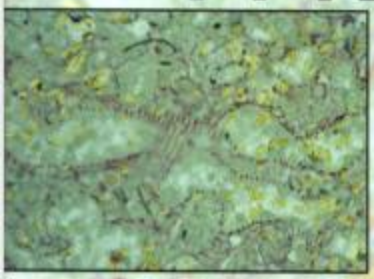
# Illustrated pathology of THE SPLEEN



Bridget S. Wilkins and Dennis H. Wright

# Illustrated pathology of the spleen

The major aim of this book is to de-mystify splenic pathology for non-specialist consultants and trainees in histopathology. The interpretation of pathological changes in the human spleen provides a significant challenge to the skills of even the most highly trained histopathologists. This comprehensive account of the most important and frequently encountered pathological conditions affecting the spleen is highly illustrated in colour throughout, with numerous detailed macroscopic and microscopic specimens. Emphasis is placed upon the importance of adequate clinical information and technical preparation of splenectomy specimens in obtaining the best possible histopathological assessment of the tissue. A systematic, analytical approach to interpretation of pathological changes is used throughout the book. This should enable readers to feel confident in their assessment of significant processes within the spleen

This volume serves as an illustrated atlas, as a bench manual, as a text and as a source of selected reference. It will be an indispensable guide.

**Dennis Wright** was Professor of Pathology at Southampton University from 1972, when the medical school was founded, until his retirement in 1995. Since then he has held the title of Emeritus Professor. Previously, he had been a Reader in Pathology at Birmingham University, preceded by a Readership at Makerere University in Uganda. He is a past President of the European Haematopathology Society, his election to this post being a reflection of his international reputation in the field of lympho-reticular pathology. He has maintained an interest in splenic pathology, particularly lymphomas involving the spleen, for many years.

**Bridget Wilkins** is a Clinical Senior Lecturer in Pathology at Southampton University and an Honorary Consultant Histopathologist for the Southampton University Hospitals NHS Trust. Previously, she has held posts as a Clinical Lecturer and a Clinical Research Fellow at Southampton University. She specializes in haematopathology, with particular interests in pathology of the spleen and bone marrow. She teaches regularly throughout the UK on these topics, emphasizing an analytical and functional approach to histological interpretation.

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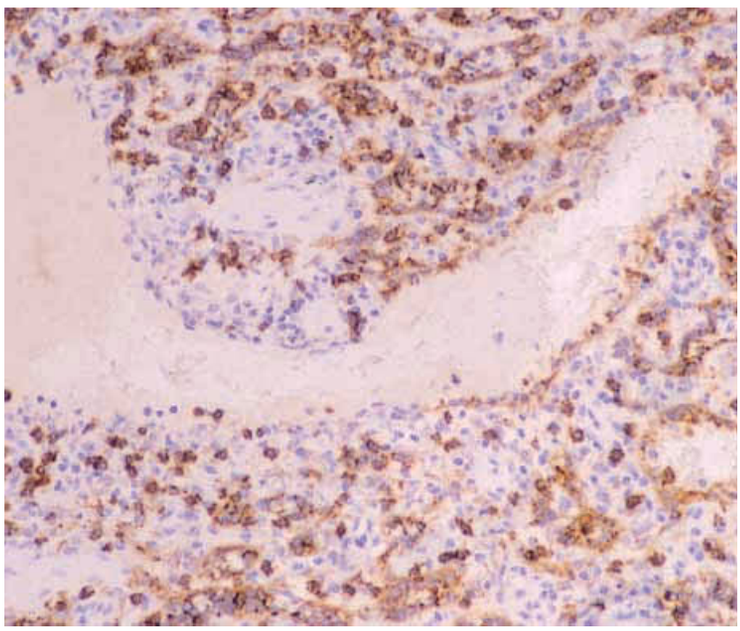

CAMBRIDGE
UNIVERSITY PRESS

PUBLISHED BY CAMBRIDGE UNIVERSITY PRESS (VIRTUAL PUBLISHING)
FOR AND ON BEHALF OF THE PRESS SYNDICATE OF THE UNIVERSITY OF CAMBRIDGE
The Pitt Building, Trumpington Street, Cambridge CB2 IRP
40 West 20th Street, New York, NY 10011-4211, USA
477 Williamstown Road, Port Melbourne, VIC 3207, Australia

http://www.cambridge.org



First published in printed format 2000

A catalogue record for the original printed book is available
from the British Library and from the Library of Congress
Original ISBN 0 521 62227 1 hardback

ISBN 0 511 01963 7 virtual (netLibrary Edition)

# Contents

# Preface

The interpretation of splenic pathology is perceived as difficult by many histopathologists. Few diseases arise primarily within the spleen, and most pathology seen at this site represents involvement of the organ by processes originating elsewhere and/or also affecting other tissues in the body. Primary diagnosis has often been made from haematological, microbiological or histopathological investigations of other tissues by the time splenectomy is performed. The role of the histopathologist in most cases is to provide confirmation of the known, or suspected, diagnosis and to exclude unsuspected pathology.

Splenic pathology is an area of shared interest for histopathologists, haematologists, oncologists and surgeons. The key to successful interpretation of splenic pathology lies in obtaining adequate clinical information and in ensuring optimal tissue fixation. Dialogue between the various clinicians involved in diagnosis and care of individual patients is required to achieve these factors. ithout this dialogue, no amount of expertise in splenic histology can provide full answers to clinical problems.

In this book, we have aimed to present a succinct, comprehensive account of those aspects of splenic pathology likely to be encountered by diagnostic histopathologists. e hope we have provided useful guidance to permit confident recognition of normal and non-specifically reactive histological appearances in the spleen. e have also attempted to convey principles of

systematic analysis which can be applied to achieve diagnoses following recognition of broad categories of abnormality affecting individual splenic compartments. The use of immunohistochemistry, cytogenetic and molecular genetic investigations to supplement morphological analysis is described where appropriate.

This book is aimed primarily for the use of general histopathologists at consultant and trainee levels. e hope that it will also be of value for histopathologists with specialist expertise in haematopathology, and to haematologists with an interest in this area.

e acknowledge that pathology in the spleen remains a challenging area for histopathological interpretation. For the foreseeable future, there will be occasions when no satisfactory, complete diagnosis can be made. None the less, we hope this book will convey some of our enthusiasm for the subject and that it will help make day-to-day practice of splenic diagnosis more rewarding for our fellow histopathologists. e hope also that some of our readers will feel encouraged to investigate further those aspects of splenic involvement by disease which remain unsolved at present.

Bridget ilkins

Dennis right

# Acknowledgements

e are grateful to the many colleagues who have shared their interesting and challenging cases of splenic pathology with us in recent years. The opportunity we have gained, through them, to extend our experience of splenic pathology and see examples of rare disorders involving the spleen, has contributed significantly to our interest in the subject and our impetus to write this book.

Thanks are due to Duncan Kempson and, in particular, Stephen Bottoms from the Medical Illustration Department at Southampton General Hospital, who provided invaluable assistance with photography of macroscopic specimens. e should also like to thank Julia Berry for assistance with typing.

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Chapter 1

# Introduction

## Why is splenectomy performed?

Splenectomy is frequently incidental, performed because of technical necessity in gaining adequate surgical access to the stomach, left side of the colon, left kidney or aorta. Under these circumstances, there may be no distinctive pathology present related to the primary cause for surgery. However, such spleens may not be absolutely normal, and it can be difficult to decide with confidence what are acceptable secondary reactive changes and what might constitute significant co-existent splenic pathology.

Splenectomy may also be performed primarily for therapy. This is obviously the case when the spleen is ruptured following abdominal trauma, a potentially life-threatening situation. It is important to know that splenectomy may none the less be delayed for hours or even days following the traumatic event, since haemorrhage from a damaged spleen does not always occur immediately after the injury, may be minor, or may cause relatively slow development of an intra-abdominal haematoma. Even after a delay of a few hours following the initial injury, changes may be present in the spleen as a response to the injury. Since, in some cases, splenic rupture may follow mild trauma because the organ is abnormal, it is important to know whether any changes present are the result, or part of the cause, of splenic injury.

Splenectomy of a primarily therapeutic

nature is also performed for hypersplenism, most commonly because of excessive red cell destruction in auto-immune haemolytic anaemia (AIHA) or platelet destruction in idiopathic thrombocytopenic purpura (ITP). In ITP, the spleen is often a source of anti-platelet antibody production so that splenectomy also reduces the underlying auto-immune response. Spleens enlarged for a variety of unrelated reasons may also become hyperfunctional in destruction of peripheral blood cells and require removal for control of cytopenias.

Splenectomy for control of symptoms (pain, abdominal swelling) may be performed in any condition giving rise to a massively enlarged spleen, with or without hypersplenism. Examples include myelofibrosis and other chronic myeloproliferative disorders, in which splenectomy also serves the useful purpose of removing a considerable bulk of neoplastic haemopoietic tissue. It is debatable whether or not splenectomy carries a significant risk of removing a major source of productive, normal haemopoiesis in such patients.

Removal of the predominant site of disease involvement may be achieved by splenectomy in certain lymphomas, particularly splenic marginal zone lymphoma/splenic lymphoma with villous lymphocytes. Despite bone marrow and blood involvement in most of these patients, long-term partial or complete remission can be achieved in many by splenectomy. The procedure may also be of value to confirm the precise diagnosis in other patients with known lymphomas in whom splenomegaly is prominent and the precise classification is in doubt.

Splenectomy may be performed primarily for diagnostic purposes when there is unexplained enlargement or unexplained focal pathology such as a cyst or a solid tumour. Associated with many different pathologies underlying such changes, there may be hypersplenism or, less often, hyposplenism.

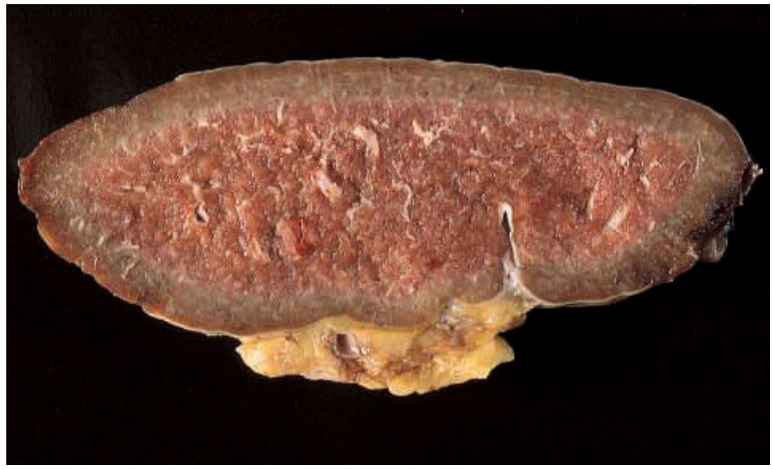

**Fig. 1.1.** Slice of spleen showing the effects of partial fixation following immersion of the intact organ in formalin for 24 hours. Subcapsular tissue is reasonably well fixed to a depth of approximately 5 mm in this example, but the central parenchyma remains unfixed.

**Fig. 1.2.** Slice of spleen, similar to that in Fig. 1.1, showing the effects of further fixation for 24 hours after slicing, the spleen having been received unsliced, immersed in formalin. There is a clear line of demarcation between the original zone of fixation and the area of secondary fixation. Histology from the central area would be suboptimal, despite now appearing fixed.

Where the spleen has been removed primarily for diagnostic or therapeutic purposes, it can be very frustrating for the pathologist to find that there is apparently little correlation between any visible morphological abnormalities and the documented clinical disease. There may be little or nothing detectable in the spleen histologically which can be regarded as abnormal, or there may be obvious abnormalities which cannot be attributed with confidence to a specific cause.

## Why is splenic pathology perceived to be difficult?

Pathological changes which would cause no diagnostic problem in another tissue may seem less straightforward when encountered in the spleen. There are a variety of reasons for this, some genuinely related to a current relative lack of understanding of normal physiological processes within splenic components and their corresponding morphology. However, a major problem is often the poor quality of histological material obtained from splenectomies, compounded by inadequate supporting clinical information.

Whatever their size, whether suspected to be pathological or not, spleens usually arrive in histopathology departments having spent several hours immersed in fixative but otherwise untouched. The result is immediately apparent when the first cut is made through the organ: a rind of tissue 0.5 cm thick beneath the capsule is fixed, while all deeper tissue is red and already partly autolysed due to inadequate penetration of fixative (Fig. 1.1). Whatever is done subsequently by way of slicing and further fixation (Figs. 1.2 and 1.3), the damage has been done and the resulting histological sections will show good tissue preservation only in the immediate subcapsular area.

There is no doubt that spleen is difficult

tissue in which to achieve good fixation, since its high content of red blood cells is inhibitory to adequate penetration of fixative solutions. However, much of this problem can be avoided by prompt delivery of the unfixed organ to the histopathology laboratory so that primary fixation can be achieved after careful slicing of the tissue.

A second important problem is that spleens sent for histopathological analysis are often accompanied by minimal, or no, clinical details, particularly in cases where removal has been considered by the surgeon to be incidental to the major surgical purpose (nephrectomy, gastrectomy, abdominal aneurysm repair, etc.). Surgeons performing splenectomy for diagnostic purposes are often acting on behalf of other clinicians and may have no detailed knowledge of the patient's underlying disease. When the spleen has been traumatized, there may be no indication of whether minor intra-operative trauma or a major road traffic accident was the cause. In cases of splenic trauma due to major abdominal injury, the time interval between injury and surgery is rarely stated. These pieces of information are of fundamental importance. An injury to the spleen, like a wound in any other tissue, may vary from insignificant to extensive and will undergo sequential reparative changes during the days following injury.

Thirdly, many pathologists lack a clear understanding of normal splenic anatomy and physiology. We rarely see genuinely normal spleens as surgical specimens, and rapid post-mortem autolysis results in generally poor morphological preservation of the spleen at autopsy.

Normal spleens from experimental animals and spleens obtained at defined time-points following immunological challenge or injury in such animals offer insight into splenic pathophysiology. However, the anatomy and functions of the spleen differ considerably between species, and data

**Fig. 1.3.** Slice of tissue prepared from a spleen which had been received fresh and sliced at 1 cm intervals prior to immersion in formalin. Twenty-four hours later, each portion of spleen was re-sliced at 5 mm thickness. A cross-section through one such piece is shown. This photograph emphasizes the slow penetration of formalin into splenic tissue, even after appropriate initial handling of the organ.

from experimental animals cannot be assumed to reflect accurately processes occurring in humans. For instance, the spleen in rodents is a major haemopoietic organ throughout life, while there is little evidence to support such a function for the normal fetal or adult human spleen. Rodent spleens also differ significantly in structure from those of humans, particularly in the organization of white pulp marginal zones and the perifollicular vasculature. The spleens of cats and dogs have an important physiological role as a reservoir for blood cells. They have considerable amounts of capsular and trabecular smooth muscle to assist this function, and normal spleen volume may vary widely in these species. Similar reservoir activity in the human spleen is usually evidence of underlying pathology causing undesirable sequestration of blood elements as, for example, in hereditary spherocytosis.

## What does the surgeon or physician want from the pathologist?

For incidental splenectomies, the surgeon requires answers to two main questions. First, is there involvement of the spleen (and/or splenic hilar lymph nodes) by primary pathology directly related to the cause of surgery? For example, the presence or absence of metastasis or direct spread from a gastric or colonic carcinoma should, obviously, be reported. Secondly, is there any significant coincidental pathology present or can any variations from normal appearances be explained by the spleen's proximity to the primary site of disease? It is this second question which can be particularly difficult to answer with confidence.

For therapeutic splenectomies, confirmation of the diagnosis and exclusion of additional pathology are the major requirements from pathological examination. It is also important to comment

specifically upon the presence of capsular damage or other features suggestive that spillage of splenic tissue could have occurred during surgery. Such spillage may lead to splenosis and subsequent return of the disease or symptoms for which splenectomy was performed.

In the case of splenectomy undertaken for diagnostic purposes, elucidation of the primary abnormality (e.g. the cause of unexplained splenomegaly) is clearly required but any additional pathology and/or evidence of injury should also be recorded, for the reasons outlined above.

## What information does the pathologist require?

Patient identification data, including date of birth or age, are essential. Age is particularly important as splenic function, and consequently morphology, varies throughout life. The purpose of splenectomy should be stated clearly. If it is incidental, details of the primary diagnosis and surgical procedure are required. If there has been abdominal trauma, its nature and timing are important, for the reasons described earlier.

Where there is known or suspected primary pathology, its nature should be stated, as a guide to organizing ancillary investigations (see below) as well as to assist in morphological assessment. Details of any concurrent diseases should also be known. For conditions such as AIHA and ITP, it is important to know whether the patient has been receiving corticosteroid therapy, and at what dosage, prior to surgery. Prolonged administration of corticosteroids for these, and other, disorders may have profoundly suppressive effects on splenic white pulp, as on lymphoid tissue elsewhere in the body.

Since the introduction of vaccines to protect against pneumococcal and *Haemophilus influenzae* type B infections, it is also desirable to know whether these have

been administered and when. The spleen is a major site for the production of immune responses to such encapsulated organisms, and non-vaccinated subjects are at significant risk of infection by these agents following splenectomy. Consequently, with the exception of emergency and unanticipated cases, most patients undergoing splenectomy will have been pre-vaccinated. Specific morphological changes in the spleen secondary to vaccination have not been described in the published literature. Only by careful recording of vaccination details will it be possible, over time, to acquire experience of vaccinated versus non-vaccinated appearances in otherwise equivalent splenectomy specimens and determine the extent of any changes which can confidently be ascribed to vaccination per se.

## How can fixation and processing be optimized to give the best possible histology?

Ideally, all spleens should be transported directly to the histopathology laboratory as soon as possible after removal from the patient. They should be sent unfixed in a dry container, accompanied by informative clinical details as described above.

On receipt in the laboratory, the spleen should be weighed, minus any associated blood clot but otherwise undisturbed, since slicing may cause significant loss of blood (and hence weight) from a congested spleen. Very large spleens may be weighed in their containers using bathroom scales. The weight of an equivalent empty container is subtracted to obtain the actual weight of the spleen. Measurements of splenic vertical length, medio-lateral width and antero-posterior depth are desirable, but normal values for these parameters are highly variable. The state of the capsule (intact or disrupted) should be noted,

together with any abnormal appearances such as sugar-icing or subcapsular haematoma. The presence or absence of hilar lymph nodes (plus their number and size, if present) and any hilar vascular abnormalities should be described.

The spleen should then be sliced at 0.5–1.0 cm intervals (Fig. 1.4) and the parenchyma described. Particular features to note are congestion, prominence of white pulp nodules, haemorrhagic foci, infarctions, cysts and any obvious tumour masses. The value of macroscopic assessment in splenectomy specimens has been inadequately emphasized in the past. Much useful information can be gained from careful examination of the sliced tissue before and after fixation, as we shall demonstrate in subsequent chapters.

Following examination, the slices should be rinsed by gentle agitation in a large volume of 10% neutral-buffered formalin (or other fixative, according to local practice), to remove excessive blood. They should then be submerged, preferably flat and interleaved with absorbent paper, in clean fixative solution for 24 hours. It is important to use a generously sized container and abundant fixative solution so that the fluid can penetrate freely between individual slices which are not packed tightly together. After 24 hours, although fixation will be incomplete, tissue blocks of 2–3 mm in thickness should be prepared from selected slices and put to fix in cassettes (to avoid distortion of the tissue pieces) for a further 24 hours. In our experience, this procedure yields histological sections of better quality than those obtained by blocking tissue straight from fresh slices of spleen. Also, slicing spleen thinly for insertion into cassettes is much easier after partial fixation has made the tissue somewhat more rigid than it is in its fresh state.

If it is unavoidable that a spleen is sent to the laboratory in fixative, it should be measured, described, sliced and fixed further as

**Fig. 1.4.** Partly sliced spleen demonstrating the preparation of complete horizontal cross-sections through the organ at 0.5 to 1 cm intervals.

outlined above, as soon as possible. Fixation will be best in the subcapsular tissue and blocks should be taken from here, unless this is not representative (e.g. in the case of a focal lesion deep within the organ). Tissue from the centre of the spleen, even after further fixation, will yield suboptimal histology. Any hilar lymph nodes present should be described and sampled.

We recommend that several tissue blocks should be taken for histological examination, even in the case of an incidental splenectomy. A useful guide is to sample the superior and inferior borders, the hilum and the lateral convex border of the spleen as a minimum. In this way, individual pathologists can easily accumulate reference material upon which to base their appreciation of normal and abnormal splenic morphology. We perform Giemsa, reticulin, Perls' and periodic acid-Schiff's stains on one block in addition to routine H&E staining.

## Ancillary investigations

Well-fixed splenic tissue can be stained successfully using the tinctorial methods mentioned above. In addition, immunohistochemistry can be performed as for other formalin-fixed, paraffin-embedded tissues, with no special modifications. Where antigen retrieval methods such as proteolysis or wet heat techniques (microwave oven or pressure cooker heating in appropriate buffered solutions) are required, slightly longer exposure times may be needed, reflecting the relatively prolonged fixation to which spleens are usually subjected.

When the spleen has been received fresh, small pieces can be removed under sterile conditions to disperse cells into suitable media for immunostaining, biochemical, cytogenetic and molecular genetic analysis. It may also be useful to make imprints from

small pieces of tissue for rapid cytological assessment. Imprinting is made easier by drying the tissue very gently on a piece of fibre-free filter paper before touching it onto the glass slide(s). This removes some of the blood from the tissue surface and permits better assessment of nucleated cells of interest, uncontaminated by red blood cells.

Such investigations may be appropriate at present in cases of suspected lymphoma, myeloproliferative or histiocyte storage disorders. As understanding of the cellular mechanisms underlying these and other diseases will undoubtedly continue to improve, requirement for such techniques to be performed on splenectomy specimens is likely to expand.

## Splenic biopsy, fine needle aspiration and laparoscopic splenectomy

Historically, there has been a reluctance to perform needle biopsies for histological or cytological analysis of the spleen, because of the significant risk of haemorrhage following puncture of the spleen's delicate capsule. With improved imaging techniques to guide the insertion of biopsy needles, however, sampling of solid lesions within the spleen has been practised increasingly in recent years. There is a need for pathologists to understand the potential uses and pitfalls of splenic needle biopsy samples. As with all aspects of splenic pathology, a key factor is awareness of the variety of normal appearances which may be present in such small samples, ranging from trabecular connective tissue components to normal lymphoid structures and different subcompartments of red pulp.

To complicate matters further for the histopathologist, techniques for laparoscopic splenectomy are becoming established in the surgical repertoire (Delaitre & Pitre, 1997; Trias et al., 1998). With such methods, splenic tissue may be removed piecemeal or

by aspiration via laparoscopic instruments. This has profound implications for the nature of any splenic tissue which may be submitted subsequently for histological analysis. To date, laparoscopic splenectomy has been used mainly in cases where diagnosis is not primarily at issue and where it is considered desirable to preserve some splenic function, e.g. in hereditary spherocytosis and ITP in young patients (Watson et al., 1997). However, there can be issues of differential diagnosis even in some of these patients (such as the possibility of HIV-associated thrombocytopenia mimicking ITP). It remains to be determined, as pathological experience of tissue from such procedures increases, whether it will pose major new diagnostic challenges in an already difficult field. Laparoscopic and open partial splenectomy techniques are also in development (Petroianu et al., 1997).

## Implications of splenectomy for the patient

Splenectomy is not without hazard. Apart from technical risks specifically associated with the surgical procedures employed to remove the spleen, lack of splenic function post-splenectomy leaves patients at increased risk of infection, particularly by encapsulated bacteria. The risk is greatest in children and young adults but is not insignificant in older individuals (see Meekes et al., 1996; Working Party of the British Committee for Standards in Haematology Clinical Haematology Task Force, 1996). It is not yet known whether vaccination against pneumococci, meningococci and *Haemophilus influenzae* will have a major impact on the occurrence of post-splenectomy infections.

It is unclear whether useful splenic function is lost by the removal of a spleen massively replaced by lymphoid or haemopoietic malignancy and it is also not known

whether valuable haemopoiesis is lost in the latter situation. Functional studies to attempt to define splenic haemopoietic function in such patients are, as yet, imperfect.

## Summary

Provided with high-quality histological material and good clinical background information it is possible to make a rational assessment of spleen structure and any pathological changes present. In the following chapters, it is our aim to provide a systematic overview of splenic pathology in the context of its relationship to normal structure, functions and appearances of the organ.

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Chapter 2

# Normal structure, development and functions of the spleen

## Anatomy and vascular supply

The normal spleen lies in the left hypochondrium, adjacent to the tail of the pancreas, the greater curve of the stomach and the upper pole of the left kidney. It weighs approximately 10 grams at birth, gaining the full adult weight of 150 grams by 15 years. With enlargement from any cause, it expands caudally and medially and may extend across the midline to reach the right iliac fossa. The shape of the normal spleen is a compressed ovoid, with a convex lateral surface and concave medial (hilar) aspect. The upper pole is usually more rounded and bulky than the lower. The antero-medial border is sometimes irregular and, in some individuals, bears one or more notches which may become prominent if the spleen enlarges. Ectopic splenic location and splenunculi are described in Chapter 9.

Blood supply is via the splenic artery, which branches from the coeliac plexus and passes along the superior border of the pancreas. It often follows a tortuous course in older individuals and its wall may become heavily calcified. The splenic vein drains blood from the organ to the portal vein by a parallel route. Lymphatic channels from the spleen also follow this route. Afferent lymphatics have not been described, which should not be surprising in view of the spleen's key role as a filter and immune response organ for the bloodstream. There is marked variation in the extent of branching of the splenic artery and vein at the

splenic hilum, a feature which is of considerable surgical importance. The only significance for the pathologist is that it should be recognized as normal anatomical variation.

Within the spleen, the blood supply and drainage are segmental in distribution, with polar branches (superior and inferior) and varying numbers of central branches. Each segment of splenic tissue has a perpendicular orientation relative to the long axis of the organ and their structures have been studied by corrosion casting techniques (Redmond et al., 1994). The existence of avascular planes between adjacent segments remains controversial but offers the prospect of partial splenectomy, and hence preservation of splenic function, in suitable patients.

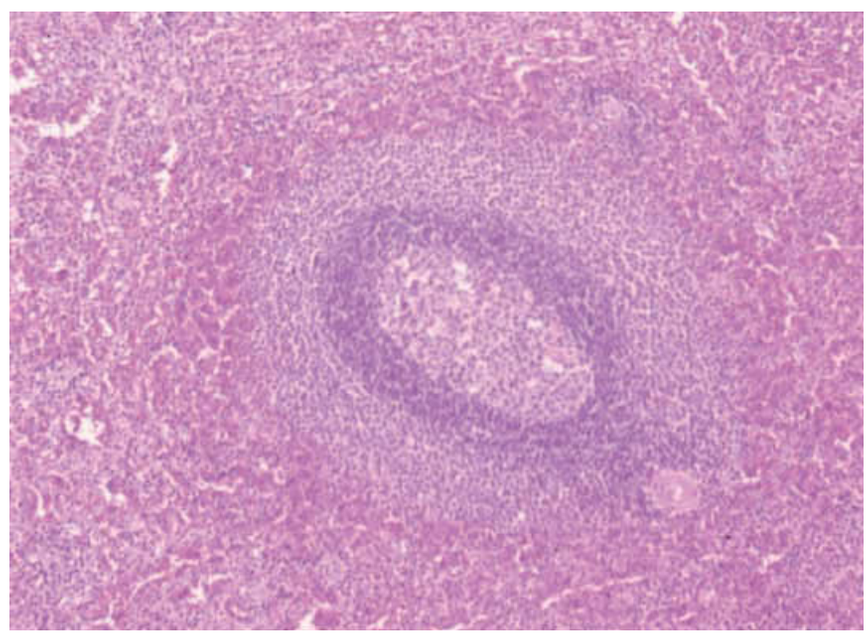
A thin fibrous capsule encloses the spleen. From this, connective tissue trabeculae extend into the parenchyma of the organ towards the hilum. In occasional individuals, prominent trabeculae can be seen orientated circumferentially a short distance beneath the capsule. Branches of the splenic vein and artery, passing to and from the hilum, accompany these trabeculae. The penicillar arteries and arterioles are formed from arterial branches which extend outwards from trabeculae into the splenic parenchyma. Most of the lymphoid tissue of the spleen is organized into distinct white pulp structures (see below) suspended within a loose meshwork of red pulp by the scaffold afforded by penicillar arterioles and connective tissue trabeculae.

## Normal micro-anatomy of the spleen

### White pulp

White pulp consists of peri-arterial and peri-arteriolar lymphoid sheaths (PALS), expanded at intervals by nodular lymphoid follicles (Fig. 2.1). The PALS is an organized

**Fig. 2.1.** H&E stained section showing a white pulp nodule with well-defined germinal centre, mantle zone and marginal zone extending from the adjacent peri-arteriolar lymphoid sheath.



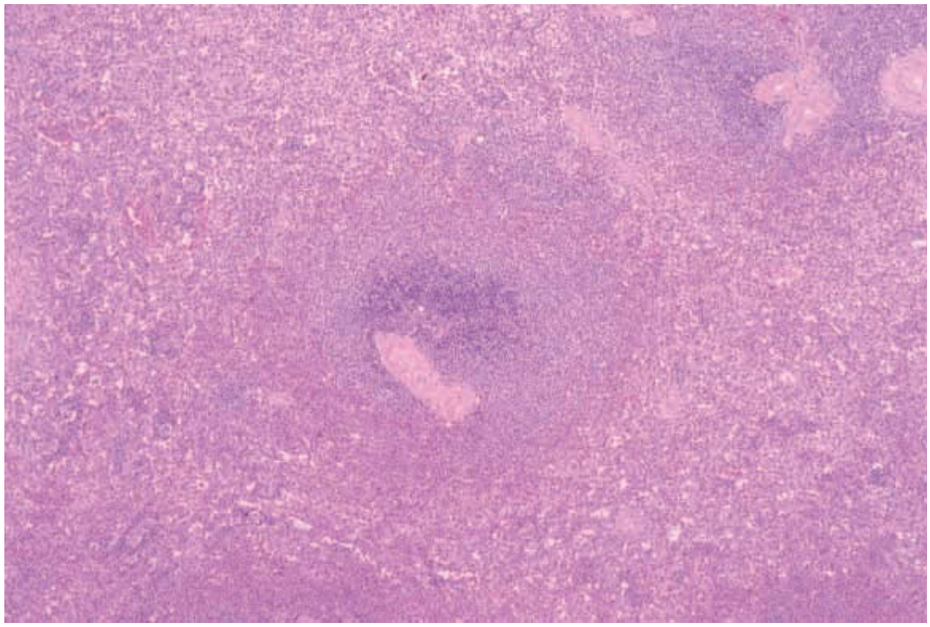


**Fig. 2.2.** H&E stained section showing unstimulated white pulp nodule with central small, densely packed lymphocytes and peripheral marginal zone of paler lymphoid cells.

T cell compartment which is clearly demarcated from the adjacent red pulp. Cells within the PALS are predominantly CD4-positive T cells: relatively few CD8-positive cells are normally found here. White pulp B cell follicles form intermittent expansions within the PALS, the latter being highly attenuated or even undetectable over the follicular surface.

An unstimulated B cell follicle consists of a nodule of small B lymphocytes surrounded by a rim of marginal zone B cells which are larger, have more abundant cytoplasm and are more heterogeneous than the central cells (Fig. 2.2). The small B lymphocytes have the characteristics of mantle zone cells, expressing surface IgM and IgD. Marginal zones are not unique to the spleen but in other lymphoid tissues they are generally inconspicuous. Marginal zone B cells are functionally, as well as cytologically, heterogeneous, but a major subpopulation is responsible for generating T-independent immune responses to carbohydrate antigens such as bacterial capsular polysaccharides (Bruyn et al. 1992; Llopis et al., 1996). Small numbers of T cells are also present within marginal zones.

With antigenic stimulation, inactive B cell follicles develop germinal centres and clearly demarcated mantle zones, as seen in nodal lymphoid tissue. The extent of these changes, and of the marginal zone expansion which may accompany them, varies considerably. Such variations presumably reflect the precise nature and concentration of the antigen(s) encountered, the time course of exposure and individual factors regulating the immune response. Reactive changes affecting the various components of red and white pulp are described in detail in later sections.

## Red pulp

Red pulp consists of filtering areas, composed of sinusoids and cords, and

non-filtering areas occupied by capillaries, venules and small amounts of supporting connective tissue (Chamberlain, 1990). Sinusoids are lined by endothelial cells, historically believed to be of macrophage origin and called littoral cells (from the Latin *littoralis* = by the shore). These cells are unique among endothelia in expressing the T cell-associated antigen CD8 (Timens & Poppema, 1985). The role of CD8 expression in this context is unknown. Sinusoidal endothelial cells are supported by 'barrel hoop' fibres of reticulin and separated by cords of Billroth (Fig. 2.3). The resident cells of splenic cords are dendritic macrophages and small numbers of fibroblasts. Cords contain, in addition, varying numbers of transitory cells, including red blood cells, polymorphonuclear leukocytes, monocytes, lymphocytes and plasma cells. T cells expressing CD8 are present as a normal constituent of red pulp cords, scattered singly amongst cordal macrophages. Red pulp should therefore be regarded as a distinct T-zone within the spleen, even though the CD8-positive T cells are not organized into a compact structure equivalent to the PALS. Only small numbers of CD4-positive T lymphocytes are normally found within red pulp.

At the interface between red pulp and marginal zones, sinusoids are replaced by an indistinct network of peri-follicular capillaries which surrounds each white pulp nodule (Chamberlain, 1990; Kashimura & Fujita, 1987). This site is probably important for antigen presentation to lymphocytes of the white pulp. It appears to be unique to human spleen, but it is not known whether it is directly equivalent to the well-defined capillary network found internal to the marginal zone in rodents (Steiniger et al., 1997). A reticular meshwork of dendritic cells expressing alpha-smooth muscle actin ($\alpha$-SMA) has been demonstrated at this site (Satoh et al., 1997; Wilkins et al., 1995 *a,b*), possibly corresponding with a previously

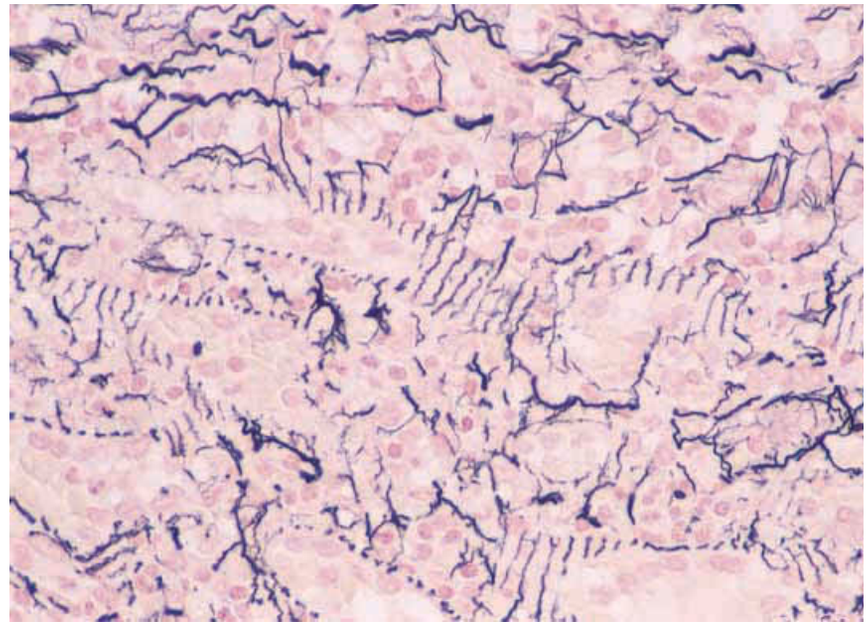


**Fig. 2.3.** Reticulin staining of red pulp cords and sinusoids showing 'barrel hoop' fibres supporting individual sinusoids. Gordon & Sweet's silver stain for reticulin.

reported population of cytokeratin-positive peri-follicular cells (Doglioni et al., 1990). In our experience, peri-marginal cells expressing α-SMA are continuous with a dense reticular meshwork underlying PALS T-zones. They appear therefore, to form a scaffold for white pulp structures and to define boundaries between these structures and the adjacent red pulp.

## Development of the spleen during embryonic and fetal life

The spleen begins to develop from a group of foregut mesodermal proliferations at approximately 5 weeks of gestation. These foci rapidly condense together and become vascularized, eventually fusing to form a single organ. This multifocal origin accounts for the occasional finding of a cluster of small spleens in miscarried fetuses instead of a fused organ. Marked variation in splenic shape between individuals, including variation in the degree of surface notching, probably reflects variation in the number and size of individual segments contributing to the final organ (Redmond et al., 1994). Multi-focal origin is presumably also the basis of the segmental organization of the spleen's internal vasculature.

White pulp structures are well developed by the 18th week of gestation but cordal and sinusoidal architecture is not fully established until later. Red pulp consists of relatively solid, sparsely vascularized connective tissue between weeks 15 and 20, becoming progressively richer in sinusoids and cordal macrophages during the final trimester of pregnancy.

There has been much controversy about the haemopoietic function of the spleen in human embryonic and fetal life. Its small size, particularly relative to the liver, throughout most of gestation, argues against its making a significant contribution to haemopoiesis. The relatively late development

of mature cordal and sinusoidal structures within the red pulp also makes it unlikely that significant haemopoietic function occurs at this site (Timens et al., 1987). Histological examination of fetal spleens at 17 to 20 weeks' gestation, the predicted time of potential maximum splenic haemopoiesis (before hepatic and, later, medullary haemopoiesis become maximal), shows very few granulocytic and early erythroid precursors (Wilkins et al., 1994). The only haemopoietic cells found in significant numbers are mid- to late normoblasts (also present in the circulation at this stage of gestation). This implies that such cells may be trafficking passively through the red pulp vascular bed or, possibly, that the splenic red pulp provides a permissive environment for terminal differentiation of erythroid precursors. These findings are quite different from those seen in 'incidental' splenic extramedullary haemopoiesis (EMH) and EMH associated with chronic myeloproliferative disorders in adult life, in which erythroid and (particularly in the latter) granulopoietic cells of all maturational stages are found, together with megakaryocytes.

The data from which a role for the human spleen in fetal haemopoiesis has been inferred come mainly from animal studies, predominantly studies of rodents. Haemopoiesis in rodents is organized very differently from that in humans, with the spleen acting as a haemopoietic organ throughout life. It is inappropriate therefore, to assume that findings in rodents are applicable to humans.

White pulp usually appears inactive from the time of its development to birth, lacking germinal centres in B cell nodules. Little is known about the patterns of change which occur as infants rapidly gain exposure to a wide range of antigens in early life. However, in spleens examined from cases of sudden infant death syndrome, white pulp nodules frequently appear stimulated, with prominent reactive germinal centres and

T zone hyperplasia (authors' unpublished observations; see also Valdés-Dapena et al., 1993). These reactive appearances are likely to reflect the age group most affected, coinciding with recent vaccination (measles, mumps and rubella) and with the fact that infections, albeit generally minor and heterogeneous in type, are often documented or suspected in these babies.

