTE LYMPHOBLASTIC LEUKEMIA

Splenic involvement by acute myeloid leukemia (AML) and acute lymphoblastic leukemias (ALL) is not uncommon (see Chapters 14 and 15). The spleen is typically normal in size, although splenomegaly can be seen. Involvement and expansion of the red pulp by leukemic blasts is characteristic, and a rare but significant complication of both ALL and AML is rupture of the spleen, which has been reported in cases with extensive leukemic involvement. Involvement of the spleen is almost always seen in conjunction with peripheral blood or bone marrow findings that yield morphologic and immunophenotypic data for classification. In exceedingly rare cases, myeloid sarcoma involving the spleen can be a presenting feature of AML.

## LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

Large granular lymphocytic (LGL) leukemia can also secondarily involve the spleen, and up to 50% or more of patients will exhibit clinically detectable splenomegaly (see Chapter 13). Histologically, there is expansion of the red pulp with an intrasinusoidal infiltration by large granular lymphocytes; these cells are best appreciated on smear or touch preparation. Lymphoid germinal center hyperplasia is commonly seen, and in most cases preservation of the white pulp boundaries is maintained; expansion of the mantle zone by LGLs is also a feature. These neoplastic cells generally express CD3, CD8, perforin, and granzyme B. Although they characteristically express CD57 in the blood and bone marrow, they frequently lack expression of CD57 in the spleen. Interestingly, CD5 is lost in many cases of splenic involvement. Even though it is a clonal neoplastic disease associated with cytopenias, LGL leukemia is a relatively indolent disorder.

## LYMPHOMAS SECONDARILY INVOLVING THE SPLEEN

As leukemias can secondarily involve the spleen, so too can lymphomas from nodal or extranodal sites. The clinical features of lymphoma involvement in the spleen are diverse, but with extensive involvement the end-result is the same: splenomegaly. Although these disorders are described in other chapters, the characteristic splenic features are provided here.

## DIFFUSE LARGE B-CELL LYMPHOMA

Diffuse large B-cell lymphoma (DLBCL) is a common non-Hodgkin lymphoma that can secondarily involve the spleen (see Chapter 8). Classically, large nodules of firm white fish flesh–like tissue expand the spleen and may extend beyond the capsule. Regional lymph nodes are typically involved and often enlarged. Histologic sections of the spleen show an expansion of the white pulp by nodules and diffuse sheets of large atypical centroblastic or immunoblastic lymphoid cells with clearing of nuclear chromatin and distinct nucleoli; mitoses are generally abundant. Immunophenotypically, the cells are positive for CD45 and for B-cell antigens such as CD20, CD79a, PAX5, and CD19. As in cases of DLBCL in other anatomic sites, germinal center markers such as BCL6

and CD10 are variably expressed, and a subset of cases may express CD5 and lack germinal center markers. The lymphoma cells are monotypic, and immunoglobulin light chain restriction can often be demonstrated.

In addition, DLBCL may arise de novo or can result from transformation of a low-grade splenic lymphoma such as splenic marginal zone lymphoma (13% of patients in one series). However, in these cases peripheral nodes, rather than the splenic and hilar lymph nodes, are the usual sites of transformation.

Less commonly, diffuse large B-cell lymphoma develops as a splenic red pulp proliferation. Such cases appear to be the splenic manifestation of intravascular large B-cell lymphoma. Importantly, these cases may be confused with red pulp involvement by acute or chronic leukemia, but immunophenotyping studies are useful in proving the mature B-cell lineage of the proliferation.

## LYMPHOPLASMACYTIC LYMPHOMA

Lymphoplasmacytic lymphoma (LPL) typically involves both the splenic white and red pulp and often in a diffuse pattern, although the infiltrate may form distinct small nodules in the red pulp (see Chapter 7). Periarteriolar aggregates of plasmacytoid cells, small lymphocytes, plasma cells, and a variable number of immunoblasts are consistent with LPL and Dutcher bodies, increased mast cells, and hemosiderin may also be features. Rarely, LPL has been associated with Hodgkin lymphoma or progression to diffuse large B-cell lymphoma. Currently, LPL is a diagnosis of exclusion; therefore other lymphomas in the differential diagnosis to rule out are splenic marginal zone lymphoma with plasmacytoid differentiation, CLL/SLL, and mantle cell lymphoma. The most frequently overlapping entity is splenic marginal zone lymphoma; however, SMZL shows a biphasic pattern with paler cells suggesting marginal zone differentiation.

## MANTLE CELL LYMPHOMA

Splenomegaly is a common clinical finding in mantle cell lymphoma (MCL) due to dense infiltration of tumor cells. A number of patterns including mantle zone hyperplasia, pseudo-nodular growth, and diffuse infiltration can be seen (see Chapter 7). The red pulp cords and sinuses are typically infiltrated. Tumor cells are characteristically monotonous and small with an irregular cleaved nucleus; however, morphologic variants (blastoid, pleomorphic, small cell, and marginal zone–like) may also be seen. The immunophenotype remains characteristic and cells express CD5, CD19, FMC-7, CD43, and cyclin-D1 and lack CD10 and BCL6. The various growth patterns of MCL introduce SMZL, follicular lymphoma, CLL/SLL, and reactive follicular hyperplasia into the differential diagnosis; however, cyclin-D1 positivity by immunohistochemistry establishes the diagnosis of MCL. In addition, detection of bright CD20 and immunoglobulin light chain expression are useful in excluding CLL/SLL.

Of note, a variant of MCL that lacks nodal involvement but rather shows peripheral blood and frequent splenic involvement has been described. Such cases have hypermutated immunoglobulin heavy chain variable region genes, appear to lack SOX11, and have an indolent clinical course.

## FOLLICULAR LYMPHOMA

Follicular lymphoma commonly involves the spleen; however, splenomegaly may not be apparent (see Chapter 7). Typically the white pulp is expanded throughout the spleen, and mass lesions are absent unless large cell transformation has occurred. The splenic cut surface demonstrates numerous small discrete white nodules, which histologically are often irregular in shape and may be surrounded by a residual mantle and marginal zones (Figure 22-7). As in other anatomic locations, the lymphoma cells are a variable composition of cleaved centrocytes and centroblasts and may also resemble marginal zone type cells in the periphery of nodules. Germinal center marker expression is retained (CD10 and BCL6), and in most cases aberrant expression of BCL2 in follicles is seen; however, in at least one study of splenic follicular lymphoma in which the presenting disease was primarily in the spleen, BCL2 expression was lost or diminished in a significant number of cases. Interestingly, many cases may also show patterns of involvement by in situ follicular lymphoma. Molecular analysis for t(14;18) *IGH@/BCL2* or *BCL6* translocations have made the diagnosis more definitive. Misinterpreting an expanded marginal zone component in splenic involvement by follicular lymphoma as splenic marginal zone lymphoma is a differential diagnostic pitfall. however, follicular lymphoma is a more systemic disease when compared to splenic marginal zone lymphoma. In addition, identification of central white pulp cells with the characteristic mix of small cleaved and large cells of follicular lymphoma is the best diagnostic clue to follicular lymphoma over splenic marginal zone lymphoma and should lead to immunophenotyping studies and clinical evaluation for further evidence of follicular lymphoma.

## HODGKIN LYMPHOMA

The spleen is the most common extranodal site of involvement by Hodgkin lymphoma, and early studies found that more than one third of patients have splenic involvement (see Chapter 11). Typically, the spleen is enlarged and the cut surface shows several white fleshy

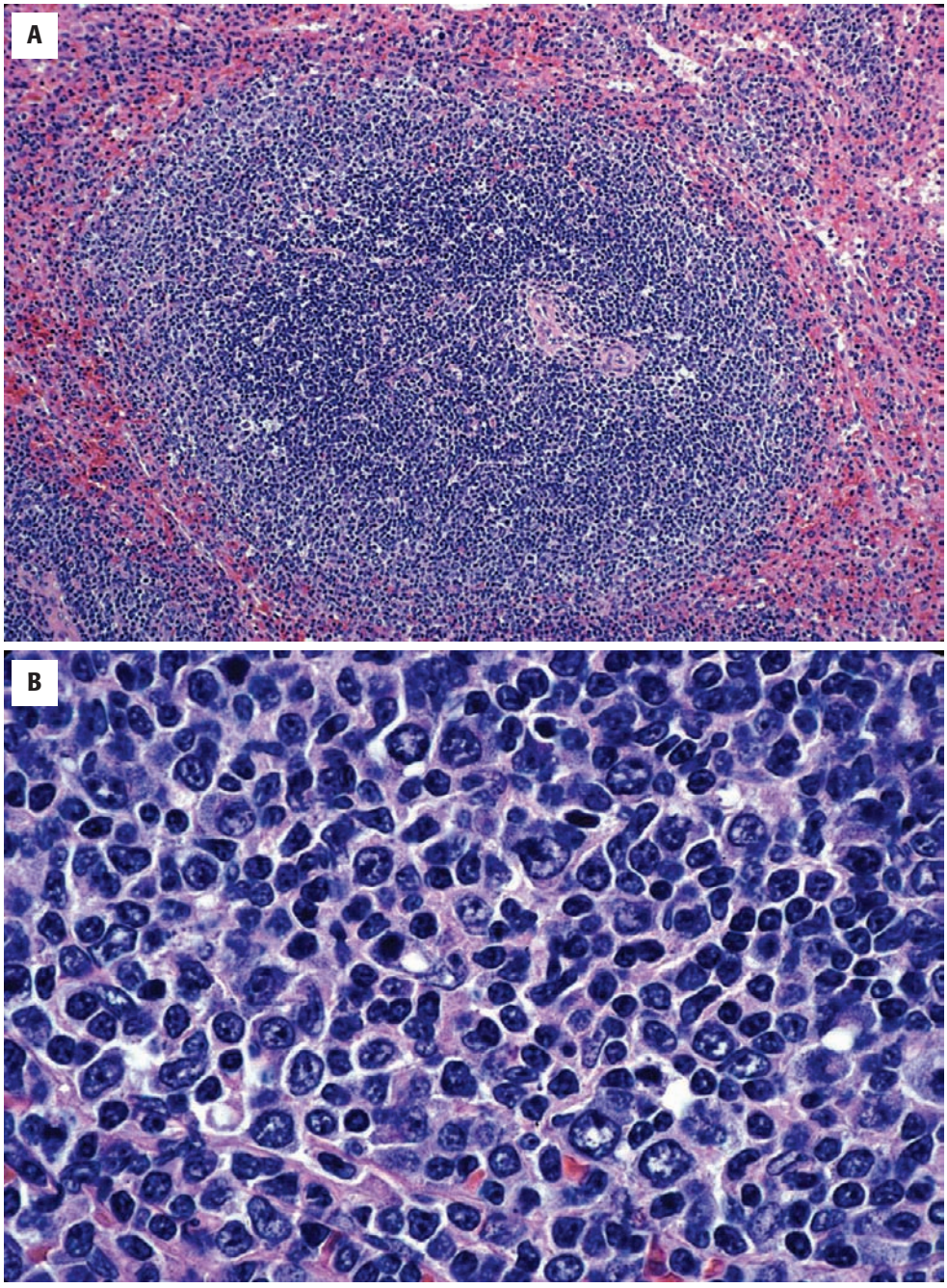


**FIGURE 22-7**

Follicular lymphoma involving the spleen. **A,** The white pulp is expanded, and there is an expanded outer marginal zone that suggests splenic marginal zone lymphoma. **B,** The central white pulp cells show a mix of small cleaved and large cells typical of follicular lymphoma. These B cells also expressed CD10 and BCL2, confirming the diagnosis of follicular lymphoma.

tumor nodules. Lesions characteristically first involve the periarteriolar lymphoid sheath and marginal zones of the white pulp. Although the nodular sclerosis subtype is the most common to involve the spleen, other subtypes can be seen as well, with the exception of nodular lymphocyte predominant Hodgkin lymphoma, which typically does not involve the spleen. Subtyping of Hodgkin lymphoma, other than classical type versus lymphocyte predominant type, should not be performed on splenic tissue as bands of fibrosis are not specific to the nodular sclerosis type in this organ. In addition, some cases can be associated with granulomas, but the presence of granulomas alone without identifiable tumor cells should not be used as an indicator of involvement by Hodgkin lymphoma. Finally, splenic involvement is an indicator of advanced disease and is usually associated with involvement of the marrow or liver.

## OTHER HEMATOPOIETIC PROLIFERATIONS OF THE SPLEEN

### MASTOCYTOSIS

Although mastocytosis (see Chapter 20) describes a group of biologically distinct diseases, all are recognized causes of splenomegaly. Mast cells can invade the red pulp, white pulp, or both and form aggregates typically in the red pulp around trabeculae and the capsule (Figure 22-8). When invasion of the white pulp is present, mast cells are found in the follicular and parafollicular regions. A characteristic feature of splenic mastocytosis is fibrosis, which is often grossly evident on cut section and tends to surround the mast cell aggregates, imparting a lobular appearance. Mast cells often display cytoplasmic projections in tissue sections, raising the differential diagnosis of hairy cell leukemia; however, the presence of patchy cellular aggregates is

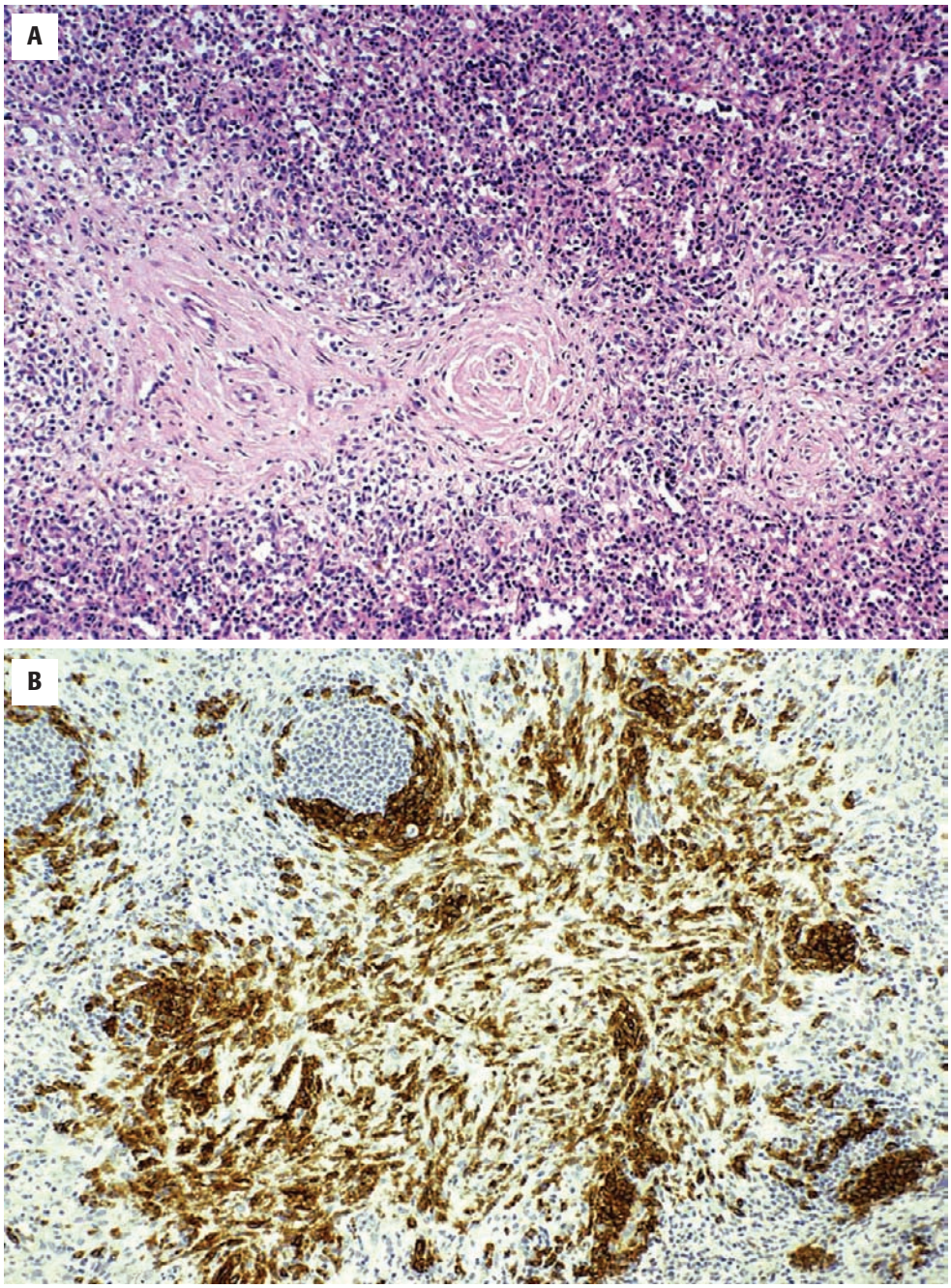


**FIGURE 22-8**

Mastocytosis. **A,** Patchy areas of fibrosis are present. Within the fibrosis are eosinophils and mast cells with abundant pale or clear staining cytoplasm. **B,** These cells stain for CD117 (shown here) and tryptase, and they usually show aberrant coexpression of CD25 on tissue sections.

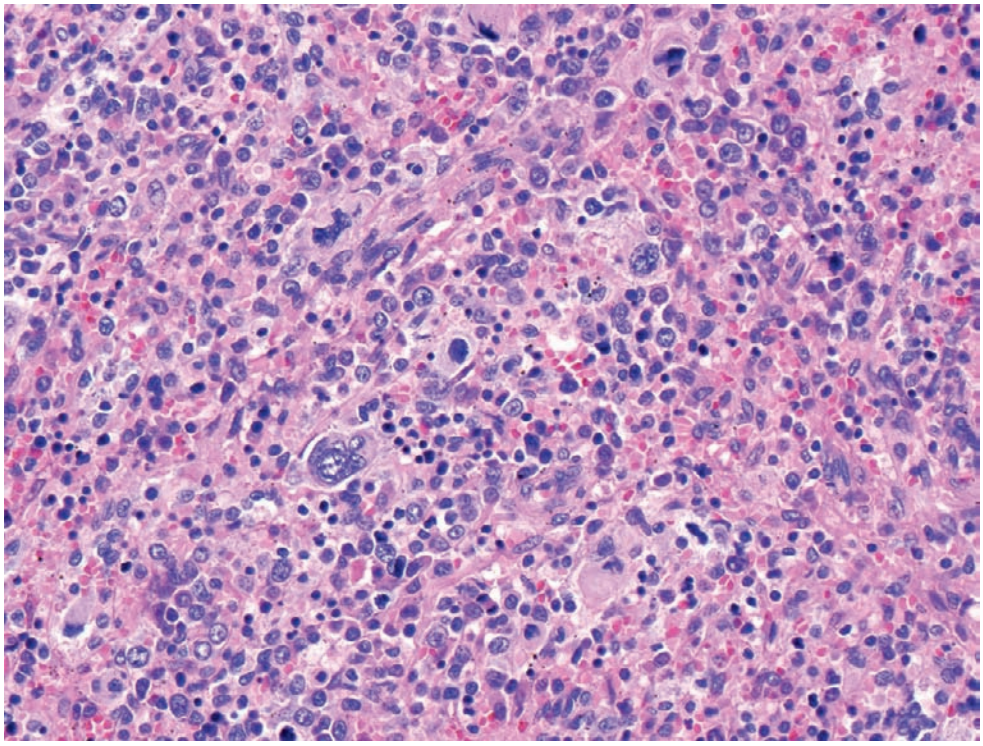

**FIGURE 22-9**

Primary myelofibrosis in the spleen. The spleen is usually massively enlarged with expansion of the red pulp by marrow elements, including many atypical megakaryocytes.

characteristic of mastocytosis and is rarely seen in hairy cell leukemia involving the spleen. A definitive diagnosis is made by immunohistochemistry with cells positive for mast cell tryptase and CD117. Aberrant expression of CD25 and CD2 is often seen.

## MYELOPROLIFERATIVE NEOPLASMS

Myeloproliferative neoplasms, while not originating in the spleen, frequently involve it. For example, in the case of primary myelofibrosis, massive splenic enlargement is common because of the production of defective red blood cells and platelets that are secondarily removed by the spleen, resulting in splenomegaly and extramedullary hematopoiesis (Figure 22-9). Splenic infarction can occur at later stages of myelofibrosis (50% of cases) and is often with associated abdominal pain.

## VASCULAR AND OTHER NONHEMATOPOIETIC PRIMARY SPLENIC TUMORS

Vascular tumors are the most common tumors of the spleen (Table 22-1) that typically involve the red pulp. They may be diffuse or form a tumor mass and are usually found incidentally on imaging.

## SPLENIC HEMANGIOMA

Splenic hemangiomas are benign tumors that are also usually asymptomatic, but may cause splenomegaly, abdominal pain, and hypersplenism. Most hemangiomas are localized and form single or multiple tumor nodules that contain cystic blood-filled spaces (Figure 22-10); these spaces are lined by endothelial cells, and papillary projections may occur in areas along with thrombi. The tumor nodules are usually surrounded by fibrosis and may show calcification. Plain abdominal radiographs, computed tomographic scans, and sonograms are nonspecific, but all show discrete solid and cystic masses, often with evidence of calcification. Diffuse hemangiomatosis of the spleen is less common and often is associated with systemic hemangiomatosis. Massive splenomegaly and coagulopathies are characteristic in these cases. Diffuse hemangiomatosis differs from peliosis by the presence of intervening fibrosis in hemangiomatosis, which is not a feature of peliosis. The differential diagnosis also includes lymphangioma and primary splenic cyst; however, localized lymphangiomas and primary splenic cysts of the spleen usually contain proteinaceous fluid rather than the blood of a hemangioma. In addition, diffuse lymphangiomatosis may be localized to the spleen, but is usually a systemic process and most commonly occurs in children and young adults with massive splenomegaly.

**TABLE 22-1**

**Vascular and Other Nonhematopoietic Primary Splenic Tumors**

| Entity | Clinical Features | Pathologic Findings | Immunophenotype | Prognosis |
|---|---|---|---|---|
| Hamartoma | Rare, predominantly elderly, cytopenias common | Nodular lesion; median size, 5 cm; numerous slitlike vascular channels lined by plump-flattened endothelial cells without white pulp | $CD8^+$, $vWF^+$, $CD31^+$, $CD34^{+/-}$, $CD21^-$, $CD68^-$ | Benign, but possible risk of rupture in larger lesions |
| Hemangioma | Common benign tumor of the spleen, generally asymptomatic | Nonencapsulated, <2 cm, vascular channels separated by red pulp and fibrous septae | $CD31^+$, $CD34^+$, $vWF^+$, $CD21^-$, $CD68^-$, $CD8^-$ | Benign, but there can be a risk of rupture |
| Littoral cell angioma | Rare, often incidental finding | Numerous channel-like vascular spaces lined by plump cells that surround fibrovascular cores and luminal macrophages | $CD31^+$, $vWF^+$, $CD163^+$, $CD68^+$, $CD21^{+/-}$, $CD34^-$, $CD8^-$ | Benign, reported association with secondary malignancies |
| Lymphangioma | Rare, generally an isolated finding | Often subcapsular, variably sized cystic spaces with flat, bland endothelium filled with proteinaceous fluid | $CD31^+$, $CD34^{+/-}$, $CD21^-$, $CD8^-$, $D2\text{-}40^+$ | May recur if not completely excised |
| Sclerosing angiomatoid nodular transformation | Rare, occurs in elderly (>50 years), generally asymptomatic; female-to-male ratio, 2:1 | Red-tan, unencapsulated mass composed of nodules with slitlike, round, vascular spaces lined by plump endothelial cells and pericytes surrounded by densely collagenous fibrotic or fibrinoid granulomatous tissue | Three vascular patterns: $CD34^-$, $CD31^+$, $CD8^+$ sinusoids; $CD34^+$, $CD31^+$, $CD8^-$ capillaries; $CD34^-$, $CD31^+$, $CD8^-$ veins; CD68 expression is variable | Indolent and benign with no tendency for recurrence after splenectomy |
| Angiosarcoma | Most common nonlymphoid malignancy of the spleen | Typically multifocal, with irregular anastomosing vascular channels with marked atypia, frequent mitoses, and invasion of surrounding stroma | $CD31^+$, $CD34^+$ | Malignant lesion with high rate of dissemination |
| Splenic inflammatory pseudotumor (inflammatory pseudotumor–like follicular dendritic cell sarcoma) | Rare, with fever and abdominal pain | Scarlike lesion composed of myofibroblastic spindle cells with mixed inflammatory cells (lymphocytes, plasma cells, eosinophils) | Spindle cells: $EBV^+$, $SMA^+$, $Desmin^+$, $vimentin^+$, $CD68^{-/+}$, $S100^{-/+}$, $CD21^{+/-}$, $CD34^-$, $CD8^-$, $ALK1^-$ | Indolent, responsive to splenectomy, incomplete resection may recur as follicular dendritic cell tumor |

## LITTORAL CELL ANGIOMA

Littoral cell angiomas are unique to the spleen. They may occur at any age and usually cause mild to moderate splenomegaly. Littoral cell angioma is a tumor presumably derived from the littoral cells lining the sinus channels, but the tumor immunophenotype differs slightly from this presumed normal counterpart. Grossly, the spleen shows diffuse multinodularity with spongy, dark red nodules that can measure up to 9 cm in diameter; rarely, it develops as a single, large mass. Histologically, the vascular spaces are lined by plump cells with nuclear enlargement and often show papillary areas with lining cells sloughing into the vascular spaces (Figure 22-11). The lining cells of littoral cell angioma have a unique immunophenotype, expressing vascular, histiocytic, and dendritic-associated markers CD31, CD68, CD163, and at least focal CD21. The actual lining cells are $CD34^-$, and they do not express CD8, unlike normal splenic sinus lining cells. The differential diagnosis includes hemangioma and angiosarcoma; however, hemangiomas of the spleen express CD34 and usually lack the nuclear enlargement of littoral cell angioma. In addition, angiosarcomas show more cytologic atypia than littoral cell angiomas as well as mitotic figures and necrosis—features that are not present in littoral cell angioma. Most cases of littoral cell angioma are treated with splenectomy without recurrence. Rare cases with foci containing a solid clear cell proliferation have metastasized many years later, and such cases probably represent a rare entity termed *littoral cell hemangioendothelioma*.

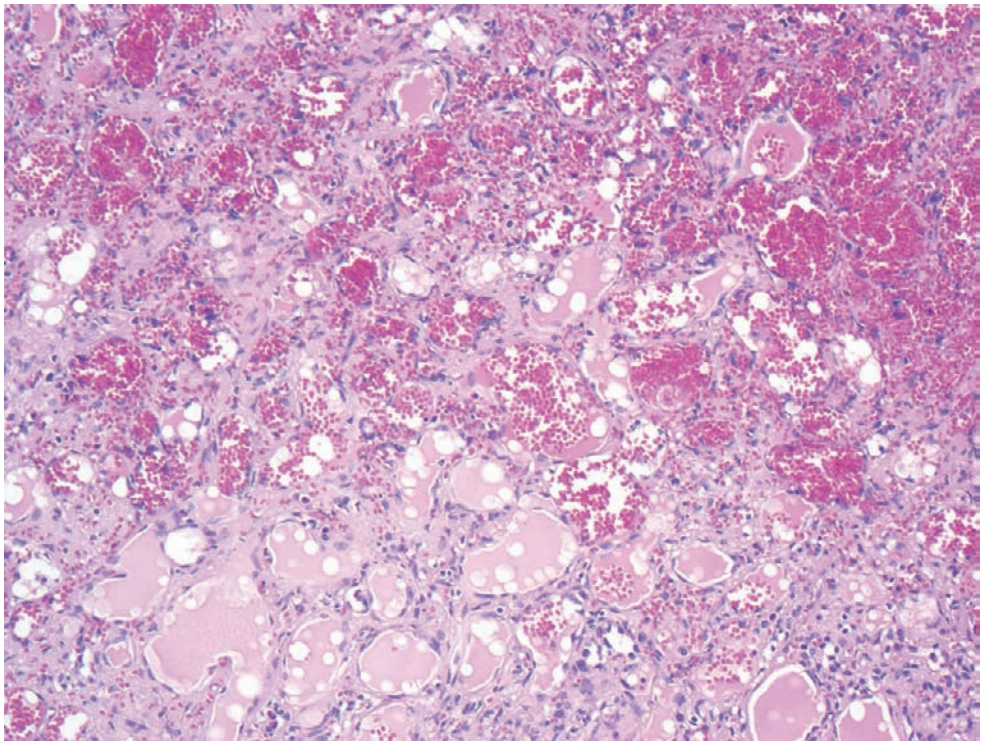

**FIGURE 22-10**

Hemangioma. These proliferations can form a distinct mass or diffusely involve the spleen. They show numerous, variably sized vessels with intervening fibrosis without atypia or necrosis.

**LITTORAL CELL ANGIOMA—FACT SHEET**

**Clinical Features**
- Median age 49 years; no gender preference
- Occasionally splenomegaly with thrombocytopenia or anemia
- Reported association with secondary malignancies

**Gross Findings**
- Splenomegaly with diffuse multinodularity with spongy, dark red nodules

**Morphology**
- Anastomosing vascular channels with irregular lumina
- Channels lined by plump cells with nuclear enlargement and variable hemophagocytosis

**Immunophenotype**
- $CD31^+$, $CD163^+$, $vWF^+$, $CD68^+$, $CD21^{+/-}$, $CD34^-$, $CD8^-$

**Prognosis and Therapy**
- Splenectomy for therapy
- A benign disease with variable prognosis given the associated possible comorbidities

**Differential Diagnosis**
- Hemangioma
- Angiosarcoma

## SPLENIC ANGIOSARCOMA

Splenic angiosarcoma is a rare tumor that occurs most commonly in adults; fewer than 200 cases have been reported. It is usually associated with splenomegaly, abdominal pain, and cytopenias, and splenic rupture can be seen in up to 30% of cases. Because most angiosarcomas involving the spleen are high-grade sarcomas with dissemination, it is often difficult to determine whether the splenic tumor is primary or secondary. The tumor typically forms an infiltrating mass but can also form a network of anastomosing vascular channels lined by atypical hobnail cells, with high mitotic activity and necrosis. Areas of cystic hemorrhage are sometimes present. Many cases may be difficult to differentiate from other high-grade sarcomas, and immunohistochemical detection of vascular antigen expression (e.g., CD31, CD34, von Willebrand factor) is necessary to diagnose such cases. The differential diagnosis includes littoral cell angioma, cavernous hemangioma, normal splenic sinuses, or other sarcomas such as Kaposi sarcoma. Immunohistochemical studies for CD34, CD8, or human herpes virus 8 can usually distinguish angiosarcoma from littoral cell angioma and Kaposi sarcoma, and the presence of necrosis or mitotic activity essentially excludes littoral cell angioma. High-grade angiosarcomas involving the spleen have a generally poor

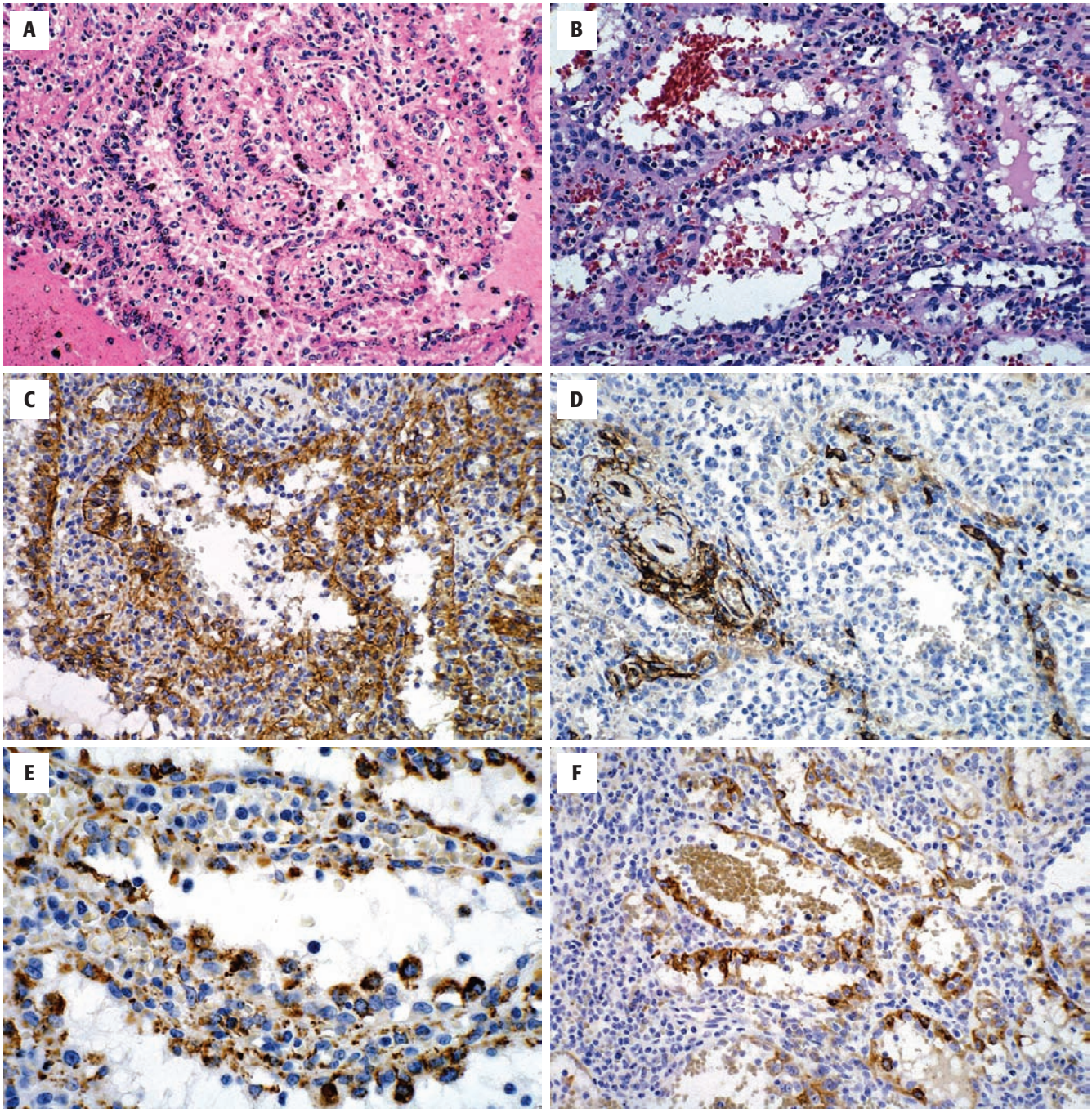


**FIGURE 22-11**

Littoral cell angioma. **A,** These tumors form hemorrhagic masses with a usually papillary histologic appearance. **B,** The lining cells show nuclear enlargement with cells often sloughing into the vascular spaces, but do not show necrosis. The lining cells express CD31 **(C),** but not CD34 **(D),** and focally express CD68 **(E)** and CD21 **(F).**

prognosis, and most patients die of disease within 1 year of diagnosis; however, rare cares with long-term survival following splenectomy are reported.

## SPLENIC LYMPHANGIOMA

Lymphangiomas of the spleen are uncommon tumors that develop as an isolated nodule or diffusely infiltrate the spleen, often in the setting of a patient with lymphangiomatosis. Localized tumors are subcapsular, whereas diffuse proliferations can involve the entire spleen. Three histologic categories are recognized: cystic, cavernous, and simple or capillary. Cystic lymphangiomas are most common and show thin-walled cysts of variable size filled with serous fluid (Figure 22-12). The endothelial cells are positive for CD31 and D2-40 and often focally positive for CD34, but

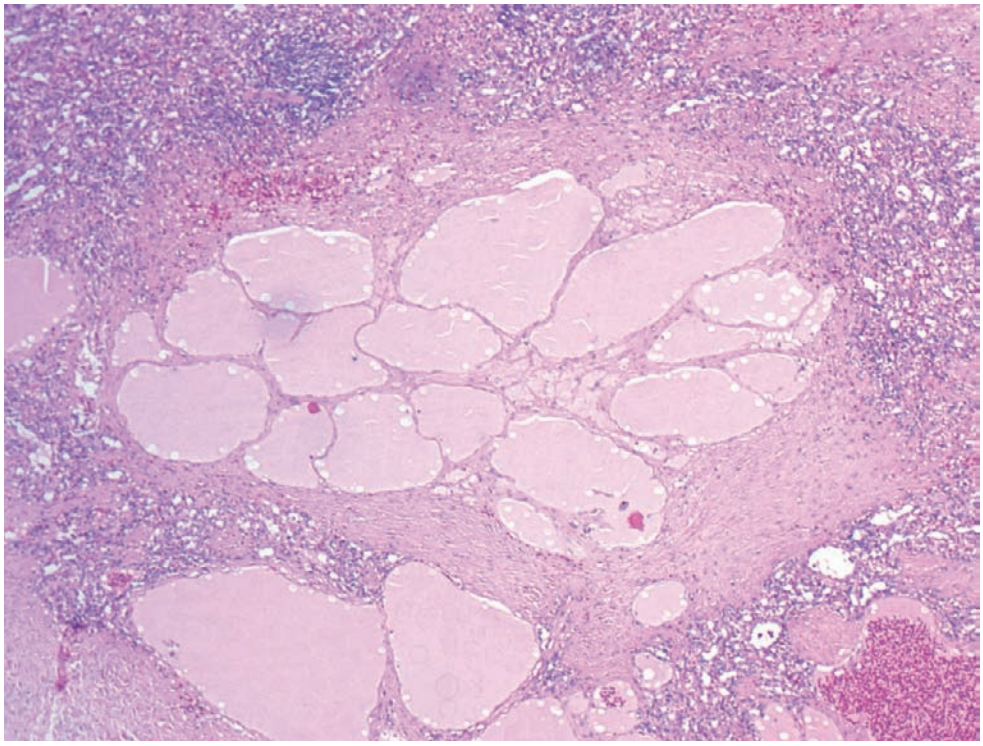

**FIGURE 22-12**

Lymphangioma. These proliferations are often subcapsular and consist of multiple, small, dilated fluid filled spaces without atypia.

negative for CD21 and CD68. These lesions are benign and often are found incidentally and require no treatment.

## SCLEROSING ANGIOMATOID NODULAR TRANSFORMATION OF THE SPLEEN

Sclerosing angiomatoid nodular transformation (SANT) is a recently classified non-neoplastic vascular lesion of the spleen. The median age at presentation is 54 years, but ranges from 22 to 74 years, and a female predominance is seen (female-to-male ratio, 2:1). Although most patients are asymptomatic with a mass found only incidentally on imaging, as many as 16% of patients may complain of abdominal pain. Interestingly, a small subset of patients may have leukocytosis, an elevated erythrocyte sedimentation rate, and a polyclonal gammopathy.

Grossly, the spleen is generally normal to slightly enlarged in size and on cut cross-section reveals a single unencapsulated mass composed of multiple red-brown nodules separated by stellate fibrous stroma (Figure 22-13). Microscopic examination shows that some nodules are surrounded by densely collagenous fibrotic tissue, whereas other nodules are circumscribed by a fibrinoid granulomatous rim. Within the nodules, numerous slitlike round vascular spaces are seen that are lined by plump endothelial cells and pericytes. Rare mitoses can be seen, but cellular atypia should not be prominent.

### SCLEROSING ANGIOMATOID NODULAR TRANSFORMATION OF THE SPLEEN—FACT SHEET

**Clinical Features**

- Average age 54 years old; female predominance (2:1)
- Sixteen percent of patients present with abdominal pain
- May have leukocytosis, elevated erythrocyte sedimentation rate, and polyclonal gammopathy

**Morphology**

- Nodules composed of slitlike, round vascular spaces lined by plump endothelial cells and pericytes
- Rim of fibrosclerotic or fibrinous granulomatous tissue
- Myofibroblasts with a mixed inflammatory infiltrate within internodular regions

**Immunophenotype**

- Three staining patterns
  - Splenic sinusoidal $CD34^{-}CD31^{+}CD8^{+}$ endothelial cells
  - Capillary-like $CD34^{+}CD31^{+}CD8^{-}$ endothelial cells
  - Venous $CD34^{-}CD31^{+}CD8^{-}$ endothelial cells

**Differential Diagnosis**

- Other vascular tumors of the spleen

**Prognosis and Therapy**

- Indolent with no tendency for recurrence after splenectomy

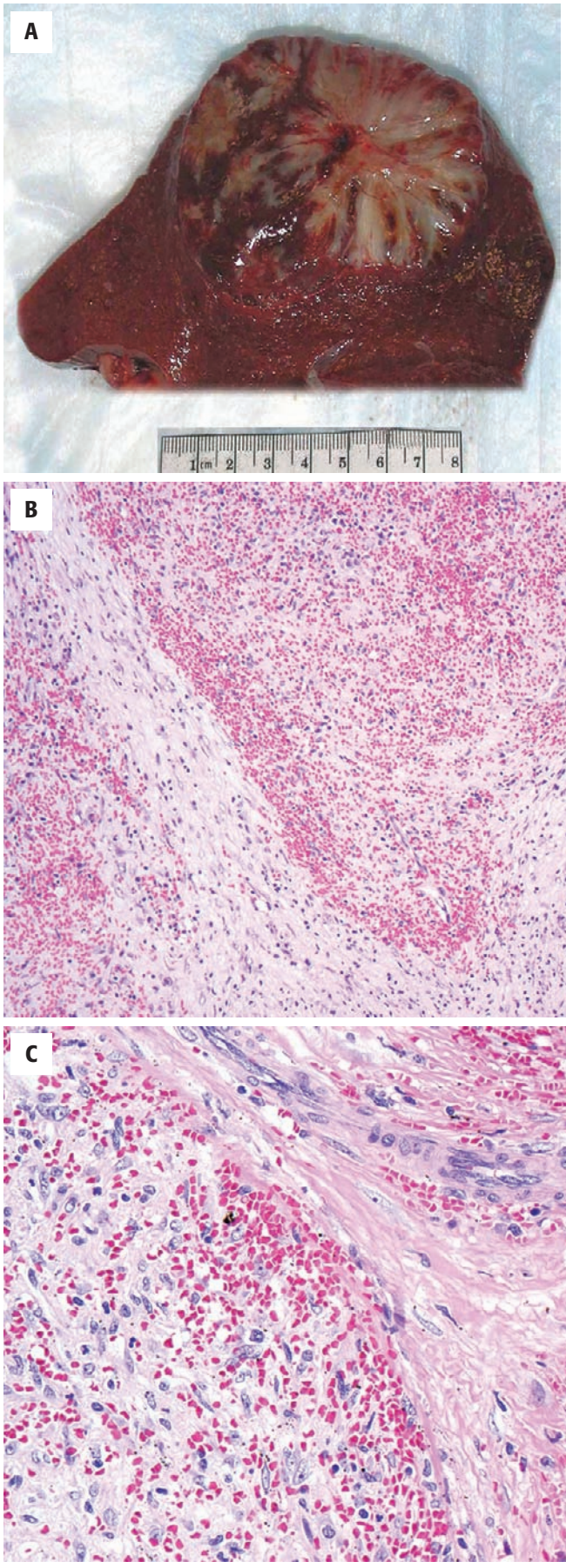


**FIGURE 22-13**

Splenic angiomatoid nodular transformation. **A,** Grossly, these proliferations are distinct but lobulated. **B,** Microscopically, there are multiple hemorrhagic nodules with small vessels and intervening fibrosis **(C)** containing larger vessels. (**A,** Courtesy of Raj Dewar, MD, Boston, Mass.)

The fibrosclerotic internodular spaces are composed of myofibroblasts and a mixed inflammatory infiltrate of lymphocytes, plasma cells, and macrophages.

The vessels of SANT show three staining patterns: a splenic sinusoidal immunophenotype with vessels lined by $CD34^{-}CD31^{+}CD8^{+}$ endothelial cells, a capillary-like immunophenotype with vessels lined by $CD34^{+}CD31^{+}CD8^{-}$ endothelial cells, and a venous immunophenotype with vessels lined by $CD34^{-}CD31^{+}CD8^{-}$ endothelium. Expression of CD68 within nodules can also be demonstrated. These staining patterns are reminiscent of normal vasculature of red pulp vessels. A background of scattered IgG4 positive plasma cells in the fibrosclerotic stroma is also seen.

The differential diagnosis of SANT includes other vascular lesions of the spleen, nodular transformation of the splenic red pulp in response to metastatic carcinoma, and inflammatory pseudotumor. The nodular pattern with three different vessel types differentiates this proliferation from the others in the differential diagnosis. These proliferations are considered indolent with no tendency for recurrence after splenectomy.

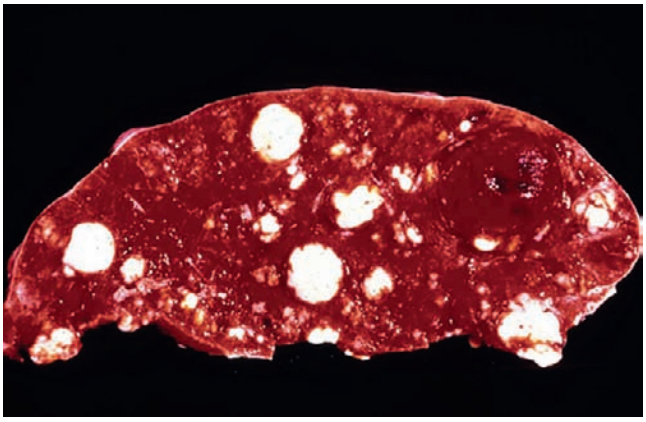

**FIGURE 22-14**

Spleen involved by diffuse large B-cell lymphoma and hamartoma. The multiple tan-white nodules are characteristic gross features of splenic involvement by large B-cell lymphoma and classical Hodgkin lymphoma. The large, red nodule in the *upper right* with a bulging cut surface is the typical presentation of splenic hamartoma, which may be an incidental finding in spleens removed for other reasons.

## PELIOSIS OF THE SPLEEN

Peliosis of the spleen is a rare proliferation of dilated blood-filled cavities. It is more common in the liver, but the spleen is occasionally a site of disease. Secondary conditions such as infections, particularly tuberculosis, malignant conditions such as lymphomas and leukemias, and drug use as in chemotherapy can be associated with peliosis. Sections of the spleen demonstrate multiple round to oval blood-filled cysts with or without endothelial lining cells. Peliosis differs from hemangiomas or hemangiomatosis by the lack of intervening fibrosis and in peliosis, the dilated vascular spaces are separated by normal-appearing splenic red and white pulp. Finally, peliosis may be associated with spontaneous splenic rupture.

## SPLENIC HAMARTOMA

Hamartoma of the spleen is a rare benign nodular lesion with an incidence of 0.13% and equal occurrence in males and females. It appears most commonly in older patients, but up to 20% of cases can occur in children. Clinically, patients can have splenomegaly, thrombocytopenia, or other symptoms of hypersplenism; however, 50% of patients will be asymptomatic. These lesions are generally less than 3 cm in size but can reach 18 cm and form a red, bulging tumor on cut sections of the gross spleen (Figure 22-14). On histologic analysis, they are indistinct lesions that mimic normal red pulp; they show numerous slitlike vascular channels lined by plump to flattened endothelial cells with absence of normal red pulp cord, lymphatic or organized white pulp elements. The cells lining the spaces show an immunophenotype of normal sinusoids with expression of CD8 and CD31; CD34 expression is variable. These cells lack CD21 and CD68, although $CD21^{+}$ follicular dendritic cell aggregates of the white pulp are present within the hamartoma. The differential diagnosis includes inflammatory pseudotumor, SANT, and other vascular tumors, but the lack of a distinct tumor nodule on histologic sections is a distinct characteristic finding

**SPLENIC HAMARTOMA—FACT SHEET**

**Clinical Features**
- Fifty percent of patients are symptomatic with splenomegaly, thrombocytopenia, or other symptoms of hypersplenism

**Morphology**
- Indistinct lesion that mimics normal red pulp
- Numerous slitlike, vascular channels lined by plump to flattened endothelial cells
- Absence of normal red pulp cord, lymphatic or organized white pulp elements

**Immunophenotype**
- $CD8^{+}$, $CD31^{+}$, $CD34^{+/-}$, $CD21^{-}$, $CD68^{-}$

**Prognosis and Therapy**
- Generally benign, although large lesions have risk of rupture
- Splenectomy for symptomatic hamartomas

**Differential Diagnosis**
- Other vascular tumors of the spleen
- Inflammatory pseudotumor of the spleen

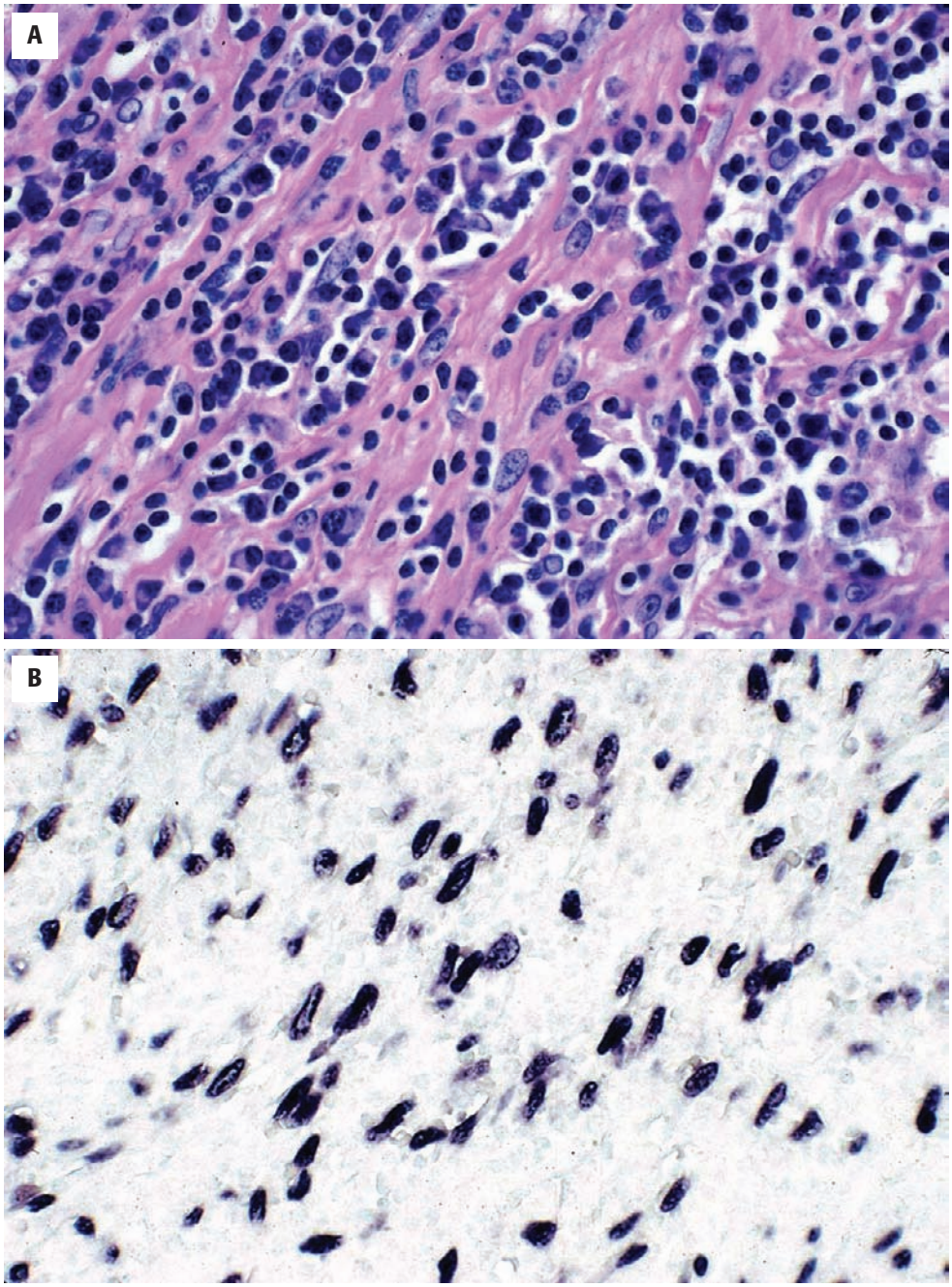


**FIGURE 22-15**

Splenic inflammatory pseudotumor. **A,** These tumors form distinct splenic nodules that contain a mixture of spindled cells with fibrosis, lymphocytes, and plasma cells. They may also contain histiocytes, eosinophils, and neutrophils. **B,** The spindled cells positive for Epstein-Barr virus, as shown here by in situ hybridization for EBER1, and usually also express CD21.

of splenic hamartoma. Although most cases are benign in progression, there can be a risk of rupture of large lesions. Splenectomy is the most frequent treatment of symptomatic hamartomas.

## INFLAMMATORY PSEUDOTUMOR OF THE SPLEEN

Splenic and hepatic inflammatory pseudotumors (also known as *inflammatory pseudotumor–like follicular dendritic cell sarcomas*) are unique to these locations and differ from inflammatory pseudotumors or inflammatory myofibroblastic tumors at other sites. These tumors develop as fibroinflammatory processes, often with fever and abdominal pain; a subset of cases will be associated with a concomitant malignancy either in association or in isolation. The gross appearance of the spleen demonstrates a well-defined, firm, yellow-tan lesion without capsular extension; occasionally multiple nodules are seen. Histologically, the lesion appears as a bland spindle cell myofibroblastic proliferation with interspersed variable amounts of collagenous stroma and intervening abundant inflammatory cells including lymphocytes, plasma cells, histiocytes, and eosinophils (Figure 22-15). Foci of necrosis may be seen. Unlike inflammatory pseudotumors in other organs, the spindled cells in the spleen are positive for EBV and may express smooth muscle actin and vimentin. Spindle cells in most tumors express CD21. S100, CD68, or CD30 may be positive in some cells. CD34 and CD8 are not expressed. All cases of splenic inflammatory pseudotumor are negative for ALK1 in contrast to inflammatory myofibroblastic tumor of soft tissue, which is generally positive for ALK1. Complete splenectomy is curative for symptomatic cases, but partial resection can lead to recurrence or metastasis as a follicular dendritic cell tumor.

**INFLAMMATORY PSEUDOTUMOR OF THE SPLEEN (INFLAMMATORY PSEUDOTUMOR–LIKE FOLLICULAR DENDRITIC CELL SARCOMA)—FACT SHEET**

**Clinical Features**
- Often presents with fever and abdominal pain
- A subset of cases are associated with a concomitant malignancy

**Morphology**
- Numerous bland myofibroblastic spindle cells
- Interspersed collagenous stroma
- Intervening abundant inflammatory cells

**Immunophenotype**
- EBV$^{+}$, CD21$^{+}$, smooth muscle actin (SMA)$^{+/-}$, vimentin$^{+/-}$, S100$^{+/-}$, CD68$^{+/-}$, CD30$^{+/-}$, CD34$^{-}$, CD8$^{-}$, ALK1$^{-}$

**Prognosis and Therapy**
- Splenectomy is curative.
- Partially resected tumors may recur or metastasize as follicular dendritic cell tumors.

**Differential Diagnosis**
- Vascular tumors of the spleen
- Metastatic carcinoma

### SUGGESTED READING

The complete reference list is available online at www.expertconsult.com.

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23

# Flow Cytometric Principles in Hematopathology

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## INTRODUCTION

The importance of immunophenotyping in diagnostic hematopathology was first emphasized by the Revised European-American Lymphoma classification, and more recently by the 2001 and 2008 World Health Organization (WHO) classifications of hematolymphoid neoplasms. In these classifications, nearly all myeloid and lymphoid malignancies are defined, at least in part, by the antigenic features of the neoplastic cells. Flow cytometry (FC) is an immunophenotyping technique in which suspensions of living cells are stained with specific, fluorescently labeled antibodies and then analyzed with a flow cytometer. In hematopathology practice, these cell suspensions are derived from blood, bone marrow, body fluids, or fresh solid tissue samples. Parallel advances in our understanding of the basic biology of hematopoietic malignancies, in the technological attributes of flow cytometers, and in the development of reagents for assessing cellular antigen expression have allowed FC to attain particular prominence in the diagnosis of these malignancies. In addition, FC can identify prognostically relevant antigens and potential therapeutic targets in a number of diseases.

## TECHNICAL CONCEPTS AND METHODS IN THE FLOW CYTOMETRIC EVALUATION OF HEMATOLYMPHOID NEOPLASMS

### THE FLOW CYTOMETER

The flow cytometer consists of three main components—fluidics, optics, and electronics—in association with a desktop computer. The fluidic system includes the pumps used to aspirate the cell suspension into the cytometer and the tubing through which cells are propelled through the cytometer. After being aspirated into the cytometer, the specimen is surrounded by a stream of buffered saline (i.e., sheath fluid) introduced into the instrument at higher pressure than the specimen, such that the cells in the specimen assume a roughly single-file position because of the phenomenon of hydrodynamic focusing. The focused stream then reaches the flow cell, a quartz cuvette where the cells are illuminated by light from one or more lasers. Ultimately, the fluid stream is directed to a waste receptacle.

The optical system includes: (1) the lasers used to excite the fluorescent dyes conjugated to the antibodies used in the assay, (2) the system for conveying the laser light to the flow cell, (3) the system for conveying the emitted fluorescent light from the cells to specific detectors, and (4) the detectors themselves. The detectors are usually photomultiplier tubes (PMTs) that convert photons to electrical impulses. The light emitted by fluorescently labeled cells is conveyed to the PMTs via a combination of dichroic mirrors that allow light of defined wavelengths to pass while reflecting light of other wavelengths, and optical filters that further narrow the wavelengths reaching a PMT. In newer cytometers, fiber-optic cables may help to convey the emitted light to the appropriate detector clusters, whereas the light travels in air in older cytometers.

The electronics system measures the electrical impulses generated by the PMTs and converts these measurements to digital information that is gathered and interpreted by the analysis software. The associated computer system directly interfaces with the flow cytometer and controls its functions. In most newer cytometer systems, data analysis can be done either on the computer connected to the flow cytometer or on other computers accessing the data via a central server (i.e., off-line analysis).

### CLINICAL INDICATIONS FOR FLOW CYTOMETRY

Specific clinical indications for FC in the evaluation for hematolymphoid neoplasia are defined in the 2006 Bethesda International Consensus Recommendations on the Flow Cytometric Immunophenotypic Analysis of

Hematolymphoid Neoplasia. There are four major clinical indications for FC in the laboratory evaluation of hematopoietic neoplasms. First, FC is central to the diagnosis and classification of hematopoietic neoplasms, defining cell lineage in the chronic lymphoproliferative disorders and acute leukemias and becoming the gold standard for identifying paroxysmal nocturnal hemoglobinuria. The 2006 consensus recommendations also recognized FC as a useful adjunct in the workup for myelodysplastic syndromes and myeloproliferative neoplasms, including chronic myelomonocytic leukemia. Second, as antigens associated with disease prognosis in hematopoietic neoplasms are identified (e.g., ZAP-70 expression in chronic lymphocytic leukemia and small lymphocytic lymphoma), FC has been used to measure such antigens. Third, with the advent of therapies targeted at specific antigens in hematopoietic neoplasms (e.g., anti-CD20 therapy in B-cell lymphomas, anti-CD52 therapy in T-cell lymphomas), FC has been used to identify potential therapeutic targets. Fourth, FC has been used extensively to look for and quantify residual disease after therapy. This quantification of residual disease includes minimal residual disease, which usually is defined as less than 1% involvement by neoplastic cells.

## SPECIMEN REQUIREMENTS FOR FLOW CYTOMETRY

In the clinical laboratory, specimens amenable to FC include fresh, unfixed peripheral blood, bone marrow, body fluids, and finely minced solid tissue such as lymph node biopsy or spleen. For tissue and cerebrospinal fluid (CSF) specimens, immediate immersion into tissue culture medium, such as RPMI 1640 supplemented with 10% fetal bovine serum, 1% glutamine, and 1% penicillin-streptomycin antibiotics, is required to maximize cell viability. If the tissue or CSF is likely to reach the FC laboratory in 24 or hours or less, the cells may be maintained at room temperature in medium. If delivery to the FC laboratory is likely to take more than 24 hours, or if the specimen may be exposed to relatively high ambient temperatures as during the summer months, the sample container should be shipped with a packet of wet ice. Cell suspensions should never be subjected to extremes of temperature (less than 0° C or greater than 37° C), and should therefore never be transported with dry ice.

Peripheral blood and bone marrow aspirates must be anticoagulated before transport to the flow cytometry lab. The most common anticoagulants include ethylenediamine tetraacetic acid and heparin, both of which allow high-quality FC evaluation, although other anticoagulants such as acid citrate dextrose (ACD) and citrate do not preclude FC. Peripheral blood, bone marrow, and non-CSF body fluid specimens are typically transported and stored at room temperature.

## SPECIMEN PROCESSING

Universal biohazard precautions should be taken with all specimens in the FC laboratory. Tissue that is minced to create cell suspensions should be filtered through a 40-μM or 60-μM cell strainer to remove large particles that may clog the cytometer tubing. After specimens have been incubated with antibodies and washed, but before they are analyzed on the flow cytometer, the specimens should be fixed in 1% paraformaldehyde or formaldehyde, which both stabilizes antigen-antibody interactions by introducing cross-links and inactivates infectious agents. Adding bleach to the instrument's waste container at a final concentration of 10%, and daily purging of the fluidic system with 10% bleach at the time of instrument shutdown, further minimize biohazard risks.

A zero-tolerance policy should exist for specimen mix-ups in the FC laboratory, to minimize the chance that incorrect patient data are reported. Procedures to minimize the possibility of mix-ups can include fastidious labeling of specimen containers and associated paperwork, double-checking of all specimens at multiple points during processing, and separate processing of specimens to minimize the risk of cross-contamination.

In any specimen containing a large amount of peripheral blood, the erythrocytes should be removed before introducing the specimen into the flow cytometer. Most clinical FC laboratories lyse the red blood cells at some point during specimen processing, using either a commercially available reagent or a homemade ammonium chloride solution. In many laboratories, antibodies are incubated with the cells in the presence of erythrocytes, and the erythrocytes are lysed and bound antibodies fixed to the cells of interest at the end of the preparation step (the so-called whole blood technique), just before evaluating the cell suspension on the flow cytometer. An alternative method is up-front bulk lysis of the red blood cells, which is less time efficient than the whole blood method and has the potential to lyse some leukocytes in addition to erythrocytes. Therefore up-front bulk lysis is best avoided in specimens with small numbers of leukocytes, such as CSF and scanty tissue biopsy specimens. Note that erythrocyte lysis techniques, when applied to bone marrow specimens, destroy the great majority of the nucleated erythroid precursors, compromising the ability to evaluate these cells.

The majority of antigens currently evaluated in clinical FC are cell surface associated, but nuclear antigens (e.g., terminal deoxynucleotidyl transferase [TdT] in lymphoblasts) or cytoplasmic antigens (e.g., myeloperoxidase in myeloid blasts) also can be evaluated by FC. Table 23-1 contains a list of antigens commonly evaluated in the workup for hematolymphoid neoplasia. When both cell surface and cytoplasmic antigens are evaluated in the same assay, the surface staining is

**TABLE 23-1**

**Antigens Commonly Evaluated in Diagnostic Flow Cytometry**

| Antigen | Key Normal Hematopoietic Cell Type |
|---|---|
| CD1a | Immature T cells (common thymocytes), Langerhans cells |
| CD2 | Pan–T cell/NK cell |
| CD3 (surface) | Pan–T cell |
| CD4 | Helper/inducer T cells |
| CD5 | Pan–T cell, subset of B cells |
| CD7 | Pan–T cell/NK cell |
| CD8 | Cytotoxic/suppressor T cell |
| CD9 | B cells, platelets, and megakaryocytes |
| CD10 | Immature and germinal center B cells, neutrophils, follicular helper T cells |
| CD11b | Maturing and mature myelomonocytic cells, NK cells |
| CD11c | Maturing and mature myelomonocytic cells, myeloid dendritic cells, some B cells |
| CD13 | Pan-myeloid cells |
| CD14 | Maturing and mature monocytes, mature neutrophils |
| CD15 | Maturing and mature myelomonocytic cells |
| CD16 | Late-stage neutrophil, NK cells |
| CD19 | Pan-B cell (including immature forms and plasma cells) |
| CD20 | Maturing and mature B cells |
| CD22 | Maturing and mature B cells |
| CD23 | Activated B cells |
| CD25 | Activated T and B cells |
| CD30 | Activated T and B cells |
| CD33 | Pan-myeloid cell |
| CD34 | Myeloid and lymphoid blasts, stem cells |
| CD38 | Plasma cells, blasts, activated B and T cells, NK cells, monocytes |
| CD41 | Platelets and megakaryocytes |
| CD45 | Leukocyte common antigen (all hematopoietic cells except later erythroids) |
| CD56 | NK cells, activated T cells |
| CD57 | Subset of cytotoxic and suppressor T cells |
| CD61 | Platelets and megakaryocytes |
| CD64 | Monocytes, immature granulocytes, activated neutrophils |
| CD103 | Enteric T cells |
| CD117/c-kit | Myeloid blasts, promyelocytes, proerythroblasts, mast cells |
| CD123 | Basophils, plasmacytoid dendritic cells, some blasts |
| CD133 | Immature progenitor cells |
| BCL-2 | Most mature B cells except germinal center cells, most T cells |
| HLA-DR | Myeloid blasts, all B cells, activated T cells, monocytes, dendritic cells |
| FMC7 | Variety of mature B cells (a CD20 epitope) |
| κ Light chain | Mature B cells, plasma cells |
| λ Light chain | Mature B cells, plasma cells |
| TdT | Immature B and T cells |
| Zap-70 | T and NK cells |

*NK,* Natural killer; *TdT,* terminal deoxynucleotidyl transferase.

performed first. The cells are then fixed and permeabilized with reagents for these purposes and then stained for the cytoplasmic antigens.

## INSTRUMENT CONFIGURATION AND QUALITY CONTROL

The type of flow cytometer in the laboratory dictates the number of antigens evaluated simultaneously. Single-laser flow cytometers can evaluate three to five antigens simultaneously in addition to the generic light-scatter properties of forward scatter (proportional to cell size) and side scatter (also known as *orthogonal* or *90-degree light scatter* and proportional to cytoplasmic abundance or granularity). Two-laser instruments can usually evaluate six to eight antigens simultaneously, depending on the PMT configuration, whereas three-laser instruments are typically required to evaluate nine or more antigens simultaneously. The feasibility of nine- and ten-color FC for leukemia–lymphoma immunophenotyping has been demonstrated, and this technology is currently being used by a small number of clinical laboratories. The simultaneous assessment of such a large number of antigens minimizes the number of tubes of cells and antibodies that must be set up, and it is of particular benefit for the analysis of scanty specimens. In addition to saving technologist time, which is typically the most valuable commodity in the FC laboratory, nine- and ten-color FC offers additional cost savings by minimizing redundancy of antibody and other reagent usage across tubes and by maximizing the efficiency of flow cytometer use.

Regardless of the type of flow cytometer, quality control (QC) measures must be performed and documented on a daily, weekly, and monthly basis to ensure optimal instrument performance. Daily QC usually uses brightly fluorescent, 4- to 6-μM plastic microbeads to help ensure that the voltages allotted to the individual PMTs are adequate to detect the expected level of fluorescence, and to confirm adequate laser power and alignment. The overriding principle for optimizing PMT voltage is to maximize the signal-to-noise ratio for that detector. A second daily QC function is a standard nine-color T cell analysis assay on a commercially available, well-characterized, stabilized whole blood preparation, and confirmation that the proportions of the various cell populations fall within the published ranges for the preparation. Less frequent QC measures include: (1) confirming linearity of detectable fluorescence by using a series of microbeads having known fluorescence properties ranging from negative to very bright; (2) confirming the reproducibility of fluorescence in multiple replicate assays of a single specimen; and (3) confirming an acceptably low level of specimen carryover from one tube to the next. (Less than 0.1% carryover should be sought, which is usually achievable if an adequate

amount of blank sheath fluid is run through the cytometer between the collection of individual tubes of cells and antibodies.) Annual or semiannual preventive maintenance by a service representative of the cytometer manufacturer should be performed and documented.

## ANTIBODIES: COMPENSATION AND PANEL DESIGN

Each of the fluorochromes used in FC has a well-characterized absorption and emission spectrum that extends over a range of wavelengths (Figure 23-1). The simultaneous use of multiple antibodies conjugated to different fluorochromes therefore results in some degree of spillover, in which a portion of the fluorescence from a given fluorochrome is detected by a PMT targeted for a different fluorochrome. As a result of spillover, the fluorescence detected by each PMT actually represents the sum of the fluorescence from multiple fluorochromes. The majority of detected fluorescence almost always comes from the fluorochrome the PMT was designed to detect, but significant contributions may come from other fluorochromes because of spillover. To adjust for spillover, a mathematical correction known as *compensation* (or *color compensation*) is applied routinely to all multiparametric FC data. As more antibodies are used in individual tubes (e.g., 6- to 10-color analysis), the potential for compensation artifacts increases. A detailed discussion of compensation is beyond the scope of this chapter, but it is important to recognize that proper compensation is another critical QC function in the FC laboratory.