## Functional correlates of red and white pulp structure

### Red pulp

The intimate association of sinusoids, lined by fenestrated endothelium, and cords containing resident macrophages in the red pulp offers the ideal environment for detection, retention and destruction of unwanted material from the blood circulation. Endothelial cells as well as macrophages in this tissue have phagocytic capacity when activated, enhancing the ability of red pulp to remove particulate matter from the circulation. Many specialized texts describe the complex dynamics of circulatory pathways through the splenic red pulp (e.g. Chamberlain, 1990). Most of these accounts are based on data from animal studies and may not be directly applicable to human spleen, since red pulp vascularization, particularly in rodents, differs significantly. However, specific studies of human splenic vascular anatomy and flow have been reported and do support the general principle of dual circulatory patterns as found in other species. The so-called 'closed' circulatory pathway provides rapid transit of blood through the red pulp via connections between sinusoids and efferent capillaries (Fig. 2.4). The 'open' circulatory pathway operates when blood components leave sinusoids via fenestrae in the endothelium and percolate through the cords prior to entering efferent capillaries (Fig. 2.5).

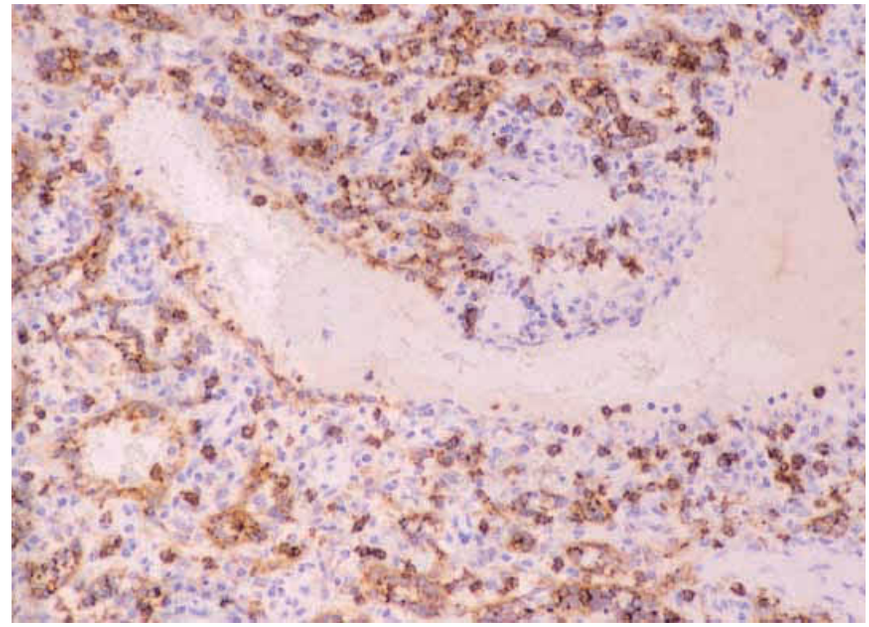


**Fig. 2.4.** Closed circulatory pathway of the spleen. Section immunostained to demonstrate CD8 expressed by sinusoidal endothelium (left-hand side of picture). The sinusoidal lumen is in direct continuity with a dilated capillary lumen with CD8-negative endothelium on the right-hand side of the picture. Strepavidin–biotin complex immunoperoxidase technique.

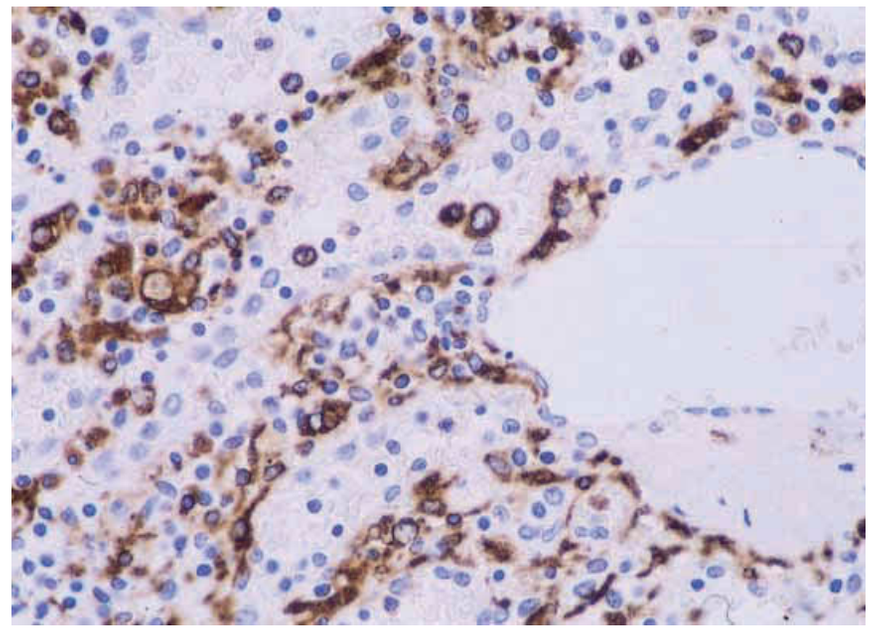


**Fig. 2.5.** Open circulatory pathway of the splenic red pulp. Section immunostained to demonstrate CD68 expression by cordal macrophages. A prominent cord, recognizable by its content of dendritic-appearing macrophages expressing CD68, opens directly into a dilated capillary channel (right-hand side of picture). Streptavidin–biotin complex immunoperoxidase technique.

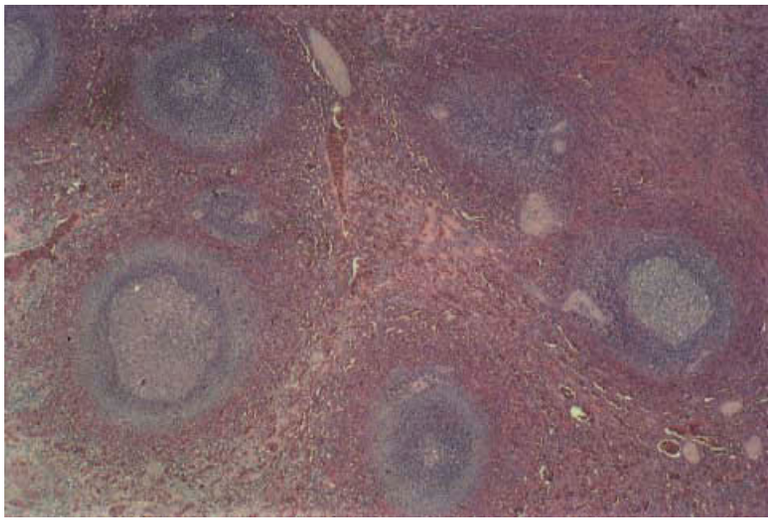
Within cords, macrophages with complex dendritic morphology provide both a structural scaffold and a large potential surface area for interaction with passing blood elements. Sequestration of cells such as red blood cells (RBC) and platelets may occur, presumably reflecting adhesive interactions due to altered surface properties of the latter. With appropriate stimulation, phagocytosis of sequestered material follows.

### Peri-follicular zone

Adjacent to the interface between white and red pulp, the red pulp vascular pattern is altered and spill-over of white pulp lymphocytes (or retention of lymphocytes prior to their entry into white pulp) is often present. Sinusoids empty into a complex network of capillaries which surrounds each white pulp nodule. The prominence of this area varies greatly between individual spleens, but it is frequently congested with RBC, giving the appearance in H&E-stained sections of red haloes around lymphoid nodules (Fig. 2.6). Close examination and immunostaining reveal that neutrophil polymorphs are also abundant in this peri-follicular vascular network and that the reticular meshwork of spindle cells expressing $\alpha$-SMA, described above, underlies it. It is not clear whether a continuous endothelial surface connects peri-follicular sinusoids directly to the capillaries in this area. Alternatively, there may be discontinuity, with blood permeating freely through the meshwork of $\alpha$-SMA-positive spindle cells before entering capillary lumens.

The functions of this meshwork of $\alpha$-SMA-positive spindle cells are unknown but, as well as surrounding B cell nodules of the white pulp, it is continuous with a diffuse reticular array of similar cells underlying the PALS T zones. Hence, it could represent a white pulp scaffold which becomes reversibly stretched around B cell follicles as they expand and contract within the PALS.

**Fig. 2.6.** H&E stained section demonstrating prominent red flares around a group of highly reactive white pulp nodules, representing peri-follicular red pulp intensely congested with red blood cells.



### White pulp

Information about routes of lymphocyte migration through the human spleen is scanty and most understanding of this topic has come from studies conducted in rodents (Liu et al., 1991). The presence of a well-defined marginal zone capillary in such animals, for which there is no direct human equivalent, may limit the value of this information for the human situation. However, the purposes of lymphocyte traffic through the spleen are not fundamentally different between these species, and rodent data currently provide the best available model for what occurs in humans. The description below is derived from such animal studies.

Lymphocytes and antigens enter the spleen via its arterial supply and reach the white pulp by migration into the marginal zones of white pulp nodules from the marginal zone capillary (presumably via perifollicular capillaries in humans). Antigen is phagocytosed and transported to the PALS by antigen-presenting cells (APC). T cells transit rapidly from marginal zones to the PALS. If these cells recognize antigen on the surfaces of APC within the PALS, they proliferate and remain at this site, primed for B cell interactions. B cells migrate from the marginal zone more slowly and enter the outer aspect of the PALS where they interact with primed T cells. Initial B cell proliferation in response to this interaction is extra-follicular, giving rise to short-lived plasma cells. Activated effector T cells may leave the spleen or enter the follicles. As these reactions are progressing, antigen–antibody complexes are actively transported to be deposited upon follicular dendritic cells, where they may remain for many months providing long-lasting antigen-specific stimulation for recirculating lymphocytes.

Activated B cells migrate from extra-follic-

ular foci of proliferation into follicles to form germinal centres, where selection and somatic hypermutation occur in association with further proliferation. Centrocytes which interact successfully with activated T cells in the germinal centres either leave the spleen to become relatively long-lived plasma cells at other sites in the body or migrate back into the marginal zones to become memory cells there. As in germinal centres within other secondary lymphoid tissues, most B cells which fail to be selected by successful antigen binding and T cell interactions die by apoptosis.

In the case of T-independent antigens such as bacterial capsular polysaccharides, B cells still follow the route of migration to the PALS via the marginal zone to proliferate in extra-follicular foci, but the marginal zone itself appears to be a major site for antibody production and the route by which colonization of marginal zones occurs after initial B cell proliferation is not known.

## Abnormalities of splenic function

Atrophy of the spleen is sometimes associated with clinical evidence of deficient function (hyposplenism) and, conversely, enlargement may be associated with excessive function (hypersplenism). However, the relationship between splenic size and function is more complex than this, since a normal sized spleen may be either hypo- or hypersplenic and hyposplenism can be present when the spleen is greatly enlarged. Many pathologists, including ourselves, find it difficult to relate specific morphological abnormalities within the spleen to clinical or haematological features of hyposplenism and hypersplenism. Therefore, to conclude this section, we have attempted to clarify the definitions of these altered functional states and some of the diseases with which they are associated.

## Hyposplenism

Reduced or absent function of the spleen is most commonly caused by surgical removal of the organ. Whatever the cause of hyposplenism, the major features reflect reduction in red pulp functions although an immunological deficit due to lack of aspects of white pulp activity may also occur. The predominance of clinically evident effects of red pulp failure is probably due to the age of the patients affected. Most hyposplenism occurs in adults, after the major immune responses of the body have been established; in infants and children, hyposplenism is associated with marked immunological deficits.

Non-iatrogenic causes of hyposplenism include congenital absence or hypoplasia of the spleen, reduction in splenic tissue volume (due to atrophy and/or infarction) and infiltration or replacement of splenic tissue by abnormal components such as lymphoma cells, which impede normal functioning. Causes of hyposplenism are summarized in Table 2.1.

Clinical features of hyposplenism are primarily haematological. Failure of the pitting function of cordal macrophages results in retention of a variety of inclusions within red blood cells. Howell–Jolly bodies, which are nuclear remnants within red cell cytoplasm, are perhaps the most widely recognized of these. Heinz bodies caused by oxidative damage to haemoglobin and Pappenheimer bodies due to abnormal iron storage are also well known to haematologists. Red cell shape is also abnormal, with target cells and acanthocytes visible in blood films. Quantification of red cell membrane defects termed 'pits' has been used in recent years to assess the severity of hyposplenism in individual patients. These membrane defects are normally removed through pitting activity of cordal macrophages, and their accumulation over the

**Table 2.1.** *Causes of hypo- and hypersplenism*

| Causes of hyposplenism | Causes of hypersplenism |
|---|---|
| Congenital hypoplasia/aplasia | Hereditary red cell membrane disorders |
| Inherited immunodeficiency syndromes | Hereditary red cell enzyme disorders |
| Prematurity and extreme old age | Thalassaemias |
| | Sickle cell disease and other haemoglobinopathies |
| AIDS | |
| Malaria | Malaria |
| Tropical sprue | Tropical splenomegaly syndrome |
| | Leishmaniasis |
| Coeliac disease | Tuberculosis |
| Crohn's disease and ulcerative colitis | |
| Rheumatoid arthritis | Auto-immune haemolytic anaemia |
| Hypopituitarism | Idiopathic thrombocytopenic purpura |
| Hypothyroidism | Evans' syndrome |
| Chronic alcoholism | Auto-immune neutropenia |
| Nephrotic syndrome | Felty's syndrome |
| Sickle cell disease | Megaloblastic anaemia |
| Vasculitides and SLE | Hyperthyroidism |
| Thrombo-embolism | |
| Infarction | Gaucher's disease |
| | Niemann–Pick disease |
| Macrophage storage disorders | Ceroid histiocytosis and other storage disorders |
| Amyloidosis | |
| Sarcoidosis | Chronic myeloproliferative disorders |
| | Acute leukaemias |
| Chronic myeloproliferative disorders | Lymphomas (especially if RP extensively involved) |
| Acute leukaemias | Langerhans' cell histiocytosis |
| Lymphomas | Vascular tumours |
| Metastatic carcinomas | Hamartomas |
| Splenic cysts | Peliosis |
| | Splenic cysts |
| Corticosteroid therapy | |
| Cytotoxic chemotherapy | Portal hypertension |
| Irradiation | • Chronic liver disease |
| Intravenous $\gamma$-globulin administration | • Portal vein occlusion |
| Chronic graft-vs.-host disease | • Hepatic vein obstruction |
| | Splenic vein obstruction |
| Splenectomy or partial splenectomy | |
| Therapeutic splenic embolization | |

lifespan of circulating erythrocytes correlates well with the severity of other features of hyposplenism.

Transient leukocytosis and thrombocytosis may occur following splenectomy. Usually the peripheral white blood cell and platelet counts return to normal within one month of surgery unless splenectomy has been performed for treatment of an underlying haematological disorder. Secondary erythrocytosis may persist following splenectomy for auto-immune haemolytic anaemia or hereditary spherocytosis. Potentially life-threatening thrombocytosis may develop after splenectomy for essential thrombocytosis or polycythaemia rubra vera.

## Hypersplenism

An adequate definition of hypersplenism has proved elusive since the concept first originated. In the broadest sense, the term refers to increased splenic activity and, as with hyposplenism, the aspects of function under consideration are predominantly those involving red pulp. Hypersplenism is characterized by splenomegaly and peripheral blood cytopenias affecting one or more cell lines, accompanied by compensatory bone marrow hyperplasia. These features reflect increased retention and destruction of blood cells from the circulation. However, there are other causes of this combination of abnormalities and, conversely, not all may be found in some cases of otherwise typical hypersplenism. Ultimately, the ‘acid test’ of a diagnosis of hypersplenism in any particular patient is whether or not the abnormalities are corrected following splenectomy. Since this information cannot be known until some time after removal of the organ, it is not particularly helpful to the histopathologist when

dealing with the spleen immediately following surgery.

The concept of hypersplenism has been complicated further by attempts to define primary and secondary forms. The term 'primary hypersplenism' describes hypersplenism occurring in the absence of a recognizable cause for cytopenia(s) in the spleen, bone marrow or in the circulating blood cells themselves. Whether primary hypersplenism exists at all remains controversial and, to us as pathologists, the idea seems unsatisfactory. Secondary hypersplenism, on the other hand, accounts for the great majority of examples of this disorder. Hereditary red cell abnormalities and auto-immune cytopenias are examples of conditions in which underlying blood cell alterations lead to increased demand for splenic red pulp activity. Examples of primary abnormalities within the splenic tissue causing hypersplenism are more difficult to understand in mechanistic terms. Red pulp infiltration by lymphoid or myeloid proliferations, storage disorders affecting cordal macrophages, vascular tumours and peliosis may all be associated with hypersplenism. Indeed, almost any cause of splenomegaly may cause hypersplenism.

Impairment of fast transit circulatory pathways through the red pulp as a result of infiltration, outflow obstruction or altered anatomy has been proposed to account for increased splenic sequestration in these diverse conditions (Mackie & Shepherd, 1994). Table 2.1 includes a summary of causes of hypersplenism.

It is obvious that the conditions listed above and in Table 2.1 include disorders in which, if red pulp involvement is severe, hyposplenism may occur rather than over activity. The final twist in the tale is that, since hypersplenism is itself a cause of splenic enlargement, a vicious spiral of increasing splenomegaly and worsening cytopenias may develop in some patients.

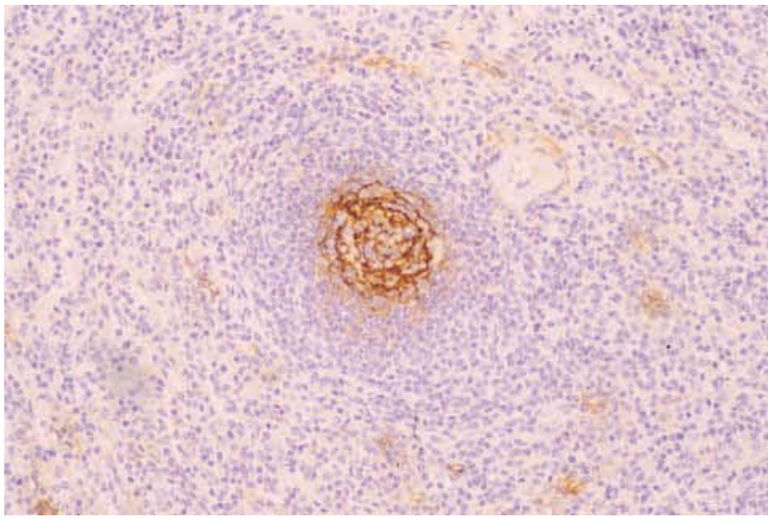
## An immunohistochemical inventory of the spleen

### Introduction

Reference will be made throughout this book to the immunophenotypes of cell populations within red and white pulp areas of the spleen. A number of studies have been conducted using frozen or paraffin sections to characterize cell populations within the various compartments of the human spleen (Timens & Poppema, 1985; van Krieken & te Velde, 1986). These have demonstrated aspects of normal splenic architecture and cell distribution, increasing our understanding of normal and pathological splenic function. Here, we shall present a description of immunophenotypic characteristics in unstimulated and non-specifically reactive spleens. It is not exhaustive but includes reference to a wide range of antigens that can be demonstrated reliably using formalin-fixed, paraffin-embedded spleen tissue. Antibodies reactive with all of these antigens are commercially available.

Uses of immunohistochemistry for the diagnosis of lymphoid and myeloid proliferations and in other specific disorders are described in later chapters, as appropriate.

**Fig. 2.7.** Expression of the low-affinity nerve growth factor receptor (p75) by follicular dendritic cells within a white pulp nodule. Note that peri-follicular capillaries are also highlighted due to expression of p75 by adventitial cells. Steptavidin–biotin complex immunoperoxidase technique.



### White pulp

#### Germinal centres (GC)

These show essentially similar features to those found in other organized lymphoid tissues. They have a scaffold of follicular dendritic cells which express CD21, CD23, CD35 and the p75 low-affinity nerve growth factor receptor (Fig. 2.7).

The centrocytes and centroblasts of reactive GC have a B cell phenotype (e.g. CD20 and CD79a positive). They express CD10 and CD75, but not CD5. They have a high proliferation fraction as demonstrated by Ki67 antigen expression but lack BCL-2 protein. Numerous small T lymphocytes

may be present within GC; these can be demonstrated by their expression of CD3 and CD5. They are usually predominantly CD4-positive, CD8-negative and most of them express CD45RO. The latter is a marker of antigen-experienced T cells (Beverley, 1990). Tingible body macrophages in reactive GC express CD68.

### Mantle zones

As in other lymphoid tissues, mantle zones consist predominantly of small B lymphocytes which are CD5 positive. Most of these cells co-express surface IgM and IgD. They react positively with the antibody DBA.44 (Fig. 2.8), which is used in lymphoma diagnosis as a marker of hairy cell leukaemia (Al Saah et al., 1989). A proportion of cells also expresses CD23 but no CD10 is expressed by mantle zone B cells. Scattered T lymphocytes are present, as in GC. There is very little proliferative activity in mantle zones.

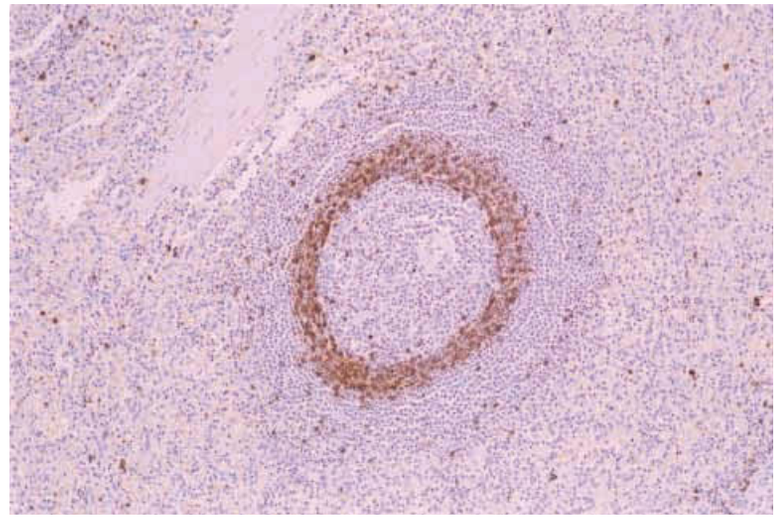


**Fig. 2.8.** White pulp mantle zone clearly delineated by immunostaining using the monoclonal antibody DBA.44. Steptavidin–biotin complex immunoperoxidase technique.

### Marginal zones

These consist of cytologically heterogeneous B lymphoid cells, some of which have monocytoid or plasmacytoid features. Scattered mature plasma cells are also present. Cytoplasmic and surface IgM expression is detectable in the more plasmacytoid cells. Only a minority of cells expresses IgD. Also present in the marginal zone are scattered blast cells, which express CD75 and Ki67 antigen, and a few T lymphocytes. Expression of CD35 by marginal zone B cells can also be demonstrated in paraffin sections (Fig. 2.9). Unfortunately, no antigens absolutely specific for marginal zone cells have yet proved reliably demonstrable in fixed tissue. In reactive spleens, but not in lymphomas, antibodies reactive with CD79a have the useful property of staining GC, mantles and marginal zones differentially (Fig. 2.10).

### PALS

The PALS consists predominantly of small T lymphocytes (CD3 and CD5 positive)

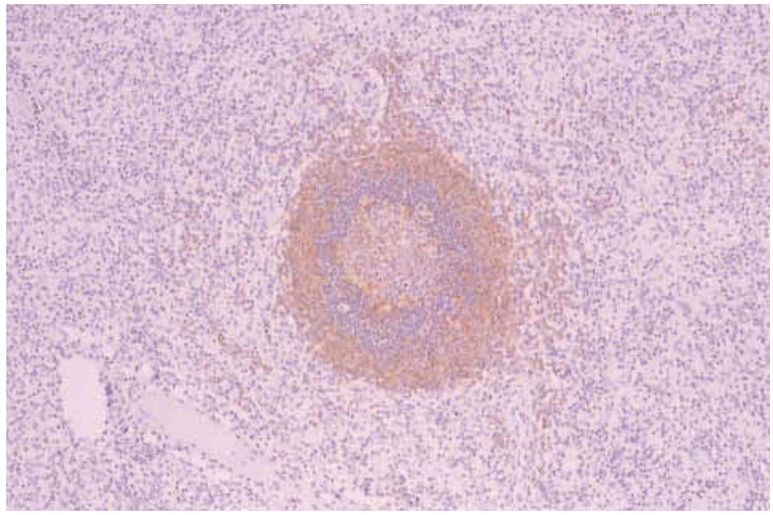


**Fig. 2.9.** White pulp nodule with central area of GC and mantle-type lymphocytes and prominent positive reaction of peripheral marginal zone cells with a monoclonal antibody directed against CD35. In normal and reactive spleens, this antibody discriminates well between mantle and marginal zones. However, it does not distinguish reliably between neoplastic mantle and marginal zone B-cells. Streptavidin–biotin complex immunoperoxidase technique using monoclonal antibody E11, reactive with CD35.

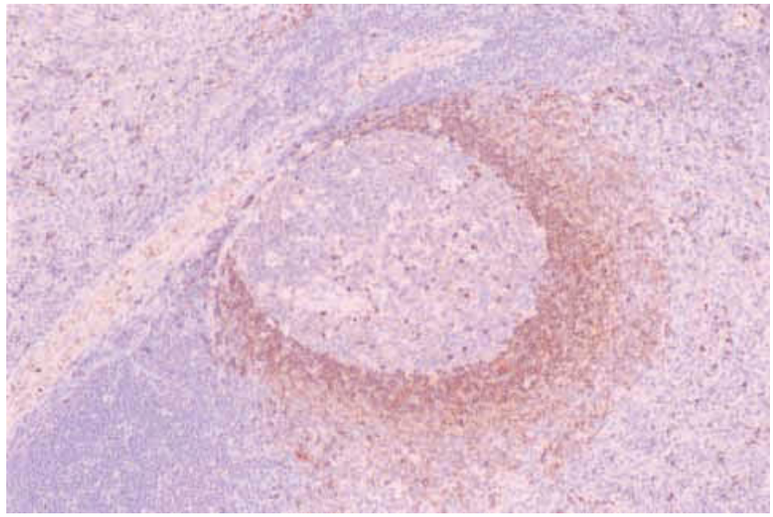


**Fig. 2.10.** Reactive white pulp nodule immunostained for CD79a. Differential staining of the germinal centre (weak) mantle zone (strong) and marginal zone (intermediate) is evident. Streptavidin–biotin complex immunoperoxidase technique.

expressing CD4. Usually no more than 25–30% of PALS T cells are CD8 positive (Fig. 2.11). Scattered T blasts may be present, which express Ki67 antigen; some of these also express the activation marker CD30. These can bear a passing resemblance to mononuclear Hodgkin's disease cells but are smaller than the latter, with less prominent nucleoli.

Supporting the PALS is a dendritic network of cells expressing $\alpha$-SMA. This is continuous with the peri-follicular meshwork described below. Also present in the PALS are abundant interdigitating reticulum cells, which express S100 protein.

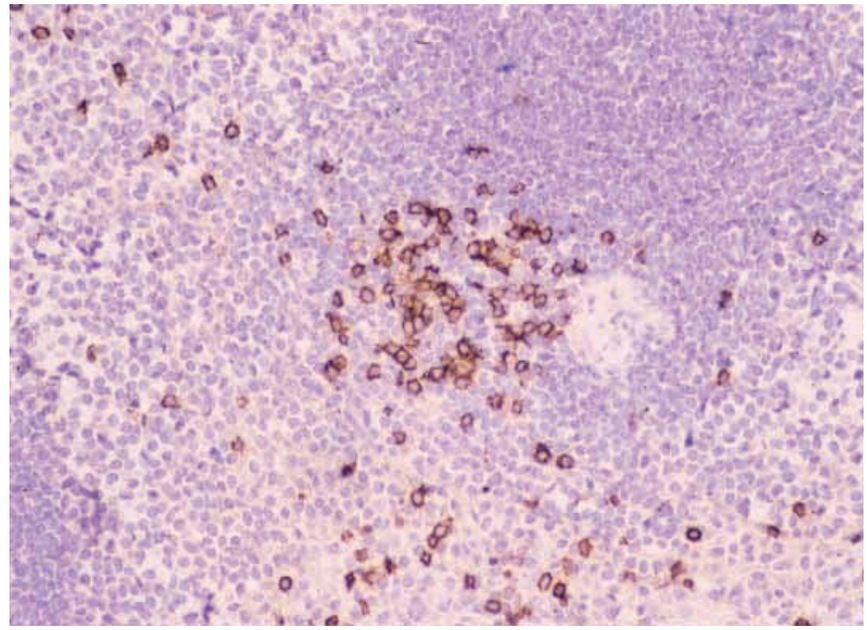


**Fig. 2.11.** PALS immunostained for CD8, showing only a modest proportion of cells present in the T zone expressing this antigen. Most of the T cells at this site are normally CD4 positive. Streptavidin–biotin complex immunoperoxidase technique.

## Peri-follicular zones

The characteristic features of the peri-follicular zone in human spleen are its meshwork of $\alpha$-SMA-positive dendritic cells (Fig. 2.12) and its open vascular structure. The latter is best demonstrated by CD8 staining of sinusoidal endothelium but can also be seen in sections immunostained using antibodies reactive with a variety of other endothelial antigens. The difference in sinusoidal structure in this area compared with red pulp elsewhere accounts for the peri-follicular accumulation of red blood cells and neutrophils, which is seen in some reactive spleens.

## Red pulp

### Cords

These contain spindle cells expressing $\alpha$-SMA and macrophages expressing CD68 (Fig. 2.13). In addition to these resident cells, extra-cellular matrix is present, containing fibronectin, laminin, collagen IV and other components.

A wide variety of red and white blood cells pass through the cords to and from the bloodstream. These can be identified by their expression of lineage-associated antigens; glycophorins A and C (red cells); CD15

and calprotectin (mature granulocytes and monocytes); CD42b and CD61 (megakaryocytes and platelets); T and B cell markers and, for plasma cells; CD138 (syndecan), p63 (a rough endoplasmic reticulum-associated protein reactive with the antibody VS38c; Turley et al. 1994) and immunoglobulin light and heavy chains.

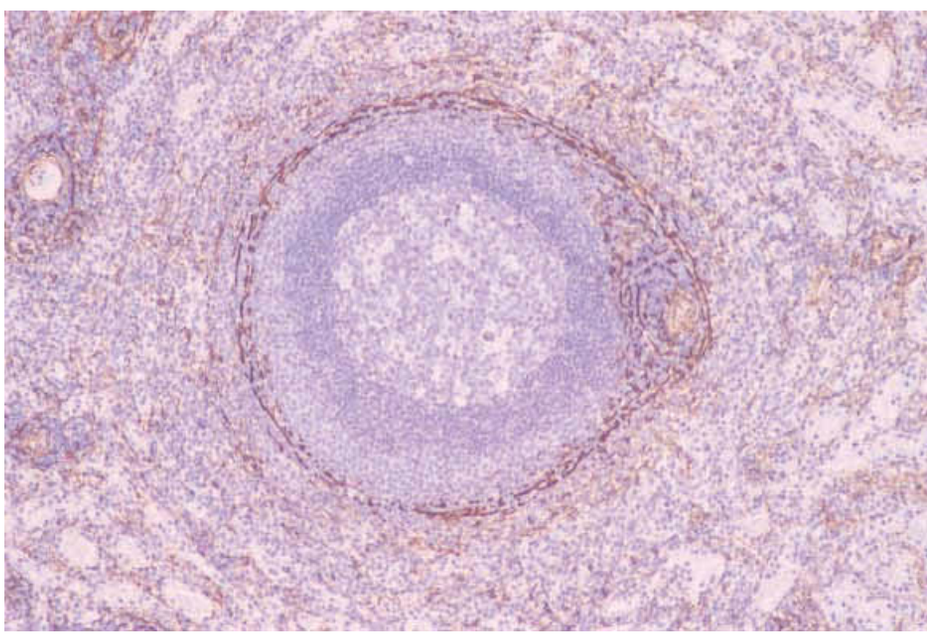
### Sinusoids

The splenic sinusoids are lined by endothelium expressing von Willebrand Factor (VWF), CD31 and vascular cell adhesion molecule-1 (VCAM-1; CD106). Sinusoidal endothelium reacts with *Ulex europaeus* lectin but expression of CD34 is weak or absent. The most distinctive immunophenotypic property of this endothelium, however, is its CD8 expression (Fig. 2.14).

### Capillaries

Capillary endothelium in the non-filtering areas of splenic red pulp, and in the white pulp, has the typical immunophenotype of endothelia in most body tissues (VWF, CD31, CD34 and CD106 positive; reactive with *Ulex europaeus* lectin)

**Fig. 2.12.** Reactive white pulp nodule showing peri-follicular meshwork of dendritic cells expressing alpha smooth muscle actin ($\alpha$-SMA). Streptavidin–biotin complex immunoperoxidase technique.



## Connective tissues

Capsular and trabecular fibroblasts are, not surprisingly, strongly vimentin positive. Despite the lack of evidence for significant reservoir function in human spleens, cells expressing alpha-smooth muscle actin (and hence potentially myofibroblastic in nature) are present within the capsule and trabeculae in moderate to high numbers; their functions are currently unknown.

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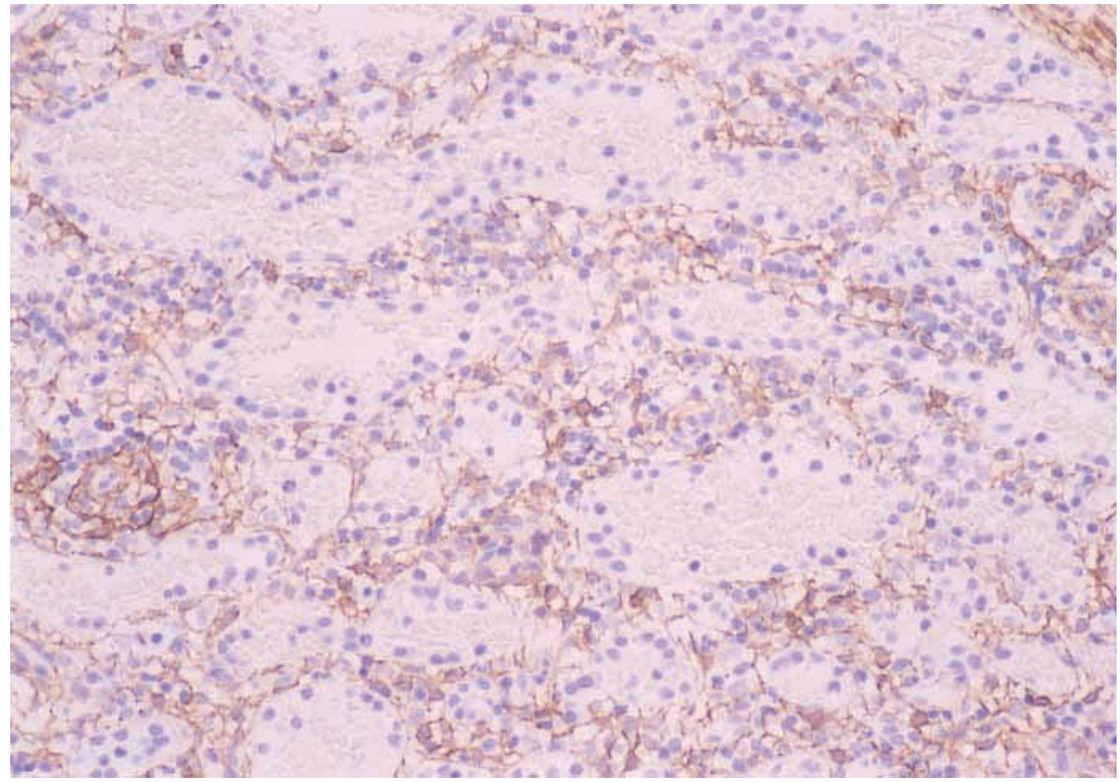
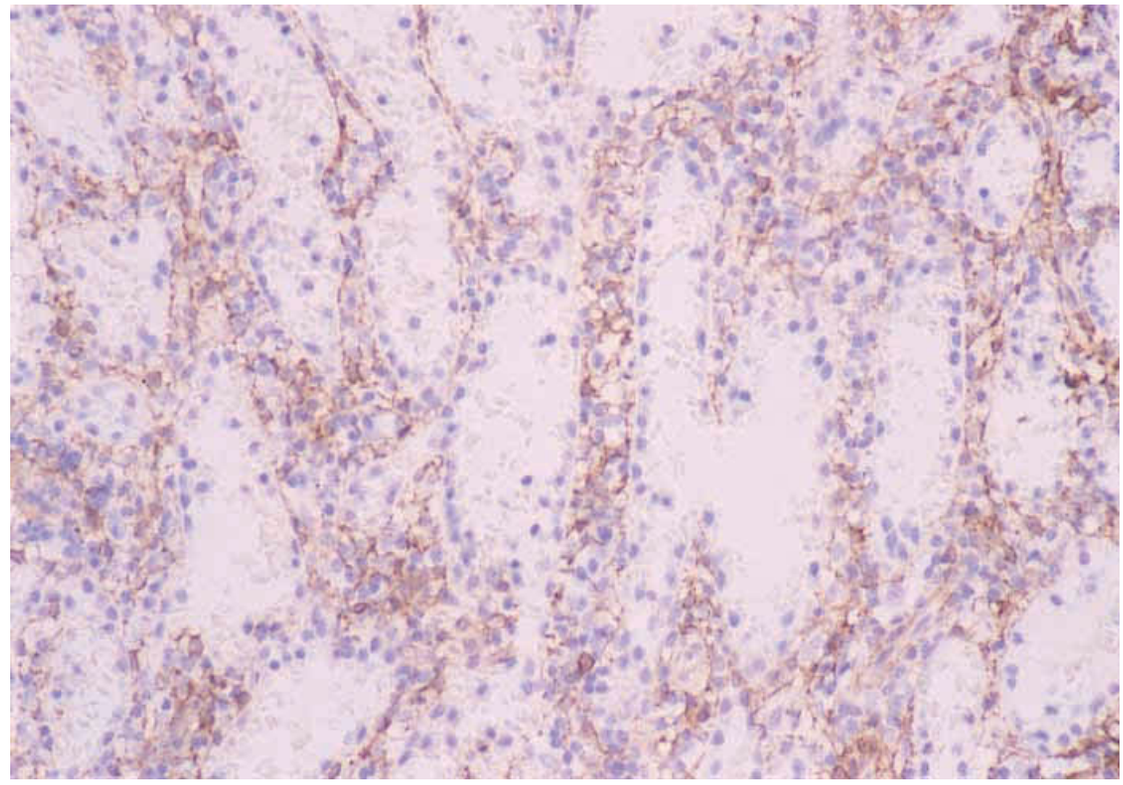


(*a*)



(*b*)

**Fig. 2.13.** (*a*) Expression of $\alpha$-SMA by dendritic, presumably myofibroblastic cells within splenic red pulp cords. (*b*) Macrophages with dendritic morphology within red pulp cords, immunostained for CD68. Streptavidin–biotin complex immunoperoxidase technique.

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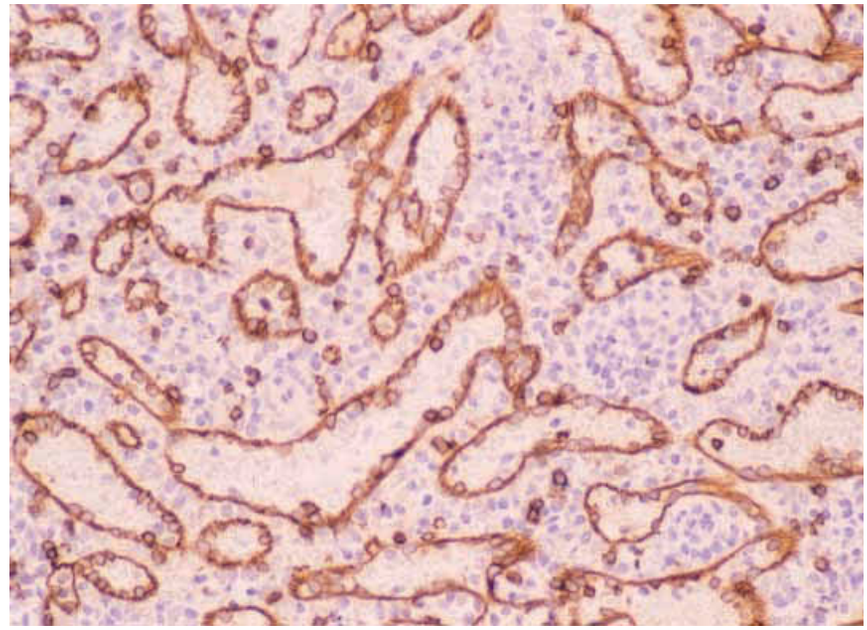


**Fig. 2.14.** Red pulp sinusoids immunostained to demonstrate expression of CD8 by sinusoidal endothelium. No other endothelium within the body expresses this antigen and its function on these cells is unknown. Streptavidin–biotin complex immunoperoxidase technique.

Chapter 3

# Post-traumatic and incidentally removed spleens

## Introduction

Inclusion of these topics in a chapter of their own represents a departure from the traditional pattern of description in diagnostic pathology. However, a high proportion of splenectomies fall into these categories and are often dismissed, erroneously, as normal. We feel that to offer a specific approach to these types of splenectomy is important. Features can be discerned, at least in well-fixed tissue, which will enable the reader to make a confident distinction between insignificant changes, changes secondary to surgery or trauma and those which reflect significant underlying pathology.

## Spleens removed incidentally and those removed following trauma are different

In the category of traumatized spleens are included those removed following blunt abdominal injury (caused by a fall, road traffic accident or other crush injury) and open trauma (e.g. due to a gunshot or stab wound). Spleens removed for these reasons require a slightly different approach from those removed purely for technical reasons during abdominal surgery. The latter specimens are usually removed because of requirement to gain access to a difficult surgical site, inability to preserve splenic blood supply or because capsular damage has

been caused accidentally or unavoidably during the surgical procedure. Depending on the nature of the injury which the patient has suffered, pathological changes are more likely to be present in the first group of spleens than in the second, incidental, group.

Support for the concept that incidental and post-traumatic splenectomies should be regarded as distinct, if overlapping, pathological entities is provided by elegant morphometric studies undertaken by van Krieken and colleagues in the 1980s. These investigators found significant differences between the total weights and aspects of white pulp reactive change in spleens removed in the two clinical contexts (van Krieken et al., 1983). Their post-traumatic spleens were, on average, heavier than incidentally removed spleens and contained larger germinal centres and marginal zones. Their cases did not include any spleens with specific, identifiable pathology of the types which may predispose to rupture upon minor trauma or spontaneously and they excluded any influence of age upon their statistical analysis. Van Krieken and his co-authors challenged the assumption that, apart from the direct effects of parenchymal disruption, post-traumatic spleens are normal. They suggested that subclinical immune stimulation causing lymphoid hyperplasia in the spleen predisposes to rupture. Therefore, although the spleen may be disrupted by massive or penetrating trauma when entirely normal, it may be spared in lesser traumatic events unless it is vulnerable due to coincidental changes. These findings have been supported by a further, recent study by Farhi and Ashfaq (1996). However, the alternative hypothesis that such reactive changes are a response to trauma remains to be studied, as discussed earlier. It also remains arguable whether splenic enlargement with reactive white pulp constitutes a physiological or patho-

logical state in such cases, but these changes are certainly not synonymous with the spleen's resting or unstimulated appearances.

Unfortunately, as discussed above, no systematic study has been made of the time-course of splenic changes following abdominal trauma to investigate the alternative hypothesis that they develop as a response to parenchymal disruption. As an additional complication to thinking on this subject, it is undoubtedly true that some incidental splenectomy specimens, even when not enlarged, show white pulp lymphoid hyperplasia for which no specific cause is apparent.

## An approach to analysis of traumatized or incidental splenectomy specimens

### Obtain as much clinical information as possible

It is sometimes entirely unclear from the brief comments recorded on histology request forms whether trauma was sustained pre- or intra-operatively. This is an important issue to clarify, since spleens subjected to intra-operative trauma are more appropriately categorized as incidental splenectomies. It is rare, in cases following blunt abdominal trauma, for any estimate of the time interval between injury and surgery to be provided. This is also important information to obtain, since quite pronounced secondary reactive changes can develop around traumatized areas of splenic parenchyma during the hours (and days, in occasional cases of so-called 'delayed rupture') following injury. Other valuable information includes description of whether the spleen appeared normal or not at the time of surgery, with specific details of any abnormalities noticed at that time.

### Expect to see pathological changes

There is a temptation to expect (or hope!) that such spleens will show no abnormality. It is much more rewarding to approach each case with the assumption that something abnormal may be found. The major likely pathologies can then be excluded systematically in order to conclude that the spleen truly has no clinically significant abnormality. As a general principle with spleens removed following abdominal injury, the less significant the trauma, the more likely it is that some underlying splenic pathology has pre-disposed to rupture.

### Know the appearances of the normal spleen

Macroscopic appearances may be very difficult to interpret in a badly traumatized spleen. The tissue may be received in several pieces and there may be a large amount of clotted blood within it or attached to its surface. Major capsular and parenchymal abnormalities, if present, should still be visible, and the combined weight of fragments (excluding as much blood clot as possible) provides a reasonable estimate of the original spleen weight. It should be remembered that accumulation of intrasplenic haematoma may add significantly to this, however, and increases up to double the normal spleen weight may be apparent solely from this cause.

For normal splenic histology, refer to Chapter 2 (see also van Krieken & te Velde, 1988; van Krieken et al., 1985 *a,b*). It may be useful to keep a section of normal spleen handy for comparison with 'active' cases.

### Interrogate the sections systematically

Look in turn at capsule, trabeculae, PALS, white pulp B cell nodules (germinal centres, mantle zones and marginal zones), perifol-

**Table 3.1.** *Non-specific reactive features within individual compartments of the spleen*

| | |
|---|---|
| Germinal centres | Expansion with active immune stimulation; hyalinosis upon involution |
| Mantle zones | Solid nodules in unstimulated white pulp developing into well-defined zones around germinal centres upon immune stimulation. May be attenuated if GC very large and active |
| Marginal zones | Become expanded, forming wider zones around periphery of WP nodules upon immune stimulation. Very low proliferative activity except for scattered blast cells which may be evident in the most prominent marginal zones – ?expansion by accumulation/retention of trafficking cells |
| Peri-arteriolar lymphoid sheaths | Expansion accompanied by 'moth-eaten' appearance due to increased size and number of inter-digitating reticulum cells (similar to dermatopathic change in lymph nodes) |
| Peri-follicular zones | 'Flares' representing congestion of peri-follicular sinusoids by RBC in spleens with marked WP stimulation. Mechanism unknown |
| Red pulp sinusoids | Prominence of endothelium with cuboidal or 'hob-nail' appearances |
| Red pulp cords | Increased cellularity due to accumulation of a variety of inflammatory cells |
| Non-filtering areas of red pulp | Peri-capillary plasma cell clusters |

licular zones, red pulp (capillaries, cords and sinuses). See Table 3.1.

## Know the changes which can be safely assumed to be innocent

Most such changes are reactive in nature, reflecting red and white pulp responses to injury or immunological stimulation. The likelihood of finding prominent reactive germinal centres in white pulp is less in extreme old age than in younger patients and, if the spleen from an elderly individual does contain highly stimulated white pulp, the possibility of underlying pathology should be considered carefully. However, even spleens removed during surgery for gastric, colonic or renal carcinomas may be surprisingly reactive in some older patients, for unknown reasons.

The most common white pulp reactive change seen in traumatized or incidentally removed spleens is marginal zone expansion (Fig. 3.1). It is less common to see prominent germinal centres. Where the marginal zone is expanded there is commonly congestion of the peri-follicular zone. In the red pulp, endothelial cells may appear prominent and cuboidal (Fig. 3.2). A diffuse red pulp lymphocytosis may be present but is not severe; it may be accompanied by increased numbers of plasma cells, usually clustered around capillaries (Fig. 3.3). These features have no specificity on their own (see below). Although it is difficult to accumulate accurate data regarding the time-course of their development, they appear more prominent in post-traumatic spleens where there has been a delay of many hours to a few days between injury and surgery.

Incidental splenectomy generally accompanies surgery performed in later life, for gastro-intestinal or renal neoplasia, or for aortic aneurysm. Spleen size is often reduced in very elderly patients but in those under the age of 70 years it should be

questioned whether this is attributable to age alone (van Krieken and te Velde, 1988). Connective tissue trabeculae in such spleens may appear prominent due to reduction in other splenic components. White pulp nodules and PALS are atrophic; trabeculae may appear to be bare rather than having their normal cuff of T lymphocytes and B cell nodules may be small throughout the tissue. Such atrophic B cell nodules contain no germinal centres or, at most, hyalinized remnants of involuted germinal centres. The cells comprising these nodules are of mantle type (small lymphocytes) and marginal zone differentiation is usually inconspicuous.

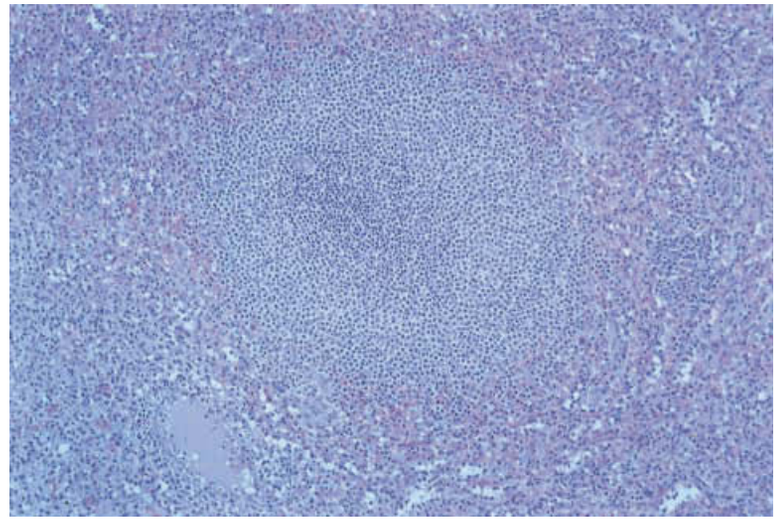


**Fig. 3.1.** White pulp nodule from incidentally removed spleen showing marginal zone expansion without germinal centre formation. H&E stain.

### Know the sort of pathology which may silently cause rupture

In cases of minimal abdominal trauma, the splenic features may overlap with those seen in spontaneous rupture. In both cases there is likely to be underlying pathology predisposing to capsular disruption (see below). Although such ruptures may occur in normal-sized spleens, one should be particularly suspicious of underlying pathology in spleens enlarged to more than twice normal size.

## Reactive changes in individual compartments of the spleen

### White pulp

Upon antigenic stimulation, splenic lymphoid follicles undergo enlargement and the development of germinal centres, just as do follicles in lymph nodes. The central small lymphocytes of the follicle are displaced peripherally to form a mantle zone. In addition, the marginal zone frequently expands, becomes more cellular and often is seen to contain occasional blast cells resembling centroblasts (Fig. 3.4). The PALS

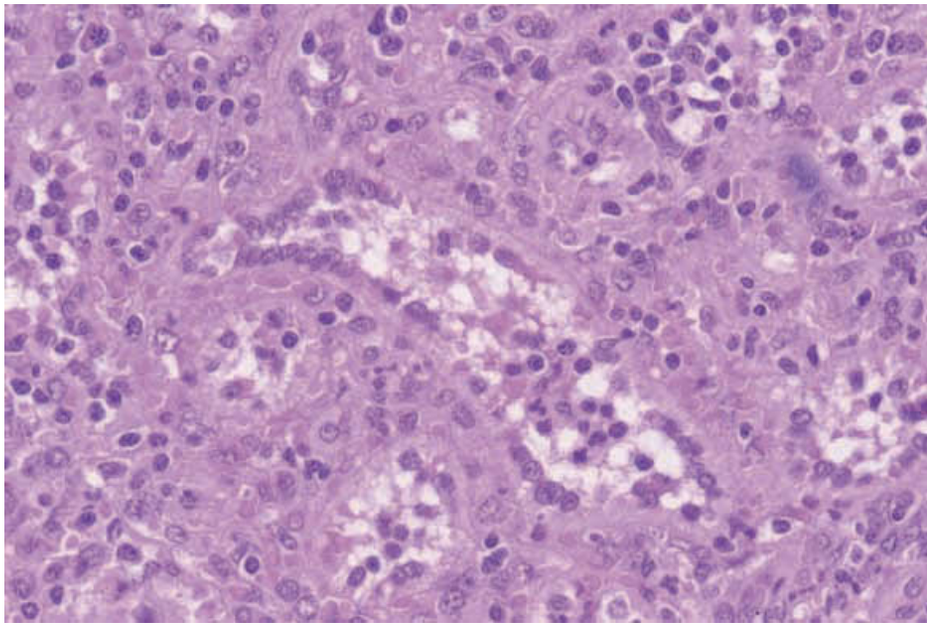


**Fig. 3.2.** Cuboidal appearance of reactive red pulp sinusoidal endothelium. This change is non-specific and may be seen in spleens removed incidentally or for a variety of pathological conditions. H&E stain.

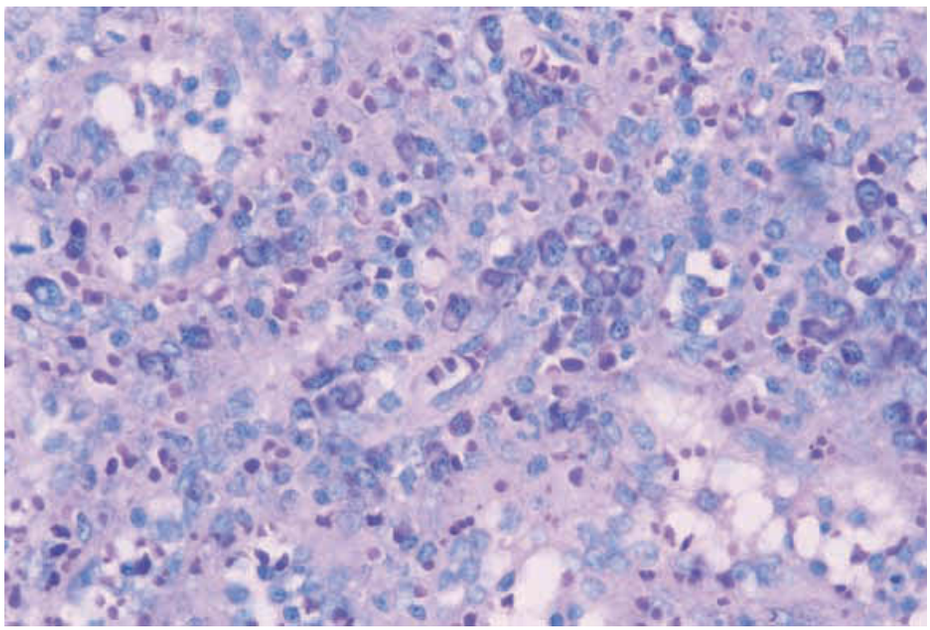


**Fig. 3.3.** Red pulp plasmacytosis with plasma cells clustered around a small capillary. Giemsa stain.

also expands and may gain small numbers of blast cells, but changes in this compartment are often difficult to appreciate in H&E stained sections. The relative predominance of germinal centre formation, marginal zone expansion or T-zone enlargement varies greatly between specimens, but is usually consistent throughout the parenchyma of incidentally removed spleens. In cases of splenectomy following relatively localized trauma to the organ, white pulp reactive changes may be more pronounced at the periphery of the area of disrupted parenchyma.

The variable nature of the changes presumably reflects differences in the precise nature and timing of the stimulus between cases. In incidentally removed spleens, white pulp changes presumed to represent late stages and involution of immune responses are frequently seen (Neiman & Orazi, 1999), characterized by germinal centre shrinkage and hyalinization (Fig. 3.5). The hyaline material is strongly PAS-positive, reacts like fibrin or fibrinoid material with stains such as Martius Scarlet Blue and may be accompanied by abundant nuclear dust. Such white pulp atrophy is more commonly seen in spleens from older individuals, and reactive expansion of the white pulp may be more pronounced in younger individuals. However, exceptions to these generalizations are common and van Krieken et al., in their series of incidentally removed spleens, found no evidence of statistically significant reduction in the reactivity of individual white pulp nodules with old age, as discussed earlier.

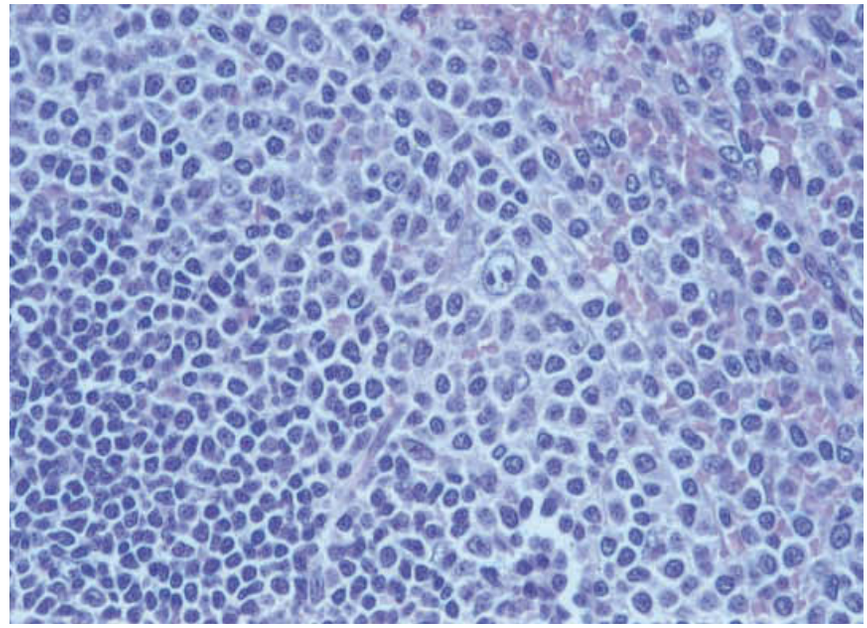
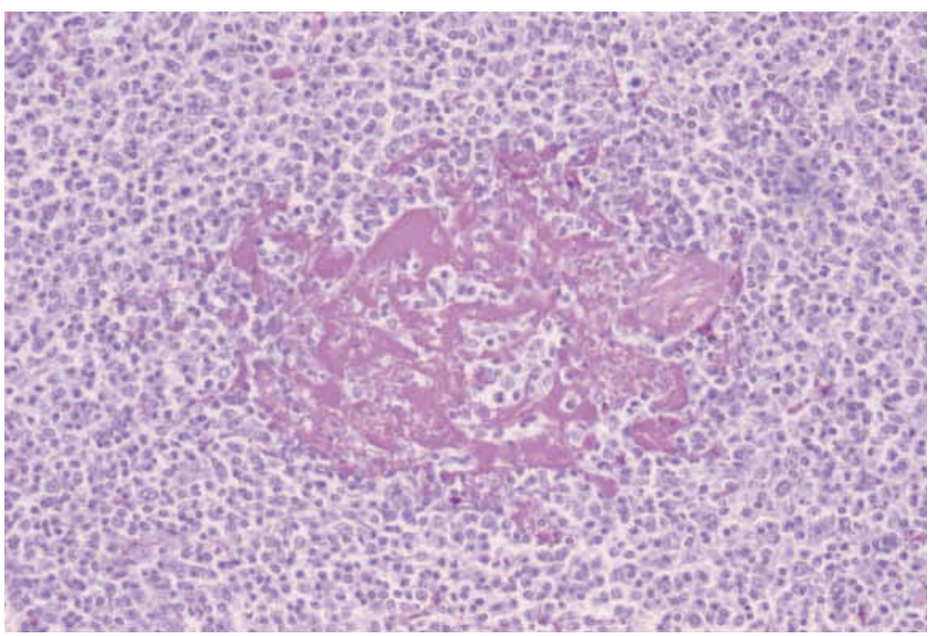


**Fig. 3.4.** Reactive marginal zone showing a large, centroblast-like cell among the smaller, typical marginal zone cells. Small, compact mantle zone cells are present towards the lower left-hand side of the picture.

### Red pulp

Red pulp changes are also heterogeneous and some of the features which correlate with specific pathological processes are described below. Non-specific reactive changes include plasmacytosis and CD8-positive T cell lymphocytosis within cords.

**Fig. 3.5.** Involuting white pulp germinal centre showing prominent hyaline material, highlighted by periodic acid-Schiff's (PAS) stain.



These features are accompanied by congestion of peri-follicular red pulp capillaries by neutrophils and red blood cells. Cuboidal enlargement of endothelial cells lining sinusoids has been mentioned earlier and is illustrated in Fig. 3.2.

Splenic cords become thickened and fibrotic in conditions of prolonged portal hypertension, as may be seen in incidental splenectomies performed during surgery to create porto-systemic shunts in patients with cirrhosis.

## Spontaneous rupture of the spleen

Splenic enlargement from any cause may be associated with apparently spontaneous rupture. Considering how thin the splenic capsule is, it is remarkable how infrequently rupture of even a massively enlarged spleen occurs. Pain and abdominal swelling, with or without infarctions in the organ, are more common presenting features of extreme splenomegaly.

Spontaneous rupture of an entirely normal spleen is probably extremely rare, although it has been reported in association with anticoagulant therapy (Soyer et al., 1976), presumably secondary to intrasplenic haemorrhage caused by impaired haemostasis. When splenic rupture is apparently spontaneous therefore, the presumption must be that underlying pathology is present which has predisposed to the event. Table 3.2 lists some of the more common associations of spontaneous splenic rupture. As a general principle, diffuse pathology is more likely to lead to rupture than any focal lesion; simple splenic cysts and focal deposits of large cell lymphoma or metastatic tumour are rarely associated with spontaneous splenic rupture. Necrosis within tumour deposits or abscess formation secondary to septicaemia or infected embolism increases the risk of rupture. Areas of splenic infarction from any cause,

particularly if extensive, may occasionally become disrupted.

Pathological processes affecting predominantly the red pulp (e.g. infiltration by haemopoietic cells in myeloproliferative disorders or sinusoidal disruption in peliosis) carry a greater risk of rupture than do white pulp disorders. Generalized white pulp expansion, as in many low grade non-Hodgkin's lymphomas, will tend to reduce the relative proportion of fragile sinusoidal tissue in the spleen, reducing both the likelihood and the severity of any parenchymal haemorrhage.

Rupture may also be associated with inflammatory injury weakening the parenchyma and/or capsule; this is probably the mechansism underlying splenic rupture in septicaemia and other infectious conditions involving the spleen, such as that caused by Epstein–Barr virus (see Chapter 5). Inflammatory vascular injury in conditions such as polyarteritis nodosum and systemic lupus erythematosus, which affect larger blood vessels and spare the sinusoids, predispose to infarction rather than rupture.

**Table 3.2.** *Causes of spontaneous rupture of the spleen*

| |
|---|
| *Infections and inflammation:* |
| Epstein–Barr virus (infectious mononucleosis) |
| Influenza |
| Rubella |
| Human immunodeficiency virus |
| Infective endocarditis |
| Tuberculosis |
| Malaria |
| *Diffuse infiltration (especially of red pulp):* |
| Acute leukaemias |
| Lymphomas (esp. hairy cell leukaemia) |
| Chronic myeloproliferative disorders |
| Amyloidosis |
| *Focal tumours/tumour-like lesions/miscellaneous:* |
| Peliosis |
| Hamartomas |
| Vascular tumours |
| Cysts |
| Infarctions |

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Chapter 4

# The spleen in hereditary blood cell abnormalities and auto-immune disorders

## Introduction

Although primary splenic pathology is uncommon underlying disorders in which the spleen is responsible for excessive destruction of blood cells, splenectomy may be performed for control of symptoms (usually anaemia and/or thrombocytopenia). The spleen in such conditions is usually modestly enlarged, up to twice or three times normal weight. Only rarely is major splenomegaly of 1000 g or more the cause for removal.

Excessive red blood cell destruction occurs in dyserythropoietic states such as hereditary spherocytosis (HS), hereditary elliptocytosis (HE), various inherited red cell enzyme deficiencies, sickle cell disease and thalassaemias. In these conditions, mechanically or metabolically abnormal red blood cells are removed prematurely from the circulation by splenic sequestration.

In auto-immune haemolytic anaemia (AIHA) and idiopathic thrombocytopenia (ITP), antibody-coated red cells and platelets are phagocytosed by splenic macrophages. Occasionally auto-immune red cell and platelet destruction occur together in the same patient; this combination is sometimes referred to as Evans' syndrome.

Variable cytopenias may also occur in association with splenic enlargement from any cause, although the mechanisms underlying hypersplenism in splenomegaly are often unclear.

## Vaccination before splenectomy

In recent years, pre-splenectomy vaccination against *Streptococcus pneumoniae*, type B *Haemophilus influenzae* and *Neisseria meningitidis* groups A and C has been introduced into routine surgical practice (Mayon-White, 1994). It is likely that inoculation against *N. meningitidis* group B will also be undertaken in future, once an appropriate vaccine becomes available. These developments are significant for histopathologists because the vaccines specifically stimulate immune reactions for which the spleen is important (directed against bacterial capsular polysaccharides, as discussed in Chapter 2) and are therefore likely to produce morphological alterations in the spleen. Pathologists must learn to recognize vaccine-related changes in order to avoid attributing them to underlying splenic pathology. As yet, insufficient data exist to permit accurate distinction between vaccine and pathological effects in most cases. In our hospital, vaccination is performed routinely 2 weeks prior to planned splenectomy. Spleens from HS patients represent a useful group in which to study possible vaccine effects because there is unlikely to be any lymphomatous white pulp involvement to confuse interpretation and the individuals will not have received any immunosuppressive treatment, such as glucocorticoids. We have found no significant differences in spleen weights or histological appearances between HS spleens retrieved from our archive from more than 10 years ago (pre-vaccine) and age-matched cases known to have received pneumococcal and haemophilus vaccination. However, we have only been able to study ten cases in each category and more formal studies are needed to ascertain the validity of these findings.

Successful vaccination may be undertaken post-operatively in patients requiring emergency splenectomy or non-anticipated

removal of the spleen during unrelated surgery. In these cases the spleen will obviously have appearances representing the unvaccinated state. It is of interest to note that such immunization can be achieved despite removal of the splenic marginal zone compartment which is believed to be so important for T cell-independent reactions. This may suggest that epitopes recognized in the vaccine are different from those targeted by the immune system in natural exposure to these organisms. Alternatively, marginal zones elsewhere in the body (e.g. in mucosa-associated lymphoid tissue) may make a significant contribution.

## Sickle cell anaemia and thalassaemias

Splenectomy is rarely performed in patients with these inherited disorders of haemoglobin synthesis. Patients suffer chronic haemolysis due to destruction of their abnormally haemoglobinized cells and the splenic changes which result have some shared features. However, important differences also occur, reflecting differences in the nature of red cell deformation caused. There is also variation in the degree of red cell destruction between heterozygotes and homozygotes with each type of disease, a situation complicated by factors such as the occurrence of several forms of $\alpha$-thalassaemia heterozygosity and combinations of thalassaemia traits or other haemoglobinopathies with sickle cell heterozygosity. Subtle differences which may occur between the splenic changes in such individuals are beyond the scope of this text. Further details of the clinical, haematological and molecular genetic aspects of these conditions may be found in a recent overview of the topic by Weatherall (1999).

In sickle cell anaemia, the haemoglobin $\beta$-chain is abnormal due to a point mutation which causes the sixth amino acid

of the protein molecule, normally glutamic acid, to be substituted by valine. Under hypoxic conditions, the abnormal $\beta$-chains form stable tactoids (liquid crystals) and cause RBC to adopt the characteristic sickle shape which gives the disorder its name.

Destruction of deformed red cells in sickle cell anaemia occurs in the spleen in early years of the patients' lives, with splenomegaly resulting from massive sequestration of sickled cells within splenic sinusoids and cords (Fig. 4.1). The presence of these cells occludes the red pulp circulation, reducing the oxygen tension further and hence promoting additional sludging and sequestration of abnormal red cells. Despite splenic enlargement, the normal functions of the organ may be inefficient due to overload with abnormal red cells. Consequently, features of hyposplenism (particularly persistence of red cell inclusions such as Howell–Jolly bodies) may occur. Episodes of rapid splenic enlargement accompany sequestration crises in which sudden anaemia results from massive red cell pooling in the spleen.

Over time, however, splenic enlargement declines and atrophy of the organ supervenes as a result of repeated infarctions, with consequent worsening of hyposplenism. Infarcted areas are replaced by irregular bands of fibrous tissue; the spleen undergoes progressive scarring and atrophy with large amounts of haemosiderin present. Heterozygotes generally have less severe anaemia and consequently less abnormality of splenic function and morphology.

In thalassaemias, a variety of mutations result in relative or absolute failure to synthesize either $\alpha$ or $\beta$ haemoglobin chains. Haemoglobin precipitation in the thalassaemias occurs due to formation of unstable tetramers containing abnormal proportions of each chain. Excessive pitting activity by cordal macrophages attempting to remove the resultant haemoglobin inclusions

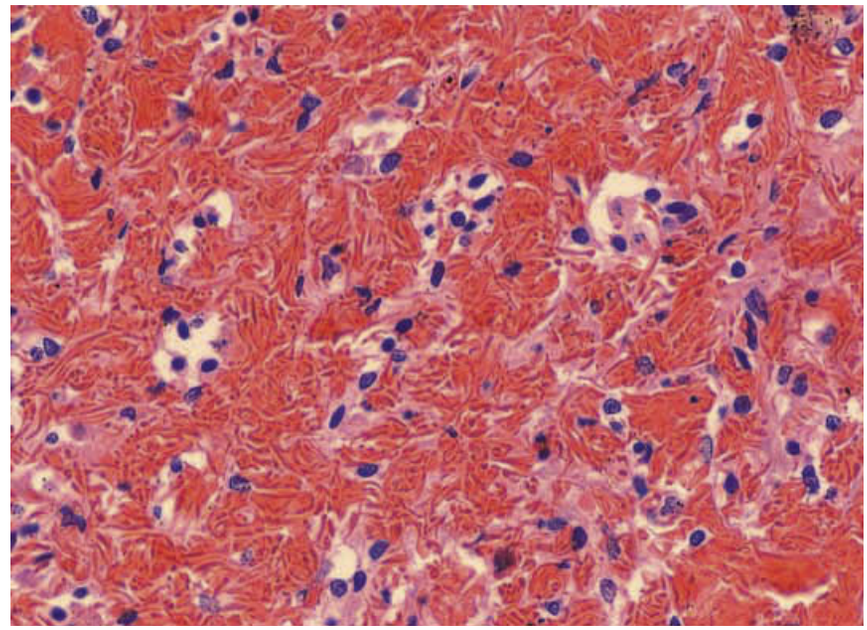


**Fig. 4.1.** Accumulation of sickled red blood cells within sinuosoids and cords of the spleen in sickle cell anaemia. H&E. Photomicrograph kindly provided by Professor Sebastian Lucas, United Medical and Dental Schools, St Thomas' Hospital Campus, London.

causes spherocytosis due to decreased surface area:volume ratio of red cells. These spherocytes are less deformable than normal red cells and are sequestered within red pulp cords. Not surprisingly, in thalassaemias the splenic appearances resemble those seen in hereditary and other forms of spherocytosis (see below). Splenic enlargement is usually more persistent in thalassaemia patients than in individuals with sickle cell disease because infarction and subsequent fibrosis are uncommon in the absence of the vascular blockade caused by sickling.

## Hereditary spherocytosis and autoimmune blood cell destruction

Hereditary spherocytosis (HS), autoimmune haemolytic anaemia (AIHA) and idiopathic thrombocytopenic purpura (ITP) are unrelated disorders in which sequestration and/or phagocytosis of cellular elements from the blood occur. Patients usually present with symptoms due to lack of red cells or platelets (or both, in Evans' syndrome), with little or only modest splenic enlargement (up to 500 g). Splenectomy in HS may be undertaken at the time of cholecystectomy in those individuals who develop pigment gallstones, or to prolong red cell survival in individuals with severe anaemia. Similar features occur in hereditary elliptocytosis and various red cell enzyme defects but are generally less severe.

In AIHA and ITP, splenectomy may also be undertaken to prolong the lifespan of red cells or platelets, respectively, in patients whose symptoms are refractory to immunosuppressive treatment with corticosteroids. In addition, splenectomy may be performed in some patients with AIHA/ITP to investigate the possibility of underlying lymphoproliferative disease, particularly if the splenic weight seems disproportionately

high for the degree of haematological disturbance.

The justification for considering these conditions together in this section is that systematic comparison of their splenic changes illustrates how the normal spleen responds to mechanically (HS) or immunologically (AIHA, ITP) abnormal cells in the bloodstream. In essence, these disorders represent exaggerated examples of normal splenic function.

## The nature of HS, AIHA and ITP

Hereditary spherocytosis and related conditions such as hereditary elliptocytosis arise as a result of mutations affecting genes encoding elements of the red cell cytoskeleton. Proteins contributing to maintenance of the normal biconcave red cell shape are particularly important in this regard (e.g. spectrin, ankyrin, band 3). The clinical syndrome of HS may arise from an underlying genetic abnormality in any of these proteins plus, rarely, some others. Hereditary elliptocytosis is usually due to spectrin defects. Inherited abnormalities of components of metabolic pathways, for example, red cell pyruvate kinase deficiency, can also lead to premature senescence and consequent excessive sequestration by the spleen (Luzzatto & Gordon-Smith, 1999; Tse & Lux, 1999).

Auto-immune haemolytic anaemia and ITP result from auto-antibody coating of red cells and platelets, respectively. The target antigens are usually unknown, although in some cases of AIHA epitopes of Rhesus antigens have been shown to be involved (Barker et al., 1997). The underlying cause of the development of auto-immune reactions to these targets is also frequently mysterious, particularly in the case of ITP. An associated non-Hodgkin's lymphoma is present in a proportion of

**Fig. 4.2.** Cross-section of normal-sized spleen from a patient with ITP. In this case, white pulp is unstimulated and the parenchyma has a uniform red/brown appearance.

**Fig. 4.3.** Cross-section of spleen from a patient with ITP showing diffuse, miliary nodularity throughout the parenchyma secondary to the white pulp stimulation, which is present in this case.

individuals, predominantly in those with AIHA but also occasionally with ITP (Sokol et al., 1992). These are usually low grade tumours and there may, or may not, be direct involvement of the spleen itself by the neoplastic cells. Auto-antibodies associated with such lymphomas are usually polyclonal, produced as a result of dysregulation of reactive lymphoid cells rather than being a monoclonal product of the neoplastic lymphoid cells themselves. An ITP-like syndrome can occur in association with infection by the human immunodeficiency virus (HIV); this is discussed further in Chapter 5.

## Macroscopic appearances of the spleen in HS, AIHA and ITP

The spleen is rarely massively enlarged in these conditions unless underlying pathology such as lymphoma is also present. In HS and AIHA there is usually intense congestion of the parenchyma, which appears firm, meaty and dark red. There may, or may not, be sufficient white pulp expansion to produce macroscopically evident pin-point nodules of white tissue throughout the parenchyma. As will be discussed below, white pulp hyperplasia is a common feature in HS as well as AIHA, for reasons which are unclear. Spleens from patients with ITP may appear entirely normal or may also show delicate miliary nodularity due to white pulp expansion (Figs. 4.2 and 4.3). They do not usually have significant congestion with red cells and therefore lack the meaty red parenchymal appearance seen in AIHA or HS; its presence would suggest Evans' syndrome.

What follows below is a systematic comparison of the predominant microscopic features in HS, AIHA and ITP.

## Red pulp

### The hallmark of hereditary spherocytosis is red cell sequestration

In HS and other abnormalities of red cell shape/metabolism leading to reduced red cell lifespan, the predominant splenic change is widening of cords by sequestered red cells (Fig. 4.4). Most of these red cells are retained in the loose meshwork of the cords and relatively few of them are found as phagocytosed particles within macrophage cytoplasm. Cordal macrophages retain their dendritic morphology and become more widely separated than normal by the accumulated red cells (Fig. 4.5). There is evidence that phagocytosis by endothelial cells lining splenic sinusoids can occur (Schiffman et al., 1988) but red cells can rarely be seen within their cytoplasm. The amount of haemosiderin deposition in macrophages is usually small. Sinusoidal lumens appear relatively empty.

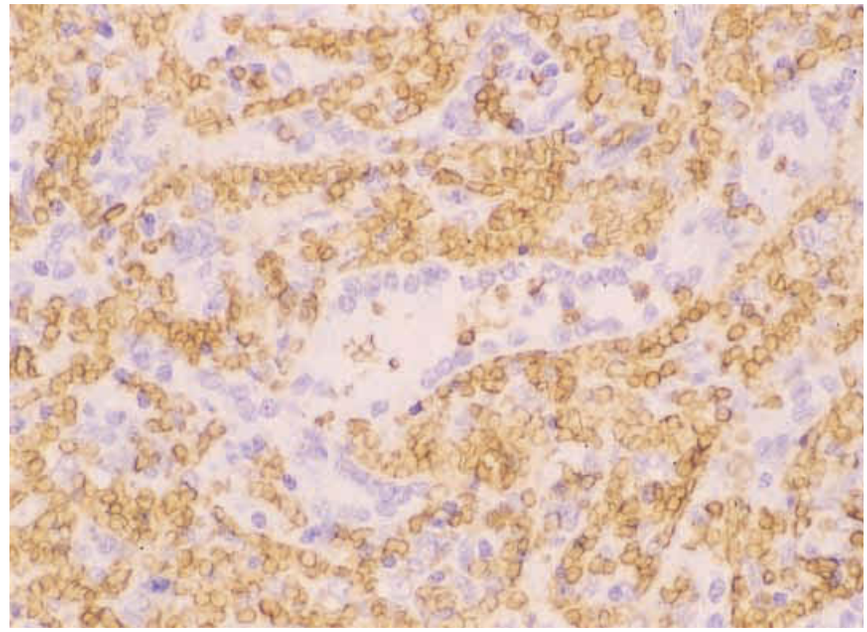


**Fig. 4.4.** Red blood cells sequestered within splenic cords in hereditary spherocytosis. The central sinusoid is relatively empty and the cells are preferentially retained within cords. Section immunostained for glycophorin A using monoclonal antibody BRIC 101, kindly donated by Dr D. Anstee, South West Blood Transfusion Service, Bristol.