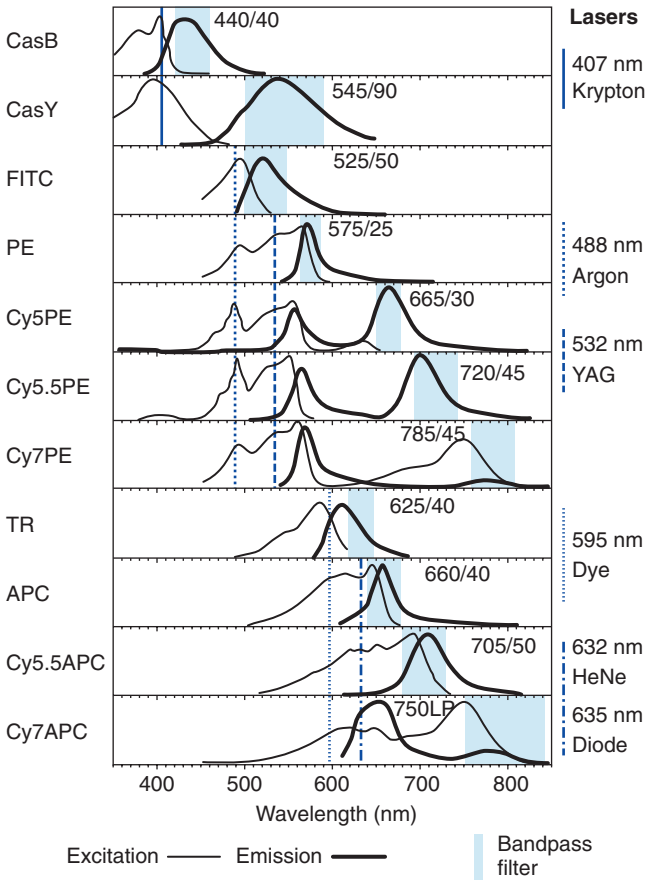


**FIGURE 23-1**

Emission spectra of a range of fluorochromes (fluorophores) excited by violet (407 nm), blue (488 nm), green (532 nm), yellow (595 nm), and red (632 to 635 nm) lasers. The horizontal axis represents wavelength. The vertical lines along the right of the figure depict the wavelengths of commonly used lasers (shown on horizontal axis). For each fluorochrome (shown on vertical axis), the curve on the left represents the excitation/absorption spectrum, the darker and thicker curve on the right represents the emission spectrum, and the blue shaded region represents the width of the emission spectrum commonly detected by the bandpass filter targeting that fluorochrome. The variable overlap of the emission spectra (i.e., spillover), particularly among adjacent fluorochromes, necessitates the analytical technique of compensation. *(From Baumgarth N, Roederer M: A practical approach to multicolor flow cytometry for immunophenotyping,* J Immunol Methods *243:77-97, 2000.)*

In clinical FC, antibodies are typically used in defined combinations, or panels, to answer specific questions about specific cell populations. Most laboratories performing leukemia–lymphoma immunophenotyping have an acute leukemia panel to distinguish acute myeloid leukemia from acute lymphoid leukemia and a lymphoma panel to distinguish benign from malignant lymphoid tissue. At a minimum, antibody panels should measure sufficient antigens to distinguish normal–benign from abnormal–neoplastic cell populations with a high degree of sensitivity and specificity. See Table 23-2 for a list of antigens important in the evaluation of specific hematopoietic cell populations, many of which are included in the published recommendations from the 2006 Bethesda International Consensus Conference.

The quality of FC data not only depends on the specific antigens evaluated, but also on the specific fluorochromes conjugated to specific antibodies and on the ways in which antibodies are used together. For example, when a weakly expressed antigen is sought, such as an aberrantly expressed lymphoid antigen on myeloid blasts, conjugation of the relevant antibody to a bright fluorochrome, such as phycoerythrin, can maximize the chance of detecting the antigen. Conversely, it is often unwise to use a bright fluorochrome to detect a strongly expressed antigen, because the bright fluorescence emission from this strategy will likely create compensation problems resulting from spillover.

When a specimen is received in the FC laboratory, the pathologist or FC technologist must consider the underlying clinical question in deciding which antibodies and panels to use in the evaluation. When the clinical question is limited, such as whether a bone marrow aspirate from a patient with known B-cell non-Hodgkin lymphoma has evidence of disease, then it is appropriate to perform a limited study focused primarily on the cell population in question, such as the mature B cells. If such a limited evaluation is to be performed, it is important to have an antibody to the leukocyte common

**TABLE 23-2**

**Useful Antigens in the Evaluation of Specific Hematopoietic Cell Populations**

| Myeloid Blast | Granulocyte and Monocyte | Immature B Cell | Mature B Cell | Plasma Cell | Immature T Cell | Mature T/NK Cell |
|---|---|---|---|---|---|---|
| CD13 | CD4 | CD19 | CD19 | CD19 | CD1a | CD2 |
| CD33 | CD10 | CD10 | CD20* | CD20* | CD2 | CD3 |
| CD34 | CD11b | CD20* | CD10 | CD38 | CD3 | CD4 |
| CD38 | CD13 | CD22* | CD38 | CD45* | CD4 | CD5 |
| CD45* | CD14† | CD34 | CD45* | CD138 | CD5 | CD7 |
| CD117 | CD15 | CD38 | κ | CD56‡ | CD7 | CD8 |
| HLA-DR | CD16† | CD45* | λ | CD117‡ | CD8 | CD10 |
| CD2‡ | CD24† | HLA-DR | CD5‡ | Cyκ§ | CD10 | CD16 |
| CD5‡ | CD33 | κ | CD11c¶ | Cyλ§ | CD34 | CD45* |
| CD7‡ | CD38 | λ | CD22‡,¶ | | CD38 | CD56 |
| CD11b‡ | CD45* | TdT§ | CD23¶ | | CD45* | TCR-b¶ |
| CD15‡ | CD64 | CD13‡ | CD25¶ | | TdT§ | KIRs¶ |
| CD19‡ | HLA-DR | CD33‡ | CD103‡,¶ | | CD13‡ | CD25* |
| CD56‡ | CD56‡ | CyCD79a§ | FMC7¶ | | CD33‡ | CD30* |
| CyMPO§ | | | CyZAP-70‡,§ | | CD117‡ | CD52* |
| | | | CyBCL-2§ | | CyCD3§ | |

*Denotes antigens of potential therapeutic value.
†Denotes glycosylphosphatidylinositol-linked antigens useful in evaluation for paroxysmal nocturnal hemoglobinuria, in addition to CD59 evaluation on erythrocytes.
‡Denotes aberrantly expressed, nonlineage antigen.
§Denotes intracellular antigens assessed in permeabilized cells.
¶Denotes additional antigens to help classify mature lymphoid neoplasms.

antigen CD45 in the assay, so that unexpected expansions of cell populations warranting additional FC evaluation, such as blasts or monocytes, will be recognized. When the clinical question is much broader, for example, ruling out a hematolymphoid neoplasm in the bone marrow of a patient with pancytopenia and no known malignancy, then a broader evaluation of the myeloid, lymphoid, and plasmacytic lineages is likely to be appropriate. If there is clinical concern for a lymphoproliferative disorder in a patient without a prior diagnosis, it is prudent to evaluate the B, T, and natural killer (NK) cells whenever possible. However, because B-cell lymphomas are much more common than T-cell or NK-cell lymphomas (particularly in Western patient populations), it is reasonable to rule out a B-cell malignancy before evaluating the T-cells if a limited amount of specimen is available.

## DATA ACQUISITION

The overall data collection process by which antibody-stained cells are propelled through the flow cytometer, illuminated by the lasers, and detected by the PMTs is known as *acquisition*. The number of cells (also known as *events*) required for evaluation depends on both the purpose of the flow cytometric assay and on the nature of the specimen. For example, when evaluating a lymph node that is replaced by lymphoma, a relatively low number of viable cells (e.g., 10,000) can be acquired per aliquot (or tube) of cells and antibodies, because this relatively low number of cells will allow adequate characterization of the neoplastic population. In contrast, when characterizing a low-frequency cell population (e.g., myeloid blasts in most bone marrow specimens), a much larger number of viable cells (e.g., 100,000) should be collected. When looking for very low-level bone marrow or peripheral blood involvement by a neoplastic population (so-called minimal residual disease), such as after therapy, it is reasonable to collect 300,000 to 500,000 viable cells. Note that the discussion of appropriate cell numbers to collect must be limited to *viable* cells, because extraneous particles such as nonviable cells or debris or nonlysed erythrocytes will be counted as particles by the cytometer, along with the viable cells of interest.

If adequate viable cells are available, it is common to collect at least 100,000 per tube for diagnostic specimens. At least 300,000 cells are usually acquired in most post-therapy settings, but in a leukemia, lymphoma, or myeloma patient being considered for stem cell harvest for autologous stem cell transplantation, at least 500,000 cells will be acquired from bone marrow or blood specimens to maximize the ability to identify an

occult neoplastic population capable of contaminating collected stem cells. Assuming that the presence of 50 neoplastic cells in an aliquot will enable confident identification of this population, the evaluation of 500,000 cells offers the ability to detect the neoplastic population at a frequency of 1 in 10,000 cells, or 0.01%.

## DATA ANALYSIS

FC data analysis requires specialized computer programs designed for this purpose. Such programs are available for both PC and Macintosh platforms, and they ideally permit adequate evaluation of both QC and specimen data. At a minimum, such programs should be able to perform compensation, generate two-dimensional histograms (also known as *two-parameter dot plots* or *scatter plots*) of the data, and enumerate the various cell populations of interest for reporting purposes.

The process of targeting the analysis to the cell populations of interest is known as *gating*. The first gating step is always the exclusion of multicell aggregates (two-cell doublets and higher-order aggregates) from the analysis (Figure 23-2, A), because flow cytometric evaluation must be restricted to individual leukocytes (i.e., singlets). The second gating step is the exclusion (i.e., gating out) of nonviable cells from the analysis. Because nonviable cells typically have a marked decrease in forward scatter (FS) because of membrane damage from cellular degeneration, FS-versus–side scatter (SS) gating (see Figure 23-2, B) is a simple and effective way to exclude nonviable cells. FS-versus-SS gating also excludes many nonlysed erythrocytes because of their small size. An alternative method for removing nonviable cells from the analysis is the addition of a DNA-binding dye, such as 7-amino-actinomycinD (i.e., 7-AAD, which is well excited by 488-nm lasers) or 4',6-diamidino-2-phenylindole, dihydrochloride (i.e., DAPI, which is well excited by 405-nm lasers), to the cell suspension just before acquisition on the flow cytometer. These dyes penetrate the damaged plasma membranes of nonviable cells, but are excluded from viable cells. Cells demonstrating the characteristic fluorescence of these dyes are excluded from further evaluation by gating. Our third gating step is the separation of cells according to broad cell type. Because viable

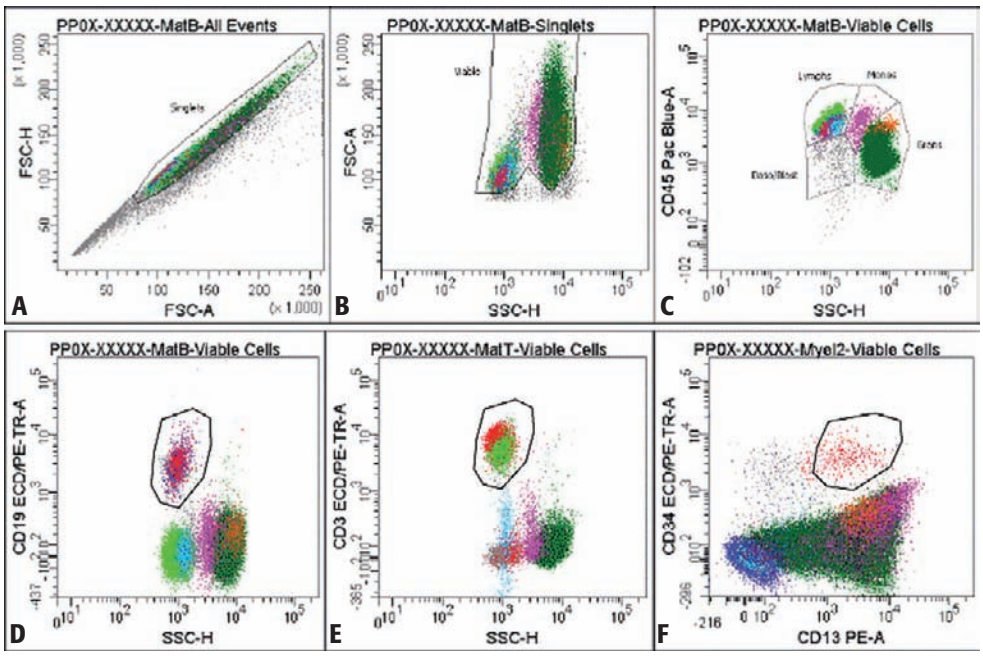


**FIGURE 23-2**

Preliminary gating strategy for a complex cell mixture such as bone marrow **(A-C)** and lineage-specific gating to target specific cell populations **(D-F).** If the flow cytometer allows concurrent measurement of both forward scatter height (FSC-H) and forward scatter area (FSC-A), then gating on events on the 45-degree line defined by these two parameters limits the analysis to single cells (i.e., singlets). For cell doublets and higher-order aggregates, the FSC-A value exceeds the FSC-H value, such that aggregates fall to the right of this 45-degree line when FSC-H and FSC-A are measured on the *y* and *x* axes, respectively **(A).** Once singlets are gated, a reasonable second step is to use FSC versus side-scatter SSC gating to limit the analysis to viable single cells retaining relatively high FSC **(B).** Side scatter (SSC) may be measured on either a log or linear scale, with log SSC measurement providing a more compressed view of the data than linear SSC. Thereafter, the viable hematopoietic cell populations can be well separated for analysis by CD45 versus SSC gating **(C).** Lineage-specific gating is particularly useful when one or two antigens can identify an entire cell lineage, such as surface CD19 for B-lymphoid cells, including B-lymphoblasts and plasma cells **(D),** surface CD3 for mature T cells **(E),** and surface CD34 and CD13 for myeloid blasts **(F).** However, neoplastic cells with abnormally decreased expression of the gating antigens might not be identified by this strategy, necessitating an alternative gating strategy to ensure that such cells will not be overlooked. As with all the subsequent figures, antigen-associated fluorescence in this figure is displayed on a "logicle" scale that transforms the appearance of negative to low-positive fluorescence data, without altering the data values or summary statistics computed from the data (see text for details).

lymphocytes, monocytes, and granulocytes typically show reproducible differences in their combined FS and SS characteristics, FS-versus-SS gating can be used effectively to separate these three cell populations in peripheral blood. This gating strategy becomes less effective when applied to bone marrow, because one particularly important bone marrow population—the blasts—has FS-versus-SS characteristics that overlap both the lymphocytes and monocytes. As a result, many laboratories use CD45-versus-SS gating to separate the various bone marrow cell populations, including the blasts (see Figure 23-2, C). The fourth gating step, which is particularly useful when looking for a mature B-cell or T-cell lymphoproliferative disorder, is lineage-specific gating (see Figure 23-2, D-F). In this gating strategy, evaluation of a pan–B-cell antigen such as CD19, or a pan–T-cell antigen such as CD3, restricts the analysis to these lymphoid populations. An inherent limitation of lineage-specific gating is that B- or T-cell neoplasms with aberrant loss of CD19 or CD3, respectively, will not be identified by this gating procedure. Therefore a more generic gating step, such as CD45-versus-SS gating to identify all the lymphocytes, which can then be evaluated for aberrant antigenic loss, should always be used in conjunction with lineage-specific gating.

A variety of antigenic abnormalities may be observed during the FC evaluation of malignant hematopoietic cell populations. These abnormalities typically contrast with the highly regular and reproducible patterns of antigen expression seen in benign hematopoietic cell populations and include: (1) abnormal increases or decreases in the levels of expression of antigens normally on the cells of interest, including complete loss of expression (e.g., aberrant loss of CD7 on the neoplastic $CD4^+$ T cells of mycosis fungoides–Sézary syndrome); (2) abnormally homogeneous expression of antigens that normally show coordinate variation in expression in a population of interest (e.g., abnormally homogeneous expression of CD34 and CD38 on myeloid blasts in myelodysplasia or acute myeloid leukemia); (3) asynchronous antigen expression in which the timing of antigen expression during a maturational process is abnormal (e.g., asynchronous expression of CD13 and CD16 during neutrophil maturation in myelodysplasia); and (4) aberrant expression of nonlineage antigens (e.g., aberrant expression of the T cell–associated antigen CD7 on leukemic myeloid blasts). To appreciate these abnormalities, the flow cytometrist must thoroughly understand the normal patterns of antigen expression in the cell populations of interest. Thought should be given to the numerical scale used to display each FC parameter. FS is typically presented on a linear scale. SS may be presented on a linear or a log scale, with the latter offering a relatively compressed view of the data. Antigen-associated fluorescence may be presented on a traditional four-logarithm scale or, as some authors have recommended in recent years, on a modified log scale (so-called "logicle display") that does NOT alter the actual data values or summary statistics computed from the data. Functionally, logicle display employs a hyperbolic sine function that transforms the appearance of the compensated FC data by centering antigen-negative and low-positive populations on a linear scale around a "0" point on the relevant axis while effectively retaining logarithmic scaling for antigen-positive populations with moderate- to high-level positivity. In many cases, logicle scaling improves one's certainty about the nature of negative- and low-positive populations.

### DATA REPORTING

Clinical leukemia and lymphoma immunophenotyping data are typically reported in one of two ways. The less informative way is to report the percentage of cells in the population of interest that is considered positive for each of the antigens evaluated. Because such lists of antigens do not provide a unifying description of the abnormal cell populations in the specimen, a much more useful way to report FC data is to describe the detailed immunophenotype of each abnormal cell population in a free-text format in the FC report, including the proportion each population represents of the total viable cells. In describing levels of antigen expression associated with abnormal cell populations, the preferred terms are *high-level* and *low-level* instead of *bright* and *dim,* because the latter two terms describe levels of fluorescence associated with bound antibodies, which are surrogates for actual levels of antigen expression.

It is common to report a CD45-versus-SS–based differential count of the viable leukocytes in the specimen (i.e., the proportions of lymphocytes, monocytes, granulocytes, blasts, and plasma cells). Finally, key two-dimensional histograms of the FC data may be included in the report, which provides a snapshot of a neoplastic immunophenotype that can be of great help in following the patient's disease when subsequent specimens are received.

## B-LYMPHOID NEOPLASMS

### INTRODUCTION

B-lymphoid neoplasms are best understood in the context of normal B-cell maturation in the marrow (Figure 23-3). Precursor B-cell neoplasms correspond to the pro–B-cell and pre–B-cell, or B-lymphoblast, stages. Mature B-cell neoplasms correspond to the early–naive (pregerminal center), germinal center, and postgerminal center B-cell stages. Plasma-cell neoplasms correspond to the terminal stage of B-cell maturation.

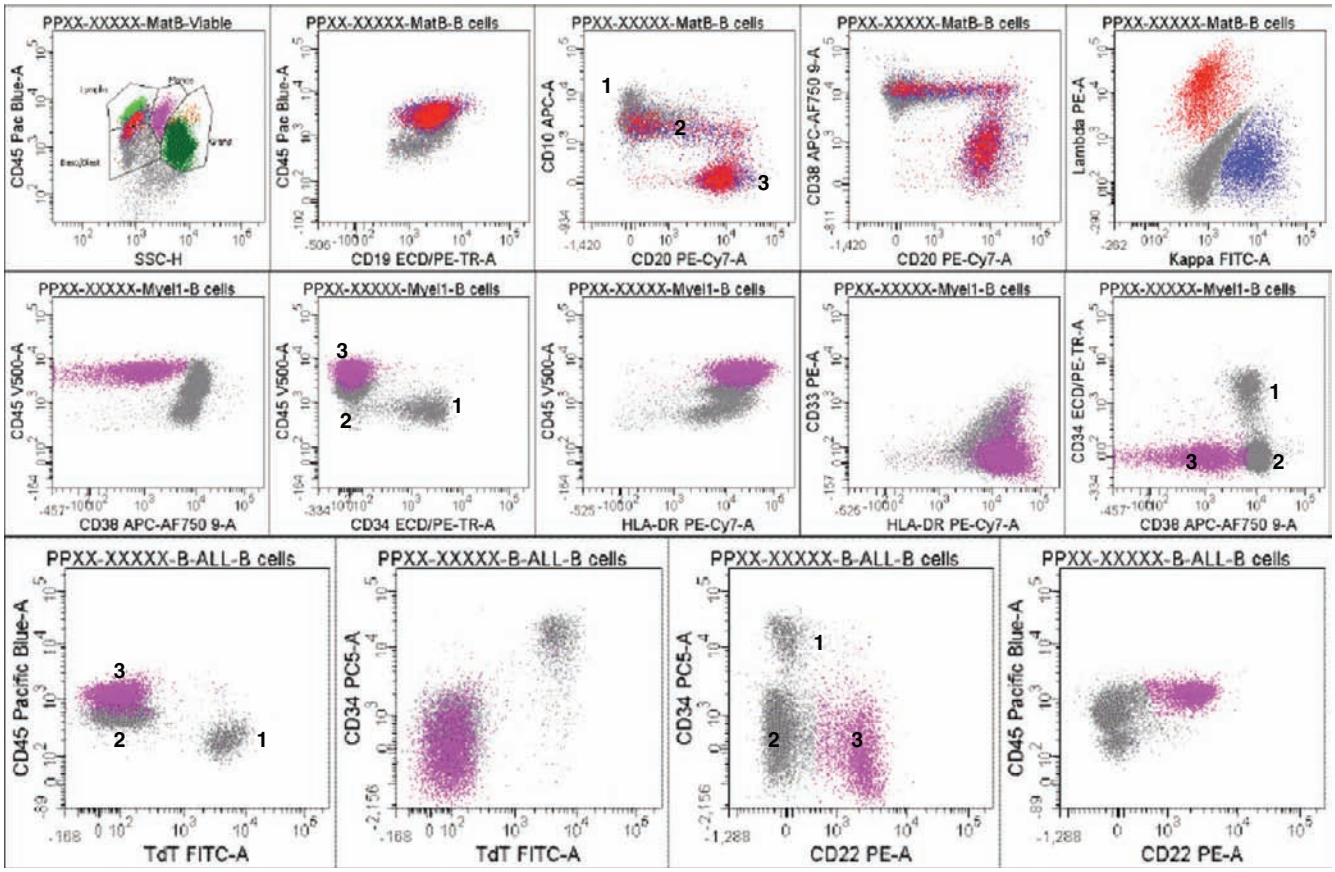


**FIGURE 23-3**

Antigen expression during normal B-cell maturation in the bone marrow. These nine-color flow cytometry data show the three distinct phases of normal B-cell maturation. The most immature phase (population 1) is characterized by the lowest level of CD45 and CD19, no CD20, high-level CD10, intermediate-high CD38, coexpression of CD34 and terminal deoxynucleotidyl transferase (TdT), and no surface light chains. The intermediate immature phase (population 2) is characterized by intermediate-level CD45 and CD19, gradual acquisition of CD20, intermediate-level CD10, intermediate-high CD38, and no CD34, or TdT, and negative to low surface light chains. The mature, naive phase (population 3) is characterized by high-level CD45, intermediate-level CD19 and CD20, low-to-negative CD38, either κ or λ surface light chains (colored blue and red, respectively, when shown as separate κ- or λ-expressing populations, or lavender when all the mature B cells are shown as a single population), and no CD10, CD34, or TdT. Maturation proceeds in an orderly fashion from population 1 to population 2 to population 3.

## PRECURSOR B-LYMPHOID NEOPLASMS

According to the 2008 WHO classification, precursor B-cell neoplasms are referred to generically as *B-lymphoblastic leukemias–lymphomas* (B-LBL); under the 2001 WHO classification, these were called *precursor B-lymphoblastic leukemias–lymphomas*. Most cases develop as leukemias involving the bone marrow and blood, and lymphomatous presentation is rare. B-LBL subtypes with a favorable prognosis include those bearing the t(12;21)(p12;q22) or hyperdiploidy with greater than 50 chromosomes. Subtypes with an unfavorable prognosis include cases having the t(9;22)(q34;q11), the t(1;19)(q23;p13), or the t(4;11)(q21;q23). Virtually all B-LBLs express the B-cell–associated antigen CD19, in addition to human leukocyte antigen (HLA)-DR and the lymphoblast-associated antigen TdT (Figure 23-4). Most B-LBLs express CD10 (the common acute lymphoblastic leukemia antigen) or CALLA and the blast-associated antigen CD34, and most lack surface light chains. CD45 expression is typically low to occasionally negative.

In addition to yielding prognostic information, certain karyotypes have characteristic immunophenotypes. The t(12;21) is associated with relatively low-level CD9 and no expression of the mature B-cell antigen CD20. Hyperdiploid cases frequently lack CD45. The t(9;22) is associated with aberrant expression of myeloid antigens, such as CD13 and CD33, and occasional lack of CD45. The t(1;19)(q23;p13) is associated with a lack of CD34. The t(4;11) is associated with loss of CD10 expression (CALLA-negative pre–B-ALL), lack of CD20, and frequent aberrant expression of the myeloid-associated antigen CD15.

## MATURE B-CELL NEOPLASMS: GENERAL FEATURES

Almost all mature B-cell neoplasms express CD19 and CD20 at some level. Most cases show restricted cell

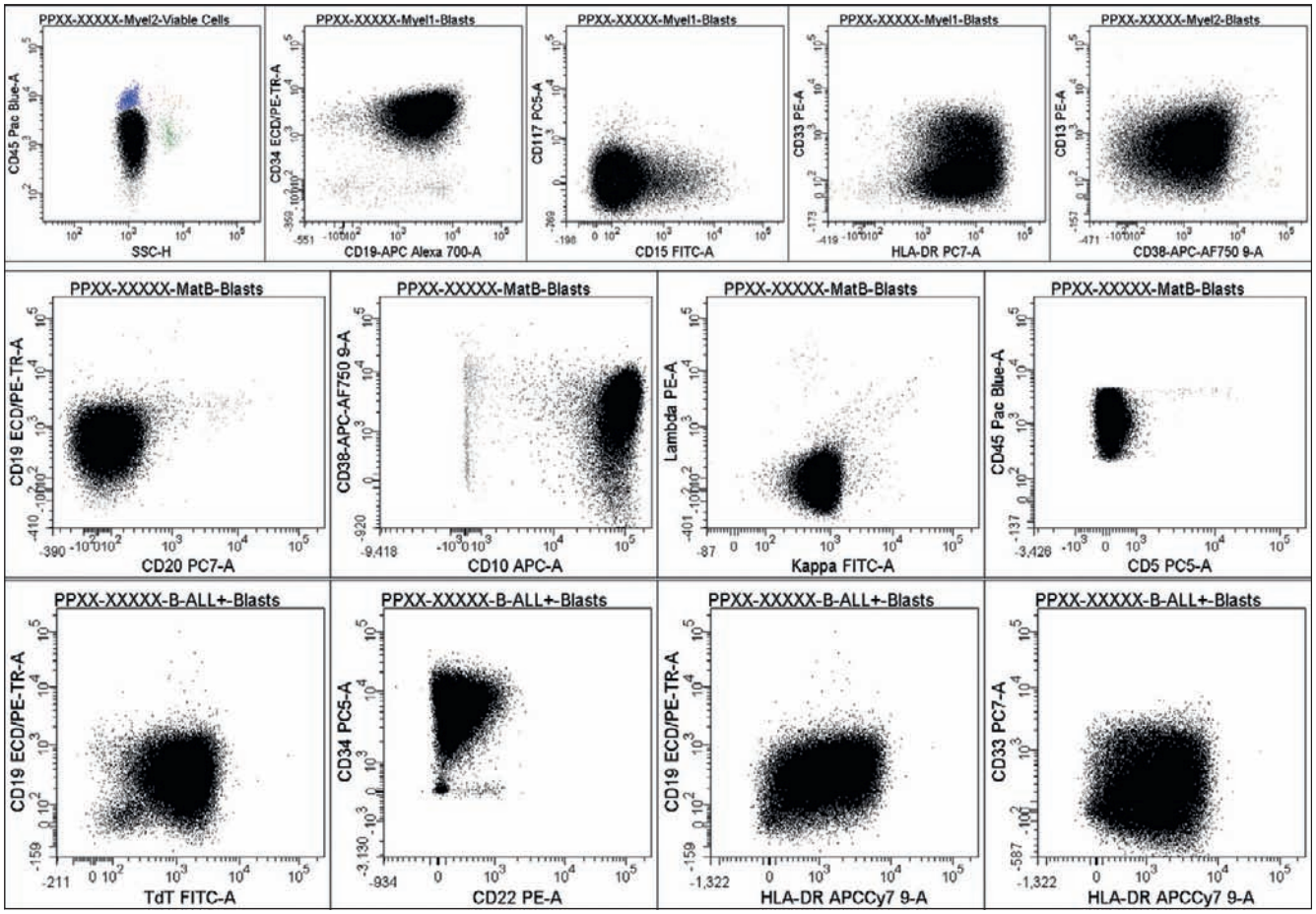


**FIGURE 23-4**

Precursor B-lymphoblastic leukemia/lymphoma (B-ALL). These nine-color flow cytometry data show the leukemic B-lymphoblasts (colored black) to express uniform CD34 and terminal deoxynucleotidyl transferase, low CD22, abnormally low-level CD19 and CD38, abnormally high-level CD10, aberrant low-level but uniform expression of the myeloid-associated antigen CD13, aberrant low-level expression of the myeloid-associated antigens CD15 and CD33 on small subsets, and no CD20 or surface light chains. Aberrant myeloid antigen expression is relatively common in B-ALL bearing the Philadelphia chromosome, which was the genotype of this case.

surface expression of κ or λ light chains, allowing clonality to be inferred easily by FC, but a minority of cases demonstrate a loss of surface light chains, requiring evaluation of cytoplasmic light chains to provide formal evidence of clonality. Cytoplasmic light chain evaluation may not be essential to prove malignancy if the remainder of the surface immunophenotype is unequivocally aberrant. However, in cases with apparent loss of surface light-chain expression but an otherwise normal surface immunophenotype, formal evidence of clonality should be obtained from cytoplasmic light-chain evaluation, because benign B-cell populations, particularly germinal center B-cells, occasionally downregulate surface light-chain expression to such an extent that they appear surface light chain–negative.

CD20 is currently such an important therapeutic target in the treatment of B-cell malignancies that evaluation of its expression is required in any FC evaluation of B cells. In patients recently treated with anti-CD20 antibodies, FC typically fails to identify a $CD20^+$ cell population, owing to the therapeutic antibody coating of CD20 on B-cells. In patients remotely treated with anti-CD20 antibodies, lymphoma recurrences occasionally lack surface CD20, presumably because of the selection of a $CD20^-$ subclone following therapy. Note that rare, mature B-cell neoplasms will appear $CD20^-$ by flow cytometry using antibodies to extracellular epitopes, but $CD20^+$ by immunohistochemistry (IHC), which typically uses clone L26 against an intracellular epitope; Such cases, which likely contain alterations in the extracellular epitopes recognized by the FC antibodies, should be considered $CD20^+$ for therapeutic purposes. Therefore CD20 IHC should be performed on any available paraffin-embedded tissue for all mature B-cell neoplasms that appear $CD20^-$ by FC. Additional potential therapeutic targets in mature B-cell neoplasms include CD22 and CD52.

## CHRONIC LYMPHOCYTIC LEUKEMIA–SMALL LYMPHOCYTIC LYMPHOMA

A major feature of chronic lymphocytic leukemia–small lymphocytic lymphoma (CLL/SLL) is aberrant coexpression of the T cell–associated antigen CD5

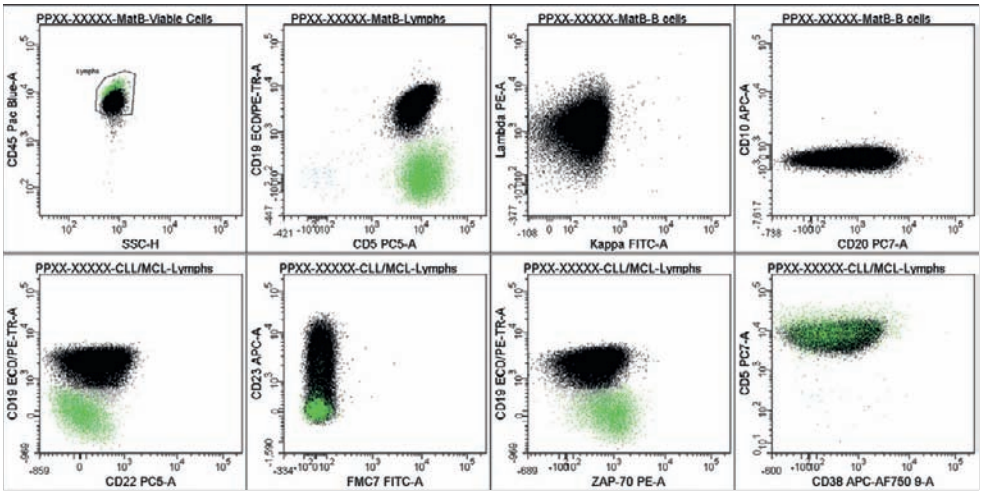


**FIGURE 23-5**

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). These nine-color flow cytometry data show a typical CLL/SLL population (colored black) coexpressing CD19 and aberrant CD5, with low-level λ-restricted surface light chain, low-level CD20 and CD22, variable CD23, and no FMC7. In addition, the CLL/SLL cells express both ZAP-70 and CD38, which are adverse prognostic markers.

and T cell–myeloid-associated antigen CD43 on the neoplastic B cells, along with the B cell–associated antigens CD19 and CD20 (Figure 23-5). There is usually diminished expression (compared to normal mature B cells) of a variety of mature B cell–associated antigens—including surface light chains, CD20, CD22, and CD79b—but some cases show an activated phenotype with higher levels of these antigens. Variable CD23 is typically expressed, with little or no FMC7 antigen (FMC7 is believed to represent an epitope of CD20). Cases bearing unmutated immunoglobulin genes, a feature associated with an adverse prognosis, frequently express CD38 or cytoplasmic ZAP-70, or both. Because CLL/SLL is a common disease, occasional patients show two different CLL/SLL clones. In CLL/SLL progression, the expanded component of larger, prolymphocyte-like cells often shows a relative decrease in CD5 and CD23 expression, and a relative increase in surface light chains, CD20, CD22, CD79b, and FMC7.

## FOLLICULAR LYMPHOMA

The majority of follicular lymphoma (FL) cases coexpress the germinal center–associated antigen CD10 in addition to CD19, CD20, restricted light chains, and cytoplasmic BCL-2 (Figure 23-6). CD19 often is expressed at abnormally low levels. Surface light chain expression is often brighter than in CLL/SLL, although some cases show aberrant loss of surface light chains. Low-grade follicular lymphomas (grades 1 and 2 out of 3 in the WHO system) tend to show relatively small cell size by FS and SS light characteristics, whereas grade 3 follicular lymphomas tend to show a spectrum of larger cell size.

## HAIRY CELL LEUKEMIA

Hairy cell leukemia (HCL) cells show increased SS because of their relatively abundant cytoplasm, high-level CD19 and CD20 compared with normal B cells, and restricted surface light chains. The key to making the diagnosis of classical HCL is identifying coexpression of CD103, CD25, and relatively high-level CD22 and CD11c (Figure 23-7). Classical HCL also typically expresses CD123, expresses CD10 in a minority of cases, and is associated with monocytopenia. A distinct subset of HCL-like cases, formerly referred to as *hairy cell variants* and currently referred to as *splenic B-cell lymphoma, not otherwise specified* under the 2008 WHO classification, typically lacks CD25 and CD123, with an otherwise HCL-like phenotype.

## MANTLE CELL LYMPHOMA

Mantle cell lymphoma (MCL), like CLL/SLL, is characterized by coexpression of CD5 and CD43 on the neoplastic B cells, along with CD19, CD20, and restricted light chains. However, in contrast to CLL/SLL, MCL typically shows moderate to high-level expression of surface light chains, CD20, CD22, and CD79b, with coexpression of FMC7 and little or no CD23. Rare cases express little or no CD5 or CD43; they also may express low CD10. Flow cytometric evaluation of nuclear cyclin D1 expression, the protein overexpressed as a result of the characteristic t(11;14)(q13;q32) of MCL, is not in common clinical usage; therefore, the diagnosis of MCL is usually confirmed by cyclin D1 IHC, t(11;14) fluorescence in situ hybridization, or cytogenetics.

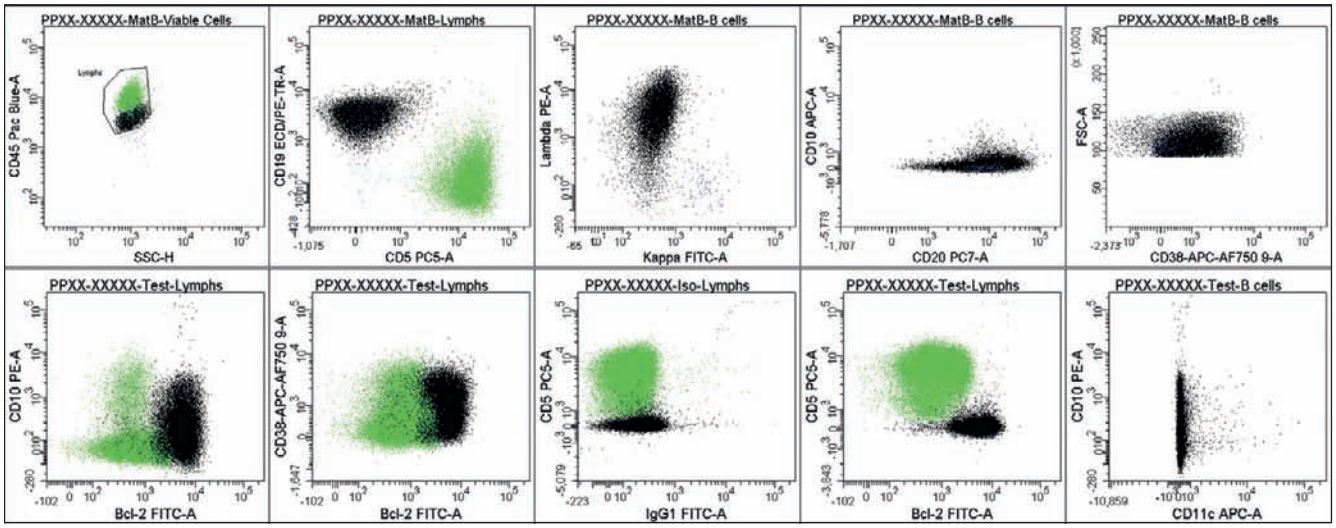


**FIGURE 23-6**

Follicular lymphoma. These nine-color flow cytometry data show the common coexpression of low-level CD10 and CD38 on the λ-restricted neoplastic B cells (colored black), intermediate-level CD20, and clear cytoplasmic BCL-2 overexpression compared to the green-colored T cells. Cytoplasmic antigen evaluation is one of the few instances in which we run a separate isotype-matched control (an IgG1-FITC control for the BCL-2-FITC antibody). The lack of CD11c expression on the neoplastic B cells rules out the formal alternative of CD10-positive hairy cell leukemia.

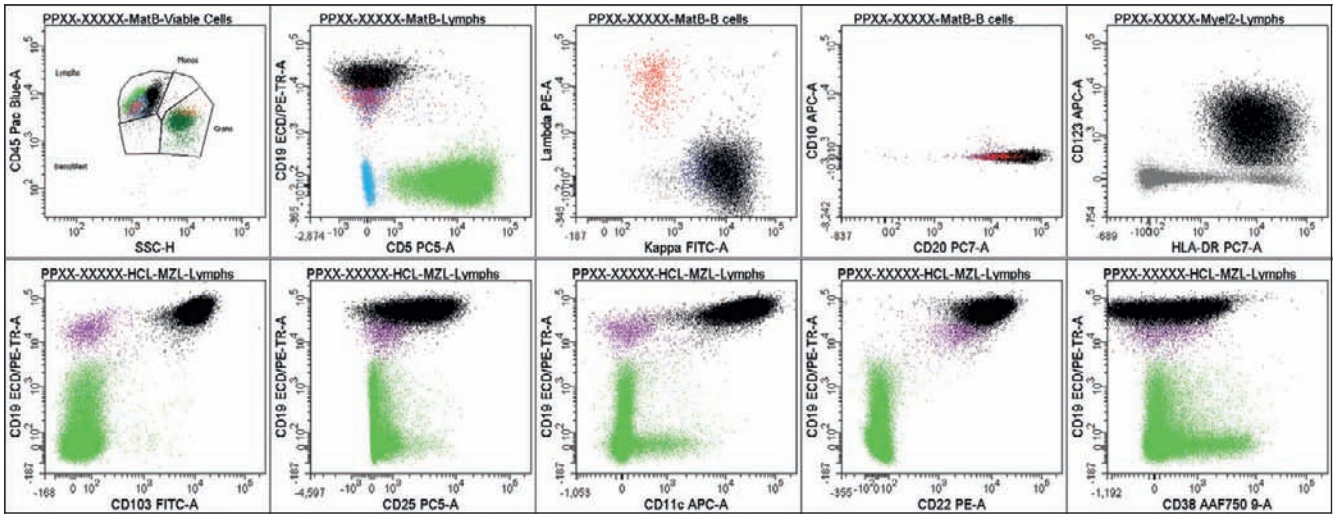


**FIGURE 23-7**

Hairy cell leukemia. Hairy cell leukemia has a distinctive immunophenotype enabling the flow cytometric identification of small populations of these cells (less than 0.01% in some cases). These nine-color flow cytometry data show the neoplastic B cells (colored black) with the characteristic increase in side scatter (corresponding to the increased cytoplasm in the cells), expression of high-level CD19, CD20, CD22, and CD11c, and the disease-defining coexpression of CD103 and CD25. CD123 expression is also characteristic of (classical) hairy cell leukemia. Hairy cell leukemia variant typically lacks expression of both CD25 and CD123.

## DIFFUSE LARGE B-CELL LYMPHOMA

Diffuse large B-cell lymphoma (DLBCL) generally shows increased cell size based on FS and SS characteristics, although degenerating or partially necrotic specimens may show an artifactual decrease of both light scatter parameters. CD19, CD20, and restricted light chains are generally expressed at moderate to high levels, but can be unusually low, as with decreased CD20 in immunoblastic lymphomas because of plasmacytoid differentiation. When present, CD10 expression suggests a germinal center origin of the neoplastic cells. Certain subtypes of DLBCL recognized by the WHO classification have distinctive immunophenotypes: (1) intravascular large B-cell lymphoma often shows aberrant CD5 coexpression; (2) T-cell/histiocyte–rich large B-cell lymphoma generally shows a prominent benign background T-cell population, few normal small B cells, and few if any detectable neoplastic large B cells; (3) primary mediastinal large B-cell lymphoma often expresses CD23, with

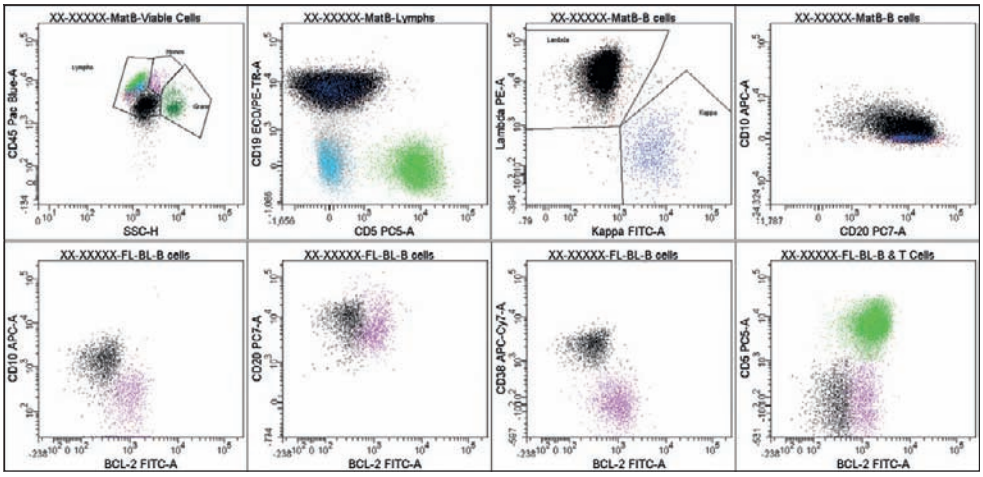


**FIGURE 23-8**

Burkitt lymphoma. These nine-color flow cytometry data show the characteristic features of Burkitt lymphoma (neoplastic cells colored black), including abnormally decreased CD45 (for mature B cells), coexpression of CD19, CD20, CD10, CD38, and restricted surface light chains (λ), and no cytoplasmic BCL-2 in comparison to the normal B cells (colored lavender) and T cells (colored green).

loss of surface HLA-DR and loss of both surface and cytoplasmic light chains; and (4) primary effusion lymphoma generally demonstrates a loss of B-cell–associated antigens such as CD19 and CD20, but may express CD45, CD38, CD30, or restricted cytoplasmic light chains.

## BURKITT LYMPHOMA

Burkitt lymphoma typically demonstrates intermediate, rather than large, cell size based on the light-scatter properties, often with a prominent nonviable population because of the very high apoptotic rate among the tumor cells. There is moderate to high-level expression of CD19, CD20, CD38, and restricted light chains, along with variable CD10 (Figure 23-8). In contrast to most FLs and many DLBCLs, cytoplasmic BCL-2 is not expressed in Burkitt lymphoma.

# PLASMA CELL NEOPLASMS

Plasma cells characteristically express higher-level CD38 than any other cell in the bone marrow, and they show variably increased cell size, making them relatively easy to identify by FC. Antibodies to CD138 (syndecan-1) can help to identify abnormal plasma cells in cases showing abnormally decreased CD38. In almost all cases, plasma cell neoplasms express restricted cytoplasmic light and heavy chains identifiable by FC in permeabilized specimens. In practice, many clinical flow cytometry laboratories that evaluate plasma cell clonality do so by examining light chain expression alone, relying on serum or urine immunofixation, or both, to identify the heavy chain component. In rare cases, neoplastic plasma cells express restricted surface light chains in addition to cytoplasmic light chains, or they show aberrant loss of both surface light and cytoplasmic chain expression. The majority of true plasma cell neoplasms (rather than the neoplastic plasma cells of B cell non-Hodgkin lymphomas with plasmacytic differentiation) have abnormal surface immunophenotypes, with decreased to absent CD19 and CD45, occasionally decreased CD38, frequent aberrant CD56, and occasional aberrant CD117/c-kit or other antigens (Figure 23-9).

# T-LYMPHOID AND NATURAL KILLER-CELL NEOPLASMS

## INTRODUCTION

T-lymphoid neoplasms, like B-lymphoid neoplasms, can be understood in the context of normal T-cell maturation, which occurs in the thymus and is illustrated by benign T cells in the setting of thymoma (Figure 23-10, A). Normal mature T cells have an identical immunophenotype in the blood and bone marrow (see Figure 23-10, B). Precursor T-cell neoplasms correspond to the prothymocyte and immature, common, and mature thymocyte stages. Mature T-cell neoplasms usually derive from single-positive (i.e., $CD4^+$ or $CD8^+$) T-cells expressing α-β T-cell antigen receptors (TCRs), although rare γ-δ T-cell malignancies occur. NK cells represent a lymphoid lineage distinct from T cells and, unlike T cells, do not have rearranged T-cell antigen receptors.

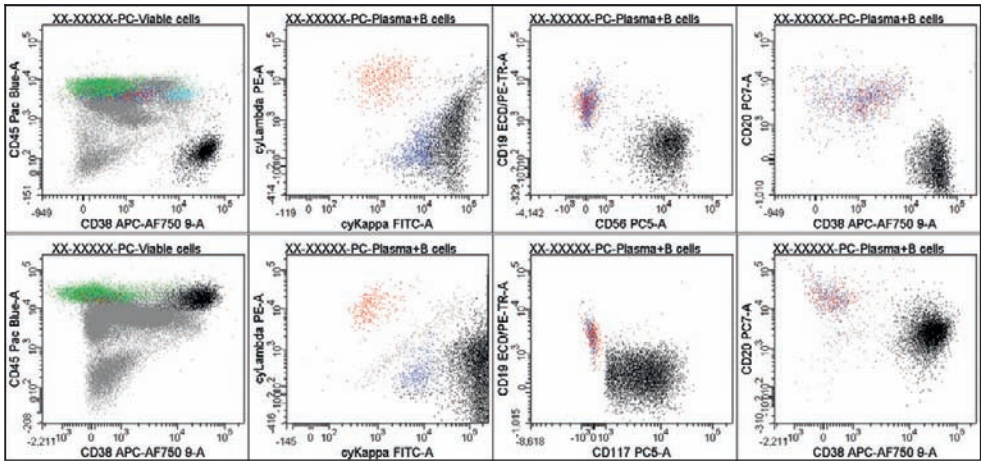


**FIGURE 23-9**

Two cases of plasma cell myeloma. These eight-color flow cytometry data show a typical case of plasma cell myeloma in the upper panel (neoplastic cells colored black), with characteristic high-level CD38 expression, high-level cytoplasmic light chain restriction (κ), aberrant loss of both CD45 and CD19, aberrant coexpression of high-level CD56, and no CD20. The *lower panel* shows an unusual case with high-level cytoplasmic (κ) light chain restriction, abnormally high-level CD45 and CD20, aberrant loss of CD19, and aberrant coexpression of CD117. Looking for the characteristic plasma cell expression of CD138, without CD22, can be helpful in distinguishing unusual myelomas from B-cell lymphomas with unusually high-level CD38 expression (the latter will usually be $CD138^{low/-}$ and $CD22^{+}$).

## PRECURSOR T-CELL NEOPLASMS

According to the 2008 WHO classification, precursor T-cell neoplasms are referred to generically as *T-lymphoblastic leukemias/lymphomas* (T-LBL; under the 2001 WHO classification, these were called *precursor T-lymphoblastic leukemias/lymphomas*). Compared to B-LBL, T-LBL is much more likely to present with soft-tissue involvement, particularly in the anterior mediastinum/thymus. Like B-LBL, T-LBL consists of a number of genetically distinct entities, many of which are thought to derive from different stages of early T-cell maturation; all these stages typically express TdT, CD99, and CD7. The least mature T-LBLs, which are also called early T-cell precursor leukemias and are associated with a particularly poor prognosis, are thought to derive from prothymocytes, which express TdT, HLA-DR, CD34, and CD7 (Figure 23-11). Somewhat more mature T-LBLs may correspond to the immature thymocyte stage, at which time HLA-DR and CD34 are lost and CD2, CD5, and cytoplasmic CD3 are acquired. T-LBLs of the common thymocyte stage typically show dual expression of CD4 and CD8 and express the common thymocyte antigen CD1a at some level. Finally, occasional T-LBLs have a mature thymocyte immunophenotype, with single positivity for CD4 or CD8 and acquisition of surface CD3. The identification of immunophenotypic aberrancy among immature T cells, such as abnormally homogeneous expression of normal immature T-cell antigens or aberrant expression of non–T-cell–associated antigens, such as CD 13, CD33, or CD117, can be helpful in distinguishing mediastinal T-LBL from thymoma or thymic hyperplasia, because the benign immature T cells of thymoma or thymic hyperplasia show a normal maturational spectrum of antigen expression. CD79a expression has been described in both T-LBL and benign thymocytes.

## MATURE T-CELL AND NATURAL KILLER CELL NEOPLASMS: GENERAL FEATURES

With the exception of γ-δ T-cell lymphomas and NK-cell lymphomas, the entities discussed in this section involve α-β T cells. These neoplasms all lack CD34, TdT, and CD1a. Historically, prominent abnormal increases or decreases in levels of expression of T-cell–associated surface antigens have been used as surrogates for clonality in the evaluation of T-cell neoplasms. In this approach, care must be taken to avoid overinterpreting changes in surface antigen expression, because certain benign T-cell populations show characteristic decreases in surface antigen expression. For example, both benign and malignant large granular lymphocytes of the ($CD8^{+}$) T-cell type characteristically show a decreased level of CD5 compared with other $CD8^{+}$ T cells, such that mildly decreased CD5 among a subset of the $CD8^{+}$ T cells is not sufficient to make the diagnosis of T-cell large granular lymphocytic leukemia. Similarly, $CD4^{+}$ memory T cells characteristically show diminished CD7 expression compared with other T cells.

Antibodies to 24 different TCR-β isoforms are commercially available, enabling identification of restricted TCR-β usage in approximately 70% of neoplasms derived from α-β T cells. As a result, clonality now can be proved by FC in the majority of T-cell neoplasms. For

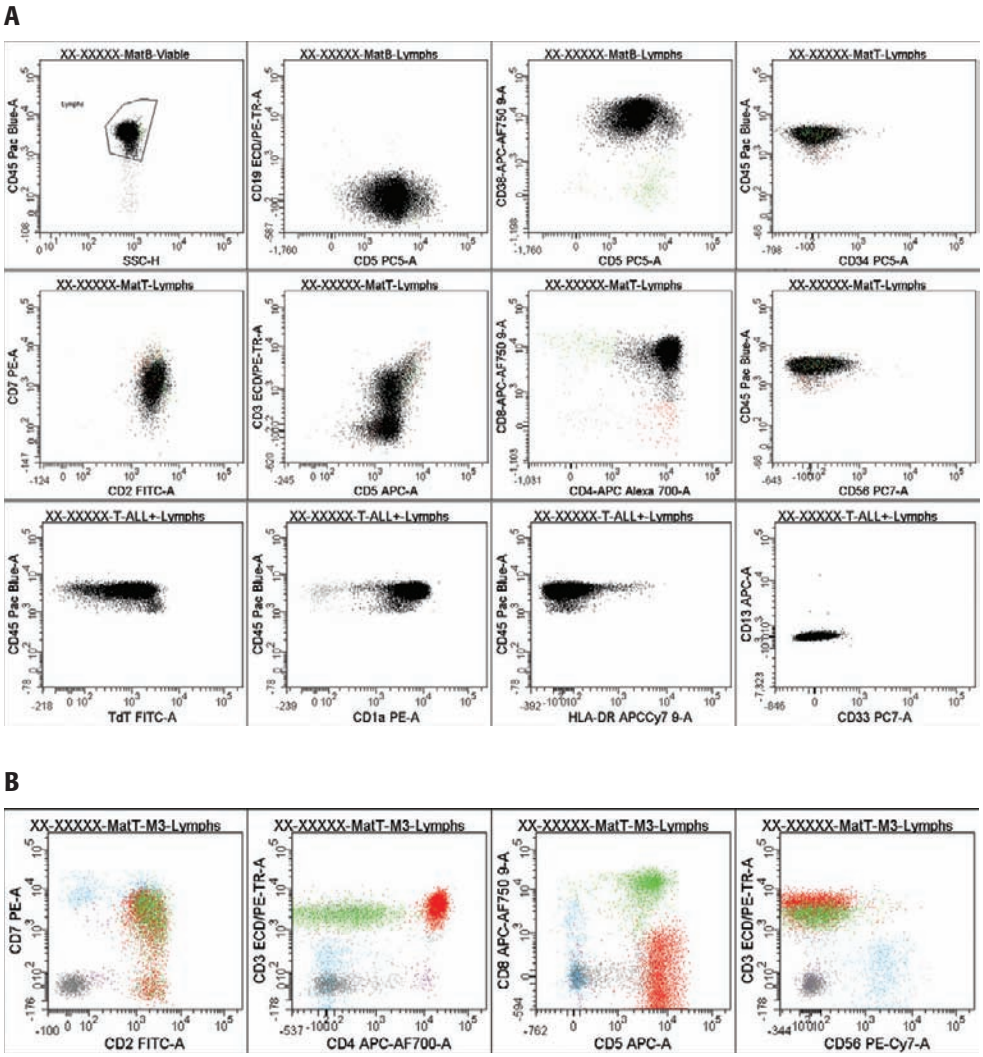


**FIGURE 23-10**

Normal immature T cells in a case of thymoma **(A)** and normal mature T cells in the bone marrow **(B).** Although the common lymphoid stem cell arises in the bone marrow, most T cell maturation occurs in the thymus. The nine-color flow cytometry data in **A** show a predominant population of normal immature T cells of common thymocyte stage (colored black) with characteristic coexpression of CD4, CD8, CD1a, and terminal deoxynucleotidyl transferase. The rare, mature, single $CD4^+$ and single $CD8^+$ T cells are colored red and green, respectively, in the middle panel. Among the normal bone marrow T cells in **B,** the $CD4^+$ and $CD8^+$ cells are also colored red and green, respectively. The light blue–colored cells are normal natural killer cells, and the few lavender cells are monocytes in the gate. Normal peripheral blood T and natural killer cells have immunophenotypes identical to those of their bone marrow counterparts.

the other 30% or so of α-β T-cell neoplasms, a lack of reactivity of the tumor cells with any of the 24 antibodies allows clonality to be inferred. Importantly, because clonal T-cell expansions can be seen in benign immunologic reactions, FC demonstration of T-cell clonality must be interpreted in the overall context of the case before a diagnosis of T-cell malignancy is made. In laboratories not using TCR-β antibodies, proof of T-cell clonality will require TCR gene polymerase chain reaction evaluation.

Note that the majority of the mature T-cell neoplasms express CD52, an important target in salvage therapy of these tumors. Expression of CD25 and CD30, which are other potential therapeutic targets, is less common in T-cell neoplasms.

## T-CELL PROLYMPHOCYTIC LEUKEMIA

T-cell prolymphocytic leukemia (T-PLL) typically involves clonal T cells expressing CD2, CD3, CD5, and CD7, although one or more of these antigens, or rarely CD45 (Figure 23-12), may be expressed at abnormal levels. Sixty percent of cases are reported to be $CD4^+$ and $CD8^-$, 25% of cases coexpress CD4 and CD8, and 15% of cases are $CD8^+$ and $CD4^-$. Coexpression of CD4 and

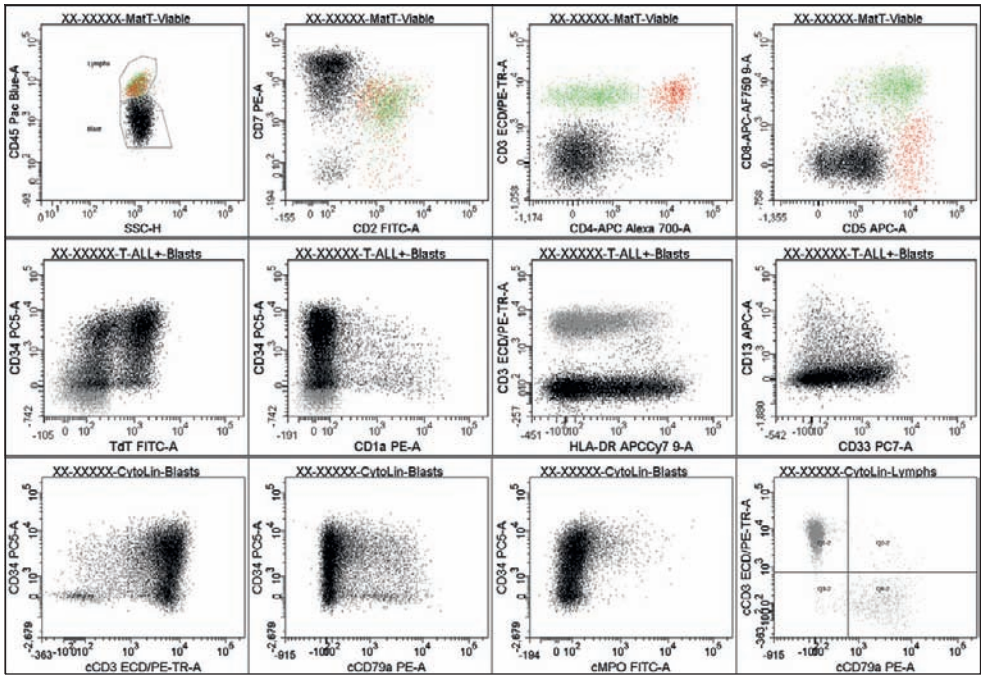


**FIGURE 23-11**

Precursor T-lymphoblastic leukemia–lymphoma of prothymocyte stage (early T-cell precursor leukemia). Precursor T-ALL can show immunophenotypic features reminiscent of any stage during early T cell development. In this case, the leukemic T-lymphoblasts (colored black in these nine-color flow cytometry data) display a prothymocyte phenotype, with coexpression of CD34, terminal deoxynucleotidyl transferase (TdT), cytoplasmic CD3, high-level CD7, low-level CD5, low-level aberrant CD33 on a subset, and no surface CD1a, CD3, CD4, or CD8.

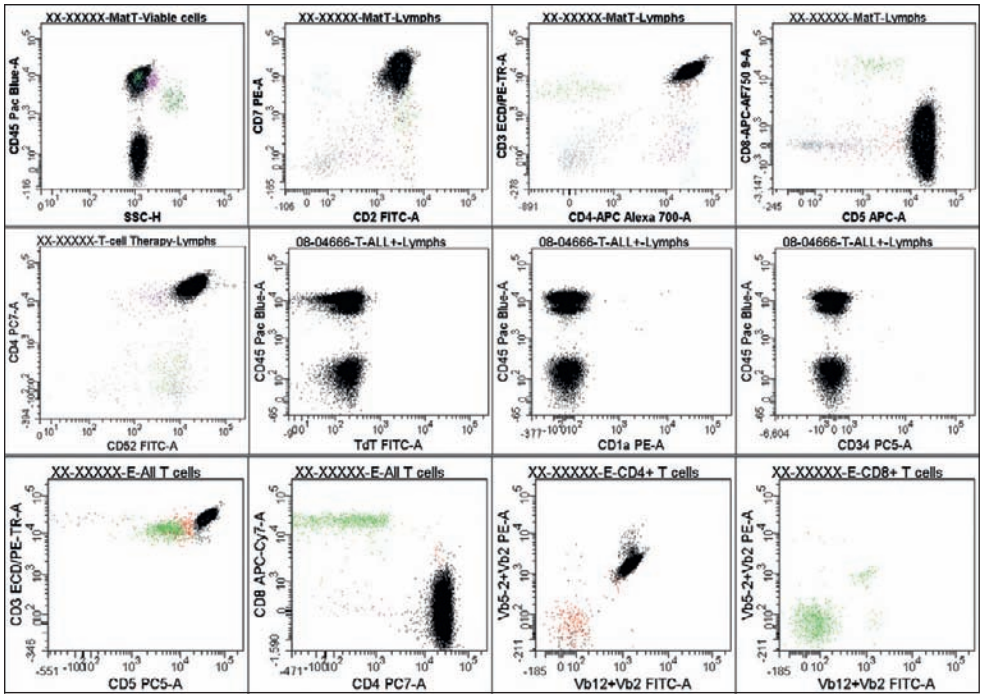


**FIGURE 23-12**

T cell prolymphocytic leukemia. The neoplastic $CD4^+$ T cells in this nine-color analysis (colored black) show slightly increased expression of CD3, CD5, and CD7 compared to the few normal T cells, and include a discrete subset with aberrant loss of CD45. Flow cytometric evaluation of TCR-β expression showed V-β-2-restriction (lower row of flow histograms), confirming monoclonality.

CD8 is relatively unique to T-PLL among mature T cell neoplasms, which helps to distinguish it from other mature T cell neoplasms of small to intermediate cell size. Formal confirmation of the diagnosis of T-PLL can be achieved by cytogenetic or fluorescence in situ hybridization demonstration of the characteristic inv(14) or t(14;14) involving the *TCL1* gene, demonstration of one of the common abnormalities of chromosome 8, or both. *TCL1* expression is seen with IHC in more than 80% of cases and can be used to confirm the diagnosis in paraffin-embedded tissue, because *TCL1* expression in T cells is specific for T-PLL.

## T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

The majority of T-cell large granular lymphocytic leukemia cases are clonal $CD8^+$ T cell neoplasms showing variable loss of CD5 and often decreased CD7, commonly with CD57 coexpression. Rare T-cell large granular lymphocytic leukemia variants express CD4, occasionally together with CD8. There is commonly a history of prolonged cytopenias in these patients, most commonly neutropenia, but occasionally anemia or thrombocytopenia, or both. Underlying autoimmune disorders are common, particularly rheumatoid arthritis.

## MYCOSIS FUNGOIDES (SÉZARY SYNDROME)

The neoplastic cells in mycosis fungoides and (Sézary syndrome) are usually clonal $CD4^+$ T cells showing loss of CD7 and CD26 expression and occasional increases or decreases in the levels of other pan–T cell antigens. Rare cases express CD8. Transforming cases may express CD30.

## PERIPHERAL T-CELL LYMPHOMA, UNSPECIFIED

No consistent immunophenotypic pattern exists in peripheral T-cell lymphomas (PTCLs), although most cases are clonal CD4+ T-cell neoplasms, and aberrant loss or gain of one or more T-cell–associated antigens is common. Fewer cases express CD8, and rare cases coexpress CD4 and CD8. Some peripheral T-cell lymphoma cases express variable CD30.

## ADULT T-CELL LEUKEMIA–LYMPHOMA

The neoplastic cells in adult T-cell leukemia–lymphoma are typically clonal $CD4^+$ T cells expressing CD25 and showing aberrant loss of CD7. Proteins produced by the causative agent of adult T-cell leukemia–lymphoma, human T-lymphotropic virus-1, currently are not being assayed in clinical FC.

## ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

The neoplastic cells in angioimmunoblastic T-cell lymphoma are typically $CD4^+$ T cells that often demonstrate aberrant loss of surface CD3 or CD7 expression, or both, with retention of surface CD5 (Figure 23-13). In the majority of cases, the neoplastic cells show some degree of CD10 expression, a unique feature among T-cell malignancies that reflects the follicular T helper cell origin of the neoplastic cells in angioimmunoblastic T-cell lymphoma. In a subset of cases, a simultaneous clonal B cell population may be detected, presumably because of underlying immunologic dysregulation and reactivation of Epstein-Barr virus in the clonal B cells.

## HEPATOSPLENIC T-CELL LYMPHOMA

The neoplastic cells in this disorder are usually γ-δ T cells, although a minority of cases expresses the α-β TCR. The neoplastic cells typically express CD2, CD3, CD7, and CD56, without CD4, CD5, or CD8. In contrast, benign γ-δ T cells usually express variable low-level CD5 and CD8. Because the neoplastic cells do not express the α-β TCR, they cannot be identified as clonal using antibodies to TCR-β isoforms. Formal proof of clonality in these cases requires molecular evaluation of the TCR-γ or δ chains.

## ANAPLASTIC LARGE-CELL LYMPHOMA

With the exception of the small-cell variant, the neoplastic cells in anaplastic large cell lymphoma are typically very large in size as assessed by FS and SS, often requiring gating strategies including the maximal FS values in two-dimensional dot plots. Moderate to high-level CD30 typically is seen by FC, as is expression of the T-cell activation–associated antigen HLA-DR (Figure 23-14) and frequently CD25. Expression of CD30 has taken on therapeutic significance with the recent approval of the anti-CD30 monomethyl auristatin E conjugate brentuximab vedotin for Hodgkin lymphoma and anaplastic large cell lymphoma. Surface CD2 and CD4 can be identified with flow cytometry, although the cells are usually negative for surface CD3, CD5, and CD7. Note that the systemic and cutaneous forms of anaplastic large cell lymphoma, although biologically distinct neoplasms, have relatively similar immunophenotypes by FC, such that clinical and immunohistochemical criteria, including evaluation of ALK and EMA expression, are required to distinguish between these entities.