### The hallmark of auto-immune haemolysis is intrasinusoidal red cell phagocytosis

In AIHA, by contrast, phagocytosis of antibody-coated red cells occurs predominantly within sinusoids and to a lesser extent within the adjacent cords. Macrophages containing phagocytosed red cells and debris are prominent within sinusoidal lumens so that the latter appear partly or completely filled by such cells, which are distinctly different from circulating red and white blood cells (Fig. 4.6). The cordal macrophages remain predominantly dendritic in appearance but scattered single round/oval forms are found, containing cell debris (Fig. 4.7). Occasional small epithelioid granulomata may be formed within the red pulp or at the interface between red and white pulps. Iron deposition in cordal and intra-sinusoidal macrophages is usually prominent in cases of chronic auto-immune

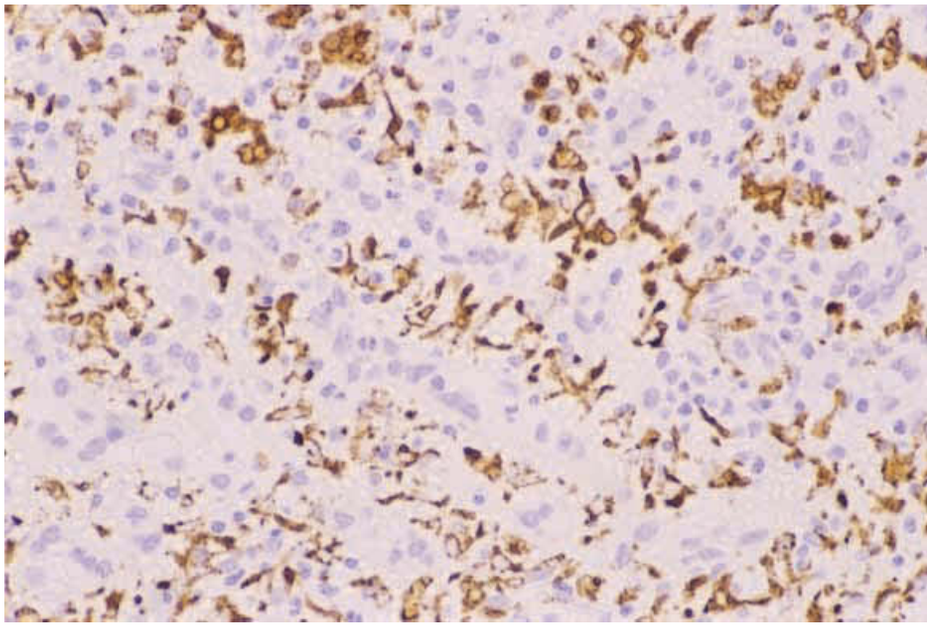
haemolysis; the longer the patient's clinical history, the more haemosiderin is likely to be present.

### The hallmark of auto-immune platelet destruction is phagocytosis by cordal macrophages

The phagocytosis of antibody-coated platelets occurs predominantly within cordal macrophages. These macrophages in ITP are converted from their normal dendritic morphology into rounded cells with superficial resemblance to the abnormal macrophages found in a variety of metabolic storage disorders (Fig. 4.8). They are often accompanied by plasmacytosis in the red pulp and by scattered oleogranulomas. In occasional cases, the appearances may be so florid that they genuinely mimic those of Niemann–Pick disease or one of the so-called 'ceroid histiocytoses' (see Chapter 8).

No reported data exist to indicate whether or not individuals so affected have a *forme fruste* of any of these inherited enzyme deficiencies. However, it may be speculated that their normal reserves of lysosomal enzymes, required for digestion of phagocytosed platelets, have been exhausted by particularly heavy demand.

**Fig. 4.5.** Splenic cordal macrophages in hereditary spherocytosis demonstrated by immunostaining for CD68. Individual macrophages retain their dendritic morphology, and a prominent 'feathery' pattern is produced by widening of the cords due to sequestration of red cells. Streptavidin–biotin complex immunoperoxidase technique, using monoclonal anti-CD68 antibody PG-M1.



## White pulp

### White pulp changes are variable in auto-immune haemolysis and platelet destruction

White pulp changes in HS, AIHA and ITP can be very confusing. One might predict absence of white pulp reactive changes in HS, it being a mechanical rather than an immune disorder, while spleens in AIHA and ITP might be expected to show evidence of immune stimulation.

Spleens from patients with ITP or AIHA may have floridly reactive white pulp but, in

many cases, appear unstimulated or even show white pulp atrophy. This variation presumably reflects differences in disease activity between individuals, often further modified by steroid therapy (Kristensen & Jensen, 1985; Hassan & Neiman, 1985). Patients in whom splenectomy is performed are usually those for whom medical therapy has been unsuccessful. Therefore, steroids may have been used in high doses for prolonged periods in attempts to control disease symptoms before splenectomy was undertaken.

The appearances of non-Hodgkin's lymphomas within the spleen, which may be associated with AIHA or ITP as discussed above, are described in Chapter 6.

### The spleen in HS shows marked white pulp stimulation

Spleens removed from patients with HS typically show florid white pulp reactive changes, particularly marginal zone expansion (Fig. 4.9). The reason for this is unknown, but it may be that sequestered red cells, held in prolonged contact with splenic antigen-presenting cells, provoke an immune reaction to their highly carbohydrate-rich surface antigens.

Table 4.1 summarizes the main features of splenic histology in HS, AIHA and ITP.

## Felty's syndrome

A combination of rheumatoid arthritis, splenomegaly and neutropenia (with or without anaemia or thrombocytopenia) comprises Felty's syndrome. Typical Felty's syndrome occurs in patients who have severe joint disease of long duration, high titres of IgM rheumatoid factor and sometimes also anti-nuclear antibodies. Hypersplenism plus possible auto-immune destruction of cells probably accounts for the cytopenias and splenectomy generally

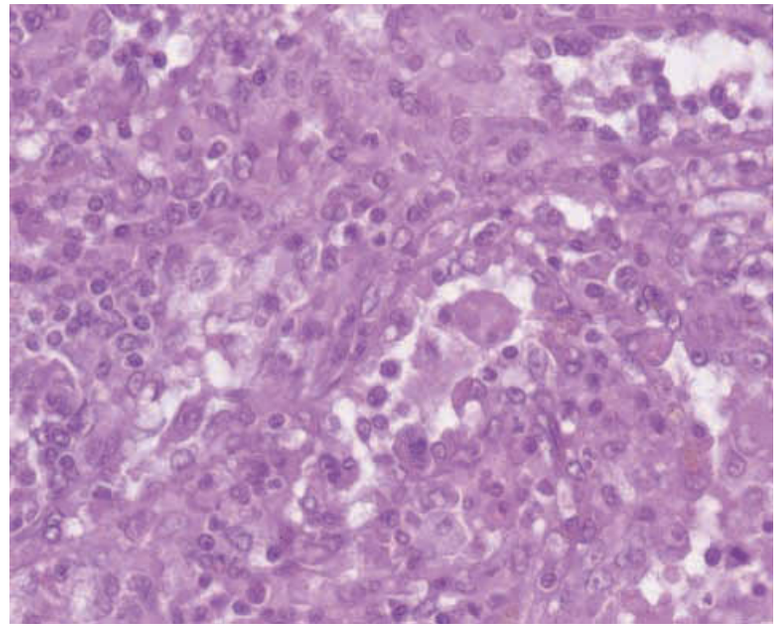
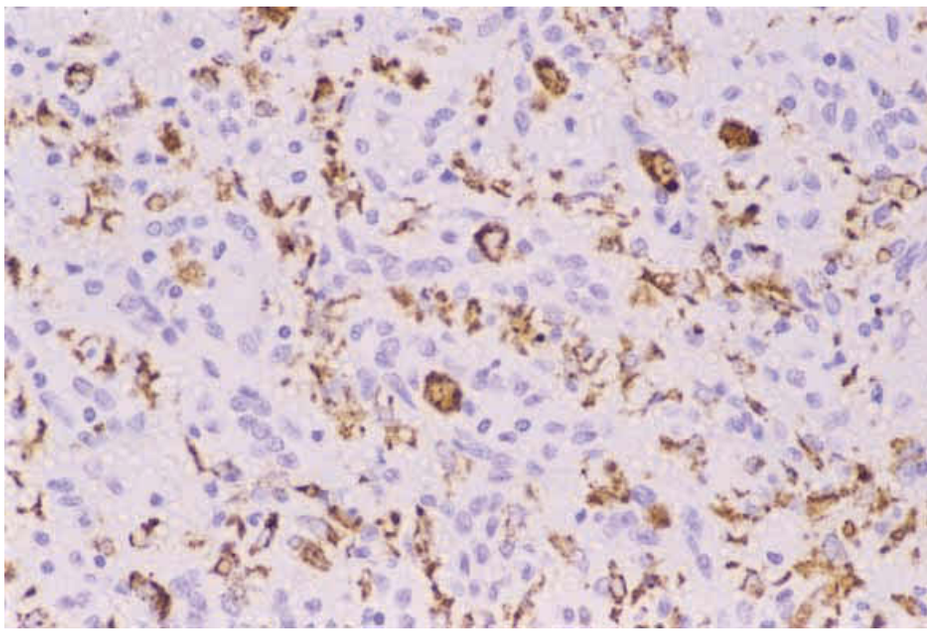


**Fig. 4.6.** In auto-immune haemolytic anaemia, prominent erythrophagocytosis occurs within sinusoids and large, phagocytic macrophages are prominent within sinusoidal lumens. H&E stain.

**Fig. 4.7.** Immunostaining of CD68 in auto-immune haemolytic anaemia shows relatively normal dendritic cordal macrophages and scattered large, round intrasinusoidal macrophages corresponding to cells such as that shown in Fig. 4.6. Streptavidin–biotin complex immunoperoxidase technique using monoclonal anti-CD68 antibody PG-M1.

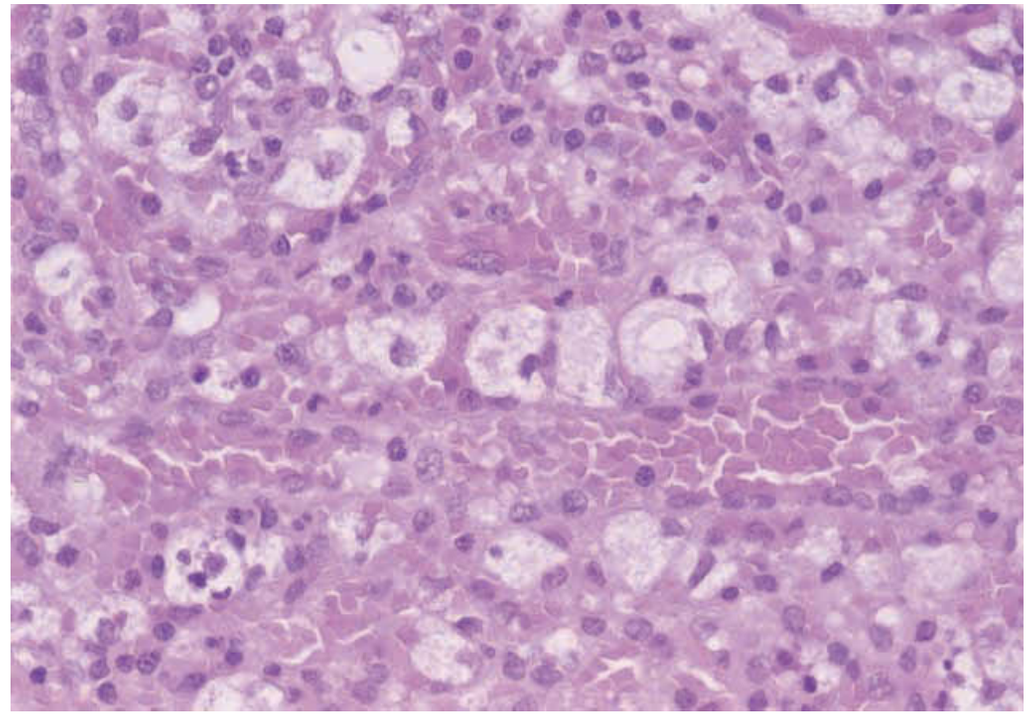


leads to improvement. The histological features in such cases are non-specific and mainly reflect hypersplenism. A red pulp plasmacytosis, either diffuse or clustered around capillaries in the non-filtering areas, may be present (Fig. 4.10). These plasma cells are polyclonal. The white pulp may appear stimulated but will be relatively inactive or even atrophic in patients who have received high dose corticosteroids or other immunosuppressive treatment.

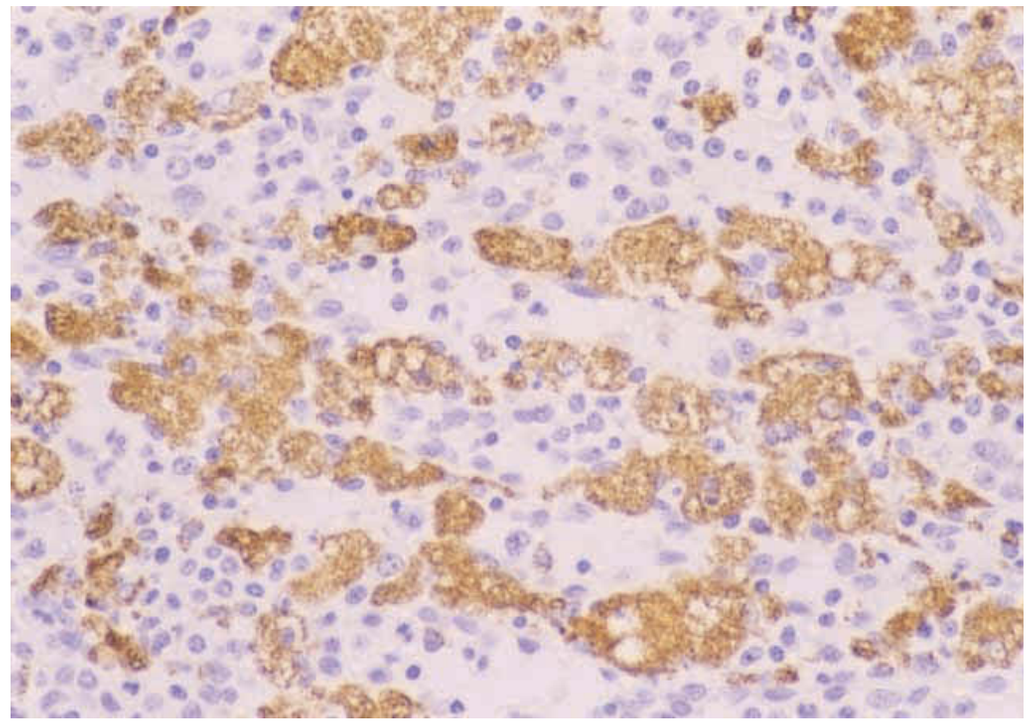
In recent years, a subgroup of patients with atypical Felty's syndrome has been recognized in whom joint manifestations and splenomegaly have developed contemporaneously. A high proportion of these patients has increased circulating large granular lymphocytes (LGL), with complex immunophenotypic patterns indicative of T cell, natural killer (NK) cell or hybrid 'NK-like' T cell differentiation (Loughran et al., 1985). In the majority of cases studied, these LGL have been found to be monoclonal and they are considered to be a manifestation of LGL leukaemia. Splenic red pulp is diffusely, but variably, infiltrated by these cells in LGL leukaemia (described further in Chapter 6). The precise mechanism underlying the development of cytopenias in these patients is unclear; only moderate improvement follows splenectomy and it has been suggested that T or NK cell-mediated suppression of haemopoiesis is the major underlying factor, rather than hypersplenism or auto-immune destruction.

## Systemic lupus erythematosus

The spleen may be enlarged and there may be hypersplenism in systemic lupus erythematosus (SLE). The histological appearances may resemble AIHA, ITP or both, since both of these auto-immune phenomena may occur as part of the spectrum of disease in SLE. Since patients are likely to have been treated with high doses of



(*a*)



(*b*)

**Fig. 4.8.** (*a*) Prominent foam cell change within cordal macrophages in ITP. (H&E stain). (*b*) Cordal macrophages in ITP immunostained to demonstrate CD68, again showing prominent foam cell change affecting most cordal macrophages. (Compare with Figs. 4.5 and 4.7.)

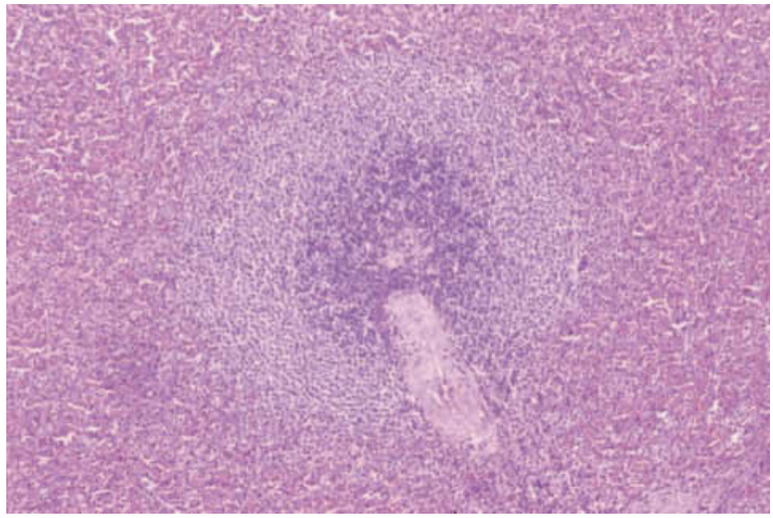


**Fig. 4.9.** White pulp nodule from the spleen of a patient with hereditary spherocytosis. This nodule, particularly its marginal zone, shows evidence of immunological stimulation, despite the essentially mechanical basis of HS. H&E stain.

corticosteroids, the white pulp is often atrophic. In addition, vasculitic features may be seen, with onion-skin fibrosis and fibrinoid necrosis of penicillar arterioles. Splenic infarctions, usually small, may occur as a result of the formation of micro-emboli; over time such infarctions may become organized into Gamna–Gandy bodies (see McWilliams & Hardman, 1995).

## Polyarteritis nodosum

Although systemic vasculitis does not often involve the spleen, involvement by polyarteritis nodosum (PAN) and other forms of non-infectious vasculitis has been described (Neiman & Orazi, 1999). In PAN, involvement is said to take the form of localized vascular swellings at arteriolar bifurcations, often with aneurysm formation. The vessel walls show fibrinoid necrosis and acute inflammation, with secondary thromboses leading to multiple infarctions.

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**Table 4.1.** *Comparison of microscopic features between hereditary spherocytosis, auto-immune haemolytic anaemia and idiopathic thrombocytopenic purpura*

| | Hereditary spherocytosis | Auto-immune haemolytic anaemia | Idiopathic thrombocytopenic purpura |
|---|---|---|---|
| Germinal centres | ± | 0 to +++ | 0 to +++ |
| Mantle zones | + to ++ | + to ++ | + to ++ |
| Marginal zones | +++ | ± to ++ | ± to ++ |
| Peri-arteriolar lymphoid sheaths | + | ± to ++ | ± to ++ |
| Peri-follicular areas | Not usually conspicuous | Congestion with red cells and neutrophils may be conspicuous in cases with marked WP stimulation | Congestion may be conspicuous in cases with highly reactive WP, as in AIHA |
| Red pulp sinusoid lumens | Usually appear empty, unless spleen is also passively congested | Contain abundant large, round/oval macrophages; some show erythrophagocytosis or containing haemosiderin | May contain a few large, round/oval macrophages |
| Red pulp sinusoidal endothelium | Usually prominent and cuboidal | Variable; often prominent and cuboidal | Variable; sometimes prominent and cuboidal |
| Red pulp cords | Dendritic processes of cordal macrophages form feathery patterns enclosing sequestered RBC | Dendritic processes of cordal macrophages have normal distribution. Occasional large, round macrophages present | Cordal macrophages show varying degrees of conversion to foam cells containing lipid ± ceroid |
| Red pulp non-filtering areas | Usually normal | Peri-capillary plasmacytosis common | Peri-capillary plasmacytosis common |

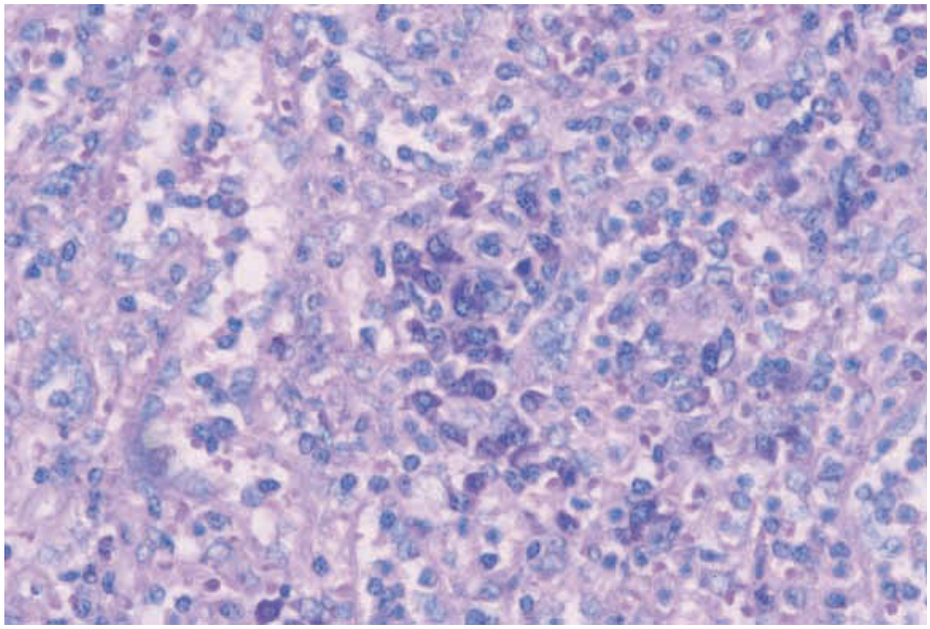

**Fig. 4.10.** Cluster of plasma cells within the splenic red pulp of a patient with Felty's syndrome. Giemsa stain.




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Chapter 5

# The spleen in immunodeficiency and systemic infections

## Inherited immunodeficiency syndromes

### General features

Splenectomy is rarely performed in patients with inherited immunodeficiency syndromes but, since deaths may occur in early life due to infection, spleens from such individuals may be seen at post-mortem examination. In those syndromes associated with less severe immunodeficiency, splenectomy may occasionally be performed incidentally during intra-abdominal surgery or the spleen may require removal following trauma, just as in any other individual. Therefore, it is useful to have an understanding of the pathological changes likely to be present due to underlying immunodeficiency. See Singer (1998) and Janeway et al. (1999) for overviews of clinico-pathological and immunological aspects of these conditions.

In general, red pulp cords and sinusoids are anatomically and functionally normal. In combined immune deficiency due to defective production of the T and B lymphoid cell lineages from early precursor cells, absence of the PALS and B cell follicles will be seen. Small B lymphocytes, plasma cells and CD8-positive T cells will also be absent from red pulp cords but this may only be detectable by immunohistochemistry. Immunodeficiency syndromes associated with this pattern of splenic lymphoid hypoplasia present as severe combined

immunodeficiency (SCID) syndromes and have heterogeneous underlying genetic defects, such as cytokine receptor $\gamma$-chain deficiency in X-linked SCID.

### Inherited immunodeficiency states predominantly affecting T cells

Selective depletion of T cells in the PALS and cords accompanies pure T cell deficiency such as that in DiGeorge's syndrome. This syndrome results from failure of thymic development and hence absence of the micro-environment required for T cell development. Susceptibility to infection in DiGeorge's syndrome is not limited to viral or other intracellular infections because T cell help for B cell responses is deficient in addition to absence of cytotoxic T cell activity. In DiGeorge's syndrome therefore, the splenic white pulp follicles will be small and unstimulated (primary follicles) with no germinal centre production. Curiously, despite the role of marginal zone B cells in responses to T cell-independent antigens, marginal zones are not spared in DiGeorge's syndrome (Fig. 5.1), supporting the concept that even these reactions do, in fact, require interaction with T cells to allow the responding B cells to proliferate (discussed in Chapter 2).

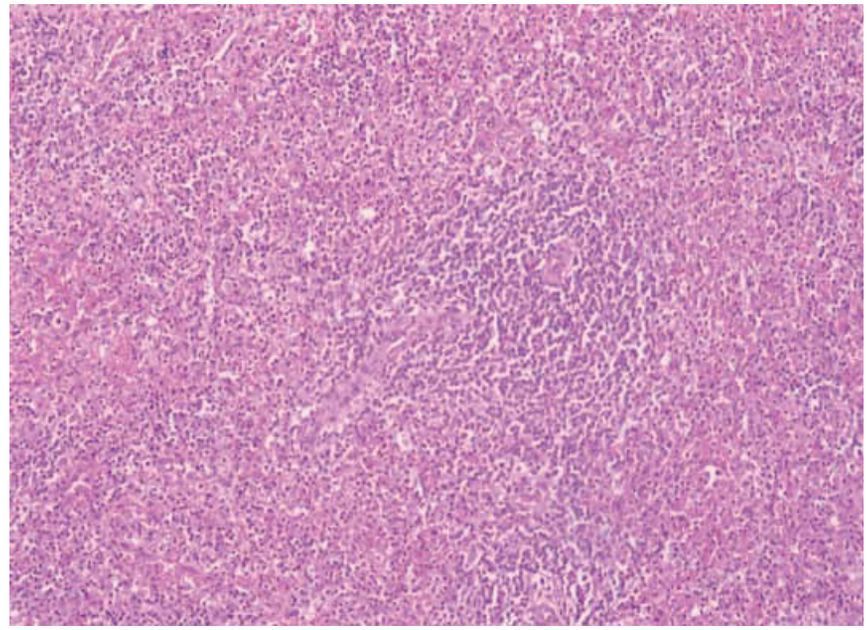


**Fig. 5.1.** Severe white pulp atrophy affecting all components in DiGeorge's syndrome. H&E stain.

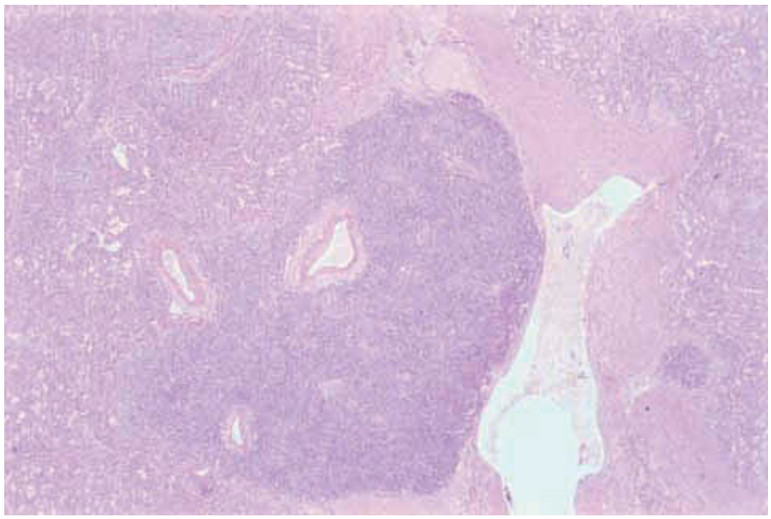
### Inherited immunodeficiency states predominantly affecting B cells

Lymphocyte developmental defects affecting predominantly B cell function include Bruton's X-linked agammaglobulinaemia and selective IgA deficiency. The latter is the most common inherited form of immunodeficiency (approximately 1 in 800 people are affected) and is associated with chronic lung disease, possibly reflecting increased susceptibility to a variety of pulmonary pathogens. The splenic white pulp has a normal composition in selective IgA deficiency, as one would predict for a disor-

der affecting predominantly mucosal immunity. However, the presence of sarcoid-like granulomas has been described, which may represent macrophages responding to antigen(s), normally eliminated by mucosal IgA responses, which have entered the general circulation to reach the spleen. Bruton's agammaglobulinaemia is caused by absence of a protein tyrosine kinase in early B cells which leads to failure of maturation at the pre-B cell stage. Splenic white pulp is severely atrophic, with B cell follicles virtually absent.

Failure of the immunoglobulin heavy chain gene class-switching mechanism occurs in the X-linked hyper-IgM syndrome, causing severe lack of all immunoglobulins other than IgM and IgD. The underlying defect is actually deficient expression of CD40–ligand by T cells and the B cells themselves are not intrinsically abnormal (Korthauer et al., 1993). The abnormal T cells are unable to bind to CD40 expressed on B cell surfaces. This interaction normally provides an essential co-stimulatory signal for B cell proliferation, class-switching and affinity maturation of immunoglobulins in response to T-dependent antigens. B cells in the hyper-IgM syndrome secrete low affinity IgM antibodies in response to bacterial pathogens; only their responses to T-independent antigens are normal. Florid T zone hyperplasia accompanies germinal centre atrophy and marginal zone expansion (Fig. 5.2).

**Fig. 5.2.** Florid T-zone expansion in a patient with hyper-IgM syndrome. H&E stain.



## Acquired immunodeficiency due to drugs or irradiation

Exposure to corticosteroid agents and other immunosuppressive drugs for treatment of auto-immune diseases causes varying degrees of impairment of immune functions. Similar effects will result from immunosuppressive therapy employed following

transplantation and from cytotoxic chemotherapy or radiotherapy used in the treatment of neoplasia. As in the case of inherited immunodeficiencies, the spleen is not often removed from patients exposed to such treatments and no specific pathological changes associated with particular treatment modalities have been described. Overall, in keeping with the degree of lymphocyte depletion throughout the body, T and B cell components of the splenic white pulp will appear unstimulated or even atrophic, with low spleen weight if atrophy is severe. If immunosuppression is severe, opportunistic infection may occur and should be sought, as in human immunodeficiency virus infection (see below). Splenic enlargement in immunocompromised patients should raise suspicion of opportunistic infection, particularly by mycobacteria, or involvement by the underlying disease in the case of leukaemia or lymphoma treated by chemotherapy.

## Acquired immunodeficiency syndrome and the spleen

### Changes occur in the spleen in early stages of HIV-associated disease

Mild or moderate splenomegaly is not uncommon in individuals infected with human immunodeficiency virus (HIV) (Klatt & Meyer 1987; Mathew et al., 1989; Niedt & Schinella 1985). In many cases, the cause is unclear and, when it occurs relatively early in the course of HIV-associated disease, the process may be analogous with the persistent lymph node enlargement which occurs in some patients as a prodrome to the development of acquired immunodeficiency syndrome (AIDS) (Ewing et al., 1985; Falk et al., 1988; Piris et al., 1987). The histological findings are variable and non-specific; there may be evidence of white pulp stimulation but features of

atrophy may already be present. A diffuse, polyclonal, red pulp plasmacytosis has been described in some reported series (Falk et al., 1988; Klatt & Meyer, 1987).

### An ITP-like syndrome can accompany HIV infection

It is important to be aware that a picture resembling idiopathic thrombocytopenic purpura (ITP), usually with highly reactive white pulp, may be seen in early HIV infection (Marti et al., 1993). Since, at this stage, HIV infection may not be suspected, it is important to be aware of this possibility when examining spleens from individuals, said to have ITP, who have any lifestyle risks for HIV infection. Such patients have usually undergone splenectomy for intractable thrombocytopenia attributed to steroid-resistant ITP. Because high dose corticosteroid treatment will often have been used pre-operatively in these patients, as in those with true ITP, atrophic features are easily dismissed as resulting from this treatment and red pulp changes may be minimal (see Chapter 4). Features of opportunistic infection should be sought carefully by macroscopic examination of the spleen (looking for focal necrotizing granulomatous lesions evident to the naked eye within the parenchyma) and study of multiple histological sections.

The occurrence of this ITP-like syndrome supports the hypothesis that auto-immune platelet destruction contributes to the thrombocytopenia found in some HIV-positive individuals (Dominguez et al., 1994). Splenic appearances may resemble true ITP quite closely. A useful point to note in attempting to distinguish ITP from HIV-associated thrombocytopenia is that, in HIV-associated thrombocytopenia, there is frequently an increased number of CD8-positive T cells in the red pulp. This probably occurs as part of a generalized CD8-positive T lymphocytosis which occurs

as numbers of CD4-positive T cells decline (Oksenhendler et al., 1992). Significantly increased red pulp CD8-positive T cells are not a feature of ITP.

## Splenic atrophy accompanies progression of HIV infection to AIDS

With progression of HIV infection to AIDS, the picture in the spleen is generally one of worsening white pulp atrophy, modified by infections and/or neoplasia. Few studies have specifically compared the spleen in early and late stages of HIV infection. In one post-mortem study of spleens from Côte d'Ivoire, a country with endemic HIV infection, more severe white pulp atrophy was observed, accompanied by an absolute increase in the number of CD8-positive T cells in the red pulp and PALS, in patients dying from advanced AIDS than in HIV-positive individuals without AIDS, who had died from incidental causes (Figs. 5.3 and 5.4). In this study, the increase in splenic CD8-positive cells correlated with reduction in circulating CD4-positive T cells (Chandler et al., 1994).

In patients with advanced AIDS, the spleen becomes severely atrophic as B cell depletion follows loss of CD4-positive T cells. In some patients, secondary expansion of CD8-positive T cell numbers is sufficiently extreme as to mimic neoplasia. Such severe reactions may be genetically determined, as association with several human leukocyte antigen (HLA) haplotypes has been shown (HLA-A1, HLA-B2, HLA-DR3 and HLA-DR5) (Itescu et al., 1990, Oksenhendler et al., 1992).

## Opportunistic infections often involve the spleen in AIDS

Secondary infections, including disseminated tuberculosis, *Mycobacterium avium-intracellulare* (MAI) infection, histoplasmosis, cryptococcosis and

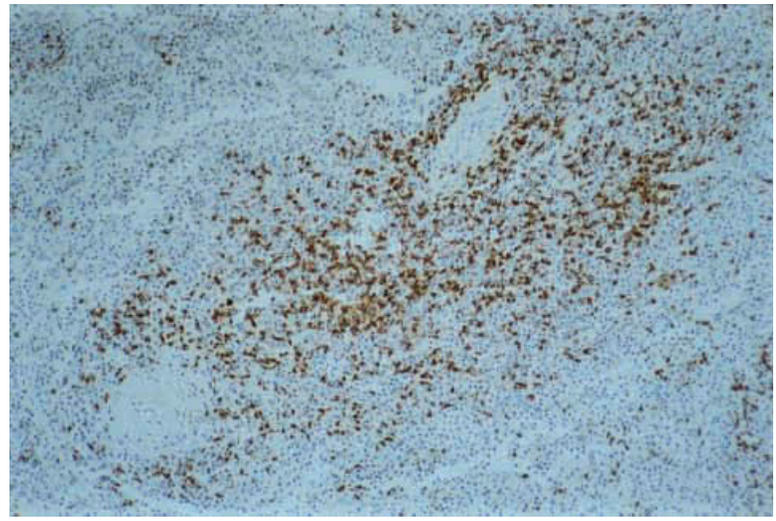
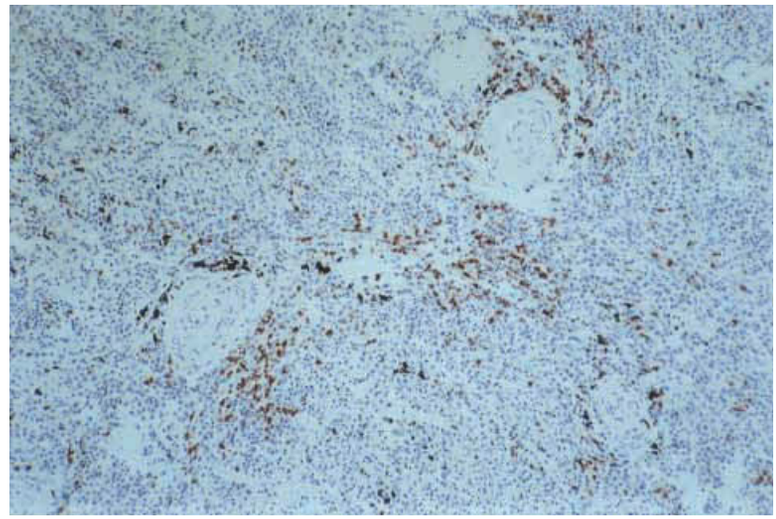


**Fig. 5.3.** White pulp area from an HIV-positive patient with early disease. Immunostained using an anti-CD45RO monoclonal antibody to demonstrate T cells in the PALS. The T cells are reduced in number and more dispersed than normal. B cell nodules were infrequent, small and atrophic-appearing in this patient. Streptavidin–biotin complex immunoperoxidase technique using monoclonal antibody OPD4, reactive with CD45RO.

**Fig. 5.4.** White pulp area from a patient with advanced AIDS, immunostained as for Fig. 5.3, showing severe reduction in PALS T cells. Increased red pulp T cells were present in this patient but cannot be appreciated because the expanded CD8 positive T cell population is CD45RO negative.

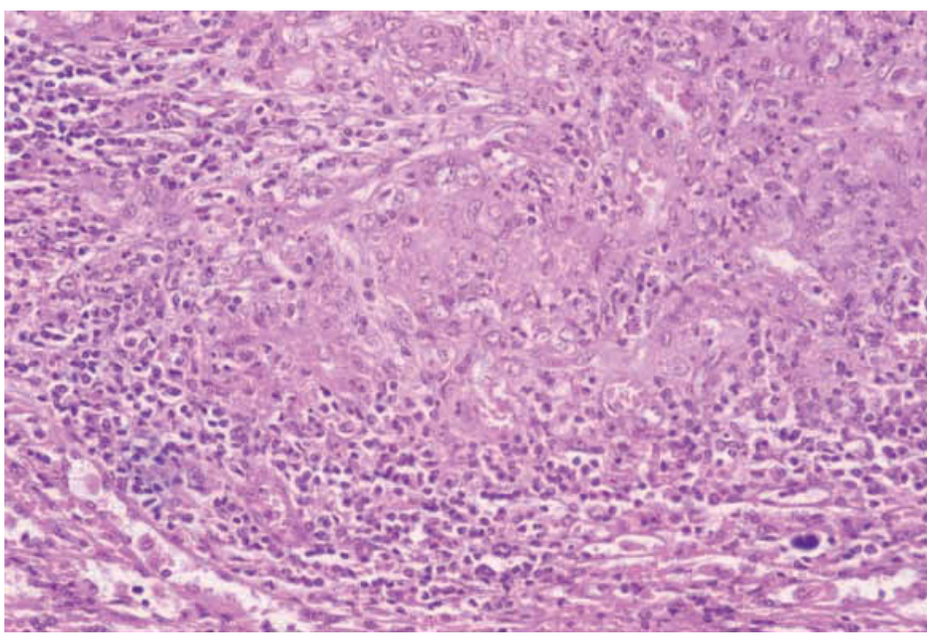


leishmaniasis, may cause splenomegaly in patients with more advanced disease and full-blown AIDS (Klatt & Meyer 1987; Pedro-Botet et al., 1991). Granulomas in tuberculosis are often less well formed than normal in immunocompromised individuals, reflecting deficient T cell function. In MAI infection, the usual picture is one of generalized red pulp infiltration by plump histiocytes, with or without discrete granuloma formation. Occasionally, however, MAI may give rise to multinodular or diffuse infiltrates of spindle-shaped cells mimicking myofibroblasts. This pattern has been termed 'mycobacterial spindle cell pseudotumour' (Kumar et al., 1994) and should not be mistaken for an inflammatory pseudotumour or true stromal neoplasm (see Chapter 8).

Infections with certain encapsulated bacteria (e.g. *Streptococcus pneumoniae*), for which asplenic individuals are known to have an increased risk, are also increased in AIDS patients, presumably as a manifestation of hyposplenism accompanying severe white pulp atrophy (Grotto & Costa, 1991).

*Bartonella henselae* (previously called *Rochalimaea henselae*), the agent responsible for cat-scratch disease in immunocompetent individuals, can cause disseminated bacillary angiomatosis in AIDS patients and some other immunocompromised persons (Warnke et al., 1995). Skin, lymph nodes and spleen are common sites of involvement. Within the spleen, coalescent, pale nodules are formed which may be more than 1 cm in diameter. These resemble tuberculous granulomas or deposits of lymphoma macroscopically. Caseation is absent. Microscopically, the nodules can be seen to lie within red pulp and consist of vascular proliferations lined by plump endothelium, with abundant eosinophilic material in the stroma between vascular channels (Fig. 5.5). Neutrophils may be present but macrophages are usually few in number and granuloma formation within

**Fig. 5.5.** Nodular lesion of bacillary angiomatosis from the spleen of a patient with AIDS. The nodule consists predominantly of proliferating vascular channels, inflammatory cells including many neutrophils (not fully appreciable at this magnification) and abundant extracellular eosinophilic material. H&E stain.



the lesions is uncommon. The eosinophilic stromal material contains aggregates of the *B. henselae* organisms, which are demonstrable by Giemsa stain and by silver staining techniques such as the Warthin–Starry or Dieterle methods.

### AIDS-associated tumours may involve the spleen

AIDS-associated lymphomas and Kaposi's sarcoma may also involve the spleen, although rarely presenting primarily at this site. The lymphomas are predominantly large cell or Burkitt-like tumours with features similar to those arising at other sites in association with HIV infection (Ferry & Harris, 1997). Likewise, Kaposi's sarcoma involving the spleen resembles equivalent tumours elsewhere; it develops in association with connective tissue trabeculae and their enclosed vessels. The spindle cell proliferation extends from these sites into the adjacent parenchyma but distinct tumour formation is rare. Involvement is often multifocal (Falk et al., 1988).

## Systemic infections involving the spleen in immunocompetent individuals

### Epstein–Barr virus

Splenectomy may be performed in EBV infection either because of spontaneous rupture or for diagnosis of mild/moderate splenomegaly of unknown cause. It is rare for the spleen to weigh more than 500 g as a result of EBV infection alone. Macroscopically, the capsule and parenchyma appear normal, unless features of trauma are present secondary to rupture. It is not known why EBV infection predisposes to spontaneous rupture or disruption associated with minimal trauma; the splenic capsule shows no obvious pathology to

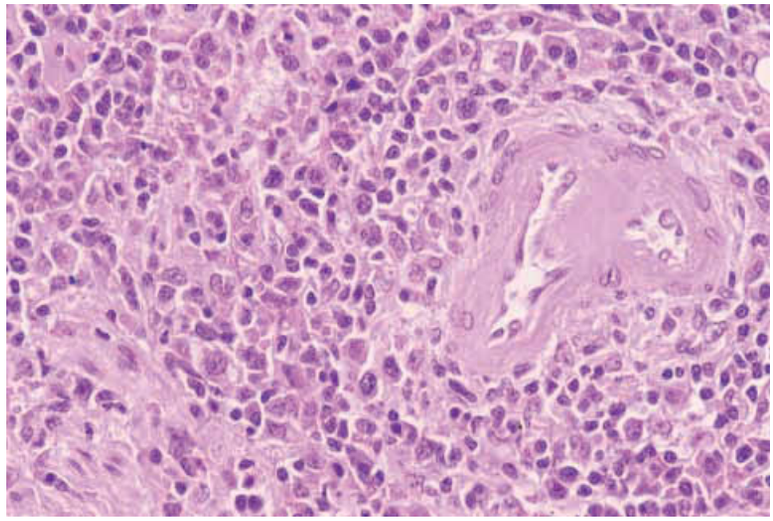
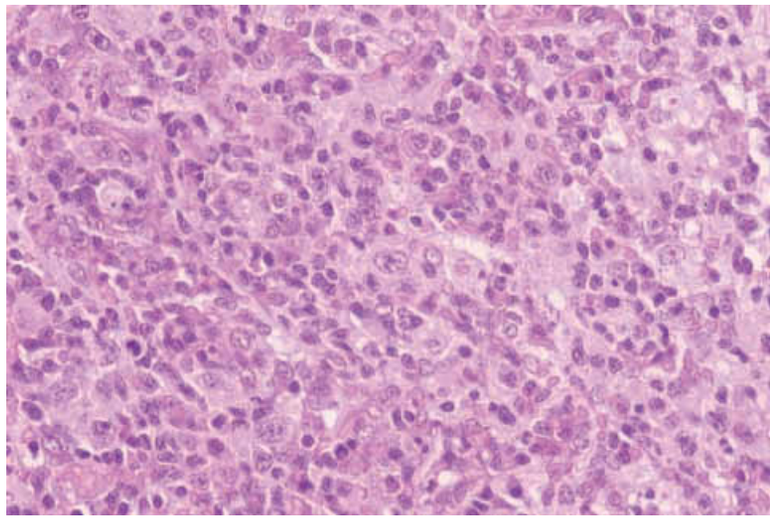


**Fig. 5.6.** PALS from a patient with acute infection by Epstein–Barr virus showing reduced content of small lymphocytes and replacement by a pleomorphic mixture of cells including blast cells. H&E stain.

**Fig. 5.7.** Red pulp from a patient with EBV infection showing increased cellularity within cords and sinusoids. The mixture of cells is heterogeneous, including plasma cells and blast cells. H&E stain.



suggest intrinsic weakness or inflammatory injury.

White pulp nodules in EBV infection appear unstimulated. The PALS contains reduced numbers of small lymphocytes and instead is expanded by a mixture of immunoblastic and plasmablastic cells (Fig. 5.6). Similar cells admixed with abundant mature plasma cells and intermediate forms expand the red pulp cords, spilling into sinusoids (Fig. 5.7). The blastic cells are a mixture of polyclonal T and B lymphoid blasts and the plasma cells are also polytypic.

## Tuberculosis

Splenic involvement is almost always a manifestation of miliary TB, infection occurring by bloodstream spread. It is mainly seen nowadays in immunocompromised individuals such as patients with AIDS, as mentioned above, or those receiving immunosuppressive treatment such as corticosteroids in high dosage. Splenomegaly is usually moderate but occasionally massive. Macroscopically, miliary nodularity is present throughout the parenchyma, superficially mimicking white pulp hyperplasia or low grade non-Hodgkin's lymphoma. Careful examination reveals that individual nodules are cream-coloured rather than grey/white as in white pulp expansion and they are less uniformly distributed than is the case with processes causing generalized white pulp expansion. The nodules also show more variation in size than those of reactive white pulp hyperplasia. Caseation may be evident, particularly in large nodules.

Microscopically, nodules have the typical appearances of granulomas caused by *M. tuberculosis* and are found preferentially within red pulp.

## Malaria

Palpable splenomegaly is found in 30% or more of otherwise healthy individuals in

tropical Africa, compared with less than 5% in Western countries. The major cause is malaria. Splenomegaly in childhood in endemic malarial areas is a manifestation of semi-immunity. It diminishes as resistance to malaria is acquired but stabilizes at approximately twice the average normal Western spleen weight (Chellappa, 1994).

In acute *Plasmodium falciparum* infection the spleen is intensely congested with parasitized RBC. White pulp nodules are prominent and reactive, accompanied by fibrinoid necrosis in some cases (Fig. 5.8). In chronic infection and benign malaria variants (*P. vivax, P. malariae* and *P. ovale*), red pulp expansion with abundant haemozoin in cordal macrophages is the major feature and white pulp reactive changes are less marked. The amount of malarial pigment increases progressively with prolonged or repeated episodes of infection and clears only slowly if individuals are removed from exposure to re-infection, or as they acquire immunity. As discussed in Chapter 4, sequestration of infected red cells leads to splenic changes similar to those seen in thalassaemia or spherocytosis. In addition, parasitized cells tend to agglutinate and foci of infarction occur due to blockage of small blood vessels within the spleen; spontaneous rupture may result. With time, fibrosis of necrotic areas occurs and may become confluent, the spleen eventually becoming severely shrunken. Malarial haemozoin pigment is usually reduced in such fibrotic 'end-stage' spleens but haemosiderin may be abundant due to preceding haemorrhagic infarction.

A small proportion of malaria sufferers in endemic areas may get 'tropical splenomegaly syndrome' in which progressive splenic enlargement occurs, usually in association with a lesser degree of hepatomegaly (Chellappa, 1994). This syndrome is probably multifactorial in origin. It does not appear to correlate with development of portal hypertension and an abnormal

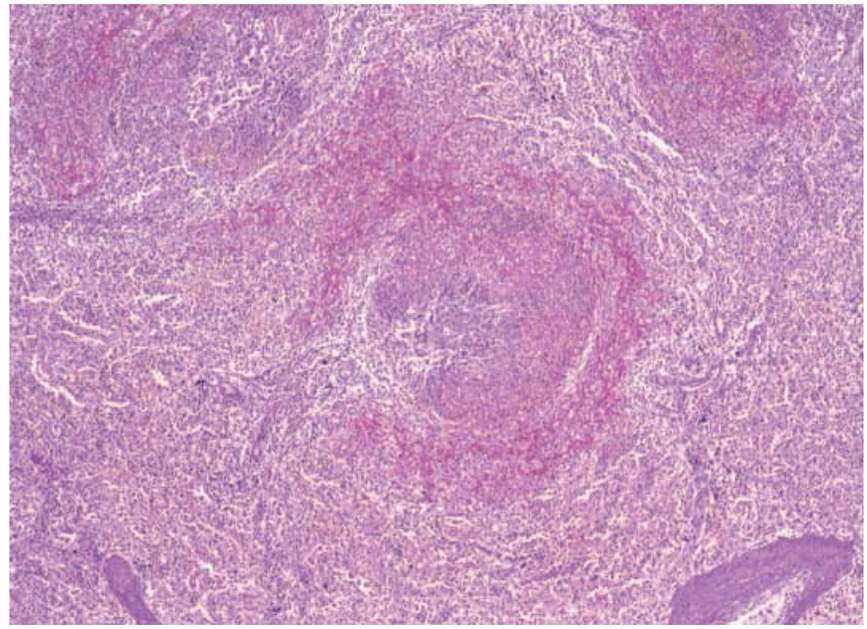


**Fig. 5.8.** Spleen from a young African patient with fulminant malaria due to *Plasmodium falciparum.* The germinal centre is completely necrotic and abundant deposition of fibrinoid material is evident, particularly in marginal and perifollicular zones. Martius Scarlet Blue stain.

immune response to malarial parasites has been postulated as one cause. In addition to massive splenomegaly, patients usually have anaemia, marked polyclonal elevation of serum IgM and very high titres of anti-malarial antibodies. Splenomegaly in tropical areas may also occur as a result of visceral leishmaniasis, trypanosomiasis, schistosomiasis, haemoglobinopathies and thalassaemias, as well as disorders more commonly seen in Western populations.

### Leishmaniasis

Visceral leishmaniasis, caused by infection with *Leishmania donovani*, typically causes hepato-splenomegaly with fever, malaise, weight loss, lymphadenopathy and cytopenias. The degree of splenomegaly may be massive. Cytoplasm of splenic cordal macrophages becomes filled with parasites, best seen in Giemsa stained sections.The enlarged, plump macrophages should not be misinterpreted as storage cells (see Chapter 8).

At high magnification, the individual Leishmania organisms can be recognized by the 'double dot' appearance of their nucleus and adjacent kinetoplast. Although visceral leishmaniasis may occur in immunocompetent individuals, it also occurs as an opportunistic infection in immunodeficiency states, including AIDS.

### Septicaemia due to other infectious agents

By virtue of the spleen's position in the systemic circulation, and its filtering function, almost any blood-borne infection may lead to splenomegaly. Those which are minor and/or short-lived do not usually give cause for splenectomy or biopsy although they may occasionally be encountered by pathologists during post-mortem examinations. Due to rapid post-mortem autolysis, enlargement, congestion and severe

softening are the usual features; the so-called 'diffluent spleen'. Distinguishing features relating to specific causative organisms are lacking. Fibrinoid necrosis of germinal centres has been described in fulminant septicaemia and other causes of systemic shock in children (Howat & Variend, 1986). The mechanism underlying this phenomenon is unknown. Similar changes also occur in fulminant falciparum malaria, as described above.

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Chapter 6

# Lymphomas involving the spleen

## Introduction

The spleen may be involved in all subtypes of lymphoma. In some cases, e.g. follicle centre cell lymphoma, it may rarely be the dominant or presenting feature. In others, e.g. mycosis fungoides, it is usually a late manifestation, most commonly discovered at post-mortem examination. In some lymphomas, splenic involvement with splenomegaly is a characteristic feature, e.g. hairy cell leukaemia, splenic marginal zone lymphoma and hepatosplenic $\gamma\delta$-T cell lymphoma. Primary lymphomas of the spleen, with the exception of these three entities, are very rare and do not fall into a single subtype. Most such cases are likely to be early dominant manifestations of more generalized disease.

Distinction between subtypes of splenic lymphoma, particularly the small B cell lymphomas, may be difficult. This difficulty is often compounded by poor fixation (see Chapter 1). Reference to the clinical and haematological features of the case is usually of great help. However, this information is not always readily available to the pathologist. Tables 6.1 and 6.2 show a scheme that will allow a pathologist to identify approximately the subtype of lymphoma using the macroscopic appearances and main micro-anatomical features. Precise diagnosis will depend on these micro-anatomical appearances together with cell morphology, immunophenotype and, in some instances, molecular genetic characteristics.

**Table 6.1.** *Macroscopic appearances of lymphomas involving the spleen*

| |
|---|
| *Prominent white pulp nodules* |
| Follicle centre cell lymphoma |
| Mantle cell lymphoma |
| B-CLL |
| Lymphoplasmacytoid lymphoma |
| Marginal zone lymphoma |
| *Homogeneous red pulp expansion with absent or inconspicuous white pulp* |
| Hairy cell leukaemia (often with blood lakes) |
| Most T cell lymphomas |
| *Circumscribed or confluent tumour masses and areas of necrosis and/or fibrosis* |
| Large B cell lymphoma (very rarely, high grade T cell lymphoma) |
| Burkitt's lymphoma |
| Hodgkin's disease |

It is always worth searching for lymph nodes at the hilum of the spleen and taking representative blocks. Subtypes of malignant lymphoma are usually easier to identify in involved lymph nodes than in the spleen itself. For example, follicle centre cell lymphomas and mantle cell lymphomas may show marginal zone differentiation in the spleen and thus be confused with splenic marginal zone lymphoma. Marginal zone differentiation in these lymphomas is less likely to be present in the hilar lymph nodes (Piris et al., 1998).

## B cell small lymphocytic lymphoma/chronic lymphocytic leukaemia (B-CLL)

B-CLL is a systemic disease with blood and bone marrow involvement in the majority of cases. Rarely, splenectomy may be performed for diagnostic purposes. More commonly in this disease, it is performed to relieve symptoms of enlargement or as part of the management of haemolytic anaemia.

B-CLL primarily involves the white pulp of the spleen (Fig. 6.1). However, lymphoma cells spill out into the red pulp, leading to fusion of white pulp infiltrates with areas of diffuse splenic tumour (Figs. 6.2 and 6.3). Tumour cells have the morphology of small lymphocytes with round, heterochromatic nuclei. These are interspersed with cells having more open, delicate chromatin and visible nucleoli that have been variously designated as prolymphocytes or para-immunoblasts (Fig. 6.4). We prefer the term para-immunoblasts, to avoid confusion with prolymphocytic leukaemia. The latter is a different disease, clinically and immunophenotypically, from B-CLL. Para-immunoblasts may form aggregates, the so-called 'proliferation centres', but these may be less obvious in the spleen than in lymph nodes. Recognition of para-immunoblasts is important in the

**Table 6.2.** *Microscopic features of lymphomas involving the spleen*

| | |
|---|---|
| *Prominent/expanded white pulp nodules* | |
| Follicle centre cell lymphoma | |
| Mantle cell lymphoma | |
| B-CLL | |
| Lymphoplasmacytoid lymphoma | |
| Splenic marginal zone lymphoma | |
| *Residual non-neoplastic germinal centres (bcl-2 negative) at centres of nodules* | |
| Splenic marginal zone lymphoma (usually) | |
| Mantle cell lymphoma (sometimes) | |
| *Expansion of marginal zones* | |
| Splenic marginal zone lymphoma | |
| Mantle cell lymphoma | all may occasionally show marginal zone differentiation |
| FCCL | |
| B-CLL | |
| *Spill-over of tumour cells into red pulp* | |
| B-CLL (often pronounced) | |
| Lymphoplasmacytoid lymphoma (often pronounced) | |
| Mantle cell lymphoma (variable) | |
| Splenic marginal zone lymphoma (variable – may be inconspicuous) | |
| FCCL (variable – often inconspicuous, rarely diffuse) | |
| *Red pulp expansion with absent or inconspicuous white pulp nodules* | |
| Hairy cell leukaemia (often with blood lakes) | |
| Most T cell lymphomas (PALS may be infiltrated preferentially in some cases) | |
| *Tumour masses with intervening areas of normal/reactive splenic parenchyma* | |
| Large B cell lymphomas (very rarely, high grade T cell lymphomas) | |
| Hodgkin's disease | |

**Fig. 6.1.** B-lymphocytic lymphoma (B-CLL) of spleen. The tumour primarily involves white pulp, giving a nodular appearance to the cut surface. In some areas, white pulp nodules coalesce to form larger aggregates.

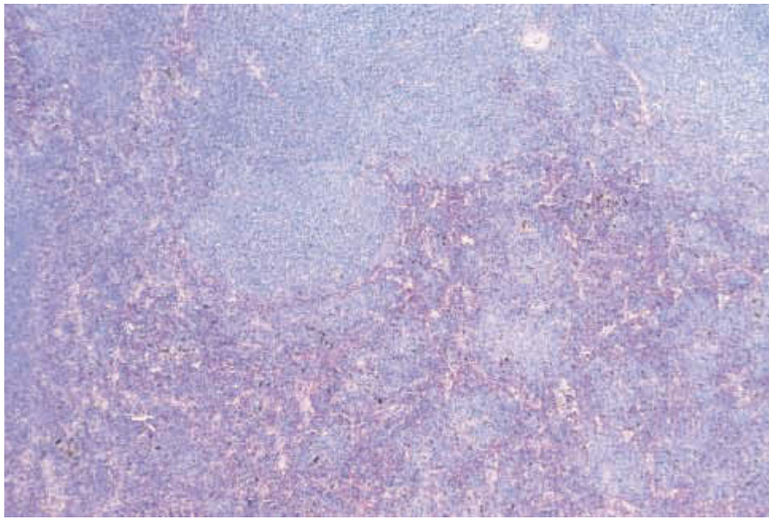


**Fig. 6.2.** B-CLL of spleen. Low power view showing enlarged white pulp nodules plus considerable spillage of lymphoma cells into the red pulp. H&E stain.

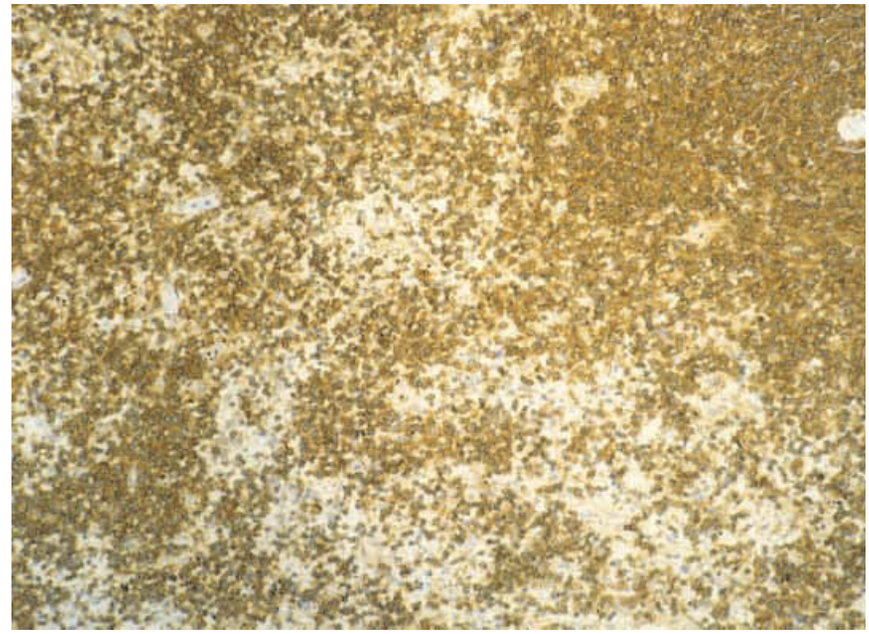


**Fig. 6.3.** B-CLL of spleen immunostained for CD20. Spillage of lymphoma cells into the red pulp is highlighted by this stain. Streptavidin–biotin complex immunoperoxidase technique.

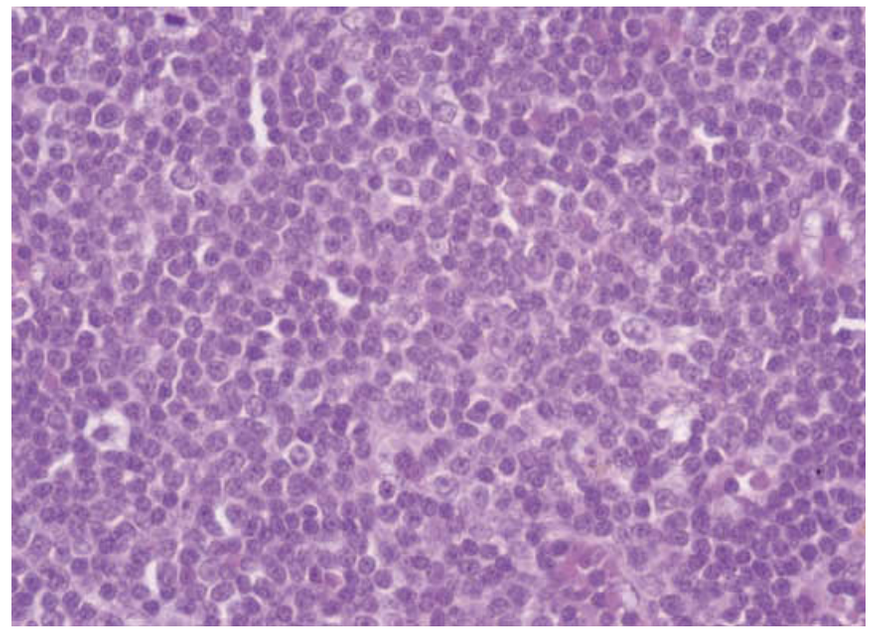


**Fig. 6.4.** B-CLL of spleen. High power view showing that the tumour is composed predominantly of small lymphocytes. Interspersed among these are larger cells with one or more nucleoli visible; these scattered para-immunoblasts are characteristic of B-CLL.

morphological distinction between B-CLL and some cases of mantle cell lymphoma.

### Immunophenotype

B-CLL cells show weak expression of surface IgM (SIgM) and occasionally express SIgD. The B cell-associated antigens CD19, CD20 and CD79a are expressed, although CD79b and CD22 are usually weak or undetectable. Not all of these antigens can be demonstrated in fixed tissue, but their expression may be sought using peripheral blood or bone marrow. The tumour cells are CD10-negative but express CD5, CD23 and CD43. Expression of CD23 is useful in the differentiation of B-CLL from mantle cell lymphoma.

### Genetic features

The genetic analysis of B-CLL indicates that this is a heterogeneous disease (Hamblin & Oscier, 1997). Trisomy 12 is found in 10–15% of cases by routine cytogenetic analysis and in 20% of patients using interphase fluorescence *in situ* hybridization (FISH). Only a proportion of cells belonging to the malignant clone (identified by immunoglobulin phenotype) exhibits trisomy 12, indicating that acquisition of this abnormality is a secondary genetic event.

Deletions of chromosome 13q14 are found in 20% of B-CLL by cytogenetic analysis and up to 60% using interphase FISH. Deletions of chromosome 11q23, associated with bulky, progressive disease and a poor prognosis are found in less than 5% of patients cytogenetically but in 20% by interphase FISH. Mutations of the p53 gene occur in 10–15%.

Immunoglobulin heavy and light chain genes are rearranged in B-CLL. More than half of patients with B-CLL show somatic mutations of the Ig genes, indicating that these tumours have been exposed to the mutational influences of the germinal

centre. Such cases are more likely to be BINET stage A, with stable disease, typical lymphocyte morphology and to have 13q14 deletions. The absence of somatic mutations is associated with progressive disease, atypical morphology and trisomy 12.

## B-prolymphocytic leukaemia

B-prolymphocytic leukaemia is an uncommon disease. Patients present with high peripheral blood white cell counts and splenomegaly. Peripheral lymphadenopathy is rare and therefore it is uncommon for histopathologists to encounter this disease unless splenectomy is performed. The diagnosis is usually made by haematologists on the basis of the blood and bone marrow findings.

Growth of the neoplasm in the spleen is usually nodular but may be diffuse. The tumour cells have round nuclei with open chromatin and often exhibit a prominent central nucleolus (Fig. 6.5). They have weakly basophilic cytoplasm which is more abundant than that seen in B-CLL.

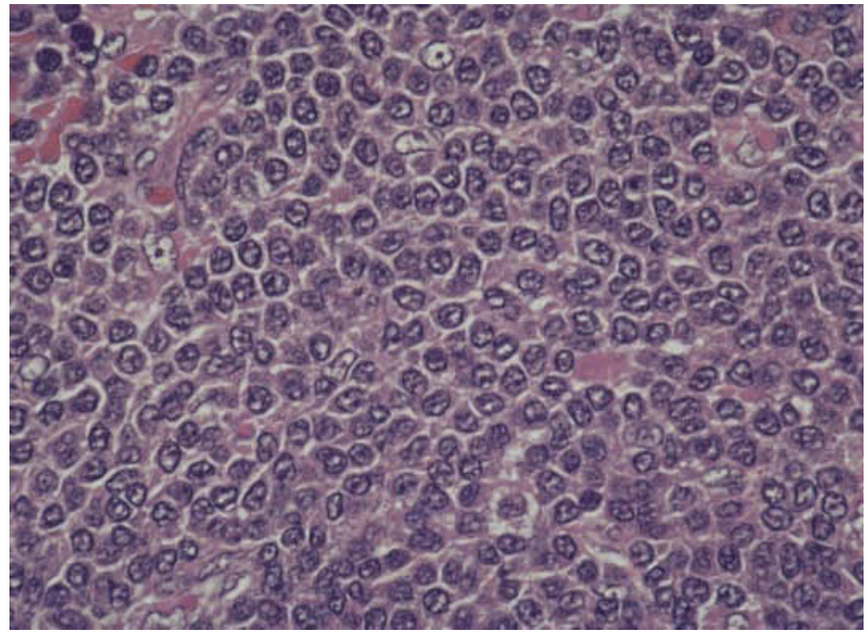


**Fig. 6.5.** B-prolymphocytic leukaemia. The tumour cells have more open chromatin than B-CLL and many show a central nucleolus. H&E stain.

### Immunophenotype

The neoplastic cells express strong SIg and are often CD5-negative.

## Mantle cell lymphoma (MCL)

Mantle cell lymphoma has been defined as a distinct entity in recent years (Banks et al., 1992). Patients may present with lymphadenopathy or with tumour at extranodal sites. Tumours of Waldeyer's ring or of the gastrointestinal tract occur, with multiple lymphomatous polyps in the intestine (lymphomatous polyposis) being a characteristic but uncommon manifestation. Patients may present with splenomegaly (Fig. 6.6), usually accompanied by bone

**Fig. 6.6.** Enlarged spleen from a patient with mantle cell lymphoma. Note the ill-defined white pulp nodules.

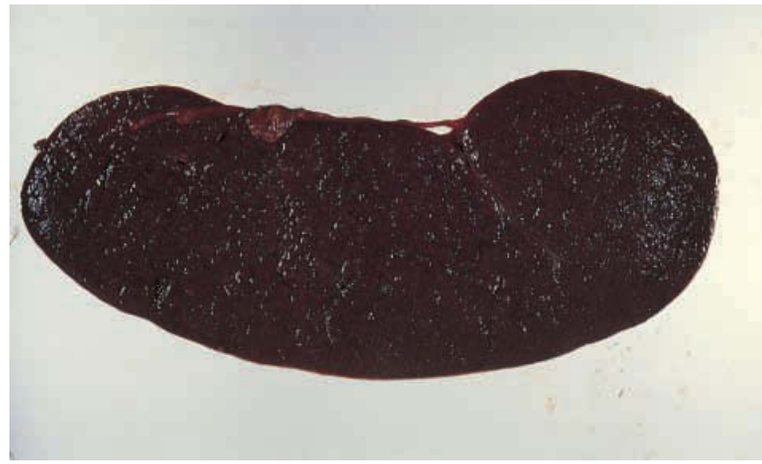
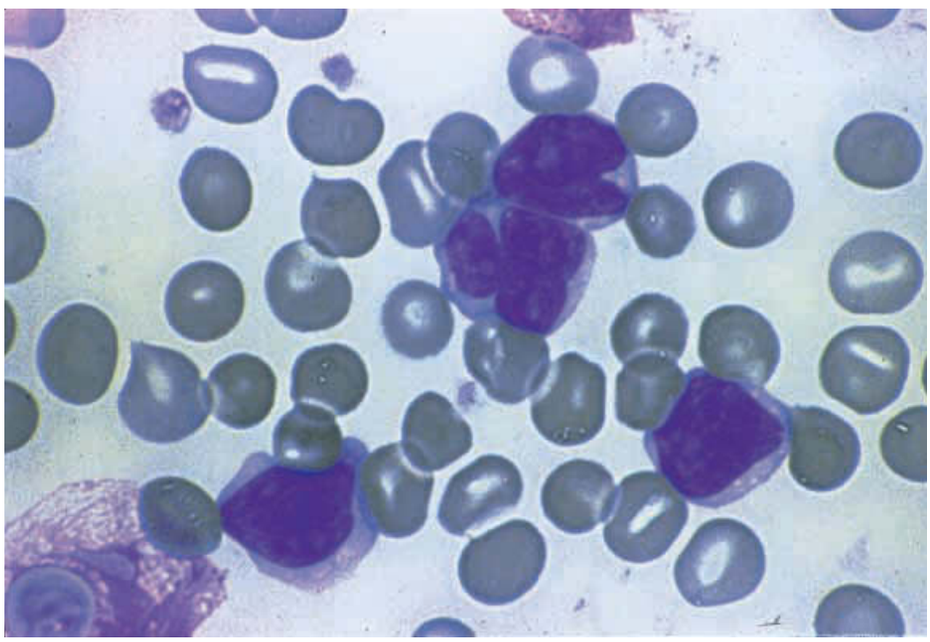


**Fig. 6.7.** Blood film from a patient with mantle cell lymphoma. Characteristic cleaved lymphocytes are seen in the peripheral blood.



marrow infiltration and often by overspill of neoplastic cells into the peripheral blood (Fig. 6.7). Splenectomy may be performed for diagnostic purposes or to relieve symptoms of splenomegaly.

Involvement of the spleen by MCL initially affects the white pulp (Fig. 6.8), where the tumour may surround reactive germinal centres giving a 'mantle zone' pattern. Eventually, the white pulp is overrun by tumour cells, which also extend out into the red pulp giving rise to diffuse infiltration. The neoplastic cells are larger than small lymphocytes and often have angulated nuclei (Fig. 6.9). The cytoplasm is usually inconspicuous and, in Giemsa-stained sections, has a greyish blue colour. Tumour cells usually show little mitotic activity. However, blastic transformation may occur and such cases have a high mitotic index. The cells of this blastic variant have open nuclei with delicate chromatin and may be difficult to distinguish from lymphoblastic lymphoma by morphology alone. Less commonly, transformation to pleomorphic (sarcomatoid) tumours may occur.

The differentiation of MCL from B-CLL by morphology alone can be difficult. The finding of para-immunoblasts identifies B-CLL. Mantle cell lymphomas often contain large histiocytes with eosinophilic cytoplasm, but this feature is characteristic rather than diagnostic.

A proportion of MCL involving the white pulp shows marginal zone differentiation (Piris et al., 1998) (Fig. 6.10). In such cases, cells in the marginal zones appear larger and have more abundant, pale cytoplasm than those in the centres of the nodules. The micro-environment in the marginal zone is thought to induce these morphological changes. Such cases must be distinguished from marginal zone lymphoma, which is usually a more indolent disease. In MCL, cells at the centres of lymphoid nodules will have the morphology and

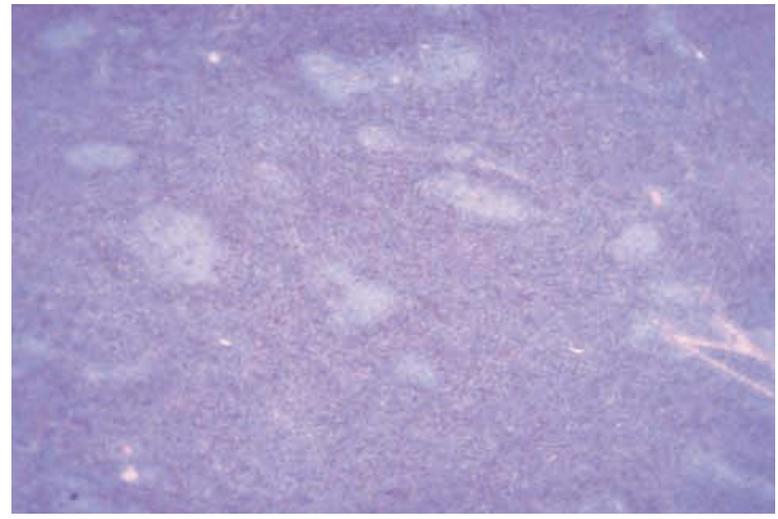


**Fig. 6.8.** Mantle cell lymphoma of spleen. Low power view showing expansion of white pulp nodules and spillage of tumour cells into red pulp. H&E stain.

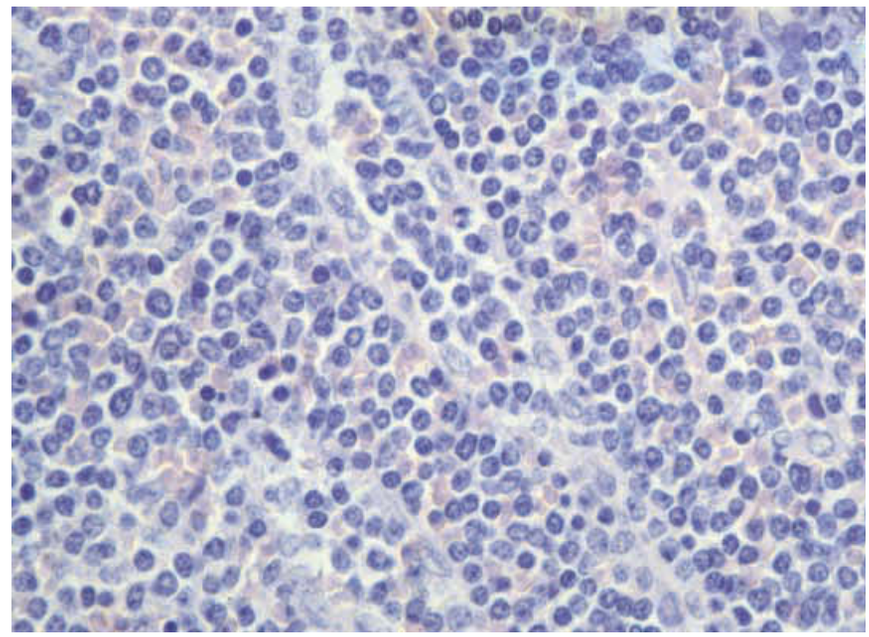


**Fig. 6.9.** Mantle cell lymphoma of spleen. The tumour cells have nuclei which appear more angulated than lymphocytic lymphoma and para-immunoblasts are not seen. H&E stain.

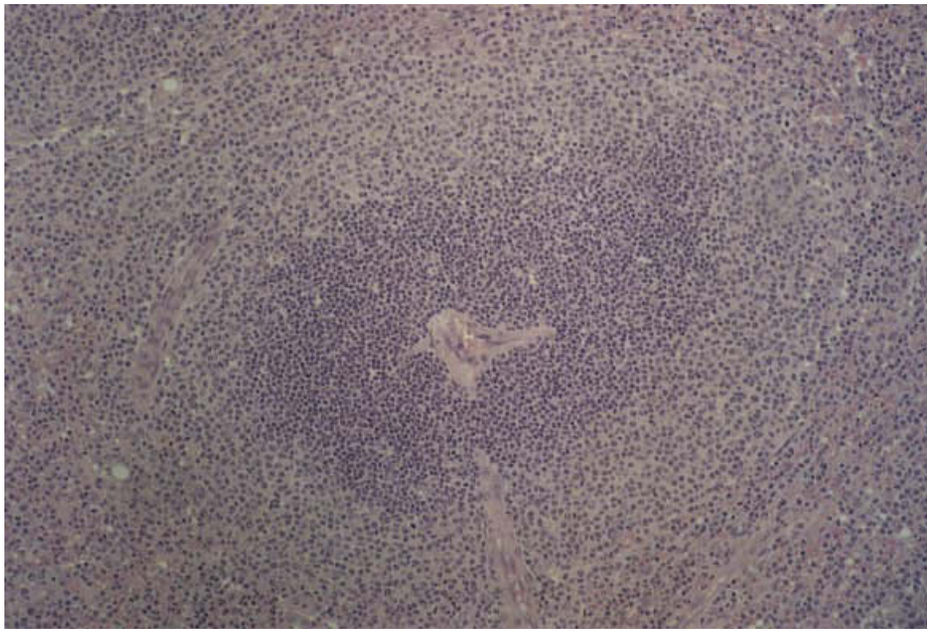


**Fig. 6.10.** Mantle cell lymphoma of spleen showing marginal zone differentiation. Note the central nodule of darkly staining mantle-cells surrounded by a halo of paler cells. These paler cells have the nuclear characteristics and immunophenotype of mantle-cells, but have more abundant cytoplasm reminiscent of marginal zone cells. H&E stain.

immunophenotype of MCL whereas, in marginal zone lymphoma, they will be occupied either by reactive germinal centres or by small lymphocytes.

### Immunophenotype

The tumour cells of MCL express SIg, usually IgM and IgD. Lambda light chain is expressed more frequently than kappa. The B cell-associated antigens CD19, CD20, CD22 and CD79a are expressed. In common with B-CLL, the tumour cells are positive for CD5 and CD43 but, by contrast with B-CLL, they do not express CD23. Expression of CD10 is variable.

### Genetic features

The translocation t(11;14)(q13;q32) characterizes MCL. It brings the cyclin D1 gene on chromosome 11 under the influence of the immunoglobulin promoter on chromosome 14 and leads to over-expression of cyclin D1 (Bosch et al., 1994). Cyclin D1, together with cyclin-dependent kinase 4 (CDK4), phosphorylates retinoblastoma protein (pRB), allowing transition from the G1 to S phase of the cell cycle. Deletions of the gene encoding p16/CDKN2 which, in normal cells, suppresses the effects of CDK4/cyclin D1 on pRB are associated with disease progression in MCL and are positively correlated with the proliferative activity of the tumour (Dreyling et al., 1997). Immunohistochemical detection of cyclin D1 in the nuclei of tumour cells provides a reliable and almost specific marker for the identification of MCL (Fig. 6.11).