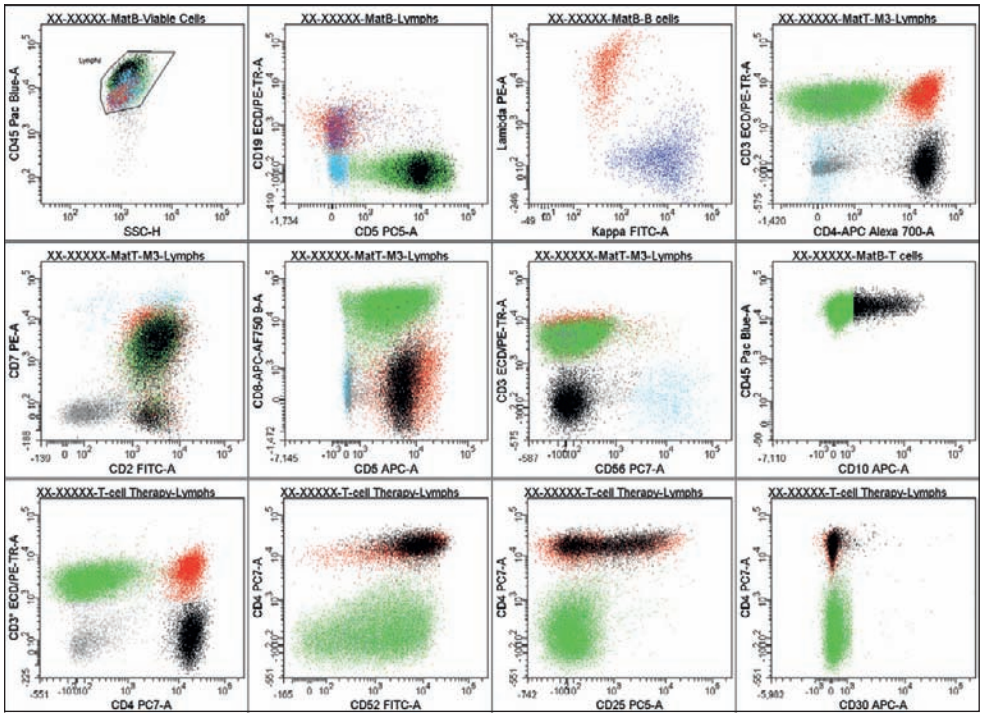


**FIGURE 23-13**

Angioimmunoblastic T-cell lymphoma partially involving a lymph node. The neoplastic $CD4^+$ T cells (colored black in this nine-color analysis) show aberrant loss of surface CD3 expression (seen with two different anti-CD3 antibodies), loss of CD7 on a subset, relatively normal levels of CD2, CD4, and CD5, and low-level CD10 on a subset, which are all common findings. It is routine to evaluate expression of the potential therapeutic targets CD52 and CD25 in all cases containing a significant abnormal T cell population.

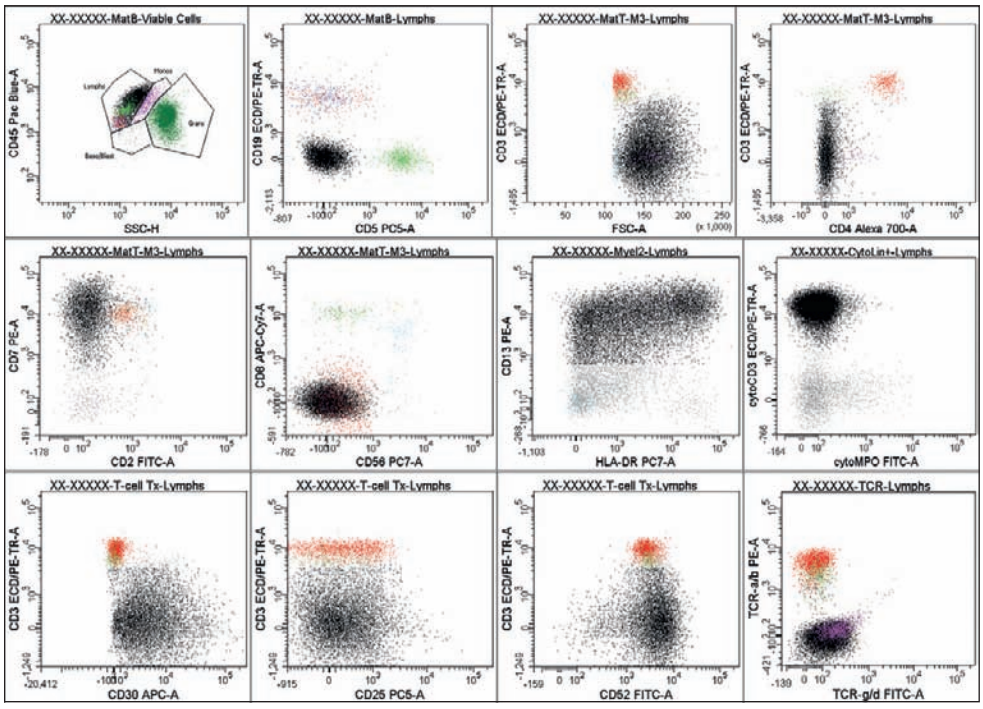


**FIGURE 23-14**

Anaplastic large cell lymphoma, ALK-positive, involving peripheral blood. The high-level coexpression of CD45, CD7, and cytoplasmic CD3, with variable CD30 and lack of cytoplasmic myeloperoxidase, suggesting that the neoplastic cells (colored black in this nine-color analysis) were mature T cells. As in this case, aberrant CD13 expression is relatively common in ALCL, particularly ALK-positive cases. Immunohistochemistry on a concurrent lymph node biopsy demonstrated coexpression of CD30 and ALK on the tumor cells, confirming the diagnosis.

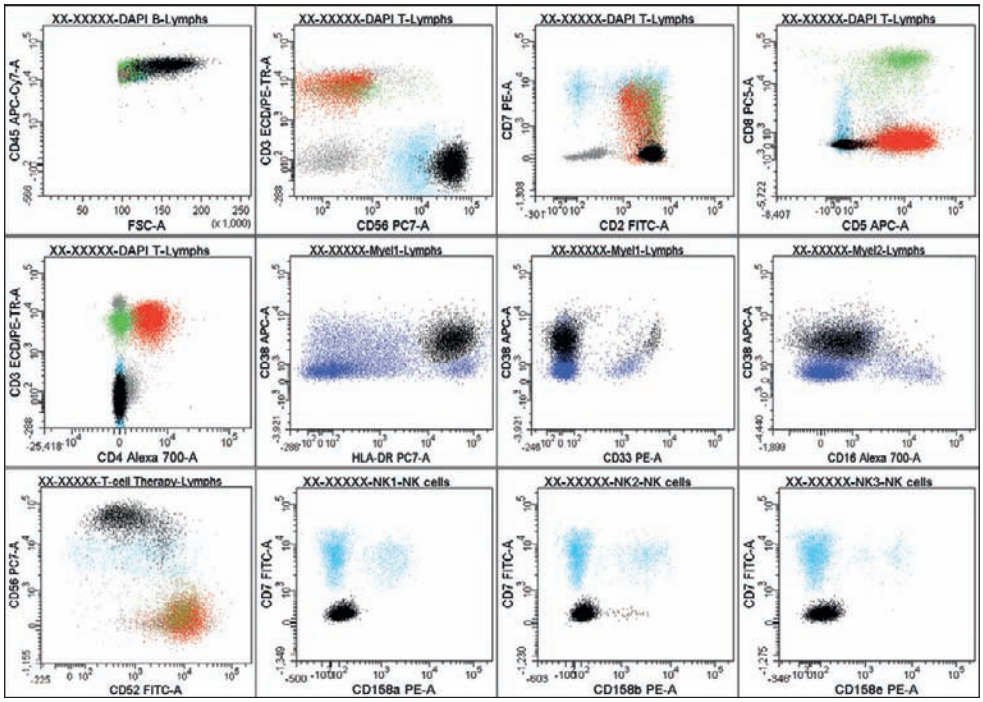


**FIGURE 23-15**

Aggressive natural killer cell leukemia. These nine-color flow cytometry data from a bone marrow aspirate show the black-colored neoplastic population to express normal CD2 and CD38, abnormally high-level CD56 and HLA-DR, and aberrant loss of CD7, CD8, and CD16. The neoplastic cells were negative for the three different KIR isoforms examined (CD158a, CD158b, and CD158e, bottom row of images), implying clonality. The increased forward scatter of these cells suggests larger size than residual normal natural killer cells (colored light blue). EBER1 in situ hybridization on the marrow biopsy confirmed that the tumor cells were positive for Epstein-Barr virus.

## AGGRESSIVE NATURAL KILLER CELL LEUKEMIA AND NASAL-TYPE EXTRANODAL NATURAL KILLER CELL LYMPHOMA

The clinical settings of aggressive NK-cell leukemia and nasal-type NK-cell lymphoma are different, but the immunophenotypes of the neoplastic cells are identical in these two aggressive malignancies. The neoplastic cells typically show an aberrant NK-cell immunophenotype, with the characteristic lack of surface CD3 expression and frequent loss of one or more NK-cell–associated antigens, including CD2, CD7, CD8, CD16, and CD56 (Figure 23-15). Analogous to TCR-β evaluation in T-cells, NK-cell clonality can be assessed by flow cytometric evaluation of the killer inhibitory receptor (KIR) antigens. These antigens are expressed in a mutually exclusive manner on NK cells, similar to the TCR genes in T-cells. In benign or polyclonal NK-cell populations, flow cytometric evaluation of the three KIRs p158a, p158b, and p158e/KIR p70 typically reveal expression of one of these antigens in 50% to 80% of the NK cells. Therefore restricted KIR expression, or a complete lack of KIR expression, suggests clonality among an NK-cell population. As with the flow cytometric identification of T cells, the flow cytometric identification of NK-cell clonality does not prove malignancy, and the flow cytometric findings must be interpreted in the overall context of the case, including evaluation for Epstein-Barr virus infection, before a diagnosis of NK-cell malignancy is made.

# MYELOID STEM CELL NEOPLASMS

## LOW-GRADE MYELODYSPLASTIC SYNDROMES AND CHRONIC-PHASE MYELOPROLIFERATIVE DISORDERS

Multiple studies have demonstrated that cases of myelodysplastic syndrome (MDS) and chronic-phase myeloproliferative disorders, including chronic myelomonocytic leukemia (CMML), frequently manifest abnormal antigen expression on the myeloid blasts or maturing granulocytes and monocytes. In clinical FC laboratories in which the patterns of antigen expression during normal myeloid maturation in the bone marrow (Figure 23-16, A) or benign regeneration of the myeloid series following chemotherapy or other injury to the marrow (see Figure 23-16, B) are well understood, abnormalities in these patterns can be used to support

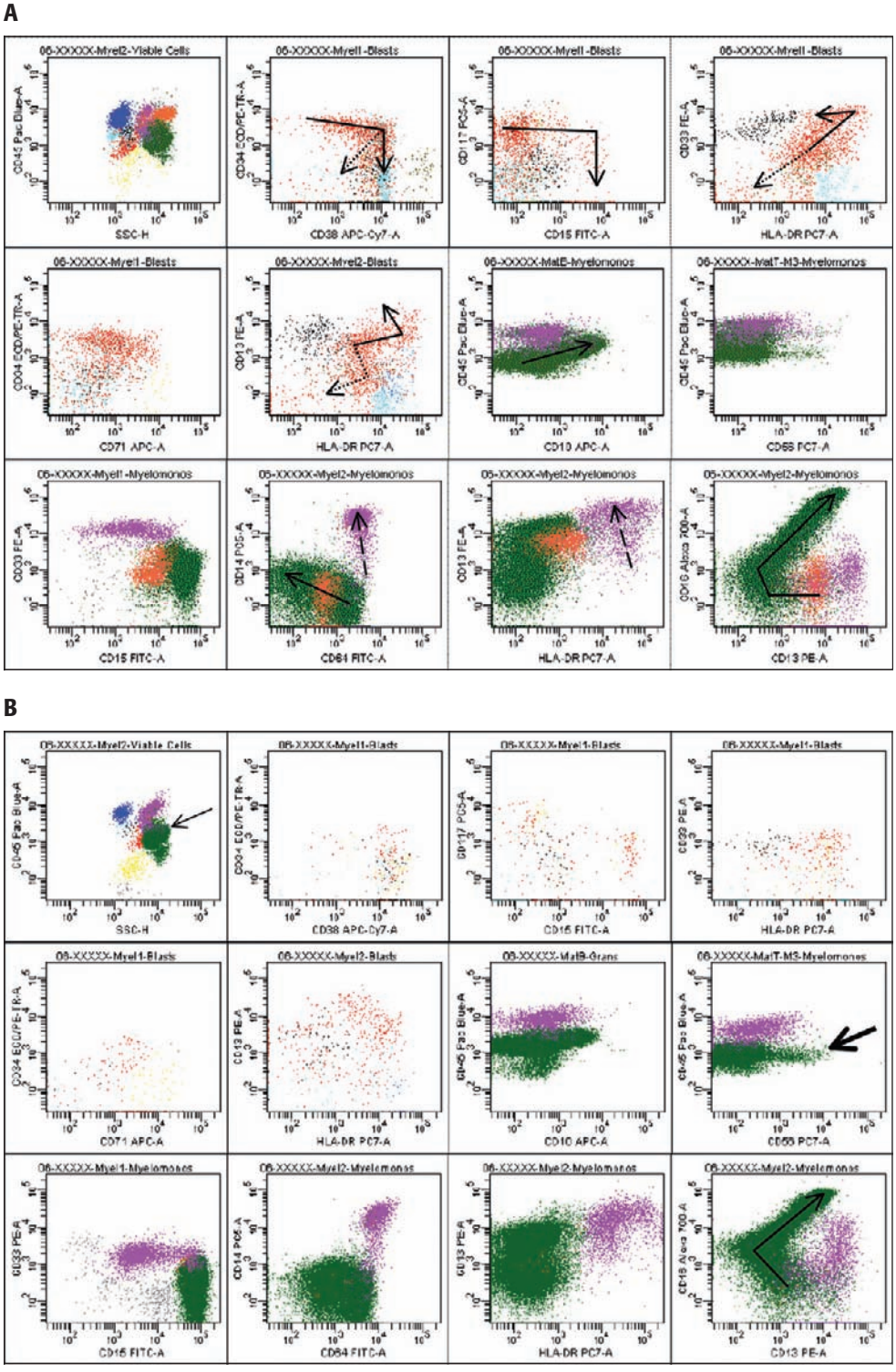


**FIGURE 23-16**

Normal maturation of myeloid lineage cells in the bone marrow **(A)** or benign abnormal maturation with myeloid series regeneration following cytotoxic chemotherapy **(B).** Both figures show nine-color FC data, with the myeloid blasts colored red, the neutrophil series cells colored green, the eosinophils colored orange, and the monocytes colored lavender. **A,** Among the myeloid blasts, the *solid arrows* denote normal maturational progression to neutrophil series cells or monocytes, and the *dashed lines* denote progression to erythroid precursors. Among the neutrophil and monocyte series, maturation proceeds in the direction of the *solid* and *dashed arrows,* respectively. These patterns are highly reproducible across normal bone marrow specimens. **B,** The *thin arrow* in the *upper left* dot-plot highlights abnormally homogeneous CD45 expression among regenerating neutrophil series cells. The *thick arrow* in the *right-middle* dot plot shows the low-level aberrant CD56 commonly seen among these cells. The *bent arrow* in the *right lower* dot-plot shows the characteristic dyssynchronous (i.e., right-angled) relationship between CD13 and CD16 expression.

the diagnosis of MDS or chronic-phase myeloproliferative disorders in patients suspected of having these diagnoses based on clinical and morphologic grounds. This additional immunophenotypic information can be particularly helpful in morphologically equivocal cases in which cytogenetic studies fail to detect a clonal karyotypic abnormality. In the examples shown in Figure 23-17, the constellation of antigenic abnormalities among the myeloid blasts and maturing granulocytes and monocytes are consistent with the diagnoses of MDS (refractory cytopenia with multilineage dysplasia by morphology) and CMML-1 with eosinophilia, respectively. Note that both cases did not show significantly expanded myeloid blast populations. Importantly, in all cases of MDS or MPD, clinical, morphologic, and cytogenetic–molecular correlation are required to render a specific WHO diagnosis.

## ACUTE MYELOID LEUKEMIA WITH THE t(8;21)(q22;q22)

In addition to a markedly expanded myeloid blast population by CD45 versus side-scatter gating, this acute myeloid leukemia (AML) (Figure 23-18) typically shows a relatively prominent population of maturing granulocytes and is considered a form of AML M2 under the French-American-British (FAB) classification. CD34 typically is expressed at a high level, often with aberrant CD15, which is normally a more mature myeloid antigen not expressed on blasts. The pan-myeloid antigen CD33 typically is expressed weakly, whereas HLA-DR and the myeloid-associated antigens CD13 and cytoplasmic myeloperoxidase generally are expressed at a high level. There is frequent aberrant expression of the B-cell antigen CD19 or the T/NK-cell antigen CD56, or both; the lymphoblast antigen TdT also may be aberrantly expressed.

## ACUTE MYELOID LEUKEMIA WITH THE t(15;17)(q24;q21)

Acute myeloid leukemia with the t(15;17)(q24;q21), also known as *acute promyelocytic leukemia* (APL; or AML M3 under the FAB classification), is a leukemic proliferation of promyelocytes, characteristically expressing CD13, CD33, CD117 (c-kit), and CD9, with loss of HLA-DR (Figure 23-19). Most cases lack CD34, but in the hypogranular form of APL (approximately 30% of cases), low-level CD34 on a minority of the leukemic cells is common. The more mature myeloid-associated antigen CD15 is expressed much more weakly on neoplastic promyelocytes than on normal promyelocytes, which is useful in distinguishing APL from markedly left-shifted benign marrow. Aberrant CD56 and CD2 expression are relatively common in APL.

## ACUTE MYELOID LEUKEMIA WITH inv(16)(p13;q22) OR t(16;16)(p13;q22)

This leukemia, which is AML M4eo under the FAB classification, typically shows a prominent $CD34^+$ myeloblast population, and evidence of both monocytic and granulocytic differentiation by CD45 versus SS gating. The myeloid blasts may show aberrant coexpression of CD15 or CD2 and, by evaluation of CD45 versus SS, generally form a maturational continuum with both the monocytes and granulocytes. The monocytic component shows relatively strong expression of CD33, CD64, and HLA-DR and acquisition of CD14 on the more mature forms. The neutrophilic component expresses relatively weak CD33 and CD64, without HLA-DR, and acquires both CD13 and CD16 with maturation. A variably expanded eosinophil population, with relatively bright CD45 and increased SS, but without the CD16 expression seen on neutrophils, is characteristic.

## ACUTE MYELOID LEUKEMIA, NOT OTHERWISE CATEGORIZED

FC can be helpful in subclassifying AMLs without recurrent cytogenetic abnormalities. For example, the diagnosis of AML, minimally differentiated under the WHO classification (AML M0 under the FAB classification), which by definition lacks myeloperoxidase reactivity by cytochemistry, depends on FC to establish myeloid lineage. In those occasional cases of acute myelomonocytic or acute monocytic–monoblastic leukemia showing little nonspecific esterase reactivity by cytochemistry, FC can confirm monocytic differentiation. Finally, FC identification of megakaryocyte-associated antigens, such as CD41, CD61, or both, on leukemic myeloid blasts provides important support for the diagnosis of acute megakaryoblastic leukemia (AML M7 under the FAB classification), and helps to distinguish it from AML M0.

## BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

This aggressive neoplasm, which almost always involves the skin and usually involves the bone marrow, was termed *blastic NK cell lymphoma* in the 2000 WHO classification, and hematodermic $CD4^+CD56^+$ neoplasm in the 2005 WHO-European Organisation for Research and Treatment of Cancer (EORTC) classification of cutaneous hematolymphoid neoplasms. It is currently believed that this neoplasm derives from precursors of plasmacytoid dendritic cells that usually show aberrant coexpression of CD4, CD43, CD56, CD123, and TCL1.

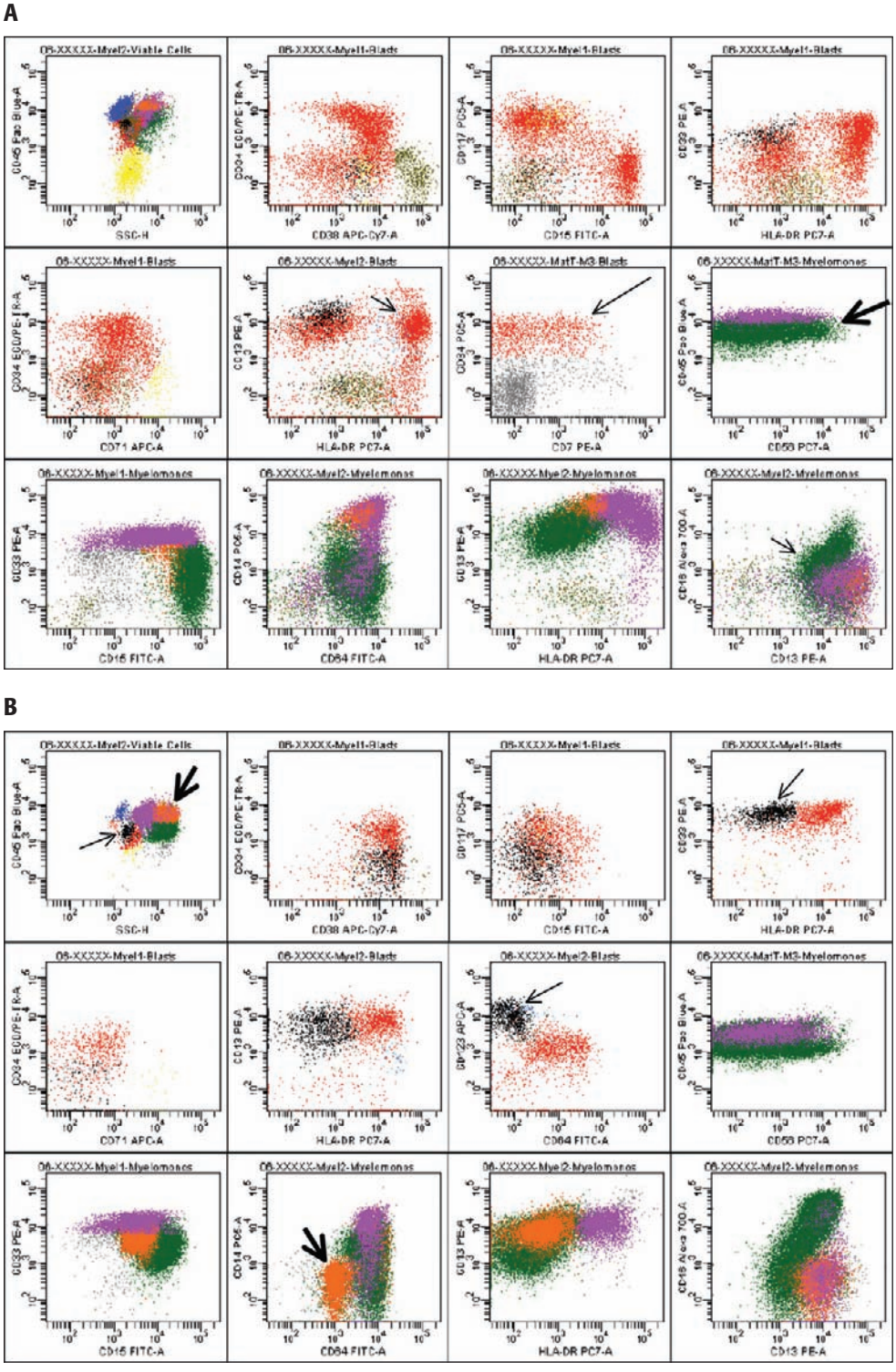


**FIGURE 23-17**

Low-grade myeloid stem cell neoplasms. **A** and **B,** Nine-color flow cytometry data from cases of refractory cytopenia with multilineage dysplasia (RCMD) and chronic myelomonocytic leukemia-1 with eosinophilia (CMML-1-eo), respectively. The cell lineages are colored as in Figure 23-16. Basophils are colored black, and nonlysed erythroid precursors are colored yellow. Immunophenotypic abnormalities in the RCMD case include abnormally increased CD13 on all the myeloid lineages (*short thin arrows* in the two dot-plots showing CD13 expression), aberrant CD7 expression *(long thin arrow)* and abnormally homogeneous overall antigen expression on the myeloid blasts, increased CD14 on the neutrophil series, and aberrant low-level CD56 on both the maturing granulocytes and monocytes *(thick arrow)*. In the CMML-1-eo case, the *thin arrow* identifies the mildly expanded basophils, and the *thick arrow* identifies the significantly expanded eosinophils. Immunophenotypic abnormalities in this case include abnormally increased CD13 on all the myeloid lineages, abnormally decreased CD34 and homogeneous overall antigen expression on the myeloid blasts, increased CD14 on the neutrophil series, and aberrant low-level CD56 on both the maturing granulocytes and monocytes.

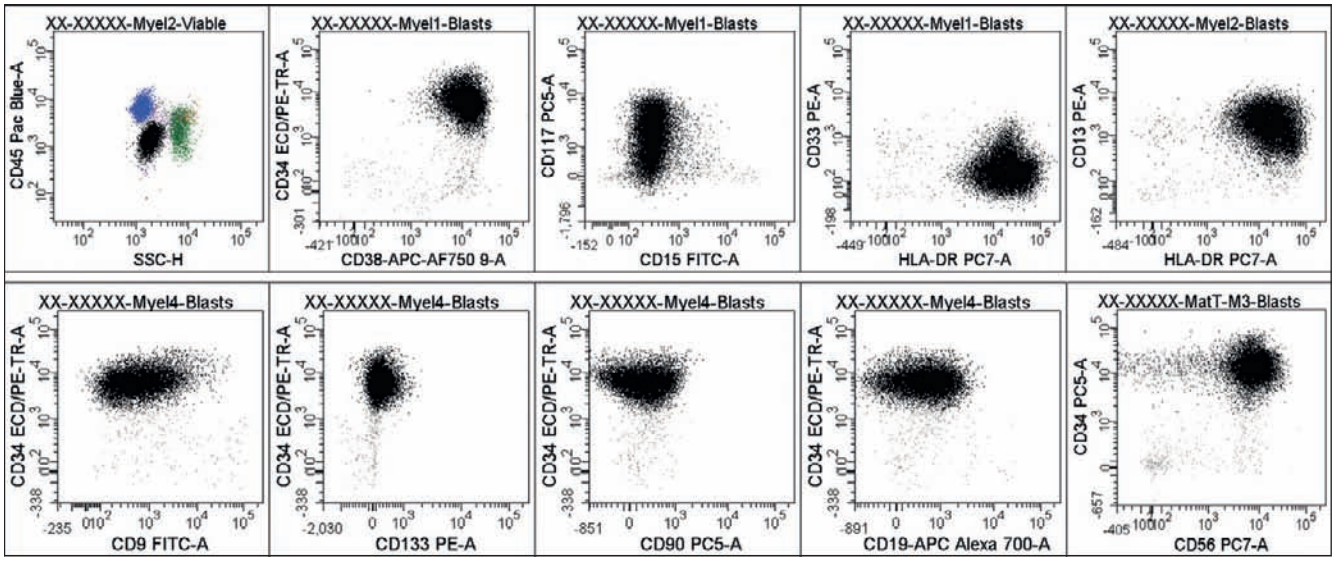


**FIGURE 23-18**

Acute myeloid leukemia with the t(8;21)(q22;q22). The nine-color flow cytometry data show many of the characteristic features of the leukemic blasts (colored black), including abnormally increased CD34 and decreased CD33 expression, aberrant low-level CD19, and aberrant CD56 (unusually high level in this case).

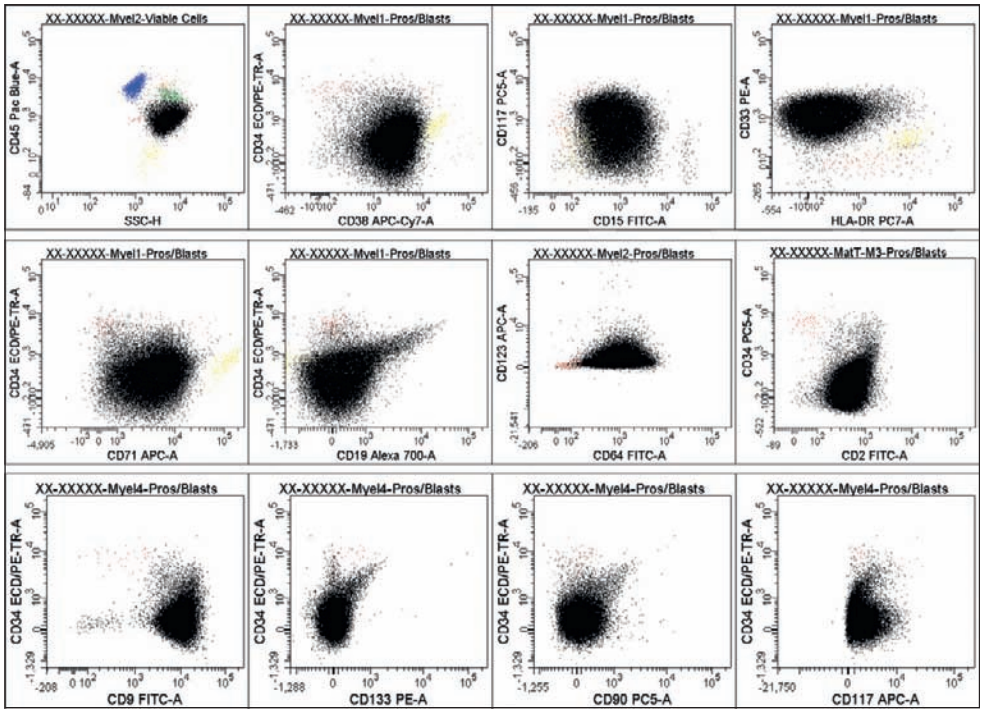


**FIGURE 23-19**

Acute myeloid leukemia with the t(15;17)(q24;q21). The nine-color flow cytometry data show the characteristic features of the leukemic promyelocytes (colored black), including minimal CD34 and human leukocyte antigen (HLA)-DR expression, abnormally low-level CD15 (compared to normal promyelocytes) with retention of CD117/c-kit and CD64, high-level CD9, and aberrant low-level CD2. As in most cases and in normal promyelocytes, the leukemic cells in this case also expressed low-level CD4.

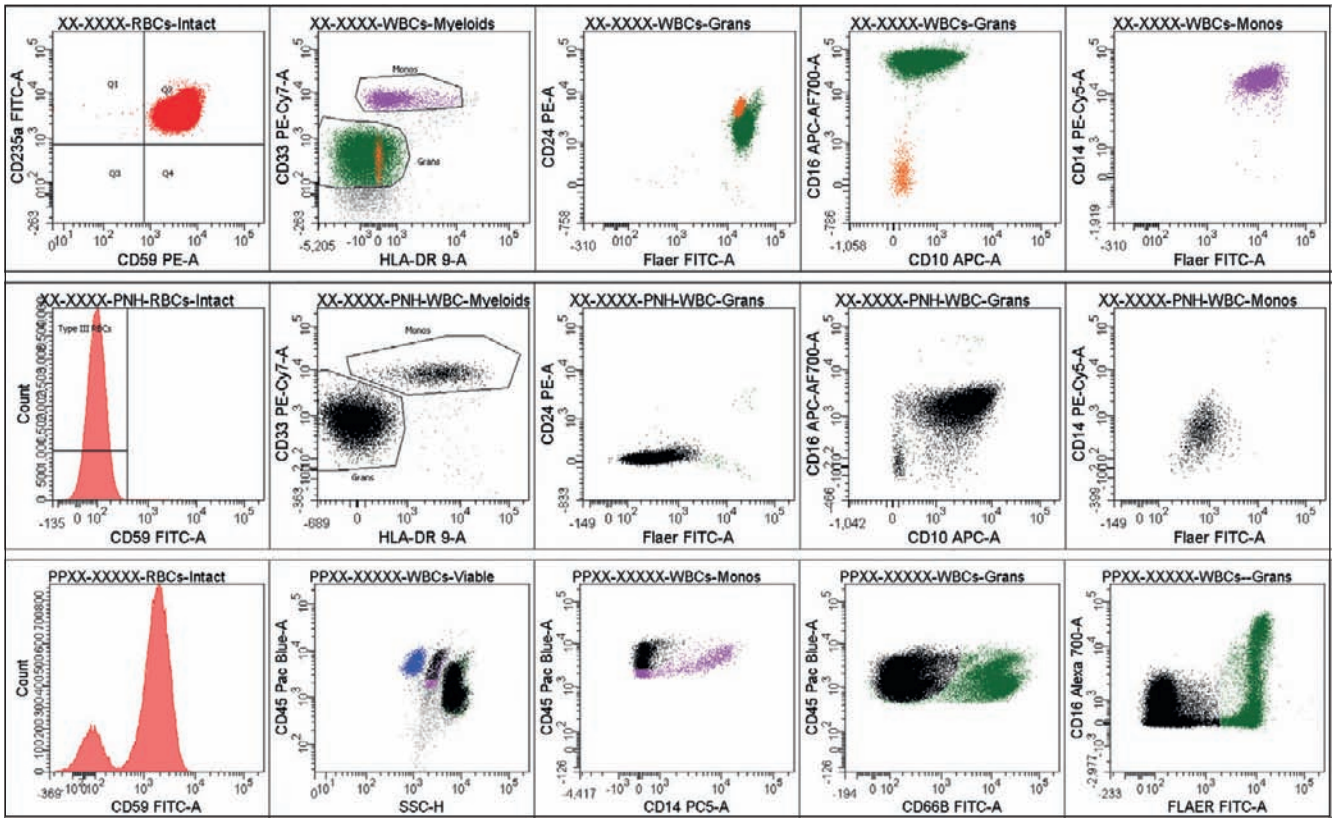


**FIGURE 23-20**

Evaluation for paroxysmal nocturnal hemoglobinuria (PNH). In these images, erythrocytes are red, neutrophil series cells green, eosinophils orange, and monocytes lavender. The *top row* depicts a normal peripheral blood specimen, negative for evidence of PNH. In the two-color erythrocyte evaluation, CD235a (glycophorin A) is a gating reagent used to limit the analysis to intact erythrocytes, whereas CD59 is the glycosyl-phosphatidylinositol (GPI)-linked protein evaluated on these cells. In the seven- or eight-color evaluations of granulocytes and monocytes, CD33 and HLA-DR are gating reagents used to restrict the analysis to intact cells of these two lineages, although an alternative approach is to use CD15 and side scatter to separate these lineages. Among the granulocytes, CD45, CD10, and side scatter can be used to separate neutrophils from eosinophils. Leukocyte GPI-linked proteins that we routinely evaluate include CD24, CD16, and CD66b on neutrophils and CD14 on monocytes. The FLAER reagent (fluorescently-labeled aerolysin, a bacterial toxin that binds to GPI linkages on all leukocytes) is included in all leukocyte PNH assays. *The middle row* shows peripheral blood from a patient with an extremely large PNH clone representing greater than 98% of the erythrocytes (CD59-negative "type III" cells), neutrophils (negative for FLAER and CD24, with the characteristically decreased but not absent CD16), and monocytes (negative for FLAER and CD14); the GPI-negative leukocytes are colored black in the images. The *bottom row* shows a bone marrow aspirate from a different patient with prolonged anemia, submitted to rule out myelodysplasia. The diagnosis of PNH was strongly suggested by the loss of CD59 on a significant minority of the erythrocytes and the prominently expanded mature (high-level $CD45^+$) neutrophil and monocyte populations with loss of CD16/CD66b/FLAER binding and CD14/FLAER binding, respectively (abnormal populations both colored black in the images). This case shows that PNH clones can be readily identified in bone marrow aspirates, but note that peripheral blood evaluation is required to assess baseline PNH clone size for the purpose of following the disease.

Antigens less commonly expressed include CD7, CD33, CD68, and TdT. CD34, CD117, and Epstein-Barr virus–associated antigens are not expressed.

## ACUTE LEUKEMIAS OF AMBIGUOUS LINEAGE

The classification of these rare acute leukemias was significantly revised between the 2000 and 2008 WHO systems. According to the 2008 WHO classification, acute undifferentiated leukemia lacks antigens specific for B-lymphoid origin (strong CD19 with strong CD10 or cytoplasmic CD79a or CD22, or weak CD19 with at least two of the following: strong CD10 or cytoplasmic CD79a or CD22), T-lymphoid origin (cytoplasmic or surface CD3), myeloid origin (cytoplasmic myeloperoxidase), or monocytic origin (at least two of the following: nonspecific esterase, CD11c, CD14, CD64, or lysozyme). Acute undifferentiated leukemia blasts often express HLA-DR, CD34, or CD38 and may express TdT. Mixed phenotype acute leukemia with the t(9;22)(q34;q11.2) is defined by the presence of a BCR-ABL1 rearrangement and coexpression of myeloid-associated antigens with either B-lymphoid antigens (most common) or T-lymphoid antigens (less common). Mixed-phenotype acute leukemia with the t(v;11q23) is defined by the presence of a chromosomal translocation involving the *MLL* gene and usually includes a $CD10^-$ B-lymphoblast population with frequent coexpression of CD15, and a separate population of leukemic myeloid cells, commonly with monocytic differentiation. In addition to the

latter two genetically defined acute leukemias, the 2008 WHO classification also includes mixed phenotype acute leukemia, not otherwise specified, of either B-myeloid or T-myeloid lineage. Rarely, the blasts in these ambiguous cases coexpress markers for all three lineages.

## PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

This clonal myeloid stem cell abnormality results from the loss of cell surface glycosyl-phosphatidylinositol (GPI)-linked proteins because of acquired mutation of the *pigA* gene, leading to abnormal susceptibility to complement-mediated lysis among erythrocytes and other features of the disease, including predisposition to thrombosis. The most common GPI-linked proteins assayed to identify affected cells include CD59 on erythrocytes, CD14 monocytes, and CD16, CD24, and CD66b on neutrophils. Because of the heterogeneity of CD14 and CD16 expression among immature monocytes and neutrophil lineage cells, respectively, evaluation for paroxysmal nocturnal hemoglobinuria (PNH) is best performed on peripheral blood, where only mature forms of these cell lineages are expected to occur. Over the past several years, a number of studies have shown that fluorescently labeled aerolysin protein from the bacterium *Pseudomonas aeruginosa* (FLAER) is a sensitive and specific reagent for detecting GPI-linked proteins on leukocytes (but not erythrocytes), such that PNH monocytes and granulocytes show abnormally decreased ability to bind FLAER. Serial evaluation of the size of the PNH monocyte and neutrophil populations in the peripheral blood is used to provide surrogate information about marrow involvement by the PNH clone. Evaluation of $CD59^-$ erythrocyte populations is less useful for this purpose, because affected erythrocytes are more sensitive to complement-mediated lysis than are leukocytes; therefore the size of the $CD59^-$ erythrocyte population in the blood typically underestimates the size of the PNH clone in the bone marrow. Flow cytometric evaluation of GPI-linked proteins in peripheral blood from a normal individual, and in blood and bone marrow from two different PNH patients, is shown in Figure 23-20. It is important to remember that detection of a PNH-type clone is not diagnostic of clinical PNH, and small PNH clones can be seen in settings such as myelodysplasia and other bone marrow failure disorders such as aplastic anemia. Indeed PNH-type clones can be detected in normal individuals (on the order of 1 in $10^6$ cells when extremely sensitive assays are used). A sensitivity of 1% is sufficient for most clinical purposes, although high-sensitivity assays with the ability to detect 1 in $10^4$ cells do exist, particularly in research settings. Suggested guidelines for performing PNH assays by flow cytometry have been published and may serve to help standardize testing in clinical laboratories.

## CONCLUSION

The central importance of immunophenotyping in the current diagnosis and classification of hematolymphoid diseases makes an understanding of antigen expression patterns in benign and neoplastic settings imperative for any pathologist who diagnoses these diseases and for any hematologist or oncologist who treats these diseases. An understanding of both the advantages and the potential pitfalls of FC evaluation is likewise important for all physicians involved in the diagnosis and treatment of these diseases. Although FC is used primarily in diagnosis and disease monitoring, it will assume greater importance in therapeutic decision-making as more potential therapeutic targets are identified by basic research on hematopoietic disease.

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The complete reference list is available online at www.expertconsult.com.

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24

# Molecular Diagnosis in Hematopathology

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## INTRODUCTION

Molecular investigation of the hematopoietic neoplasms has contributed significantly to the current understanding of the genetic basis of cancer, and progress in this dynamic field continues unabated. In many regards, hematolymphoid malignancies represent an ideal model for these biologic studies, because the candidate cells are readily extracted from the patient and can be manipulated in the laboratory to produce viable single-cell preparations for the purposes of immunophenotypic and genetic characterization. Accordingly, a large body of knowledge concerning recurrent genetic abnormalities in leukemias and lymphomas has been accumulated, leading to the integral incorporation of tumor genetics in the current World Health Organization (WHO) classification of the hematopoietic and lymphoid tumors. Importantly, this new taxonomy brings together elements of morphology, phenotype, and genotype, underscoring the vital contribution of molecular genetic investigations to the diagnosis, subclassification, and prognosis of these diverse neoplasms. The role of classical cytogenetics in this process has been invaluable; however, the advent of sophisticated molecular techniques has provided certain advantages over standard karyotyping, including rapidity, the ability to use a variety of tissue preparations (e.g., fresh cells or fixed, paraffin-embedded material), and enhanced detection sensitivity. Nevertheless, cytogenetic evaluation is often a critical component of hematopoietic tumor diagnosis and remains complementary to the current and developing collection of molecular tools.

The detection of these genetic aberrancies by molecular diagnostic methods should ideally be accurate, precise, and rapid, in order for results to be incorporated in synchrony with clinical and pathologic data. The diagnostician must appreciate the basic molecular pathobiology of these abnormalities, the clinical purpose of detection (e.g., diagnosis versus residual disease monitoring), and be familiar with the advantages and limitations of various molecular methods. This chapter summarizes the key technical considerations, basic molecular biology, specific tumor associations, and application of molecular diagnostic approaches to the classification, prognosis, and monitoring of hematolymphoid cancers. Beyond the setting of neoplastic hematopathology, molecular investigations currently constitute a substantial and growing component of diagnostic procedures performed in laboratories focused on hemostasis–thrombosis, red blood cell (RBC) disorders, and hemoglobinopathies. Although coagulation system–related applications are beyond the scope of this chapter, a brief summary of molecular diagnostics in the evaluation of non-neoplastic erythrocyte and hemoglobin abnormalities is provided.

## COMMON METHODS USED IN THE MOLECULAR DIAGNOSIS OF HEMATOLYMPHOID NEOPLASMS

### STANDARD POLYMERASE CHAIN REACTION TECHNIQUES AND SAMPLE CONSIDERATIONS

The ubiquitous technique of polymerase chain reaction (PCR) is used as a standalone analytic procedure or as one component of more complex methodologic assays in the majority of molecular diagnostic laboratory operations. PCR using oligonucleotide primers can be performed using genomic DNA or messenger RNA transcripts as templates, in the latter case requiring a reverse transcription step to generate complementary DNA (cDNA) prior to amplification (i.e., reverse transcriptase [RT] PCR). A myriad of PCR variations have been developed (e.g., allele-specific, asymmetric, use of modified oligonucleotides) to enhance analytic specificity according to particular assay requirements. Detection of PCR-amplified products can be achieved by a number of methods, including gel electrophoresis,

or capillary electrophoresis. In the latter approach, PCR primers are typically covalently labeled with a fluorescent dye moiety to permit highly accurate fragment length determination following laser excitation of the capillary gel matrix. Routine PCR methods are of great utility in hematopathology, including the determination of B- or T-cell monoclonality, and the detection of chromosomal translocations characteristic of many leukemias and lymphomas. Translocation events can either produce a novel chimeric gene, which is subsequently transcribed as a unique fusion messenger RNA (mRNA) species (this is typical of the acute leukemias), or result in aberrant overexpression of a highly regulated proto-oncogene because of its juxtaposition with an unrelated region of DNA containing a strong transcriptional promoter or enhancer (this is most often seen in the lymphomas). Other types of genetic rearrangements (e.g., small insertion and deletion events, point mutation changes in genes) can be detected by standard PCR methods, along with additional post-PCR procedures such as fluorescent capillary electrophoresis, restriction enzyme digestion, oligonucleotide probe hybridization, or high-resolution melting curve analysis using DNA intercalating dyes. Downstream applications of automated, fluorescent DNA sequencing by the Sanger method, or alternatively by pyrosequencing, also rely on high-quality PCR amplification products. Finally, other non-PCR or isothermal amplification techniques have been used in the molecular diagnostic setting, including ligation-mediated amplification and nucleic acid sequence based amplification, but these alternatives occupy a relatively small niche in relation to the abundance of PCR-based methods.

A variety of tissue or sample sources can be used for the molecular genetic investigation of leukemia and lymphoma, including fresh or frozen tissues and cells and fixed paraffin-embedded tissue material. Blood and bone marrow specimens should be obtained in anticoagulant (e.g., ethylenediamine tetraacetic acid or heparin) to ensure that the leukocytic component is adequately available for DNA or RNA extraction. Paraffin-embedded tissue blocks are a common source of material for DNA PCR assays, although fixed tissues are more prone to nucleic acid degradation. While technically more difficult, RNA can also be obtained from paraffin tissue; however, the substrate quality is less optimal compared with RNA from fresh or cryopreserved cells. Analyzable DNA (and sometimes RNA) can also be reliably obtained from air-dried, glass slide smears or touch preparations of blood, marrow, or tissue. In this case, unstained slides are preferred, because commonly used histologic and hematologic dyes can adversely inhibit successful PCR. Regardless of tissue source, the molecular diagnostic laboratory must have adequate procedures to ensure the quality and integrity of each individual sample. Such measures include assessing the amount, purity, and adequacy of amplification of the obtained nucleic acids. RNA samples are particularly labile and subject to rapid degradation by RNAses. Furthermore, given the sensitive nature of PCR technique, isolation of nucleic acid preparations must be done carefully to avoid sample cross-contamination. Rigorous separation of pre-PCR (nucleic acid isolation) from post-PCR (analysis) areas of the laboratory is also important to prevent preanalytic sample contamination arising from amplified PCR products. Therefore standard amplification controls used in all PCR assays should include: relevant positive and negative nucleic acid controls to ensure specificity, an internal control (i.e., a housekeeping gene) of sufficient fragment size to ensure integrity of the RNA or DNA within the expected amplicon size range, and a "no template" control to assess for possible contamination.

## REAL-TIME QUANTITATIVE PCR METHODOLOGY AND GENERAL ASPECTS OF MINIMAL RESIDUAL DISEASE DETECTION

Specialized real-time quantitative PCR (RQ-PCR) platforms have also become invaluable in the molecular diagnostic evaluation of several hematolymphoid neoplasms. RQ-PCR is performed in an automated, multiple-sample, closed-system format in which the initial amount of a specific nucleic acid target can be quantified with exceptional precision. Commercially available RQ-PCR instruments are based on two major technologies: the 5′ fluorescent nuclease assay, also known as *Taqman* (Applied Biosystems, Foster City, Calif.), and the dual fluorescent probe hybridization assay based on the concept of fluorescence resonance energy transfer (Roche LightCycler; Roche Molecular Diagnostics, Indianapolis, Ind.). A summary of these competing RQ-PCR methods is illustrated schematically in Figure 24-1. Regardless of the technologic approach, the ability of each platform to deliver highly reproducible quantitative PCR results is highly similar. Although standard end-point PCR methods can be designed to identify the presence of very small amounts of a target nucleic acid, the plateau phase of PCR product detection (e.g., by gel electrophoresis) often does not reliably reflect the starting concentration of the species of interest, mainly because of irregular late cycle amplification effects on PCR efficiency. In contrast, measurement of the initial quantity of a specific mRNA or DNA species by RQ-PCR is possible by relying on the rapid detection of PCR product during the early exponential phase of PCR (see Figure 24-1). The initial appearance of a fluorescent signal above background at the so-called threshold cycle (Ct) during this phase of PCR is directly and quantitatively related to the starting amount of the target DNA or RNA in the sample of interest. A standard curve prepared by plotting Ct numbers against serial 10-fold

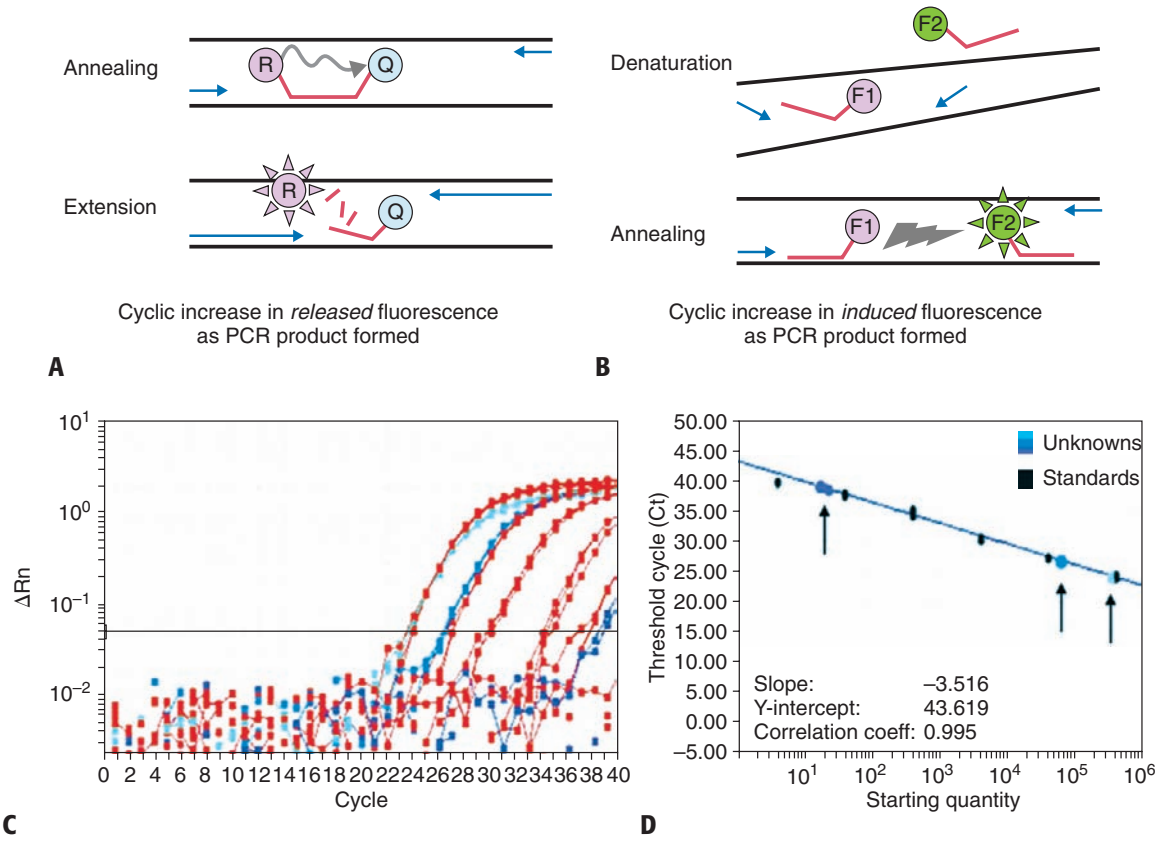



**FIGURE 24-1**

Basic principles of automated real time quantitative polymerase chain reaction analysis (RQ-PCR). **A,** A representation of the fluorescent 5′ nuclease or TaqMan RQ-PCR approach (Applied Biosystems, Foster City, Calif.). In this methodology, a fluorescently labeled oligonucleotide probe is designed to bind specifically to a target region of DNA. The target region is flanked by PCR primers *(blue arrows)* that bind at a slightly lower annealing temperature (typically 5° to 10° C) than the probe. In the intact state, fluorescence emission excited from the reporter dye *(R)* is quenched by the adjacent quencher *(Q)* conjugate. During the primer extension step in the PCR cycle, the 5′-3′ nuclease activity of Taq DNA polymerase degrades the probe molecule on this strand, resulting in an increase in reporter fluorescence. As more PCR product is made with each cycle, more fluorescence emission is produced and can be measured precisely. The amount of initial detectable fluorescence above background in the early exponential phase of the PCR is quantitatively related to the starting amount of target RNA or DNA. **B,** A different technology based on dual hybridization probes (LightCycler; Roche Diagnostics, Indianapolis, Ind.). In this procedure, two singly labeled fluorescent probes are directed to a specific, nonoverlapping target region of DNA. When bound to the target and in spatial proximity, one fluorophore *(F1)* is able to excite the second fluorochrome *(F2)* by the physical process of fluorescence resonance energy transfer. The second dye emits fluorescence at a longer wavelength, which again can be sensitively measured. In this case, the detection interval for maximal fluorescent emission can be expected to occur at the annealing step of a PCR cycle, and the increase in fluorescence is also monitored over the course of the PCR and detected during the exponential phase. Both TaqMan and LightCycler automated platforms accomplish similar goals in quantitative PCR analysis and both achieve comparable levels of analytic sensitivity in various applications. **C** and **D,** Typical data obtained with these technologies (results from TaqMan type analysis are shown). **C,** A series of dilution standards of a cloned BCR-ABL1 template and three patient samples with different concentrations of BCR-ABL1 cDNA (following reverse transcription of RNA). The *y* axis represents relative fluorescence intensity changes (above baseline), and the *x* axis indicates PCR cycle number. The *black horizontal line* represents a user-defined threshold to identify early exponential phase fluorescence sample emission. Each dilution *(red traces)* can be visualized as sufficient PCR product is formed and the shape of each plot indicates the efficiency of amplification for each sample. Patient sample traces are indicated in shades of blue. Importantly, the threshold cycle (Ct) or crossing point defines the earliest time at which fluorescent PCR product is detected in the exponential phase. This value is quantitatively related to the amount of target nucleic acid. Each standard and test sample is analyzed in triplicate with remarkable reproducibility. This feature underscores the extremely high precision and usually low interrun variance observed with well-constructed automated RQ-PCR experiments. By plotting the obtained Ct values (cycle number) against the known log dilutions of the standards, a linear relationship is derived as shown by the black dots in **D.** The unknown samples are indicated by the corresponding color dots *(vertical arrows),* which lie along the standard curve line. The amounts of BCR-ABL1 transcript in each unknown can then be determined directly from this standard curve with high accuracy and precision over a wide dynamic range (5 to 6 logs). RQ-PCR performance can be affected by sample and experimental conditions, including nucleic acid degradation, or in the case of RNA templates, poor reverse transcription quality before the RQ-PCR amplification. Also critical to analysis, the expression value of the target nucleic acid is normalized to that of a housekeeping gene or transcript, in order to correct for variations in nucleic acid integrity, reverse transcription efficiency, or reagent concentrations.

dilutions of a given target molecule should in theory produce a linear relationship with a slope of –3.3 (assuming 100% PCR efficiency).

One method of quantifying a nucleic acid of interest in an unknown sample using RQ-PCR is to directly read the concentration from a standard curve generated from coamplified serial dilutions of a positive control template, thus deriving an absolute measurement of the species. The standard curve method still requires that a quantitative unit value for a particular target be normalized to that of an amplified, unrelated housekeeping gene or transcript, in order to compensate for sample and PCR condition variability. Alternatively, one can choose to measure the relative quantity of a specific PCR

product by comparison with a reference standard or "calibrator", again after normalizing both the unknown sample and calibrator amplification Ct results to an unrelated gene or transcript. The comparative approach assumes that the sample gene target and calibrator amplify with equal PCR efficiency as the normalizing control gene, in which case-relative changes in the normalized quantity between the unknown sample and calibrator can be reproducibly measured. The comparative method is also known as the $2^{-\Delta\Delta Ct}$ method reflecting its mathematical derivation. If carefully validated, the requirement for a formal standard curve with each experiment can be avoided, although the quantitative results are expressed as a fold or percentage change relative to the calibrator, rather than a discrete quantitative number. Current RQ-PCR methods can routinely resolve target quantities across a 5- to 6-log dynamic range of detection sensitivity. Notably, for RNA analytes, a reverse transcription step is performed before the actual RQ-PCR; the reverse transcription reaction is assumed to be close to 100% efficient, such that the subsequent RQ-PCR accurately reflects the mRNA target abundance in the sample. Poor reverse transcription efficiency can thus adversely affect the reliability of RQ-PCR data. Additional considerations for successful and robust RQ-PCR assays include the appropriate choice of standards (e.g., serial dilutions of positive control cell line DNA, RNA, or cloned template of a particular genetic target), adequate controls (negative and no-template samples) and selection of the normalizing or housekeeping genes. Normalization controls for RNA-based RQ-PCR are typically selected to possess relatively stable gene expression levels in both tumor cells and background cells, to have degradation characteristics similar to the target mRNA, and to show minimal tendency for significant deviation between clinical subjects. If adequately constructed, RQ-PCR analyses can demonstrate remarkable reproducibility, in addition to high analytical sensitivity. In addition to obvious applications in post-therapy minimal residual disease (MRD) detection, RQ-PCR can also be used to identify and quantify single base changes in nucleic acids for allelotyping assays.

The sensitive measurement of MRD using RQ-PCR is an integral component in the management of several hematologic cancers. The rationale for MRD evaluation is to provide more definitive prognostic or predictive information for individual patients in order to confirm treatment success, or conversely determine the need for additional intervention. Beyond the concerns inherent in developing high-quality technical RQ-PCR assays, a variety of other issues are germane to MRD determination, such as the sample source (i.e., blood versus bone marrow), the frequency of monitoring required, and the biologic characteristics of the hematolymphoid tumor being evaluated. In general, serial MRD assessments at key time intervals after treatment are most likely to provide the best predictive data on biologic tumor behavior in a given patient, rather than single measurements at only one or two time-points. Whereas elimination of molecular residual disease (i.e., PCR negativity) is a desirable major goal of treatment (e.g., acute promyelocytic leukemia), it is also well known that some long-term leukemia survivors (e.g., patients with *BCR-ABL1* chronic myeloid leukemia or *RUNX1-RUNX1T1* acute myeloid leukemia) still harbor detectable molecular MRD, albeit at low levels. Because it is not possible to definitively predict which patients have low-level MRD consisting of dormant residual tumor cells versus those with minor tumor populations capable of reconstituting overt disease relapse, any low-abundance PCR-positive result must be considered as potentially significant. The ability of RQ-PCR technology to reproducibly assess temporal disease level fluctuations in individual patients is therefore of obvious benefit in this situation.

The detection of rare events by sensitive RQ-PCR methods is subject to a variety of additional difficulties. At the analytical limits of assay detection, it is possible to encounter stochastic effects such that a sample may test positive in one reaction aliquot, but negative in a duplicate one or in a later sample from the same patient, despite the same minimal level of disease in each case. It is important that laboratories performing molecular MRD detection establish consistent definitions of both the maximal limit of reproducibility and limit of target detection for each quantitative assay. Outlying or spurious results can then be better interpreted in light of these precision standards. Of note, several leukemia-associated fusion gene transcripts (such as the *BCR-ABL1* abnormality) have been recently described at very low levels, but with relatively high prevalence in the blood of healthy individuals. These data are consistent with the hypothesis that an abnormal gene fusion is necessary, but likely insufficient on its own for the production of overt leukemia. Although these intriguing findings would appear to potentially complicate the interpretation of RQ-PCR MRD analyses in leukemia patients, the detection of such low abundance fusion transcripts in normal subjects has required the use of modified PCR methods with extended sensitivities, typically one or two orders of magnitude greater than the maximal limits obtained by current RQ-PCR assays (i.e., $10^{-5}$ to $10^{-6}$). Therefore the chance of identifying a confounding positive PCR result arising from possible rare bystander cells should be negligible in a well-designed RQ-PCR assay.

## SOUTHERN BLOT HYBRIDIZATION

Southern blot hybridization (SBH) technique retains a role in the molecular diagnostic laboratory for the analysis of relatively large (kilobase) scale alterations in genomic DNA. SBH involves digestion of high-molecular-weight DNA with site-specific restriction

endonucleases, followed by size separation of the DNA by gel electrophoresis and transfer of the nucleic acid fragments onto a nylon or nitrocellulose membrane. A particular region of immobilized DNA can then be interrogated by stringent hybridization with a specific double-stranded genomic probe, which has been labeled with a radioisotope (e.g., $P^{32}$) or by a nonisotopic reagent (e.g., for chemiluminescent detection). Exposure of the resultant band pattern on radiographic film reveals the presence of any atypical rearrangements at the genetic locus in question, relative to the expected or germline (unaltered) band configuration. SBH has been most widely applied to detect clonotypic rearrangements of the antigen receptor genes in abnormal B- or T-cell lymphoid proliferations, but assays to evaluate specific oncogenes (e.g., *MLL, BCL2, CCND1/BCL1*) have also been described. Visual interpretation of exposed blots is usually straightforward; however, occasional difficulties can arise because of degraded DNA quality or incomplete enzymatic digestion of DNA or when novel pathologic bands comigrate with germline fragments. The multistep, technically demanding requirements of SBH also increase the possibility of analytic delays, because errors in procedure are seldom identified before the blot is finally exposed. Of note, sample sources for SBH analysis are limited to fresh or frozen cells, because of the requirement for high-quality, long-strand genomic DNA. For these reasons and with the advent of more comprehensive fluorescence in situ hybridization (FISH) or PCR-based methods, SBH is less frequently performed in molecular hematopathology laboratories.

## FLUORESCENCE IN SITU HYBRIDIZATION

Conventional cytogenetic analysis remains the standard method to identify numerical and structural chromosomal aberrations in tumors. However, the limited resolution of chromosome-specific banding obtained by Giemsa techniques (GTG- or G-banding) makes the recognition and interpretation of masked or cryptic chromosome aberrations difficult to ascertain and therefore potentially inaccurate. During the past 2 decades several techniques have been developed—FISH, multiplex FISH, spectral karyotyping, and array comparative genomic hybridization (A-CGH)—combining traditional cytogenetic methods and modern molecular genetics in the discipline of molecular cytogenetics. The use of these various techniques enhances the resolution of both numerical and structural chromosomal aberrations (especially those that are complex or subtle), bridging the gap between conventional chromosomal band analysis and molecular genetic studies.

FISH essentially involves the base-pairing of fluorescently labeled nucleic acid probes to complementary DNA sequences in tissue or cell preparations, followed by direct visualization of probe-specific, intranuclear signals using fluorescence microscopy. The probes used are either primarily labeled with a fluorochrome, or are alternatively conjugated to a carrier molecule (e.g., biotin or digoxigenin), which can subsequently bind avidly to a secondary fluorescent molecule (e.g., fluorochrome-labeled antibody or streptavidin). A large number of different probes designed to identify specific chromosomes and parts thereof are commercially available for diagnostic purposes, with the choice of probe dependent upon the particular application in question. Therefore FISH provides a targeted approach for revealing pathologically or prognostically relevant genetic alterations in a particular type of neoplasm. The most commonly used probes in FISH analysis of hematologic malignancies are repetitive sequence probes, locus-specific probes, and whole chromosome paints.

Repetitive sequence probes target chromosome-specific satellite sequences of pericentromeric heterochromatin (i.e., centromere-specific probes) or unique DNA sequences at the ends of all chromosomes (i.e., subtelomeric probes). These probes are useful when numerical aberrations carry information of diagnostic or prognostic relevance, as in subgroups of childhood acute lymphoblastic leukemia or the myelodysplastic syndromes. Subtelomeric probes are helpful for the improved detection of terminal translocations in metaphases that escape the resolution level of karyotypic G-banding.

Locus-specific probes target sequences normally present as only one copy in the haploid genome. Because these probes target single-copy DNA sequences, probe size needs to be considered. First, the larger the target sequence, the more efficient the hybridization. Second, the signals need to be large enough (at least 30 Kb) to be detected by routine fluorescence microscopy. Single-copy probes cloned in cosmid, yeast artificial chromosome, P1-derived artificial chromosome, or bacterial artificial chromosome vectors all give reliable and reproducible hybridization results. The main utility of locus-specific probes in hematologic malignancies is the detection of deletions, inversions, and translocations that are often disease specific. Two-color FISH experiments are performed routinely for this purpose, and commercial probes for most specific chromosomal rearrangements are readily available. Locus-specific FISH technique is highly versatile, and many different probe strategies can be designed to optimize the detection of an abnormality, depending on the clinical application. Variations such as extra-signal FISH, or dual-color–dual-fusion FISH (D-FISH) translocation probe methods have been developed to increase the sensitivity and specificity of FISH detection. Another strategy of particular utility is use of so-called break-apart probes (BAPs) to target genes with multiple possible translocation partners (e.g., the *MLL* gene). In this setting, a typical dual-color–dual-fusion FISH assay directed at one particular translocation would miss a variant translocation. A BAP, however, can detect the presence of a

translocation involving a given gene locus independent of the specific partner gene, although the nature of the translocated partner would not be known without additional investigations.

Finally, whole chromosome paints represent a cocktail of DNA fragments targeting nearly all the nonrepetitive sequences in the entire chromosome. Because they cover such a large region of DNA, these multiple probes would yield more diffuse signals in interphase nuclei; therefore their application is primarily restricted to metaphase spreads for resolving complex structural alterations. Small intrachromosomal changes such as deletions, duplications, or inversions may remain undetectable with this approach. Whole chromosome paints form the basis for advanced applications such as multiplex FISH and spectral karyotyping.

Karyotype analysis in hematologic malignancies is often hampered by poor chromosome morphology or a low number of requisite tumor metaphases. Despite improvements in high-resolution banding and culture methods to detect chromosomally abnormal cells, many hematologic tumors are still uninformative when evaluated by conventional chromosome analysis. One of the greatest advances in molecular cytogenetics facilitated by FISH has been the ability to use intact, nondividing cells as DNA targets. Consequently, this feature enables the analysis of a larger number of cells and has considerable added advantages for assessing hematologic malignancies in which the proliferative activity is low, or when the mitotic cells are reactive in nature and do not represent the neoplastic clone. In several aspects, FISH protocols are relatively straightforward and analogous to those of tissue immunohistochemistry, the latter being widely applied in surgical pathology laboratories. However, FISH provides quantitative data and is therefore amenable to more objective interpretation. The development of new image acquisition devices in parallel with the development of highly sophisticated computer algorithms has made the automation of FISH signal analysis feasible. Direct visual evaluation of fluorescence signals is tedious, time-consuming, and traditionally limited to a 200-cell count minimum. Automation increases efficiency and improves the sensitivity of interphase FISH analysis by permitting evaluation of a larger number of cells. Despite these advantageous technical attributes, one major drawback of FISH is that long-term storage of assay slides is not currently possible. Even with proper freezer storage and avoidance of exposure to light, fluorescent hybridization signals are subject to fading over several months. Currently, permanent records can only be established if chromogenic (rather than fluorescent) in situ hybridization detection methods are used, or through storage of FISH results through digital capture and imaging.

Because culture for mitotic phase cells is not a necessity, FISH is applicable to a variety of specimen types, including fresh or frozen tissue, cytologic preparations (e.g., fine-needle aspiration), air-dried unstained slides, and formalin-fixed paraffin-embedded tissues. For nonarchival samples, a disaggregated cell preparation is initially made and fixed in methanol acetic acid solution. These cell pellets can be stored indefinitely at –20°C for future use. FISH on archival material is more technically challenging and can be performed using either thin (4 to 6 μm) paraffin sections or intact nuclei extracted from thick (50 μm) sections, such as those prepared for DNA flow cytometric analysis. Thin-section technique is relatively simple, uses less tissue, and preserves architecture (e.g., for correlation with hematoxylin and eosin–stained slides); however, overlapping or truncation of cells can interfere with accurate scoring of nuclei. The isolation of individual nuclei from thick sections has helped to circumvent some of these problems. However, the thick-section approach requires a larger amount of tissue, is time consuming, and is laborious. In addition, FISH results cannot be correlated easily with the area of interest on hematoxylin and eosin–stained slides. Recent modifications using tissue cores from paraffin blocks combine the best aspects of the above-mentioned thick and thin section techniques. Tissue cores use minimal material and can be directed to the area of interest while producing individual nuclei in which FISH signals are bright, planar, and easy to score. In any FISH assay, nuclear DNA is globally stained with a dim fluorescent dye, such as 4′,6-diamidino-2-phenylindole, to permit localization of the nuclei by microscopy. The concept of experimental controls in FISH analyses essentially involves determination of the false-positive rate. False-negative results (no observed signals) should be obvious and will relate most often to technical errors (e.g., probe not added or poor probe quality). Laboratories performing FISH testing must determine the false-positive rate for each assay individually and in turn, for each type of tissue or specimen used in that assay, based on a sufficiently large number of control samples lacking the genetic abnormality of interest. This validation is required because signals from partially overlapping nuclei can lead to erroneously high pseudo-positive result scores for fusion probe sets. The laboratory must establish the overall percentage of such signals in a series of true-negative samples, and the upper limit for false positivity of an assay is usually expressed as the average plus three standard deviations. A rigorous application and understanding of these criteria is important to avoid overcalling of FISH data, which could lead to serious errors in patient diagnosis and clinical management. Finally, the individual interpreting the FISH results must be aware of the occurrence and significance of possible variant signal patterns; one such example when using a dual color–dual fusion probe set is the deletion of a genetic locus on one derivative chromosome in a chromosomal translocation, giving rise to only a single probe fusion event, rather than the expected positive pattern of two fusion signals.

## ASSAY SENSITIVITY AND SPECIFICITY IN MOLECULAR DIAGNOSTICS

Clinical application of laboratory methods to detect chromosomal or genetic aberrations in the hematolymphoid disorders requires a clear understanding of assay sensitivity and specificity, in order to determine the relative utility and limitations of various seemingly complementary techniques in routine practice. Most commonly, reference is made to the concept of analytic sensitivity. This measurement is important and is defined as the ability of a given assay to detect the lowest amount or concentration of a substance when it is present in a test sample. For molecular (DNA or RNA) analytes, typical analytic sensitivity can attain very low limits of detection (e.g., $10^{-3}$-$10^{-6}$), in contrast to microscopically based platforms such as FISH and conventional karyotyping (Table 24-1). Good analytic sensitivity is often associated with diagnostic or clinical sensitivity, the latter being the ability of an assay to detect a particular clinical condition (signified by the presence of the target analyte) in a general population of subjects. However, analytic and diagnostic sensitivity can diverge if, for example, the analyte in question is not uniformly represented (because of cell or tissue distribution effects, temporal or ethnic variations), leading to a situation in which a clinical test may have exquisite detection sensitivity, yet may miss some patients with the condition of interest (i.e., analytic false negativity). Analytic specificity concerns the ability of an assay to only identify the target analyte, even in heterogeneous samples with other molecules having potentially overlapping features. For most molecular assays, high analytic specificity can be readily achieved by relying on the highly complementary nature of DNA fragment binding, as well as additional technical maneuvers to enhance these nucleic acid interactions, or amplify positive signals. Clinical or diagnostic specificity refers to the ability of a test to accurately determine individuals that do not have the disease or condition in question. Once again, these two parameters are linked but can show a significant lack of concordance if, for example, a truly negative specimen is affected by external contamination with the nucleic acid of interest or if related molecules in the sample are not discriminated by the assay (i.e., analytic false positivity). Amplification-based assays are especially prone to false-positive results, an unfortunate potential by-product of high analytic sensitivity. Scrupulous control of contamination is therefore essential in any molecular diagnostic laboratory performing PCR analyses.

When designing molecular cytogenetic or molecular genetic diagnostic tests with high analytic and potential clinical utility, the hematopathologist must understand these concepts thoroughly and include sufficient data in validation studies to mitigate potential concerns regarding sensitivity and specificity. As a result, many elements require careful integration in the development of a molecular diagnostic application including: (1) an understanding of the molecular genetic basis of a particular disease; (2) detailed knowledge of and practical experience with molecular assay tools, including their relative analytic sensitivities and appropriate applications; (3) careful consideration of preanalytic variables; (4) recognition of potential interferences; (5) clinical and biologic disease variation; (6) tissue or cell type distribution of target analytes; and (7) effects of therapeutic intervention.

**TABLE 24-1**

**Relative Sensitivity of Standard Methods Used to Detect Genetic Abnormalities in Leukemias and Lymphomas**

| Method | Analytic Sensitivity | Notes |
|---|---|---|
| Cytogenetics | 5-10 × $10^{-2}$ | • Global genomic assessment of numerical and large structural chromosome defects including partial or whole gains, losses, duplications, translocations<br>• Laborious; requires fresh, sterile cells for metaphase analysis<br>• Difficult or impossible to detect smaller scale alterations in DNA (i.e., less than several megabases) |
| Fluorescence in situ hybridization | 1-5 × $10^{-2}$ | • Targeted assessment of large regional genomic abnormalities (submegabase range)<br>• Technically straightforward and rapid; can frequently be performed on interphase nuclei<br>• Applicable to a variety of tissue sources including paraffin-embedded biopsies<br>• Requires rigorous quality standards to avoid false-positive results at low levels of the abnormality in question |
| Southern blot hybridization | 5-10 × $10^{-2}$ | • Targeted assessment of structural changes in genomic DNA (kilobase range)<br>• Technically laborious and time-intensive<br>• Requires samples that preserve high-molecular-weight DNA (e.g., fresh or frozen cells) |
| Polymerase chain reaction (PCR) | $10^{-4}$ to $10^{-6}$ | • Targeted assessment of small genomic or messenger RNA abnormalities (hundreds of base pairs)<br>• Technically straightforward and rapid; often require post-PCR detection procedures with variable analytic sensitivities (e.g., DNA sequencing)<br>• Applicable to a broad range of sample types, including fixed paraffin-embedded tissues<br>• Best method for high sensitivity minimal residual disease monitoring, especially using real time quantitative PCR technique |

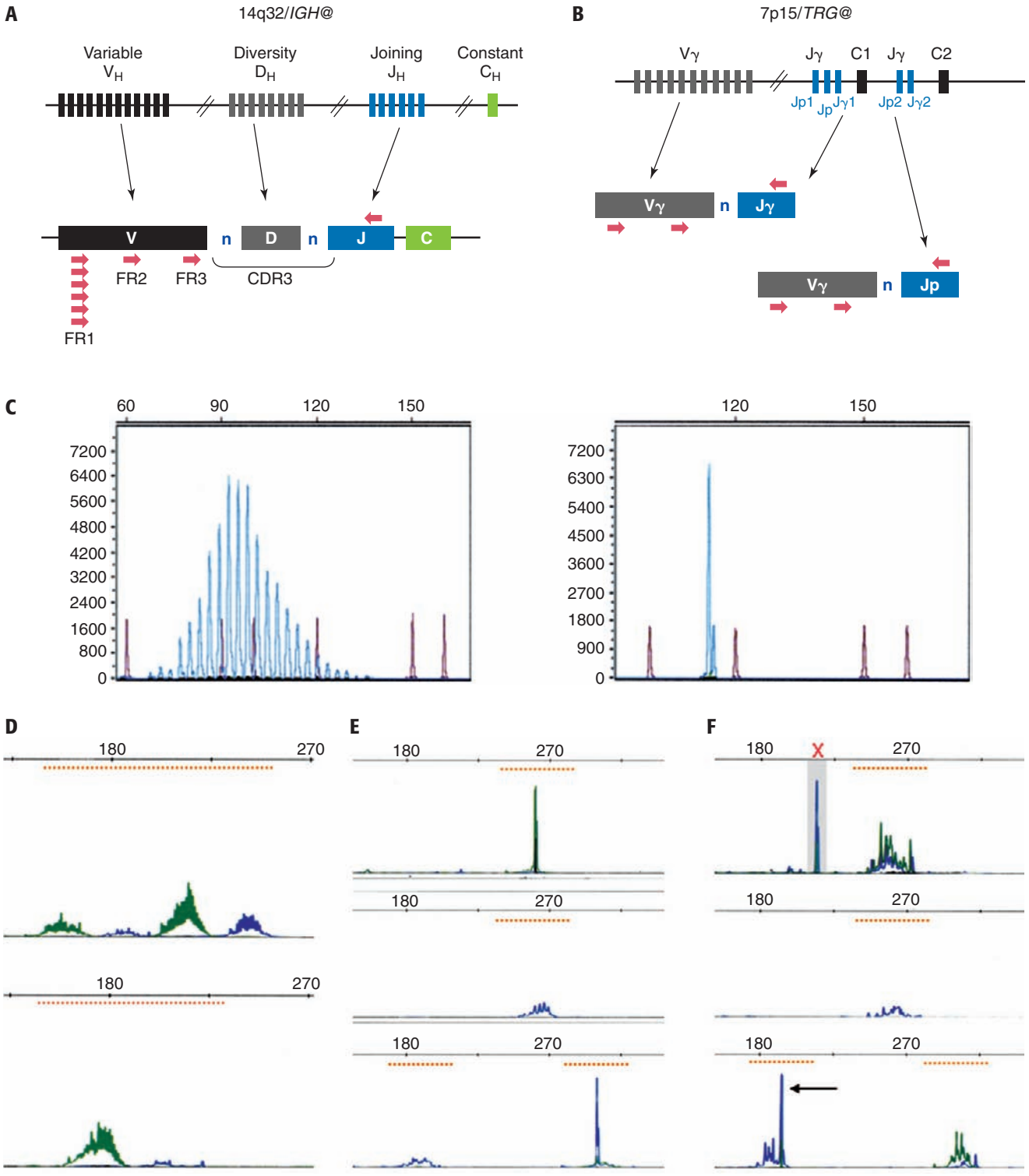
## DETECTION OF CLONAL VERSUS NONCLONAL B-CELL AND T-CELL POPULATIONS IN LYMPHOID PROLIFERATIONS

The concept of clonality is central to the cellular definition of cancer and is microscopically represented by characteristic morphologic alterations or by the presence of nonrandom karyotypic abnormalities in a tumor. Lymphoid neoplasms are unique in that the clonal nature of these proliferations can also be readily demonstrated by immunophenotypic or molecular genetic methods. In the primary diagnostic setting, the clonal status of an atypical B-cell lymphoid proliferation can usually be determined by deviations in the ratio of expressed surface or cytoplasmic light chains, as assessed by flow cytometry, immunohistochemistry, or RNA in situ hybridization. However, a number of distinct diagnostic situations require the application of molecular genetic clonality analysis. These situations include lymphoid processes with indeterminate immunophenotypic features for clonality, many T-cell proliferations, or suspected neoplastic lymphoid expansions that pose difficulty for routine immunophenotypic investigation.

The presence of unique immunoglobulin or T-cell receptor antigen receptor proteins on the surface of mature lymphocytes is a product of the somatic gene rearrangement process occurring early in the development of B- and T-cells in the bone marrow and thymus respectively. The antigen receptor genes consist of groups of many variable (V), diversity (D), joining (J), and constant (C) gene segments, each separated by large intervening regions of noncoding DNA. D-segment regions are integral to only the immunoglobulin heavy chain (*IGH@*) and T-cell receptor β (*TRB@*) loci, but are not present in the immunoglobulin light chain (*IGK@*, *IGL@*) or T-cell receptor γ (*TRG@*) genes. In a given lymphoid cell, rearrangements bring together V, (D), J and C gene segments across many kilobases of DNA in the antigen receptor gene loci, by a process of coordinated double-strand DNA breakage and recombination mediated by the recombinase activating gene (RAG) system and a variety of DNA repair enzymes. The resulting relatively compact V(D)JC coding cassettes consist of rearranged DNA sequences that are distinct in both size and sequence characteristics in each lymphocyte. The remarkable assortment of antigen receptor proteins produced among lymphocytes is thus essentially mediated by the combinatorial pool of hundreds of individual V, D, J, and C gene segments. The addition of a variable number of nontemplated ("n") nucleotides to the junctional ends of the V and D gene segments (i.e., VnDnJ) by the enzyme terminal deoxynucleotidyl transferase (TdT) during the rearrangement process further dramatically increases sequence diversity (Figure 24-2). As such, in a mixture of cells, every B or T lymphocyte harbors an individual receptor fingerprint in both DNA and peptide composition, which comprises a polyclonal population. The expression of heterodimeric antigen receptors on the surface of individual B and T cells (i.e., heavy or light chains on B cells and α-β or γ-δ chains on T cells) ensures a tremendous range for potential antigen recognition, with differential binding affinities.

**FIGURE 24-2**

Detection of clonality in lymphoid populations by polymerase chain reaction (PCR) analysis of antigen receptor gene rearrangements. **A,** Schematic demonstration of the basic process of somatic rearrangement of the immunoglobulin heavy chain (*IGH@*) locus at chromosome 14(q32). Selection and rearrangement of *IGH@* individual variable region ($V_H$), diversity ($D_H$) and joining ($J_H$) exon segments is initiated over a large span of DNA to form a relatively compact VDJ coding unit. The substantial combinatorial diversity of many possible V, D, and J segments is further increased by terminal deoxynucleotidyl transferase, which inserts a random number of nontemplated *(n)* nucleotides at the exon segment junction sites. The constant region ($C_H$) segment completes the heavy chain gene-coding unit and confers effector specificity to the antibody molecule. It should be noted that each B cell has two *IGH@* alleles and two of each of the light chain genes. Genomic rearrangements proceed at the second allele if the first is unsuccessful for protein production. Following a functional *IGH@* rearrangement, similar recombination events occur at the immunoglobulin light chain genes (i.e., *IGK* and *IGL*). The T-cell receptor γ *(TRG)* gene **(B),** located at chromosome 7(p15), is less complex with only 11 Vγ segments capable of functional rearrangements and no D segments, although there are two J-segment loci (Jp and Jγ) and two constant regions. The T-cell receptor β locus *(TRB)*, though not shown in the diagram, is highly complex but demonstrates the same structural rearrangement features as *IGH@*, *TRG,* and the light chain genes. Although antigen receptor gene rearrangements are unique in any individual B or T cell, regions of nucleic acids in many V- and J-region exon segments show sequence homology, allowing the use of consensus oligonucleotide primers that flank the rearrangement junction (i.e., VDJ or VJ) sites. Therefore gene rearrangements occurring at immunoglobulin and T-cell receptor gene loci in a lymphoid proliferation can be amplified and detected by PCR techniques. This approach is depicted by small *red arrows* in **A** and **B.** A limited set of PCR primers directed to consensus sites in the *IGH@* framework regions (FR1, FR2, FR3) and a reverse J-exon primer can effectively amplify most rearrangements in B cells by specifically targeting the highly diverse hypervariable complementary determining region 3 (CDR3). For *TRG,* a set of Vγ primers in combination with two J-region consensus primers can amplify the majority of gene rearrangements in T cells. By also combining multiple-primer PCR strategies for *IGK* and *TRB* loci (not shown), extensive coverage of nearly all possible antigen receptor gene rearrangements can be achieved in lymphoid cell populations. **C,** Typical data from *IGH@* analysis using fluorescently tagged PCR primers and capillary electrophoresis readout (*y* axis, relative PCR product fluorescent intensity; *x* axis, fragment size in base pairs [bp]; small red peaks show size-standard markers). The *left image* displays a polyclonal pattern of amplicon fragment lengths producing a normal (bell-shaped) distribution over a defined size range. In contrast, the *right image* shows a single peak population characteristic of a monoclonal B-cell population. In some instances, two monoclonal peaks can be observed, indicative of biallelic clonal rearrangements. Panels **D** to **F** illustrate sample T-cell PCR results (*dotted orange lines* indicate expected amplicon size distribution ranges for each primer set amplification). **D,** An example of polyclonal rearrangements detected at the *TRG* locus with two multiplex primer sets. **E,** *TRB* PCR data using three primer cocktails, with two distinct monoclonal peak populations identified (*upper* and *lower tracings*). **F,** A prominent peak population in a polyclonal T-cell background detected by *TRB* PCR *(arrow, lower tracing)*. Note the polyclonal distributions with the other two primer sets (the *upper* electropherogram shows an additional peak in shaded area marked by an *X*, falling outside the expected size range, and is due to nonspecific DNA amplification). The situation shown in **F** leads to difficulties in interpretation because the preferentially amplified atypical *TRB* peak may represent the presence of a true monoclonal subpopulation or a skewed benign population distribution. Such results are not infrequent with T-cell PCR studies and are designated *equivocal* or *not definitive* for a clonal T-cell population. Careful clinical and pathologic correlation is needed to avoid overinterpretation.


A
14q32/IGH@
Variable
$V_H$
Diversity
$D_H$
Joining
$J_H$
Constant
$C_H$
V
n
D
n
J
C
FR1
FR2
FR3
CDR3
B
7p15/TRG@
Vγ
Jγ
C1
Jγ
C2
Jp1
Jp
Jγ1
Jp2
Jγ2
Vγ
n
Jγ
Vγ
n
Jp
C
60
90
120
150
7200
6400
5600
4800
4200
3200
2400
1600
800
0
120
150
7200
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Although there is evidently a large spectrum of individual gene rearrangement sequences arising from these recombination events, the coding region segments of the many possible V(D)J recombinations can be detected by relatively simple PCR techniques. Despite considerable sequence heterogeneity, amplification across the V(D)J region is feasible because of the presence of relatively conserved DNA sequences within families of V and J segments that flank the rearrangement site, permitting the use of consensus V and J region primers. In this manner, a polyclonal B- or T-cell population containing many unique immunoglobulin or T-cell receptor gene rearrangements can be visualized following PCR as a normal (bell-shaped) distribution of amplified fragments within a defined size range. Conversely, a monoclonal lymphoid proliferation, arising from a single clonal cell proliferation, produces only one or possibly two single-size fragments, the latter situation reflecting the occurrence of bi-allelic rearrangements of the particular antigen receptor gene analyzed. These concepts are illustrated in Figure 24-2.

The immunoglobulin heavy chain gene (*IGH@*) located on chromosome 14(q32) and the T-cell receptor γ gene (*TRG@*) on chromosome 7(p15) are the most commonly targeted antigen receptor loci for B- and T-cell clonality assessment by PCR methods. The *IGH@* gene consists of 70 to 80 functional $V_H$-region exons, 20 to 30 $D_H$ segments, and six $J_H$ segments. Most $V_H$-segments are characterized by relatively conserved nucleotide sequences encoding three structural peptide or framework regions (FRs) of the antibody molecule, which in turn are separated by more diverse intervening sequences encoding three complementarity determining regions responsible for antigen binding (epitope recognition). Similar to $V_H$ framework regions, nearly all $J_H$ exons also share partial sequence homology. The $V_H$-FR and $J_H$ gene segments thus constitute templates for binding by consensus PCR primers (see Figure 24-2). Notably, although these primers are not specific for any particular *IGH@* gene rearrangement, this strategy produces amplicons that include the hypervariable third complementarity determining region, which contain the greatest sequence and fragment length diversity and give rise to the normal distribution of PCR products in a polyclonal B-cell process (see Figure 24-2). The *TRG@* locus is less complex by comparison, containing 11 V-region exons (grouped as four families) and five J-region segments. Nonetheless, sequence- and fragment-length differences remain significant enough (owing to segmental recombination and terminal deoxynucleotidyl transferase activity), to create a practically wide distribution of rearrangements in polyclonal T-cell populations. Again, a limited set of consensus and segment-specific primers can be used to efficiently amplify *TRG@* gene rearrangements by PCR (see Figure 24-2). Following PCR amplification, post-PCR analysis of *IGH@* or *TRG@* products can be achieved by agarose or polyacrylamide gel electrophoresis; however, fluorescent capillary electrophoresis has become commonplace in light of its superior resolution and precise fragment sizing capabilities (see Figure 24-2). One clear advantage of PCR methodology for antigen receptor gene studies is the ability to use a variety of tissue sources, including fresh or frozen cells, extracted DNA, cells from glass slides, or paraffin-embedded samples.

PCR methods represent a powerful means of determining whether a lymphoid proliferation is monoclonal (i.e., neoplastic) or polyclonal (i.e., benign), but the technique is associated with potentially serious errors involving both clinical sensitivity and specificity. For example, consensus primer PCR methods cannot detect all possible V(D)J rearrangements in the antigen receptor genes. A related problem concerns the failed detection of *IGH@* rearrangements in B-cells that have experienced a germinal center environment. Germinal center (and postgerminal center) B cells often have additional acquired mutations in the immunoglobulin genes resulting from the process of somatic hypermutation (SHM). SHM increases the affinity of antibody interaction for cognate antigenic epitopes, but during this event the induced genetic mutations may extend into conserved regions that are recognized and bound by consensus primers. These situations of incomplete gene rearrangement coverage and SHM can lead to false-negative results for true monoclonal *IGH@* rearrangements and a corresponding loss of clinical sensitivity, even if the analytic sensitivity of the method is acceptable. False-negative PCR results can be significantly diminished by the use of multiple primers directed to different conserved $V_H$ framework regions or families, in combination with assessment of the *IGK@* locus. Similarly, for the analysis of T-cell receptor gene rearrangements, targeting both the *TRG@* and *TRB@* loci can increase the positive detection rate for monoclonal T-cell populations. Conversely, PCR-based assays can suffer from false positivity arising from sample contamination (e.g., carryover of tissue fragments or amplified products from an unrelated sample). Again, control of contamination in the molecular laboratory is critical to prevent this occurrence. For T-cell receptor PCR studies, another risk of potential false-positive interpretation can occur because of physiologic or iatrogenic circumstances. T-cell repertoire restriction is found in some clinical settings (e.g., autoimmune diseases, after transplantation) and with normal aging. Apparent clonal or oligoclonal T-cell populations can be identified by PCR in these settings, requiring careful correlation with clinical history and other laboratory or morphologic data to avoid overcalling a pseudoclonal result. The use of peak-height criteria has also been proposed to improve confidence for interpreting clonotypic T-cell PCR results; however, considerable overlap between true monoclonal (neoplastic) and atypical benign amplification patterns is often encountered in practice. Finally, the analytic

(dilutional) sensitivity of PCR for B- and T-cell clonality determination using consensus primer strategies deserves mention. Although PCR is a highly sensitive technical modality, the practical lower detection limit of a monoclonal lymphoid population in a tissue sample is nominally 1% to 5%. This level of sensitivity can vary significantly depending on the particular clonal antigen receptor gene rearrangement and the amount of accompanying polyclonal lymphocytic background in a given case. For specialized indications, such as post-therapy MRD monitoring, PCR sensitivity can be markedly increased by designing patient clone-specific primer and oligonucleotide probe reagents to detect MRD in follow-up samples.

To complete the discussion on lymphoid molecular clonality evaluation, the merits and drawbacks of SBH should be considered. SBH can identify relatively large-scale rearrangements of single-copy genomic DNA at the antigen receptor gene loci, in contrast to the detection of amplified, small, rearranged V(D)J region nucleotide fragments by PCR technique. The nature of SBH technique and the ability to interrogate substantially larger regions of genomic DNA for pathologic alterations enables the identification of nearly all possible clonal immunoglobulin and T-cell receptor gene rearrangements in lymphoid tumors; this correspondingly lowers the risk of false negative results, as long as the assay is performed within its analytic sensitivity range. The *IGH@*, *IGK@,* and *TRB@* loci are the most informative antigen receptor genes for SBH clonality assessment. Despite the apparent utility of this method, SBH has been used less frequently in the routine molecular diagnostic investigation of the lymphomas and lymphoid leukemias for reasons stated previously. In addition, SBH has an analytic sensitivity of optimally 5% to 10% for the detection of monoclonal B- or T-cell populations, typically inferior to that of standard PCR assays. The more refined development of multi-primer and multi-locus antigen receptor gene PCR approaches, as advocated by the BIOMED-2 consortium and related studies, has largely led to a decrease in the routine application of SBH, although many laboratories retain the capacity to perform this analysis to occasionally complement PCR-based clonality methods.

## MOLECULAR DIAGNOSTIC EVALUATION OF NON-HODGKIN LYMPHOMAS: DETECTION OF SPECIFIC GENETIC ABNORMALITIES FOR DIAGNOSIS AND CLASSIFICATION

The pathobiology of the non-Hodgkin lymphomas (NHLs) has been greatly aided by the recognition and characterization of recurrent, nonrandom chromosomal translocation events (Table 24-2). For B-cell NHL in particular, such translocations result in the placement of a protooncogene adjacent to one of the transcriptionally active immunoglobulin genes, most commonly *IGH@*. In turn, the normally tightly regulated oncogene becomes aberrantly activated, leading to unchecked cellular proliferation or protection from normal cellular apoptosis thresholds. In certain other subtypes of lymphoma (e.g., some extranodal marginal zone lymphomas, anaplastic large cell lymphoma), translocations produce a chimeric fusion gene with its corresponding hybrid mRNA transcript and novel oncoprotein. In this situation, pathophysiologic effects arise in part from disruption of key cellular signaling pathways, mediated by aberrant activity of the chimeric protein. In B-cell lymphomas, various mechanisms have been proposed to explain the occurrence of translocation-associated gene rearrangements. These mechanisms include abnormal targeting of the RAG recombinase machinery during physiologic immunoglobulin gene rearrangements, aberrant resolution of double-strand breaks during germinal center processes (e.g., somatic hypermutation, class switch recombination) mediated in part by the enzyme activation-induced cytidine deaminase, and fragile DNA intermediates prone to breakage and errant recombination.

The identification of characteristic cytogenetic abnormalities is not always essential for establishing a diagnosis of NHL. Most often, a correct diagnosis and subclassification is reached by a combination of morphology and immunophenotypic studies. Furthermore, the detection of expressed oncoproteins by immunohistochemistry provides a simple alternative method of confirming the presence of an underlying specific molecular genetic event in some types of NHL (e.g., cyclin D1 expression in mantle cell lymphoma and ALK expression in anaplastic large cell lymphoma). Nonetheless, there are many circumstances in which the presence or absence of these genetic anomalies can be critical for correct disease diagnosis and classification. The detection of lymphoma-associated genetic abnormalities can be achieved by cytogenetics, FISH analysis, or PCR-based methods. Cytogenetics is often limited by the unavailability of fresh tissue for sterile culture. PCR assays relying on clustering of genomic breakpoints have been developed to detect some gene fusion abnormalities in NHL, but this approach in general suffers from suboptimal clinical sensitivity because many cases have large and inconstant break-site regions precluding the use of standard PCR techniques. FISH methods in contrast are robust in this regard and are most often applied to reveal the presence of translocation events in NHL.

### T(14;18)/BCL2-IGH ABNORMALITY IN FOLLICULAR AND LARGE B-CELL LYMPHOMAS

The t(14;18)(q32;q21)/*BCL2-IGH@* abnormality is prototypic of lymphoma translocations and occurs in

**TABLE 24-2**

**Common Genetic Abnormalities in the Non-Hodgkin Lymphomas Detected by Molecular Diagnostic Methods**

| Genetic Abnormality | Disease Associations | Molecular Pathogenesis* | Molecular Diagnostic Detection | Notes |
|---|---|---|---|---|
| t(14;18)/*BCL2-IGH@* | Follicular lymphoma (90%); diffuse large B-cell lymphoma (20%-30%) | Overexpression of antiapoptotic *BCL2* protein | FISH, DNA PCR | PCR for *BCL2* MBR locus rearrangements detects ≈ 60% to 70% of cases; multiple primer sets required to increase overall detection |
| t(11;14)/*CCND1-IGH@* | Mantle cell lymphoma (≈100%) | Overexpression of G1-phase cell cycle protein cyclin D1 | FISH, DNA PCR | PCR for *BCL1* MTC locus rearrangements detects <50% of cases; cyclin D1 overexpression by immunohistochemistry is also diagnostic in correct pathologic setting |
| t(8;14)/*MYC-IGH@* and variant *MYC* translocations | Burkitt lymphoma (100%); diffuse large B-cell lymphoma (5%-10%) | Overexpression of potent early response mitogenic transcriptional factor *MYC* | FISH | *MYC* translocations also occur in subsets of other aggressive B-cell lymphomas (e.g., large cell transformation of low grade lymphoma, HIV-related); *MYC* rearrangements also present in a subset of B-cell lymphomas with high-grade cytology and concurrent *BCL2* or *BCL6* (double hit) gene rearrangements; *MYC* translocation in DLBCL associated with poor outcome |
| t(11;18)/*API2-MALT1* t(14;18)/*MALT1-IGH@* | Extranodal marginal zone lymphomas (≈20%-30%) | Upregulation of NF-κB signal transduction pathway activity | FISH, RT-PCR (for API2-MALT1 mRNA) | Frequency of specific translocations varies with different anatomic sites |
| t(2;5)/*NPM1-ALK* and other *ALK* translocation variants | T-cell anaplastic large cell lymphoma (60%) | Constitutive activation and abnormal localization of Alk tyrosine kinase | FISH, RT-PCR (for NPM1-ALK mRNA) | *ALK* protein detection by immunohistochemistry is also diagnostic (staining patterns vary according to translocation type); rare large B-cell lymphomas with *CLTC-ALK* gene fusion |
| inv14 or t(14;14)/*TCL1A* gene rearrangement | T-cell prolymphocytic leukemia (80%) | Overexpression of oncogenic transcription factor | FISH | *TCL1A* protein detected by immunohistochemistry in most cases; rare variant translocations involving *MTCP1* also seen in T-PLL |

*DLBCL,* Diffuse large B-cell lymphoma; *FISH,* fluorescence in situ hybridization; *MBR,* major breakpoint region; *MTC,* major translocation cluster; *NF-κB,* nuclear factor κ B; *RT-PCR,* reverse transcriptase polymerase chain reaction; *T-PLL,* T-cell prolymphocytic leukemia.
*Major pathophysiologic effect resulting from genetic lesion.

approximately 90% of follicular lymphomas (FLs) and up to one third of diffuse large B-cell lymphomas (DLBCL). The juxtaposition of the *BCL2* gene with the transcriptionally active *IGH@* leads to enforced expression of the *BCL2* protein, a potent antiapoptotic factor. At the genomic level, breakpoints in the *BCL2* gene are most often highly clustered in the distal part of the third noncoding exon, known as the *major breakpoint region* (MBR), whereas the break-fusion sites in the *IGH@* gene are in the vicinity of the JH exons (Figure 24-3). Additional *BCL2* breakpoint sites have also been identified loosely grouped downstream of the MBR and have been designated as 3′ MBR, intermediate cluster region (icr), minor cluster region (mcr), and 5′ mcr. PCR approaches using extracted tumor DNA can detect nearly 65% of *BCL2-IGH@* translocation rearrangements using a primer situated 5′ to the MBR and a consensus J-region *IGH@* primer (see Figure 24-3). Additional primer sets targeting the mcr and icr can identify these additional breakpoint sites, raising the clinical sensitivity of PCR technique above 80%; however, a significant minority of *BCL2* breakpoints remains undetectable, even with these more comprehensive protocols. Notably, as is the case for any PCR assay involving fixed, paraffin-embedded material, both the analytic and clinical sensitivity will be adversely affected because of the effects of DNA degradation. D-FISH using probes spanning the constant through variable regions of the *IGH@* locus on 14(q32) and the entire *BCL2* gene on 18(q21) is highly sensitive and specific

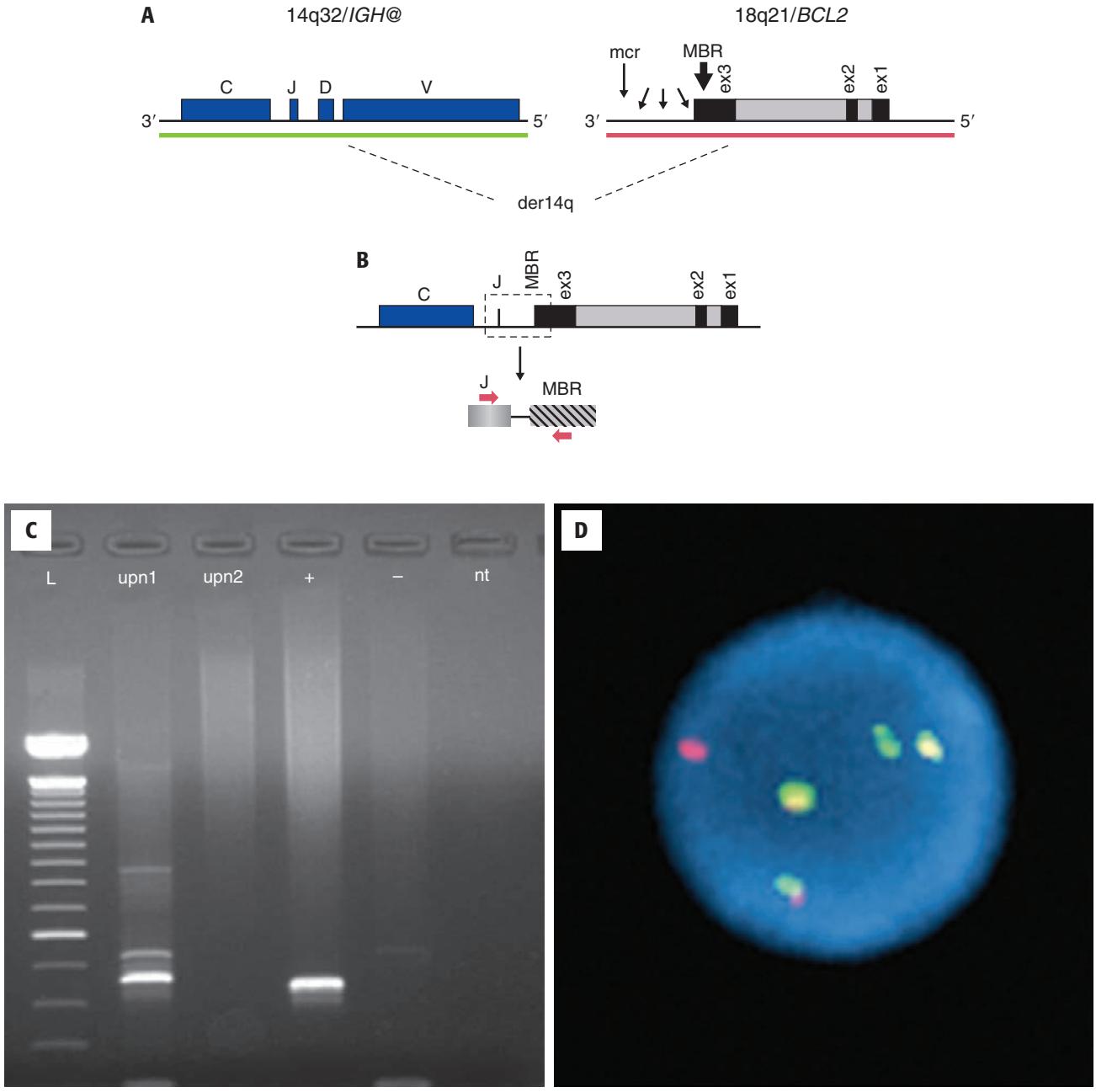



**FIGURE 24-3**

The t(14;18)/*BCL2-IGH@* abnormality. **A,** The arrangement of *IGH@* and *BCL2* genes on their respective chromosomes. Most genomic breakpoints are tightly grouped in the major breakpoint region (MBR) of *BCL2*, situated in the terminal aspect of the noncoding exon 3. A small number of break-sites are distributed in the minor cluster region (mcr). Additional loose cluster areas for breakpoints include the 3′ MBR, 5′ mcr, and the intermediate cluster region; these loci are indicated by *small arrows* between the MBR and mcr. Breakpoints in the *IGH@* gene are located within the $J_H$ region. Fluorescence in situ hybridization (FISH) probes for *IGH@ (green bar)* typically span the entire locus, whereas probes for *BCL2* region *(red bar)* span the entire gene as well as several hundred kilobases in both 5′ and 3′ directions, thus covering the MBR through the mcr. **B,** The molecular consequence of *BCL2-IGH@* juxtaposition. By using a consensus $J_H$ primer, along with an oligonucleotide primer placed just upstream of the MBR site *(small red arrows),* approximately two thirds of *BCL2* gene translocations can be detected by DNA polymerase chain reaction (PCR). PCR approaches to detect mcr and icr region breakpoints can further increase the PCR detection rate. **C,** The t(14;18)/ *BCL2-IGH@* in a case of follicular lymphoma detected by MBR-$J_H$ PCR. Gel lanes are as follows: 100 bp ladder (L); patient positive result (upn1); patient negative result (upn2); positive control (+); negative control (–); no DNA template (nt). Note that the patient-positive sample demonstrates a dense band slightly larger in size compared with the positive control, consistent with the variability in *BCL2* and *IGH@*-$J_H$ break-sites in each individual case. **D,** Interphase dual color–dual fusion probe FISH (D-FISH) in a cell with the t(14;18)/*BCL2-IGH@* translocation. The two yellow fusion signals represent the reciprocally translocated *IGH@* and *BCL2* genes on the respective der(14) and der(18) chromosomes. Single red and green signals represent the remaining normal genomic regions. An extra *IGH@* locus green signal is also present in this case. *(FISH image courtesy of Abdul Al-Saadi, MD, William Beaumont Hospital, Royal Oak, Mich.)*

for detecting this translocation. FISH is also reliable using tissue sections or nuclei extracted from paraffin-embedded samples.

Detection of the *BCL2-IGH@* can be useful to differentiate follicular lymphoma from atypical reactive follicular hyperplasia in occasionally difficult biopsies, especially when immunophenotyping and immunoglobulin gene rearrangement studies are not informative. More commonly, the presence of this genetic abnormality is used to establish the diagnosis of follicular lymphoma and exclude other small B-lymphocyte neoplasms with potentially overlapping morphologic features. In this regard, it is important to note that *BCL2* protein overexpression is commonly observed in many other types of NHL, resulting from genetic or epigenetic mechanisms distinct from the *BCL2-IGH@*. The presence of *BCL2* protein overexpression is therefore not specific for lymphoma subclassification. A small number of FLs lack the t(14;18)/*BCL2-IGH@,* and these cases are often associated with centroblastic (i.e., grade 3) cytology and a relatively distinct molecular pathogenesis, including *BCL6* gene translocations. In DLBCL, the *BCL2-IGH@* has been related to a subgroup of cases with a germinal center B-cell gene expression signature, which is associated with relatively favorable outcome. However, the actual effects of this translocation on DLBCL prognosis remain controversial. More importantly, the detection of *BCL2* gene rearrangements in conjunction with *MYC* gene translocation identifies a subset of highly aggressive DLBCL cases with high-grade cytomorphology (see also the section on t[8;14] abnormality in Burkitt lymphoma and B-cell lymphomas with high-grade morphologic features).

## T(11;14)/*CCND1-IGH@* ABNORMALITY IN MANTLE CELL LYMPHOMA

The t(11;14)(q13;q32)/CCND1-IGH@ abnormality is observed in mantle cell lymphoma (MCL), as well as a subset of multiple myelomas. While legacy studies examining the t(11;14) described its presence in occasional cases of chronic lymphocytic leukemia (CLL), B-cell prolymphocytic leukemia, and splenic marginal zone lymphoma, most occurrences represent morphologic variants of MCL. As a result, the t(11;14) can be considered essentially pathognomonic of MCL in the differential diagnosis of B-cell NHL. This translocation places the CCND1 genetic locus in proximity to the JH-region of the *IGH@* gene (Figure 24-4). The genomic breakpoints on 11(q13) are typically located at a substantial centromeric distance to CCND1, at a site termed the *BCL1 locus*. CCND1 encodes the G1-phase cell cycle protein cyclin D1, which becomes highly overexpressed as a result of the t(11;14). The three D-type cyclins (D1, D2, and D3) promote early G1-phase entry to the cell cycle by binding to specific cyclin dependent kinases (cdk4, cdk6), leading to inactivation of retinoblastoma protein, transition to S-phase and subsequent commitment to mitosis. Cyclin D1 (in contrast to its related counterparts) is notable for not being normally expressed in normal B-cell proliferation; therefore overexpression of cyclin D1 through a CCND1 translocation is a hallmark of essentially all cases of MCL. Consequently, CCND1 deregulation is thought to result in unchecked cell cycle entry, leading to increased cellular proliferation in MCL. Data from gene expression microarray analysis confirm a prominent proliferation and antiapoptosis signature that seems to underlie both the pathogenesis of this disease and the observation that patients with MCL have a distinctly adverse outlook compared with other small B-cell neoplasms generally.

From the molecular diagnostic standpoint, approximately 50% of the breakpoints in the 11(q13)/*BCL1* locus aggregate in a region known as the major translocation cluster (MTC; see Figure 24-4), located approximately 120 kb centromeric from the *CCND1* gene. The remaining breakpoints can occur anywhere downstream from the MTC, including immediately 5′ of the *CCND1* gene. Given the substantial breakpoint heterogeneity in this area of 11(q13) and the lack of significant breakpoint clustering outside of the MTC, DNA PCR-based methods to detect the t(11;14) cannot achieve high detection sensitivity. The use of a *BCL1* MTC region primer with a consensus $J_H$-region *IGH@* primer detects slightly less than half of locus rearrangements overall and is further diminished in paraffin tissue samples. SBH has been used historically to identify *BCL1* gene rearrangements; however, probes for SBH are not widely available, and the technique requires fresh tissue for high quality DNA. Quantitative RT-PCR evaluation of relative cyclin D1 mRNA expression levels has also been proposed as a method to assist in the diagnosis of MCL. Despite the evident frustration in developing a robust molecular genetic approach for *CCND1* rearrangements, D-FISH using two *IGH@* region probes and a large *CCND1/BCL1* region probe can detect virtually all t(11;14) events in MCL (see Figure 24-4). Along with the general advantages of the dual fusion strategy, the 11(q13) probe can simultaneously identify *CCND1* gene locus amplification in addition to the presence of the t(11;14). *CCND1* amplification has been associated with the more aggressive blastoid variant of MCL. Immunohistochemistry using antibodies directed to cyclin D1 has become a widespread ancillary test in establishing a diagnosis of MCL. Regardless, FISH analysis for *CCND1* gene rearrangements retains an important function for the specificity of MCL diagnosis, given that aberrant cyclin D1 expression can be seen in other subtypes of B-cell neoplasia (e.g., hairy cell leukemia, rare DLBCL) in the absence of the t(11;14) or *CCND1* alteration. Very rare examples of morphologic and phenotypic MCL have been recently described lacking abnormalities of *CCND1*. These cases share similar

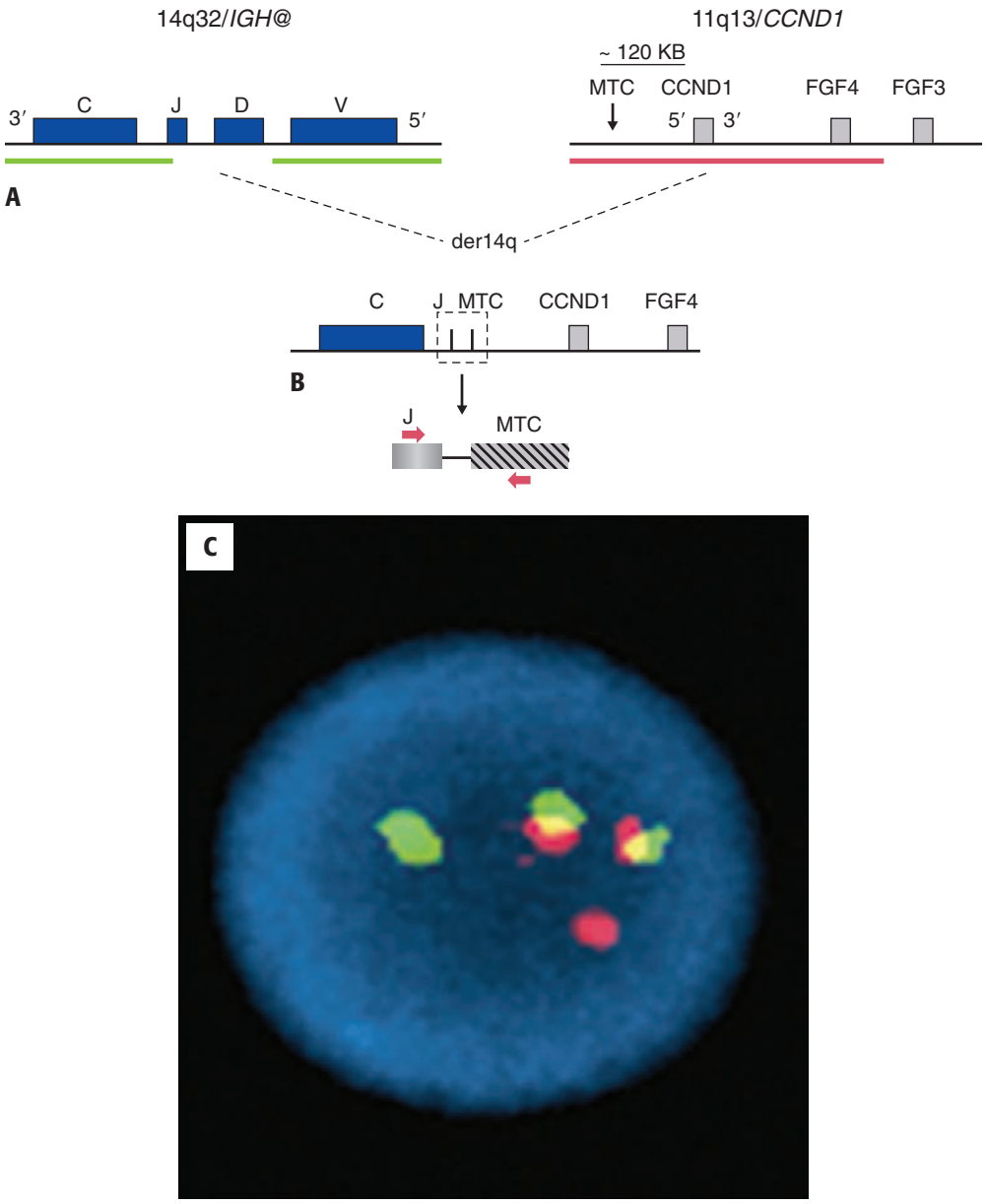



**FIGURE 24-4**

The t(11;14)/*CCND1-IGH@* abnormality. **A,** The arrangement of *IGH@* and *CCND1* (cyclin D1 gene) loci on their respective chromosomes. *CCND1*-related breakpoints occur over a large genomic region of 11(q13) referred to as the *BCL1 locus*. Approximately 50% of *BCL1* breakage abnormalities occur at the major translocation cluster (MTC); however, the remainder show wide distribution without significant additional clustering. *CCND1* is located more than 100 kb away from the MTC and becomes deregulated via fusion with *IGH@* gene $J_H$ region on 14(q32). Fluorescence in situ hybridization (FISH) probes for *IGH@ (green bars)* typically hybridize to areas on either side of the *IGH@* breakpoint region, whereas probes for *CCND1 (red bar)* span several hundred kilobases of the genomic locus, including the MTC. **B,** A schematic polymerase chain reaction strategy for detecting the subset of *CCND1*(*BCL1*)*-IGH@* rearrangements occurring in the MTC, using consensus *BCL1* and *IGH@*-$J_H$ primers *(small red arrows)*. **C,** Interphase D-FISH analysis for the t(11;14)/*CCND1-IGH@*. Two yellow fusion signals represent reciprocally translocated *IGH@* and *CCND1* genes on the respective der(14) and der(18) chromosomes. Single red and green signals represent the remaining normal genomic regions. *(FISH image courtesy of Abdul Al-Saadi, MD, William Beaumont Hospital, Royal Oak, Mich.)*

gene expression profile features with typical MCL, but may show alternate translocations of related *CCND2* or *CCND3* genes and overexpression of cyclins D2 or D3. Aberrant expression of the neurogenic transcription factor SOX11 is strongly associated with MCL, and its detection by immunohistochemistry is potentially promising in positively identifying cases lacking *CCND1* rearrangements.

## ABNORMALITIES OF *MALT1, BCL10,* AND *FOXP1* GENES IN THE EXTRANODAL MARGINAL ZONE LYMPHOMAS

The extranodal marginal zone (B-cell) lymphomas (ENMZLs) or mucosa-associated lymphoid tissue lymphomas are a group of indolent B-cell tumors encompassing a variety of anatomic sites and several tumor

genetic abnormalities. Many of these lymphomas are associated with a background or preceding phase of tissue inflammation, often with accompanying reactive lymphoid hyperplasia. In some cases the inciting predisposition is known, as in the case of *Helicobacter pylori* infection and its relationship with gastric ENMZL. Although numerical chromosome imbalances (e.g., trisomies of 3 and 18) are often observed in ENMZL, several recurrent chromosomal translocations have been identified in these lymphomas. The t(11;18)(q21;q21) is most frequently observed in gastric and pulmonary ENMZL, with a much lower incidence in orbital and salivary gland sites. This translocation results in the formation of the *API2-MALT1* gene fusion. *API2* (or *IAP2*) encodes an apoptosis inhibitor protein, whereas the *MALT1* (or *MLT1*) gene produces a paracaspase protein. The chimeric *API2-MALT1* is transcribed and creates a novel oncogenic protein. A second, less frequent translocation in ENMZL is the t(14;18)(q32;q21) generating the *MALT1-IGH@* abnormality, with resultant deregulation of *MALT1* gene expression. The *MALT1-IGH@* has been associated mainly with orbital, salivary gland, lung, and cutaneous ENMZL, but it is seen infrequently in gastrointestinal primary sites. The genomic breakpoints on chromosome 18q21 are only slightly centromeric to the *BCL2* locus, such that classical karyotyping studies cannot differentiate between the *BCL2* translocation of follicular lymphoma and the *MALT1* rearrangement in ENMZL. FISH technique can, however, accurately distinguish these submicroscopic differences. A third, rare t(1;14)(p22;q32) event has been found in gastric and pulmonary ENMZL and is characterized by the *BCL10-IGH@* genetic fusion with overexpression of BCL10 protein. Remarkably, although substantial diversity is present in the anatomic location and genetic features of ENMZL, a common molecular pathogenesis has emerged regarding these translocation events. Both BCL10 and MALT1 are cytosolic adaptor proteins involved in antigen receptor-induced activation of the nuclear factor κ B (NF-κB) signal transduction pathway in normal B-cells. In ENMZL, these translocations effectively deregulate either MALT1 or BCL10, inducing constitutive activation of the NF-κB pathway. Altered NF-κB effects on cellular proliferation and survival are considered critical to promoting tumor cell growth. The centrality of the NF-κB pathway in these lymphomas also suggests the possibility of developing targeted therapies. Finally, but likely not last in this expanding list, the *FOXP1-IGH@* anomaly arising from the t(3;14)(p14.1;q32) has been identified in a small number of ENMZL and large B-cell lymphomas often in extranodal (e.g., gastric) sites. FOXP1 is a factor involved in the regulation of the RAG recombinase proteins responsible for mediating antigen receptor gene recombination in developing B-cells.

Overall, FISH methods are best suited to identify the common translocations in ENMZL. *MALT1* gene abnormalities derived from the t(11;18) or t(14;18) abnormalities can be ascertained by separate D-FISH strategies. The chimeric API2-MALT1 mRNA can be detected with RT-PCR; however, because of substantial breakpoint heterogeneity in both of these genes, one of several fusion transcripts could be present in any given case of ENMZL, requiring the use of multiple PCR primers. The requirement for sufficient RNA quantity and quality in the face of often limited (or fixed) tissue biopsy material also significantly limits the broad applicability of RT-PCR analysis. Molecular diagnostic evaluation is not routinely sought in ENMZL, although some situations may require specific investigations. For gastric ENMZL, identification of the t(11;18)/*API2-MALT1* abnormality is clinically important in that positive tumors do not respond to antibiotic-mediated eradication of *H. pylori* infection, a therapeutic option that is otherwise often successful in a significant proportion of translocation-negative cases. Small extranodal tissue biopsies involved by low-grade B-cell NHL (B-NHL) can sometimes pose a difficult differential diagnosis, and FISH studies in this setting can be helpful for subclassification. Given that the probes developed for *BCL2* and *MALT1* FISH analyses are not overlapping, this approach can differentiate between these t(14;18)-related abnormalities, which is a distinction of potential clinical importance. Probably more common in practice, extranodal tissue specimens with suspicious lymphoid populations are often assessed for the presence of monoclonal immunoglobulin gene rearrangements by PCR to support a definitive diagnosis of lymphoma, or to check for minimal persistence of lymphoma following treatment. Molecular clonality studies with positive results in this scenario require careful clinical and morphologic correlation, in light of some reports describing clonal B-cell populations in apparently reactive lymphoid hyperplasias, or cases with delayed clearance of true molecular disease in histologically negative post-therapy specimens.

## MOLECULAR AND CYTOGENETIC PROGNOSTIC MARKERS IN CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA

Diagnostic evaluation of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) represents another example of the rapid progress in the identification and application of genetic markers for prognosis. From the perspective of the molecular cytogenetic and molecular genetic laboratories, several assays are becoming more frequently incorporated in the initial assessment of CLL/SLL. FISH analysis is commonly performed to detect changes in specific chromosomes: −13 or 13q−, 11q−, +12 and 17p−. Of these, 11q− and 17p− are associated with adverse outcomes, characterized by risk

of early disease progression or therapeutic failure. Trisomy 12 is considered an intermediate-risk prognostic marker, and sole abnormalities of chromosome 13 appear to connote a more indolent disease course. The significance of 11q− and 17p− is related in part to monoallelic loss of the tumor suppressor genes *ATM* and *TP53* respectively in the regions of chromosomal deletion. For 17p−, which occurs in approximately 5% of patients with untreated CLL/SLL, point mutation inactivation of the remaining *TP53* gene is almost always present, indicating biallelic loss of p53 function. Data are incomplete for 11q−, but a similar complete loss of *ATM* can be expected. Patients undergoing therapy or who experience a transition to more aggressive disease have an even higher prevalence of 17p−/*TP53* mutation tumor genotype. Patients with 17p− disease tend to progress earlier with poor responses to purine nucleoside analog drugs, and thus require alternative treatment options. Detection of the 17p− abnormality in CLL/SLL, and perhaps more so the presence of *TP53* mutation, is of critical importance clinically. In addition to FISH for the 17p− (often performed as part of a CLL panel), *TP53* mutation testing can be accomplished with DNA PCR and direct Sanger sequencing of exons 4 to 9. In addition to FISH testing for genomic anomalies, the status of SHM in the rearranged immunoglobulin heavy chain gene variable region ($V_H$) provides another marker of prognosis in CLL/SLL. A substantial proportion of patients with CLL demonstrate nucleotide deviations from germline *IGH@* sequences indicative of SHM, implying that these tumor B cells have experienced a germinal center environment. Extensive studies have shown that a $V_H$ mutation load greater than 2% from germline is associated with significantly better outcome (mutated CLL) versus patients with SHM less than 2% (unmutated CLL). Determination of *IGH@* $V_H$-region mutations is not trivial and requires an optimized PCR assay using either DNA or RNA, followed by direct sequencing and database comparison to a reference human *IGH@* germline sequence library (e.g. http://imgt.cines.fr). With the exception of *TP53* gene mutation, the actual effects of any single prognostic factor like SHM status in CLL/SLL may become more difficult to ascertain for a given patient when integrated with numerous other analyzed markers.

## T(8;14)/*MYC-IGH@* ABNORMALITY IN BURKITT LYMPHOMA AND B-CELL LYMPHOMAS WITH HIGH-GRADE MORPHOLOGIC FEATURES

Burkitt lymphoma (BL) is an uncommon, yet distinctive and highly aggressive B-NHL. BL is classically associated with the t(8;14)(q24;q32) cytogenetic anomaly resulting in the fusion of the *MYC* and *IGH@* genes, but less commonly *MYC* can be involved with translocations to the *IGK* and *IGL* immunoglobulin light chain genes on 2(p12) or 22(q11), respectively. The normal cMyc gene product is a highly regulated early response transcription factor that coordinates mitogenic stimuli with nuclear responses for DNA synthesis and replication. *MYC* gene translocation thereby leads to marked overexpression of cMyc and upregulation of many genes driving cell proliferation, although experimental evidence also indicates a role for concurrent loss of normal apoptotic control in sustaining the neoplastic process. The break-sites in *MYC* are variable and occur over a large genomic span. The so-called endemic (or African) type of BL is characterized by a strong association with chronic Epstein-Barr virus (EBV) infection and demonstrates a molecular pattern of *MYC* breakpoints far upstream of the gene and *IGH@* breakpoint within the $J_H$-region. Sporadic BL cases show genomic breaks involving the immediate 5′ region of *MYC* and the heavy chain switch region of *IGH@*. Sporadic BL is infrequently EBV positive. The observed variation in *IGH@* locus breakpoints suggests that the translocation occurs in B cells at slightly different maturational stages in the endemic versus sporadic types, although there is no apparent clinical relevance to this phenomenon. Recent data implicate activation-induced cytidine deaminase in the pathogenesis of *MYC-IGH@* translocations.

The definitive diagnosis of BL requires demonstration of *MYC* rearrangement and, more specifically, the presence of a translocation to the *IGH@* or light chain loci. The breakpoints occurring in *MYC* and *IGH@* are not amenable to standard DNA PCR methods because of the large intervening DNA regions and lack of significant break-site clustering. Fortunately, FISH strategies are highly sensitive for the detection of these *MYC* translocations. BAP FISH technique can be used to rapidly identify *MYC* gene disruption, in conjunction with a D-FISH approach to specifically identify the *MYC-IGH@* fusion (Figure 24-5). Notably, *MYC* gene rearrangements are also found in several other examples of aggressive B-NHL, including a minority of de novo diffuse large B-cell lymphomas, some large cell or blastoid transformations of indolent B-cell lymphomas, HIV/AIDS-associated lymphomas, and some monomorphic posttransplant lymphoproliferative disorders. In addition, uncommon cases of high-grade B-cell lymphomas that have variable morphologic overlap with BL have been described and are currently difficult to precisely classify. Such tumors for now occupy a gray zone category between true BL and DLBCL and tend to respond poorly to standard DLBCL therapy. These rapidly progressive B-cell lymphomas are genetically heterogeneous; however, a significant subset harbor *MYC* translocations along with rearrangements of *BCL2* or *BCL6* genes (so-called double-hit lymphomas) and can be identified using appropriate FISH probes.

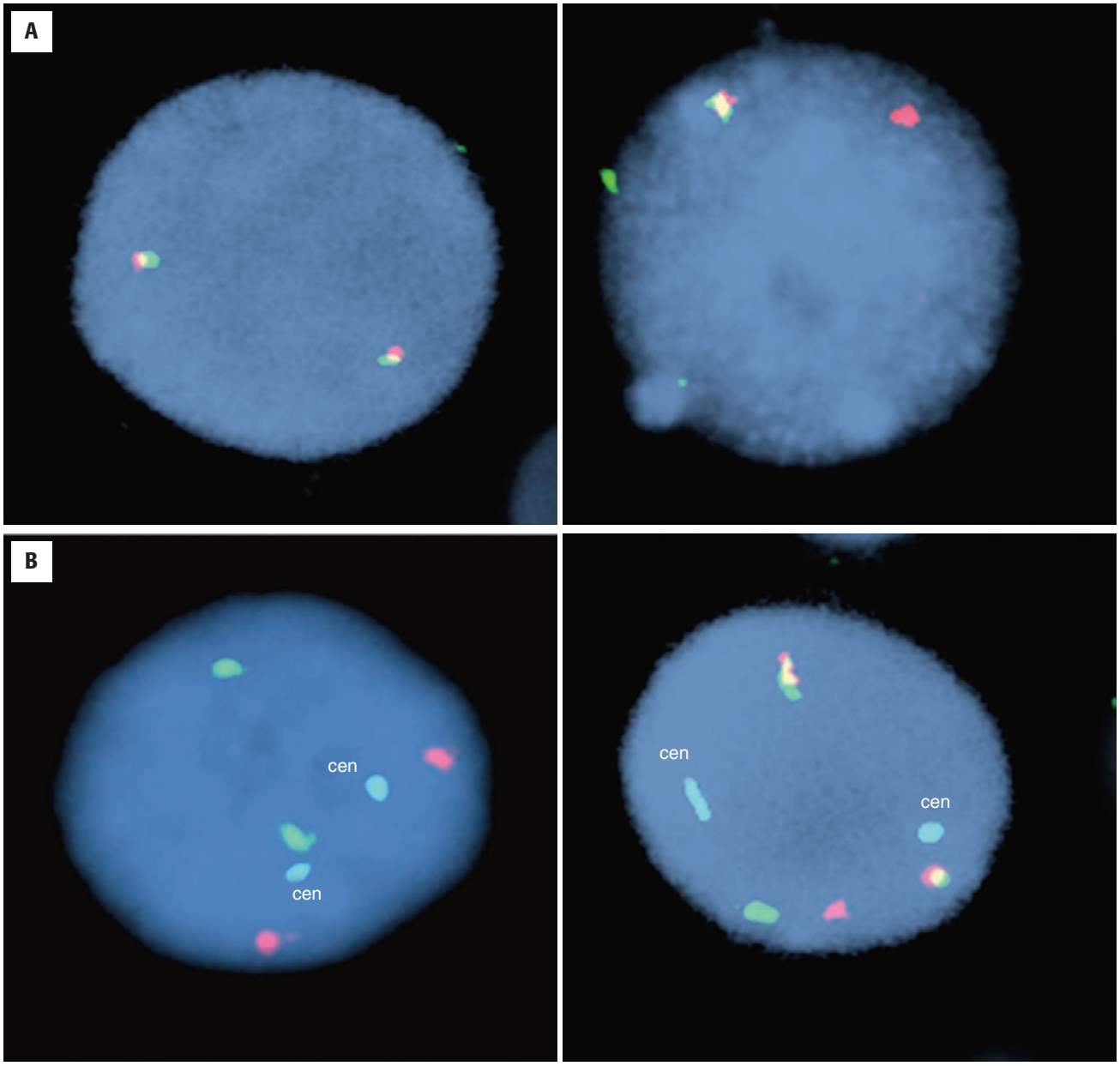



**FIGURE 24-5**

Detection of *MYC* gene rearrangements. *MYC* gene locus abnormalities on chromosome 8(q24) can be detected as a disruption of intact *MYC* or as specific translocation events. **A,** *MYC* break-apart probe (BAP) interphase fluorescence in situ hybridization (FISH) analysis. The *left image* displays intact dual color probes directed at 5′ and 3′ regions of the *MYC* locus, generating two distinct yellow fusion signals in a normal cell. The *right image* of a Burkitt lymphoma cell shows one intact fusion signal (normal) and separate single red and green fluorescence dots, indicating that one locus of the *MYC* has been rearranged, implying *MYC* gene translocation; however, the nature of the underlying translocation is not revealed by BAP-FISH. **B,** A D-FISH method to detect *MYC-IGH@* gene fusion arising from the t(8;14) abnormality. The *left image* shows a normal cell pattern with two red and two green signals (normal *IGH@* and *MYC* loci respectively). The *aqua-colored dots* labeled *cen* represent chromosome 8 centromeric probes. The *right image* of a Burkitt lymphoma cell displays two yellow fusion signals indicating reciprocally translocated *IGH@* and *MYC* genes on the respective der(14) and der(8) chromosomes. Single red and green signals represent the remaining normal genomic regions. *(FISH images courtesy of Rhett P. Ketterling, MD, Division of Cytogenetics, Mayo Clinic, Rochester, Minn.)*

## *BCL6, MYC,* AND *ALK* GENE ABNORMALITIES IN DIFFUSE LARGE B-CELL LYMPHOMAS AND INSIGHTS FROM GENE EXPRESSION PROFILING STUDIES

The DLBCLs are grouped by common if somewhat heteromorphous cytologic features, but accumulating evidence indicates substantial tumor genetic heterogeneity underlying the observed variability in clinical outcomes noted in this disease. Several recurrent cytogenetic abnormalities have been identified in DLBCL, including the t(14;18)/*BCL2-IGH@* (described with follicular lymphoma) and rearrangements of the *BCL6, MYC,* and *ALK* genes. *BCL6* is located on chromosome 3(q27) and can become deregulated by translocation to the *IGH@* in a t(3;14)(q27;q32) event. However, *BCL6* is also known to become aberrantly activated by promoter substitutions arising from translocations with many other

nonimmunoglobulin genes. Overall, approximately one third of DLBCLs show the presence of a *BCL6* rearrangement; an even higher proportion reveal somatic mutations involving the 5′ regulatory region of *BCL6,* sometimes concurrent with gene translocation. The *BCL6* gene product is a critical transcription factor responsible for normal secondary follicle formation during the germinal center reaction in lymphoid tissues and for proper T-cell–dependent antibody responses upon exposure to antigen. Accordingly, *BCL6* is strongly expressed by germinal center B cells (GCBs), but is not present in naive or post-GCBs. *BCL6* is thought to protect normal GCBs during the affinity maturation process (i.e., somatic hypermutation) in part by downregulating proapoptotic stimuli elicited by physiologic DNA double-strand breakage. *BCL6* is overexpressed in a large subset of B-cell NHL, most notably tumors that are related to normal germinal center (GC) counterparts (e.g., FL, many DLBCLs, BL). The pathophysiology of *BCL6* deregulation is not fully understood, but the sustained GC-like state may provide an environment in which susceptible B cells can develop additional genetic abnormalities promoting lymphomagenesis. The clinical significance of *BCL6* gene rearrangements in DLBCL remains controversial, with clinical studies demonstrating both improved and worse outcome associations. *BCL6* gene abnormalities may not be independently prognostic in the era of rituximab (anti-CD20 antibody) use but appear to track with other potentially adverse clinical and biologic features. *BCL6* rearrangements in DLBCL can be detected using BAP FISH technique, but this is infrequently performed in the diagnostic setting.

*MYC* gene rearrangements occur in approximately 5% to 10% of typical DLBCL through translocations to immunoglobulin or occasionally nonimmunoglobulin genes. The presence of an *MYC* translocation confers a significantly worse outcome, and because the morphologic and immunophenotypic features of *MYC*-rearranged DLBCL are not distinctive, routine FISH evaluation of the *MYC* locus in de novo cases has been advocated by some investigators. Other rare, large B-cell lymphomas are recognized with an unusual morphology and phenotype, featuring immunoblastic (or plasmablastic) cytology, cytoplasmic immunoglobulin A positivity, plasmacytic cell marker profile, and CD30 negativity. These lymphomas have rearrangements of the *ALK* gene arising from t(2;17)(p23;q23)/*CLTC-ALK*, or occasionally t(2;5)(p23;q35)/*NPM1-ALK* abnormalities (the *ALK* gene is discussed further with anaplastic large cell lymphoma). To establish the diagnosis of *ALK*-positive large B-cell lymphoma, the presence of *ALK* gene rearrangements can be demonstrated with BAP FISH analysis or indirectly by immunohistochemical detection of aberrant Alk protein expression. The pattern of ALK expression in *CLTC-ALK*–positive tumors is distinctive, showing a punctate membranous distribution reflecting localization in clathrin-coated pits.

The application of microarray gene expression profiling (GEP) in DLBCL revealed a general dichotomy between lymphomas with GCB and activated B-cell (ABC) signatures, with attendant prognostic implications. GCB DLBCLs were shown to have better survival and treatment response compared with ABC DLBCLs. Subsequent studies have examined smaller subsets of expressed genes and have independently shown similar classification structures and outcome features. For example, expression of genes including *BCL6, LMO2, HGAL,* and *CD10* have been strongly correlated with GCB-type lymphoma, whereas *MUM1/IRF4, CD44, XBP1,* and others connote an ABC phenotype. The prognostic differences between these two types of DLBCL are maintained, despite inclusion of rituximab in chemoimmunotherapy protocols for DLBCL. It has been difficult to standardize ancillary studies in clinical laboratories (e.g., by immunohistochemistry) to accurately recapitulate the GEP-derived divisions. Because gene expression microarray technology is not easily incorporated into the routine diagnostic molecular hematopathology setting, widespread adoption of GCB/ABC DLBCL molecular subtyping has been difficult to achieve. Other GEP studies in DLBCL have also indicated different and nonoverlapping subgroups, highlighting the challenge of large-scale studies and complex data sets. While the diagnostic utility of GEP analysis awaits further refinement, key insights into DLBCL pathogenesis nonetheless continue to emerge. An important example is the identification of deregulated NF-κB pathway activity in many ABC type DLBCLs, which may be related to mutations in upstream proteins like CARD11 or dysfunctional components of the B-cell receptor. Clearly, the effects of GEP and other highly complex investigative genomic approaches continue to be indispensable for unraveling disordered pathways in DLBCL and revealing new diagnostic, predictive, and therapeutic molecular targets.

## *ALK, TCL1A,* AND *IRF4* GENES IN T-CELL NON-HODGKIN LYMPHOMAS

Substantially less is understood about the molecular pathogenesis of the mature (post-thymic) T-cell lymphomas. One significant exception is the subset of systemic anaplastic large-cell lymphoma (ALCL), *ALK*-positive, which is most commonly characterized by the t(2;5)(p23;q35) genetic abnormality. This genetic event couples the nucleophosmin gene *(NPM1)* to a novel tyrosine kinase gene designated *anaplastic lymphoma kinase (ALK)*. NPM1 is a multifunctional protein involved in nuclear-cytoplasmic shuttling of ribosomal units, cell cycle regulation through stabilization of p53 protein, and maintaining proper chromosome alignment during mitosis. ALK is a tyrosine kinase protein that is not expressed in normal lymphocytes. The resultant

*NPM1-ALK* fusion gene produces a chimeric protein causing constitutive deregulation of ALK and consequent aberrant phosphorylation of downstream signaling target proteins. Other translocations involving ALK characterize this group of ALCL, including the t(1;2)(q25;p23)/*TPM3-ALK*, t(2;3)(p23;q35)/*TFG-ALK* and inv(2)(p23q35)/*ATIC-ALK* abnormalities. A key feature of *ALK* gene fusion products in ALCL is that the partner gene in each case dictates the intracellular distribution, and hence activity, of the ALK moiety. Determination of *ALK* gene rearrangement status in a case of morphologic and phenotypic ALCL is critical, because *ALK*-positive neoplasms are characterized by favorable treatment responses and outcome, in contrast to the relatively poor prognosis for pathologically identical cases of *ALK*-negative ALCL. *ALK* gene rearrangements are best identified using a BAP FISH strategy, one advantage of which is the applicability to paraffin-embedded fixed-tissue biopsies. Identification of the specific involved partner gene or chromosome is not necessary, as no particular prognostic significance has been ascribed to the known translocation variants. The NPM1-ALK chimeric mRNA can be detected by RT-PCR, and some reports using long-range genomic DNA PCR methods have been described; however, these approaches are not sufficiently comprehensive for diagnosis. The technically simple application of ALK immunohistochemistry largely obviates the need for molecular cytogenetic evaluation in many cases, and the cellular pattern of ALK distribution can provide some information concerning the nature of the ALK translocation.