## Follicle centre cell lymphoma

Follicle centre cell lymphomas (FCCL) usually present with lymphadenopathy. Most patients have stage III or IV disease at presentation. Occasional patients have

splenomegaly and undergo splenectomy for diagnostic purposes or to relieve symptoms. Follicle centre cell lymphomas involve the white pulp, with much less permeation of the red pulp than is seen in B-CLL or MCL, although discrete red pulp satellite nodules may develop. The prominent white pulp nodules can usually be seen on macroscopic examination of the spleen (Fig. 6.12).

In the spleen, FCCL have the same cytomorphology as at other sites. The neoplastic follicles are composed predominantly of centrocytes with a variable proportion of centroblasts (Figs. 6.13 and 6.14). These are accompanied by a network of follicular dendritic cells, best seen by immunohistochemistry, and small T lymphocytes. The neoplastic follicles are each surrounded by a mantle zone of small lymphocytes, of variable prominence. Like MCL, FCCL may show marginal zone differentiation (Alkan et al., 1996). In such cases, the mantle is usually inconspicuous but may be highlighted by immunostaining for IgD.

The main differential diagnoses of FCCL in the spleen are MCL and splenic marginal zone lymphoma (SMZL), particularly in those cases that show marginal zone differentiation. The presence of centroblasts in FCCL clearly differentiates this tumour from MCL and the latter can be identified positively by nuclear staining for cyclin D1. Follicle centres, when present in SMZL, are reactive or atrophic rather than neoplastic. These can usually be distinguished on morphology but, as at other sites, staining for bcl-2 and Ki67 antigen can help. Bcl-2 staining is negative in reactive follicle centre cells and positive in most neoplastic follicles. The proliferative index demonstrated by Ki67 immunostaining is usually much higher in reactive than neoplastic follicles.

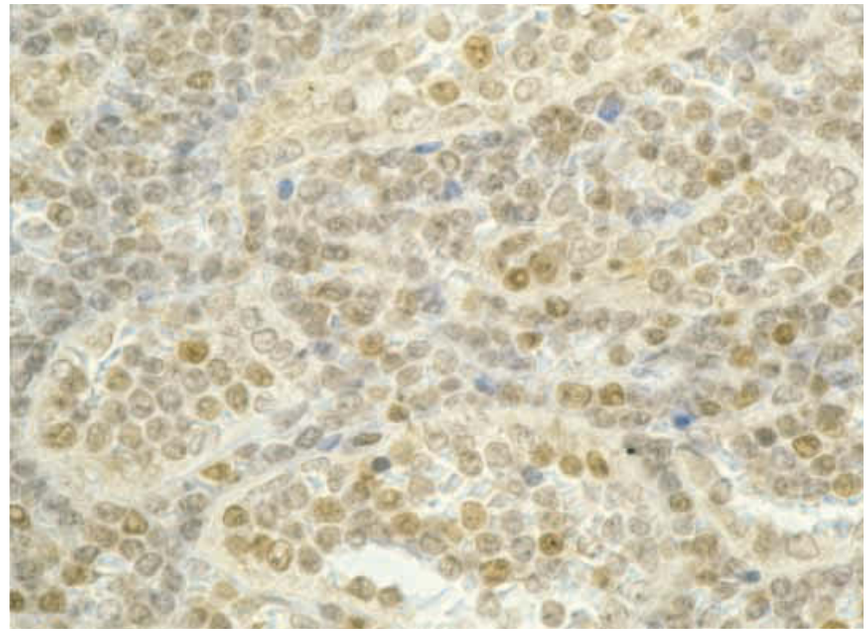


**Fig. 6.11.** Mantle cell lymphoma of spleen involving red pulp. This section has been immunostained to demonstrate cyclin D1. This antigen is expressed within nuclei and has high specificity for mantle cell lymphoma. Streptavidin–biotin complex immunoperoxidase technique.

### Immunophenotype

The neoplastic cells of FCCL are usually SIg-positive and frequently express cytoplasmic

Ig (CIg). They express the B cell-associated antigens CD19, CD20, CD22 and CD79a. CD10 is often expressed but the tumour cells are negative for CD5 and CD23. The majority of FCCL express bcl-2 protein, a feature that distinguishes them from reactive follicular proliferations but not from other low grade B cell lymphomas. Immunohistochemical staining for CD21 or other dendritic cell markers often identifies a tight meshwork of follicular dendritic cells in the neoplastic follicles. In most tumours there is also a substantial population of T cells expressing CD3 and CD4 within neoplastic follicles.

### Genetic features

Most, but not all, FCCL are characterized by t(14;18)(q32;q31). This translocation brings the bcl-2 gene on chromosome 18 under the influence of the Ig heavy chain gene promoter on chromosome 14 and leads to constitutive expression of bcl-2 protein. This, in turn, protects FCCL cells from apoptosis. Other genetic alterations, including mutations of the p53 gene, are associated with transformation of FCCL to higher grade lymphomas.

The Ig genes of FCCL show a high level of somatic mutation, with ongoing mutations in the complementarity-determining regions suggestive of antigen drive. Approximately 5% of FCCL have crippling mutations of the Ig genes and therefore do not express Ig.

## Splenic marginal zone lymphoma

This lymphoma is recognized as a provisional entity in the REAL classification (Harris et al., 1994). In a proportion of cases, characteristic villous lymphocytes are present in the peripheral blood that have led to confusion, historically, with hairy cell leukaemia (Fig. 6.15). The

**Fig. 6.12.** Follicle centre cell lymphoma. High power view of cut surface of spleen showing prominent, discrete white pulp nodules.

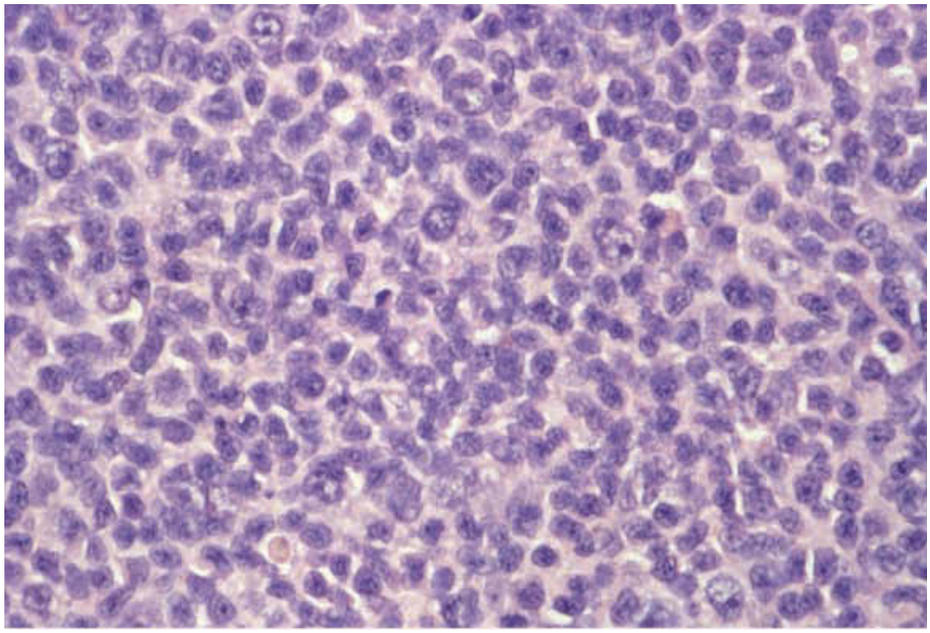


**Fig. 6.13.** Follicle centre cell lymphoma of spleen. This is a Grade I tumour showing predominantly centrocytes and occasional centroblasts. H&E stain.

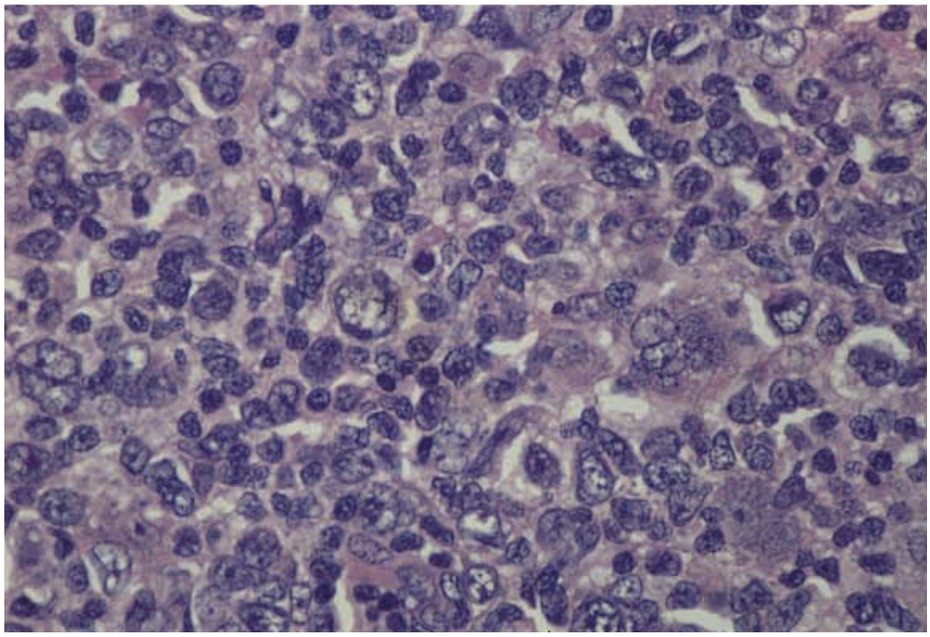


**Fig. 6.14.** Follicle centre cell lymphoma of spleen. This is an example of a Grade III tumour with abundant centroblasts, some of which have multi-lobated nuclei. H&E stain.

clinico-pathological entity of splenic lymphoma with villous lymphocytes (SLVL) is now believed to represent a predominant variant of SMZL with leukaemic overspill (Isaacson et al., 1994).

Although SMZL shares cytological and immunophenotypic characteristics with extranodal marginal zone lymphomas arising from mucosa-associated lymphoid tissue (MALT), these are distinct clinico-pathological entities. Splenic marginal zone lymphoma usually, but not always, presents with significant splenomegaly (Pawade et al., 1995; Rosso et al., 1995; Schmid et al., 1992) and has bone marrow involvement at diagnosis. However, it follows an indolent course and may show prolonged remission following splenectomy, even in patients presenting with the features of SLVL.

Histologically, SMZL occupies the white pulp of the spleen with small satellite nodules in the red pulp (Fig. 6.16). The centres of the white pulp nodules may be occupied by reactive or atrophic germinal centres or by non-neoplastic small lymphocytes. Tumour cells are distributed in the marginal zone, outside the mantle. Their nuclei are larger and have more open chromatin than those of mantle zone cells (Fig. 6.17). They also have relatively more abundant, pale cytoplasm than mantle zone cells. Scattered throughout the tumour, individual medium-sized blast cells are present with two to four nucleoli.

Cells similar to those in the white pulp tumour masses frequently form small aggregates in the red pulp. These satellite nodules often have a cluster of epithelioid histiocytes at their centres (Pawade et al., 1995) (Fig. 6.18). When the tumour has haematological features of SLVL, red pulp involvement may be more extensive and diffuse. If SMZL involves splenic hilar lymph nodes, it typically shows the nodular appearance described in peripheral lymph node involvement by this neoplasm (Mollejo et al., 1997).

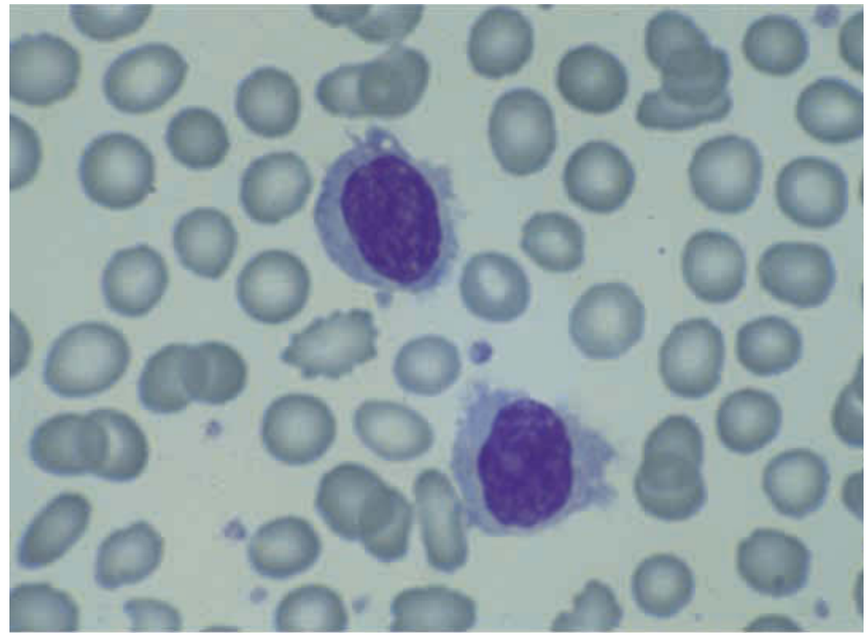


**Fig. 6.15.** Blood film from a patient with splenic marginal zone lymphoma showing villous lymphocytes. These cells have abundant, pale cytoplasm showing villous projections at one pole of the cell. A nucleolus is visible in one of the cells.

### Immunophenotype

The neoplastic cells of SMZL express SIg and, in approximately half of the cases, CIg. The B cell-associated antigens CD19, CD20, CD22 and CD79a are expressed, but the tumours are usually negative for CD5, CD10 and CD23.

### Genetic features

No characteristic cytogenetic abnormalities have yet been detected in SMZL. The Ig genes are re-arranged and hypermutated, as in post-germinal centre cells (Zhu et al., 1995).

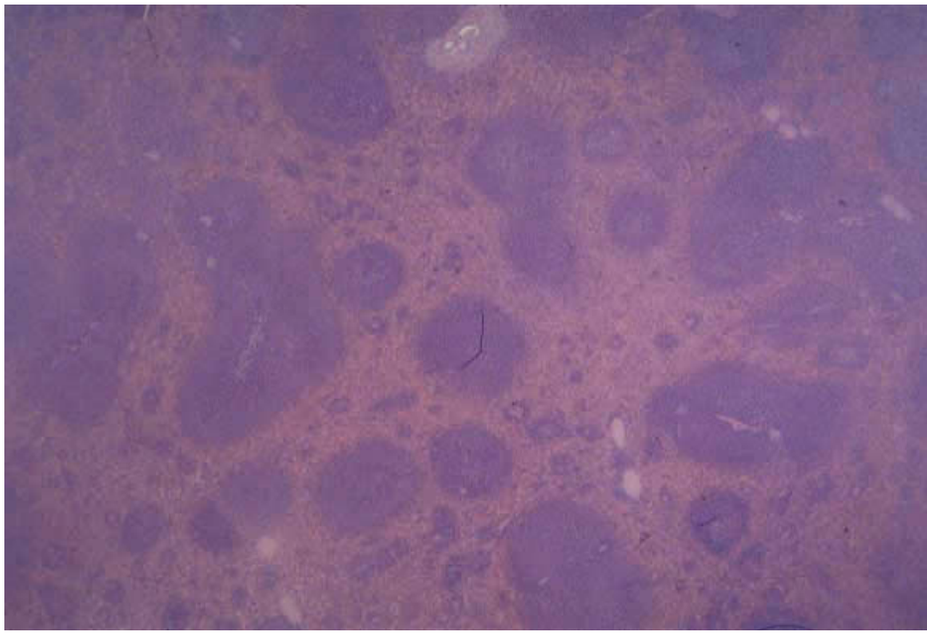
## Lymphoplasmacytoid lymphoma/immunocytoma

Plasma cell differentiation may be seen in a number of lymphomas, including extranodal marginal zone lymphomas, B-CLL and, less commonly, FCCL. In the past, such cases may have been classified as lymphoplasmacytic lymphomas or immunocytomas, but these terms should now be reserved for tumours composed of mixtures of small lymphocytes, lymphoplasmacytoid cells and plasma cells without characteristics of other low grade B cell lymphomas. In the Kiel classification, the term lymphoplasmacytoid lymphoma was used for a tumour in which the cells resembled lymphocytes but contained Dutcher bodies (see below). These lymphomas are CD5-positive and are better regarded as part of the spectrum of B-CLL.

Splenic infiltration by sheets of mature plasma cells or plasmablasts is seen occasionally in cases of multiple myeloma with extensively disseminated disease. However, the appearances are identical to those at other sites and are not considered further here.

Lymphoplasmacytoid lymphomas (LPL), as defined by the REAL classification, may

**Fig. 6.16.** Splenic marginal zone lymphoma. Low power view showing expansion of white pulp nodules and numerous satellite nodules within red pulp. H&E stain.



present with splenomegaly in the absence of lymphadenopathy and are often associated with the clinical syndrome of Waldenström's macroglobulinaemia.

Lymphoplasmacytoid lymphoma may involve the white pulp of the spleen but typically shows considerable overspill into the red pulp. The tumours are composed of a mixture of small lymphocytes, plasmacytoid cells and plasma cells, more easily identified in Giemsa than in H&E stained sections. Blast cells, often with a prominent single nucleolus, are found scattered throughout the infiltrate but proliferation centres are not formed. Large aggregates or sheets of such blast cells indicate transformation to a higher grade lymphoma (usually, an immunoblastic large B cell lymphoma). A variable number of tumour cells may contain Ig inclusions that often indent the nucleus, forming apparently intra-nuclear inclusions (Dutcher bodies). Since most cases of LPL secrete IgM, these inclusions are usually strongly PAS-positive (Fig. 6.19).

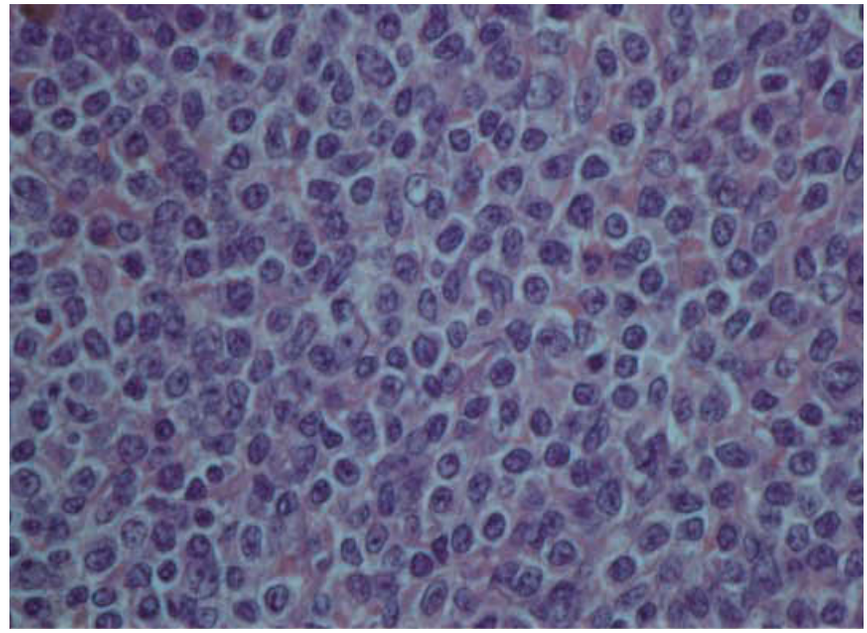


**Fig. 6.17.** Splenic marginal zone lymphoma. High power view showing the characteristics of tumour cells in the marginal zone. They have larger and more open nuclei than small lymphocytes of the mantle zone. In addition, they have more abundant, pale cytoplasm. H&E stain.

### Immunophenotype

The tumour cells of LPL express SIg and CIg, usually of the IgM isotype. The B cell markers CD19, CD20, CD22 and CD79a are positive but the tumour cells lack expression of CD5 and CD10.

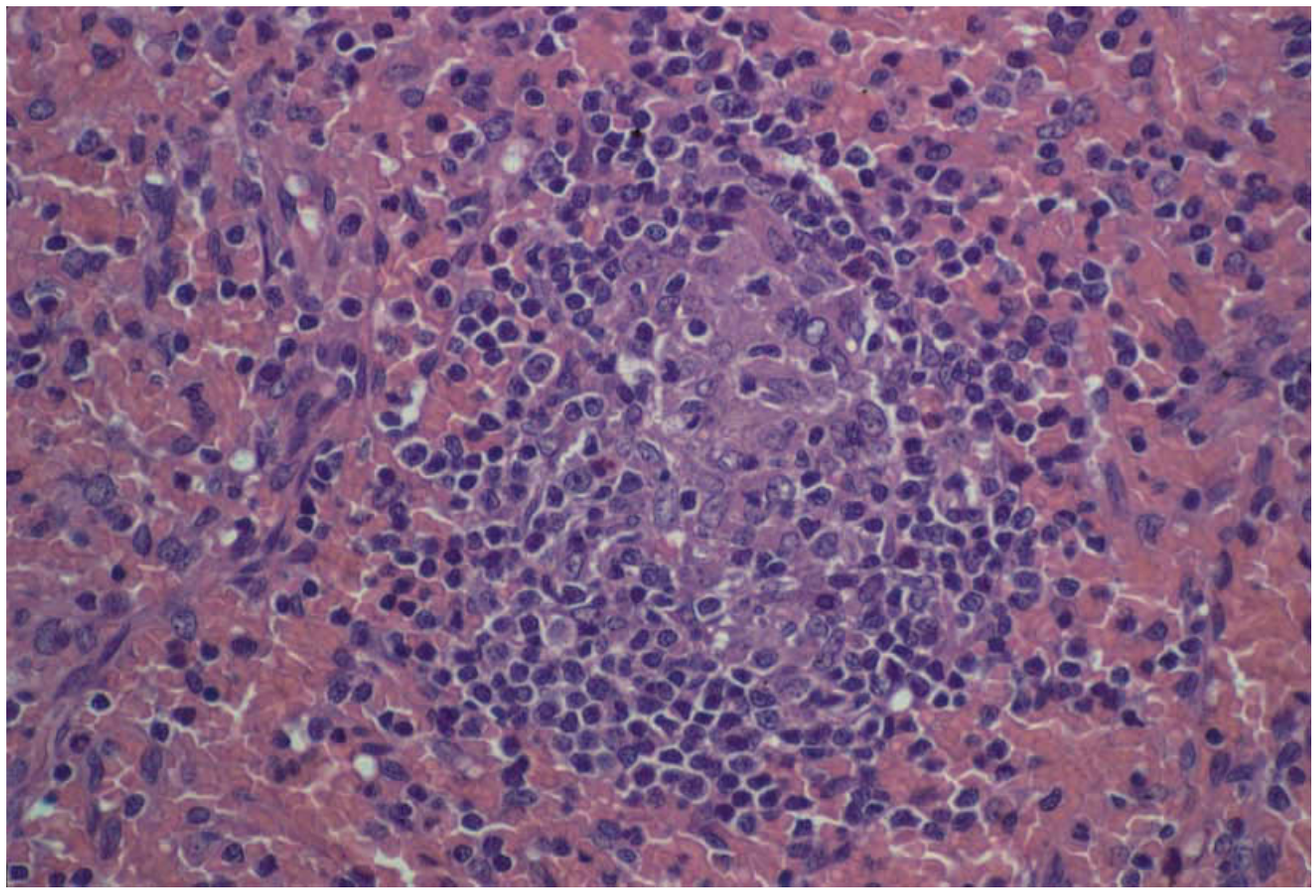
### Genetic features

The t(9;14)(p13;q32) chromosomal translocation has been associated with LPL. This translocation juxtaposes the PAX-5 gene at 9p13 and the Ig regulatory elements at 14q32, leading to dysregulation of the PAX-5 gene (Iida et al., 1996).

## Hairy cell leukaemia

Hairy cell leukaemia (HCL) is usually an indolent disease. Patients present with

**Fig. 6.18.** Splenic marginal zone lymphoma. High power view of a satellite nodule within red pulp. Note the cluster of pink-staining epithelioid macrophages at the centre of the nodule. H&E stain.



cytopenias and infections associated with depressed immunity. Splenomegaly is common but peripheral lymphadenopathy is rare and, when present, may be due to opportunistic infection rather than tumour. Splenectomy is often followed by remission of disease and used to be adopted as a therapeutic measure. In recent years, however, splenectomy has largely been replaced by medical treatment.

Most patients have characteristic hairy cells in their peripheral blood (Fig. 6.20). These cells are usually positive for tartrate-resistant acid phosphatase and, at an ultrastructural level, they can be shown to contain characteristic ribosomal lamellae.

By contrast with all other low grade B cell lymphomas, HCL primarily involves the red pulp of the spleen. The white pulp follicles are displaced by tumour cells and, in many instances, are not detectable macroscopically or microscopically. The cut surface of the spleen has a homogeneous red, beefy appearance, often with variably sized areas of haemorrhage present, representing blood lakes (see below).

In histological sections, the cells of HCL are slightly larger than small lymphocytes, with more oval and more euchromatic nuclei. The nuclei are surrounded by a halo of indistinct, pale cytoplasm and thus appear less closely packed than any similar infiltrate of small lymphocytes (Fig. 6.21). The tumour cells can be seen infiltrating both the sinusoids and cords of the splenic red pulp.

Blood lakes, which are characteristic of HCL, usually appear to be lined by a single layer of hairy cells (Fig. 6.22). This appearance led to the suggestion that hairy cells adherent to the sinusoidal lining damaged the barrel-hoop fibres, leading to sinusoidal ectasia and eventually to blood lake formation. However, inability to demonstrate sinusoidal lining cells in blood lakes fails to support this hypothesis. It appears more likely that such lakes originate from hae-

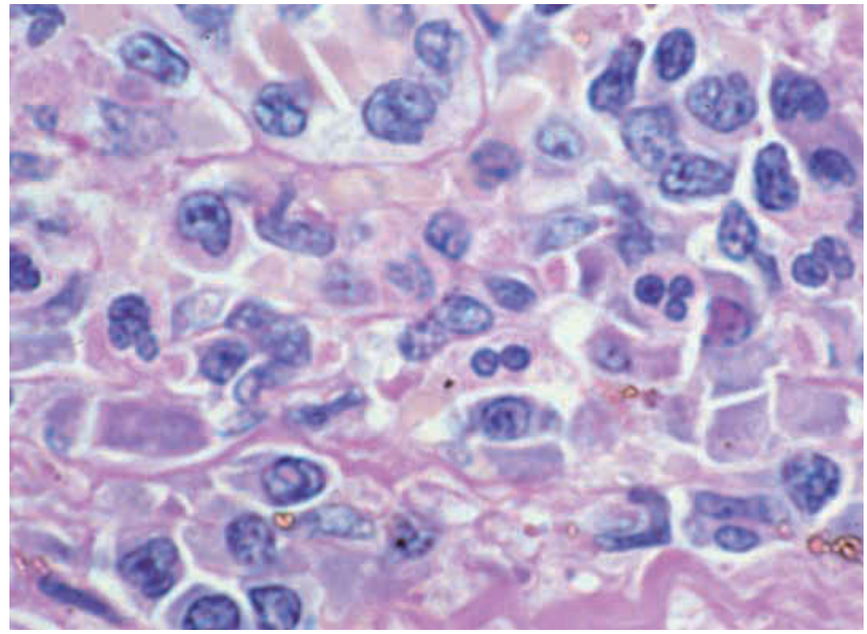


**Fig. 6.19.** Lymphoplasmacytoid lymphoma of spleen from a patient with Waldenström's macroglobulinaemia. This section has been stained with PAS; note the PAS-positive immunoglobulin inclusions with their apparently intranuclear localization (Dutcher bodies).

morrhages within the red pulp cords (Fig. 6.23), presumably as a result of haemodynamic disturbances caused by the infiltrating tumour cells. Ectatic sinusoids are seen in other tissues infiltrated by HCL, including liver, bone marrow and lymph nodes.

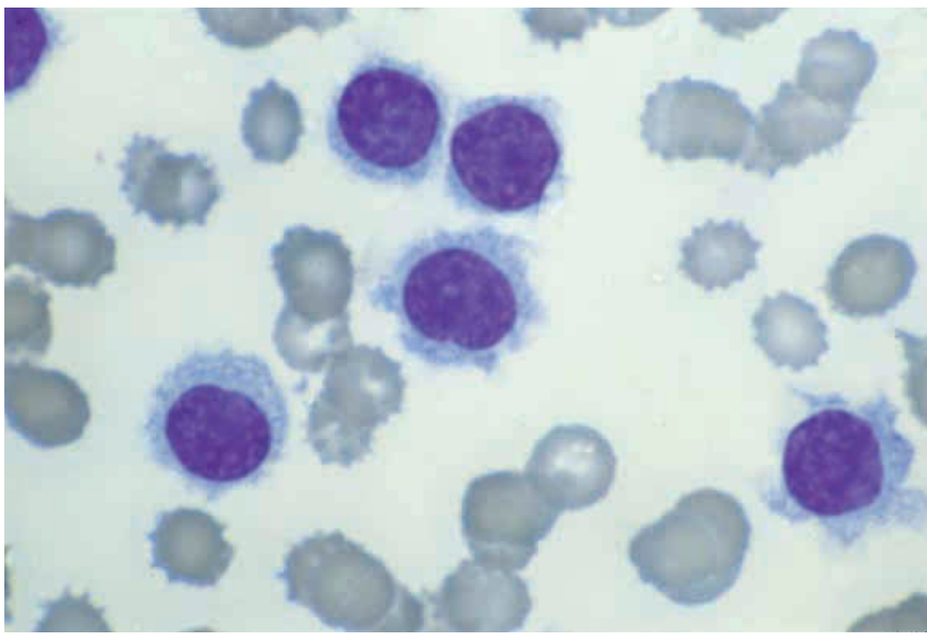
### Immunophenotype

The tumour cells of HCL express SIg. They are positive for the B cell markers CD19, CD20, CD22 and CD79a. They also express CD11c, the interleukin-2-receptor CD25 and the mucosal lymphocyte marker CD103. They lack expression of CD5 and CD10 (Moller et al., 1990; Visser et al., 1989) but they react with the monoclonal antibody DBA44 which was developed to enhance their detection in paraffin sections (Al Saati et al., 1989).

### Genetic features

No characteristic chromosomal abnormalities have been associated with HCL. The Ig genes are clonally re-arranged.

**Fig. 6.20.** Blood film from a patient with hairy cell leukaemia showing typical hairy cells with abundant pale cytoplasm and surface projections.



## Large B cell lymphomas

Large B cell lymphomas in the spleen usually cause sizeable tumour masses rather than showing the diffuse, 'miliary' infiltration characteristic of most small B cell lymphomas (Fig. 6.24). Rarely, large B cell lymphomas arise in the spleen as a result of transformation from a pre-existing small B cell lymphoma and therefore show additional features of low grade tumour in the background parenchyma. The tumour masses of large B cell lymphoma are usually well demarcated and may show areas of necrosis. The histological appearances are non-uniform, in part due to morphological diversity of the tumour cells and, in part to variation in the nature and intensity of

accompanying non-neoplastic cells. Thus, tumours equivalent to T cell-rich large B cell lymphomas at other sites may be seen.

Immunophenotypic and genetic features of large B cell lymphomas in the spleen are essentially the same as those in such tumours at nodal sites (for further details, see Harris et al., 1994; Isaacson & Norton, 1994).

Pleomorphic T cell lymphomas composed of medium-sized and large cells may also rarely cause tumour nodules but typically show diffuse infiltration within the spleen (Stroup et al., 1992). Tumours expressing CD4 are somewhat more likely to form microscopic, irregular masses involving the PALS and those expressing CD8 tend to infiltrate red pulp structures diffusely. However, there is considerable overlap. Their features are otherwise equivalent to those found at other sites in the body.

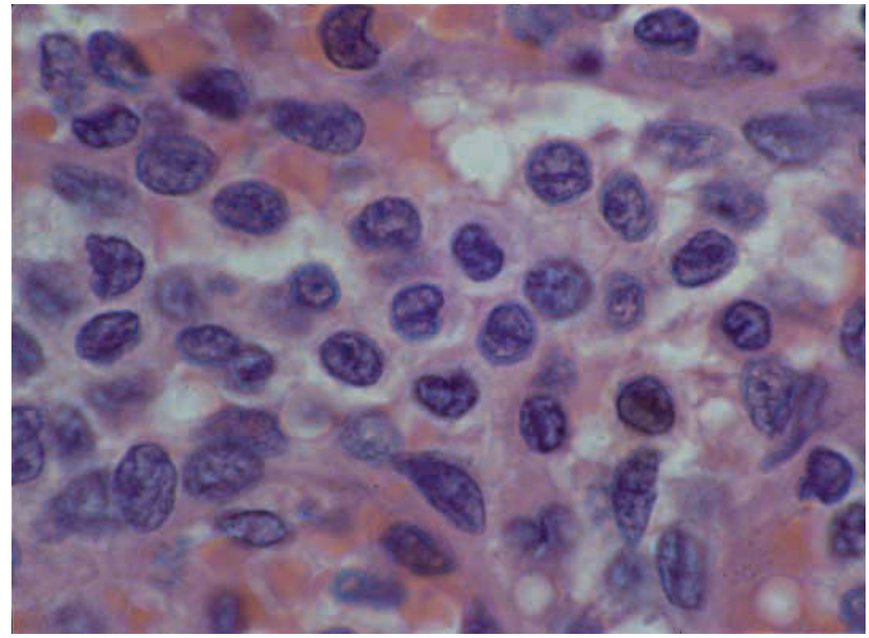


**Fig. 6.21.** Hairy cell leukaemia of spleen. High power view to show characteristic nuclear grooving of tumour cells and the abundant, clear cytoplasm of these cells. H&E stain.

## T cell prolymphocytic leukaemia

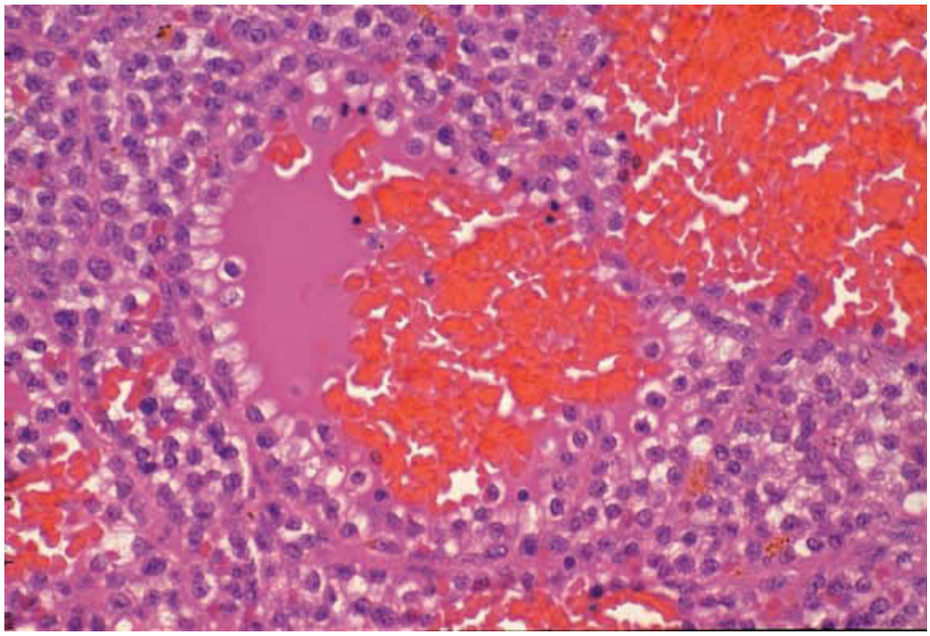
There is probably no direct T cell equivalent of B-CLL and T cell lymphocytic leukaemias are rare. The circulating lymphocytes may be small but in many cases they show nuclear irregularity with visible nucleoli (Fig. 6.25). For this reason, and because they pursue a somewhat less indolent course than most cases of B-CLL, T cell lymphocytic leukaemias are categorized as prolymphocytic leukaemias (T-PLL), with small and larger cell variants.

The cells of T-PLL do not have cytoplasmic granules. In addition to bone marrow involvement, infiltration of splenic red pulp and hepatic sinusoids is common. Involvement of lymph nodes is predominantly paracortical and the infiltrates lack proliferation centres.

### Immunophenotype

The neoplastic cells of T-PLL express CD2, CD3, CD5 and CD7. Not all of these antigens

**Fig. 6.22.** Hairy cell leukaemia of spleen showing blood lake lined by hairy cells. The clear halos around these cells are characteristic. H&E stain.



can be demonstrated reliably in paraffin sections but they can be shown by immunofluorescent staining of blood or bone marrow cells. Most cases express CD4 or, less commonly, both CD4 and CD8 (Matutes et al., 1991).

### Genetic features

Three-quarters of cases show a characteristic inv14(q11;q32). Trisomy 8q has also been reported. The T cell receptor genes show clonal rearrangement.

## Large granular lymphocyte leukaemia, T cell and NK cell types

This disease is characterized by the presence in blood and bone marrow of large granular lymphocytes having either cytotoxic T cell or natural killer (NK) cell characteristics. The lymphoid cells have a variable nucleus-to-cytoplasm ratio with discernible nucleoli and multiple azurophilic granules within pale blue cytoplasm (Fig. 6.26). Patients usually have a moderate peripheral blood lymphocytosis, often accompanied by anaemia and neutropenia; in some patients the picture resembles Felty's syndrome (see Chapter 4). Splenomegaly is found in the cytotoxic T cell type but is not a feature of the NK cell variant.

T cells infiltrate the splenic red pulp and frequently leave the white pulp intact (Fig. 6.27). Infiltration is also seen in the sinusoids of the liver (Agnarsson et al., 1989).

### Immunophenotype

Tumours of the T cell type usually express CD2, CD3, CD8, CD16 and CD57. They show expression of the T cell $\alpha\beta$ receptor. Tumours of the NK cell type may express CD2, CD16, CD56 and CD57 but do not show T cell receptor expression.

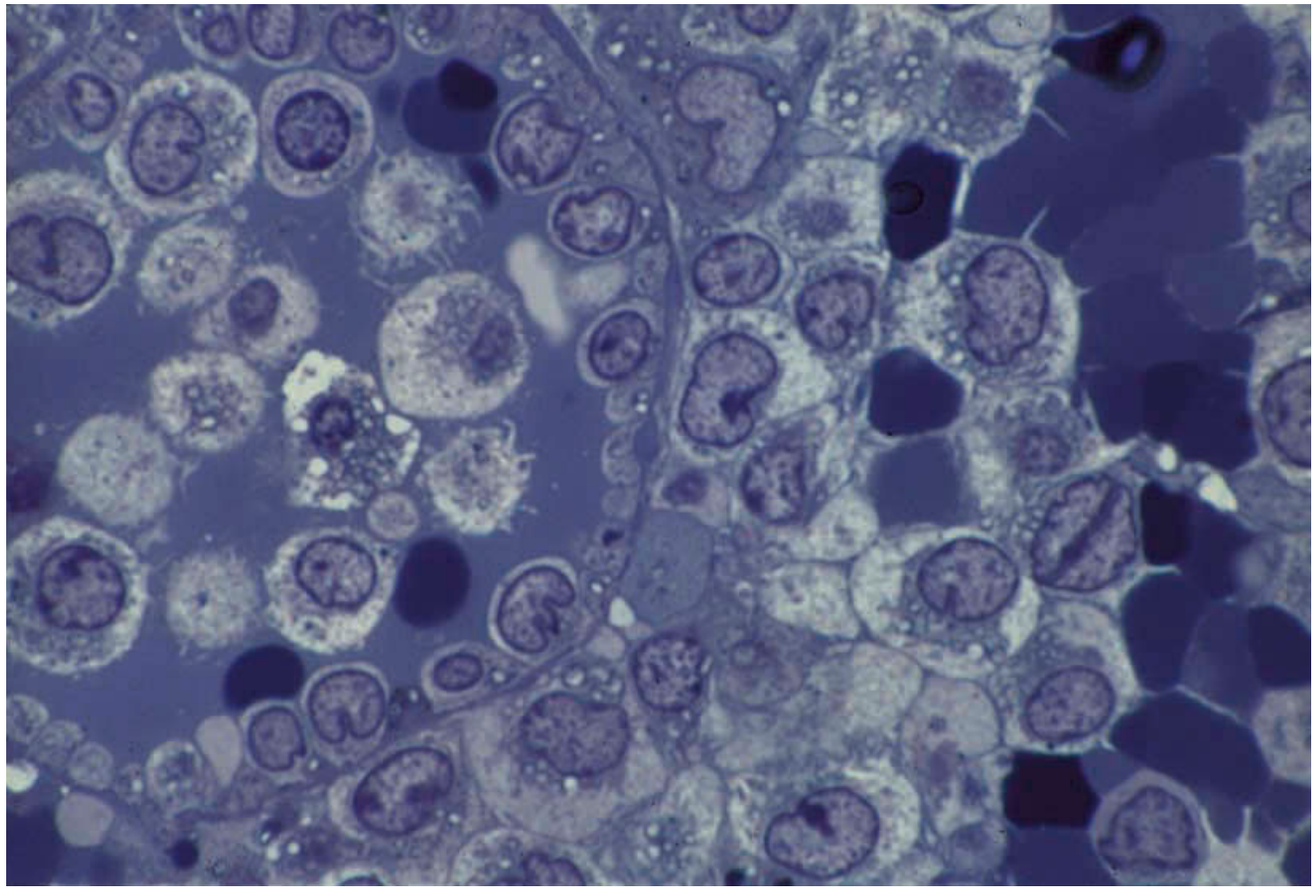


**Fig. 6.23.** Hairy cell leukaemia of spleen. Plastic-embedded section stained with toluidine blue to show hairy cells within a sinusoid and splenic cord. A small haemorrhage is present within the splenic cord (right-hand side of picture) suggesting that this might be the site of origin of blood lakes, rather than their originating from sinusoidal ectasia.

**Fig. 6.24.** Large B cell lymphoma of spleen. The tumour forms large, well-defined nodules within the splenic tissue with relatively normal intervening parenchyma.

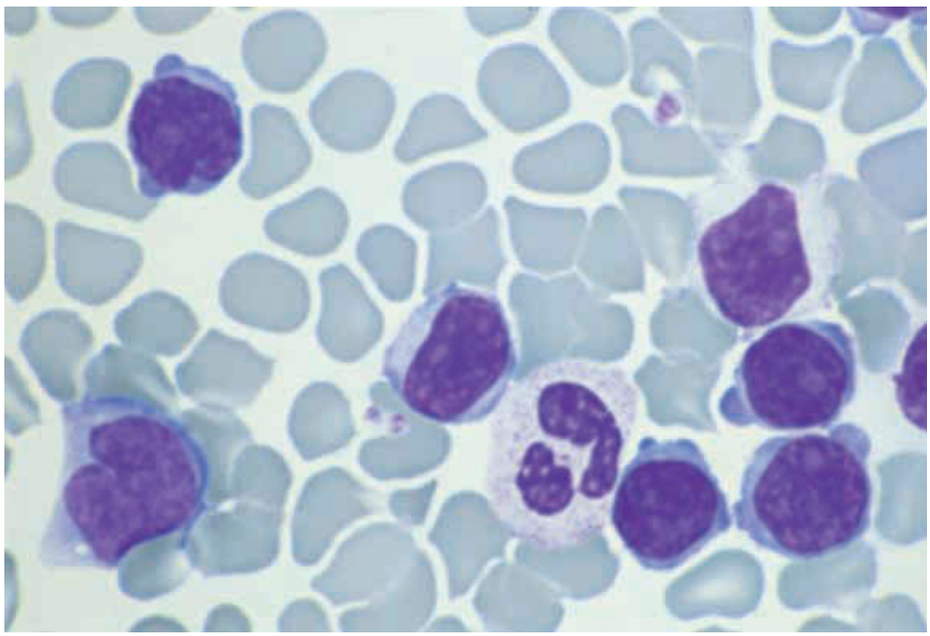


**Fig. 6.25.** T-prolymphocytic leukaemia. Peripheral blood film showing several tumour cells, most of which have visible nucleoli. Blebbing of the weakly basophilic cytoplasm is characteristic.

### Genetic features

Tumours of T cell, but not NK cell origin show clonal rearrangements of T cell receptor genes.

## Splenic T cell lymphoma with erythrophagocytosis

Although not recognized as an entity in the REAL classification, this is a distinctive morphological variant of high grade T cell lymphoma in the spleen. The neoplasm corresponds broadly to the condition designated 'histiocytic medullary reticulosis' by Scott and Robb-Smith (1939) and later called malignant histiocytosis by Rappaport (1966). Patients present with fever, weight loss, cytopenias and hepatosplenomegaly. The splenic red pulp is diffusely infiltrated by pleomorphic medium-sized and large tumour cells, either singly or in small aggregates. Tumour cells also aggregate at the periphery of atrophic white pulp follicles. This infiltrate is accompanied by intense erythrophagocytosis by non-neoplastic histiocytes (Fig. 6.28).

## Hepatosplenic $\gamma\delta$ T cell lymphoma

This lymphoma is recognized as a provisional entity in the REAL classification. It is a rare disease, occurring predominantly in young adults, and showing a marked male preponderance. Patients present with fever, hepatosplenomegaly and cytopenias. The disease usually runs an aggressive course with few long-term survivors (Cooke et al., 1996; Farcet et al., 1990).

Neoplastic cells infiltrate the cords and, particularly, sinusoids of the splenic red pulp (Fig. 6.29). White pulp structures are often completely effaced by this infiltrate. The tumour cells are of medium size with round or angulated nuclei and inconspicu-

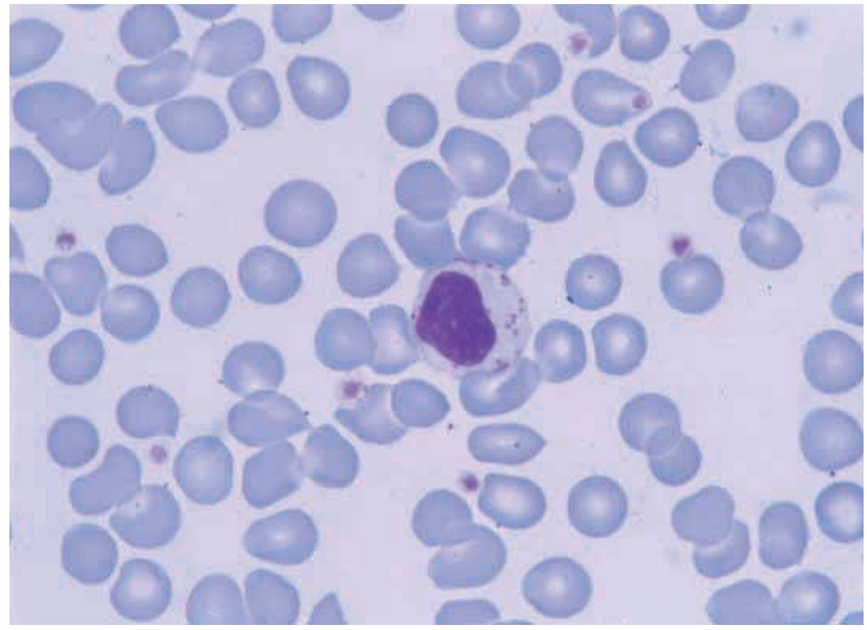


**Fig. 6.26.** Large granular lymphocytic leukaemia. Blood film showing the large granular lymphoid cells with their characteristic azurophilic granules.

ous nucleoli (Fig. 6.30). Their cytoplasm shows weak to moderate basophilia. Sinusoidal infiltration is characteristic in the liver and bone marrow as well as in the spleen. Within sinusoids, the tumour cells often appear cohesive.

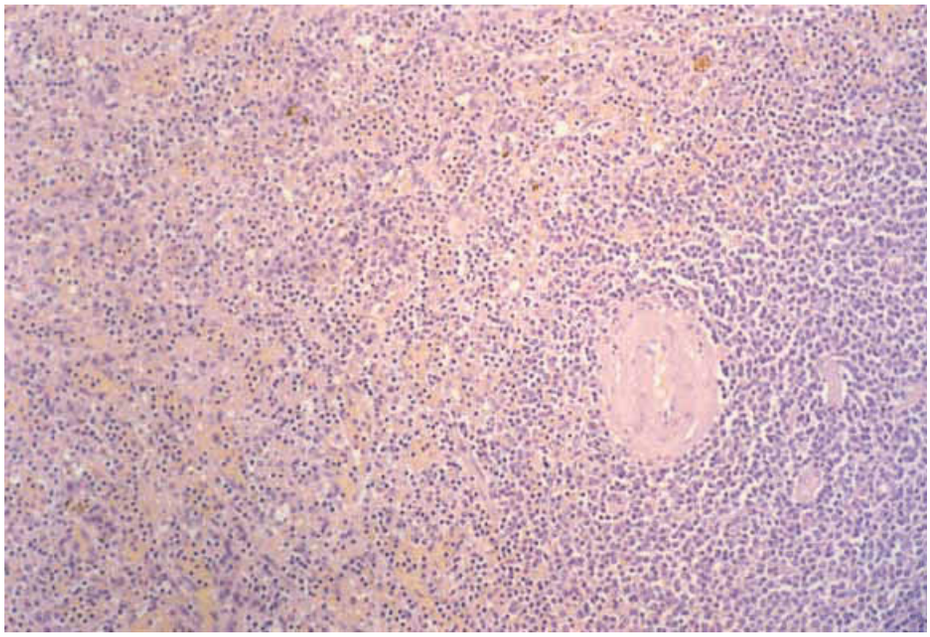
### Immunophenotype

The neoplastic cells express CD3 and CD56. They usually give a positive reaction for the precursor cytotoxic granule protein recognized by the antibody TIA-1. However, they usually lack the more specific cytotoxic granule protein granzyme B. They typically express the $\gamma\delta$ T cell receptor, although rare cases expressing the $\alpha\beta$ receptor have been reported.

### Genetic features

Isochromosome 7q is a characteristic, but not specific, marker found in many of these tumours. The T cell receptor $\gamma\delta$ genes are rearranged and clonal.

**Fig. 6.27.** Large granular lymphocytic leukaemia. Section of spleen showing diffuse infiltration of red pulp by tumour cells, predominantly intra-sinusoidal. H&E stain.



## Hodgkin's disease

In the past, it was common practice to subject patients with Hodgkin's disease (HD) to a staging laparotomy to determine the extent of disease present. During this procedure, various lymph node groups were sampled, a liver biopsy was performed and the spleen was removed. Critical examination of the spleen was central to the procedure since, in the absence of splenic tumour, involvement of the liver and bone marrow was almost never found. In more recent times, improved imaging techniques and management regimes have removed the need for staging laparotomy in HD, although diagnostic splenectomy is still performed occasionally.

The spleen may be moderately enlarged in HD, even without evidence of tumour infiltration. During the era of staging

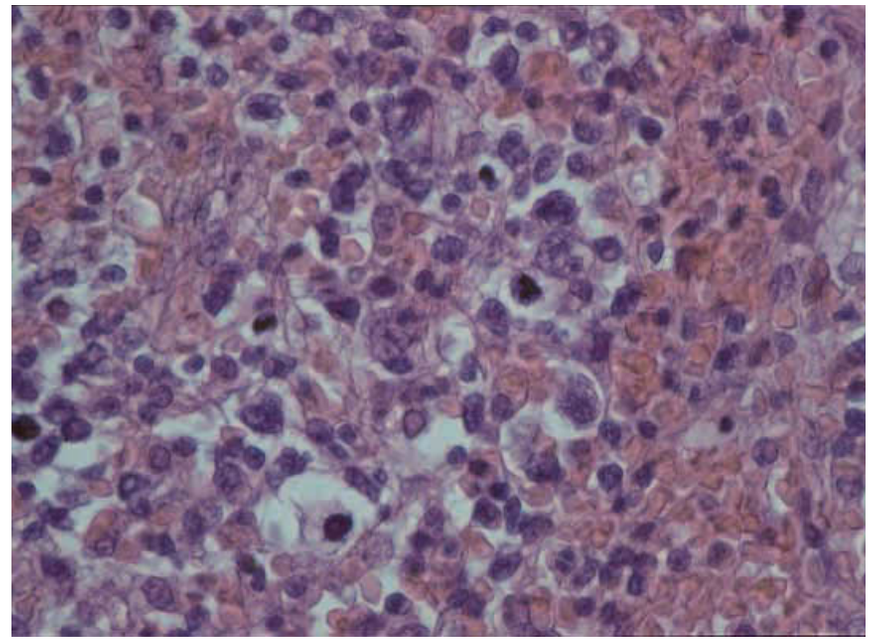


**Fig. 6.28.** Splenic T-cell lymphoma with erythrophagocytosis. Pleomorphic tumour cells are seen accompanied by reactive macrophages showing erythrophagocytosis. Tumour cells themselves are not phagocytic. H&E stain.

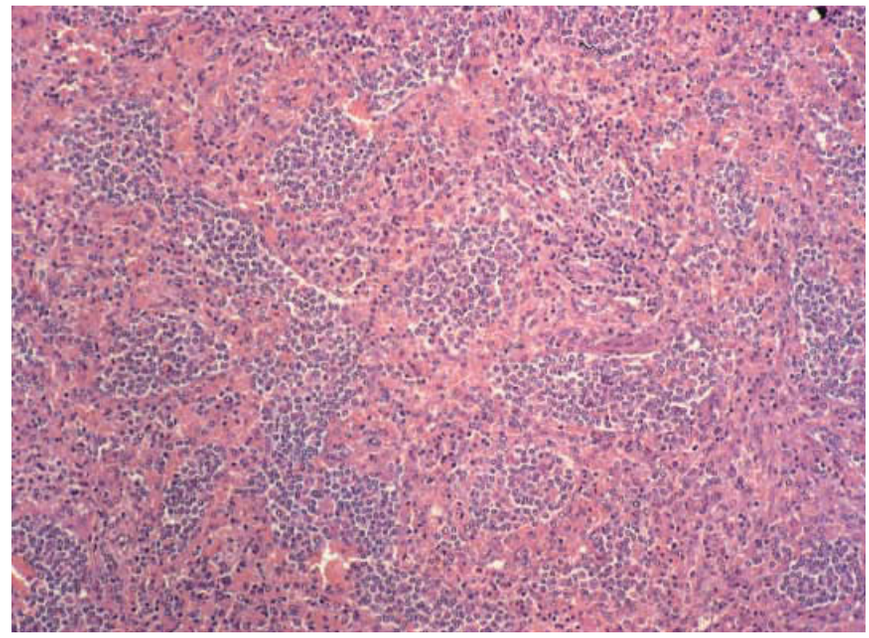


**Fig. 6.29.** Hepatosplenic $\gamma\delta$-T-cell lymphoma. Splenic red pulp showing intra-sinusoidal spread of tumour cells with relative sparing of the intervening cords. H&E stain.

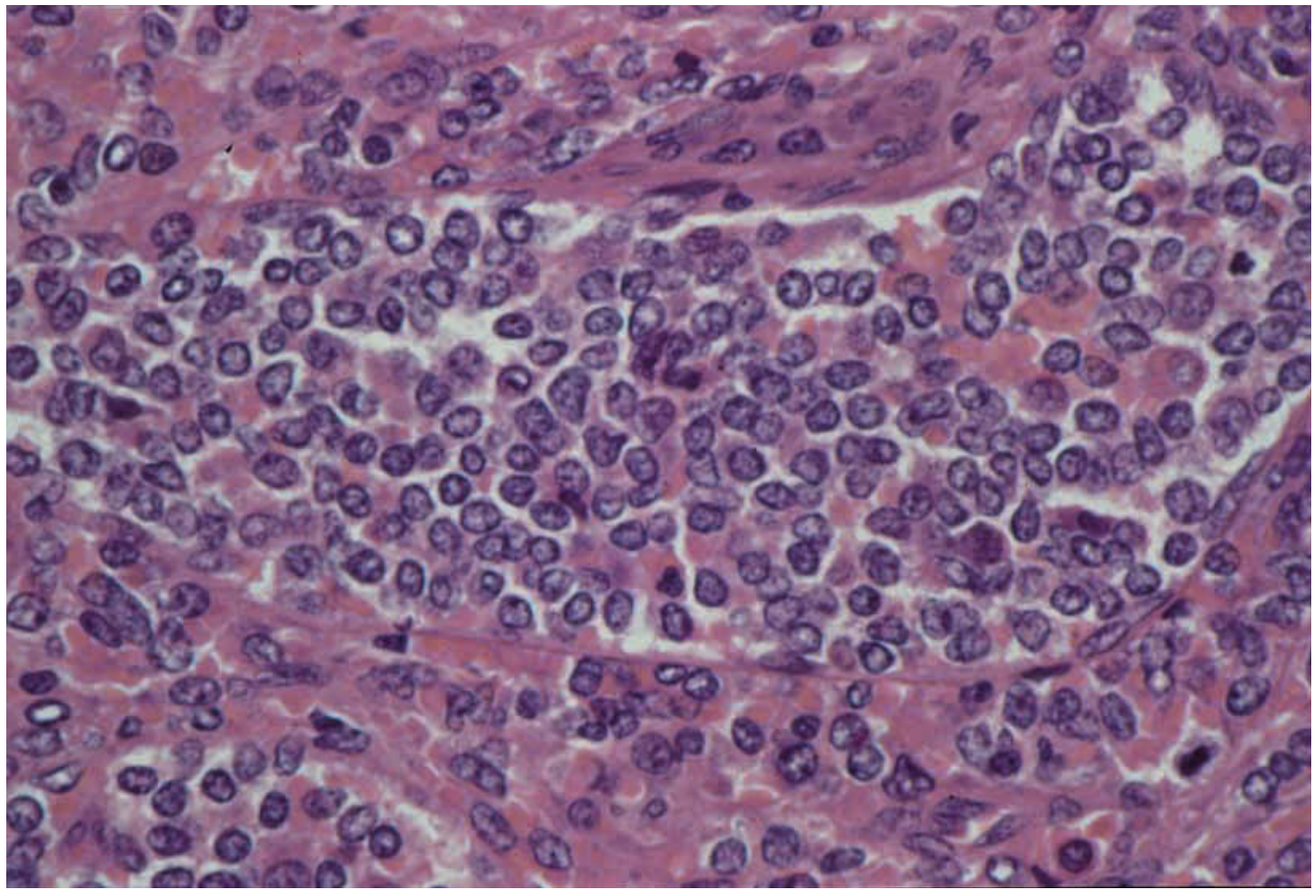


**Fig. 6.30.** Hepatosplenic $\gamma\delta$-T-cell lymphoma. Higher power view of the case shown in Fig. 6.29, demonstrating the cytological features of the intra-sinusoidal medium-sized tumour cells. H&E stain.

laparotomy, it was found that spleens weighing 600 g or more usually contained tumour. Macroscopically, deposits of HD may be seen as localized or more diffuse (but still relatively well-defined) tumour masses of varied size (Fig. 6.31). These may contain areas of necrosis or fibrosis. White pulp nodules in non-involved areas of the spleen, particularly in young individuals, are often enlarged and clearly visible to the naked eye.

Histologically, HD in the spleen is similar to that in lymph nodes and at other sites. The essential feature is the presence of Reed–Sternberg (RS) cells or their lacunar variants in an appropriate reactive cellular setting for one of the subtypes of classical HD. Extensive necrosis may occasionally make their recognition difficult. It is usual nowadays to confirm the diagnosis using immunohistochemistry for B and T cell-associated antigens, CD30 and CD15. This excludes T cell-rich B cell lymphomas and rare T cell lymphomas that may mimic HD in the spleen.

The residual non-involved spleen in HD often shows hyperplasia of the white pulp with prominent marginal zones. In spleens removed at staging laparotomy, it was not uncommon to find epithelioid or giant cell granulomas, typically within the PALS, in the absence of identifiable involvement by HD (Fig. 6.32). Similar granulomas were found in the portal areas of the liver and are still seen today in staging bone marrow biopsies. The presence of these granulomas was initially a cause of concern as to whether they represented occult or regressed tumour. However, it has been shown that they are probably a response to altered cytokine production in HD patients and that they may be of good prognostic significance (Sacks et al., 1978).

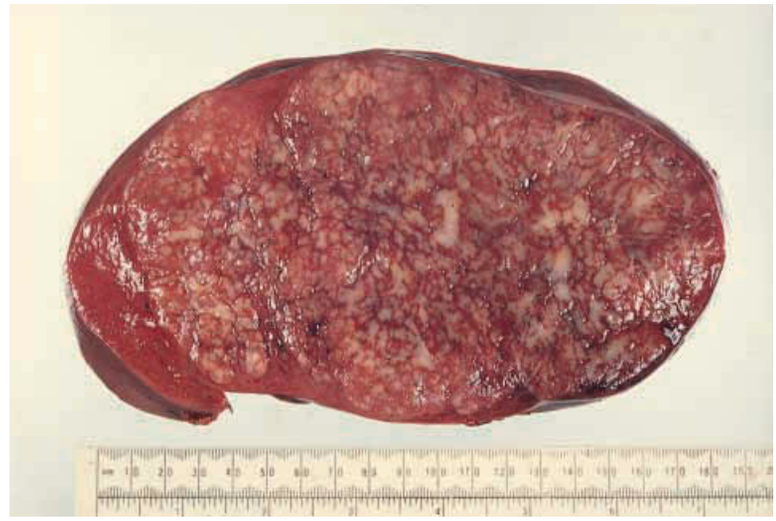


**Fig. 6.31.** Spleen removed at staging laparotomy of a patient with Hodgkin's disease. This spleen is extensively involved by tumour but small areas of relatively normal splenic tissue can be seen between coalescent tumour masses.

### Immunophenotype

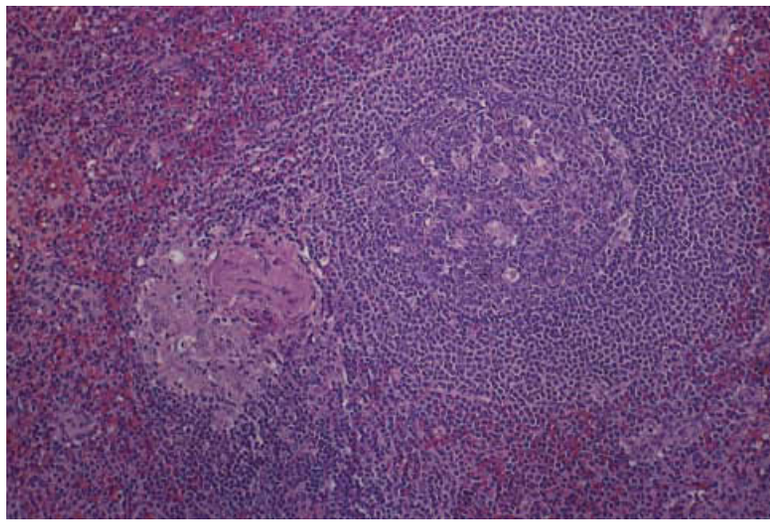
Reed–Sternberg cells and other Hodgkin's cell variants are CD45-negative. They can be

shown to express B cell-associated antigens and, less commonly, antigens associated with T cell differentiation. Expression of these antigens is variable, generally weak and demonstrable in only a minority of the cells. It can be difficult to assess expression of T cell membrane antigens because of tight rosetting of RS cells and their variants by reactive T lymphocytes. The majority of tumour cells in HD express the activation marker CD30 on their membranes and/or in the Golgi (paranuclear) region. A similar pattern of CD15 expression is usually present.

### Genetic features

Although RS and other HD tumour cells have multiple cytogenetic aberrations, no specific abnormalities have been detected. The majority of cases studied have shown rearranged and hypermutated Ig genes, consistent with a post-germinal centre B cell origin. However, the Ig genes are usually crippled by mutations that prevent the production of immunoglobulin molecules.

**Fig. 6.32.** Cluster of epithelioid macrophages in the peri-arteriolar T cell area of the white pulp from a spleen removed at staging laparotomy for Hodgkin's disease. There was no evidence of direct involvement of this spleen by Hodgkin's disease. H&E stain.



## Nodular lymphocyte and histiocyte predominant Hodgkin's disease (nodular L&H HD)

This entity is worthy of brief mention if only because of the rarity with which the spleen is involved. Historically, nodular L&H HD was regarded as a variant of true HD and staging laparotomy was performed in some patients as for other subtypes of HD. Typically, nodular L&H HD is low stage disease and the spleens were not involved. However, involvement was observed in a small proportion of cases, raising the question of whether such patients had a different form of disease.

In recent years, it has become accepted that nodular L&H HD is a B cell lymphoma quite distinct from classical HD. Review of

cases originally diagnosed as disseminated nodular L&H HD has shown that many are better classified as a newly recognized classical HD variant, for which the term 'lymphocyte-rich classical HD' has been coined (Harris et al., 1994; Sextro et al., 1996).

We have recently reviewed three involved spleens from staging laparotomies performed in patients with diagnoses of nodular L&H HD. In each case, the splenic appearances were those of lymphocyte-rich classical HD, with CD30 positivity and weak or absent expression of B cell-associated antigens by neoplastic cells (authors' unpublished observations). In one case, the patient had recurrent stage IV classical HD with lymphocyte-rich appearances 12 years after the original diagnosis. The other two patients had no evidence of recurrent disease 10 and 16 years, respectively, after their staging laparotomies. The original diagnostic lymph node biopsies were available for review in two cases only; the first showed lymphocyte-rich classical HD and the second was more in keeping with nodular sclerosing HD.

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Chapter 7

# The spleen in myeloproliferative disorders

## Splenic extramedullary haemopoiesis

### Different forms of splenic extramedullary haemopoiesis have different significance

The occurrence of significant splenic extramedullary haemopoiesis (EMH) as a normal process in human fetal life is questionable (see Chapter 2), but there is no doubt that small foci of EMH are common incidental findings in the liver and spleen throughout post-natal life. Such foci are generally of no clinical significance. They usually consist of clusters of nucleated erythroid cells within sinusoids. Single, apparently mature, megakaryocytes may also be seen in these tissues but it is doubtful whether these should be regarded as representing haemopoiesis. Megakaryocytes are known to enter the circulation and lodge in small vessels at a variety of sites around the body (including the lungs) as part of their normal, mature behaviour. Pathologists must be able to recognize such incidental examples of extramedullary haemopoietic cell location so that they do not mistake them for any significant abnormality.

Splenic EMH is significant when it occurs as a manifestation of a myeloproliferative disorder or leukaemia, or secondary to bone marrow replacement by fibrosis or metastasis. Under these circumstances, EMH preferentially involves the spleen, with or without detectable involvement of the liver. The spleen is usually enlarged (sometimes

massively) and the underlying diagnosis may already be known from a bone marrow biopsy. Splenectomy is usually performed for relief of symptoms (abdominal mass, pain and/or hypersplenism) in these patients rather than for diagnostic purposes. The role of the pathologist is to confirm that the appearances are consistent with the bone marrow diagnosis and to exclude any additional pathology.

### Incidental splenic EMH

A spleen enlarged and/or reactive for any reason may be found to contain small foci of erythropoiesis within the red pulp, with occasional megakaryocytes and, less commonly, foci of granulopoiesis also present. In such spleens, the appearances of the differentiating haemopoietic cells are essentially normal (O'Keane et al., 1989). Maturation to terminally differentiated erythrocytes and granulocytes is seen.

Erythrocyte clusters are preferentially localized within red pulp sinusoids (Fig. 7.1) and Giemsa staining highlights their presence by demonstrating the marked cytoplasmic basophilia of pro-erythroblasts, early and intermediate normoblasts. Late normoblasts are recognizable by virtue of their small, extremely dense nuclei. These are approximately equal in size to that of a normal mantle lymphocyte and their outlines are typically circular, or slightly oval, with no irregularities or indentations.

Granulopoiesis tends to occur at the interface between the PALS and red pulp and also centred upon red pulp capillaries. However, the presence of any more than occasional promyelocytes and myelocytes should raise concern about the possibility of a significant associated myeloproliferative or myelodysplastic disorder. Mature neutrophils and, in smaller numbers, eosinophils are normal components of splenic red pulp. Their presence alone is not an indication of EMH of either an incidental or

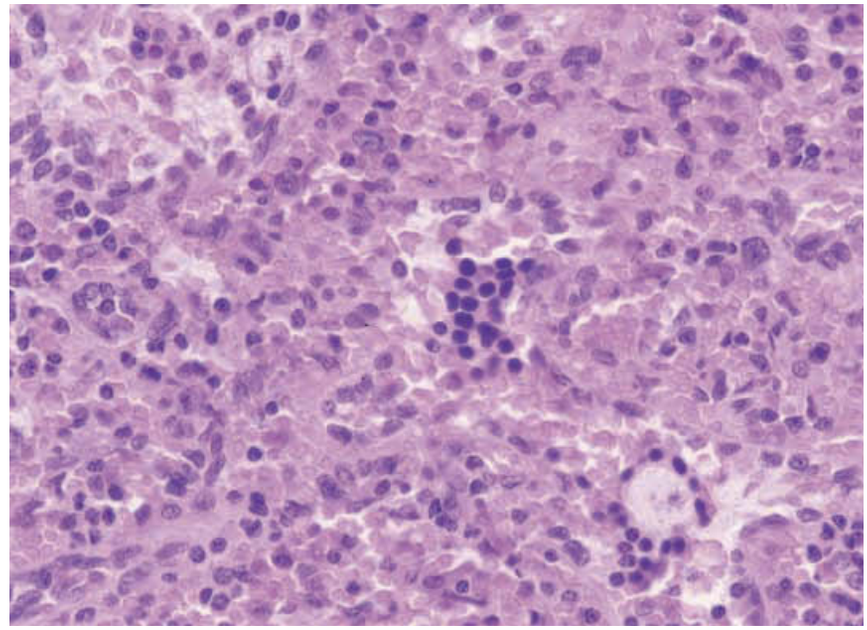


**Fig. 7.1.** Incidental EMH. Intra-sinusoidal cluster of relatively late erythroid precursor cells. H&E stain.

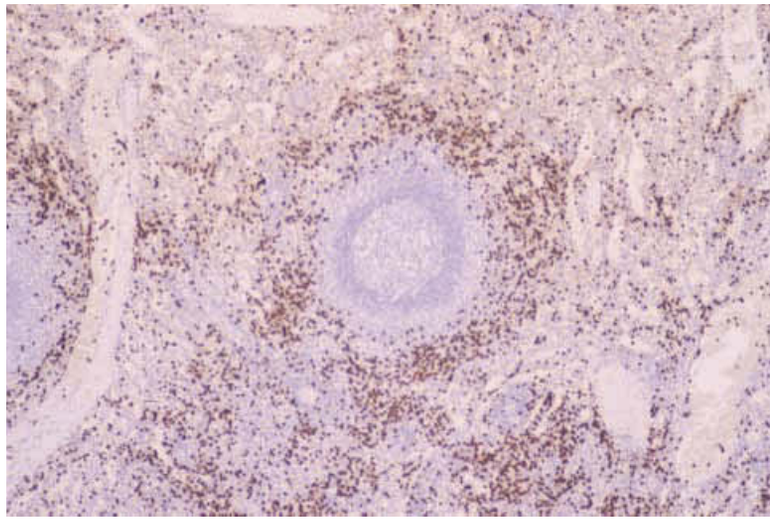
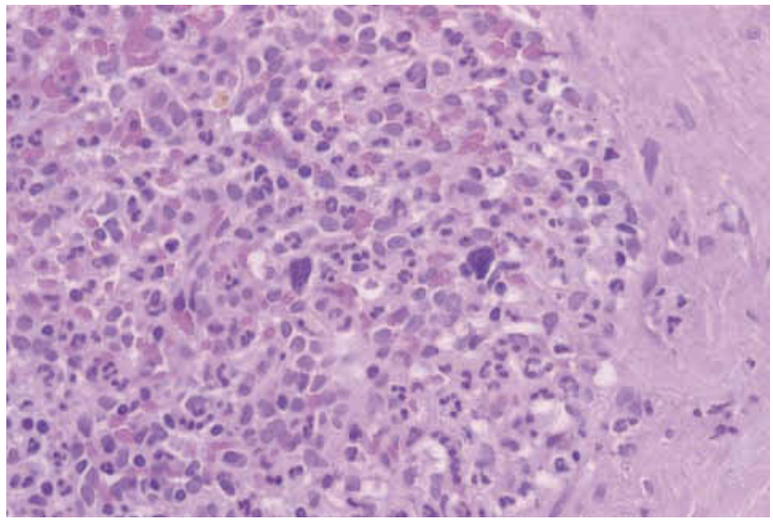


**Fig. 7.2.** Accumulation of neutrophils surrounding a reactive white pulp nodule. Streptavidin–biotin complex immunoperoxidase technique using monoclonal antibody MAC 387, reactive with calprotectin expressed by mature neutrophil granulocytes.

**Fig. 7.3.** Incidental megakaryocytes within the splenic red pulp. These examples show minimal cytoplasm and appear to represent bare nuclei. Such end-stage megakaryocytes are common, entirely normal findings scattered in small numbers within splenic red pulp. H&E stain.



significant nature; in reactive spleens, it is not uncommon to see accumulation of neutrophils in the peri-follicular zones surrounding white pulp nodules (Fig. 7.2).

Megakaryocytes, when present incidentally in the spleen, may be found within cords or sinusoids (Fig. 7.3). They have the appearances of mature cells, with normal numbers of nuclear lobules (approximately 5–10 per cell) and abundant, weakly PAS-positive, granular cytoplasm. They are dispersed singly. Occasional end-stage 'bare' megakaryocyte remnants may also be found in splenic sinusoids.

Similar, incidental, foci of EMH may occasionally be found in liver, lung and other tissues. This is not surprising, since small numbers of haemopoietic stem cells are normally present in the circulation and presumably these become lodged in small blood vessels at a variety of sites. It is probable that spleen and liver are preferentially involved because sinusoidal endothelium, in particular, is able to support differentiation or, at least permit survival, of immature haemopoietic cells. What factors determine whether haemopoietic cells survive at these sites or undergo apoptosis are unknown, but are presumed to include the activation of intrinsic cell programmes for completion of differentiation as well as signals provided by the local micro-environment.

## Why does incidental splenic EMH occur?

Two alternative explanations for this type of splenic EMH in post-natal life have been proposed (Damashek, 1952; Bowdler, 1990). First, it has been suggested that haemopoietic stem cells remain dormant in the spleen from fetal life onwards and can be re-activated under a variety of conditions. Unless the concept of normal fetal splenic EMH is accepted, this seems unlikely. There is no evidence that haemopoietic stem cells are normally present in fetal or post-natal

spleens in numbers greater than those in the circulation. Secondly, it is proposed that haemopoietic stem cells may be displaced into the circulation under conditions of bone marrow 'stress', including fibrosis from a variety of causes, and home to the spleen where they find a supportive micro-environment for subsequent proliferation and differentiation. Mobilization of bone marrow stem cells into the circulation can certainly occur in a variety of circumstances, including iatrogenic manipulation for peripheral blood stem cell harvesting for transplantation. Lineage-committed haemopoietic cells with proliferative potential are also mobilized into the circulation and it remains to be determined whether it is these cells completing their differentiation programmes, rather than true stem cells, which are represented by incidental splenic EMH.

### Pathological splenic EMH in primary disorders of haemopoiesis

The second, and more significant, type of adult EMH is essentially a form of metastasis. It is found in patients with chronic myeloproliferative disorders, including essential thrombocythaemia (ET), polycythaemia rubra vera (PRV), chronic granulocytic leukaemia (CGL) and myelofibrosis. At diagnosis, splenic enlargement is greatest in CGL and myelofibrosis, with only 70% of patients with PRV and 50% of those with ET having palpable spleens, usually of modest proportions (Mackie & Shepherd, 1994). Occasionally, the spleen is also enlarged by EMH in myelodysplastic syndromes. Splenectomy specimens from such patients are relatively common in surgical pathology practice. Even in patients who initially have no, or modest, splenomegaly, splenic enlargement may become massive and be poorly controlled by cytotoxic drugs or irradiation.

In the chronic myeloproliferative disor-

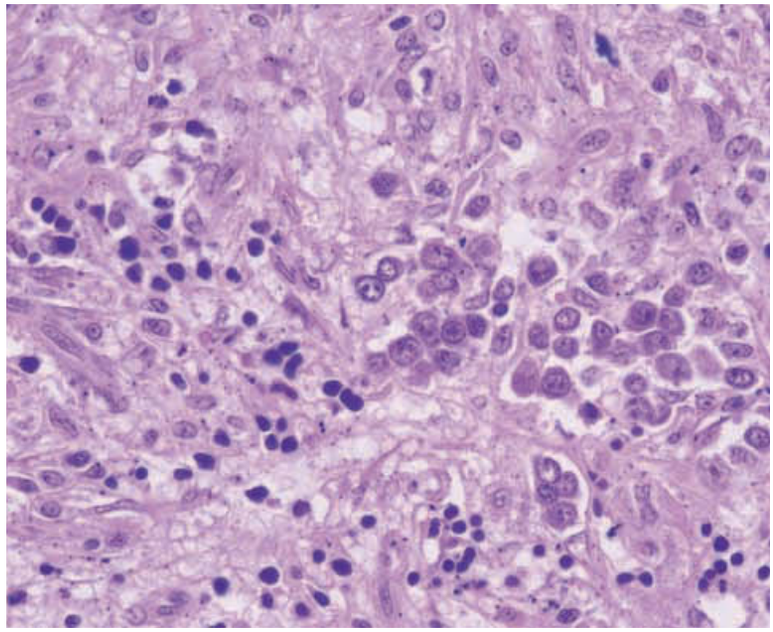


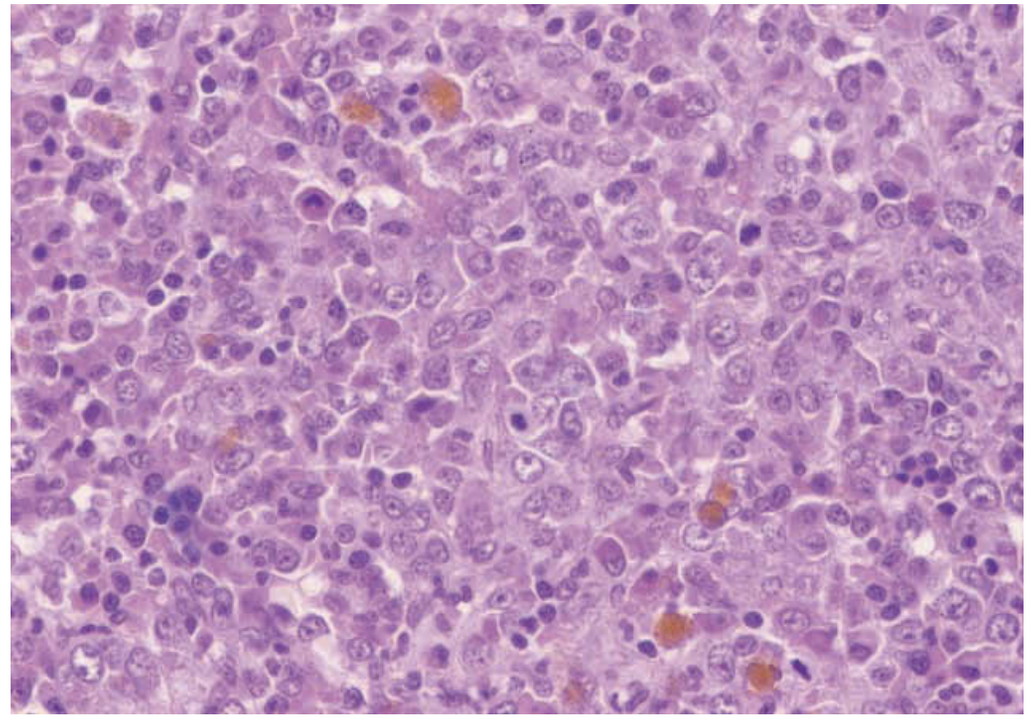
**Fig. 7.4.** Cluster of dysplastic erythroid cells within a red pulp sinusoid. Most of these cells are larger than the late normoblasts which can be seen at the left-hand side of the picture and their nuclei have a more open chromatin pattern. These appearances are typical of the megaloblast-like abnormality of erythroid differentiation commonly seen in spleens involved by chronic myeloproliferative disorders. H&E stain.

ders, splenic involvement may be extensive (Wolf et al., 1988). With the exception of advanced myelofibrosis, in which little intramedullary haemopoiesis remains for comparison, haemopoietic cells in the spleen usually appear similar to those in the bone marrow. The different lineages are present in approximately the same proportions at the two sites. Predominance of immature granulocytic and erythroid cells, megaloblast-like erythroid dysplasia and megakaryocyte abnormalities are seen in the spleen (Figs. 7.4 and 7.5) (Wilkins et al., 1994) just as in the bone marrow (Bain et al., 1996).