Primary cutaneous T-cell lymphomas comprise a spectrum of disorders, which include $CD30^+$ large-cell lesions. Molecular genetic studies have revealed the presence of interferon regulatory factor 4 (*IRF4*; also known as *MUM1*) gene translocations in a substantial subset (20% to 50%) of primary cutaneous anaplastic large cell lymphoma (C-ALCL). *IRF4* translocations were also detected in rare cases of peripheral T-cell lymphoma (PTCL) with a cytotoxic phenotype, and in single cases each of systemic *ALK*-negative ALCL and PTCL, unspecified. The cytotoxic PTCL cases were characterized by a recurrent t(6;14)(p25;q11) and FISH evidence for *IRF4* translocation to the *TRA* locus, but the latter gene was not involved in C-ALCL or remaining systemic cases. In addition, none of these tumors showed *ALK* gene rearrangements. Although *IRF4* gene abnormalities (e.g., locus amplification) have been described previously and MUM1/IRF4 protein expression is relatively common in peripheral T-cell lymphomas (especially ALCL), the presence of *IRF4* gene rearrangement appears to be a novel finding, with relatively high specificity for C-ALCL.

T-cell prolymphocytic leukemia (T-PLL) is a rare peripheral T-cell malignancy with typically widespread anatomic distribution. Despite having some distinctive cytomorphologic and immunophenotypic features, T-PLL is essentially characterized by rearrangements of the *TCL1A* gene. In 80% of T-PLLs, *TCL1A* is altered and abnormally activated by an inv14(q11q32) or t(14;14)(q11;q32) event. In either case, the *TCL1A* protooncogene is brought in proximity to the T-cell receptor α locus *(TRA)* on 14(q11). This pathogenetic process leads to overexpression of TCL1A, a transcription factor that is not normally present in mature T cells. *TCL1A* gene rearrangement is also not observed in other types of peripheral T-cell lymphoma, constituting a relatively specific genetic marker for T-PLL. FISH analysis can detect rearrangements of this gene arising from either the inversion or translocation anomalies. Immunohistochemistry can also be used to identify overexpression of the protein. Of note, a small group of T-PLLs demonstrate intact *TCL1A* alleles, but instead harbor the alternate translocation events t(X;14)(q28;q11) or t(X;7)(q28;q35), which involve fusion of a *TCL1A*-related gene *MTCP1* to either the *TRA* or T-cell receptor β (*TRB*) loci, respectively.

The pace of steady investigations in the far less frequent area of PTCLs, unspecified, has also uncovered a recurrent translocation t(5;9) involving two tyrosine kinase genes, inducible T-cell kinase (*ITK*) on chromosome 5q33, and spleen tyrosine kinase (*SYK*) on chromosome 9q22. The *ITK-SYK* gene fusion occurs in almost 20% of PTCLs and is associated with overexpression of the nonreceptor SYK signal transduction kinase, which is a potent mediator of immune receptor activation in lymphoid cells. The discovery of this translocation and the corresponding finding that a large number of PTCLs are associated with SYK overexpression (though uncommonly related to the *ITK-SYK* abnormality) has focused interest on aberrant T-cell receptor signaling pathways as potential therapeutic targets in this aggressive group of non-Hodgkin lymphomas.

## MOLECULAR DIAGNOSTIC EVALUATION OF ACUTE MYELOID LEUKEMIAS: DETECTION OF SPECIFIC GENETIC ABNORMALITIES FOR DIAGNOSIS AND CLASSIFICATION

Recurrent or nonrandom cytogenetic abnormalities have been associated with particular morphologic and phenotypic features and have been incorporated into the diagnosis of acute myeloid leukemia (AML) for many years. Most recently, these pathogenetic events have become disease-defining for a significant subset of AML (Table 24-3). Two large general classes of genetic anomalies are currently recognized in AML: structural rearrangement of chromosome regions by (usually) balanced translocations and mutational alterations of single genes. While many of the former group have been well characterized, single-gene abnormalities represent a

**TABLE 24-3**

**Common Genetic Abnormalities in the Acute Leukemias Detected by Molecular Diagnostic Methods**

| Genetic Abnormality | Disease Associations | Molecular Pathogenesis* | Molecular Diagnostic Detection† | Notes |
|---|---|---|---|---|
| ***Acute Myeloid Leukemias*** | | | | |
| t(15;17)/*PML-RARA* | Acute promyelocytic leukemia (100%) | Chimeric fusion protein; interference with normal myeloid maturation and differentiation | RT-PCR for PML-RARA mRNA; RQ-PCR for MRD detection<br>FISH | RT-PCR required to define PML-RARA transcript type; favorable prognosis genetic marker (good responses to ATRA therapy); MRD evaluation by quantitative RT-PCR has prognostic value after therapy; rare APL-like tumors have variant *RARA* translocations and may be unresponsive to ATRA |
| t(8;21)/*RUNX1-RUNXT1* inv(16) or t(16;16)/*CBFB-MYH11* | Acute myeloid leukemia with maturation (10%); acute myelomonocytic leukemia with abnormal eosinophils (10%) | Chimeric fusion protein affecting the CBF transcriptional regulatory pathway; aberrant effects on myeloid cell proliferation and differentiation | RT-PCR for RUNX1-RUNXT1 and CBFB-MYH11; RQ-PCR for MRD detection<br>FISH | Favorable prognosis genetic markers; associated with responsiveness to Ara-C based chemotherapy; accompanying *KIT* gene mutations may be associated with poor outcome |
| 11(q23)/*MLL* | Acute myeloid leukemia with monocytic differentiation (5% of adults; slightly higher in pediatric AML); subset of therapy-related AML (after DNA topoisomerase II agent exposure) | Chimeric fusion protein interrupting normal hematopoietic maturation via *HOX* gene dysregulation | FISH | Most common event is t(9;11), although many *MLL* translocation partners are known; intermediate prognosis marker in de novo AML; therapy-related AML has a poor outcome |
| *FLT3* mutations | Acute myeloid leukemia (20%-30%), typically with normal cytogenetics | Constitutive activity of Flt3 tyrosine kinase with increased cell proliferation | DNA PCR and fragment sizing of exon 14/15 region to detect ITDs; PCR and restriction enzyme digestion to detect D835 point mutations | *FLT3*-ITD is an unfavorable prognostic marker (ITD:wild type allelic ratio may be more informative); effect of D835 mutation not entirely clear |
| *NPM1* mutations | Acute myeloid leukemia (50%-60%), typically with normal cytogenetics | Truncated cytoplasmic form of NPM1 with loss of normal cell cycle regulation | DNA PCR of exon 12 region and fragment sizing or high resolution melting analysis, allele-specific PCR, or PCR and sequencing | *NPM1* mutation is a favorable prognostic marker in the absence of *FLT3* ITD mutation |
| *CEBPA* mutations | AML (10%), typically with normal cytogenetics | Mutations alter normal function of *CEBPA* in granulocytic maturation | RT-PCR for CEBPA mRNA and sequencing | Biallelic *CEBPA* mutation is a favorable prognostic marker in the absence of *FLT3* mutation |
| ***B-cell Lymphoblastic Leukemia/Lymphoma*** | | | | |
| t(9;22)/*BCR-ABL1* | $Ph^+$ B-cell acute lymphoblastic leukemia in adults (20%-25%) and children (<5%) | Chimeric fusion protein with deregulation of Abl tyrosine kinase; effects on cell proliferation, apoptosis, cell adhesion | RT-PCR for BCR-ABL1 mRNA; RQ-PCR for MRD detection<br>FISH | Poor prognosis genetic marker; FISH is highly specific, but RT-PCR is required to define the precise BCR-ABL1 transcript type (p190 or p210); MRD evaluation by RQ-PCR has prognostic value after therapy |
| t(12;21)/*ETV6-RUNX1* | Childhood B-precursor acute lymphoblastic leukemia (20%) | Chimeric fusion protein; disruption of CBF transcriptional regulatory pathway and abnormal effects on normal ETV6 transcriptional functions | RT-PCR for ETV6-RUNX1 mRNA<br>FISH | Favorable prognosis genetic marker; t(12;21) is cytogenetically cryptic; loss of remaining *ETV6* allele often occurs |
| t(1;19)/*TCF3-PBX1* | Childhood pre-B cell ALL (<5%) | Chimeric fusion protein; interference with normal early B-cell development | RT-PCR for TCF3-PBX1 mRNA<br>FISH | Unfavorable prognosis genetic marker, but can be overcome with more intensive therapy |
| 11q23/*MLL* translocations | Childhood (mainly infant) B-lineage ALL (5%) | Chimeric fusion protein interrupting normal hematopoietic maturation via *HOX* gene dysregulation | FISH<br>RT-PCR for specific gene fusion transcripts (e.g., MLL-AFF1) | t(4;11)/*MLL-AFF1* and t(11;19)/*MLL-MLLT1* are most common; FISH is preferable for *MLL* locus rearrangement detection because of numerous rearranging partner genes and *MLL* breakpoint heterogeneity; unfavorable prognosis genetic marker in infant ALL; also adverse finding in older children |

*ALL,* Acute lymphoblastic leukemia; *AML,* acute myeloid leukemia; *ATRA,* all-*trans* retinoic acid; *CBF,* core binding factor; *FISH,* fluorescence in situ hybridization; *ITD,* internal tandem duplication; *MRD,* minimal residual disease; *mRNA,* messenger RNA; *PCR,* polymerase chain reaction; *Ph,* Philadelphia; *RQ-PCR,* real-time quantitative PCR; *RT-PCR,* reverse transcriptase polymerase chain reaction.

*Major pathophysiologic effect resulting from genetic lesion.

†Most frequent molecular techniques used for detection.

growing and biologically complex area of basic and clinical research. In fact, the expanding spectrum of pathogenetic single-gene alterations has provided further substantiation to the hypothesis that many, if not most, AMLs develop from a combination of class I and class II–type mutational events. In this schema, class I mutations confer a proliferative or survival advantage to leukemic cells, whereas class II changes produce adverse effects abrogating normal cellular differentiation.

Chromosome translocations in AML most often result in juxtaposition of one genetic locus with another unrelated gene, leading to the formation of a fusion protooncogene and transcription of a chimeric mRNA species that retains partially functional, but aberrantly regulated features of both individual genes. Leukemogenic gene fusions frequently involve at least one partner gene with a critical transcriptional role in normal hematopoiesis. The resulting altered chimeric protein therefore manifests widespread pathophysiologic effects on cell differentiation, maturation, and apoptosis in the AML stem or progenitor cell. Many but not all chimeric gene fusion events thus represent examples of class II mutations. Chromosomal translocations can be detected by cytogenetic and FISH techniques, but RT-PCR methods with primers spanning breakpoint-fusion regions are also commonly used to sensitively and specifically identify the resulting novel chimeric mRNA products at diagnosis in some subtypes of AML (e.g., acute promyelocytic leukemia). Leukemia-specific transcripts also serve as invaluable markers for detecting minimal residual disease using RQ-PCR methods in patients who have received curative-intent therapy. Known single-gene mutations in AML typically affect transcriptional regulation of normal stem–progenitor cell hematopoiesis or result in deregulation of cellular proliferation by constitutively activating a proto-oncogene. Point mutation or small insertion or deletion changes involving specific genes can produce either class I (e.g., *FLT3*) or class II (e.g., *CEBPA*) type cellular alterations. The following discussion of genetic abnormalities in AML will focus on the most common and clinically important tumors that constitute discrete diagnostic entities in the most recent WHO classification of hematolymphoid neoplasms. Although there are many additional, rare types of AML defined by specific translocations and new single-gene mutations are being identified at a rapid pace, the relevance of these findings to current molecular hematopathology clinical practice is either relatively minimal or not sufficiently understood.

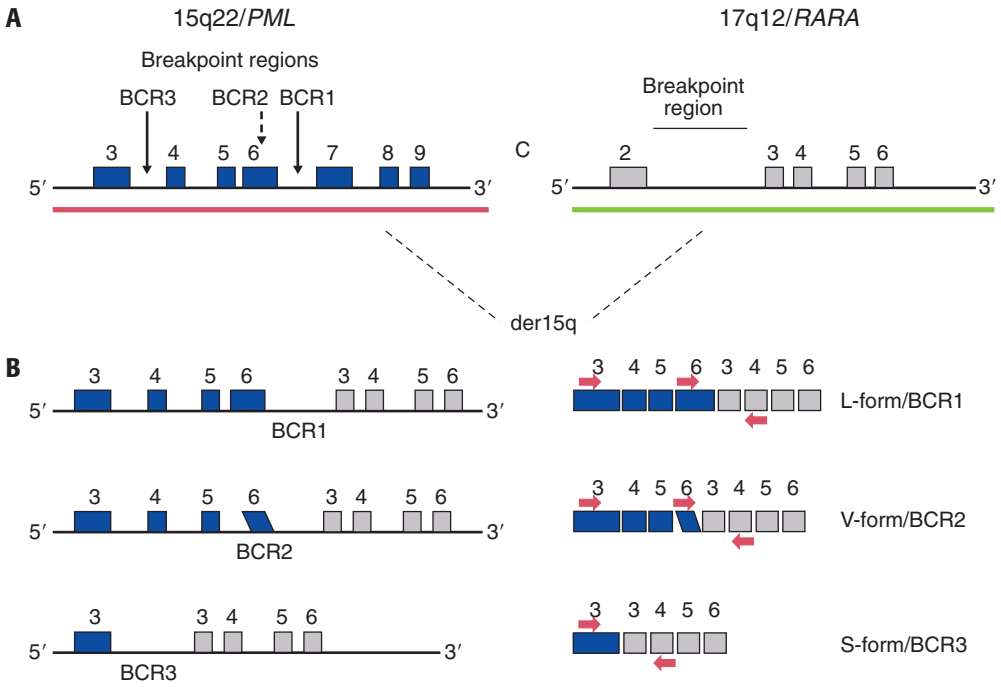
## ACUTE MYELOID LEUKEMIA WITH RECURRENT CHROMOSOMAL TRANSLOCATION ABNORMALITIES

### T(15;17)/*PML-RARA* ABNORMALITY IN ACUTE PROMYELOCYTIC LEUKEMIA

Acute promyelocytic leukemia (APL), accounting for 5% to 10% of de novo AML, is characterized by the presence of the t(15;17)(q24;q21) abnormality, resulting in the formation of a novel fusion gene *PML-RARA* on the derivative chromosome 15q (Figure 24-6). The *RARA* gene encodes the α receptor (RARα) for the ligand retinoic acid or vitamin A; the retinoic acid receptor signaling pathway functions in normal cell differentiation. The normal *PML* gene product is a DNA-binding zinc finger protein, which in association with a

**FIGURE 24-6**

The t(15;17)/*PML-RARA* abnormality in acute promyelocytic leukemia. **A,** The arrangement of *PML* and *RARA* genes on their respective chromosomes. Exons are numbered in each gene (gene maps are not shown to proper genomic scale). Genomic breakpoints in the *RARA* locus occur invariantly in intron 2; this results in consistent fusion of *RARA* exon 3 to the *PML* gene. Break-sites in *PML* are heterogeneous and can involve one of three breakpoint cluster regions (BCR) in any given patient with acute promyelocytic leukemia (APL): in intron 6 (BCR1), within exon 6 (BCR2) or in intron 3 (BCR3). Of these, the BCR1 and BCR3 breakpoints are by far the most common, accounting for more than 90% of APL cases. Fluorescence in situ hybridization (FISH) probes for the *PML* and *RARA* genes (*red* and *green bars,* respectively) typically span the entire genomic loci, including all breakpoint regions, and are designed to extend several hundred kilobases telomeric to each gene (not fully shown here). **B,** The three possible *PML-RARA* gene fusions *(left side)* and corresponding chimeric mRNA species *(right side).* Because the BCR1 type *PML-RARA* fusion results in the longest transcript, it is also known as the *long* (L) form, in contrast to the BCR3 product, which conversely is designated the *short* (S) form. The BCR2 event, owing to the inconstant intraexonic break-fusion location in each case, is also known as the *variable* (V) form transcript. Reverse transcriptase polymerase chain reaction (RT-PCR) approaches to specifically amplify these various PML-RARA fusion messenger RNA species are schematically depicted by *small red arrows* representing PCR primer locations. It is evident that the PML exon 3 primer can also detect both L-form and V-form transcript types, generating correspondingly larger amplicon fragment sizes. **C,** Representative results of RT-PCR analysis for the PML-RARA fusion mRNA. The lane designations appear in duplicate and are as follows: 100 bp ladder (L); L-form/BCR1 (1); V-form/BCR2 (2); S-form/BCR3 (3); negative control (–); no template (nt). The second through fifth lanes from the left show results obtained with a PML exon 6 and reverse RARA exon 4 primer set. Note that the V-form product is slightly smaller than the adjacent L-form product, consistent with a break-fusion event occurring within exon 6 of PML, generating a shortened transcript. Because there is no corresponding exon 6 primer site in the S-form fusion, no PCR product is produced with this primer set in this lane. The last five lanes of the gel show amplification results with a PML exon 3 primer and the same RARA primer. All three transcript variants can be identified with this approach, although the L-form and V-form products are much larger in size. A further finding with this latter primer set is the presence of multiple bands in L-form and V-form lanes. The additional smaller sized PCR products arise from alternative splicing of exon 5, as well as both exons 5 and 6, in the longer PML-RARA transcript. This finding is seen in all cases of APL with L-form and V-form messenger RNA types. **D,** Interphase D-FISH analysis in a cell with the t(15;17)/*PML-RARA* translocation: the two yellow (or juxtaposed red-green) fusion signals represent the reciprocally translocated *PML* and *RARA* genes on the respective der(15) and der(17) chromosomes; single red and green signals represent the remaining normal genomic regions. Rare cases of APL-like acute myeloid leukemia may have alternate gene fusions involving *RARA,* and FISH can suggest this possibility by revealing *RARA* breakage in the absence of *PML-RARA* fusion. Although either FISH or RT-PCR methods are excellent for initial diagnosis of APL, FISH is not advisable for postconsolidation monitoring of *PML-RARA* minimal residual disease. The assessment of minimal residual disease, which has predictive significance for relapse, requires optimized qualitative, or better, quantitative RT-PCR analysis. *(FISH image courtesy of Ryan Knudson, MBA, and Rhett P. Ketterling, MD, Division of Cytogenetics, Mayo Clinic, Rochester, Minn.)*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Breakpoint regions
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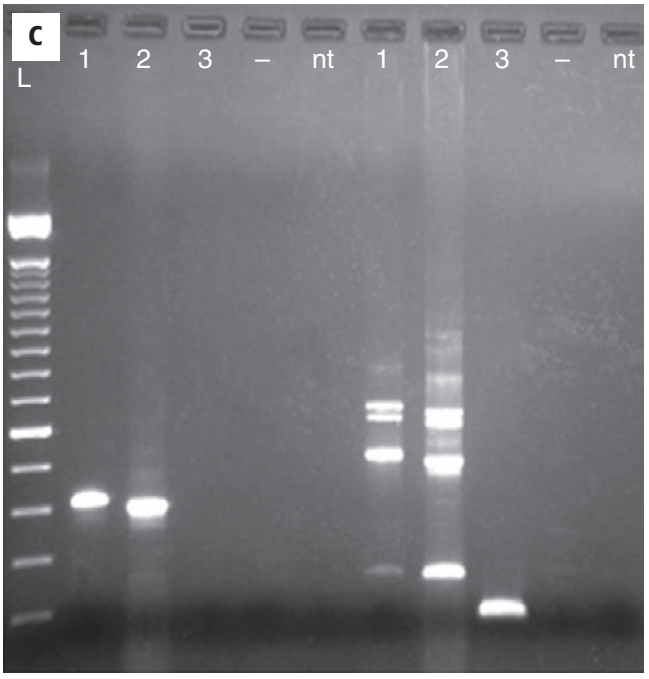
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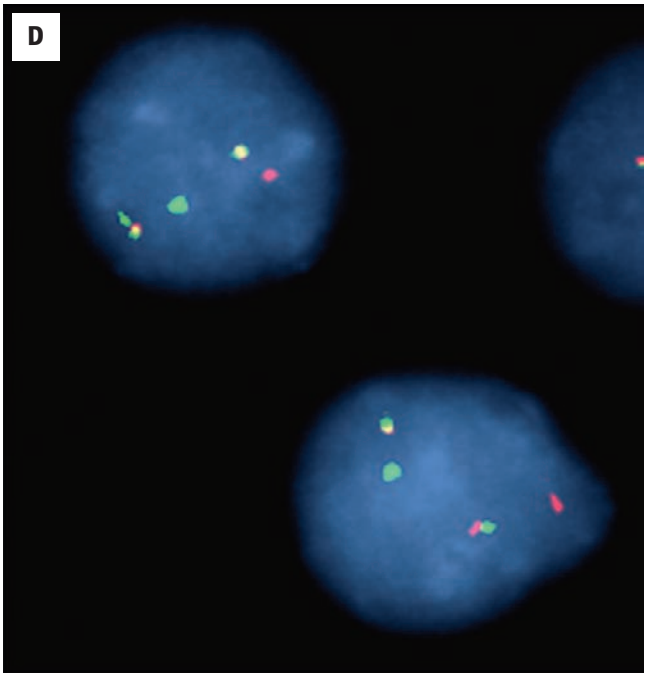
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macromolecular nuclear body complex, appears to have a regulatory influence on transcription, apoptosis, and immune surveillance. Through interference with normal RARα and possibly PML pathway functions, the chimeric PML-RARα protein results in a maturation block at the promyelocyte stage of myeloid differentiation, with the accumulation of neoplastic blasts and promyelocytes. Most importantly, the leukemogenic effects of the PML-RARα oncoprotein can be overcome by administering pharmacologic doses of all-*trans* retinoic acid (ATRA). Initial monotherapy with ATRA induces rapid terminal differentiation of APL cells and in conjunction with subsequent cytotoxic chemotherapy, serves to eradicate the tumor in the majority of patients. In normal bone marrow hematopoietic cells, unbound retinoic acid receptor associates with histone deacetylase and other members of a nuclear protein corepressor complex. This corepressor unit reduces the accessibility of chromatin to transcription factors (and thus locus-specific transcriptional activity), but is reversible in the presence of physiologic concentrations of retinoic acid. In APL, the PML-RARα oncoprotein greatly stabilizes and enhances the state of transcriptional repression by this multiprotein complex; however, therapeutic doses of ATRA can relieve the cellular differentiation block. This relatively low-toxicity "cytodifferentiative" therapeutic approach remains a sought-after paradigm for the management of other acute leukemias and underscores the importance of understanding these neoplasms at the biochemical and molecular genetic level.

The molecular genetic anatomy of the *PML-RARA* abnormality is illustrated in Figure 24-6. One of three PML-RARA fusion mRNA transcripts can result from the t(15;17) event in a given patient with APL, based on breakpoint heterogeneity occurring within the *PML* gene. The two most frequent transcripts are designated BCR1 (also long [L]-form) and BCR3 (also short [S]-form), arising from breakpoints in *PML* intron 6 and intron 3, respectively; together, these transcript types are encountered in almost 95% of APL cases. The rare BCR2 (or variable [V]-form) fusion mRNA results from unusual breakpoint events that occur within exon 6 of the *PML* gene. The BCR2/V-form transcript differs in size slightly from the BCR1/L-form, because of a variably truncated portion of PML exon 6. *RARA* breaksites invariably involve intron 2, joining exon 3 and the remainder of the gene to *PML* in each case. Amplification of PML-RARA transcripts is readily achieved using qualitative RT-PCR methods, as schematically indicated in Figure 24-6. Interpretation of the PCR results using gel electrophoresis is straightforward, although alternative exon splicing in the longer BCR1 and BCR2 transcripts can produce more complex band patterns. RT-PCR analysis is a rapid and highly specific means of confirming the t(15;17) in patients with suggested APL, in whom early treatment with ATRA can prevent the development of catastrophic APL-associated coagulopathy. RT-PCR is advantageous to establish the particular PML-RARA mRNA type for subsequent molecular residual disease detection. The t(15;17)/*PML-RARA* abnormality can also be detected by FISH technique using dual color–dual fusion probes. FISH methods are also rapid and of high utility for initial diagnosis of APL; however, both FISH and RT-PCR should be considered complementary in this regard. FISH is also useful in identifying rare cases of APL-like leukemias, which share morphologic and immunophenotypic similarities with classical APL, but have alternative translocations of *RARA* to genes other than *PML*. These variant AML types are characterized by t(11;17)/ *ZBTB16(PLZF)-RARA*, t(11;17)/*NUMA1-RARA*, t(5;17)/*NPM1-RARA* or t(17;17)/*STAT5B-RARA* abnormalities. Notably, myeloid leukemias with *ZBTB16(PLZF)-RARA* and *STAT5B-RARA* are not responsive to the differentiating effects of ATRA and have a poor clinical outcome. FISH results for *PML-RARA* would be negative in these uncommon cases of AML; however, the presence of *RARA* region breakage is helpful for distinction from true APL and for further subclassification.

Although APL is a potentially curable type of AML, relapses are not infrequent. Typically, molecular reappearance of the PML-RARA mRNA precedes hematologic relapse by several weeks to months, thus enabling a window for early detection and secondary intervention. Qualitative RT-PCR–based molecular monitoring, with a typical analytic sensitivity of $10^{-3}$ to $10^{-4}$, was initially found to be powerful for predicting relapse in individual patients with APL. The timing of molecular MRD evaluation (i.e., phase of disease therapy) is important in this regard. Patients evaluated for PML-RARA transcripts at the end of induction are often found to be positive, and the predictive value for later disease relapse at this time point is unreliable. By the end of consolidation therapy, however, conversion to or persistence of PCR positivity is strongly predictive of relapse in patients who have apparently achieved a clinical complete response. The positive predictive value for overt relapse is also highest in those patients repeatedly testing positive for PML-RARA by RT-PCR on consecutive occasions. Nonetheless, it is also recognized that a significant number of patients with a single negative PCR assessment at the end of consolidation therapy will also suffer relapse, indicating the value of ongoing postconsolidation MRD assessment. Thus a major therapeutic goal in APL is the achievement and maintenance of molecular remission (i.e., PCR-negative status). Recent efforts have focused on increasing the precision of PML-RARA detection while also improving assay sensitivity with the use of reverse transcription RQ-PCR methodology. The results from several clinical studies have not only shown the benefit of using RQ-PCR to detect MRD and predict relapse risk, but have also underscored the ability to successfully treat patients with molecular disease using

second-line therapeutic options (e.g., arsenic trioxide [$As_2O_3$]) and prevent recurrence of hematologic disease. Although guidelines have not been firmly established for PML-RARA transcript monitoring in APL, common sampling time points for RQ-PCR include the end of induction, end of consolidation, then every 2 to 3 months for the first year after therapy, as this is the window during which most relapses occur. More frequent post-consolidation phase MRD evaluation is often performed in patients deemed to be at high risk (e.g., persistent positive PCR status). Of note, PML-RARA monitoring by RQ-PCR technique should be performed on bone marrow aspirate samples, because the sensitivity of transcript detection after treatment onset is 1 to 2 logs lower than in peripheral blood. Beyond diagnosis, the role of FISH for MRD monitoring in APL is virtually nonexistent, because the sensitivity required for prognostic and clinical decision purposes cannot be reliably attained at sample time points beyond induction therapy.

## ABNORMALITIES OF THE CORE BINDING FACTOR GENES IN ACUTE MYELOID LEUKEMIA

Genetic abnormalities involving the heterodimeric core binding factor (CBF) transcriptional complex are relatively common in the acute leukemias. CBF is a critical transcription factor composed of α and β peptide subunits and is involved in the coordinate regulation of numerous cellular processes involved in normal hematopoietic cell differentiation. CBF binds to its cognate core enhancer DNA motif and facilitates access of other transcriptional regulators to genes associated with lymphoid and myeloid maturation. Two translocation events, the t(8;21)(q22;q22) and inv(16)(q13q22) or related t(16;16)(q13;q22), are found in approximately 15% to 20% of de novo AML and target the components of CBF. The t(8;21) is often associated with AML with maturation, whereas the inv(16) and t(16;16) are strongly correlated with the morphology of acute myelomonocytic leukemia with abnormal eosinophils. The t(8;21) abnormality results in the formation of a fusion gene *RUNX1-RUNX1T1* (alternate nomenclature for *RUNX1* includes *CBFA2* or *AML1,* and for *RUNX1T1*, *ETO,* or *MTG8*). The *RUNX1* gene encodes the DNA binding α-subunit of CBF (CBFα), whereas the normal *RUNX1T1* gene produces an unrelated transcription factor. The inv(16) and t(16;16) both create a chimeric fusion gene involving *CBFB* (which produces the β-subunit of CBF [CBFβ]) and a smooth muscle myosin heavy chain gene *MHY11*. Despite differences in morphologic and cytogenetic features, both *RUNX1-RUNX1T1* and *CBFB-MYH11* AML subtypes share in common the disruption of normal CBF transcriptional activity, leading to defective cellular differentiation and leukemogenesis. Clinically, these two genetically defined AML subtypes have been associated with relatively favorable chemotherapeutic responses to cytarabine-based treatment, compared to de novo AML in general.

The *RUNX1-RUNX1T1* gene fusion essentially produces a single chimeric mRNA species that is readily detectable by standard RT-PCR methods. In contrast, *CBF*β*-MYH11* is more complex based mainly on substantial breakpoint heterogeneity in the *MYH11* gene, resulting in at least 10 described fusion mRNA species. Nevertheless, the majority (90% to 95%) of inv(16) and t(16;16) AML cases demonstrate one common CBFβ-MYH11 transcript (type A), with less frequent chimeric mRNA types accounting for the remainder. RT-PCR assays have been used to detect the common type A transcript, as well as many of the rare variant CBFβ-MYH11 forms. The genetic locus rearrangements in both t(8;21) and inv(16) are also readily amenable to detection using standard FISH techniques. FISH is especially helpful for diagnosing inv(16) and the related t(16;16) abnormalities, which can be more subtle and difficult to identify by standard karyotype banding. Of note, a substantial subset of CBF AML (approximately 10% to 20%) also has concurrent point mutations in the *KIT* gene, which encodes the tyrosine kinase receptor for stem cell factor. Mutations often involve the common D816 codon in exon 17 (also altered in most cases of systemic mastocytosis) or exon 8. The presence of *KIT* mutations has been associated with poor outcome in some, but not all clinical studies. The value of post-therapeutic molecular monitoring in patients with CBF-associated AML has been examined in several studies. Patients with the RUNX1-RUNX1T1 abnormality can have very low levels of leukemic mRNA detected in the bone marrow, even in individuals who have achieved stable long-term remission. The CBFβ-MYH11 type A transcript also serves as a tumor-specific marker of disease persistence, although the rapidity of hematologic relapse is sometimes not correlated well with relative transcript levels. In either case, serial time-point measurements using RQ-PCR technique are required for most accurate prediction of relapse risk.

## RARE RECURRENT TRANSLOCATION ABNORMALITIES ACUTE MYELOID LEUKEMIA

Several uncommon AML variants are defined by an association with specific translocation events that can be detected by routine karyotyping and each account for less than 5% of de novo cases of AML. These include the t(9;11)/*MLLT3-MLL*, t(6;9)/*DEK-NUP214*, inv(3) or t(3;3)/*RPN1-EVI1,* and t(1;22)/*RBM15-MKL1* abnormalities, each with associated clinicopathologic correlations. *MLL* gene rearrangements in AML are slightly more frequently encountered in pediatric patients. The *MLL* gene locus is commonly evaluated for rearrangements in AML using BAP probe FISH technique, although this approach does not define the *MLL*

partner gene. More comprehensive *MLL* D-FISH assays have also been described to identify specific translocations in this setting. The role of molecular diagnostic evaluation in other rare translocation AML subtypes is limited beyond morphology and standard cytogenetics studies.

## ACUTE MYELOID LEUKEMIA WITH SINGLE-GENE MUTATION ABNORMALITIES

A growing number of single-gene mutation abnormalities have been associated with all genetic subtypes of AML, but in general their prognostic relevance has been mainly determined in the context of cytogenetically normal (CN) AML. The most common and best characterized abnormalities include mutations of the *FLT3, NPM1,* and *CEBPA* genes. Some mutation events induce discrete small insertions or partial segmental duplications of a gene coding region, whereas others cause single point mutations, which can be distributed widely over a large portion of a gene. Therefore different molecular techniques are often required for comprehensive mutation screening. Furthermore, the pathogenic effect of one gene mutation may be significantly influenced by concomitant mutations in other genes, indicating that these alterations are increasingly not considered as standalone prognostic factors, but require interpretation in the context of one another. New mutations are continually being added to the spectrum in CN AML (e.g., *RUNX1, KIT, WT1, IDH1, IDH2,* partial tandem duplications of *MLL,* or *MLL*-PTD) and a major challenge will be to integrate potentially multiple interacting gene mutations into a rational framework for prognostic assessment in these leukemias. Accordingly, this effort will depend on appropriately powered correlative clinical studies with robust molecular methods and, critically, high-quality bioinformatics analyses. The field of investigation becomes even more complex with the addition of gene overexpression abnormalities (e.g., *BAALC, ERG*) and the burgeoning new field of microRNA transcriptional–translational regulation. Aside from the inherently difficult challenge of evaluating the clinical significance of continuously variable biomarkers, new molecular diagnostic approaches will have to be developed to ensure accuracy and reproducibility. This section focuses on the major single-gene mutations in CN AML associated with clinical outcome.

### ABNORMALITIES OF THE *FLT3* GENE

The *FLT3* gene located on chromosome 13(q12) encodes a transmembrane receptor tyrosine kinase that is highly regulated during normal hematopoiesis. Through interaction with its ligand, *FLT3* signaling is an important part of the complex system of stem–progenitor cell maintenance and lineage propagation. Mutations of the *FLT3* gene are relatively common in AML and most frequently occur as an internal tandem duplication (ITD) of the juxtamembrane coding region. The open reading frame of the gene is maintained despite this partial duplication abnormality, and the net result is ligand-independent, constitutive activation of the receptor. Less often, a heterozygous point mutation of codon D835 can be found (in the absence of an ITD), affecting the activation loop region of the protein. Together, these two *FLT3* mutation events are found in approximately 20% to 30% of de novo adult AML, with most patients having normal cytogenetics. *FLT3* mutations are also described in a smaller proportion of childhood AML. *FLT3* gene abnormalities are not restricted to CN AML and can be seen in other genetically defined subtypes of AML, notably in APL. Therefore the presence of *FLT3* alteration is not considered to be a disease-defining genetic finding, but more so a key pathogenetic feature of leukemogenesis. Nonetheless, the prognostic significance of *FLT3* mutation is most apparent in CN AML. *FLT3*-positive AML cases with normal karyotype are associated with poor treatment response, increased relapse risk, and decreased survival. More specifically, this prognostic effect is clearly defined for *FLT3* ITD abnormalities, especially if the ratio of *FLT* mutated to *FLT3* wild type alleles is high. The effect of *FLT3* D835 alteration is less well understood and remains somewhat controversial. Interestingly, in APL the presence of a concurrent *FLT3* mutation correlates with higher initial white blood cell count, but does not appear to predict an adverse outcome. This finding indicates that, beyond its role as a cooperating mutation in some APL cases, *FLT3* does not otherwise substantially affect the response of neoplastic blasts and promyelocytes to current therapies. Thus *FLT3* testing in APL is not recommended.

*FLT3* ITD mutations occur in exon 14/15 regions of the gene and are detected by targeted PCR of sample DNA, followed by accurate fragment size identification (e.g., by fluorescent capillary electrophoresis). The *FLT3* ITD is seen as a larger amplicon shifted away from the expected wild type product. Although detection of the ITD abnormality is relatively straightforward, a greater degree of assay refinement is required to reproducibly evaluate mutated-to–wild type ratios at diagnosis, although this semiquantitative type of analysis is more often undertaken in the research setting at present. Point mutations involving the D835 site can be identified by several methods, such as allele-specific PCR, DNA sequencing, or restriction enzyme digestion of the PCR product.

### ABNORMALITIES OF THE *NPM1* GENE

The gene *NPM1*, located on chromosome 5q35, encodes nucleophosmin, a nuclear-cytoplasmic shuttle protein

previously described in the section on *ALK*-positive anaplastic large cell lymphoma. *NPM1* is mutated in 50% to 60% of CN AML, and such cases are often characterized by CD34 antigen negativity and monocytic differentiation. Alterations of *NPM1* in CN AML are heterozygous and occur in exon 12, consisting of small insertion events. The mutations lead to frame shifts in the C-terminal region of the translated protein, which encompasses the nuclear localization signal. Consequently, abnormal NPM1 protein accumulates in the cytoplasm of leukemic cells, abrogating its normal regulatory functions affecting the cell cycle and mitosis. More than 25 distinct *NPM1* mutant alleles have been described in CN AML, but the majority of these (approximately 80%) involve a common TCTG tetranucleotide insertion (type A). Because *NPM1* mutations alter the length of the exon 12 nucleotide sequence, PCR with fragment length product analysis (e.g., by fluorescent capillary electrophoresis) provides a powerful way to identify this abnormality. PCR methods using DNA as a template require the use of at least one intron-spanning primer in order to prevent amplification from known *NPM1* pseudogenes. The presence of *NPM1* mutation is associated with relatively favorable clinical outcome in CN AML; however, this relationship is highly dependent on the status of the *FLT3* gene. Concurrent mutation of *FLT3* (found in more than one third of *NPM1* mutated cases) nullifies the favorable prognostic effect of *NPM1* and in fact confers a similar outlook as for *FLT3* mutation alone. Therefore clinical molecular diagnostic testing for *NPM1* must be performed in conjunction with *FLT3* mutation evaluation. Fortunately, similar molecular techniques can be used to identify both gene mutations using leukemic cell DNA. The analytic sensitivity for typical fluorescent PCR product fragment length analysis is in the range of 5% to 10%; this level may be marginally adequate with some patient samples (e.g., a low blast count peripheral blood) or in some cases of AML with clonal heterogeneity (e.g., a small subclone with the mutation). Additional methods for detecting *NPM1* mutations include direct sequencing or fluorescent dye melting curve analysis of PCR products. Some investigators have developed RQ-PCR methods to very sensitively detect specific *NPM1* mutations (e.g., the type A insertion) for minimal disease monitoring; standard diagnostic assays should not be used for this purpose.

## ABNORMALITIES OF THE *CEBPA* GENE

The *CEBPA* gene produces an important transcription factor responsible for promoting granulocytic maturation, as well as other regulatory cellular functions. Mutations in *CEBPA* (located on chromosome 19q13.1) are observed in approximately 10% of de novo AML patients, most of whom have normal cytogenetics. In these individuals, the presence of *CEBPA* mutations is associated with favorable treatment responses and survival. Recent data indicate that the beneficial effect of *CEBPA* alterations is limited to cases showing biallelic mutations of the gene; heterozygous mutations do not appear to have the same effect on improved outcomes, although these may still be cooperative with other gene mutations in the process of leukemogenesis. Furthermore, the simultaneous presence of the *FLT3* ITD abnormality, though uncommon, is a dominantly adverse prognostic finding in *CEBPA* mutated CN AML. For reasons that are not well appreciated, *NPM1* gene mutations are relatively rarely encountered in AML with *CEBPA* mutations. Because *CEBPA* mutations can be distributed throughout the gene, but mostly in 5′ and 3′ coding regions, RT-PCR of *CEBPA* mRNA and direct sequencing of the PCR-amplified products is currently the most comprehensive technique used for detection, although denaturing high-performance liquid chromatography has also been advocated for rapid mutation screening.

## EFFECTS OF OTHER SINGLE GENE ABNORMALITIES IN ACUTE MYELOID LEUKEMIA AND THE CHALLENGE OF MULTI-GENE MUTATION ANALYSIS

Apace with technologic advances in laboratory medicine, the number of additional genetic mutations in the large subgroup of CN AML continues to expand. Partial tandem duplications of the *MLL* gene (*MLL*-PTD), and mutations of the Wilms tumor *(WT1),* core binding factor α *(RUNX1)* and isocitrate dehydrogenase 1 and 2 *(IDH1, IDH2)* genes have each been found in approximately 5% to 10% of de novo AML, typically with normal cytogenetics. All these genetic changes have been linked to poor clinical outcome. Notably for the *IDH* genes, recent data have shown a significant adverse effect of missense point mutations apparently within the subset of patients with mutated *NPM1* and absence of *FLT3* ITD. *IDH* mutations are localized to exon 4 in either gene and nearly always involve codon 132 of *IDH1*, or codons 140 or 172 of *IDH2*, thus providing for relatively simple detection by allele-specific PCR, high-resolution melting curve analysis, denaturing high-performance liquid chromatography, or direct sequencing of PCR products. As is evident in discussing the molecular diagnostic evaluation of CN AML, the degree of prognostic effects of any individual gene mutation will necessitate thorough multivariate analytic evaluation, particularly for mutational changes that appear to show a substantial survival or treatment benefit. Given the current status of therapeutic management for AML, the clinical utility of combined single-gene mutation prognostic markers serves to identify "better risk" individuals who might benefit from

chemotherapy alone (e.g., *CEBPA* or *NPM1* positive), versus others at high risk for failure and disease relapse, in whom allogeneic stem cell transplantation for eligible patients may offer a life-saving alternative. Finally, because of the differing molecular nature and distribution of such mutations and the potential for subclonal heterogeneity in AML, a variety of methodologic approaches are presently required in the diagnostic laboratory to maximize detection sensitivity and specificity (both analytic and clinical). Ideally, adoption of a single platform for molecular diagnosis and prognosis of AML would achieve greater uniformity; the development and application of next-generation high-throughput DNA sequencing holds much promise in this regard and can generate sufficient depth of genomic coverage to address the problems inherent to heterogeneous subpopulations of leukemic cells. This powerful technology is also briefly summarized in the section on Emerging Technologies.

## MOLECULAR DIAGNOSTIC EVALUATION OF ACUTE LYMPHOBLASTIC LEUKEMIAS: DETECTION OF SPECIFIC GENETIC ABNORMALITIES FOR DIAGNOSIS AND CLASSIFICATION

### B-CELL LYMPHOBLASTIC LEUKEMIA–LYMPHOMA

The molecular genetics of B-cell lymphoblastic neoplasms (precursor B-cell acute lymphoblastic leukemia [B-ALL]), especially in the pediatric population, represent a paradigm for linking tumor genetics with clinical outcomes. In childhood B-ALL, genomic alterations can be segregated according to the presence of numeric or structural chromosomal abnormalities. These pathogenetic events have distinct prognostic value and can help to define patient subsets with differential risk of relapse. Among adult patients, the profile of genetic aberrations is dissimilar and less well understood; correspondingly, the natural biologic behavior and therapeutic approach in adults are different. This section is primarily focused on the utility of cytogenetic and molecular diagnostic assessment of childhood B-ALL, with appropriate reference to the adult counterpart when diagnostically relevant. Genetic abnormalities in T-cell lymphoblastic leukemia–lymphoma (T-ALL) continue to be better defined and include translocations of *HOX* genes with the T-cell receptor gene loci, *NOTCH1* gene mutations, *TAL1* oncogene activation, *JAK1* mutations, and rare examples of gene fusions involving *ABL1*. However, these abnormalities have not yet become commonly integrated in the diagnostic or prognostic assessment of T-ALL and are not further detailed in this section.

### COMMON GENETIC ABNORMALITIES IN CHILDHOOD B-ALL

Childhood B-ALL cases with chromosomal aneuploidy (i.e., hyperdiploid or hypodiploid) are strongly associated with clinical outcome. Patients with hyperdiploid karyotype, defined by a chromosome complement greater than 52, are associated with highly favorable treatment responses and long-term remission. More specifically, those tumors with trisomic copies of chromosomes 4, 10, and 17 are considered to have an excellent prognosis. This subgroup represents approximately one third of patients. Conversely, the presence of hypodiploid chromosome number (less than 44) defines a small group of individuals with aggressive disease and treatment failure. These patients may be considered as early candidates for allogeneic stem cell transplantation. The presence of tumor aneuploidy can be determined by standard cytogenetics or application of multiple FISH probes, or it can be inferred from flow cytometric DNA ploidy analysis of lymphoblast cell nuclei with targeted enumeration probe FISH studies to assess for specific (trisomic) chromosome anomalies.

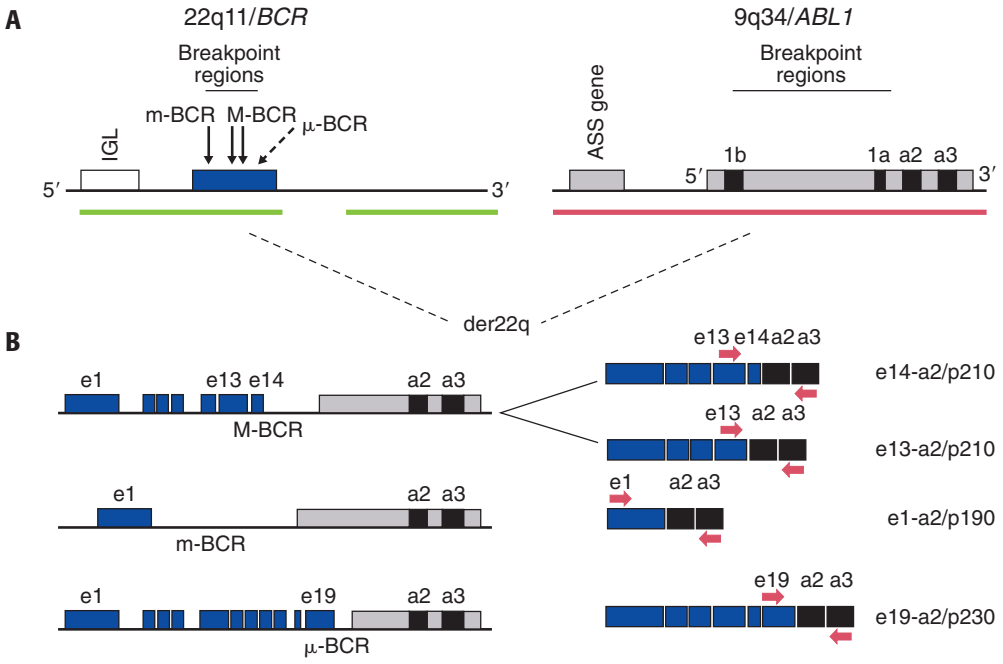
Recurrent chromosome translocations are also relatively common in childhood acute lymphoblastic leukemia (ALL) and four of these account for approximately 25% of cases (see Table 24-3). Representing 3% of B-ALL in children and 20% to 25% of adult patients, the t(9;22)(q34;q11)/*BCR-ABL1* abnormality (Philadelphia [Ph] chromosome) is associated with a very poor prognosis typified by primary chemoresistance and high relapse risk. Approximately 80% of pediatric and two thirds of adult Ph-positive B-ALLs produce a BCR-ABL1 fusion mRNA termed *e1-a2,* resulting in a 190-kD oncoprotein. Remaining cases are associated with a p210 protein and corresponding e13-a2 or e14-a2 BCR-ABL1 transcripts. The molecular anatomy of the *BCR-ABL1* fusion gene is discussed in more detail in the following section on chronic myeloid leukemia (CML) and is illustrated in Figure 24-7. The adverse outcome of *BCR-ABL1*–positive B-ALL also appears to be enhanced by the presence of *IKZF1* gene deletions and (rarely) nucleotide point mutations. *IKZF1* encodes a zinc finger DNA binding protein, Ikaros, which is a critical transcription factor for normal lymphoid cell development. Recently, *IKZF1* deletions have also been identified in some Ph-negative B-ALL cases with poor outcomes, which share gene expression signature alterations in common with *BCR-ABL1* positive tumors, suggesting that loss of function of the *IKZF1* gene product itself underlies a fundamentally aggressive disease pathobiology. The identification of additional cooperating mutations (e.g., *CRLF2*, *JAK* genes) in Ph-negative B-ALL with *IKZF1* deletions has helped to define a greater degree of genetic complexity than previously appreciated among the subgroup of pediatric patients with poor treatment response.

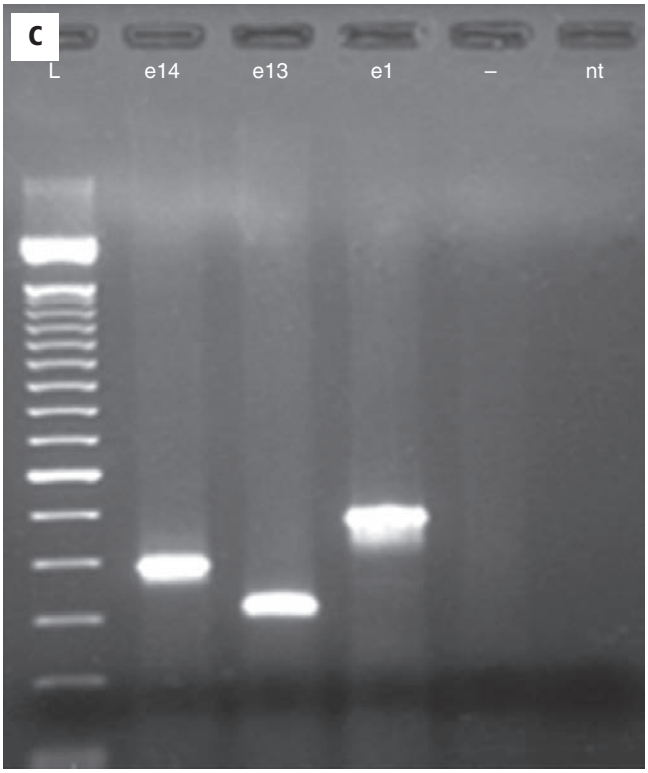
The t(12;21)(p13;q22)/*ETV6-RUNX1* (previously *TEL-AML1*) is the most common single genetic abnormality in childhood B-ALL (approximately 20% of patients at diagnosis), but this abnormality is virtually absent in adult B-lineage ALL. Significantly, *ETV6-RUNX1*–positive patients have a favorable clinical outcome following chemotherapy, similar to the group with hyperdiploidy and specific (i.e., good prognosis) chromosome trisomies. The occurrence of late relapses in *ETV6-RUNX1* B-ALL is a concern for a subset of these individuals, although secondary treatment responses are often successful. The *ETV6* gene, which encodes an Ets family transcription factor, is joined with *RUNX1* to form the chimeric fusion gene on chromosome 12p. The hybrid ETV6-RUNX1 protein disrupts the normal function of CBF transcriptional activity and, as with the t(8;21)/*RUNX1-RUNX1T1* in AML, this leads to profound alterations in progenitor cell proliferation and differentiation control. In addition, a large proportion of *ETV6-RUNX1* lymphoblastic leukemias also have deletions of the remaining *ETV6* allele, indicating that complete inactivation of ETV6 function may also be pathogenetically important. In the majority of t(12;21)-positive B-ALLs, the translocation is cytogenetically cryptic, requiring either RT-PCR or FISH molecular methods for detection. Breakpoints in the *ETV6* gene are generally invariant within intron 5; however, the *RUNX1* gene can show heterogeneity both in genomic break-site location and by alternative splicing of RUNX1 exons in the chimeric mRNA. This situation leads to as few as one and potentially as many as four ETV6-RUNX1 fusion transcripts that can be amplified and detected by RT-PCR technique in any given patient. The most frequent types, present in nearly all cases, involve fusion of ETV6 exon 5 with exons 2 or 3 of RUNX1. FISH technique also readily detects the *ETV6-RUNX1* translocation in pediatric B-ALL, and this approach further enables the simultaneous identification of an accompanying deletion of the nontranslocated *ETV6* allele, if present.

Slightly less than 5% of pediatric B-lineage ALL, most commonly with a pre-B cell (cytoplasmic immunoglobulin M positive) immunophenotype, harbor an unbalanced t(1;19)(q23;p13) with the resultant formation of a chimeric *TCF3-PBX1* oncogene (the *TCF3* gene is also known as *E2A*). In keeping with the gene fusion concept in the pathogenesis of many leukemias, this abnormality joins elements of two disparate transcription factors to create a novel leukemogenic TCF3-PBX1 protein. TCF3 is an important mediator of normal lymphoid cell and myocyte development, whereas PBX1 is a DNA binding protein that is not expressed in normal lymphocytes. Leukemia patients with this translocation fusion gene are considered to have more aggressive disease biology, although once identified, more intensive treatment options can achieve long-term remission in most cases. A single chimeric TCF3-PBX1 mRNA is produced in nearly all cases from the consistent intronic breakpoint regions in both genes, and this transcript can be readily detected by RT-PCR analysis; FISH technique can also be used to detect the genomic abnormality. Rare occurrences of a balanced t(1;19)(q23;p13) also occur in B-ALL and do not involve the *TCF3* and *PBX1* genes, rather they create novel *DAZAP1-MEF2D* and reciprocal *MEF2D-DAZAP1* gene fusions associated with poor outcome.

The *MLL* gene (also known as *ALL1, HRX1*), located on chromosome 11(q23), is implicated in approximately 5% of childhood leukemias, although the majority of these cases occur in infants younger than 1 year. Infantile acute leukemias with *MLL* gene rearrangements are of B-cell lineage and manifest in a clinically aggressive fashion with a dismal prognosis, particularly when associated with a poor therapeutic steroid response. The *MLL* gene becomes juxtaposed in such cases with another gene locus, resulting in the formation of a chimeric gene product with concomitant disruption of MLL function. In normal mammalian cells, the MLL protein differentially regulates a set of homeobox (or *HOX*) genes, which are variably responsible for coordinated skeletal development during embryogenesis and, significantly, normal post-fetal hematopoietic progenitor cell development. Accumulating evidence suggests that MLL fusion proteins can promote leukemogenesis by aberrantly deregulating certain *HOX* gene subsets in hematopoietic stem cells. The central role of *MLL* in this neoplastic process is underscored by the numerous (greater than 50) described reciprocal pairings at other partner genetic loci. Of these, the t(4;11)(q21;q23) is most commonly encountered in infantile ALL, resulting in the formation of the *MLL-AFF1* fusion. In this event, the *MLL* gene exhibits substantial genomic breakpoint heterogeneity encompassing a large region containing exons 5 to 11. Likewise, the *AFF1* gene (also known as *AF4*) also shows variability of breakpoint location among B-ALL cases. A second translocation, the t(11;19)/*MLLT1*(or *ENL*)-*MLL* abnormality, is also encountered in infant B-ALL, as well as lymphoblastic and acute myeloid leukemias in older children and adults. Although one MLL fusion transcript type is associated with any given leukemic tumor, the propensity for many possible chimeric transcripts arising from *MLL* gene fusion events coupled with the large number of potential rearranging partner genes in *MLL* translocations creates significant challenges for detection. Multiplex RT-PCR approaches have been designed to identify many different *MLL* gene fusions using a single platform; however, comprehensive molecular evaluation of *MLL* in childhood leukemias by PCR methods remains problematic due to both biologic and technical complexity. FISH technique based on a BAP probe strategy (with probes spanning either side of the breakpoint cluster region in the *MLL* gene) is well suited for the rapid identification of *MLL* translocations. This strategy is

diagnostically sensitive for identifying *MLL* rearrangements regardless of the translocation partner. D-FISH probe strategies can then be used to specifically define the more common translocation variants. FISH analysis also discriminates between true 11(q23)/*MLL* locus rearrangements and genetic recombinations not involving *MLL*, but clustering within the 11(q22-q25) region; this distinction may be difficult to discern by routine cytogenetic analysis. On occasion, distal deletions of the *MLL* locus may accompany a true *MLL* gene translocation event with a resulting "deletion only" signal pattern suggested by BAP FISH technique. In these situations, confirmation of an abnormal *MLL* gene fusion can be obtained by targeted D-FISH or RT-PCR analysis.



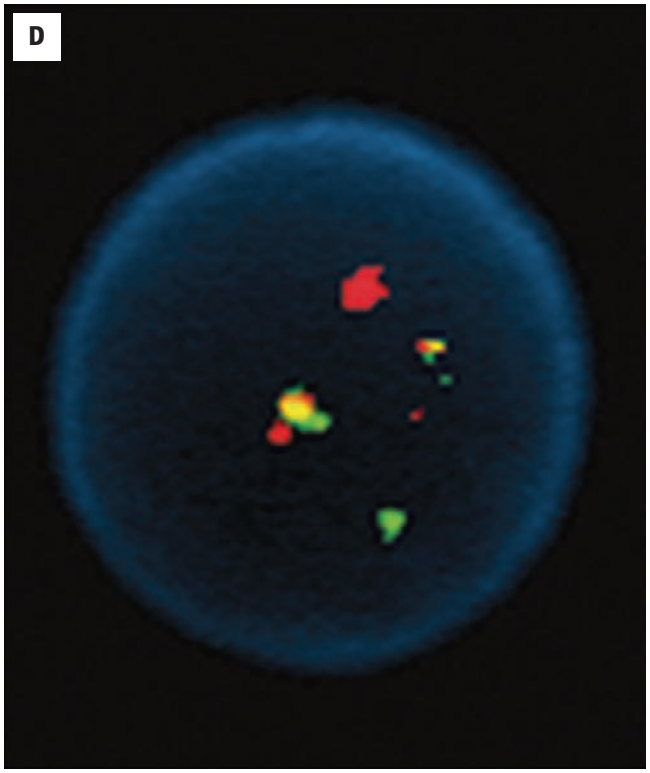




**FIGURE 24-7**

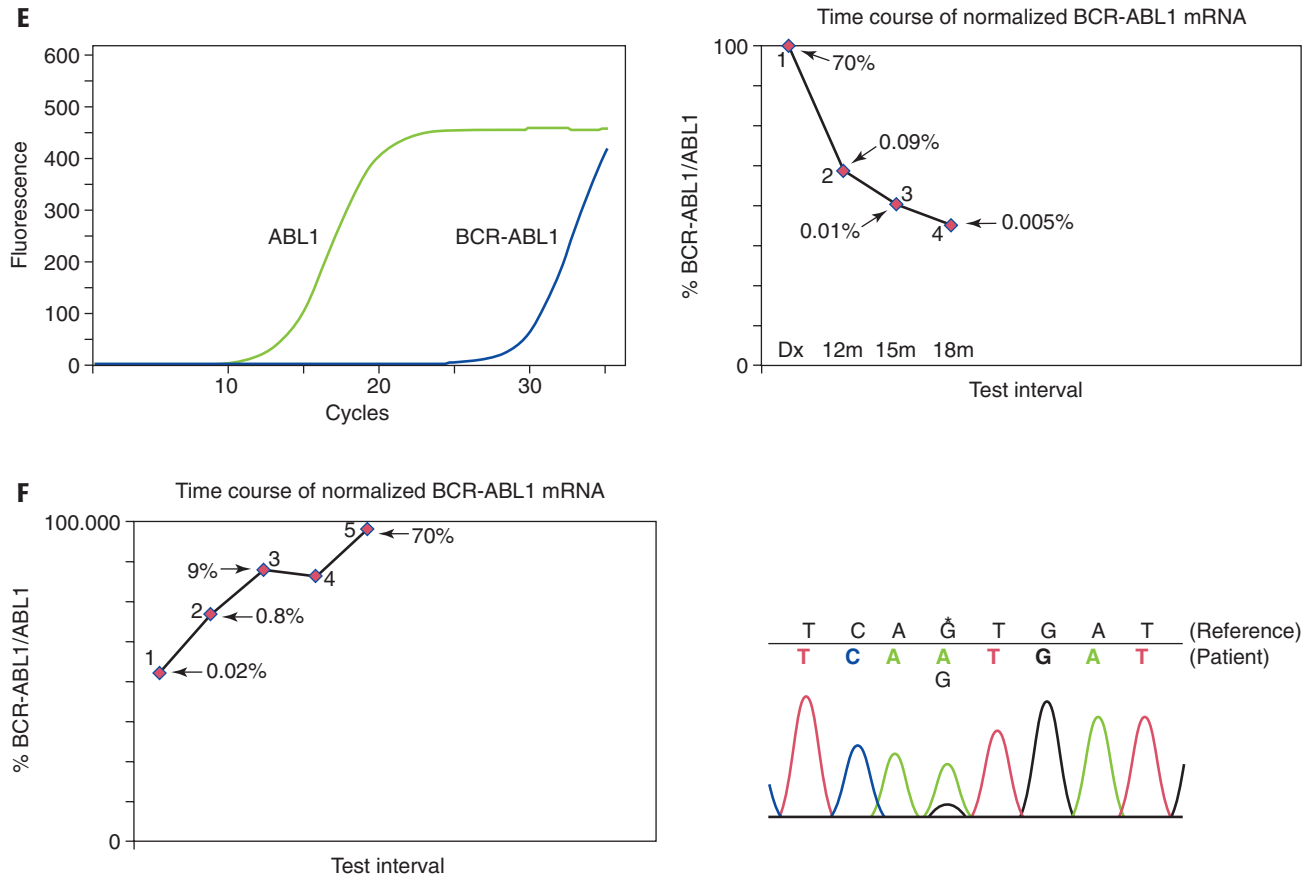



**FIGURE 24-7, cont'd**

The t(9;22)/*BCR-ABL* abnormality. **A,** The arrangement of *BCR* and *ABL1* genes on their respective chromosomes. Other genes (e.g., the immunoglobulin λ light chain gene, *IGL*) are also shown for relative orientation (gene maps are not shown to proper genomic scale, especially *BCR*, which is represented as a single locus rather than the individual exons). Genomic breakpoints in *ABL1* occur variably over a large region of DNA representing intron 1 of the gene. However, the subsequent juxtaposition with *BCR* results in the consistent presence of *ABL1* exon 2 (a2) in the *BCR-ABL1* fusion gene. *BCR* breakpoints are heterogeneous. Essentially all cases of chronic myeloid leukemia (CML) harbor breakpoints in the major breakpoint cluster region (M-BCR), whereas the majority of adult Ph+ B-lineage acute lymphoblastic leukemia (Ph+ ALL) and most cases of pediatric Ph+ ALL are characterized by break-sites occurring in the minor breakpoint cluster region (m-BCR). However, nearly one third of adult and a small minority of pediatric Ph+ ALL may also manifest M-BCR type breakpoints. A third breakpoint region, the μ-BCR, is situated farther 3′ in the *BCR* gene and is associated with a rare predominantly neutrophilic form of CML. Fluorescence in situ hybridization (FISH) probes for the *BCR (green bars)* typically span several hundred kilobases on either side of the gene, whereas probes for *ABL1 (red bar)* span the entire gene as well as several hundred kilobases centromeric to cover the argininosuccinate synthetase (*ASS*) gene. Deletion of the 5′ region of the reciprocal *ABL1-BCR,* which includes the latter genetic locus, occurs on the der 9(34) in a subset of CML patients and has been linked in some studies to adverse prognosis. **B,** The common resulting *BCR-ABL1* fusion gene structures on the der 22(q11). M-BCR locus breakpoints result in the fusion of either *BCR* exon 14 or exon 13 to *ABL1* exon a2. The resultant e14-a2 or e13-a2 chimeric messenger RNA (mRNA) species *(right side)* encode a p210-kD BCR-ABL1 oncoprotein. In contrast, the M-BCR breakpoint joins the *BCR* exon e1 with *ABL1* exon a2 to form the e1-a2 transcript and a corresponding p190-kD product. The e19-a2 chimeric fusion also produces a functional leukemic protein, p230. Oligonucleotide primers can detect each of these common BCR-ABL1 mRNA species by reverse-transcriptase polymerase chain reaction (RT-PCR) technique *(small red arrows).* A single e13 primer can identify both e14-a2 and e13-a2 transcript forms, whereas separate primer reactions are required to identify the e1-a2 and e19-a2 mRNA fusions. **C,** Gel image of qualitative RT-PCR for BCR-ABL1 chimeric mRNA. Lanes are as follows: 100-bp ladder (L); e14-a2 fusion (e14); e13-a2 fusion (e13); e1-a2 fusion (e1); negative control (–); no template (nt). Although the native e14-a2 and e13-a2 transcripts are longer than the e1-a2 mRNA, the placement of primers results in a larger e1-a2 amplicon length by PCR. **D,** Interphase D-FISH analysis in a cell with the t(9;22)/*BCR-ABL1* translocation. The two yellow fusion signals represent the reciprocally translocated *ABL1* and *BCR* genes on the respective der(9) and der(22) chromosomes; single red and green signals represent the remaining normal genomic regions. Alternate FISH patterns affecting one of the fused signals may be encountered with D-FISH method if 5′ or 3′ deletions occur on the der(9) chromosome. **E,** The *left panel* illustrates results from real-time quantitative (RQ-PCR) analysis for BCR-ABL1 mRNA in a patient successfully treated with the tyrosine kinase inhibitor (TKI) imatinib mesylate (*blue trace* is patient BCR-ABL1 transcript; *green trace* is an amplification plot of endogenous sample ABL1 mRNA, for normalization and calculation of a quantitative BCR-ABL1/ABL1% ratio). The graphical data *(right)* demonstrates a desirable response over time to TKI therapy in CML. Following initiation of drug treatment at diagnosis, the patient achieved rapid complete cytogenetic response (not shown), followed by a greater than 3-log reduction (i.e., less than 0.1%) of BCR-ABL1/ABL1 level by 12 months. This situation is associated with low risk of disease progression. Conversely, **F** shows a graph of a patient with rapidly increasing normalized BCR-ABL1 transcript amounts *(left);* within the first two consecutive time-points, an increase of greater than 2 logs is documented. A serial increase in BCR-ABL1 mRNA of 0.5 log (or greater) magnitude suggests imatinib resistance because of acquired *ABL1* region kinase domain mutations (KDM) in the *BCR-ABL1* oncogene. KDM analysis of the patient sample *(right)* by long transcript BCR-ABL1 RT-PCR and sequencing demonstrates the pan-resistant T315I abnormality (reverse strand sequence is shown with a G>A nucleotide change, indicated with an asterisk, corresponding to a C>T substitution at codon 315). *(FISH image courtesy of Abdul Al-Saadi, MD, William Beaumont Hospital, Royal Oak, Mich.)*

Next-generation resequencing of the 11(q23) region is a promising new approach for comprehensive single-pass evaluation of potentially numerous *MLL* gene rearrangements in these leukemias.

Further underscoring the centrality of MLL dysregulation in leukemogenesis is the finding of *MLL* translocations in the subgroup of therapy-related, or secondary AML following treatment with DNA topoisomerase II–inhibiting agents (e.g., etoposide, daunorubicin). Therapy-related AML is associated with a poor prognosis and, remarkably, the gene breakage sites situated in the 3′ region of the *MLL* breakpoint cluster overlap with those of de novo infant *MLL*-positive B-ALL. Several studies have examined the underlying basis for greater genetic fragility in this genomic region, and this has led to speculation regarding the role of in utero exposure to exogenous substances that can potentiate *MLL* gene breakage and translocation, leading to rapid development of B-ALL. The concept of leukemia initiating in the prenatal period is supported by intriguing investigations backtracking identical *MLL* gene rearrangements in individual patient leukemias to their corresponding neonatal blood samples. This finding has similarly been demonstrated in older children with *ETV6-RUNX1* B-ALL and other types of ALL and AML in childhood. However, the latency period to presentation differs between subtypes of genetically defined B-ALL, suggesting that promoting mutations or environmental influences have an important role in leukemogenesis. In addition, healthy newborn or umbilical cord blood population screening studies have shown an appreciable prevalence (approximately 0.1% to 1%) of leukemia-associated translocations events, albeit at low quantitative levels (less than $10^{-4}$). These data again indicate the requirement for cooperating oncogenic mutations and a conducive background in the generation of overt leukemia. Nevertheless, the realization that at least some childhood leukemias have prenatal origins has spurred interest in identifying risk-modifying factors. In this regard, recent evidence shows that low-penetrance population genetic variations in some genes (e.g., *IKZF1*) may increase the risk of developing ALL.

## RISK STRATIFICATION IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AND THE EFFECTS OF MINIMAL RESIDUAL DISEASE ASSESSMENT

The presence of predictive molecular markers in pediatric ALL has significantly augmented the development of more refined risk-adapted therapy practices. This approach is based on the premise of achieving maximum cure rate while minimizing toxic effects and late sequelae such as treatment-related neoplasms. For B-lineage ALL in particular, initial clinical risk assessment (e.g., age, white blood cell and blast count, extramedullary involvement) is further modified by the presence and type of tumor genetic abnormalities. Patients can then be differentiated by low, standard, high, or very high risk of relapse. The presence of the *BCR-ABL1* abnormality, hypodiploid karyotype, or therapeutic induction failure constitutes a very high-risk patient subgroup. Conversely, B-ALL cases with hyperdiploidy (i.e., greater than 52 chromosomes and favorable trisomies), or the *ETV6-RUNX1* gene fusion are considered good prognostic factors. Early classification (by end-induction treatment phase) thereby influences the type and duration of chemotherapy for a given patient. This strategy of tumor genetic risk stratification is used in slightly different formats by most large children's cancer groups. Useful as these parameters have been for more appropriately stratifying patients earlier to receive optimized therapy, a significant minority (20% to 30%) of children, regardless of initial prognostic subgroup, experiences treatment failure and leukemic relapse, indicating some lack of precision in the risk assessment models. Better predictive surrogate markers of biologic response and disease clearance have therefore been sought, and minimal residual disease detection has emerged as a powerful method of post-therapeutic prediction, providing a more individualized approach to outcome evaluation.

Many large clinical studies have now confirmed the power of MRD to determine relapse potential in childhood B- and T-lineage ALL. Levels of MRD as high as $10^{-2}$to $10^{-3}$ at the end of induction therapy have been shown to be highly correlated with risk of relapse, and this relationship has proved to be strongly independent of nearly all currently applied clinical and cytogenetic and molecular genetic factors in multivariate analyses. Even lower levels of MRD ($10^{-4}$or less) are apparently also associated with an incrementally increased risk of failure, compared to MRD negative status. Targets for molecular MRD measurements include clone-specific antigen receptor gene rearrangements (e.g., *IGH@*, *IGK@*, *TRG@*, *TRB@*) and chimeric fusion gene transcripts as described above. Notably in B-ALL, illegitimate gene rearrangements also occur frequently at the T-cell receptor genes, such that most B-lineage tumors have at least one and as many as two to three unique gene rearrangements available for MRD monitoring. The highly specific nature of rearranged antigen receptor Vn(D)nJ regions in lymphoblastic leukemia cells can be amplified and then sequenced for the design and synthesis of tumor-specific PCR primers and probes for use in RQ-PCR analyses. Most commonly, this method relies on a consensus region V or J primer and consensus fluorescent probes in conjunction with an allele-specific oligonucleotide primer; the latter reagent is selected to be complementary to the region of unique rearrangement sequence identifying the leukemic clone. Generation of standard curves for RQ-PCR in this setting requires serial dilutions of the patient's leukemic DNA in a nonclonal DNA background (e.g., pooled

normal donor leukocytes). The typical analytic sensitivity for antigen receptor gene rearrangement RQ-PCR is in the range of $10^{-2}$ to $10^{-5}$, depending on the characteristics of the particular target gene sequence and the degree of nonspecific competition for primers by partially homologous rearrangements in background B or T cells. In this regard, design of primer–probe combinations is relatively complicated, in that multiple antigen receptor gene targets may have to be assessed and possibly multiple primer–probe sequences made and evaluated for obtaining the best sensitivity for a given case of B-ALL or T-ALL. The comprehensive descriptions of the BIOMED-2 and related studies have provided excellent details for using antigen receptor gene approaches for MRD, although the technical complexity and difficulty in cross-institutional standardization have confined these MRD assays to dedicated laboratories with sufficient expertise. In contrast, leukemia-specific fusion gene mRNA can be detected relatively easily using reverse transcription RQ-PCR techniques, with analytic sensitivities that can be in the range of $10^{-4}$ to $10^{-6}$. However, recurrent translocations represent a minority of B-ALL cases (25% to 30%) and fewer in T-lineage tumors.

In light of the difficulties inherent in establishing consistent, specific, and sensitive enough RQ-PCR assays for molecular MRD, other investigators have opted for flow cytometric evaluation of MRD based on quantitative or qualitative alterations in antigen expression by the leukemic blasts. Flow cytometric protocols can achieve comparable analytic sensitivity to molecular methods and have a potential advantage of being more easily standardized between labs. Whether a molecular- or cell marker–based technology is used, the profound effect of MRD detection on relapse prediction is evident, and many childhood leukemia study groups have begun to incorporate MRD data as a routine component of risk assessment, enabling higher risk MRD-positive patients to be rapidly stratified into more intensive therapy arms. Timing of MRD assessment is critical for the derivation of meaningful prognostic data in childhood ALL. In general, the end of induction therapy is a significant and highly informative time point, although ongoing serial measurements can further increase the predictive value. End-induction MRD status, in conjunction with rapid versus slow blast clearance from the bone marrow, has been described as further improving relapse risk prediction for individual patients. MRD positivity later in therapy (e.g., following consolidation) also has prognostic value for relapse risk prediction; however, the sensitivity of MRD detection is poorer at these intervals. Finally, the epidemiology of molecular genetics in childhood B-ALL appears to vary in different global regions (e.g., Far East versus Western patients), again suggesting that MRD evaluation may be a more universal means of prognostication in more diverse patient populations.

## MOLECULAR DIAGNOSTIC EVALUATION OF CHRONIC MYELOID NEOPLASMS

The chronic myeloid tumors represented in this section include several neoplasms grouped by common clinicopathologic characteristics. Specific genetic abnormalities are continuing to subdivide and better categorize seemingly related members of these diseases between and within existing classifications (Table 24-4). These entities include the classic myeloproliferative neoplasms (chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, and essential thrombocythemia), myeloid and some lymphoid neoplasms associated with eosinophilia, and systemic mast cell disease.

### T(9;22)/BCR-ABL1 ABNORMALITY AND CHRONIC MYELOID LEUKEMIA

The t(9;22)(q34;q11)/*BCR-ABL1* abnormality is emblematic of the relationship between genetic mutation and carcinogenesis. Initially recognized as the Ph chromosome associated with CML nearly 50 years ago, the study of this genetic lesion over 4 decades has resulted in a detailed understanding of the pathobiology of *BCR-ABL1* leukemogenesis, as well as the recent development and success of targeted pharmacologic therapies. The *BCR-ABL1* chimeric gene is required for the diagnosis of CML and is also central to the pathogenesis of 20% to 25% of adult and 3% of childhood B-lineage ALL. Although the functional role of the normal BCR gene product is incompletely understood, the abnormal gene fusion deregulates the activity and localization of the ABL1 tyrosine kinase, producing complex effects on cellular signal transduction pathways, proliferation, apoptosis control, and cell adhesion. Two major forms of *BCR-ABL1* exist based on different breakpoint-fusion sites within the *BCR* gene (see Figure 24-7). The major breakpoint cluster region (M-BCR), encompassing a 5.8-kb DNA span, is involved in nearly all cases of CML. Breakpoints in *ABL1* occur in a large intronic region upstream of exon 2. The *BCR-ABL1* gene fusion on the derivative 22(q11) results in the production of an e14-a2 or e13-a2 fusion transcript (formerly b3-a2 or b2-a2 respectively), with a corresponding 210-kD chimeric oncoprotein (p210). Alternative splicing of *BCR* exon 14 frequently generates both e13-a2 and e14-a2 transcripts in the same leukemia. In contrast, Ph-positive B-ALL cases are associated with *BCR* breakpoints occurring in the minor breakpoint cluster region located farther 5′ in the *BCR* gene. The resultant single e1-a2 chimeric mRNA is associated with the production of a 190-kD protein (p190). However, *BCR* gene break-sites and disease phenotypes are not exactly correlated. Although the e14 (or e13)-a2 product (p210) is essentially pathognomonic of CML in the appropriate clinical and

**TABLE 24-4**

**Common Genetic Abnormalities in the Chronic Myeloid Malignancies Detected by Molecular Diagnostic Methods**

| Genetic Abnormality | Disease Associations | Molecular Pathogenesis* | Molecular Diagnostic Detection† | Notes |
|---|---|---|---|---|
| t(9;22)/*BCR-ABL1* | Chronic myeloid leukemia (100%) | Chimeric fusion protein with deregulation of *ABL* tyrosine kinase; effects on cell proliferation, apoptosis, adhesion | RT-PCR for BCR-ABL1 mRNA<br>FISH | FISH is highly specific at diagnosis, but RT-PCR is required to verify BCR-ABL1 transcript for MRD evaluation after therapy; primary goal is attainment of CCR on TKI Rx; MRD detected by RQ-PCR technique has further prognostic value: therapeutic goal is >3-log reduction in BCR-ABL1 transcript equal to MMR (<0.1% normalized transcript level on International Scale); FISH is of little value for disease monitoring following attainment of CCR; evaluation for BCR-ABL1 KDM with loss of TKI Rx response (serial increases in BCR-ABL1 level >0.5 log with loss of MMR, or a loss of CCR |
| *JAK2* exon 14 V617F and exon 12 mutations | Classical Ph-negative chronic MPNs: PV, primary myelofibrosis, essential thrombocythemia (50%-100%) | V617F is a single point mutation in autoinhibitory domain of *JAK2,* causing constitutive activation of tyrosine kinase; exon 12 mutations result in gain of function | Allele-specific DNA PCR, RQ-PCR, or high-resolution melting curve analysis for V617F; PCR and sequencing, or high-resolution melting curve analysis to detect exon 12 insertion and deletion variants | Subset of PV and advanced PMF cases show biallelic V617F mutations; idiopathic erythrocytosis cases associated with exon 12 mutations; rare cases of de novo AML, myelodysplasia (MDS) and atypical MPN/MDS may have *JAK2* V617F mutations |
| *MPL* exon 10 mutations | Rare cases of essential thrombocythemia and primary myelofibrosis | Point mutation causes constitutive activation of thrombopoietin receptor and JAK-STAT signaling; most common mutation site is W515 | DNA PCR and sequencing of exon 10 or allele-specific PCR | Although rare, this finding establishes the presence of an MPN and excludes reactive causes |
| *PDGFRA, PDGFRB,* and *FGFR1* | Myeloid and lymphoid neoplasms with associated eosinophilia | Deregulation of growth factor receptor tyrosine kinases | FISH for specific gene locus rearrangements<br>RT-PCR for FIP1L1-PDGFRA mRNA | Improves classification of a diverse group of malignancies with prominent neoplastic eosinophil component; identifies subset of chronic eosinophilic leukemias responsive to imatinib mesylate therapy |
| *KIT* | Mastocytosis | Deregulation of KIT tyrosine kinase (receptor for stem cell factor) | Allele-specific DNA PCR, RQ-PCR, or PCR and specific exon sequencing | *KIT* D816V in exon 17 is most common mutation, present in 80%-90% of systemic mastocytosis; lower frequency of detection in cutaneous mastocytosis; D816V is not responsive to imatinib therapy |

*Major pathophysiologic effect resulting from genetic lesion.
†Most frequent molecular techniques used for detection.
*CCR,* Complete cytogenetic response; *FISH,* fluorescence in situ hybridization; *KDM,* kinase domain mutation; *MDS,* myelodysplastic syndrome; *MMR,* major molecular response; *MPN,* myeloproliferative neoplasm; *MRD,* minimal residual disease; *mRNA,* messenger RNA; *PCR,* polymerase chain reaction; *Ph,* Philadelphia; *PMF,* primary myelofibrosis; *PV,* polycythemia vera; *RQ-PCR,* real time quantitative PCR; *RT-PCR,* reverse transcriptase polymerase chain reaction; *TKI,* tyrosine kinase inhibitor.

pathologic setting, unusual cases have been described with a myelomonocytic type of hematologic presentation and the e1-a2 derived p190 BCR-ABL1. By extension, approximately one third of adult and a smaller number of pediatric Ph-positive B-ALL patients are associated with *BCR* breakpoints in the M-BCR and the presence of a p210 chimeric transcript. These exceptions illustrate the importance of cellular context (e.g., pluripotent stem cell versus committed lymphoid progenitor) in the generation of *BCR-ABL1* associated diseases. A third type of *BCR-ABL1* event involves a 3′ breakpoint cluster region termed the *μ-BCR* (see Figure 24-7), resulting in the formation of an e19-a2 fusion transcript. This latter anomaly is identified in rare cases of CML exhibiting a variant of the disease with more prominent neutrophilic differentiation. Very rare alternative fusion forms of BCR-ABL1 have also been described in CML or Ph-positive B-ALL, including the e6-a2 type and also transcripts involving ABL1 exon a3 (i.e., e1-a3, e13-a3). RT-PCR assays can be appropriately designed to detect these uncommon variants.

Timely detection of the *BCR-ABL1* abnormality is critical to correctly establish the diagnosis of CML and exclude potential overlapping entities (e.g., the Ph-negative classical myeloproliferative neoplasms, or myeloproliferative–myelodysplastic processes such as chronic myelomonocytic leukemia [CMML] and atypical CML). As indicated, rapid detection of *BCR-ABL1* is also of importance in B-ALL to identify patients with high-risk disease. The schematic in Figure 24-7 illustrates the strategy for RT-PCR amplification of specific BCR-ABL1 fusion mRNA types. Both qualitative and quantitative PCR methods can be used in this regard, depending on the clinical scenario (e.g., diagnosis versus post-therapy monitoring). Standard cytogenetics and D-FISH analyses can also be valuable for diagnosis; however, the role of cytogenetics and FISH, especially in ongoing CML management, has become more circumscribed in the era of tyrosine kinase inhibitor (TKI) monotherapy. The majority of patients with CML in the chronic phase are treated with a single-agent TKI, most often imatinib mesylate, and the success of TKI therapy has led to the development of sophisticated strategies for evaluating response and risk of treatment failure. Key therapeutic milestones with imatinib include achievement of complete hematologic response and subsequently a complete cytogenetic response (CCR). Ideally the latter goal (defined by absence of the Ph chromosome in a good-quality bone marrow karyotype study) should be met within the first 12 months of therapy, following which sequential monitoring of BCR-ABL1 transcript levels by RQ-PCR technique has prognostic value (see Figure 24-7). In CML with CCR, a reduction of three logs or greater in quantitative BCR-ABL1 mRNA amount (relative to the level at diagnosis) constitutes a major molecular response (MMR), which is associated with continuous remission and low relapse risk. BCR-ABL1 transcript levels are typically expressed as a normalized ratio of BCR-ABL1 to ABL1 or another housekeeping gene transcript. Reductions in BCR-ABL1 level of at least two logs at the time of CCR has been strongly correlated with a greater propensity to continue toward a MMR. The original data correlating a decrease of three logs or greater in BCR-ABL1 with good long-term outcome using imatinib therapy were derived from a landmark international group study, and these findings have been subsequently confirmed. Efforts have therefore been undertaken to help individual clinical molecular laboratories evaluate their RQ-PCR platforms in relation to this reference standard. To achieve better uniformity (e.g., for interpreting consecutive patient results in the same lab or for comparing sample results between different labs), a normalized international scale has been proposed based on the use of standardized analytic reagents, such that initial BCR-ABL1/ABL1 levels are designated at 100% and a three logs reduction represents 0.1%. Using this system, serial RQ-PCR monitoring of BCR-ABL1 mRNA in patients with a CCR or MMR can be used to identify a loss of therapeutic drug response, which is heralded by increasing quantitative transcript levels. The minimum threshold of a significant quantitative elevation in normalized BCR-ABL1 varies based on laboratory experience; however, laboratories routinely performing these tests should be able to reproducibly detect fivefold changes in sequential samples. It should be noted that BCR-ABL1 levels are detected at similar concentrations in peripheral blood and bone marrow specimens of CML patients. The ability to use anticoagulated whole blood for RQ-PCR analysis thus reduces the morbidity associated with repeated bone marrow sampling. RQ-PCR evaluation is also clearly of value in monitoring patients who receive allogeneic stem cell transplantation as a curative procedure for CML.

Rising amounts of BCR-ABL1 precede loss of MMR or CCR, depending upon the level of transcript reduction nadir achieved, and can thus be used as a surrogate marker for inadequate treatment response. Therapeutic resistance is relatively uncommon in CML managed with TKI agents and is not fully understood, but approximately 50% of individuals in this subset develop acquired point mutations in the *ABL1* kinase domain region of the *BCR-ABL1* oncogene. Kinase domain mutations (KDMs) most frequently affect codons in the phosphorylation (P) loop, activation (A) loop, and catalytic domain (CD) or at residues known to be critical for drug contact. More than 100 KDMs have been described, and some of the most prevalent amino acid changes with documented imatinib resistance include: M244V, G250E, Q252H, Y253F/H, E255K/V (P-loop), H396P/R (A-loop), M351T, F359V (CD) and T315I, and F317L (contact sites). KDMs are usually detected by qualitative RT-PCR and direct sequencing of the *ABL1* region. Other diagnostic methods include point

mutation screening using high-resolution melting curve analysis of PCR products, pyrosequencing, or allele-specific PCR for common mutations (see Figure 24-7). One practical problem with KDM analysis is related to the quantitative BCR-ABL1 mRNA level; with lower transcript abundance (e.g., near the MMR or 0.1%), successful RT-PCR of a full length BCR-ABL1 template is more difficult to achieve for assessment of the KDM region. As a result, the analytic sensitivity of RQ-PCR is high, but it is correspondingly poorer for ABL1 KDM detection, based on the challenge of obtaining a long transcript amplification product and the inherent limits of sequencing sensitivity (approximately 25%).

Kinase domain mutations differ in their resistance profiles, such that the effects of some can be overcome by simply escalating imatinib dosage. More resistant mutation types can often be successfully overcome by switching to newer generation TKI drugs (e.g., dasatinib, nilotinib, bosutinib). Most significantly, patients with CML developing the T315I abnormality show widespread insensitivity to all currently used TKI agents. As more data accumulates with the use of various TKI protocols, resistance patterns and relative sensitivities are emerging for available drugs, leading to rational management strategies for these patients. Some studies have identified low-level mutations using more sensitive methods in patients with early chronic phase CML who have good therapeutic responses. The clinical significance of this finding is not clear, and current recommendations do not support KDM analysis at diagnosis. KDMs are also more frequently identified in CML with accelerated phase or blast crisis and complicate the use of TKI therapy when acquired in Ph-positive B-ALL.

From the foregoing discussion, it is apparent that the landscape for CML management has become complex, requiring the use of multiple evaluation methods, including cytogenetics and molecular diagnostics. Published consortium guidelines for de novo CML now comprehensively detail the parameters and timeline for identifying those at risk of TKI treatment failure and relapse risk. At diagnosis, initial bone marrow cytogenetics (or *BCR-ABL1* FISH) combined with a baseline RQ-PCR study for BCR-ABL1 transcript level is indicated. Following hematologic remission, bone marrow cytogenetics is repeated at 6-month intervals to establish the presence of CCR. RQ-PCR monitoring is then routinely instituted (e.g., at 3-month intervals) to ascertain the depth of molecular response. The role of FISH analysis in CML has become limited to initial diagnosis and possibly subsequent blood or bone marrow evaluation if karyotyping studies are unsuccessful. There is no defined rationale for FISH testing to monitor minimal disease after a CCR is attained using TKI therapy. Indications for *ABL1* KDM testing can include: serial increases in normalized BCR-ABL1 levels indicating loss of MMR or CCR, failure to achieve CCR after sufficient initial TKI treatment (e.g., by 12 months), rare instances of primary therapeutic resistance, development of blast crisis, and when considering a switch to a different TKI agent (e.g., because of drug intolerance).

## MUTATIONS OF JAK2 AND MPL IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

The classic Ph-negative chronic myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocythemia. These entities have overlapping clinical and morphologic features that suggest a common disease pathogenesis. The discovery of mutations in the *JAK2* gene has solidified this concept. Whereas precise subclassification still requires consideration of key clinical, laboratory, and histopathologic findings, the presence of a *JAK2* mutation provides strong evidence for the diagnosis of an MPN in suspected cases and is now an integral component of current WHO diagnostic criteria. The JAK family of intracellular tyrosine kinases provides a critical platform for cytokine-driven proliferative cell signaling. Binding of a potent ligand, such as erythropoietin (EPO), with its cognate heterodimeric transmembrane receptor (i.e., EPO-R), results in conformational change and phosphorylation of *JAK2*. The activated cytokine receptor/JAK2 complex can then recruit and phosphorylate one of several STAT proteins, leading to downstream effects eventuating in increased cell division. A single-point mutation (G1849T) in exon 14 replacing valine with phenylalanine at codon 617 (*JAK2* V617F) is found in the majority of MPNs. This mutation involves the pseudokinase (JH2) region of JAK2, which normally negatively regulates and inhibits autophosphorylation by the adjacent kinase domain in the absence of associated receptor-ligand activity. The amino acid substitution alters the inactive conformation of *JAK2* and abrogates its intrinsic regulatory control, resulting in autonomous cell signaling independent of receptor-cytokine engagement. The *JAK2* V617F abnormality is most prevalent in PV (95%) and PMF (75%), but is less frequently found in essential thrombocythemia (approximately 50%). The ratio of V617F allele (versus wild type *JAK2*) also varies between different individuals with the same disease, reflecting tumor heterogeneity. Furthermore, patients with advanced PV and a subset of patients with PMF have biallelic mutations arising from acquired uniparental disomy of chromosome 9(p24). The latter process entails loss of the normal 9(p24) region resulting from homologous duplication of the allele containing the *JAK2* V617F, producing a homozygous mutation state. Several features of the *JAK2* V617F—such as its varying prevalence within the MPN disease subcategories, the data derived from in vitro bone marrow cell culture experiments and familial MPN studies, the absence of the mutation in AML

transformations arising in patients with underlying PV, and the variability of observed mutation allele burden among patients—together suggest that this molecular aberration is an important factor in MPN pathogenesis, but is likely not an initiating genetic event. Conversely, murine models using retroviral transduction or conditional overexpression of *JAK2* V617F in bone marrow cells reveal that stable and transplantable myeloproliferative disease resembling the human counterpart can be produced directly, demonstrating that the exact role of the mutation remains to be fully understood.

The heterogeneous distribution of the V617F *JAK2* allele in tumor cells from different patients also indicates that relatively sensitive assays are required for detection. It has been recommended that molecular analytic assays should be able to minimally detect a level of 0.1% mutated DNA in a patient specimen. A variety of methods have been advocated for detecting the *JAK2* V617F, including high-resolution PCR product melting curve analysis, Sanger sequencing, or pyrosequencing, and variations of allele-specific PCR (e.g., amplification refractory mutation system, RQ-PCR). Of these, allele-specific PCR approaches using DNA as template reproducibly achieve the highest analytic sensitivity (Figure 24-8). Although true quantification of mutation burden is not required for diagnostic purposes, the advent of *JAK2* inhibitor therapies may change this aspect as more data emerge. It should be noted that the *JAK2* V617F is also uncommonly found (less than 5%) in patients with myelodysplasia, CMML, and de novo AML, as well as in most cases of the provisional WHO entity refractory anemia with ring sideroblasts and thrombocytosis. Therefore, the presence of a *JAK2* V617F mutation does not necessarily specify the diagnosis of a classical MPN and should not be used in the context of conclusively distinguishing MPN from other rare, atypical myeloid tumors with proliferative morphologic features. Conversely, a negative *JAK2* V617F analysis does not exclude the diagnosis of a classical MPN if definitive clinical and morphologic findings are otherwise present. The use of high-sensitivity of PCR methods may also identify apparent V617F mutations at very low levels (less than 0.1%), and the presence of rare mutated cells in healthy subjects has been described. In this regard, the finding of a low-abundance *JAK2* V617F abnormality in a patient with unexplained minimal thrombocytosis or leukocytosis could be misleading, and by extension the association between mutated allele detection and early MPN in the absence of diagnostic morphologic bone marrow pathology has been incompletely studied. Therefore each molecular diagnostic laboratory must be aware of the dynamic range of detection for *JAK2* V617F and the relative clinical utility of low-level positives when reporting such results. The importance of thorough clinical and pathologic correlation is again emphasized in this setting.

A small group of patients considered to have primary (idiopathic) erythrocytosis lack the *JAK2* V617F, but have been found instead to have small insertion–deletion events or base substitutions involving exon 12 of the gene. These cases may show more subtle histologic changes in the bone marrow compared with typical findings in PV, but also demonstrate endogenous erythroid colony formation in vitro, like classical PV. Exon 12 *JAK2*

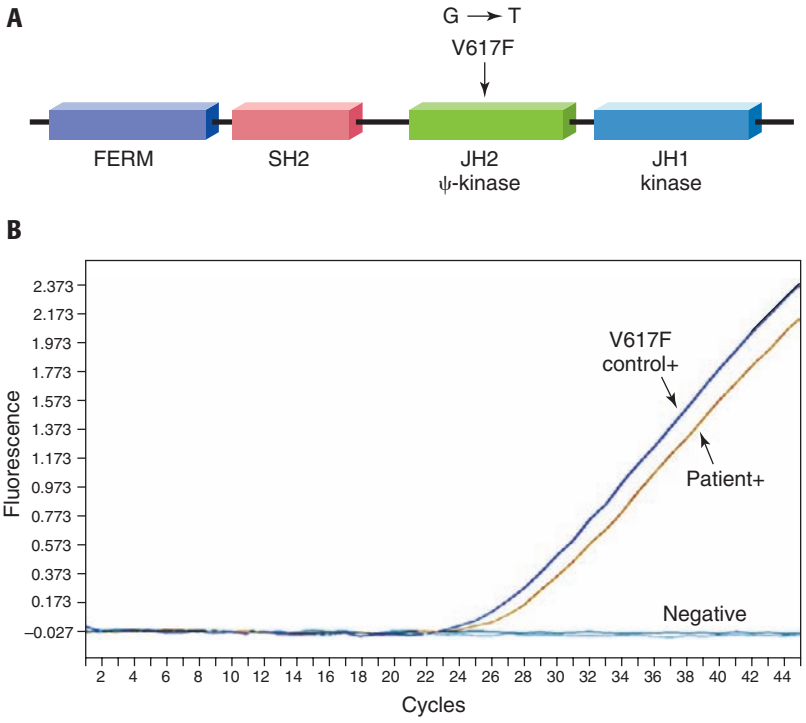



**FIGURE 24-8**

*JAK2* V617F gene mutation in Philadelphia-negative myeloproliferative neoplasms. **A,** The Jak2 tyrosine kinase, illustrating functional regions of the protein. A single G>T somatic point mutation in exon 14 of the *JAK2* gene produces an amino acid substitution of phenylalanine for valine at codon 617 (i.e., V617F). This mutation resides in the JH2 pseudokinase domain, an autoinhibitory region of the protein. As a consequence of this mutation, constitutive activation of the Jak2 tyrosine kinase and Jak-STAT signal transduction pathway occurs contributing to cell proliferation. The V617F is detected with variable frequency in classic myeloproliferative neoplasms (see text for detail). Rare patients with primary erythrocytosis can have alternate small insertion or deletion mutations in exon 12 of *JAK2*. **B,** An allele-specific real-time quantitative polymerase chain reaction (PCR) assay to detect the *JAK2* V617F using DNA extracted from a patient with polycythemia vera. A cell line positive control is present, as well as a positive patient sample demonstrating a relatively high allelic burden of *JAK2* V617F. Negative and no-template controls show no amplification. The *JAK2* patient and positive control results are normalized to a housekeeping gene (e.g., β globin) to compensate for sample and reaction factors. Allele-specific PCR is highly sensitive for detecting the V617F abnormality (e.g., less than 1% mutated allele in a wild type background). Exon 12 insertion or deletion changes require different methods for detection.

mutations are detected by direct sequencing of PCR products, allele-specific PCR, or PCR with high-resolution melting curve analysis, but these techniques are less sensitive than RQ-PCR as described for the V617F abnormality. Additional mutations of *JAK2* in MPN have been identified by more comprehensive gene sequencing studies; however, these anomalies constitute rare events of uncertain pathogenetic significance and do not justify this broader screening approach in routine molecular diagnostic evaluation. Intriguingly, recent data indicate that mutational events in other components regulating the JAK-STAT pathway may also contribute to the development of MPN. One example is the finding of somatic mutations affecting the gene encoding the LNK adaptor protein (also called SH2B3), which functions normally as a negative regulator of activated *JAK2*.

A minority of patients with PMF and essential thrombocythemia (approximately 5%) lack *JAK2* gene mutations, but instead are found to have coding alterations in the *MPL* gene. The MPL gene product is the cell surface receptor for thrombopoietin and this receptor-ligand interaction also initiates cell signaling through the JAK-STAT pathway. In this case, *MPL* mutations render the receptor itself constitutively active. *MPL* mutations mostly involve codon W515 and result in amino acid substitutions. Although the W515 site can be interrogated with allele-specific PCR methods, sequencing of *MPL* exon 10 can identify these changes and any additional rare sequence variants.

## ABNORMALITIES OF *PDGFRA, PDGFRB,* AND *FGFR1* GENES IN MYELOID AND LYMPHOID NEOPLASMS ASSOCIATED WITH EOSINOPHILIA

Progress in tumor genetic classification has improved the definition of several hematopoietic tumors that are loosely linked by the presence of accompanying neoplastic eosinophilia. These entities share common gene rearrangements resulting in the deregulation of proliferation-associated receptor tyrosine kinases. Chromosomal rearrangement at 4(q12) involving *PDGFRA* underlies the central pathogenesis in a subset of chronic eosinophilic leukemias. These patients exhibit a profound blood and bone marrow eosinophilia in which the tumor cells harbor a gene fusion between *PDGFRA* and an adjacent gene locus termed *FIP1L1* on the same chromosome. Although a chimeric transcript is produced from the *FIP1L1-PDGFRA* abnormality, the major cellular manifestation appears to be overexpression of PDGFRA receptor tyrosine kinase activity, leading to cell proliferation. The cell-specific context that results in predominant neoplastic eosinophil production has not been fully elucidated. Less commonly, the *FIP1L1-PDGFRA* can be seen in cases of T-ALL or AML with eosinophilia. Most significantly, patients with chronic eosinophilic leukemia with the *FIP1L1-PDGFRA* fusion gene exhibit a high response rate to single-agent imatinib therapy, often at a relatively low dosage. Identification of this genetic marker is thus critical to guide treatment, because patients with chronic eosinophilic leukemia lacking *PDGFRA* rearrangements are not imatinib sensitive. The intrachromosomal del4(q12q12) alteration is relatively small and thus not easily recognized by karyotyping studies; however, an intervening gene, *CHIC2,* is deleted during formation of the *FIP1L1-PDGFRA* fusion, and the loss of *CHIC2* can best be identified by locus-specific FISH technique. RT-PCR analysis has been used to detect the chimeric FIP1L1-PDGFRA mRNA transcript, but can produce false-negative results because of breakpoint heterogeneity in *FIP1L1*. The related *PDGFRB* gene, encoding the β receptor of platelet-derived growth factor is also disrupted in rare myeloid neoplasms, most often encountered as CMML with eosinophilia. In these cases, cytogenetic studies generally reveal a t(5;12)(q33;p13) resulting in formation of the fusion gene *ETV6-PDGFRB,* although several variant translocations involving *PDGFRB* have been described. *PDGFRB* locus abnormalities can be detected by interphase FISH analysis. These tumors also appear relatively sensitive to imatinib treatment. A third group of aggressive hematopoietic neoplasms with prominent eosinophilia is characterized by translocation-induced rearrangements of the *FGFR1* gene on 8(p11). *FGFR1*-associated malignancies encompass a variety of myeloid and lymphoid tumors including T-ALL, mixed phenotype acute leukemias, AML, and atypical chronic myeloproliferative neoplasms. *FGFR1* rearrangements are detectable by FISH technique or standard chromosome studies. Such cases are unfortunately imatinib resistant. From the preceding summary, hematolymphoid malignancies with a clonal eosinophilic component are morphologically diverse. Characterization of specific diseases based on abnormalities of *PDGFRA, PDGFRB,* and *FGFR1* genes is therefore valuable for more precise classification and, in some patients (e.g., *PDGFRA*- and *PDGFRB*-positive myeloid neoplasms), for guiding effective therapy with imatinib or related TKI agents.

## ABNORMALITIES OF THE *KIT* GENE IN SYSTEMIC MASTOCYTOSIS

Mast cell diseases have a wide clinical and pathobiologic spectrum, from localized, relatively indolent cutaneous lesions, to debilitating systemic presentations involving skin, visceral organs, and bone marrow. Point mutations of the *KIT* gene are highly characteristic of mastocytosis, and the large majority result in a change from aspartic acid to valine at codon 816 in exon 17 (D816V). The *KIT* gene encodes the tyrosine kinase receptor for stem cell factor, which is critically required for normal

hematopoietic stem–progenitor cell proliferation and expansion. Because mastocytosis is an uncommon disease, the frequency of the D816V abnormality is variable between studies, but also differs when considering clinical subtypes of mast cell disease (e.g., cutaneous versus systemic) or the age of onset (i.e., adult versus childhood presentations). In adults with systemic mast cell disease, the D816V is found in approximately 80% of cases, although with more sensitive molecular detection methods and cell enrichment techniques, nearly 95% of cases are mutation positive. In general, systemic mastocytosis (SM) is associated with a high prevalence of the *KIT* D816V; however, this appears to differ according to adult versus childhood (prepubertal) age of disease development (approximately 90% versus 50%). When assessing only patients with cutaneous mastocytosis, the presence of the D816V appears to be much less frequent, but this too is controversial between reported series. Notably, younger patients with SM or cutaneous mastocytosis seem to have a higher incidence of *KIT* mutations occurring at other sites in exon 17 or point mutations in exons 8, 9, or 11. Nevertheless, the largest proportion of individuals (80% to 90%) with SM harbors the D816V change permitting targeted molecular analysis as an important aspect of diagnosis.

The *KIT* D816V can be sensitively identified using allele-specific PCR and fluorescent PCR product detection. Because the mutation is heterozygous in most cases, separate primers recognizing mutant and wild type alleles at the 816 codon site can be used to both determine the presence of a mutation and ensure amplification of the DNA template. RQ-PCR analysis using allele-discriminating primers or probes can similarly be used for this purpose. High-resolution melting analysis of PCR products is an alternative technique for mutation screening, and direct sequencing (or pyrosequencing) of the amplified exon 17 region of *KIT* can also be used. Molecular assays specifically detecting the D816V anomaly will be associated with a risk for clinical false negativity, because a small percentage of SM cases will have alternative nucleotide changes at this site or involve mutations elsewhere in exon 17. Furthermore, as indicated, a subset of pediatric patients may have rare *KIT* point mutations in other exons. The focal and fibrotic nature of bone marrow mast cell lesions also may result in negative D816V results because of sampling effects. Therefore the absence of the D816V does not rule out a diagnosis of SM if clinical and pathologic findings are otherwise diagnostic. Accordingly, the presence of the *KIT* D816V is included as one of four minor WHO criteria in establishing the diagnosis of SM. However, it is apparent that the presence of a *KIT* gene mutation provides rather definitive evidence for the presence of a suspected mast cell neoplasm, although the precise subclassification will depend on the clinical presentation, morphologic tissue evaluation, and pertinent laboratory studies (e.g., serum tryptase level). The broader application of imatinib therapy for renegade tyrosine kinase activity in the hematopoietic cancers has unfortunately not been successful in SM with *KIT* mutations. The fact that imatinib is variably effective in other *KIT* mutation positive tumors (e.g., some gastrointestinal stromal tumors and melanomas) underscores the importance of additional factors such as the mutation location and cellular context in conferring sensitivity or resistance to particular TKI agents. Finally, as discussed in the section on CBF AML, mutations of *KIT* (often the D816V) occur as additional so-called class I genetic abnormalities producing increased cell proliferation and a poor outcome in these patients.

## EMERGING TRENDS AND NEW TECHNOLOGY APPLICATIONS IN MOLECULAR HEMATOPATHOLOGY

The preceding summary of how molecular and cytogenetic analyses are integrated into modern hematopathology practice illustrates that, with few exceptions (e.g., acute promyelocytic leukemia), evaluation of single genetic markers for disease diagnosis and prognosis or prediction is yielding to the requirement for simultaneous analysis of multiple tumor-specific markers. Examples such as cytogenetically normal AML, CLL/SLL, and pediatric ALL indicate that the current "state of the art" lies in the assembly of various individual genetic and phenotypic features into a framework for optimal clinical management. New technologies (gene expression profiling, whole genome scanning, high-throughput DNA sequencing) and recent discoveries in molecular genetics demonstrate that the complexity in hematolymphoid tumor biology is remarkable, and the application of large-scale, multiparameter methodologies may create new opportunities in the pursuit of truly individualized patient care. These advances require commensurate progress in novel therapeutics and appropriately scaled clinical studies to rigorously assess potential biomarkers. This section briefly summarizes the key established and emerging technology platforms that will bring new perspectives to the diagnosis and prediction of blood and lymphoid cancers.

### SINGLE NUCLEOTIDE POLYMORPHISM ARRAYS AND ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

Following the seminal effort to sequence the entire human genome, a wealth of data has been generated detailing far greater complexity in genomic structure and variation than was previously thought. Single nucleotide polymorphisms (SNPs) are the most frequent type of variation in the human genome, with approximately

10 million distributed throughout DNA. Potentially more significant, from the perspective of disease pathogenesis, has been the discovery of a large number of kilobase- to megabase-scale variations in regional DNA sequence, known as *copy number variations* (CNVs). These gains or losses can encompass genomic regions harboring many different genes. While many CNVs represent polymorphic variants, recent studies have begun to identify recurrent alterations that may be critically involved in disease causation, including cancer. The advent of large-scale automated technologies for assessing global genome architecture (i.e., SNP-A and A-CGH) has enabled more widespread application to a variety of disorders, including hematolymphoid neoplasms. These two genome-scale analysis platforms differ in design, but are largely similar in the comprehensive scope of genomic information provided. A-CGH is performed using both target (e.g., tumor) DNA and normal control (diploid) DNA samples applied to a solid substrate embedded with thousands of individual DNA probes. The type of probe can be variable, but commercially available array platforms use oligonucleotides. An advantage of A-CGH is the ability to densely tile the oligonucleotide probes so that a large proportion of total genomic coverage is potentially obtained. Equal amounts of fluorescently labeled target and control DNA are cohybridized to the array, and the signal differences and intensities can be measured, indicative of gains and losses in different regions of the target DNA. Bioinformatic software algorithms display the hybridization results in a bimodal manner of signal intensities, denoting copy number changes (gains/losses) in relation to a map of the reference genomic sequence and relative chromosome position. The power of A-CGH lies in the ability to provide genome scale information at high resolution (e.g., less than 50 to 100 kb, far superior to FISH and standard cytogenetics), but this is critically dependent on the probe density and spacing (coverage). A-CGH detects and delimits the boundaries of CNV, and because cohybridized control DNA is used, true pathologic CNV changes can be more readily distinguished from common polymorphic DNA regions in the patient sample. A-CGH also identifies areas of loss of heterozygosity (LOH), often associated with monoallelic deletion of tumor suppressor genes; however, A-CGH cannot detect copy number neutral LOH (CN-LOH), also called *uniparental disomy* (UPD). The latter genomic alteration results from duplication of an allelic region of DNA by homologous recombination with the other (maternal or paternal) allele. CN-LOH can therefore be associated with the acquisition of two identical haplocopies of a gene (or genes), but without a net loss of DNA content in the chromosomal region. CN-LOH can be a result of embryonic autozygosity (i.e., embryonic or congenital UPD) or can be somatically acquired. CN-LOH is not visible to cytogenetic or FISH analyses. A-CGH also cannot detect CN-LOH because the method is insensitive to balanced DNA changes and, unlike SNP array (SNP-A) technology, does not have an additional measure of zygosity. The sensitivity of A-CGH for detecting the presence of genomic abnormalities is variable, but typically not better than approximately 20% to 30%, indicating that for somatic changes, normal cells admixed with tumor cells can compromise analysis.

SNP-A chips also consist of arrayed oligonucleotides, but the probes correspond to allelic variants of selected SNPs distributed throughout the genome. Current high-density platforms allow the analysis of more than 900,000 loci simultaneously. SNP arrays combine both CNV analysis and SNP genotyping, the latter based on probe classification of homozygous versus heterozygous status at the given polymorphic loci. Hybridization of genomic DNA to both probe variants indicates heterozygosity, whereas signal only for one allele indicates homozygosity (or hemizygosity in the case of an allelic loss) at any given locus. In addition, the strength of the fluorescence signals allows for analysis of gene copy number. SNP-A does not require cohybridization of normal DNA to generate results. A typical SNP array analysis workflow reveals copy number profile along the chromosome (using stored data from reference DNA samples to scale fluorescence intensities to a copy number value). Segmentation algorithms are then used to computationally define regions of copy number loss and gain. In the second step, genotype analysis generates a dataset (referred to as *calls*) that identify SNP loci as either A, B, or AB (based on hybridization results with probes for allele A, allele B, or both, respectively). For each SNP, comparison with reference DNA profiles will reveal heterozygosity, homozygosity, or LOH (i.e., hemizygosity) at the interrogated site. Finally, results of all individual calls along the chromosome are statistically analyzed, identifying any genomic segments of LOH (Figure 24-9). SNP-A thus also provides a high-resolution technique for detecting unbalanced chromosomal defects such as microdeletions or gains of chromosomal material undetected by routine metaphase cytogenetic or FISH techniques. As indicated, a major advantage of SNP-A over conventional cytogenetics or CGH-A is its ability to detect CN-LOH or UPD. A diagnosis of CN-LOH is established when a homozygous constellation of genotyping calls is observed in a genomic region with diploid copy number. Recent studies based on SNP-A technology have shown a high rate of acquired UPD in several hematologic malignancies. This process occurs in PV for example, in which a biallelic *JAK2* V617F is produced by an acquired chromosome 9p CN-LOH event. The recognition of this genomic abnormality actually enabled the initial discovery of the *JAK2* V617F mutation. The sensitivity of SNP-A is similar to A-CGH (20% to 30%) and depends on the cellular heterogeneity of the sample and the analytic detection programs being used. The resolution of genomic lesions is related to the density of arrays, distribution of probes, and biostatistical algorithms used

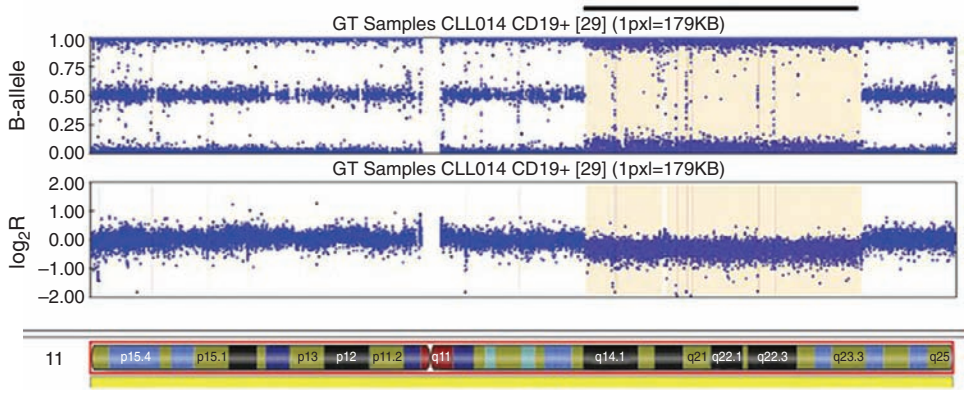



**FIGURE 24-9**

SNP-array genotyping. Example of single nucleotide polymorphism (SNP) genomic array data for chromosome 11 from a case of chronic lymphocytic leukemia (CLL), analyzed using the Illumina BeadArray system (Illumina, San Diego, Calif.) Upper scattergram shows B-allele frequency, which is essentially a distribution of calls for homozygous A or B or heterozygous AB SNPs at each interrogated site. This plot shows a large segmental region of 11q with loss of the AB allele calls (central area) and accentuated homozygous A and B intensities (delimited by *black bar*). This pattern indicates a hemizygous loss of signal (i.e., loss of heterozygosity [LOH] due to monoallelic 11q deletion). The lower scattergram displays the $\log_2$ ratio of fluorescence intensity values for the test sample after bioinformatic analysis relative to normal reference DNA data. The dip in intensity (–0.5) in the same region verifies loss of chromosomal material at 11q, consistent with a del 11(q). SNP array is also valuable to detect copy-neutral LOH (CN-LOH), arising from loss of one chromosomal region and balanced replacement with a duplicate copy of the remaining (maternal or paternal) allele. Although not present in this example, CN-LOH is recognized on SNP array as a loss of heterozygous SNP calls in a specific region, but without a net change in the $\log_2$ fluorescence ratio. The *lower image* shows a chromosome 11 idiogram for orientation. *(Courtesy of Steven A. Schichman MD, PhD, Pharmacogenomics Analysis Laboratory, Central Arkansas Veterans Healthcare System, Little Rock, Ark.)*

to detect copy number changes. The frequency of informative heterozygous loci and the nonlinear distribution of SNP markers in human DNA limit the resolution of SNP-A, which is a drawback when compared to A-CGH wherein probes can be placed more evenly and can even be targeted to specific regions of interest on individual chromosomes. More recent SNP-A platforms also incorporate additional oligonucleotides to cover SNP-poor regions and increase the sensitivity for detecting CNV, combining the advantages of both approaches. Finally, because SNP-A does not require cohybridization of paired normal patient DNA to generate results, the technique may suffer in the ability to distinguish polymorphic from pathologic regions of CNV, because an internal control is not directly and simultaneously analyzed. To circumvent this problem, concurrent analysis of a patient normal cell source (e.g., buccal cells, T-lymphocytes) can be performed on a separate array and the results compared to the target (i.e., tumor) DNA specimen. Of note, neither A-CGH nor SNP-A can resolve certain balanced chromosomal rearrangements (i.e., translocations). Other genomic analysis methods, such as cytogenetics or FISH, are still important in this regard.

## MicroRNA Profiling

MicroRNAs (miRNA) are small nonprotein encoding RNA molecules of approximately 22 nucleotides length that play an important role in posttranscriptional gene regulation. Currently, more than 1000 miRNAs known to be encoded by the human genome have been identified. MiRNAs are part of the RNA-induced silencing complex and function in most cases to repress the activity of specific mRNA molecules, either by promoting transcript degradation or by preventing translation into protein. Despite the seeming simplicity of this new class of small RNA, a single miRNA can affect the activity of multiple gene targets. Conversely, several different miRNAs can modify the function of a single gene. MiRNAs have been estimated to have a role in regulating 30% to 100% of cellular proteins by modulating gene expression levels. Consequently, miRNAs are involved in a myriad of biologic processes, including cell proliferation, apoptosis, differentiation, immune regulation, and metabolism. MiRNAs have been implicated in many benign disorders and malignant neoplasms, and more than half of miRNAs discovered to date have been located near DNA fragile sites in loci associated with LOH, common genomic breakpoints, or in regions associated with DNA amplification. Many of the miRNAs deregulated in cancers have been shown to have proto-oncogenes, tumor suppressor genes, and critical signaling pathway components as their direct targets. These findings raise the possibility that deregulation of miRNAs in concert with other classic oncogenes and tumor suppressor genes drives the behavior of cancer cells. Indeed, distinctive patterns of activation and silencing of multiple miRNAs (i.e., miRNA profiles) have been identified in certain cytogenetic and molecular subsets of leukemia. This has led to the discovery of new molecular pathways in leukemogenesis and has provided important prognostic information complementing that gained by other cytogenetic and molecular

studies. Furthermore, modification of altered miRNA expression using synthetic small RNA agents (antagomirs) could provide a viable therapeutic approach in some leukemic patients.

The potential for diagnostic and therapeutic benefits has provided a tremendous impetus in the development of high-throughput technologies for assessing miRNA profiles of cancer cells. The most common techniques for profiling miRNAs are RQ-PCR, DNA microarrays, and high-throughput RNA sequencing. There are strengths and weaknesses for each of these technologies. To analyze miRNA by RQ-PCR, RNA is converted to complementary DNA (cDNA) and amplified using a modified forward primer containing common amplification and miRNA-specific sequences, combined with a specialized miRNA-specific reverse primer (e.g., containing locked nucleic acids). For direct quantification of miRNA using RQ-PCR, a specific RNA reference is required to normalize the sample data. RQ-PCR experiments can be performed in a massively parallel fashion to determine the relative levels of potentially hundreds of miRNAs; however, this method is unlikely to be used on large numbers of samples. Microarray-based profiling of miRNA uses spotted oligonucleotide probes corresponding to specific miRNAs and relies on differential hybridization signal intensities to analyze miRNA expression levels. A potential limitation of array-based profiling is cross-hybridization of sequence-similar miRNAs, because the short lengths of these nucleic acids restricts somewhat the ability to perform optimal hybridization reactions based on common melting temperature probes or guanine-cytosine content, in order to achieve high specificity. Consequently, the best use of a microarray-based technique is in applications to screen samples and compare expression profiles amongst different cell populations. Next-generation sequencing technology appears poised to take the helm of miRNA profiling. The appeal of sequencing is the ability to look for all the miRNAs in a sample and not just those selectively printed on a chip. Presently, deep sequencing methods are the best approach for identification of different isoforms or very similar miRNAs. Major drawbacks remain the requirement for high-performance computing and bioinformatics support to analyze the data and cost. Sequencing of total expressed RNA in a sample obviously will also find degraded RNA and other small RNA species. There is also a necessity for mapping sequence reads to the genome and resolving the raw data into nonredundant transcript counts. The profiling results are therefore highly dependent on the accuracy and robustness of the biostatistical algorithms that are used.

## NEXT-GENERATION SEQUENCING

Whole genome scanning techniques such as CGH and SNP arrays have provided a multitude of data on unbalanced gene copy number aberrations and have helped to identify several genetic lesions involved in hematologic malignancies, including potential therapeutic targets. However, array-based genomic profiling provides an incomplete assessment of global genetic and genomic changes, most notably because of the inability of these technologies to provide information about translocations, very small insertion and deletion variants, and base-pair point mutations. An ideal tool for the genetic characterization of cancer is one that could simultaneously provide information about copy number variations, allelic information, somatic rearrangements, and base pair mutations in a single experiment. Furthermore, data generated from such a technology should be presented in such a way that the presence of cellular heterogeneity in the sample would not constitute an insurmountable analytical hurdle. The advent of next-generation sequencing (NGS) presents a highly promising tool for these purposes. Traditional DNA sequencing is performed with the Sanger method or an alternative approach such as pyrosequencing. DNA is usually initially prepared by PCR amplification of a targeted region of interest and analyzed as a single genomic locus per sample. Massively parallel or NGS overcomes the limited scalability of traditional Sanger sequencing by creating microreactors attaching fragmented genomic DNA molecules to be sequenced on solid surfaces or beads, allowing for millions of sequencing reactions to happen in parallel. DNA sequence is determined in situ, bypassing the need for gel separation or electrophoresis. In contrast to longer sequence reads generated from a PCR-amplified sample, NGS methods produce shorter read lengths (fewer than 400 base pairs), but can generate hundreds of megabases to gigabases of nucleotide sequence output in a single instrument run. After alignment and assembly, the short reads generated in the sequencing of each DNA molecule are counted and quantified in relation to a reference genomic database, allowing the identification of somatic mutations and accurate copy number assessments of each genomic region. In addition, by sequencing both ends of DNA fragments (paired end or mate-pair sequencing) it is possible to detect both balanced and unbalanced somatic rearrangements in a genome wide fashion. NGS provides the most comprehensive characterization of the cancer genome to date. NGS also has the power to assemble multiple reads over the same area of genomic DNA (i.e., depth of coverage), which in turn dramatically increases the sensitivity for finding rare mutation events in heterogeneous cell populations. Furthermore, complete sequencing of a tumor genomic DNA and patient nontumor DNA can compare paired samples to identify a full range of somatic alterations, including nucleotide substitutions, structural rearrangements, and copy number alterations, all in a single approach. Massively parallel sequencing can also be applied to germline DNA

for gene association studies and may constitute a paradigm shift in the way mutations that cause rare diseases or cancer predisposition can be identified. In addition to genomic DNA sequencing, NGS has also been applied to sequence RNA in several applications (e.g., transcriptomic or whole exon RNA sequencing, small–noncoding RNA sequencing). These applications have identified multiple and unsuspected mRNA splice variants, novel gene rearrangements, and novel chimeric fusion gene events. Thus, when combined with DNA sequencing data, global RNA sequencing has the potential to unravel potentially pathogenic transcriptional events.

The application of NGS has allowed cancer genomics to progress more rapidly from focused approaches to comprehensive genome-scale analyses. Despite the enthusiasm for this new vista, the vast amount of data derived from the NGS studies will require thorough efforts to identify sequence information that is clinically relevant. Given that each cancer genome may have thousands of somatic mutations, it is clear that rigorous validation of recurrent candidate alterations in large clinical studies, or by laborious biochemical and genetic laboratory investigations, will be required to separate true driver mutations from genomic noise. In addition, NGS is likely to unravel a much greater complexity of the normal human genome in terms of SNPs and polymorphic (and pathogenic) DNA copy number variants, some of which may be confined to specific tissues in an individual (i.e., genetic mosaicism). Error rates in raw sequence data produced by current NGS platforms are higher than with typical Sanger sequencing. The combination of errors arising through template preparation, sequencing library construction, the sequencing itself, subsequent alignment errors from short reads, and ever-changing DNA and RNA reference databases, together increase the possibility of significant false-positive results. For clinical applications, there is a great need to increase the accuracy of data derived from massively parallel sequencing. The requirement for high-quality bioinformatics support also presents a challenge when simultaneously analyzing data from paired tumor and matched normal tissues (e.g., for rare somatic mutation detection), and assessing samples with unknown levels of nontumor cell admixture or inherent tumor cell heterogeneity. For hematolymphoid cancers in particular, efforts can be made to substantially elucidate the latter issues by using cell enrichment techniques. These key technical and analytical issues are being addressed in new NGS platforms via continuous process streamlining, automation, chemistry refinement, and improved data management. It is evident that incremental improvements in the technology that is currently available, or the introduction of others currently in development, will make way for the transition of NGS into routine clinical diagnostics.

## MOLECULAR DIAGNOSIS OF RED BLOOD CELL DISORDERS

Abnormalities of RBCs can involve many different components of the cell. These include abnormalities of the hemoglobin molecule, disorders of hemoglobin production, abnormalities of the RBC enzymes, defects in the cellular oxygen-sensing pathway, and RBC membrane or cytoskeletal anomalies. Clinical manifestations of these disorders range from benign conditions to hemolytic anemias, erythrocytosis, impaired hemoglobin function with poor RBC survival, and thalassemic syndromes. For many of these conditions, there are well-established assays available to provide adequate diagnostic information; however, there is an increasing number of molecular assays that can be applied appropriately in specific situations, particularly if an RBC disorder is associated with clinical symptoms.

### HEMOGLOBINOPATHIES

Common methods, such as alkaline–acid hemoglobin (Hb) electrophoresis, isoelectric focusing, high-performance liquid chromatography, and capillary electrophoresis are able to identify the common (and clinically significant) Hb variants S, C, and E. Other less common variants are difficult to identify precisely because many of these have similar electrophoretic mobility or retention times depending on the given analytic platform. The combination of two or more routine techniques helps to enhance the identification of these less common Hb types; however, because the vast majority of Hb variants are clinically benign, it is not entirely necessary to definitively subclassify them by standard approaches. In contrast, variants associated with clinical symptoms (e.g., altered oxygen affinity, unstable Hb) are important to identify by molecular methods. Because of the small size of the human globin genes (three exons each, spanned by two introns), these genomic loci are amenable to PCR amplification and direct Sanger sequencing, although allele-specific probes or primers have also been used to detect relatively common mutations.

### THALASSEMIAS

#### α THALASSEMIAS

As outlined in Chapter 1, most α thalassemia mutations involve deletion of one or both α globin genes at the genetic locus on chromosome 16p13. Detection of such mutations is easily accomplished with Southern blot or gap-PCR testing—the latter technique involving primer sets normally situated far apart that are brought close together because of a deletion, enabling amplification of

the abnormality. A relatively recent method is the multiplex ligation-dependent probe amplification (MLPA) assay, which uses a series of DNA probes to hybridize at various points along the α globin gene complex. Absence of hybridization and subsequent site-specific ligation of a particular probe region indicates deletion of that area of the gene. These methods will, however, not detect point mutations responsible for nondeletional α thalassemia phenotypes. The latter abnormalities, however, can be identified by standard DNA-sequencing methods, although this is rarely necessary.

## β THALASSEMIAS

In contrast to α thalassemias, the vast majority of β thalassemias are similar to Hb variants and associated with point mutations, which introduce single nucleotide substitutions in the β globin gene located on chromosome 11p15. Most β thalassemia mutations can be detected by PCR and direct DNA sequencing of the β globin gene. In areas of the world where a small number of β thalassemia mutations are particularly common, allele-specific oligonucleotide probes or allele-specific PCR amplification may identify the majority of these cases.

## HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN AND δβ THALASSEMIA

These disorders have in common an elevation in Hb F beyond the newborn period. Similar to the case for α thalassemia mutations, these abnormalities involve large deletions in the β globin gene complex and can be detected with similar methods (Southern blot, gap-PCR, and multiplex ligation-dependent probe amplification). There are also nondeletional hereditary persistence of fetal Hb mutations that involve the promoter region of the γ genes; however, it is rarely clinically necessary to detect the exact molecular mutation in these cases.

## PRACTICAL WORKUP OF HEMOGLOBINOPATHIES AND THALASSEMIAS

For many of these situations, routine protein analytic methods are adequate to provide an appropriate answer. These methods include simple measurement of Hb $A_2$ for β thalassemia trait or evaluation of Hb variants by electrophoretic methods. Although exact identification of some Hb variants may not be possible by routine electrophoretic methods, such accuracy may not be necessary if the variant is clinically benign. Molecular identification is most helpful in situations that are clinically significant, including variants that produce hemolytic anemias or altered Hb oxygen affinity (i.e., erythrocytosis or physiologic anemia). For thalassemia syndromes, identification of the molecular abnormalities in cases of β thalassemia major or intermedia is warranted, particularly in parents undergoing prenatal counseling. Similarly, identification of α thalassemia mutations in two parents who are both microcytic is warranted to assess for the possible severity of disease in an affected child. The molecular diagnostic approach to hemoglobinopathies and thalassemic disorders thus requires an appropriate screening strategy, knowledge of clinical parameters and experience with variant globin identification. Molecular methods therefore highly complement standard protein electrophoretic techniques in specific circumstances.

## ERYTHROCYTOSIS

Most erythrocytoses are compensatory because of conditions such as chronic cardiac or pulmonary dysfunction, high-altitude dwelling, chronic smoking, sleep apnea, and morbid obesity and hypoventilation syndromes. However, when reactive and paraneoplastic conditions have been excluded and a primary neoplastic (i.e., PV) or a heritable cause is suspected, the molecular evaluation of erythrocytosis is warranted. The development of molecular tests for mutations associated with erythrocytosis has shed light on classification and has defined more clear etiologies, thereby decreasing the numbers of true idiopathic cases. With the aid of molecular techniques, many cases are confirmed as neoplastic in etiology and some erroneously assumed to be neoplastic are reclassified as heritable.

The most common cause of primary erythrocytosis is PV, an acquired somatic mutation of hematopoietic stem cells that carries a risk of evolution to myelofibrosis and acute leukemia. PV is most often diagnosed in middle aged to older individuals, but can occasionally be seen in children and young adults. It is important to differentiate PV from erythrocytoses because of inherited germ line mutations, which can be passed to progeny but do not carry the risks of clonal evolution associated with PV. The clinicopathologic and molecular evaluation of PV is discussed previously and should be undertaken in the initial stages of an evaluation of erythrocytosis, especially when the serum erythropoietin (EPO) level is low, because PV will be the cause in the overwhelming majority of cases. If PV is excluded, a heritable cause may be present.

Although much less common than PV, mutations in hemoglobin (high oxygen-affinity hemoglobin variants), hemoglobin stabilization proteins (2,3 bisphosphoglycerate [2,3 BPG] deficiency), the erythropoietin receptor and in oxygen-sensing pathway enzymes (hypoxia-inducible factor, prolyl hydroxylase domain, and von Hippel Lindau) result in polycythemia (Table 24-5). Of

**TABLE 24-5**

**Molecular Testing Available for the Evaluation of Erythrocytosis**

| Involved Gene | Inheritance | Serum Erythropoietin | p50 |
|---|---|---|---|
| *JAK2*V617F | Acquired | Decreased | NL |
| *JAK2* exon 12 | Acquired | Decreased | NL |
| *EPOR* | Dominant | Decreased to NL | NL |
| *PHD2* | Dominant | NL | NL |
| *BPGM* | Dominant | NL | Decreased |
| β globin | Dominant | Elevated to NL | Decreased |
| α globin | Dominant | Elevated to NL | Decreased |
| *HIF2α* | Dominant | Elevated to NL | NL |
| *VHL* | Recessive | Markedly elevated | NL |

*NL,* Normal range level.

**TABLE 24-6**

**Erythrocytosis Testing Stratified to Serum Erythropoietin Level***

| Decreased Erythropoietin Level | Normal Erythropoietin Level | Increased Erythropoietin Level |
|---|---|---|
| *JAK2 V617F*<br>*JAK2* exon 12<br>*EPOR* | *JAK2 V617F*<br>*JAK2* exon 12<br>p50<br>Hemoglobin electrophoresis<br>Globin gene<br>2,3 BPG enzyme assay<br>2,3 BPG mutase gene<br>Methemoglobin evaluation<br>*PHD2*<br>*HIF2α*<br>*EPOR* | p50<br>Hemoglobin electrophoresis<br>Globin gene<br>Methemoglobin evaluation<br>*VHL*<br>*HIF2α* |

*BPG,* Bisphosphoglycerate.
*Recommend test ordering in a reflexive manner beginning with more common etiologies for the clinical picture. If focused testing guided by Epo levels is negative, recommend extending the testing panel as the etiology may be multifactorial.

the heritable causes, high oxygen-affinity hemoglobin variants are the most common. Most of the remaining conditions are much less common; however, the true prevalence remains to be elucidated with wider investigation of patients previously classified as idiopathic. Serum EPO levels help to guide efficient testing choices according to the most likely etiologies (Table 24-6). The most studied genetic causes currently associated with erythrocytosis follow and are discussed separately.

## 2,3 BISPHOSPHOGLYCERATE

The RBC 2,3 BPG (also known as 2,3 DPG) molecule stabilizes the deoxygenated form of hemoglobin by allosteric binding and facilitates oxygen release at tissue sites. Decreased 2,3 BPG concentrations create increased oxygen affinity by the normal hemoglobin tetramer; therefore a deficiency is associated with a left-shifted oxygen dissociation curve (decreased p50 value) and subsequent erythrocytosis. A known mechanism of 2,3 BPG deficiency is caused by mutations in the gene coding the converting enzyme, bisphosphoglyceromutase *(BPGM)*. The *BPGM* gene is located on chromosome 7, spans 22 kb, and contains 3 exons. Point mutations and small frameshift mutations have been reported and can be identified by PCR and Sanger sequencing. The reported cases are associated with normal serum EPO.

## HIGH OXYGEN-AFFINITY HEMOGLOBIN VARIANTS

High oxygen-affinity hemoglobin variants are defined by an associated left-shifted oxygen dissociation curve (decreased p50 value), and more than 250 different mutations have been reported. Usually the result of point mutations or small deletions involving loci important in cooperative binding of α and β subunits, the heme pocket or the binding of 2,3 BPG; approximately one third of known high oxygen-affinity variants are associated with clinically evident erythrocytosis. The remaining high oxygen-affinity variants are not associated with erythrocytosis for various reasons. The degree of erythrocytosis owing to a high oxygen-affinity hemoglobin variant is usually proportional to the degree of p50 decrease; however, compound heterozygotes with concurrent β thalassemia can show a more severe polycythemia than expected. The erythrocytosis is EPO driven; therefore serum EPO levels are elevated or inappropriately normal. A thorough hemoglobin electrophoresis evaluation will identify the majority of abnormal hemoglobin variants. A subset of high oxygen-affinity variants do not resolve from normal hemoglobins by conventional methods; therefore sequencing of the β and α globin genes (discussed earlier) is warranted in negative cases to exclude these electrophoretically silent variants.

## ERYTHROPOIETIN RECEPTOR

The erythropoietin receptor (EpoR) is present on erythroid progenitor cells. When EPO binds to EpoR, apoptosis is inhibited through an intracellular signaling cascade producing increased numbers of RBCs. EpoR-modulated gene transcription is regulated by SHP1

binding. Truncating mutations in the *EPOR* gene result in the loss of the intracellular–cytoplasmic SHP1 binding domain, which translates functionally to EPO hypersensitivity and erythrocytosis (Figure 24-10, A). *EPOR* is located on the short arm of chromosome 19, spanning 7 kb and containing eight exons. Approximately 16 currently known mutations have all been localized to exon 8, are mainly stop codons, and are heterozygous. These abnormalities are detectable by PCR and direct sequencing methods. Serum EPO levels are decreased or inappropriately normal.

## MUTATIONS IN OXYGEN SENSING PATHWAY GENES

The oxygen-sensing pathway contains multiple factors that regulate RBC mass, and mutations favoring less control over RBC proliferation result in erythrocytosis (see Figure 24-10, B). The most studied components of this pathway are hypoxia-inducible factor (HIF), prolyl hydroxylase domain, and von Hippel Lindau (vHL) proteins (see Figure 24-10, B). HIF is a heterodimer protein comprised of α and β subunits, and it functions as a sensor of depleted oxygen concentration. When present, oxygen serves as a substrate for HIF α subunit degradation. In the absence of oxygen, degradation does not take place, and the α protein component is available to dimerize with the HIF β subunit (see Figure 24-10, B). In the heterodimerized state, HIF then binds to the hypoxia response elements of promoters and enhancers for more than 100 hypoxia response genes, including those for EPO, transferrin, vascular endothelial growth factor, and glucose transporter 1. The α subunit has three isoforms: HIF1α, HIF2α, and HIF3α. HIF1α is ubiquitous, whereas HIF2α is not found in as many organs and cell types. HIF3α is less understood and may have inhibitory functions. The *EPAS1* gene encoding HIF2α is located on chromosome 2, contains 15 exons, and spans 120 kb. Clinically significant mutations have been identified that appear localized within exon 12. The mutations reported have been heterozygous and result in an amino acid substitution, presumably with an abnormal gain-of-function phenotype. Most block degradation of the α subunit allowing hypoxia response elements binding to continue despite properly oxygenated conditions. *EPAS1* mutations are associated with normal or increased EPO levels.

The vHL protein functions in oxygen regulation through colocalizing the proteins necessary to rapidly destroy the oxygen sensing protein HIF, initiating its ubiquitination and proteasomal degradation. It does so by binding the HIF α subunit, although this interaction first requires prolyl hydroxylation by a specialized protein—PHD (see Figure 24-10, B). Erythrocytosis resulting from a germline missense mutation at nucleotide 598 C to T (R200W) in the *VHL* gene has been found endemically in more than 10,000 people in the Chuvash region of the former Soviet Union and is therefore named *Chuvash polycythemia* (CP). Further investigations have detected this mutation in unrelated patients from diverse ethnic groups including Asians, Caucasians, and African Americans, and haplotype studies imply a founder effect. Transmission is autosomal recessive, and patients usually have markedly high or inappropriately normal EPO levels in the presence of elevated hematocrit. Homozygous CP patients are hypersensitive to EPO, with a correspondingly increased risk for cerebrovascular and embolic complications; heterozygous relatives (carriers) are not affected. The CP mutation is not associated with the risk of neoplasias typically present in vHL syndrome (e.g., pheochromocytomas, hemangioblastomas, renal cell carcinomas). Mutations in the *VHL* gene other than the classic CP mutation have also been linked to erythrocytosis and also are associated with markedly high EPO levels. It is important to note that *VHL* mutations resulting in the vHL syndrome are mutually exclusive from the intragenic mutation causing CP. Patients with vHL syndrome are heterozygous for a germline mutation (usually 5′ from the CP mutation locus) and sporadically acquire a random somatic "second hit" in the remaining *VHL* allele resulting in complete loss of vHL function (i.e., localized homozygosity) in various tissue and organ sites, with focal tumor formation. In contrast, CP patients are germline homozygotes with a polycythemic presentation and do not suffer from tumors.

The regulation of HIFα by vHL protein-mediated ubiquitination and proteasomal degradation requires prolyl hydroxylation (see Figure 24-10, B). Enzymes important in the hydroxylation of HIF are the prolyl hydroxylase domain (PHD) proteins, which have three isoforms, PHD1, PHD2, and PHD3. The most significant isoform associated with erythrocytosis is PHD2. PHD enzymes are oxygen dependent and have an iron-containing active site; therefore, activity is modulated by low iron and ascorbic acid levels as well as by low oxygen. The *PHD2* (also *EGLN1*) gene is located on chromosome 1, spans 66 kb, and has 5 exons. Clinically significant mutations are heterozygous and have been found in exons 1 to 4. These mutations result in amino acid substitutions and are associated with inappropriately normal EPO levels. At least for *PHD2*, monoallelic mutations appear to be sufficient for a clinical phenotype, by either dominant negative activity of the mutated gene product or a gene dosage effect (haploinsufficiency).

## HEMOLYTIC ANEMIAS ASSOCIATED WITH RED BLOOD CELL ENZYME DEFICIENCIES

Deficiencies in enzymes important for RBC survival result in hemolysis of varying forms and severity. Many common clinically significant enzyme deficiencies

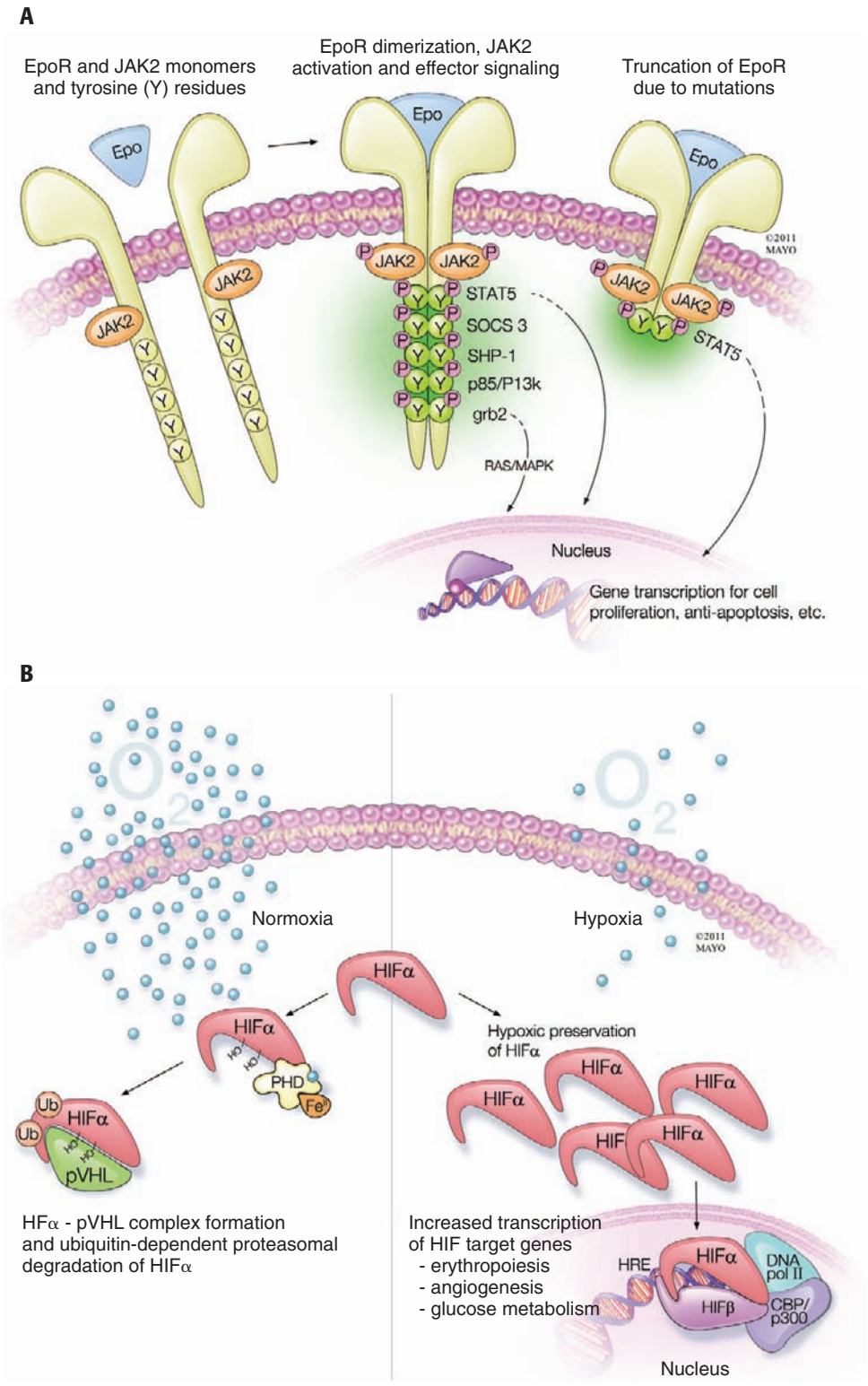



**FIGURE 24-10**

Erythropoietin receptor signaling and the oxygen sensing pathway in the regulation of erythroid cell production. **A,** Erythropoietin receptor signaling pathway. Extracellular (EPO)–erythropoietin receptor (EpoR) binding causes EpoR dimerization; this in turn activates Janus kinase 2 (JAK2) to autophosphorylate multiple sites within the cytoplasmic domain of EpoR. These phosphorylated sites allow binding of transcription factors, including STAT5, which results in the production of more red cells. The process is regulated by the binding of SHP1 to a cytoplasmic binding domain on EpoR. Polycythemia vera results from a constitutively activated JAK-STAT pathway because of a somatic gain of function mutation in JAK2. Heritable erythrocytosis has been associated with heterozygous germline *EPOR* mutations that have interrupted the binding of SHP1 to EpoR. Most result in a truncated EpoR protein resulting in the loss of the cytoplasmic SHP1 domain and are located in exon 8. **B,** Oxygen sensing pathway. In the presence of adequate oxygen, the transcription factor protein hypoxia-inducible factor α subunit (HIFα) is prepared for and ultimately degraded by a complex of enzymatic steps. Important components are oxygen-dependent hydroxylation by a family of prolyl hydroxylase domain (PHD) proteins, which in turn is required for the colocalization of ubiquitin and proteosomes by the von Hippel Lindau (vHL) protein, which results in degradation of HIFα. Under hypoxic conditions, no hydroxylation occurs and HIFα is allowed to enter the nucleus, bind to its β subunit and turn on the transcription of many genes, including *EPO*. Mutations along this pathway that allow HIFα to survive under normal oxygen concentrations have been associated with heritable erythrocytosis. Mutations in HIFα, PHD2, and vHL have been reported. *(Used with permission of Mayo Foundation for Medical Education and Research.)*

involve the glycolytic pathway, such as pyruvate kinase (PK), but by far the most common is from the hexose monophosphate shunt pathway, glucose-6-phosphate dehydrogenase (G6PD). Although G6PD deficiency is commonly associated with acute hemolytic episodes triggered by medication, illness, or fava bean ingestion, most RBC enzyme deficiencies result in a chronic nonspherocytic hemolytic anemia. Some are also associated with neurologic deficits or developmental delay. Others result in glycogen storage diseases with myopathic symptoms. Symptoms of underlying chronic hemolysis are frequently present, such as pigmented gallstones, jaundice, and splenomegaly. A thorough discussion of RBC enzyme deficiencies is beyond the scope of this chapter, but the two most common are briefly discussed.

## GLUCOSE-6-PHOSPHATE DEHYDROGENASE

G6PD is a cytosolic enzyme that produces NADPH, the electron donor molecule important in neutralizing harmful oxidizing agents. Normal conditions use 2% of the enzyme, leaving an abundant reserve. In a G6PD-deficient person, situational demand overwhelms the insufficient enzyme reserve, resulting in hemolysis. Attributed to malaria pressure, hundreds of mutations have been described with wide ranging levels of enzyme activity. The mutations are usually single base substitutions or rarely small deletions. The *G6PD* gene is located on the X chromosome, spans 18.5 kb, and contains 13 exons. Inheritance is X linked; however, because of the high prevalence of the disorder (more than 400 million people), homozygous females are common, and some heterozygous females can manifest symptoms because of mosaicism or lyonization of the X chromosome. G6PD deficiency symptoms include neonatal jaundice, acute episodic hemolysis, or chronic nonspherocytic hemolytic anemia, although many patients are asymptomatic.

## PYRUVATE KINASE

PK is an allosteric enzyme involved in glucose metabolism converting phosphoenol pyruvate to pyruvate. It exists in different isoforms coded by two genes, *PKM* on chromosome 15 and *PKLR* on chromosome 1. *PKLR* contains 12 exons, spans 10 kb, and is the gene associated with RBC PK enzyme activity. Approximately 160 mutations have been reported, usually involving alterations in substrate affinity or enzymatic stability. Occasional mutations affect the interaction of fructose 1,6-diphosphate, an allosteric activator. Inheritance is autosomal recessive, and symptoms are associated with homozygous or compound heterozygous genetic states. Simple heterozygotes for *PKLR* mutation are clinically normal, although they usually have moderately decreased measured enzyme activity levels. PK deficiency is relatively common in humans and other mammals and shows protective effects against malaria morbidity. Clinical symptoms include chronic nonspherocytic hemolytic anemia of varying degree, but can result in hydrops fetalis or severe pediatric anemia responsive to splenectomy.

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The complete reference list is available online at www.expertconsult.com.

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# Index


**A**

Accessory spleen, 634
A-CGH. *See* Array comparative genomic hybridization
Acquired immune hemolytic anemia, splenic pathology in, 639
Acquired thrombocytopenia with decreased platelet production, 90
Activated partial thromboplastin time (aPTT), 67
Acute basophilic leukemia, 443
Acute erythroid leukemia, 443, 445f
Acute leukemia of ambiguous lineage, 677-678
Acute lymphoblastic leukemia, 457-470
  B cell, 463-469, 467f-468f, 468t
    molecular genetics of, 662, 663f, 699t, 706-710
      11q23/*MLL* translocations, 708-710
      t(9;22)/*BCR-ABL1*, 706, 708f-709f
      t(12;21)/*ETV6-RUNX1*, 707-709
      t(1;19)/*TCF3-PBX1*, 707
    risk stratification in, and minimal residual disease assessment, 710-711
  clinical features of, 457-469
  cytology-histology of, 457-458, 459f-461f
  differential diagnosis of, 469
  fact sheet on, 458b
  histiocytosis following, 579
  immunophenotypes and molecular genetics of, 458-469, 463f-467f, 468t, 667, 669f
  pathology of, 457-458, 462b
  prognosis and treatment of, 469-470
  splenic involvement in, 642
  suggested readings on, 470
Acute megakaryoblastic leukemia, 443
  related to Down syndrome, 448, 448b, 449f
  with t(1;22)(p13;q13), 430-433, 432f, 433b
Acute monoblastic/monocytic leukemia, 443, 444f
Acute myeloid leukemia(s), 419
  and blastic plasmacytoid dendritic cell tumor, 450, 450b, 451f
    immunophenotypes of, 674-677
  bone marrow recovery in, post-chemotherapy, 452-456, 455f-456f
  extramedullary, 445-446. *See also* Myeloid sarcoma
  multi-gene mutation analysis in, 705-706
  myeloablative therapy and bone marrow changes in, 452, 454f, 454t
Acute myeloid leukemia(s) *(Continued)*
  with myelodysplasia-related changes, 434-438, 434b, 435f, 455
    diagnostic karyotypes in, 435-436, 436t
    differential diagnosis of, 436-438, 437f
    pathologic features of, 436b
  and myeloid proliferation related to Down syndrome, 446-448
  not otherwise specified (NOS), 440-445
    acute basophilic leukemia, 443
    acute erythroid leukemia, 443, 445f
    acute megakaryoblastic leukemia, 443
    acute monoblastic/monocytic leukemia, 443, 444f
    acute myelomonocytic leukemia, 441-443, 442f
    acute panmyelosis with myelofibrosis, 443-445, 446f
    and French-American British group classification, 440
    immunophenotypes and genetic abnormalities in, 674
    with maturation, 441, 442f
    with minimal differentiation, 440-441, 440f
    without maturation, 441, 441f
  with recurrent translocation abnormalities, 419-434, 698-706, 699t
    core binding factor genes, 422, 424, 703
    inv(16) or t(16;16)/*CBFB-MYH11*, 422-424, 422b, 423f, 703
      immunophenotypes of, 674
    inv(3) or t(3;3)/*RPN1-EVI1*, 429-430, 430b, 431f
    rare, 703-704
    t(1;22)(p13;q13); *RBM-MKL1*, 430-433, 432f, 433b
    t(6;9)(p23;q34); *DEK-NUP214*, 428-429, 429b, 429f
      differential diagnosis of, 429
    t(9;11)(p22;q23); *MLLT3-MLL*, 427-428, 427b, 428f
      differential diagnosis of, 428
    t(15;17)/*PML-RARA*, 424-427, 424b, 425f, 426b, 674, 676f, 700-703, 700f-701f
    t(8;21)/*RUNX1-RUNXT1*, 419-434, 419b-420b, 421f, 703
      differential diagnosis of, 420-422
      immunophenotypes of, 674, 676f
  with single gene abnormalities, 691f, 704-706
    *CEBPA*, 434, 705
    *FLT3*, 433, 704
    *NPM1*, 433, 704-705
  splenic involvement in, 642
  suggested readings on, 456
Acute myeloid leukemia(s) *(Continued)*
  therapy induced, 438-445, 438b, 450-452, 452t
    pathologic features of, 438b, 439f
  WHO classification (2008) of, 419, 420t
Acute myelomonocytic leukemia, 441-443, 442f
Acute panmyelosis with myelofibrosis, 443-445, 446f
Acute promyelocytic leukemia, with t(15;17)/*PML-RARA*, 424-427
  bone marrow recovery after ATRA and chemotherapy in, 455-456, 456f
  cytology-histology of, 424-425, 425f
  differential diagnosis of, 426-427
  French-American-British group classification of, 441
  immunophenotypes and genetics of, 425-426, 674, 676f, 700-703, 700f-701f
    *FLT3* abnormalities, 426
    *PML-RARA* fusion, 426
  pathologic features of, 426, 426b
Acute undifferentiated leukemia, 471-472
  clinical features of, 471-472
  cytology of, 471
  differential diagnosis of, 472
  genetics of, 471
  immunophenotype(s) of, 471
  pathology of, 471
  prognosis and treatment of, 472
ADAMTS13 activity
  assays for, 88
  normal plasma, 89
ADP/ATP ratio measurement, in platelet diagnostics, 67-68
Adult xanthogranuloma family, 577-578
  clinical features of, 577
  cytology-histology of, 578
  differential diagnosis of, 578
  immunophenotype of, 578
  prognosis and treatment of, 578
  radiologic features of, 577-578
Aggregation characteristics, of platelet disorders, 70t
Aggressive B cell lymphomas, diffuse, 261
Aggressive NK (natural killer) cell leukemia, 403, 414-416
  clinical features of, 414-416
  cytology-histology of, 410, 415, 416f
  differential diagnosis of, 415-416
  fact sheet on, 415b
  immunophenotypes and genetics of, 415, 672, 672f
Aggressive systemic mastocytosis, 599, 600f
AIDS related lymphomas, 331-346
  bone marrow involvement in, 338f, 346
  classification of, 334b
  clinical features of, 333-334


*b* indicates boxes, *f* indicates illustrations, and *t* indicates tables.

AIDS related lymphomas *(Continued)*
cytology-histology of, 333-334
Burkitt lymphoma, 334-337, 337f-338f
diffuse large B cell lymphoma, 334, 335f-336f
extranodal marginal zone B cell MALT lymphoma, 337, 339f
herpes viral related non Hodgkin lymphoma, 338-340, 342f
Hodgkin lymphoma, 343-346, 345f
multiple myeloma, 343
mycosis fungoides, 338
peripheral T/NK cell lymphoma, 337-338, 340f
plasmablastic lymphoma, 340-343, 343t, 344f
polymorphic B cell lymphoma, 343, 345f
primary effusion lymphoma, 338-340, 341f
Epstein-Barr viral infection associated with, 332
genetics of, 332-333
immunophenotype(s) of, 333-334
pathogenesis of, 332
prognosis and treatment of, 346
Alder-Reilly anomaly, 102-103, 103f
Alemtuzumab (anti-CD52 antibody), post treatment assessment of bone marrow, 200
*ALK* abnormalities, 690t
in anaplastic large T cell lymphoma, 697-698
in diffuse large B cell lymphomas, 696-697
*ALK*-positive large B cell lymphoma, 280-283
clinical features of, 280-281
differential diagnosis of, 281
fact sheet on, 281b
genetics of, 281
histology of, 281, 282f
immunophenotype(s) of, 281, 282f
prognosis and treatment of, 281-283
Allogeneic hematopoietic stem cell transplantation, for myelodysplastic syndromes, 546
Alloimmune thrombocytopenia, 81
Alpha (α) granule(s), 55-57
electronmicrograph of, 58f
in peripheral blood smear, 62f
Alpha (α) granule storage pool disorders, 76-77, 94-97
clinical features of, 76-77, 94-97
cytology-histology of, 76-77, 77b
differential diagnosis of, 77
fact sheet on, 76b
gray platelet syndrome in, 76, 94-95
platelet aggregation characteristics in, 70t
Alpha (α) heavy chain disease, 629-630
ancillary studies of, 630
clinical features of, 629
cytology-histology of, 629
prognosis and treatment of, 630
Alpha (α) thalassemia
four-gene deletion, 29. *See also* Hemoglobin (Hb) Bart hydrops fetalis
molecular diagnostic evaluation of, 721-722
two gene deletion, 31, 32f
differential diagnosis of, 34
Amyloidosis. *See* Primary amyloidosis
Anaplastic B cells, in diffuse aggressive B cell lymphoma, 262-264
Anaplastic large T cell lymphoma, 297-301
clinical features of, 297-301
cytology-histology of, 298-301, 299f
differential diagnosis of, 301
fact sheet on, 297b
immunophenotypes and genetic abnormalities in, 298-301, 300f, 670, 671f, 697-698
pathologic features of, 298b
Anemia(s). *See also under* Bone marrow failure syndromes, non-neoplastic
acquired aplastic, 160-165
of blood loss, 3
of chronic disease, 18
of chronic kidney disease, 15
of decreased red blood cell production, 3
maturation defects in, 3
proliferation defects in, 15
Diamond-Blackfan, 173
familial aplastic, 176
Fanconi, 168-173
hemolytic, 20
enzyme defective, 38
extrinsically defective, 42
hemoglobin defective, 21
membrane defective, 34
parasitic, 48
iron deficiency, 12
megaloblastic, 3-20
Anemia of chronic disease, 18
ancillary studies of, 20
clinical features of, 18-20
cytology-histology of, 18-20
bone marrow aspirate, 18-20, 19f
peripheral blood smear, 18, 19f
differential diagnosis of, 14t, 20
pathologic features of, 20b
prognosis and treatment of, 20
Anemia of chronic kidney disease, 15
clinical features of, 15-17
cytology-histology of, 15-16
bone marrow biopsy, 15-16, 17f
peripheral blood smear, 15, 16f
differential diagnosis of, 17
prognosis and treatment of, 17
Anemia of inflammation. *See* Anemia of chronic disease
Angioimmunoblastic T cell lymphoma, 293-297
clinical features of, 293-297
cytology-histology of, 293-297, 294f
differential diagnosis of, 297
fact sheet on, 295b
immunophenotypes and genetic abnormalities in, 295-297, 296f, 670, 671f
pathologic features of, 295b
Anisocytosis
in iron deficiency anemia, 12-13
in megaloblastic anemia, 6
in microangiopathic hemolysis, 87-88, 88f
Anthracosis lymphadenopathy, 148, 151f
Antibodies, for identification of histiocytes, 551t
in tissues, 551t
Antigen receptor genes, 686
Antigenic abnormalities, of malignant hematopoietic cell populations, 661
Antigens (clusters of differentiation)
evaluated in flow cytometric analysis, 656-657, 657t. *See also* Flow cytometric analysis
of histiocytes, 551t
specific, in evaluation of hematopoietic cell populations, 658-659, 659t
Aplastic anemia
acquired, 160-165
clinical features of, 160-165
cytology-histology of, 160-162
in bone marrow, 161-162, 163f
in peripheral blood, 160-161, 162f
diagnosis of, 162-165
differential diagnosis of, 165
fact sheet on, 161b
genetics of, 162-163
immunohistochemical features of, 165
immunophenotype(s) of, 163-165
pathologic features of, 161b
prognosis and treatment of, 165
secondary, 161t
familial, 176
Apoptotic neutrophils, 106
Array comparative genomic hybridization (A-CGH), 683, 717-719
Aspirin, and effect on platelet aggregation, 70t
Asplenia, 634
Ataxia telangiectasia, neoplastic lymphoproliferation in, 346-349
Atypical chronic myeloid leukemia, *BCR-ABL1* negative, 520-522
cytology-histology of, 520-521, 521f
in bone marrow, 521, 522f
differential diagnosis of, 521-522
fact sheet on, 520b
genetics of, 521-522
pathologic features of, 521b
prognosis in, 522
Auer rods
in acute promyelocytic leukemia with t(15;17)(q22;q12-21), 528f, 542
in determining bone marrow recovery after treatment of acute myeloid leukemia, 454-455
in dysplastic bone marrow, 529
Autoimmune cytopenia
bone marrow pathology in, 191
in HIV infection/AIDS myelopathy, 183
Autoimmune disorders, bone marrow pathology in, 191, 192f
Autoimmune hemolytic anemia, 42
ancillary studies of, 43
clinical features of, 42-45
cytology-histology of, 43, 44f
differential diagnosis of, 43-45
fact sheet on, 43b
forms of, 42
pathologic features of, 43b
prognosis and treatment of, 45
splenic pathology in, 639
Autoimmune lymphoproliferative syndrome, 347-348, 347f
classification of, 347t
Autoimmune myelofibrosis, 191, 192f
Autosomal dominant giant platelet disorders, 103, 103t


## B


B cell(s)
antigen expression in normal maturation process, 661, 662f
antigen (CD) panel for, 659t
B cell acute lymphoblastic leukemia, 463-469, 467f-468f, 468t. *See also* Acute lymphoblastic leukemia; B cell lymphoblastic leukemia/lymphoma
immunophenotype(s) of, 662, 663f
molecular genetic diagnostic evaluation of, 699t, 706-710
11q23/*MLL* translocations, 708-710

B cell acute lymphoblastic leukemia *(Continued)*
t(9;22)/*BCR-ABL1*, 706, 708f-709f
t(12;21)/*ETV6-RUNX1*, 707-709
t(1;19)/*TCF3-PBX1*, 707
risk stratification in, and minimal residual disease assessment, 710-711
B cell clones, molecular genetics of, 686-689
B cell leukemias, of mature lymphocytes, 387
chronic lymphocytic leukemia, 387-395
hairy cell leukemia, 397-400
hairy cell leukemia variant, 400-402
monoclonal B cell lymphocytosis, 395-396
prolymphocytic leukemia, 396-397
suggested readings on, 402
B cell lymphoblastic leukemia/lymphoma, molecular diagnostic evaluation of, 662, 663f, 699t, 706
B cell lymphomas
large, 261-292. *See also* Diffuse large B cell lymphoma
small, 205-260. *See also* Small B cell lymphomas
B cell lymphoproliferative disorders, immunophenotypes and genetics of, 394-395, 394t
B cell neoplasms
antigen expression in, 662-663
CD20 as therapeutic target in, 663
immunophenotypes and molecular genetic abnormalities of, 661-666. *See also under* Molecular diagnostic evaluation
Burkitt lymphoma, 666, 666f
chronic lymphocytic leukemia-small lymphocytic lymphoma (B cell small lymphocytic lymphoma-chronic lymphocytic leukemia), 211f, 213-214
diffuse large B cell lymphoma, 665-666
follicular lymphoma, 211f, 214-223
hairy cell leukemia, 664, 665f
mantle cell lymphoma, 664
mature B cell neoplasms, 662-663
precursor B cell neoplasms, 662, 663f
B cell prolymphocytic leukemia, 396-397
clinical features of, 396-397
cytology-histology of, 396-397, 397f
differential diagnosis of, 397
fact sheet on, 396b
genetics of, 397
immunophenotype(s) of, 396-397
pathologic features of, 396b
prognosis and treatment of, 397
B cell small lymphocytic lymphoma, 206-214
B cell small lymphocytic lymphoma-chronic lymphocytic leukemia, 206-214, 212f
clinical features of, 206-213
cytology-histology of, 206t, 207-209, 208f-209f
proliferation center, 207-209, 210f-211f
transformational, 212-213, 212f
differential diagnosis of, 213
fact sheet on, 208b
and Hodgkin lymphoma, 212-213
immunophenotypes and genetics of, 207t, 209-212, 211f, 663-664
molecular prognostic markers in, 694-695
prognosis and treatment of, 213-214
splenic involvement in, 209, 211f, 641-642, 642f
β2M, 618-619
Babesiosis, 49
cytology-histology of, 52, 53f
differential diagnosis of, 52
pathologic features of, 49b
Bacille Calmette-Guérin lymphadenitis, 128
fact sheet on, 128b
Bacterial bone marrow infections, 183-184
Bartl plasma cell grading system, 615-616, 616t
*Bartonella henselae* associated lymphadenitis, 120-124
clinical features of, 120-124
cytology-histology of, 122, 123f
differential diagnosis of, 122-124
fact sheet on, 122b
Basket cells, 107-108
Basophilic leukemia, acute, 443
Basophilic stippling, 6, 7f
in beta thalassemia minor, 32f
*BCL6* abnormalities, in diffuse large B cell lymphomas, 690t, 696-697
*BCL10* abnormalities, in extranodal marginal zone lymphomas, 690t, 693-694
Bence-Jones protein, 616
Bence-Jones proteinuria of undetermined significance, 623-624
Bernard-Soulier syndrome, 74-76, 93
clinical features of, 74-76
cytology-histology of, 74-76, 75b
differential diagnosis of, 76
fact sheet on, 74b
platelet aggregation characteristics in, 70t, 75f
prognosis and treatment of, 76
Beta (β) thalassemia intermedia, 30
Beta (β) thalassemia(s), molecular diagnostic evaluation of, 722
Beta (β) thalassemia major, 29-30
cytology-histology of, 30, 31f
differential diagnosis of, 33
Beta (β) thalassemia minor, 31, 32f
differential diagnosis of, 34
Birbeck granule(s), 571-572, 573f
2,3-Bisphosphoglycerate, 723
Bite cells, 39, 40f
Blastic plasmacytoid dendritic cell tumor, 450, 450b, 451f
immunophenotype and genetics of, 674-677
Blastomycosis, bone marrow pathology in, 185, 186f
Bleeding time test, 64
Blister cells, 22, 24f
Bone abnormalities, marrow pathology in, 194-195, 196f
Bone marrow abnormalities, non-neoplastic, 177
in bone changes, 194-195
in chemotherapy, 202
in cytokine treatments, 200-201
granulomatous, 188-189
infectious, 177-186
lymphocyte and lymphoid aggregates in, 197-200
in storage diseases, 191-194
in stromal disorders, 195-197
suggested readings on, 202
in systemic disorders, 190-191
toxin associated, 201-202
Bone marrow biopsy, in platelet diagnostics, 62-64
Bone marrow failure syndromes, non-neoplastic, 160, 173
acquired aplastic anemia, 160-165
cartilage-hair hypoplasia, 174
Bone marrow failure syndromes, non-neoplastic *(Continued)*
congenital, 168-176, 173t
congenital amegakaryocytic thrombocytopenia, 175
cyclic neutropenia, 174
Diamond-Blackfan anemia, 173
dyskeratosis congenita, 175-176
familial aplastic anemia, 176
Fanconi anemia, 168-173
myelokathexis, 175
paroxysmal nocturnal hemoglobinuria, 165-168
Pearson syndrome, 174
reticular dysgenesis, 175
severe congenital neutropenia, 174
Shwachman-Diamond syndrome, 175
suggested readings on, 176
thrombocytopenia with absent radii, 175
Bone marrow fibrosis, 195b
cytology-histology of, 196, 197f
pathologic features of, 195b
Bone marrow gelatinous transformation, 196, 198f
Bone marrow granulomas, 188-189
causes of, 188t
clinical features of, 188-189
cytology-histology of, 189, 189f
pathologic features of, 188b
Bone marrow infections, 177-186
bacterial, 183-184
clinical features of, 177
cytology-histology of, 177
fact sheet on, 177b
fungal, 184-186
parasitic and protozoal, 186
pathologic features of, 178b
viral, 178-183
Bone marrow necrosis, 195b
cytology-histology of, 196-197, 198f
disorders associated with, 197t
pathologic features of, 195b
Bone marrow stromal disorders, 195-197
cytology-histology of, 195-197, 197f
Brucellosis, reactive lymph nodes in, 124b
Burkitt lymphoma, 287-290
atypical, 290
clinical features of, 287-290
cytology-histology of, 287, 288f
differential diagnosis of, 289-290, 290t
fact sheet on, 287b
in HIV infection/AIDS, 334-337, 337f-338f
immunophenotype(s) and genetics of, 287-289, 288f, 289t, 666, 666f
molecular genetic diagnostic evaluation of, 695
post transplant, 328, 331f
prognosis and treatment of, 290
Burkitt lymphoma-leukemia, cytology of, 457-458, 461f
Burkitt-like lymphoma, 290

## C

Cartilage-hair hypoplasia, bone marrow failure in, 174
Castleman disease, 150-159
differential diagnosis of, 157-159
hyaline-vascular, 153-155
localized, 153
multicentric, 153
human herpesvirus 8 associated, 157-159, 158f, 339, 342f
plasma cell, 155-156

Cat scratch disease lymphadenopathy, 120-124
  clinical features of, 120-124
  cytology-histology of, 122, 123f
  differential diagnosis of, 122-124
  fact sheet on, 122b
*CEBPA* abnormalities, in acute myeloid leukemia, 434, 705
Centroblastic B cells, in diffuse aggressive B cell lymphoma, 262-264, 263f
Centroblasts, 225-227, 228f
Centrocyte(s), 225-227, 228f
Centrocyte-like cells, 238-241, 240f-241f
Ceroid histiocytosis, 551-552, 637-639
Charcot-Leyden crystals, eosinophil associated, 107
Chédiak-Higashi syndrome, 101-102
  dense granule storage dysfunction in, 95
  leukocytes with abnormal granules in, 101-102, 102f
Chemotherapy. *See also* Cytokine therapy; Granulocyte-colony stimulating factor (G-CSF) therapy; Myeloablative therapy
  bone marrow pathology in, 202, 202b
  post treatment recovery of bone marrow in acute myeloid leukemia, 452-456, 455f-456f
Childhood acute lymphoblastic leukemia, 463-469, 467f-468f, 468t
  genetic abnormalties in, 662, 663f, 699t, 706-710
  molecular genetic diagnostic evaluation of, 699t, 706-710
    11q23/*MLL* translocations, 708-710
    t(9;22)/*BCR-ABL1*, 706, 708f-709f
    t(12;21)/*ETV6-RUNX1*, 707-709
    t(1;19)/*TCF3-PBX1*, 707
  risk stratification in, and minimal residual disease assessment, 710-711
Choi algorithm, 264-266, 265f
Chromosome translocations. *See* Genetic abnormalities
Chronic alcoholism, bone marrow pathology in, 190, 190b
Chronic eosinophilic leukemia, 494
Chronic granulomatous disease lymphadenopathy, 130-132, 131f
  clinical features of, 130-132
  differential diagnosis of, 130-132
  fact sheet on, 130b
  macrophage cytology in, 557
Chronic lymphocytic leukemia, 387-395
  clinical features of, 387-395
  cytology-histology of, 387-394, 388f
    bone marrow, 387-389, 390f
  differential diagnosis of, 394-395, 394f, 394t
  fact sheet on, 387b
  genetics of, 392-394, 392f-393f, 392t
  Hodgkin lymphoma and, 383
  immunophenotype(s) of, 391-392, 391f
  pathologic features of, 389b
  prognosis and treatment of, 395
  scoring system for, 391, 391t
  staging systems for, 387, 388t
  transformation of, to prolymphocytic leukemia, 389
  variant, 389f
Chronic lymphocytic leukemia/small lymphocytic lymphoma. *See* B cell small lymphocytic lymphoma-chronic lymphocytic leukemia
Chronic myelogenous leukemia, 480-494
  clinical features of, 480-491
Chronic myelogenous leukemia *(Continued)*
  cytology-histology of, 481-484
    in accelerated phase, 485-487, 486f-487f
    in bilineal or biphenotypic blast phase, 489
    in blast phase, 487-489, 488f
    in bone marrow, 481-484, 483f-486f
    in extramedullary blast phase, 489
    in lymphoid blast phase, 489, 490f, 492f
    in myeloid blast phase, 487-489, 488f-490f
    in peripheral blood, 481, 482f-483f
  differential diagnosis of, 489-491
    in accelerated and blast phases, 491
    in chronic phase, 491
  fact sheet on, 481b
  immunophenotype(s) of, 484
    in accelerated phase, 487
    in lymphoid blast phase, 489
    in myeloid blast phase, 487-489, 489f
  molecular genetic diagnostic evaluation of, 708f-709f, 711-714, 712t
  pathologic features of, 482b
  prognosis and treatment of, 492-494
  t(9;22)-negative and *BCR-ABL1*-negative clone development in treatment of, 494
Chronic myeloid neoplasms, molecular genetic diagnostic evaluation of, 711-714. *See also* Myeloid neoplasms
Chronic myelomonocytic leukemia, 514-518
  clinical features of, 515-518
  cytology-histology of, 515-517, 515f-516f
    in bone marrow, 517, 517f
    extramedullary, 517
  differential diagnosis of, 517-518
  fact sheet on, 515b
  genetics of, 517-518
  pathologic features of, 516b
  prognosis in, 518
Chronic neutrophilic leukemia, 512-514
  clinical features of, 512
  cytology-histology of, 512
  differential diagnosis of, 512, 513f-514f
  fact sheet on, 512b
  pathologic features of, 513b
  prognosis and treatment of, 514
Classic Hodgkin lymphoma, 353-354, 353t. *See also* Hodgkin lymphoma
  Reed-Sternberg cell immunophenotypes in, 357-358, 357t
  small lymphocyte immunophenotypes in, 358-360, 358t
Clonal detection, in lymphoid populations, 686-689, 686f-687f
  using polymerase chain reaction technique, 688-689
  using southern blot hybridization, 689
Clusters of differentiation. *See* Antigens
CN-LOH (copy number neutral loss of heterozygosity), 717-718
Coagulation testing, in platelet diagnostics, 67
Cobalamin, in DNA and methionine synthesis, 3-4, 5f
Cobalamin deficiency, 4
Coccidioidomycosis, bone marrow pathology in, 185-186, 186f
Cold agglutinin disease. *See* Cold autoimmune hemolytic anemia
Cold autoimmune hemolytic anemia, 42-43
  cytology-histology of, 43, 44f
  differential diagnosis of, 43-45
Collagen receptor disorders, in platelets, 94
Color compensation (adjustment for spillover), 658
Common variable immunodeficiency, 349f
Congenital amegakaryocytic thrombocytopenia, 90, 175
  with radioulnar synostosis, 90
Congenital cytopenic syndromes, 173-176, 173t
Congenital dyserythropoietic anemias, 173-174
Congestive splenomegaly, 639
Cooley anemia, 29-30
Copy number neutral loss of heterozygosity (CN-LOH), 717-718, 719f
Copy number variations (CNVs), 717-718
Cotswold revision of Ann Arbor staging of Hodgkin lymphoma, 351, 351t
Cracked chromatin pattern, 387-389, 388f
Crescentic histiocytes, 140
Cryoglobulinemia, mixed (type II), 181-182
Cryptococcosis, bone marrow pathology in, 185, 186f
Cutaneous CD30 T cell lymphoproliferative disorders. *See* Primary cutaneous CD30 T cell lymphoproliferative disorders
Cutaneous diffuse large B cell lymphoma, leg type. *See* Primary cutaneous diffuse large B cell lymphoma, leg type
Cutaneous follicle center lymphoma. *See* Primary cutaneous follicle center lymphoma
Cutaneous mastocytosis, 588
  clinical features of, 588-590, 589f
  diagnostic criteria for, 592t
  histopathology of, 591-593
  WHO classification of, 591, 592t
Cutaneous T cell lymphomas, 314-318
  clinical features of, 315-318
  cytology-histology of, 315-318, 316f
  differential diagnosis of, 318
  fact sheet on, 315b
  genetics of, 318
  immunophenotype(s) of, 317-318
  pathologic features of, 317b
  rare subtypes. *See* Primary cutaneous T cell lymphomas, rare subtypes
Cyclic neutropenia, bone marrow failure in, 174
Cytogenetic analysis, sensitivity and specificity of, 685t
Cytokine therapy
  and effects in leukocytes, 106, 106f
    differential diagnosis of, 106
  non-neoplastic bone marrow abnormalities in, 200-201
    clinical features of, 200-201, 200b
    cytology-histology of, 106, 106f, 200-202, 201f
    pathologic features of, 200b
  and reduced plasma iron levels, 18
Cytomegaloviral lymphadenitis, 115-116
  clinical features of, 115-116
  cytology-histology of, 115-116, 117f
  differential diagnosis of, 116
  fact sheet on, 115b
Cytopenia with hypercellular marrow, and myelodysplastic syndrome, differential diagnosis of, 541-542
Cytopenia with normocellular or hypocellular marrow, and myelodysplastic syndrome, differential diagnosis of, 542
Cytophagic histiocytic panniculitis, 563

**D**

Dacrocytes (target cells), 22, 23f
  in Hb CC disease, 27, 28f
  in sickle cell disease, 22-26, 23f, 25f
Decreased platelet production, 90
Delayed splenic rupture, 632
Demethylating agent therapies, 545-546
Dendritic cell(s), 549-550
  cytokine influence and phenotypic changes in, 549-550
  functionally defective, 557-558
  hyperplasia of, 552, 553f
  identification of, 550, 551t
    antibodies in, 551t
  in peripheral sites, 549-550
  plasmacytoid, 549
  precursors of, 550f
  sentinel, 549-550, 552
  in tissues, 549
    antibodies in identification of, 551t
Dendritic cell sarcoma
  follicular, 584-587
    ancillary studies of, 585-586
    clinical features of, 584-587
    cytology-histology of, 585, 585f
    differential diagnosis of, 587
    immunophenotype of, 586
    pathologic features of, 585
    prognosis and treatment of, 587
  interdigitating, 582-584
    ancillary studies of, 582-584
    clinical features of, 582-584
    cytology-histology of, 582, 584f
    differential diagnosis of, 584
    immunophenotype of, 582-584
    prognosis and treatment of, 584
  not otherwise specified, 587
  pathologic features of, 581b
Dense granule storage pool disorders, 94-97
  clinical features of, 94-97
  cytology-histology of, 95-96, 95b, 96f
  differential diagnosis of, 97
  fact sheet on, 95b
  platelet aggregation characteristics in, 70t
Dense granules, 55, 58f
  and aggregation of platelets, 57
Dermatopathic lymphadenopathy, 148-150, 149f, 552, 553f
Diagnostic (Hodgkin lymphoma) cells, 354-356, 355f-356f
Diamond-Blackfan anemia, 173
Diffuse aggressive B cell lymphomas, 261
Diffuse cutaneous mastocytosis, 591
Diffuse follicular lymphoma, 224, 226f
Diffuse large B cell lymphoma, 238-241, 261
  associated with chronic inflammation, 274-276
    clinical features of, 274-275
    differential diagnosis of, 275
    fact sheet on, 275b
    genetics of, 275
    histology of, 275
    immunophenotype(s) of, 275
    prognosis and treatment of, 275-276
  Burkitt lymphoma in, 287-290
  of central nervous system. *See* Primary diffuse large B cell lymphoma of central nervous system
  fact sheet on, 262b
  in HIV infected/AIDS patients, 334, 335f-336f
    immunophenotype(s) of, 334, 336f
  in immunocompetent patients, 262t, 274-276
Diffuse large B cell lymphoma *(Continued)*
    *ALK*-positive large B cell lymphoma, 280-283
    intravascular large B cell lymphoma, 278-280
    lymphomatoid granulomatosis, 283-285
    primary mediastinal large B cell lymphoma, 276-278
  in immunocompromised patients, 262t, 334, 335f-336f
    plasmablastic lymphoma, 285-286
  immunophenotype(s) and genetics of, 665-666
  molecular genetic diagnostic evaluation of, 689-692, 690t, 691f, 696-697
  not otherwise specified, 261-267, 262t
    bone marrow involvement in, 264
    clinical features of, 261-267
    cytology-histology of, 262-264, 263f-264f
    differential diagnosis of, 266-267
    genetics of, 266, 267f
    immunophenotype(s) of, 264-266, 265f
    prognosis and treatment of, 267, 268t
  splenic involvement in, 642-643
  subtypes of, 262t, 695, 696f
    central nervous system primary diffuse large B cell lymphoma, 270-272
    cutaneous primary diffuse large B cell lymphoma, leg type, 272-273
    Epstein-Barr virus positive diffuse large B cell lymphoma of elderly, 273-274
    T cell/histiocyte-rich large B cell lymphoma, 268-270
  suggested readings on, 292
  unclassified, 290-292
Diffuse lymphocyte rich classic Hodgkin lymphoma, 379-380
  differential diagnosis of, 380
  immunophenotype(s) of, 379
  prognosis and treatment of, 380
Dimorphic red blood cells, in myelodysplastic syndrome, 526-527, 527f
Disseminated intravascular coagulation, 45, 85-86
  clinical features of, 85-86
  cytology-histology of, and laboratory studies, 86
  differential diagnosis of, 86
  prognosis and treatment of, 86
Döhle bodies, 105
Döhle body mimics
  in autosomal dominant giant platelet disorders, 103-104
  in *MYH9* macrothrombocytopenia, 72, 72f
  neutrophil inclusions in, 62
Double hit lymphoma, 291, 292f
Drepanocytes, 22, 23f
Drug induced thrombocytopenia, 81-85
  immune, 81-82
  non-immune, 85
Drug related lymphadenopathy, 143, 146f
  fact sheet on, 145b
Durie-Salmon staging system, for plasma cell myeloma, 622, 622t
Dutcher bodies, 229, 238-241
  in lymphoplasmacytic lymphoma, 255-257, 257f
Dwarf megakaryocytes, 481-484, 485f
Dyserythropoiesis, in dysplastic bone marrow, 529, 530f
  differential diagnosis of, 542-543
Dysgranulopoiesis, in dysplastic bone marrow, 526-527, 528f, 529
  differential diagnosis of, 543
Dyskeratosis congenita, bone marrow failure in, 175-176
Dysmegakaryopoiesis, in dysplastic bone marrow, 529, 531f
  differential diagnosis of, 543
Dyspoiesis, differentiation of, in myelodysplastic syndromes, 107, 107f, 202

**E**

Eccentrocytes, 39, 41f
Electronmicroscopy, in platelet diagnostics, 67
Elliptocytes, 37, 38f
  pencil cell, 12-13, 13f
Elliptocytosis, hereditary, 36
  ancillary studies of, 37
  clinical features of, 36-38
  cytology-histology of, 37, 37f
  differential diagnosis of, 38
  fact sheet on, 36b
  pathologic features of, 37b
  prognosis and treatment of, 38
Enteropathy associated T cell lymphoma, 312-314
  clinical features of, 312-314
  cytology-histology of, 312-314, 313f
  differential diagnosis of, 314
  fact sheet on, 312b
  genetics of, 314
  immunophenotype(s) of, 313f, 314
  pathologic features of, 314b
Eosinophil associated Charcot-Leyden crystals, 107
Eosinophilia, molecular genetics of myeloid and lymphoid neoplasms associated with, 712t, 716
Eosinophilic leukemia, chronic, 494
Eosinophilic microabscesses, 143, 144f
Epigenetic therapies, for myelodysplastic syndromes, 545-546
Epithelioid histiocytes, in reactive follicle, 133, 134f
Epithelioid histiocytoma, 566. *See also* Reticulohistiocytoma
Epstein syndrome, 103, 103t
Epstein-Barr positive cells, in lymphomatoid granulomatosis, 284, 284f
Epstein-Barr viral bone marrow infection, 178
  reactive lymphocytes in, peripheral blood smear, 179f
Epstein-Barr virus positive diffuse large B cell lymphoma of elderly, 273-274
  clinical features of, 273-274
  cytology-histology of, 273-274
  differential diagnosis of, 274
  fact sheet on, 274b
  genetics of, 274
  immunophenotype(s) of, 274
  prognosis and treatment of, 274
Epstein-Barr virus positive lymphoproliferative disorders of childhood, 324-326
  clinical features of, 324-326
  cytology-histology of, 325-326
  differential diagnosis of, 326
  fact sheet on, 325b
  genetics of, 326
  immunophenotype(s) of, 325-326
  pathologic features of, 325b

Erythrocytosis, 54
molecular diagnostic evaluation of, 722-723, 723t
at serum erythropoietin level, 723t
Erythroid hyperplasia, compensatory, 20, 21f
Erythroid leukemia, acute, 443, 445f
Erythropoiesis, defective, anemias in, 3
Erythropoietin, and bone marrow pathology, 201
Erythropoietin receptor, molecular diagnostics for, 723-724
Essential thrombocythemia, 501-505
clinical features of, 501-505
cytology-histology of, 502-503
in blood and bone marrow, 502-503, 503f
differential diagnosis of, 504-505
fact sheet on, 502b
genetics of, 501, 503-504, 712t, 714-716
pathologic features of, 501, 503b
prognosis and treatment of, 505
Extracavitary primary effusion lymphoma, 339, 342f
Extracutaneous mastocytoma, 602
Extranodal marginal zone B cell lymphoma, molecular genetic diagnostic evaluation of, 693-694
Extranodal marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT lymphoma), 238-244. *See also* MALT lymphoma
Extranodal NK (natural killer) cell/T cell lymphoma, nasal type, 304-307
clinical features of, 304-307
cytology-histology of, 304-305, 305f
differential diagnosis of, 305-307
fact sheet on, 304b
in HIV infection/AIDS, 337-338, 340f
immunophenotypes and genetic abnormalities in, 304-305, 306f, 672, 672f
pathologic features of, 304b

## F

Fabry disease, bone marrow pathology in, 194
Faggot cells, 424-425, 425f
Familial aplastic anemia, 176
Familial hemophagocytic lymphohistiocytosis, 558-563
clinical features of, 558-563
cytology-histology of, 561, 562f-563f
in bone marrow, 561
differential diagnosis of, 562
fact sheet on, 560b
genetics of, 561
immunophenotypes of, 561, 563f
pathologic features of, 561, 561b
prognosis and treatment of, 562-563
Familial thrombocytopenia-leukemia, 90
Fanconi anemia, 168-173
ancillary studies of, 171-172
chromosome breakage analysis of, 171-172, 172f
clinical features of, 169-173
cytology-histology of, 171
in bone marrow, 171, 171f
in peripheral blood, 171
differential diagnosis of, 172-173
fact sheet on, 170b
pathologic features of, 171b
prognosis and treatment of, 173
Fechtner syndrome, 103, 103t
Fetal hemoglobin, hereditary persistence of, 722
*FGFR1* mutations, in myeloid and lymphoid neoplasms associated with eosinophilia, 712t, 716
Fibroblastic reticular cell sarcoma, 587
Filarial lymphadenitis, 135b
*FIP1L1-PDGFRA* fusion, in hypereosinophilic syndrome and mastocytosis, 609-610
FISH. *See* Fluorescence in situ hybridization
5q minus syndrome, 505
Floral variant follicular lymphoma, 229, 232f
Flow cytometric analysis, 67, 655. *See also* Immunophenotype(s), and genetic abnormalities
antigens evaluated in, 656-657, 657t
biohazard precautions in, 656
clinical indications for, 655-656
data acquisition in, 659-660
data analysis in, 660-661, 660f
data reporting in, 661
equipment used in, 655
fluorochrome antibody panels in, 658-659, 658f
selection of, 658-659, 659t
gating in, 660-661, 660f
genetic abnormalities of malignant cell populations detected by, 661
in platelet diagnostics, 67
quality control procedures in, 657-658
scatter plots in, 660
simultaneous antigen evaluation in, 657
specimen processing in, 656-657
specimen requirements for, 656
Flower cell lymphocytes, in adult HTLV-1 associated leukemia-lymphoma, 411-412, 411f
*FLT3*, in dendritic cell development, 549
*FLT3* mutation, in acute myeloid leukemia, 433, 704
Fluorescence in situ hybridization (FISH), 683-684
image acquisition devices in development of, 684
in intact nondividing cells, 684
locus specific probes in, 683-684
repetitive sequence probes in, 683
sensitivity and specificity of, 685t
tissue and sample sources for, 684
whole chromosome probes in, 684
Foamy macrophages, 109
Folate, in DNA and methionine synthesis, 3-4, 5f
Folate assays, 10
Folate deficiency, 4
Folded tissue paper appearance, of macrophages in Gaucher disease bone marrow biopsy, 193f
Follicle lysis, lymph node, 111
Follicular dendritic cell sarcoma, 584-587
clinical features of, 584-587
cytology-histology of, 585, 585f
differential diagnosis of, 587
immunophenotype and genetics of, 585-586
pathologic features of, 585
prognosis and treatment of, 587
Follicular dendritic cell tumor, 153-155, 155f
Follicular hyperplasia
in IgG4 associated lymphadenopathy, 145-146, 147f
in Kimura disease, 143, 144f
in rheumatoid arthritis, 136, 137f
Follicular lymphoma, 223-238
clinical features of, 223-237
cytology-histology of, 206t, 224-229, 224f-226f, 228f
in collagen fibrosis type, 224-225, 227f
in diffuse type, 224, 226f
in floral variant type, 229, 232f
grading, 227, 228f
with marginal zone B cell differentiation, 229, 231f
with plasma cell differentiation, 229, 229f-230f
transformational, 235-237, 236f
variant, 227-229
differential diagnosis of, 237
extranodal involvement in, 234-235
bone marrow, 234
intestine, 235
skin, 234-235
spleen, 234, 643, 644f
fact sheet on, 224b
genetics of, 207t, 232-234
grading, 227, 228f
histiocytosis following, 579
immunophenotype(s)and genetics of, 207t, 231-232, 233f, 664, 665f
molecular genetic diagnostic evaluation of, 689-692, 690t, 691f
pediatric, 235
prognosis and treatment of, 237-238
Folliculotropic mycosis fungoides (follicular mucinosis), 315, 317
Four gene deletion α-thalassemia, 29. *See also* Hemoglobin (Hb) Bart hydrops fetalis
*FOXP1* abnormalities, in extranodal marginal zone lymphomas, 690t, 693-694
Franklin disease. *See* Gamma (γ) heavy chain disease
Fungal bone marrow infections, 184-186, 185f-186f
Fungal lymphadenitis, 132

## G

Gaisböck disease, 54
Gamma (γ) heavy chain disease, 630
ancillary studies of, 630
clinical features of, 630
pathologic features of, 630
prognosis and treatment of, 630
Gamna-Gandy bodies, 639
Gastrointestinal disorders, bone marrow pathology in, 190
Gaucher cells, 109, 110f
Gaucher disease
bone marrow pathology in, 192-194, 193f
liver sinusoidal pathology in, 557f
splenic pathology in, 637, 638f
Gene fusion transcripts, detected with RQ-PCR, 682
Gene rearrangement studies, antigen receptor, 404
Genetic abnormalities
*ALK*, 690t
in anaplastic large T cell lymphoma, 697-698
in diffuse large B cell lymphomas, 696-697
in B cell lymphoblastic leukemia/lymphoma, 662, 663f, 699t, 706-711
11q23/*MLL* translocations, 708-710
t(9;22)/*BCR-ABL1*, 706, 708f-709f

Genetic abnormalities *(Continued)*
t(12;21)/*ETV6-RUNX1*, 707-709
t(1;19)/*TCF3-PBX1*, 707
*BCL6*, in diffuse large B cell lymphomas, 690t, 696-697
*BCL10*, in extranodal marginal zone lymphomas, 690t, 693-694
in chronic myeloid neoplasms, 711, 712t
*JAK2* mutations, 714-716, 715f
*KIT* abnormalities (systemic mastocytosis), 716-717
*MPL* mutations, 716
*PDGFRA, PDGFRB, FGFR1* abnormalities, 716
t(9;22)/*BCR-ABL1* (chronic myeloid leukemia), 711-714
*FIP1L1-PDGFRA* fusion, in hypereosinophilic syndrome and mastocytosis, 609-610, 716
*FLT3*, in acute myeloid leukemia, 433, 704
*FOXP1*, in extranodal marginal zone lymphomas, 690t, 693-694
*IRF4*, in T cell non Hodgkin lymphomas, 690t, 697-698
*KIT*, in mastocytosis and non-mast neoplasms, 608-609, 608t, 609f, 716-717
*MALT1*, in extranodal marginal zone lymphomas, 690t, 693-694
*MYC*, in diffuse large B cell lymphomas, 690t, 696-697
*MYH9* associated macrothrombocytopenia, 71-74, 103-104
in non Hodgkin lymphomas, 690t
inv14 or t(14;14)/*TCL1A*, in T cell prolymphocytic leukemia, 698
t(11;18)/*API2-MALT1*, t(14;18)/*MALT1-IGH@*, in extranodal marginal zone lymphomas, 693-694
t(14;18)(q32;q21)/*BCL2-IGH@*, in follicular and large B cell lymphomas, 689-692, 691f
t(11;14)(q13;q32)/*CCND1-IGH@*, in mantle cell lymphoma, 692-693, 693f
t(8;14)(q24;q32)/*MYC-IGH@*, in Burkitt lymphoma, 695, 696f
t(2;5)/*NPM1-ALK*, in anaplastic large T cell lymphoma, 697-698
recurrent, in acute myeloid leukemias
*CEBPA*, 434, 705
core binding factor genes, 422, 424, 703
*FLT3*, 433, 704
inv(16) or t(16;16)/*CBFB-MYH11*, 422-424, 422b, 423f, 674, 703
*NPM1*, 433, 704-705
rare, 424
t(15;17)/*PML-RARA* (acute promyelocytic leukemia), 424-427, 424b, 425f, 426b, 674, 676f, 700-703, 700f-701f
t(8;21)/*RUNX1-RUNXT1*, 419-434, 419b-420b, 421f, 674, 676f, 703
*TCL1A*, in T cell non Hodgkin lymphomas, 690t, 697-698
V(D)J recombination events, 688
Genetic disorders, associated with platelet disorders, 68t
Ghost cells, 196-197, 198f
Giant bands, 6-10, 8f-9f
Giant cell formation, 552-555
Giant metamyelocytes, 6-10, 8f
Giant platelet(s), 62
Giant platelet disorders, autosomal dominant, 103, 103t
Giant pronormoblasts, in parvovirus infection, 181, 181f
Glanzmann thrombasthenia, 91-93
clinical features of, 91-93
cytology-histology of, 91-92, 92b
differential diagnosis of, 93
fact sheet on, 91b
immunophenotype(s) of, 91-92, 93f
platelet aggregation characteristics in, 70t, 92f
prognosis and treatment of, 93
Glucose-6-phosphate dehydrogenase deficiency, 38-39
ancillary studies of, 39-41
clinical features of, 39-41
cytology-histology of, 39, 40f-41f
differential diagnosis of, 41, 42f
fact sheet on, 39b
molecular diagnostic evaluation of, 726
pathologic features of, 39b
prognosis and treatment of, 41-42
Glycoprotein (GP) IIb/IIIa, deficiency or dysfunction of, 91
Glycoprotein (GP) IIb/IIIa antagonists, 62
and effect on platelet aggregation, 70t
and immune thrombocytopenia induction, 81
Glycoprotein (GP) IIb/IIIa receptor, activation of, 57, 61f
Granulocyte(s), antigen (CD) panel for, 659t
Granulocyte-colony stimulating factor (G-CSF) therapy
and blood/bone marrow changes resembling acute myeloid leukemia, 450-452, 452t, 453f
leukocyte changes caused by, 106, 106f
non-neoplastic bone marrow abnormalities in, 200-201
clinical features of, 200-201, 200b
cytology-histology of, 200-201, 201f
pathologic features of, 200b
Granulocyte-macrophage colony stimulating factor (GM-CSF), 549
Granulomatous disease lymphadenopathy. *See* Chronic granulomatous disease lymphadenopathy
Granulomatous slack skin, 315, 317
Gray platelet syndrome, 76-77, 94-95
clinical findings in, 76-77
cytology-histology of, 76-77, 77b
differential diagnosis of, 77
fact sheet on, 76b
Gray zone lymphoma, 290, 372
Greipp plasma cell grading system, 615-616, 616t
Growth centers, 207-209
Growth factor therapy. *See* Granulocyte-colony stimulating factor (G-CSF) therapy
Gumprecht cells, 107-108

**H**

HAART (highly active retroviral multiple agent therapy), 331-332
Hairy cell leukemia, 397-400, 639-640
clinical features of, 398-400
cytology-histology of, 398-400, 398f
in spleen, 398-399, 399f
differential diagnosis of, 400
fact sheet on, 398b
Hairy cell leukemia *(Continued)*
immunophenotype(s) and genetic abnormalities in, 399-400, 664, 665f
pathologic features of, 399b
Hairy cell leukemia variant, 400-402, 639-640
clinical features of, 400-402
cytology-histology of, 401-402, 401f
in spleen, 402f
differential diagnosis of, 402
fact sheet on, 400b
genetics of, 402
immunophenotype(s) of, 401-402, 664
pathologic features of, 401b
prognosis and treatment of, 402
Hans algorithm, 264-266, 265f
Haptocorrins, 4
Heavy chain disease, 629-631
alpha, 629-630
fact sheet on, 629b
gamma, 630
mu, 630-631
pathologic features of, 630b
Heinz bodies, 41
HELLP syndrome, 89
Helmet cells, 41, 42f
in microangiopathic hemolytic anemia, 45-46, 47f
Hematogones, in bone marrow pathology, 199, 199f
Hematopoietic growth factor therapy, for myelodysplastic syndromes, 545
Hematoxylin bodies, 137-138, 139f
Hemoglobin (Hb), 29
Hemoglobin (Hb) Bart hydrops fetalis, 29
cytology-histology of, 30-31
differential diagnosis of, 33
Hemoglobin (Hb) C crystals
in Hb CC disease, 27, 28f-29f
in sickle cell disease, 22-26, 25f
Hemoglobin (Hb) CC disease, 27
ancillary studies of, 27
clinical features of, 27-28
cytology-histology of, 27, 28f-29f
differential diagnosis of, 27-28
fact sheet on, 27b
pathologic features of, 27b
prognosis and treatment of, 28
Hemoglobin (Hb) H disease, 30, 32f-33f
Hemoglobin (Hb) structural disorder(s), 21
in hemoglobin CC disease, 27
in sickle cell disease, 21
in thalassemias, 28
Hemoglobin (Hb) variants, high oxygen affinity, molecular diagnostics for, 723
Hemoglobinopathies. *See* Red blood cell disorder(s), hemoglobin defective *or specific disorder or disease*
Hemolytic anemia(s), 20
acquired immune, splenic pathology in, 639
autoimmune, 42
glucose-6-phosphate dehydrogenase deficiency, 38-39
hemoglobin CC disease, 27
hereditary elliptocytosis, 36
hereditary pyropoikilocytosis, 36
hereditary spherocytosis, 34
microangiopathic, 45
molecular diagnostic evaluation of, 724-726
parasitic, 48
sickle cell disease, 21
thalassemias, 28

Hemolytic uremic syndrome, 45
Hemophagocytic syndrome, 178-181
  cytology-histology of, 180f
  fact sheet on, 179b
  malignancy associated, 179, 307, 324-325
  pathologic features of, 180b
  viral agents associated with, 178t
Heparin induced thrombocytopenia, 82-85
  clinical features of, 82-85
  cytology-histology of, 83-85, 83b
  differential diagnosis of, 85
  fact sheet on, 83b
  physiology of, 82, 82f
  pretest scoring for, 84, 84t
  prognosis and treatment of, 85
Hepatitis C viral infection, in bone marrow, 181-182
Hepatosplenic T cell lymphoma, 308-312, 639-640
  clinical features of, 310-312
  cytology-histology of, 310-312, 311f
  differential diagnosis of, 312
  fact sheet on, 310b
  immunophenotypes and genetic abnormalities in, 310-312, 311f, 670
  pathologic features of, 310b
Hereditary elliptocytosis, 36
  ancillary studies of, 37
  clinical features of, 36-38
  cytology-histology of, 37, 37f
  differential diagnosis of, 38
  fact sheet on, 36b
  pathologic features of, 37b
  prognosis and treatment of, 38
Hereditary pyropoikilocytosis, 36
  ancillary studies of, 37
  clinical features of, 36-38
  cytology-histology of, 37, 38f
  differential diagnosis of, 38
  fact sheet on, 36b
  pathologic features of, 37b
  prognosis and treatment of, 38
Hereditary spherocytosis, 34
  ancillary studies of, 35-36
  clinical features of, 34-36
  cytology-histology of, 34-35, 35f
  differential diagnosis of, 36
  pathologic features of, 35b
  prognosis and treatment of, 36
  splenic pathology in, 639
Hereditary storage diseases. *See also* Storage diseases
  lymphadenopathy in, 148
Hermansky-Pudlak syndrome, 557
  ceroid filled liver macrophages in, 557f
  dense granule storage dysfunction in, 95
Herpes simplex lymphadenitis, 116-118
  clinical features of, 116-118
  cytology-histology of, 116, 118f
  differential diagnosis of, 118
  fact sheet on, 116b
Herpes virus related non Hodgkin lymphoma, 338-340, 342f
High oxygen affinity hemoglobin variants, molecular diagnostics for, 723
Histiocyte(s), 109, 549-550. *See also* Histiocytic disorders
  functionally defective, 555-558, 557f
  identification of, 550
    antibodies in, 551t
  and macrophage activation sydrome, 558
  pathologic excess of, 552-555
  physiologic excess of, 551-552
  precursors of, 550f
  tissue, antibodies in identification of, 551t
Histiocytic disorders, 558
  classification of (Histiocyte Society, 1997; World Health Organization, 2002), 560t
  cytophagic histiocytic panniculitis, 563
  familial hemophagocytic lymphohistiocytosis, 558-563
  following acute lymphoblastic leukemia or follicular lymphoma, 579
  Langerhans and non-Langerhans histiocytosis combined, 578-579
  Langerhans cell histiocytosis, 567-575
  malignant, 579-587, 581t
  non-Langerhans cell histiocytosis, 575-578
  reticulohistiocytoma, 566-567
  Rosai-Dorfman disease, 563-566
  suggested readings on, 587
Histiocytic lesion, cells comprising, 549
Histiocytic necrotizing lymphadenitis, 138-140, 557
Histiocytic sarcoma, 579-581
  ancillary studies of, 579-580
  clinical features of, 579-580
  cytology-histology of, 579, 580f
  differential diagnosis of, 580
  fact sheet on, 579b
  immunophenotypes of, 580
  pathologic features of, 581b
  prognosis and treatment of, 581
Histiocytic sarcoma with juvenile xanthogranuloma phenotype, 586f, 587
Histone deacetylase inhibitors, 546
Histoplasmosis, bone marrow histology in, 185, 185f
HIV infection/AIDS
  hematologic changes in, 182-183, 182b
  Hodgkin lymphoma and, 343-346, 345f, 383-384, 383t
    clinical features of, 383-384
    cytology-histology of, 384
  lymphadenopathy associated with, 119-120
    clinical features of, 119-120
    cytology-histology of, 119-120
    fact sheet on, 119b
  myelopathy in, 182t, 183
    cytology-histology of, 183f
    fact sheet on, 182b
    pathologic features of, 182b
  neoplastic lymphoproliferation in, 331-346
    bone marrow involvement in, 346
    classification of, 334b
    clinical features of, 333-334
    cytology-histology of, 333-334
      Burkitt lymphoma, 334-337
      diffuse large B cell lymphoma, 334
      herpes viral related non Hodgkin lymphomas, 338-340
      Hodgkin lymphoma, 343-346
      MALT lymphoma, 337
      multiple myeloma, 343
      mycosis fungoides, 338
      peripheral T/NK cell lymphomas, 337-338
      plasmablastic lymphoma, 340-343
      polymorphic B cell lymphoma, 343
      primary effusion lymphoma, 338-340
    prognosis and treatment of, 346
Hodgkin lymphoma, 350
  and chronic lymphocytic leukemia, 383
  clinical features of, 350-354
  clinical types of, 353-354, 353t
Hodgkin lymphoma *(Continued)*
  diagnosis and staging of, 351, 351t
  diffuse lymphocyte rich classic, 379-380
  fact sheet on, 351b
  granulomatous inflammation of lymph nodes in, 128
  histology-cytology of, and classifications, 351-354, 352t, 353b, 355f-356f
  historical pathology of, 350-361
  and HIV infection/AIDS, 343-346, 345f, 383-384, 383t
    clinical features of, 383-384
    cytology-histology of, 384
  immunophenotype(s) and genetics of, 355f-356f, 356, 357t, 360-361
  lymphocyte depleted classic, 380-383
  lymphocyte rich classic, 376-379. *See also* Lymphocyte rich classic Hodgkin lymphoma
  mixed cellularity, 373-376. *See also* Mixed cellularity Hodgkin lymphoma
  nodular lymphocyte predominant, 361-366. *See also* Nodular lymphocyte predominant Hodgkin lymphoma
  nodular sclerosis, 366-373. *See also* Nodular sclerotic Hodgkin lymphoma
  post transplant, 328, 333f
  prognosis and treatment of, 354
  Reed-Sternberg cell immunophenotypes in, 355f-356f, 357-358, 357t
  Reed-Sternberg cell pathology in classification of, 354-361, 355f-356f, 357t
  small lymphocytic immunophenotypes in, 358-360, 358t
  splenic involvement in, 643-644
  suggested readings on, 384
  WHO classification of, 353
Hodkgin-like lesions, 376
Hofs, 419-420, 421f
  perinuclear, post growth factor therapy, 451-452, 453f
Hot pockets, in aplastic anemic bone marrow, 161-162, 164f
Howell-Jolly bodies, 6, 22, 23f
Howell-Jolly body mimics, 105-106, 105f
HTLV-1 viral associated T cell leukemia/lymphoma, 410-412. *See also* T cell leukemia-lymphoma associated with human T lymphotropic virus 1 (HTLV-1)
Human herpesvirus 8 associated multicentric Castleman disease, 157-159, 158f, 339, 342f
Hyaline-vascular Castleman disease, 153-155
  clinical features of, 153-155
  cytology-histology of, 153-155, 154f
  differential diagnosis of, 157
Hydroa vacciniforme-like lymphoma, 325
Hyperesoinophilic syndrome, 494
Hypersegmented neutrophil, 6, 8f
Hypertensive disorders of pregnancy, thrombocytopenia in, 89

I

Idiopathic cytopenia of uncertain significance, 541-546
Idiopathic thrombocytopenia purpura, splenic pathology in, 639
IgG4 related lymphadenopathy, 145-146, 147f
  fact sheet on, 145b

*IGH@* (immunoglobulin heavy chain gene), 688
Imatinib (Gleevec), 492
  molecular mechanism of action of, 492
  monitoring therapy with, 492
  resistance to, 492-494
  results of treatment with, 492, 493f
Immature B and T cells. *See also* Precursor lymphoid neoplasms
  antigen (CD) panel for, 659t
Immune disorders, lymphoproliferative diseases in. *See* Primary immune disorders
Immune drug-induced thrombocytopenia, 81-82
  clinical features of, 81-82
  cytology-histology of, 81
  differential diagnosis of, 81-82
  prognosis and treatment of, 82
Immune thrombocytopenic purpura, 79-80
  clinical features of, 79-80
  cytology-histology of, 79-80
    in bone marrow, 191
  differential diagnosis of, 80
  fact sheet on, 79b
  pathologic features of, 80b
  prognosis and treatment of, 80
Immunoblastic B cells, in diffuse aggressive B cell lymphoma, 262-264, 263f
Immunoblastic variant, of large B cell lymphoma, 334, 336f
Immunodeficiency related lymphoproliferative disorders, 327
  cytology-histology of, 328-331, 329t
  fact sheet on, 328b
  in HIV infection/AIDS, 331-346. *See also* AIDS related lymphomas; HIV infection/AIDS
  incidence of malignancy in, 349
  post transplantation, 327-331
  in primary immune disorders, 346-349
  suggested readings on, 349
Immunoglobulin heavy chain gene (IGH@), 688
Immunomodulator related lymphoproliferative disorders, 348, 348f
Immunomodulatory therapies, for myelodysplastic syndromes, 545
Immunophenotype(s), and genetic abnormalities, 661
  of B cell neoplasms, 661-666
    Burkitt lymphoma, 666, 666f
    chronic lymphocytic leukemia-small lymphocytic lymphoma (B cell small lymphocytic lymphoma-chronic lymphocytic leukemia), 211f, 213-214
    diffuse large B cell lymphoma, 665-666
    follicular lymphoma, 211f, 214-223
    hairy cell leukemia, 664, 665f
    mantle cell lymphoma, 664
    mature B cell neoplasms, 662-663
    precursor B cell neoplasms, 662, 663f
  of myeloid stem cell neoplasms, 672-678
    acute leukemia of ambiguous lineage, 677-678
    acute myeloid leukemia, not otherwise specified, 674
    acute myeloid leukemia with inv(16)(p13;q22) or t(16;16)(p13;q22), 674
    acute myeloid leukemia with t(8;21)(q22;q22), 674, 676f
    acute myeloid leukemia with t(15;17)(q24;q21), 674, 676f
Immunophenotype(s), and genetic abnormalities *(Continued)*
    blastic plasmacytoid dendritic cell neoplasm, 674-677
    low grade myelodysplastic syndromes and chronic myeloproliferative disorders, 672-674, 675f
    paroxysmal nocturnal hemoglobinuria, 677f, 678
  of plasma cell neoplasms, 666, 667f
  of T cell and NK(natural killer) cell neoplasms, 666-672
    adult T cell leukemia-lymphoma, 670
    aggressive NK (natural killer) cell leukemia, 672, 672f
    anaplastic large cell lymphoma, 670, 671f
    angioimmunoblastic T cell lymphoma, 670, 671f
    hepatosplenic T cell lymphoma, 670
    mycosis fungoides (Sézary syndrome), 670
    nasal-type extranodal NK (natural killer) cell lymphoma, 672, 672f
    peripheral T cell lymphoma, unspecified, 670
    precursor T cell neoplasms, 667, 669f
    T cell large granular lymphocytic leukemia, 670
    T cell prolymphocytic leukemia, 668-670, 669f
Immunophenotyping, 655, 678
  suggested readings on, 678
IMPACT (Diamed), 65, 65f
in situ follicular lymphoma, 235, 237
Indeterminate cell lesion or histiocytosis, 578
Indolent systemic mastocytosis, 596, 600f
Infectious mononucleosis lymphadenopathy, 113-115
  clinical features of, 113-115
  cytology-histology of, 113, 114f
  differential diagnosis of, 114-115
  fact sheet on, 113b
Infectious mononucleosis splenomegaly, 637
Inflammatory cytokines, and reduced plasma iron levels, 18
Inflammatory pseudotumor-like follicular dendritic cell sarcoma, 647t, 653f, 654
  fact sheet on, 654b
Interdigitating dendritic cell sarcoma, 582-584
  ancillary studies of, 582-584
  clinical features of, 582-584
  cytology-histology of, 582, 584f
  differential diagnosis of, 584
  immunophenotype of, 582-584
  prognosis and treatment of, 584
Interfollicular Hodgkin lymphoma, 374-375
Intravascular large B cell lymphoma, 278-280
  clinical features of, 278-279
  cytology-histology of, 279, 280f
  differential diagnosis of, 279
  fact sheet on, 279b
  genetics of, 279
  immunophenotype(s) of, 279
  prognosis and treatment of, 279-280
Intravascular lymphomatosis, 254-255
*IRF4* abnormalities, in T cell non Hodgkin lymphomas, 690t, 697-698
Iron deficiency anemia, 12
  ancillary studies of, 14, 14t
  clinical features of, 12-14
  cytology-histology of, 12-13, 13f
    in bone marrow, 13
  differential diagnosis of, 14
Iron deficiency anemia *(Continued)*
  fact sheet on, 13b
  pathologic features of, 13b
  prognosis and treatment of, 14-15

## J

*JAK2* mutations, in Philadelphia negative myeloproliferative neoplasms, 712t, 714-716, 715f
Juvenile chronic myelomonocytic leukemia, 518-520
  clinical features of, 518-520
  cytology-histology of, 519
  differential diagnosis of, 519-520
  fact sheet on, 519b
  genetics of, 519
  pathologic features of, 519b
  prognosis in, 520
Juvenile xanthogranuloma family, 575-577
  ancillary studies of, 576-577
  clinical features of, 575
  cytology-histology of, 575-576, 576f
  differential diagnosis of, 577
  immunophenotype of, 577
  prognosis and treatment of, 577

## K

Kawasaki disease, 120, 121b
Kikuchi disease, lymphadenopathy in, 138-142
  clinical features of, 138-142
  cytology-histology of, 140-142, 141f
  differential diagnosis of, 142
  fact sheet on, 140b
  histologic subtypes of, 140, 141f
Kikuchi-Fujimoto disease, histiocyte pathology in, 557
Kimura disease, lymphadenopathy in, 142-143
  clinical features of, 142-143
  cytology-histology of, 143, 144f
  differential diagnosis of, 143
  fact sheet on, 142b
*KIT* abnormalities, in mastocytosis and non-mast neoplasms, 608-609, 608t, 609f, 712t, 716-717
Kostmann syndrome, neutropenia in, 174

## L

L & H cells. *See* Lymphocyte predominant (LP) cells
Lacunar cells, 354-356, 355f-356f
  in nodular sclerotic Hodgkin lymphoma, 355f-356f, 367-368
Langerhans cell histiocytosis, 567-575
  ancillary studies of, 570-573
  clinical features of, 567-568
  cytology-histology of, 569-570, 570f
    in fine needle aspiration, 572-573, 574f
    in lymph liver, 572f
    in lymph node, 571f
    ultrastructural features in, 571-572, 573f
  differential diagnosis of, 573-575
  fact sheet on, 568b
  genetics of, 570-571
  immunophenotype(s) of, 572
  pathologic features of, 568-570, 569b
  prognosis and treatment of, 575
  radiologic features of, 568-575, 569f
Langerhans cell histiocytosis and juvenile xanthogranuloma, 578
Langerhans cell histiocytosis and Rosai-Dorfman disease, 578-579

Langerhans cell sarcoma, 581-582
cytology-histology of, 581-582, 583f
differential diagnosis of, 582
immunophenotype of, 581-582
prognosis and treatment of, 582
Langerhans cell-like lesions, 578
Large B cell lymphomas. *See* Diffuse large B cell lymphoma
Large T cell granular lymphocytic leukemia, 404-408
clinical features of, 404-408
cytology-histology of, 404-407, 405f
differential diagnosis of, 407-408
fact sheet on, 406b
genetics of, 407
immunophenotype(s) of, 406-407, 407f
immunophenotypes and genetic abnormalities in, 670
splenic involvement in, 642
Late onset hypogammaglobulinemia, 348-349
Leishmaniasis
bone marrow pathology in, 186, 187f
lymphadenitis in, 133
Lenalidomide, 546
Lethal midline granuloma, 304
Leukocyte adhesion deficiency, 555-557
Light transmission aggregometry, 66-67, 66f
Lipogranuloma(s), 189, 189f
in Whipple disease, 129
Littoral cell angioma, 647, 647t, 649f
fact sheet on, 648b
Loss of heterozygosity (LOH), identification of, 717-718
LP (lymphocyte predominant) cells, 354-356, 355f-356f, 359f-360f, 362-363
Lupus lymphadenitis, 136-138
cytology-histology of, 137-138, 139f
differential diagnosis of, 138
fact sheet on, 138b
Lutzner cells, 409
Lymph node anatomy, 111
Lymph node germinal center transformation, 111
Lymphadenopathy(ies)
bacille Calmette-Guérin, 128
*Bartonella henselae* associated, 120-124
in brucellosis, 124b
in Castleman disease, 150-159
in cat scratch disease, 120-124
in chronic granulomatous disease, 130-132
cytomegaloviral, 115-116
dermatopathic, 148-150
filarial, 135b
fungal, 132
herpes simplex, 116-118
HIV associated, 119-120
IgG4 related, 145-146
in infectious mononucleosis, 113-115
in Kawasaki disease, 120
in Kikuchi disease, 138-142
in Kimura disease, 142-143
in leishmaniasis, 133
in measles, 120
medication associated, 143
mycobacterial, 124-128
*Pneumocystis carinii* associated, 135b
pyogenic bacterial, 120
in rheumatoid arthritis, 133-136
in sarcoidosis, 148-150
suggested readings on, 159
in syphilis, 124
in systemic lupus erythematosus, 136-138
in toxoplasmosis, 132-133
in Whipple disease, 128-130
Lymphoblastic lymphoma, 457-470
clinical features of, 457-469
differential diagnosis of, 469
gross pathology of, 457
histology-cytology of, 457-458, 459f
immunophenotype(s) of, 463, 467f
prognosis and treatment of, 469-470
Lymphocyte depleted classic Hodgkin lymphoma, 380-383
clinical features of, 380-383
cytology-histology of, 381, 382f
differential diagnosis of, 381-383
fact sheet on, 380b
immunophenotype(s) of, 381
pathologic features of, 381b
prognosis and treatment of, 383
Lymphocyte predominant (LP) cells, 354-356, 355f-356f, 359f-360f, 362-363
Lymphocyte rich classic Hodgkin lymphoma, 376-379
clinical features of, 376-379
cytology-histology of, 376-378, 377f
differential diagnosis of, 379
fact sheet on, 378b
immunophenotype(s) of, 377f, 378-379
pathologic features of, 378b
Lymphocytic variant hypereosinophilic syndromes, 416-418
clinical features of, 416-418
cytology-histology of, 417-418
differential diagnosis of, 418
fact sheet on, 417b
genetics of, 418
immunophenotype(s) of, 417-418, 418f
Lymphocytosis, white blood cell morphology in, 107-109
Lymphoepithelial lesions, in MALT lymphoma, 238-241, 240f, 337, 339f
Lymphoepithelioid cell (Lennert) lymphoma, 301-302, 303f, 375
Lymphoid aggregates, in bone marrow pathology, 199-200
Lymphomatoid granulomatosis, 283-285
clinical features of, 283-285
cytology-histology of, 283-284, 284f
differential diagnosis of, 284-285
fact sheet on, 283b
genetics of, 284
immunophenotype(s) of, 284, 284f
prognosis and treatment of, 285
Lymphoplasmacytic lymphoma, 255-260
clinical features of, 255-260
cytology-histology of, 206t, 255-257, 255f-256f
in bone marrow, 259f
immunoglobulin inclusions, 255-257, 258f
plasma cell, 255-257, 257f
differential diagnosis of, 260
fact sheet on, 256b
genetics of, 207t, 258-260
immunophenotype(s) of, 207t, 257-258
prognosis and treatment of, 260
splenic involvement in, 643
Lymphoproliferative disorder with Hodgkin lymphoma features, 376
Lysosomal storage disorders, in histiocytes, 555-558, 557f

## M

M protein (M component), 616-618, 619f
before and after chemotherapy for plasma cell myeloma, 618
M1 macrophages, 558
M2 macrophages, 558
Macroangiopathic hemolytic anemia, 46-48, 48f
Macrocytosis, in HIV infection/AIDS, 182, 182b
Macrogametocytes, in malaria, 16, 49
Macro-ovalocytes, 6, 6f
Macrophage activation syndrome, 558, 559f
Macrophages, 549-550
activation patterns of, 558, 559f
cytokine influence on phenotype of, 549-550
functionally defective, in storage diseases, 555-558, 557f
identification of, 550
antibodies in, 551t
M1 and M2, 558
morphologic anomalies in, 109-110
pathologic excess of, 552-555
in infection and response to foreign substances, 552-555, 554f, 556f
in xanthomatous inflammation, 555, 557f
physiologic excess of, 551-552, 552f
precursors of, 550f
tissue specific, 549
antibodies in identification of, 551t
Macrothrombocytopenia(s), 71-77
Bernard-Soulier syndrome, 74-76
gray platelet syndrome, 76-77
leukocyte anomalies in, 103, 103t
Mediterranean, 77
Montreal platelet syndrome, 77
with neutrophilic inclusions, 71-74. *See also MYH9* associated macrothrombocytopenia
Malaria, 48-49
cytology-histology of, 49-52
macrogametocytes, 16
schizonts, 51f
trophozoites, 15
differential diagnosis of, 52
pathologic features of, 49b
MALT lymphoma
clinical features of, 238-244
in common variable immunodeficiency, 349, 349f
cytology-histology of, 206t, 238-242, 240f-242f
follicular, 241
in lung, 239f
lymphoepithelial, 238-241, 240f
paracortical, 241-242
differential diagnosis of, 244
fact sheet on, 239b
genetics of, 207t, 243-244, 243f
in HIV infection/AIDS, 337, 339f
immunophenotype(s) of, 207t, 242-243, 242f
molecular genetic diagnostic evaluation of, 693-694
pathogenesis of, 238, 238t
prognosis and treatment of, 244
*MALT1* abnormalities, in extranodal marginal zone lymphomas, 690t, 693-694
Mammary prostheses, lymphadenopathy associated with, 148, 150f
Mantle cell lymphoma, 214-223
clinical features of, 214-223
cytology-histology of, 206t, 215-217, 215f, 217f
in blastoid variants, 217, 219f
diffuse pattern, 214-215, 216f
mantle zone pattern, 214-215, 215f

Mantle cell lymphoma *(Continued)*
monocytoid variant, 217, 218f
nodular pattern, 214-215, 216f
differential diagnosis of, 221-223
extranodal sites involved in, 217-218
blood smear, 220f
bone marrow, 220f
intestine, 221f
spleen, 643
fact sheet on, 214b
immunophenotype(s) and genetic abnormalities in, 207t, 208, 218-221, 222f-223f, 664
molecular genetic diagnostic evaluation of, 692-693, 693f
prognosis and treatment of, 223
Mast cell(s), cytology of normal and atypical, 593-596, 594f
Mast cell leukemia, 599-602, 601f
Mast cell progenitors, 588
Mast cell sarcoma, 601f, 602
Mastocytoma, 602
Mastocytosis, 588-611
ancillary studies of, 603-610
clinical features of, 588-590, 589f
cutaneous, cytology-histology of, 591-593, 592t, 593f. *See also* Cutaneous mastocytosis
cytology-histology of, 591-603
diagnostic algorithm for, 602, 602f
differential diagnosis of, 593-596, 596t, 610
fact sheet on, 590b
genetics of, 607-610, 607f, 608t
immunophenotype(s) of, 607, 607f
prognosis and treatment of, 610-611
radiologic features of, 590-610, 590f
splenic pathology in, 645-646, 645f
suggested readings on, 611
systemic, 592t. *See also* Systemic mastocytosis
cytopathology of, 593-596, 595f
WHO classification of, 591, 592t
Mature B cell neoplasms. *See* B cell neoplasms
May-Hegglin anomaly, 103-104, 103t, 104f
Mean platelet volume (MPV), 60
Measles lymphadenitis, 120, 120f
Mediastinal large B cell lymphoma. *See* Primary mediastinal large B cell lymphoma
Medication associated lymphadenopathy, 143, 146f
fact sheet on, 145b
Mediterranean lymphoma. *See* Alpha (α) heavy chain disease
Mediterranean macrothrombocytopenia, 77
Megakaryoblastic leukemia, 443
related to Down syndrome, 448, 448b, 449f
with t(1;22)(p13;q13), 430-433, 432f, 433b
Megakaryocyte(s), 55, 56f
and platelet maturation process, 57f
Megaloblastic anemia, 3-20
ancillary studies of, 10, 11t
clinical features of, 4-12
cytology-histology of, 6-10
in bone marrow aspirate, 6-10, 8f-9f
in bone marrow biopsy, 6-10, 10f-11f
in peripheral blood film, 6, 6f-8f
differential diagnosis of, 10-12
fact sheet on, 5b
pathologic features of, 7b
prognosis and treatment of, 12
Megaloblastic changes, in leukocytes, 106-107, 107f
Metastatic carcinoma, granulomatous inflammation of lymph nodes in, 128
Methotrexate therapy, lymphoproliferative disorders in, 348, 348f, 376
Micro Typing System (DiaMed, Biorad), 84
Microabscesses
eosinophilic, 143, 144f
Pautrier, 315-317, 316f
Microangiopathic hemolysis, typical cytology of peripheral blood smear, 87-88, 88f
Microangiopathic hemolytic anemia, 45
ancillary studies of, 46
clinical features of, 45-48
cytology-histology of, 45-46, 47f
differential diagnosis of, 46-48, 48f
fact sheet on, 46b
forms of, 45
pathologic features of, 46b
prognosis and treatment of, 48
Microcytes, 6, 6f
Micronodular T cell/histiocyte-rich large B cell lymphoma, 268, 641
MicroRNA(s), 393, 719-720
MicroRNA profiling, 719-720
Microspherocytes, 37, 38f
Minimal residual disease (MRD)
assessment of, and risk stratification in childhood acute lymphoblastic leukemia, 710-711
measurement of, using RQ-PCR, 682
Mixed cell chronic lymphocytic leukemia, 387-389
Mixed cellularity Hodgkin lymphoma, 373-376
clinical features of, 373-376
cytology-histology of, 374-375, 374f
differential diagnosis of, 375-376
infectious mononucleosis and, 376
inflammatory reaction in lymph nodes and, 375-376
peripheral T cell lymphoma and, 375
T cell/histiocyte rich large B cell lymphoma and, 375
fact sheet on, 373b
immunophenotype(s) of, 375
pathologic features of, 374b
prognosis and treatment of, 376
Mixed-phenotype acute leukemia(s), 471-478
B-myeloid immunophenotype of, 476f
clinical features of, 472-477
cytology-histology of, 472-474
bilineal type, 473f
biphenic type, 473f
differential diagnosis of, 477
genetics of, 475-477, 477f
immunophenotype(s) of, 474-477, 474f, 475t, 476f
pathologic features of, 475b
prognosis and treatment of, 478
suggested readings on, 478
T-myeloid immunophenotype of, 474f
WHO classification criteria for, 440-441, 475t
*MLL* abnormalities
in childhood leukemias, 707-710
in secondary treatment related acute myeloid leukemia, 710
Molecular diagnostic evaluation. *See also* Molecular diagnostics
of acute lymphoblastic leukemias, 699t, 706-711
Molecular diagnostic evaluation *(Continued)*
of acute myeloid leukemias, 698-700, 699t. *See also* Acute myeloid leukemia
multi-gene mutation analysis in, 705-706
recurrent translocations, 700-706
core binding factor genes t(8;21)/*RUNX1-RUNXT1*, inv 16 or t(16;16)/*CBFB-MYH11*, 703
rare, 703-704
t(15;17)/*PML-RARA* abnormalities, 700-703, 700f-701f
single gene abnormalities, 704-706
*CEBPA*, 705
*FLT3*, 704
*NPM1*, 704-705
of B cell lymphoblastic leukemia/lymphoma, 699t, 706
of childhood B cell lymphoblastic leukemia, 699t, 706-710
11q23/*MLL* translocations, 708-710
t(9;22)/*BCR-ABL1*, 706, 708f-709f
t(12;21)/*ETV6-RUNX1*, 707-709
t(1;19)/*TCF3-PBX1*, 707
of chronic myeloid neoplasms, 711, 712t
*JAK2* and *MPL* mutations (Philadelphia negative myeloproliferative neoplasms), 714-716
*KIT* abnormalities (systemic mastocytosis), 716-717
*PDGFRA, PDGFRB, FGFR1* abnormalities (eosinophilia in myeloid and lymphoid neoplasms), 716
t(9;22)/*BCR-ABL1* in chronic myeloid leukemia, 707f-708f, 711-714
of non-Hodgkin lymphomas, 689, 690t. *See also specific lymphoma*
Burkitt lymphoma, 695
chronic lymphocytic leukemia/small lymphocytic lymphoma, 694-695
diffuse large B cell lymphomas, 696-697
extranodal marginal zone lymphomas, 693-694
follicular and large B cell lymphomas, 689-692
mantle cell lymphoma, 692-693
T cell lymphomas, 697-698
of red blood cell disorders, 721
erythrocytosis, 722-723, 723t, 725f
hemoglobinopathies, 721
hemolytic anemias associated with RBC enzyme deficiences, 724-726
thalassemias, 721-726
suggested readings on, 726
Molecular diagnostics, 679. *See also* Molecular diagnostic evaluation
assay sensitivity and specificity in, 685, 685t
and detection of clonal *versus* non-clonal lymphoid proliferations, 686-689, 686f-687f
emerging trends and new technology in, 717
array comparative genomic hybridization, 717-719
microRNA profiling, 719-720
next generation sequencing, 720-721
single nucleotide polymorphism arrays, 717-719
methods used in, 679-685
fluorescence in situ hybridization, 683-684

Molecular diagnostics *(Continued)*
polymerase chain reaction technique, 679-680
southern blot hybridization, 682-683
in red blood cell disorders, 721
suggested readings on, 726
Monoblastic/monocytic leukemia, acute, 443, 444f
Monoclonal B cell lymphocytosis, 395-396
diagnostic criteria for, and subclassification, 395t
Monoclonal gammopathy of undetermined significance, 623-625
ancillary studies of, 624
clinical features of, 623-625
cytology-histology of, 624
differential diagnosis of, 624-625
fact sheet on, 624b
prognosis and treatment of, 625
and progression to primary amyloidosis, 625
WHO diagnostic criteria for diagnosis of, 613t
Monoclonal immunoglobin chain deposition diseases, 627-631
ancillary studies of, 628-629
clinical features of, 628-629
cytology-histology of, 628
differential diagnosis of, 629
fact sheet on, 628b
pathologic features of, 628b
prognosis and treatment of, 629
Monoclonal mastocytosis of undetermined significance/monoclonal mastocytosis activation syndrome, 602
Monocyte(s), antigen (CD) panel for, 659t
Monocytoid B cell hyperplasia, 115-116
in cat scratch disease lymph nodes, 122
in toxoplasmosis, 133, 134f
Monocytoid B cells, in reactive lymph nodes, 111
in HIV associated lymphadenopathy, 119
Montreal platelet syndrome, 77
*MPL* mutations, in Philadelphia negative myeloproliferative neoplasms, 712t, 716
MRD. *See* Minimal residual disease
Mu (μ) heavy chain disease, 630-631
ancillary studies of, 631
clinical features of, 630
differential diagnosis of, 631
pathologic features of, 630
Mucopolysaccharidosis, 555
Alder-Reilly anomaly in, 102-103
splenic pathology in, 637-639
Multicentric Castleman disease, 153
herpes positive plasmablastic lymphoma in, 339-340, 342f
human herpesvirus 8 associated, 157-159, 158f, 339, 342f
Multiple intestinal lymphomatous polyposis, 214, 217-218, 221f, 235
Multiple myeloma, in HIV infection/AIDS, 343
Mummified cells, 354-356, 355f-356f
*MYC* abnormalities, in diffuse large B cell lymphomas, 690t, 696-697
Mycobacterial infection, of bone marrow, 184, 184f
Mycobacterial lymphadenitides, 124-128
in immunocompetent patients, 125, 125t
in immunocompromised patients, 126-128
differential diagnosis of, 126-128, 127f
Mycosis fungoides, and Sézary syndrome, 314-318. *See also* Sézary syndrome, in mycosis fungoides
clinical features of, 315-318
cytology-histology of, 315-318, 316f
differential diagnosis of, 318
fact sheet on, 315b
in HIV infection/AIDS, 338
immunophenotypes and genetic abnormalities in, 317-318, 670
pathologic features of, 317b
Myeloablative therapy, and bone marrow changes in acute myeloid leukemia, 452, 454f, 454t
Myelodysplastic syndrome(s), 523-534
of childhood, 538-540
differential diagnosis of, 538-540
refractory cytopenia and, 538, 539f
circulating blast enumeration in diagnosis of, 527, 543
classification of, 534-540
French-American-British, 534
World Health Organization 2001, 534
World Health Organization 2008, 534-538, 535t
clinical features of, 525-526, 525f
cytology-histology of, 526-529, 530f
in bone marrow, 527-529, 530f-531f
in peripheral blood, 526-527, 527f-528f
diagnostic criteria for, 541-546
differential diagnosis of, 107, 107f, 202, 541-546
blast increase in, 543
cytopenia with hypercellular marrow and, 541-542
cytopenia with normocellular or hypocellular marrow and, 542
dyserythropoiesis in, 542-543
dysgranulopoiesis in, 543
dysmegakaryopoiesis in, 543
epidemiology of, 523
fact sheet on, 524b
with fibrosis, 540-541
genetics and pathogenesis of, 523-525, 532-534, 532t
hypocellular bone marrow in, 540
and idiopathic cytopenia of uncertain significance, 541-546
immunophenotypes and genetic abnormalities in, 529-532, 672-674, 675f
medullary blast enumeration in diagnosis of, 529, 543
pathologic features of, 526b
prognosis and risk classification in, 543-545, 544t
specific (WHO 2008), 534-538
myelodysplastic syndrome, unclassifiable, 537-538
myelodysplastic syndrome associated with an isolated del(5q) chromosomal abnormality, 537, 537f
refractory anemia, 535-536
refractory anemia with ring sideroblasts, 536
refractory cytopenia with excess of blasts, 536
refractory cytopenia with multilineage dysplasia, 536
refractory cytopenia with unilineage dysplasia, 535
refractory neutropenia and refractory thrombocytopenia, 536
suggested readings on, 546
treatment of, 545-546
Myelodysplastic syndromes/myeloproliferative neoplasms, 479-480, 514-522
atypical chronic myeloid leukemia, *BCR-ABL1* negative, 520-522
chronic myelomonocytic leukemia, 514-518
juvenile chronic myelomonocytic leukemia, 518-520
unclassifiable, 522
Myelofibrosis. *See* Primary myelofibrosis
Myeloid blasts, antigen (CD) panel for, 659t
Myeloid cell maturation, 672-674
benign abnormal (following cytotoxic chemotherapy), antigen expression, 673f
normal, antigen expression, 673f
Myeloid element increase, cytokine induced, 106, 106f. *See also* Granulocyte-colony stimulating factor (G-CSF) therapy
Myeloid neoplasms
immunophenotypes and genetic abnormalities of, 672-678
acute leukemia of ambiguous lineage, 677-678
acute myeloid leukemia, not otherwise specified, 674
acute myeloid leukemia with inv(16)(p13;q22) or t(16;16)(p13;q22), 674
acute myeloid leukemia with t(8;21)(q22;q22), 674, 676f
acute myeloid leukemia with t(15;17)(q24;q21), 674, 676f
blastic plasmacytoid dendritic cell neoplasm, 674-677
low grade myelodysplastic syndromes and chronic myeloproliferative disorders, 672-674, 675f
paroxysmal nocturnal hemoglobinuria, 677f, 678
molecular genetic diagnostic evaluation of, 711
Myeloid neoplasms with eosinophilia, 494-497
clinical features of, 494-497
cytology-histology of, 495-497, 496f
differential diagnosis of, 497
fact sheet on, 495b
genetics of, 494-497
pathologic features of, 496b
prognosis and treatment of, 497
Myeloid proliferation related to Down syndrome, 446-448, 447b
acute megakaryoblastic leukemia in, 448, 449f
pathologic features of, 448b
transient abnormal myelopoiesis in, 446-448, 448f
Myeloid sarcoma, 445-446, 447f
Myelokathexis, 101, 101f
peripheral neutropenia in, 175
Myelomonocytic leukemia
acute, 441-443, 442f
chronic, 514-518
Myeloproliferative disorders
chronic, immunophenotypes and genetic abnormalities in, 672-674, 675f
platelet aggregation characteristics, 70t
Myeloproliferative neoplasms, 479-480
associated with primary thrombocytosis, 69
in overlap syndromes, 514-522
specific

Myeloproliferative neoplasms *(Continued)*
chronic myelogenous leukemia, 480-494
chronic neutrophilic leukemia, 512-514
essential thrombocythemia, 501-505
myeloid neoplasms with eosinophilia, 494-497
polycythemia vera, 497-501
primary myelofibrosis, 505-512
splenic involvement in, 646, 646f
suggested readings on, 522
unclassifiable, 514
*MYH9* associated macrothrombocytopenia, 71-74, 103-104
clinical features of, 71-74
cytology-histology of, 72-74, 72f-73f, 73b
differential diagnosis of, 74
fact sheet on, 71b
leukocyte anomalies in, 103, 103t
prognosis and treatment of, 74
Myxoid variant, of diffuse aggressive B cell lymphoma, 264, 264f

**N**

Nasal-type extranodal NK (natural killer) cell/T cell lymphoma, 304-307
clinical features of, 304-307
cytology-histology of, 304-305, 305f
differential diagnosis of, 305-307
fact sheet on, 304b
in HIV infection/AIDS, 337-338, 340f
immunophenotypes and genetic abnormalities in, 304-305, 306f, 672, 672f
pathologic features of, 304b
Necrobiosis, 106
Necrobiotic xanthogranulomas, 555
Necrotizing granuloma, 189
Necrotizing granulomatous lymphadenitis, with palisading histiocytes
in cat scratch disease, 122, 123f
in chronic granulomatous disease, 130-131, 131f
Neonatal alloimmune thrombocytopenia, 81
Neutrophilic leukemia, chronic, 512-514
Next generation sequencing (NGS), 720-721
Niemann-Pick cell, 109, 110f
Niemann-Pick disease
bone marrow pathology in, 192-194, 194f
liver sinusoidal pathology in, 557f
splenic pathology in, 180
NK (natural killer) cell(s), antigen (CD) panel for, 659t
NK (natural killer) cell enteropathy, 307
NK (natural killer) cell leukemia, aggressive, 403, 414-416
clinical features of, 414-416
cytology-histology of, 410, 415, 416f
differential diagnosis of, 415-416
fact sheet on, 415b
genetics of, 415
immunophenotypes and genetic abnormalities in, 415, 672, 672f
NK/T cell lymphoma, nasal type, 304-307. *See also* Nasal-type extranodal NK (natural killer) cell/T cell lymphoma
Nodal marginal zone B cell lymphoma, 250-255
clinical features of, 250-255
cytology-histology of, 206t, 253, 253f-254f
differential diagnosis of, 254-255
fact sheet on, 251b
genetics of, 207t, 253-254
Nodal marginal zone B cell lymphoma *(Continued)*
immunophenotype(s) of, 253
prognosis and treatment of, 255
Nodular lymphocyte predominant Hodgkin lymphoma, 353, 353t, 361-366
clinical features of, 361-366
cytology-histology of, 358-360, 359f-360f, 362-363
differential diagnosis of, 363-366
follicular lymphoma and, 365
lymphocyte rich classic Hodgkin lymphoma and, 364
mantle cell lymphoma and, 365-366
progressive transformation of germinal centers and, 363-364
T cell/histiocyte rich large B cell lymphoma and, 365
fact sheet on, 361b
immunophenotype(s) of, 359f-360f, 363
Reed-Sternberg cells, 357t, 358
small lymphocytes, 358-360, 358t
pathologic features of, 362b
prognosis and treatment of, 366
syncytial variant of, 362-363
transformation of, to large cell lymphoma, 366
Nodular lymphocyte rich classic Hodgkin lymphoma, 376-378, 377f
Nodular sclerotic Hodgkin lymphoma, 366-373
clinical features of, 366-373
cytology-histology of, 367-370, 368f
syncytial, 367-368, 369f-370f
differential diagnosis of, 371-373
anaplastic large cell lymphoma and, 371-372
diffuse large B cell lymphoma and, 372
granulomatous lymphadenitis and, 373
large B cell lymphomas and, 372
metastatic carcinoma, melanoma, primary mediastinal seminoma and, 372-373
primary mediastinal large B cell lymphoma and, 372
fact sheet on, 367b
immunophenotype(s) of, 370
pathologic features of, 371b
Non-Hodgkin lymphomas
anaplastic large T cell lymphoma, 297-301
B cell small lymphocytic lymphoma-chronic lymphocytic leukemia, 206-214
Burkitt lymphoma, 287-290
diffuse large B cell lymphomas, 261
extranodal marginal zone lymphoma, 238-244
follicular lymphoma, 223-237
mantle cell lymphoma, 214-223
molecular diagnostic evaluation of, 689, 690t
T cell lymphomas, 293
Non-Langerhans cell histiocytosis, 575-578
Nontuberculous lymphadenitis, 125-126
clinical features of, 126
cytology-histology of, 126
and tuberculous lymphadenitis, differentiation in immunocompetent patients, 125t
Normal platelet count, and platelet dysfunction, 90-98
*NPM1* abnormalities, in acute myeloid leukemia, 433, 704-705
Nucleic acid quantification, in unknown sample, using RQ-PCR, 681-682

**O**

Occult mastocytosis, 602
Osmotic fragility test, 35
Osteoporosis, bone marrow pathology in, 195
Osteosclerotic myeloma, 614
Overlap syndromes. *See* Myelodysplastic syndromes/myeloproliferative neoplasms
Owl eye cells, 354-356, 355f-356f
Oxidant induced hemolysis, 39
cytology-histology of, 39, 40f-41f
Oxygen-sensing pathway genes, molecular diagnostics for, 724, 725f

**P**

Pagetoid reticulosis, 315, 317
Panmyelosis with myelofibrosis, acute, 443-445, 446f
Pappenheimer bodies, 22
Paraimmunoblasts, 207-209
Parasitic bone marrow infections, 186
Parasitic hemolytic anemia, intracellular, 48
ancillary studies of, 52
clinical features of, 48-52
cytology-histology of, 49-52
differential diagnosis of, 52
fact sheet on, 49b
pathologic features of, 49b
prognosis and treatment of, 52-54
Paroxysmal nocturnal hemoglobinuria, 165-168
ancillary studies of, 167
clinical features of, 165-168
cytology-histology of, 166-167
in bone marrow, 167, 167b, 168f-169f
in peripheral blood, 166, 166f
differential diagnosis of, 167
fact sheet on, 166b
Ham test in, 167
immunophenotypes and genetic abnormalities in, 167, 170f, 677f, 678
prognosis and treatment of, 167-168
Paroxysmal nocturnal hemoglobinuria-aplastic anemia overlap syndrome, 165-166
Parvovirus B19 viral infection, in bone marrow, 181, 181f
Pautrier microabscesses, 315-317, 316f
*PDGFRA*, *PDGFRB, FGFR1* abnormalities, in myeloid and lymphoid neoplasms associated with eosinophilia, 712t, 716. *See also* Myeloid neoplasms with eosinophilia
PDW (platelet distribution width), 60
Pearson syndrome, bone marrow failure in, 174
Pelger-Huet anomaly, 99-101
in neutrophils, 100f
pseudo, 99-101, 100t
Pelgeroid change, 99-101
Peripheral platelet destruction, 79-81
drug induced, 81-85
in hypertensive disorders of pregnancy, 89
immune, 79-80
non-immune, 85-89
other causes of, 89-90
Peripheral T cell lymphoma(s), 293
anaplastic large, 297-301
angioimmunoblastic, 293-297
cutaneous, 314-318
enteropathy associated, 312-314

Peripheral T cell lymphoma(s) *(Continued)*
Epstein-Barr virus positive, in childhood, 324-326
extranodal natural killer cell, nasal type, 304-307
hepatosplenic, 308-312
in HIV infection/AIDS, 337-338, 340f
not otherwise specified, 301-302
clinical features of, 301-302
cytology-histology of, 301-302, 303f
differential diagnosis of, 302
fact sheet on, 301b
genetics of, 302
immunophenotypes and genetic abnormalities in, 302, 670
pathologic features of, 302b
primary cutaneous, rare subtypes, 319-324
primary cutaneous CD30, 318-319
subcutaneous panniculitis-like, 307-308
suggested readings on, 326
Perivascular cuffing, by immunoblastic cells, 334, 336f
Persitent polyclonal B cell hyperplasia, 108-109
Pertussis, reactive lymphocyte morphology in, 108, 109f
PFA-100 (Platelet Function Analyzer, Siemens), 64, 65f
Phenytoin associated lymphadenopathy, 146f
Philadelphia chromosome, 480, 480f
Philadelphia negative myeloproliferative neoplasms, 714-716, 715f
Pigmented histiocytes, in dermatopathic lymphadenopathy, 148, 149f
Pince-nez form, in granulocytes, 99, 100f
Piringer-Kuchinka lymphadenitis, 132-133
Plasma cell(s), antigen (CD) panel for, 659t
Plasma cell Castleman disease, 155-156
clinical features of, 155-156
cytology-histology of, 156, 156f
differential diagnosis of, 159
Plasma cell labeling index, 619
Plasma cell leukemia, 613-614
Plasma cell myeloma, 612-623
clinical features of, 612-623
cytology-histology of, 615-621, 617f-618f
in bone marrow, 615-616
in peripheral blood smear, 616
diagnosis of, 613
fine needle aspiration in, 619
imaging in, 614-615, 614f
laboratory findings in, 616-619
differential diagnosis of, 621-622
fact sheet on, 612b
gene expression profiling of, 621
immunophenotype(s) and genetic abnormalities in, 620-621, 621t, 666, 667f
grouping based on, 621
pathologic features of, 615b
prognosis and treatment of, 622-623, 622t
WHO diagnostic criteria for diagnosis of, 613t
Plasma cell neoplasms, 612
heavy chain disease, 629-631
immunophenotypes and genetic abnormalities of, 666, 667f
monoclonal gammopathy of undetermined significance, 623-625
monoclonal immunoglobin chain deposition diseases, 627-631
Plasma cell neoplasms *(Continued)*
plasma cell myeloma, 612-623
primary amyloidosis, 625-627
suggested readings on, 631
Plasmablastic lymphoma, 285-286
clinical features of, 285-286
cytology-histology of, 285, 286f
differential diagnosis of, 286
fact sheet on, 285b
genetics of, 286
in HIV infection/AIDS, 340-343, 343t, 344f
immunophenotype(s) of, 285-286
prognosis and treatment of, 286
Plasmacytic hyperplasia/infectious mononucleosis-like post transplantation lymphoproliferative disorder, 327-328
Plasmacytoid Burkitt cells, 336-337, 338f
Plasmacytoid dendritic cell nodules, 610
Plasmacytoid dendritic cell tumor, blastic, 450, 450b, 451f
Plasmacytoid dendritic cells, 140, 549
Plasmacytoid lymphocytes, 255-257, 257f, 259f
in diffuse aggressive B cell lymphoma, 262-264
Plasmacytoma(s), solitary, 613, 622
Platelet(s), 55-57
cytology and physiochemistry of, 55, 58f
electron micrography of, 58f
hemostatic function of, 55-56, 59f-60f
cytosolic calcium in, 57
dense granules and aggregation in, 57, 61f
evaluation of, 57-68
thrombin and collagen activation in, 56-57
maturation process of, 57f
normal morphology of, 62f
Platelet ADP receptor abnormalities, 94
Platelet aggregation assays, 66-67, 66f
Platelet associated immunoglobulins, detection of, 67
Platelet collagen receptor disorders, 94
Platelet count and peripheral blood smear, 60-62, 63f
Platelet diagnostics, 57-68
ADP/ATP ratio measurement, 67-68
bone marrow biopsy, 62-64
clinical history of patient, 57-60
coagulation testing, 67
electronmicroscopy, 67
flow cytometry, 67
platelet aggregation assay, 66-67
platelet count and peripheral blood smear, 60-62, 63f
platelet function assays, 64-65
Platelet disorders, 55-98
aggregation characteristics of, 70t
causing thrombocytopenia, 71-90
due to decreased platelet production, 90
due to peripheral platelet destruction, 79-81
large platelets, 71-77
normal sized platelets, 79
small platelets, 78-79
causing thrombocytosis, 68-71
clinical features of, 58
diagnostic categories of, 68
drugs and foods contributing to, 58-60
evaluation of, 57-68
genetic disorders associated with, 68t
with normal platelet count (qualitative), 90-98
Platelet disorders *(Continued)*
associated with other illnesses, 98
platelet release defects in, 94-98
surface glycoprotein dysfunction in, 91-94
suggested readings on, 98
Platelet distribution width (PDW), 60
Platelet function assays, 64-65, 65f
Platelet procoagulant disorders, 97
Platelet release disorders, 94-98
alpha and dense granule storage pool defects in, 94-97
platelet procoagulant defects in, 97
signal transduction defects in, 97
Platelet satellitism, 62, 63f
Platelet signal transduction disorders, 97
platelet aggregation characteristics in, 70t
Platelet surface glycoprotein(s), quantification of, 67
Platelet surface glycoprotein disorder(s), 91-94
adenosine diphosphate receptor abnormalities in, 94
in Bernard-Soulier syndrome, 74-76, 93
collagen receptor deficiencies in, 94
in Glanzmann thrombasthenia, 91-93
glycoprotein Ia/IIa deficiency in, 94
glycoprotein Ib abnormality in, 93-94
glycoprotein IIbα/Ibβ dysfunction in, 74-76
glycoprotein IIb/IIIa dysfunction in, 91-93
glycoprotein IV deficiency in, 94
glycoprotein VI deficiency in, 94
other, 94
platelet aggregation characteristics in, 70t
in platelet type von Willebrand disease, 93-94
Platelet surface glycoprotein (GP) IIb/IIIa antagonists, 62
and effect on platelet aggregation, 70t
and immune thrombocytopenia induction, 81
Platelet surface glycoprotein (GP) IIb/IIIa receptor, activation of, 57, 61f
Platelet type von Willebrand disease, 93-94
Plateletworks (Helena), 64-65
*Pneumocystis carinii* infection, bone marrow pathology in, 186, 187f
*Pneumocystis carinii* lymphadenitis, 135b
POEMS syndrome, lymphadenopathy in, 156-157
Poikilocytosis
in glucose-6-phosphate dehydrogenase deficiency, 39, 40f-41f
in iron deficiency anemia, 12-13, 13f
in megaloblastic anemia, 6, 6f
in severe beta thalassemia, 23f, 30
in sickle cell anemia, 22, 23f
Polyclonal B cell hyperplasia, persistent, 108-109
Polycythemia, 54
Polycythemia vera, 497-501
clinical features of, 497-501
cytology-histology of, 498-500, 499f-500f
in spent phase, 500
differential diagnosis of, 500-501
fact sheet on, 498b
genetics of, 500, 714-716
molecular diagnostic evaluation of, 722
pathologic features of, 498b
prognosis and treatment of, 501
Polymerase chain reaction (PCR), 679-680
in clonal detection in lymphoid populations, 686-689, 686f-687f
sensitivity and specificity of, 688-689

Polymerase chain reaction *(Continued)*
real time quantitative (RQ-PCR), 680-682, 681f
gene fusion transcripts detected with, 682
minimal residual disease measurement using, 682
sensitivity and specificity of, 685t
tissue sources for, 680
variations of, 679-680
Polymorphic B cell lymphoma, in HIV infection/AIDS, 343, 345f
Polymorphic reticulosis, 304
Popcorn cells, 354-356, 355f-356f, 359f-360f, 362-363
Post transfusion thrombocytopenia purpura, 81
Post transplantation large granular lymphocyte proliferation, 408
Post transplantation lymphoproliferative disorders, 327-331
clinical features of, 327-331
cytology-histology of
Burkitt lymphoma, 328, 331f
Hodgkin lymphoma, 328, 333f
monomorphic, 328
plasmacytic hyperplasia/infectious mononucleosis-like, 328, 329f
plasmacytoma like, 328, 332f
polymorphic, 328, 330f
T cell, 328-331
genetics of, 331
immunophenotype(s) of, 329f, 331, 331f-333f
risk factors for, 327
in younger age group, 327
Post treatment neoplasms. *See* Chemotherapy; Cytokine therapy; Granulocyte-colony stimulating factor (G-CSF) therapy; Post transplantation lymphoproliferative disorders; Therapy related myeloid neoplasms; Toxins, effects of
Precursor lymphoid neoplasms, 457-470. *See also* Acute lymphoblastic leukemia; Lymphoblastic lymphoma
B cell, immunophenotypes and genetic abnormalities of, 662, 663f
T cell, immunophenotypes and genetic abnormalities in, 667, 669f
Preeclampsia, and thrombocytopenia, 89
Primary amyloidosis, 625-627
ancillary studies of, 627
clinical features of, 625-627
cytology-histology of, 626, 626f
diagnosis of, 626
differential diagnosis of, 627
fact sheet on, 625b
pathologic features of, 627b
prognosis and treatment of, 627
Primary cutaneous CD30 T cell lymphoproliferative disorders, 318-319
clinical features of, 318-319
cytology-histology of, 319, 320f
differential diagnosis of, 319
fact sheet on, 318b
genetics of, 319
immunophenotype(s) of, 319, 320f
pathologic features of, 319b
Primary cutaneous diffuse large B cell lymphoma, leg type, 272-273
clinical features of, 272
cytology-histology of, 272, 273f
Primary cutaneous diffuse large B cell lymphoma, leg type *(Continued)*
differential diagnosis of, 272
fact sheet on, 272b
genetics of, 272
immunophenotype of, 272
prognosis and treatment of, 272-273
Primary cutaneous follicle center lymphoma, 234-235
Primary cutaneous T cell lymphoma(s), molecular genetic diagnostic evaluation of, 690t, 698
Primary cutaneous T cell lymphomas, rare subtypes, 319-324
clinical features of, 319-324
cytology-histology of, 321-324, 322f-323f
differential diagnosis of, 324
fact sheet on, 321b
genetics of, 324
immunophenotype(s) of, 321-324, 322f-323f
pathologic features of, 324b
Primary diffuse large B cell lymphoma of central nervous system, 270-272
clinical features of, 270-271
cytology-histology of, 270-271, 271f
differential diagnosis of, 271
fact sheet on, 270b
genetics of, 271
immunophenotype(s) of, 271, 271f
prognosis and treatment of, 271-272
Primary effusion lymphoma, in HIV infection/AIDS, 338-340, 341f
differential diagnosis of plasmablastic proliferation and, 340-343, 343t
HHV8 positive, 339, 342f
Primary hemophagocytic lymphohistiocytosis, 558
Primary immune disorders, lymphoproliferative diseases in, 346-349, 346b
ataxia telangiectasia, 346-349
autoimmune lymphoproliferative syndrome, 347-348
common variable immunodeficiency, 348-349
immunomodulator related lymphoproliferative disorders, 348
Primary mediastinal large B cell lymphoma, 276-278
clinical features of, 276-278
cytology-histology of, 276, 277f
differential diagnosis of, 278
fact sheet on, 276b
genetics of, 276-278
immunophenotype(s) of, 276, 277f
prognosis and treatment of, 278
Primary myelofibrosis, 505-512
clinical features of, 506-511
cytology-histology of, 506
in fibrotic phase, 506-509, 509f-510f
in prefibrotic phase, 506, 507f-508f, 508t
differential diagnosis of, 511
fact sheet on, 506b
genetics of, 505, 509, 712t, 714-716
pathologic features of, 507b
prognosis and treatment of, 511-512, 511f
Primary thrombocytosis, 69
ancillary studies of, 69, 70t
clinical features of, 69
cytology-histology of, 69
differential diagnosis of, 69
myeloproliferative neoplasms associated with, 69
Proliferation centers, 207-209
Prosthetic joint replacement, lymphadenopathy in, 148
Prothrombin time (PT), 67
Protoplatelets, 55, 56f
Protozoal bone marrow infections, 186
Pseudo-Döhle bodies. *See* Döhle body mimics
Pseudofollicles, 207-209
Pseudo-Gaucher cells, 109, 192-194
in chronic myelogenous leukemia, 481-484, 486f
Pseudo-Howell-Jolly bodies. *See* Howell-Jolly body mimics
Pseudo-Pelger-Huet anomaly, 99-101, 100t, 526-527, 528f
Pseudo-Pelger-Huet hypolobation, 99-101
Pseudothrombocytopenia, 62
cytology-histology of, 63f
Pyogenic bacterial lymphadenitis, 120
Pyothorax associated lymphoma, 274-275
Pyropoikilocytosis, hereditary, 36
ancillary studies of, 37
clinical features of, 36-38
cytology-histology of, 37, 38f
differential diagnosis of, 38
fact sheet on, 36b
pathologic features of, 37b
prognosis and treatment of, 38
Pyruvate kinase, molecular diagnostic evaluation of, 726

**Q**

Quebec platelet syndrome, 94-95

**R**

Reactive lymph nodes, 111
in bacterial infections, 120-132
bacille Calmette-Guérin lymphadenitis, 128
brucellosis, 124, 124b
cat scratch disease, 120-124
chronic granulomatous disease, 130-132
in immunocompromised patients, 126-128
mycobacterial lymphadenitis, 124-128
nontuberculous lymphadenitis, 125-126
pyogenic bacterial lymphadenitis, 120
syphilitic lymphadenitis, 124
tuberculous lymphadenitis, 125
Whipple disease, 128-130
in filarial lymphadenitis, 133
in fungal infections, 132
in immunologic disorders, 133-146
drug induced, 143
immunoglobulin G4 lymphadenopathy, 145-146
Kikuchi disease, 138-142
Kimura disease, 142-143
rheumatoid arthritis, 133-136
systemic lupus erythematosus, 136-138
nonspecific changes found in, 111, 112f
in *Pneumocystis carinii* lymphadenitis, 133
in protozoal infections, 132-133
leishmaniasis, 133
toxoplasmosis, 132-133
related to immune reaction to substances, 148-150
anthracosis, 148
dermatopathic lymphadenopathy, 148
hereditary storage diseases, 148
mammary prostheses, 148
prosthetic joint replacements, 148

Reactive lymph nodes *(Continued)*
respiratory silica exposure, 148
sarcoidosis, 148-150
in viral infections, 113-120
cytomegaloviral lymphadenitis, 115-116
herpes simplex lymphadenitis, 116-118
HIV associated lymphadenopathy, 119-120
infectious mononucleosis, 113-115
Kawasaki disease, 120
measles lymphadenitis, 120
Reactive lymphocytes
morphologic changes in, 107-109, 109f
in viral bone marrow infection, 178, 179f
Reactive lymphocytosis, 407-408
Reactive thrombocytosis, 69-71
REAL (Revised European American Lymphoma) classification, of Hodgkin lymphoma, 353
Red blood cell disorder(s), 3
categories of, 3, 4t
anemias of blood loss, 3
anemias of decreased red blood cell production, 3
hemolytic anemias, 20
enzyme defective or deficient, 38-39, 724-726
erythrocytosis in, 54, 722-723, 723t
extrinsically defective, 42
hemoglobin defective, 21, 721-726
hemoglobin CC disease, 27
sickle cell disease, 21
thalassemias, 28
hemolytic, 20
autoimmune, 42
glucose-6-phosphate dehydrogenase deficiency, 38-39
hemoglobin CC disease, 27
hereditary elliptocytosis, 36
hereditary pyropoikilocytosis, 36
hereditary spherocytosis, 34
microangiopathic, 45
parasitic, 48
sickle cell disease, 21
thalassemias, 28
inflammatory, 18
intrinsically defective, 21-54
maturation defective, 3
iron deficiency anemia, 12
megaloblastic anemia, 3-20
membrane defective, 34
hereditary elliptocytosis and hereditary pyropoikilocytosis, 36
hereditary spherocytosis, 34
molecular diagnostic evaluation of, 721
practical workup of, 722
proliferation defective, 15
anemia of chronic kidney disease, 15
suggested readings on, 54
Reed-Sternberg cells, 350
classic, 354-356, 355f-356f
in classification of classic Hodgkin lymphoma, 357-358
in classification of Hodgkin lymphoma, 354-361, 357t
Refractory anemia, 535-536
Refractory anemia with ring sideroblasts, 536
Refractory anemia with ring sideroblasts and thrombocytosis, 479-480, 522, 536
Refractory cytopenia of childhood, 534-535, 538, 539f
Refractory cytopenia with excess of blasts, 536
Refractory cytopenia with multilineage dysplasia, 536
Refractory cytopenia with unilineage dysplasia, 535
Refractory neutropenia and refractory thrombocytopenia, 536
Renal disorders, bone marrow pathology in, 190-191
Renal osteodystrophy, bone marrow biopsy, 15-16, 17f
Reticular dysgenesis, bone marrow failure in, 175
Reticulated platelets, measurement of, 67
Reticulohistiocytoma, 566-567
cytology-histology of, 566-567, 567f
prognosis and treatment of, 566-567
Rheumatoid arthritis, bone marrow pathology in, 191
Rheumatoid arthritis lymphadenopathy, 133-136
clinical features of, 133-136
cytology-histology of, 136, 137f
differential diagnosis of, 136
fact sheet on, 136b
Richter transformation, of B cell small lymphocytic lymphoma, 212-213, 212f
Rickettsial infection, of bone marrow, 184
Ring form trophozoites
in babesiosis, 52, 53f
in malaria, 49, 50f
Ring sideroblasts, 529, 530f
and differential diagnosis of myelodysplastic syndromes, 543
refractory anemia with, 536
refractory anemia with, and thrombocytosis, 479-480, 522, 536
Ristocetin induced platelet aggregation (RIPA), 67
Rituximab (anti-CD20 antibody), post treatment assessment of bone marrow, 200
Rosai-Dorfman disease, 563-566
clinical features of, 563-566
cytology-histology of, 564-566, 565f
differential diagnosis of, 566
electron microscopy in, 566
fact sheet on, 564b
immunophenotypes of, 566
pathologic features of, 564-565, 564b
prognosis and treatment of, 566
radiologic features of, 564-566
Russell bodies, 229, 238-241

**S**

Sarcoidal granuloma, 189f
Sarcoidosis, lymphadenopathy in, 148-150
clinical features of, 148-150
cytology-histology of, 148-149, 155f
differential diagnosis of, 149-150
Schistocytes
in microangiopathic hemolysis, 88f
in microangiopathic hemolytic anemia, 45-46, 47f
Schizonts, in malaria, 49, 51f
Scott syndrome, platelet aggregation characteristics in, 70t
Sea-blue histiocytes, 109-110, 551-552, 637-639, 638f
Sebastian syndrome, 103, 103t
Secondary hemophagocytic lymphohistiocytosis, 558
Secondary thrombocytosis, 69-71
clinical features of, 69-71
cytology-histology of, and laboratory studies, 71
differential diagnosis of, 71
Sentinel dendritic cells, 549-550, 552
Serum cobalamin, 10
Serum ferritin, 14
Serum free light chains, 618
Serum homocysteine, 10
Serum methylmalonic acid, 10
Serum soluble transferring receptor, 14
Severe congenital neutropenia, 174
Sézary syndrome, in mycosis fungoides, 408-410
clinical features of, 408-410
cytology-histology of, 408-409, 408f
differential diagnosis of, 410
fact sheet on, 409b
immunophenotypes and genetic abnormalities in, 409-410, 409f, 670
Shwachman-Diamond syndrome, bone marrow failure in, 175
Sickle cell(s), 22, 23f
Sickle cell disease, 21
ancillary studies of, 26
clinical features of, 21-26
cytology-histology of, 22-26
SC hemoglobin, 22-26, 25f-26f
SS hemoglobin, 22, 23f-24f
differential diagnosis of, 26
fact sheet on, 22b
genetics and structural Hb defect in, 21
prognosis and treatment of, 26-27
splenic pathology in, 639
Signet ring cell lymphoma, 227-229
Silica lymphadenopathy, 148, 151f
Silicone lymphadenopathy, 148, 150f
Single nucleotide polymorphism (SNP) arrays, 717-719, 719f
Sinus histiocytosis, 563
Sjögren syndrome, bone marrow pathology in, 191
Small B cell lymphomas, 205-206
classification of, 205
B cell small lymphocytic lymphoma, 206-214
extranodal marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT lymphoma), 238-244
follicular lymphoma, 223-238
lymphoplasmacytic lymphoma, 255-260
mantle cell lymphoma, 214-223
nodal marginal zone B cell lymphoma, 250-255
splenic marginal zone B cell lymphoma, 244-250
differential cytology of, 206t
differential genetics of, 207t
differential phenotypes of, 207t
pathologist objectives in identification of, 205-206
suggested readings on, 260
Smudge cells, 107-108, 108f
SNPs. *See* Single nucleotide polymorphism (SNP) arrays
Soccer ball pattern, 387-389, 388f
Solitary mastocytosis of skin, 593
Southern blot hybridization, 682-683
clonal detection in lymphoid populations using, 689
sensitivity and specificity of, 685t
Spherocytes, 34-35, 35f
in warm autoimmune hemolytic anemia, 44f
Spherocytosis, hereditary, 34
ancillary studies of, 35-36
clinical features of, 34-36

Spherocytosis, hereditary *(Continued)*
cytology-histology of, 34-35, 35f
differential diagnosis of, 36
pathologic features of, 35b
prognosis and treatment of, 36
Spleen, 632
anatomy and histology of, 632, 633f
Splenic abscess, 636
Splenic angiosarcoma, 647t, 648-649
Splenic B cell lymphoma, not otherwise specified, 664. *See also* Hairy cell leukemia variant
Splenic cyst, 634-635, 636f
fact sheet on, 635b
Splenic diffuse red pulp small B cell lymphoma, 245-246, 250, 251f, 640
cytology-histology of, 640, 641f
differential diagnosis of, 640
fact sheet on, 640b
immunophenotype(s) of, 252f, 640
Splenic follicular hyperplasia, 633f, 635-636
Splenic granuloma, 637
Splenic hamartoma, 647t, 652-654, 652f
fact sheet on, 652b
Splenic hemangioma, 646, 647t, 648f
Splenic inflammatory pseudotumor, 647t, 653f, 654
fact sheet on, 654b
Splenic lymphangioma, 647t, 649-650, 650f
Splenic marginal zone B cell lymphoma, 244-250, 639-640
clinical features of, 245-250
cytology-histology of, 206t, 245-246, 246f-247f
in bone marrow, 249f
in lymph node, 248f
in peripheral blood smear, 249f
differential diagnosis of, 250
fact sheet on, 245b
genetics of, 207t, 248
immunophenotype(s) of, 248
prognosis and treatment of, 250
Splenic pathology
in abnormalities, 632-635
in hematopoietic proliferations, 645-646
in hypersplenism related disorders, 639
in inflammation, 635-637
in leukemia and lymphoma, 639-641
secondary involvement, 209, 211f, 641-644, 642f
in metabolic diseases, 637-639
suggested readings on, 654
in vascular and nonhematopoietic tumors, 646-654, 647t
Splenic peliosis, 647t, 652
Splenic rupture, 632
Splenic sclerosing angiomatoid nodular transformation, 647t, 650-652
fact sheet on, 650b, 651f
Splenic tumors, 646-654, 647t
angiosarcoma, 648-649
hamartoma, 652-654
hemangioma, 646
inflammatory pseudotumor, 654
littoral cell angioma, 647
lymphangioma, 649-650
peliosis, 652
sclerosing angiomatoid nodular transformation, 650-652
Splenic-gonadal fusion, 634, 635f
Splenosis, 634
Stodtmeister form, of Pelger-Huet anomaly, 99
Storage diseases, 555-558
bone marrow pathology in, 191-194, 191t, 192b-193b
fact sheet on, 192b
hereditary, lymphadenopathy in, 148
lysosomal, 555-558
splenic pathology in, 637-639
Storage pool disorders
alpha (α) granule, 76-77, 94-97
dense granule, 94-97
platelet aggregation characteristics, 70t
Stress lymphocytosis, 108, 407-408
Structural hemoglobin disorders, 21
Subcutaneous panniculitis-like T cell lymphoma, 307-308
clinical features of, 307-308
cytology-histology of, 308, 309f
differential diagnosis of, 308
fact sheet on, 307b
genetics of, 308
immunophenotype(s) of, 308
pathologic features of, 308b
Syphilitic lymphadenitis, 124
Systemic lupus erythematosus. *See also* Lupus lymphadenitis
bone marrow pathology in, 191
Systemic mastocytosis, 588
clinical features of, 588-590
cytology-histology of, 593-596, 595f, 597f
in bone marrow, 596, 598f-599f
in gastrointestinal tract, 603, 606f
in liver, 603, 603f
in lymph nodes, 603, 604f
in spleen, 603, 605f
diagnostic criteria for, 592t
molecular genetic diagnostic evaluation of, 712t, 716-717
variants of (WHO classification), 596-602
cytology-histology of, 600f-601f
WHO classification of, 591, 592t
Systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease, 596-599, 600f

## T

T cell(s)
antigen expression in normal maturation of, 666, 668f, 686
antigen (CD) panel for, 659t
T cell and NK(natural killer) cell neoplasms
immunophenotypes and genetic abnormalities of, 666-672
adult T cell leukemia-lymphoma, 670
aggressive NK (natural killer) cell leukemia, 672, 672f
anaplastic large cell lymphoma, 670, 671f
angioimmunoblastic T cell lymphoma, 670, 671f
hepatosplenic T cell lymphoma, 670
large T cell granular lymphocytic leukemia, 670
mycosis fungoides (Sézary syndrome), 670
nasal-type extranodal NK (natural killer) cell lymphoma, 672, 672f
peripheral T cell lymphoma, unspecified, 670
precursor T cell neoplasms, 667, 669f
T cell prolymphocytic leukemia, 668-670, 669f
T cell chronic lymphocytic leukemia, clarification of entity being described, 414
T cell clones, molecular genetics of, 686-689
T cell leukemia(s), mature, 403
cytology-histology of, 403-404
genetics of, 404
immunophenotype(s) of, 403-404
in other T cell lymphomas, 416
specific, 404-418
adult T cell leukemia-lymphoma associated with human T lymphotropic virus 1 (HTLV-1), 410-412
large T cell granular lymphocytic leukemia, 404-408
NK (natural killer) cell leukemia, 414-416
prolymphocytic leukemia, 412-414
Sézary syndrome in mycosis fungoides, 408-410
suggested readings on, 418
T cell leukemia-lymphoma, adult, immunophenotypes and genetic abnormalities in, 670
T cell leukemia-lymphoma associated with human T lymphotropic virus 1 (HTLV-1), 410-412
clinical features of, 410-412
cytology-histology of, 411-412, 411f
differential diagnosis of, 412
fact sheet on, 410b
genetics of, 412
immunophenotype(s) of, 412
T cell lymphoblastic leukemia-lymphoma, antigen expression in, 667, 669f
T cell lymphoma(s)
leukemic phase of, 403-418. *See also* T cell leukemia(s)
molecular genetic diagnostic evaluation of, 697-698
peripheral, 293. *See also* Peripheral T cell lymphoma(s)
anaplastic large, 297-301
angioimmunoblastic, 293-297
cutaneous, 314-318
enteropathy associated, 312-314
Epstein-Barr virus positive, in childhood, 324-326
extranodal natural killer cell, nasal type, 304-307
hepatosplenic, 308-312
in HIV infection/AIDS, 337-338, 340f
not otherwise specified, 301-302
primary cutaneous, rare subtypes, 319-324
primary cutaneous CD30, 318-319
subcutaneous panniculitis-like, 307-308
suggested readings on, 326
post transplant, 328-331
T cell prolymphocytic leukemia, 412-414
clinical features of, 412-414
cytology-histology of, 413-414, 413f
variant, 414
differential diagnosis of, 414
fact sheet on, 413b
immunophenotypes and genetic abnormalities in, 414, 668-670, 669f
molecular genetic diagnostic evaluation of, 690t, 698
transformation from chronic lymphocytic leukemia, 389
T cell receptor γ gene (TRG@), 688
T cell/histiocyte-rich large B cell lymphoma
clinical features of, 268-270
cytology-histology of, 268, 269f
differential diagnosis of, 270
genetics of, 270
immunophenotype(s) of, 268-270, 269f

T cell/histiocyte-rich large B cell lymphoma *(Continued)*
prognosis and treatment of, 270
splenic involvement in, 268, 641
Tally algorithm, 264-266, 265f
Tangier disease, 557
splenic pathology in, 637-639
Target cells (dacrocytes), 22, 23f
in Hb CC disease, 27, 28f
in sickle cell disease, 22-26, 23f, 25f
Tattoo pigment lymphadenopathy, 148, 152f
Tay-Sachs disease, splenic pathology in, 637-639
*TCL1A* abnormalities, in T cell non Hodgkin lymphomas, 690t, 697-698
Tear drop cells, 6, 6f, 37, 38f
TEG (thromboelastograph, Haemonetics), 65, 65f
Thalassemia(s), 28
alpha (α), four-gene deletion, 29
ancillary cresyl blue studies of, 33, 33f
ancillary hemoglobin analysis of, 31-33
beta (β) intermedia, 30, 32f
beta (β) major, 29-30, 31f
beta (β) minor, 31, 32f
clinical features of, 28-30
cytology-histology of, 30-31, 31f-32f
differential diagnosis of, 14t, 33-34
fact sheet on, 30b
genetic heterogeneity of, 28-29
molecular diagnostic evaluation of, 721-726
pathologic features of, 31b
prognosis and treatment of, 34
radiologic features of, 30-34
Therapy related myeloid neoplasms, 438-445
post treatment marrow, assessment of, 200
Thienopyridines, and effect on platelet aggregation, 70t
Thrombocythemia. *See* Essential thrombocythemia
Thrombocytopenia
in decreased platelet production, 90
with decreased platelet size, 78-79
Wiskott-Aldrich syndrome, 78-79
X-linked thrombocytopenia, 78-79
drug induced, 81-85
immune destructive, 79-80
with increased platelet size, 71-77
Bernard-Soulier syndrome, 74-76
gray platelet syndrome, 76-77
Mediterranean macrothrombocytopenia, 77
Montreal platelet syndrome, 77
non-immune destructive, 85-89
disseminated intravascular coagulation, 85-86
in pregnancy, 89
thrombotic thrombocytopenia purpura, 86-89
with normal platelet size, 79
and pseudothrombocytopenia, 62
Thrombocytopenia with absent radii, 90, 175
Thrombocytosis
primary, 69
secondary, 69-71
Thrombotic thrombocytopenic purpura, 45, 86-89
clinical features of, 86-89
cytology-histology of, 45-46, 47f, 87-89, 87b, 88f
fact sheet on, 87b
prognosis and treatment of, 89
splenic pathology in, 639
Thromboxane synthesis disorder, platelet aggregation characteristics in, 70t
Toll-like receptors, 557
Toxins, effects of. *See also* Chemotherapy; Cytokine therapy
in bone marrow pathology, 201-202
and changes in leukocytes, 105-106, 105f
Toxoplasmosis, bone marrow pathology in, 186
Toxoplasmosis lymphadenopathy, 132-133
clinical features of, 132-133
cytology-histology of, 133, 134f
differential diagnosis of, 133
Transient abnormal myelopoiesis, of Down syndrome, 446-448, 448f
Translocations, gene. *See* Genetic abnormalities, *or under specific disease*
Triangulocytes, in microangiopathic hemolytic anemia, 45-46, 47f
Triple hit lymphoma, 291
Trophozoites
in babesiosis, 52, 53f
in malaria, 49, 50f
Tuberculous lymphadenitis, 125
clinical features of, 125
cytology-histology of, 125
vs nontuberculous lymphadenitis, in immunocompetent patients, 125t
T-zone lymphoma, 301-302, 303f

**U**

Unclassifiable B cell lymphoma, 290-292, 372
clinical features of, 290-292
cytology-histology of, 290-291
differential diagnosis of, 291-292
fact sheet on, 291b
genetics of, 291, 292f
immunophenotype(s) of, 291
prognosis and treatment of, 292
Uniparental disomy (UPD), 717-718
Uremia, platelet aggregation characteristics, 70t
Urticaria pigmentosa/maculopapular cutaneous mastocytosis, 591

**V**

Vacuolated lymphocytes, 104-105, 105f
disorders with, 104, 104t
Valve hemolysis, cardiac, 46-48, 48f
VerifyNow (Accumetrics), 64, 65f
Viral bone marrow infections
Epstein-Barr virus, 178
hemophagocytic syndrome in, 178-181
hepatitis C virus, 181-182
human immunodeficiency virus, 182-183. *See also* HIV infection/AIDS, myelopathy in
parvovirus, 181
Viral lymphocytosis, reactive lymphocyte morphology in, 108, 109f
V(D)J recombination events, 688
von Willebrand assay, 67
von Willebrand disease, platelet aggregation characteristics in, 70t
von Willebrand disease platelet type, 93-94
von Willebrand factor, 87
von Willebrand factor cleaving metalloproteinase, assays for, 88

**W**

Warm autoimmune hemolytic anemia, 42
cytology-histology of, 43, 44f
differential diagnosis of, 43
Warthin-Finkeldey giant cells, 120, 120f
Well differentiated systemic mastocytosis, 602
WHIM syndrome, 101
Whipple disease lymphadenopathy, 128-130
clinical features of, 128-130
cytology-histology of, 129
differential diagnosis of, 129-130
fact sheet on, 129b
White blood cell morphologic anomalies, 99
acquired, 104-109
cytokines and, 106
eosinophil Charcot-Leyden crystallization in, 107
megaloblastic and dysplastic changes in, 106-107
reactive lymphocytes and lymphocytosis in, 107-109
toxins and, 105-106
vacuolated lymphocytes in, 104
congenital, 99-104
Alder-Reilly anomaly, 102-103
Chédiak-Higashi syndrome, 101-102
May-Hegglin anomaly, 103-104
myelokathexis, 101
Pelger-Huet anomaly, 99-101
in macrophages, 109-110
suggested readings on, 110
Wiskott-Aldrich syndrome, 78-79
clinical features of, 78-79
cytology-histology of, 78, 78b
differential diagnosis of, 79
fact sheet on, 78b

**X**

Xanthogranuloma lesions
adult, 577-578
juvenile, 575-577
Xanthomatous inflammation, macrophage cytology in, 551-552, 552f, 555, 557f
X-linked thrombocytopenia, 78-79
clinical features of, 78-79
cytology-histology of, 78, 78b
differential diagnosis of, 79
fact sheet on, 78b
X-linked Wiskott-Aldrich syndrome, 557-558

**Z**

Zombie cells, 354-356, 355f-356f

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