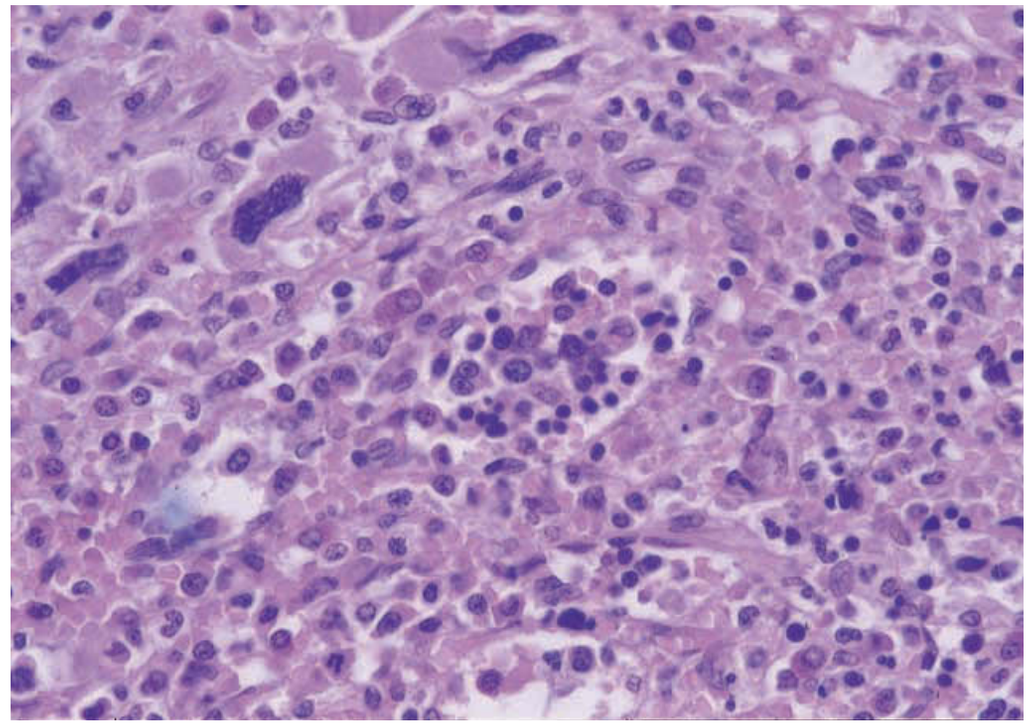
In spleens with minor or modest involvement by chronic myeloproliferative disorders, granulocyte precursors tend to be localized preferentially around connective tissue trabeculae, capillaries and larger blood vessels, or at the interface between red and white pulp. With more extensive involvement, they are found diffusely throughout the red pulp.

Megakaryocytes in ET, PRV and myelofibrosis are larger than normal with very high ploidy (often 20 or more nuclear lobules, sometimes differing widely in size) and tend to form clusters (Fig. 7.6). Splenectomy is relatively rarely performed in uncomplicated CGL, since this is one myeloproliferative disorder in which splenomegaly is usually controllable by cytotoxic therapy. However, in those patients for whom splenectomy is performed, the EMH includes megakaryocytes which are somewhat smaller than normal, with fewer nuclear lobules, identical to those in the bone marrow.

In the spleen, interaction between megakaryocyte clusters and sinusoidal endothelium may give rise to foci of peliosis (Fig. 7.7). Megakaryocyte clusters may be large enough to be detectable macroscopically as dark red nodules within the splenic parenchyma (Fig. 7.8). Such megakaryocyte tumour formation is rare at other sites of



(*a*)



(*b*)

**Fig. 7.5.** (*a*) Abundant early granulocytes within splenic red pulp, most of which have the appearances of promyelocytes and myelocytes. (*b*) Mixed population of highly atypical haemopoietic cells within splenic red pulp, including hyperchromatic megakaryocytes and a cluster of atypical erythroid cells within the sinusoid shown centrally. H&E stain.

EMH and the splenic micro-environment may somehow favour neoplastic megakaryocyte proliferation.

Occasionally, splenic EMH in chronic myeloproliferative disorders does not appear similar to the patient's bone marrow, but shows less maturation and more cytological atypia. In some cases there is complete loss of terminal differentiation, indicative of acute leukaemic transformation. In these cases, progression of disease has occurred preferentially within the spleen. Splenectomy in such cases, usually performed to relieve pain or discomfort caused by a massively enlarged spleen, does not prevent progression of the disease in the bone marrow or other sites. Myeloproliferative disorders which transform to acute leukaemia in the spleen are almost certainly going to transform elsewhere in time, if they have not done so already. It should be noted that only a minority of patients with chronic myeloproliferative disorders progress to acute myeloid or lymphoblastic leukaemia (Mackie & Shepherd, 1994). In PRV and ET, it is more common for progression to myelofibrosis, with consequent bone marrow failure, to occur.

## Systemic mastocytosis

### General features

Hepatosplenomegaly occurs in approximately half of adults presenting with systemic mastocytosis and lesser degrees of splenic involvement probably occur in the majority of cases. Macroscopically, the spleen is normal in size or moderately enlarged, with a firm texture. Parenchymal fibrosis is evident in most cases, ranging from slight accentuation of the normal delicate pattern of trabeculae to the presence of irregular, dense fibrotic patches.

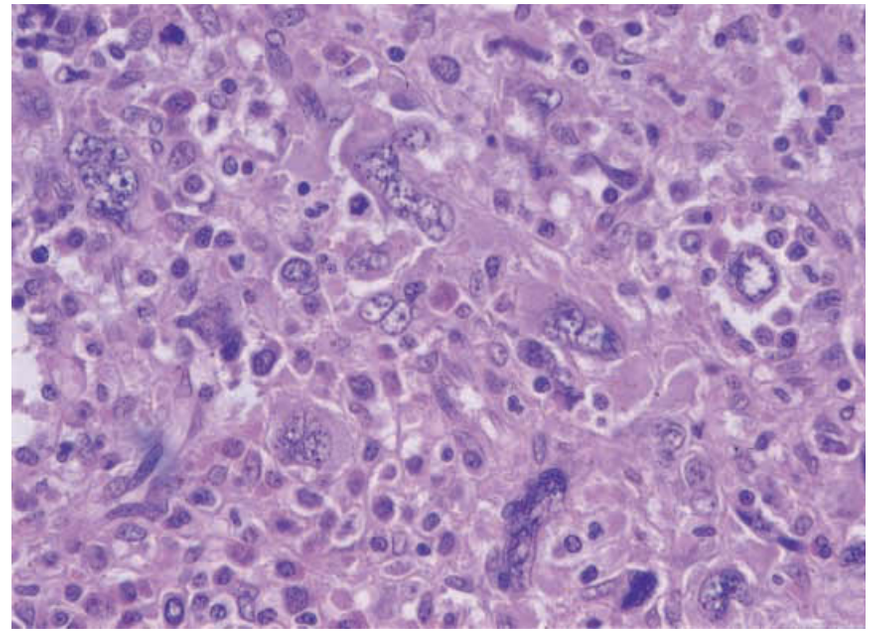


**Fig. 7.6.** Cluster of highly atypical megakaryocytes within the red pulp of a spleen from a patient with idiopathic myelofibrosis. H&E stain.

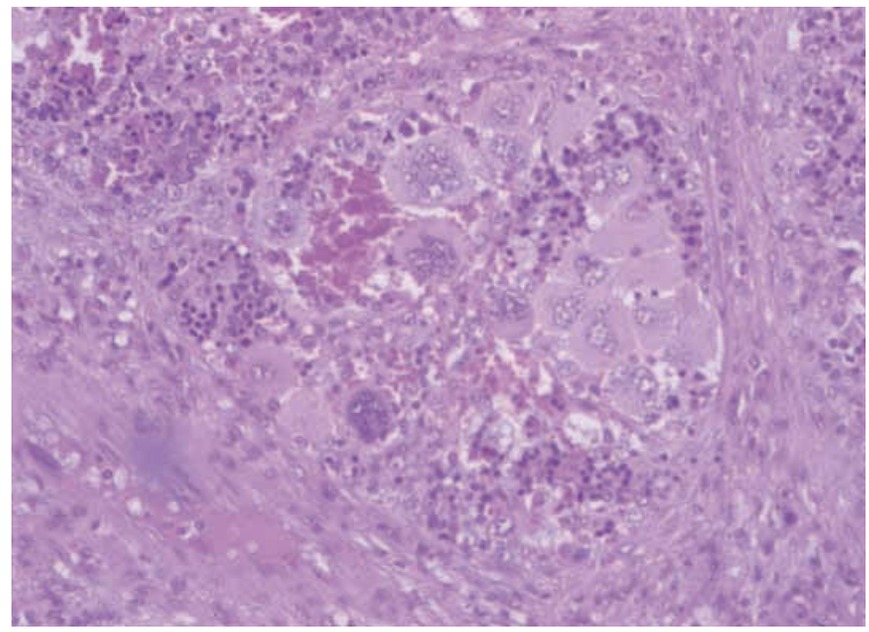


**Fig. 7.7.** Peliosis-like lesion associated with clustering of atypical megakaryocytes in the spleen of a patient with essential thrombocythaemia. H&E stain.

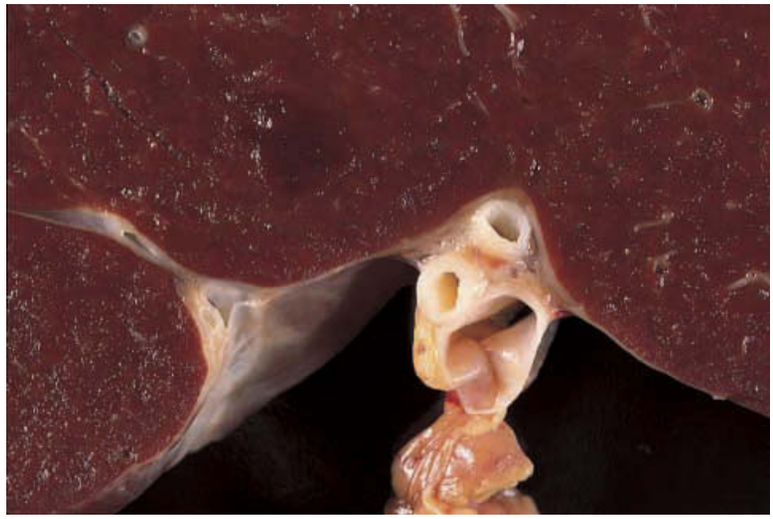


**Fig. 7.8.** Peliotic tumourlet visible macroscopically within splenic parenchyma as an ill-defined dark red nodule.

Microscopically, the hallmark of systemic mastocytosis is a sclerotic, granuloma-like lesion originating in peri-arteriolar regions or abutting connective tissue trabeculae (Fig. 7.9). Each lesion is composed of spindle cells with abundant, pale cytoplasm that may or may not contain fine basophilic granules (Fig. 7.10). Neoplastic mast cells rarely have the densely granulated cytoplasm seen in their normal counterparts. Usually their nuclei appear bland, but occasionally they are highly atypical, large and hyperchromatic. Accompanying these spindle cell infiltrates there are usually sharply demarcated collections of small lymphocytes (predominantly T cells) and scattered eosinophils. Silver staining techniques demonstrate markedly increased reticulin underlying the infiltrates, even before overt collagen fibrosis can be appreciated.

The presence of granules within the mast cells of systemic mastocytosis can be highlighted by metachromatic stains including toluidine blue, buffered thionine and Ziehl–Neelsen. Mast cell granules are also chloroacetate esterase positive. Immunohistochemistry may be misleading if the lesions are suspected to be granulomas and expression of macrophage-associated antigens is sought. Antibodies reactive with CD68, commonly employed for this purpose, will also react with mast cells. However, even the most poorly granulated cells of systemic mastocytosis can be immunostained in paraffin sections to demonstrate mast cell tryptase (Fig. 7.11). The presence of this enzyme is a highly specific marker for mast cells and monoclonal antibodies directed against it do not cross-react with macrophages. In severely fibrotic spleens from longstanding cases of systemic mastocytosis, identification of mast cell infiltrates can be very difficult without the aid of immunohistochemistry.

### Systemic mastocytosis and myeloproliferative/myelodysplastic disorders

There is an association between systemic mastocytosis, chronic myeloproliferative disorders and myelodysplastic syndromes (MDS) (Travis et al., 1989). This is not surprising, since mast cells originate from the haemopoietic stem cell, as do the cells in these other disorders. Each of these syndromes is the result of a clonal abnormality of haemopoietic stem cells. The different end-points reflect alternative patterns of altered haemopoietic differentiation resulting from different stem cell mutations.

It is uncommon for significant splenomegaly to occur in MDS but, when the two types of disease coincide, features of both MDS and systemic mastocytosis are found. As with the chronic myeloproliferative disorders described above, MDS are represented in such spleens by a red pulp infiltrate of dysplastic haemopoietic cells which resemble the mix of cells in the patient's bone marrow. Mast cell lesions are scattered throughout the spleen in predominantly perivascular and paratrabecular locations, as described above. Occasionally, the mast cell component may be more extensive and diffuse, resembling mast cell leukaemia.

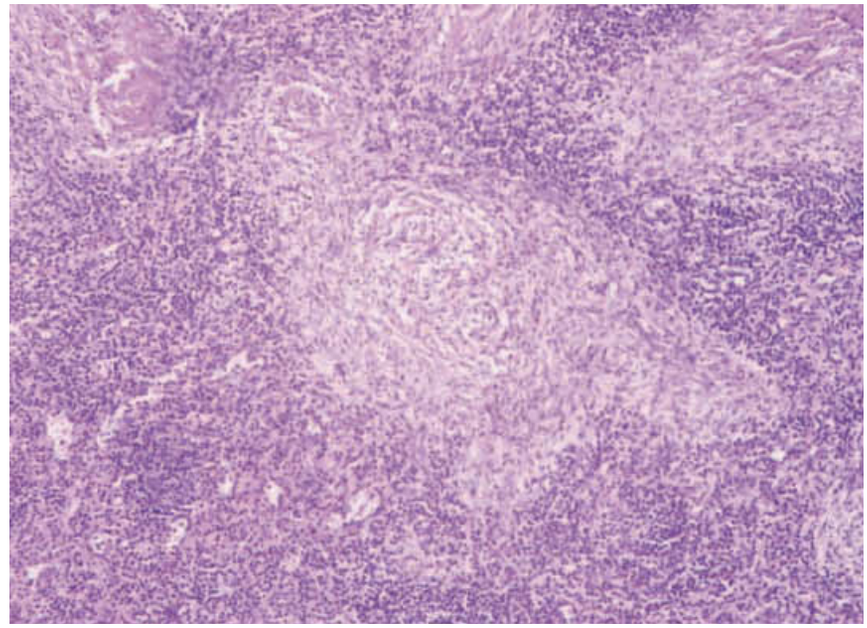


**Fig. 7.9.** Expansion of the PALS by pale, fibrotic tissue in systemic mastocytosis. H&E stain.

### Mast cell leukaemia

This may supervene as the terminal phase of systemic mastocytosis or occur without preceding symptoms of a mast cell disorder (Bain, 1999). In the spleen, leukaemic mast cell infiltrates occupy the red pulp diffusely, like any other acute leukaemia. The cells are more heterogeneous than those in uncomplicated systemic mastocytosis, with fewer spindle cells and more round or oval ones, including immature forms and blast cells. In cases evolving from systemic mastocytosis, co-existent fibrotic, spindle cell lesions will also be present.

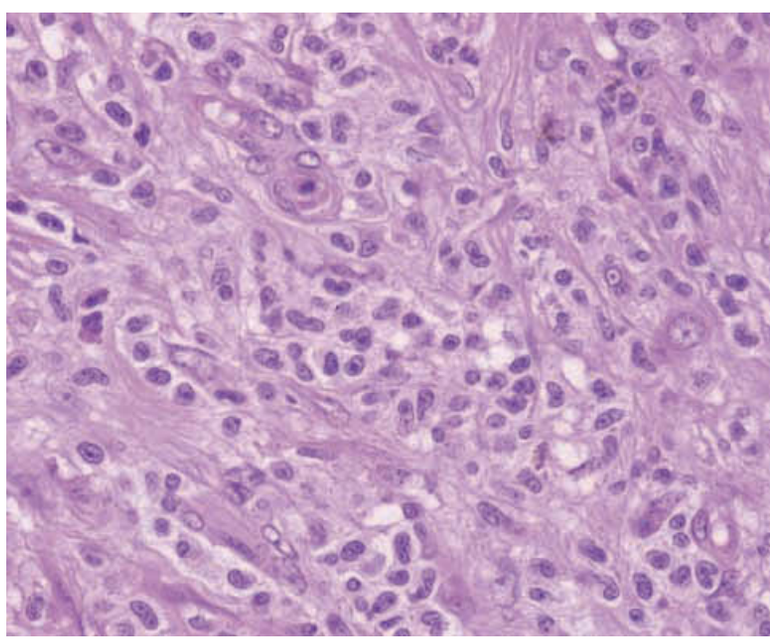
The clinical behaviour of mast cell leukaemia is variable. Some cases, with extensive blood and bone marrow involvement, follow an acute course and are appropriately treated as acute myeloid leukaemias. Other patients, usually those with few circulating mast cells, have a course more akin to a chronic myeloproliferative disorder.

## Transient abnormal myelopoiesis in association with Trisomy 21 (Down's syndrome)

A myeloproliferative condition mimicking many features of an adult chronic myeloproliferative disorder, or even acute leukaemia, may develop in the perinatal period in babies with trisomy 21 (Zipursky et al., 1997). This myeloid proliferation can be very extensive and involve a variety of extramedullary tissues, including the spleen, but it resolves spontaneously in a high proportion of cases. Criteria for a diagnosis of true acute leukaemia (which may supervene in some cases) are not entirely clear at present. Pathologists are unlikely to see such spleens due to surgical removal but they may be seen at post-mortem examination in stillborn infants or babies who die soon after birth due to other complications of the underlying trisomy.

Extramedullary infiltrates caused by this transient abnormal myelopoiesis (TAM) associated with trisomy 21 are very variable in composition. All haemopoietic cell lineages are usually represented, with at least partial differentiation to mature cells being evident. In cases which evolve into acute leukaemia, TAM typically gives rise to the rare AML-M7 subtype (acute megakaryoblastic leukaemia). The neoplastic cells in childhood AML-M7 may be scarcely, if at all, recognizable as megakaryocytes and confirmation of their nature requires demonstration of expression of platelet-associated antigens such as von

**Fig. 7.10.** High power view of the cellular infiltrate in systemic mastocytosis. Within the fibrous tissue are scattered medium-sized cells with pale cytoplasm and oval, sometimes slightly twisted nuclei. H&E stain.



Willebrand Factor, CD42b and CD61 (see Table 7.1).

## The spleen in acute myeloid and lymphoblastic leukaemias

In patients with acute leukaemias, the spleen is involved in most, if not all, cases. However, it is rarely removed for diagnostic or therapeutic purposes and is therefore likely to be examined only if the individual dies and a post-mortem examination is performed. The white pulp is generally spared. It may appear normal or atrophic, depending at least partly upon exposure of the patient to cytotoxic therapy and/or radiotherapy. The red pulp cords and sinusoids are diffusely infiltrated by blast cells. These cells have identical cytological and immunophenotypic characteristics to those in the bone marrow and blood. If immunostaining is undertaken, it should be remembered that certain antigens used commonly to characterise T cells in lymphoid proliferations are also expressed by immature myeloid cells (in particular, CD43 and the CD45RO epitope recognized by antibody UCHL-1). Table 7.1 represents a summary of antibodies which we find useful for immunohistochemical analysis of myeloid and lymphoid leukaemic infiltrates in formalin-fixed, paraffin-embedded tissues including spleen.

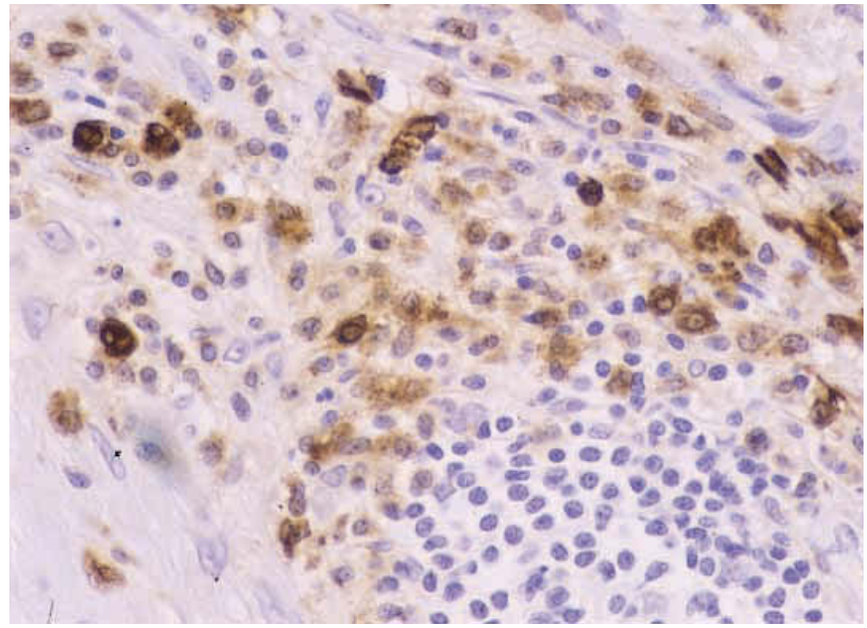


**Fig. 7.11.** Neoplastic mast cells within the lesions of systemic mastocytosis can be demonstrated by immunohistochemistry for tryptase, an enzyme associated with mast cell granules. Tryptase is demonstrable even in examples of systemic mastocytosis showing no apparent granularity by conventional metachromatic stains. Streptavidin–biotin complex immunoperoxidase stain using monoclonal antibody AA1, reactive with mast cell tryptase.

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**Table 7.1.** *Antibodies reactive with haemopoietic lineages in spleen*

| Antibody | Antigen | Gran | Mono | Eryth | Mega | Other |
|---|---|---|---|---|---|---|
| anti-1AT | A1AT | + | + | − | − | − |
| anti-Muramidase | Muramidase | + | + | − | − | − |
| KP1 | CD68 | + | + | − | ± | MP, MC |
| PG-M1 | CD68 | − | + | − | ± | MP, MC |
| NP57 | Elastase | + (early) | − | − | − | − |
| LeuM1 | CD15 | + | (+) | − | − | − |
| MAC387 | Calprotectin | + (late) | + | − | − | − |
| BRIC101 | Glyc-A | − | − | + | − | − |
| Ret40f | Glyc-C | − | − | + | − | − |
| anti-F8RAg | VWF | − | − | − | + | Endo (all) |
| Y2/51 | CD61 | − | − | − | + | − |
| mb-1 | CD79a | − | − | − | + | SmM, B cells |
| AA1 | Mast cell tryptase | − | − | − | − | MC |
| CBL419 | MBP | Eos | − | − | − | − |
| QBEnd10 | CD34 | − | − | − | − | Stem cells, Endo* |

*Key:*
Gran = granulocytes, Mono = monocytes. Eryth = erythrocytes.
Mega = megakaryocytes. MP = macrophages. MC = mast cells.
SmM = smooth muscle. Eos = eosinophils.

A1AT = alpha 1 anti-trypsin.
Glyc = glycophorin.
VWF = von Willebrand Factor.
MBP = major basic protein.
Endo = endothelium.

* CD34 is expressed weakly by sinusoidal endothelium and, depending upon fixation, expression by these cells may not be demonstrable in some cases.

*Significance*, ed. A. Bowdler, pp. 3–19.London: Chapman & Hall.

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Chapter 8

# Pathology of the splenic stroma

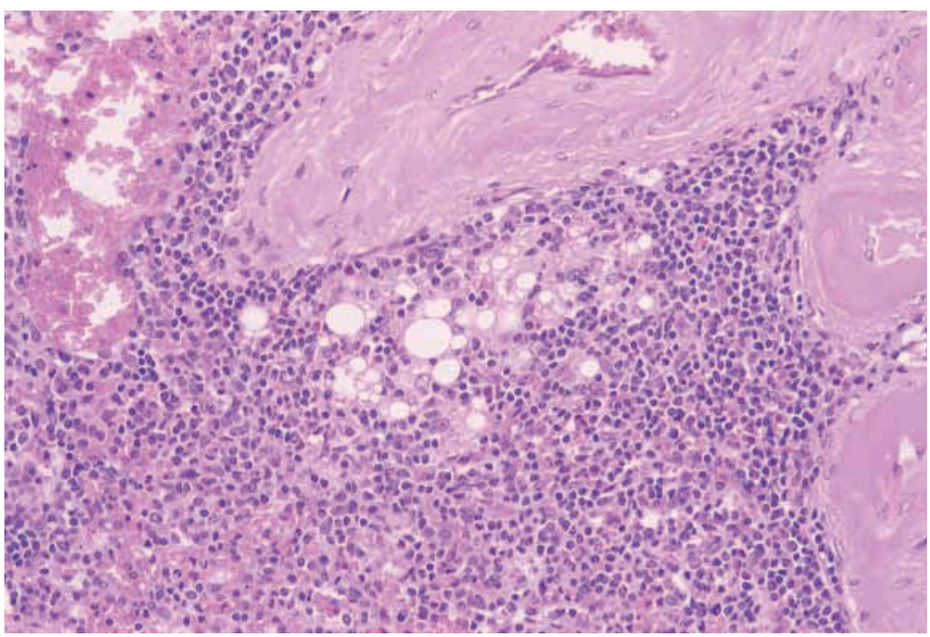
## Splenic granulomas

### 'Incidental' granulomas

Small oleogranulomas, usually located at the interface between red and white pulp, are very common incidental findings in the spleen (Fig. 8.1). Their origin is uncertain; dietary exposure to mineral oils has been suggested, or previous radiographic investigations employing lipid-based contrast media. These granulomas are of no clinical significance but they should be recognized in order to distinguish them from other, potentially important forms of granuloma and granuloma-like lesions which may occur in the spleen.

Small epithelioid granulomas may also be found incidentally. They were seen frequently in spleens removed during laparotomies performed for staging of Hodgkin's disease (HD); see Chapter 6 and Chapter 10. With the advent of computerized tomography, staging laparotomy rapidly became obsolete during the mid-1980s but unexplained, usually epithelioid, granulomas are still found in spleens removed for a wide variety of reasons.

**Fig. 8.1.** Small oleogranuloma at the edge of a splenic white pulp nodule. H&E stain.



### Significant granulomatous inflammation in the spleen

On occasions, the spleen may be massively infiltrated by sarcoid-like or caseating granulomas; sarcoidosis or mycobacterial infection should be investigated in such

cases as the obvious likely causes. Extensive sarcoid-like reaction, however, can be seen occasionally as an apparently isolated phenomenon, in the absence of any confirmatory evidence of true sarcoidosis. We have seen two patients with splenomegaly due to massive infiltration by sarcoid-like granulomas accompanied by hypogammaglobulinaemia, suggesting altered immune responsiveness to a pathogen (or other antigen) as a cause for the granulomatous response.

Tuberculosis and *M. avium-intracellulare* infection involving the spleen have been described in Chapter 5.

## Storage disorders involving cordal macrophages

### General features

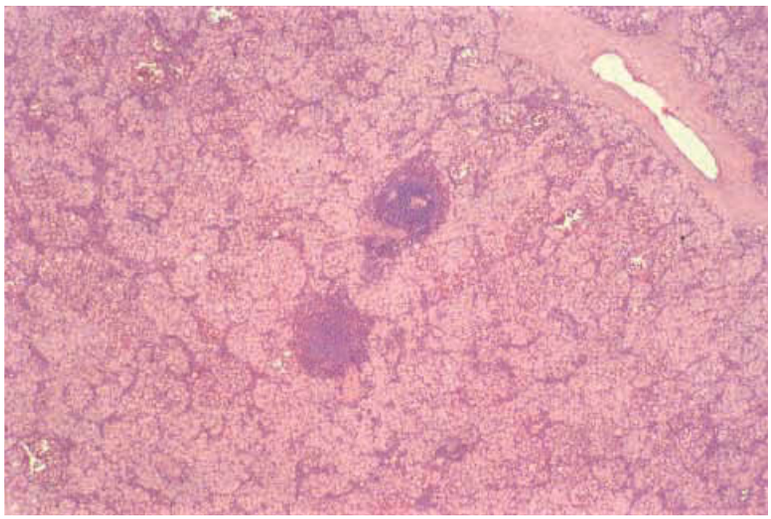
The spleen may be involved in a variety of inherited and acquired metabolic disorders resulting in impaired phagocytosis and accumulation of abnormal stored products within macrophage cytoplasm. In most inherited disorders of ganglioside, mucopolysaccharide or lipoprotein metabolism, splenic involvement is either minor or overshadowed by developmental abnormalities and/or involvement of other tissues. However, there are various circumstances in which splenomegaly or hypersplenism is the presenting feature of an unsuspected metabolic abnormality. The appearances in such cases are most likely to be those of Gaucher's disease, Niemann–Pick disease (Type C and so-called Type E) or 'ceroid histiocytosis' (sea-blue histiocyte syndrome). The degree of splenomegaly may be massive in such cases, with unremarkable macroscopic parenchymal appearances or red pulp expansion by red/orange, firm tissue, somewhat paler than normal. For further clinical and biochemical details of these and other storage disorders which may involve

the spleen, the reader is referred to specialized monographs such as those by Lake (1997) and Elleder (1994).

Microscopically, all of the storage disorders mentioned above are characterized by diffuse red pulp involvement with relatively good preservation of cordal architecture. However, many cordal macrophages lose their normal dendritic morphology and become converted into large, round or oval cells with voluminous cytoplasm. This is the predominant feature in Niemann–Pick and the ceroid histiocyte disorders. In Gaucher's disease, abundant focal, nodular macrophage aggregates are also formed which may be associated with peliosis-like lesions. Sinus endothelial cells are not affected in Gaucher's disease but may be variably abnormal in Niemann–Pick variants and in the less well-defined lipid storage disorders associated with accumulation of ceroid. Involved endothelial cells may themselves appear enlarged and foamy by light microscopy or only be detectable by recognition of stored material by electron microscopy.

The white pulp is relatively well preserved in all of these conditions, even in massively enlarged spleens. It appears structurally normal although individual white pulp nodules may be very widely separated by massive red pulp expansion (Fig. 8.2).

**Fig. 8.2.** Spleen from a patient with Gaucher's disease showing small white pulp nodules within massively expanded, pale red pulp. H&E stain.



### Gaucher's disease

This condition is due to a glucosyl ceramide degradation enzyme deficiency. Patients with Gaucher's disease develop hepatosplenomegaly and bone marrow infiltration in early adult life. Bone marrow involvement may be severe and result in cytopenias, the investigation of which leads to discovery of an abnormal spleen. Affected tissues show large macrophages with characteristic needle-like stored material which imparts a filamentous appearance to the cytoplasm as seen by light microscopy (Fig. 8.3). This appearance has been variously described as

resembling watered silk, skeins of wool or crumpled tissue paper.

In the spleen, red pulp cords are expanded by diffuse and nodular infiltrates of these Gaucher's cells. Their cytoplasmic characteristics are well seen in sections immunostained for CD68 (Fig. 8.4) and they have distinctive appearances when viewed by electron microscopy (Fig. 8.5). Perls' stain demonstrates abundant accumulated haemosiderin in many of the storage histiocytes, a finding which contrasts with Niemann–Pick disease and the other storage diseases most likely to be encountered in spleens. Haemosiderin accumulation in cordal macrophages does occur in the latter disorders but is generally much less pronounced than in Gaucher's disease.

The diagnosis of Gaucher's disease requires confirmation by enzymology, which is performed on peripheral white blood cells. 'Pseudo-Gaucher' cells in a variety of haematological conditions overlap with so-called 'sea blue histiocytes' and may have features indistinguishable from true Gaucher's cells or resemble Niemann–Pick histiocytes (Howard & Kesteven, 1993; Schaefer, 1996). The pseudo-Gaucher terminology is probably best reserved for those cells which show definite filamentous cytoplasmic appearances, using the less specific term 'ceroid histiocytes' to describe those more reminiscent of Niemann–Pick cells.

### Niemann–Pick disease

There are several clinical patterns of Niemann–Pick disease. Type A is early in onset and has severe effects upon the central nervous system as well as involving the liver, spleen and other viscera. Type B, also early in onset, involves the visceral organs without any neuropathic features. Patients with either of these forms of Niemann–Pick disease usually have hepatosplenomegaly at diagnosis, which may be

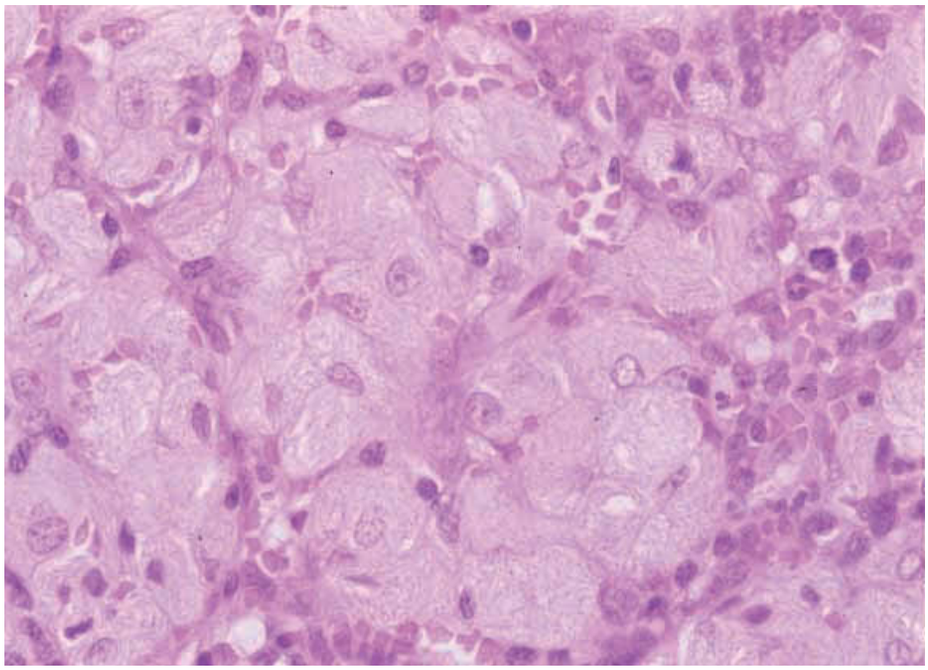


**Fig. 8.3.** Splenic red pulp macrophages in Gaucher's disease showing their abundant, pale cytoplasm. A skein-like appearance can be seen within the cytoplasm of some cells, sometimes described as resembling watered silk. H&E stain.

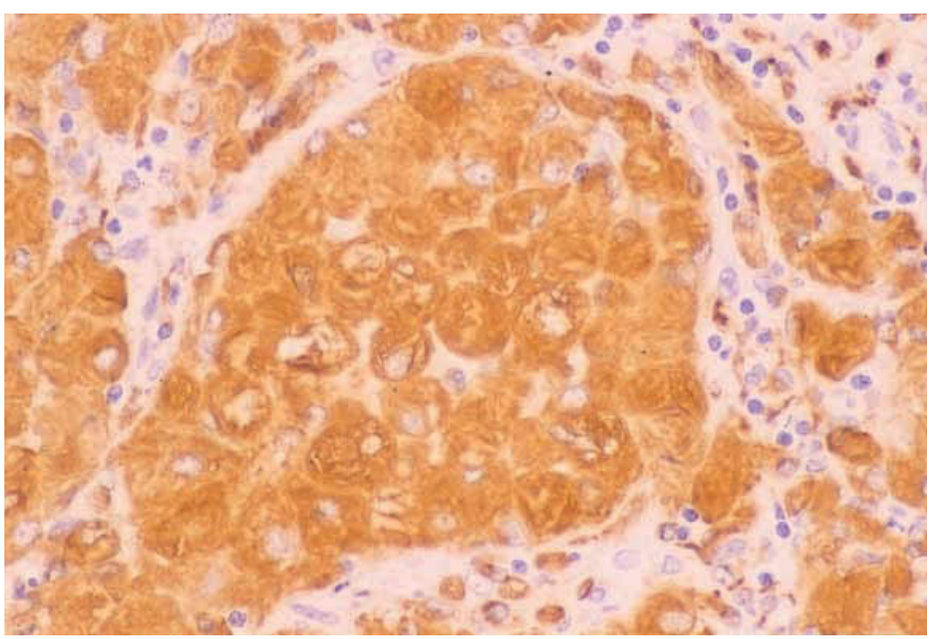


**Fig. 8.4.** Macrophages in Gaucher's disease immunostained for CD68. The cytoplasmic characteristics of the Gaucher's storage cells are highlighted by this stain. Streptavidin–biotin complex immunoperoxidase technique.

massive, but splenectomy is rarely perfomed since the overall spectrum of changes suggests the diagnosis.

Niemann–Pick disease Type E is currently thought to be a variant of Type B, presenting later in life and more insidiously. Individuals with this variant are more likely than patients with classical Type B Niemann–Pick disease to undergo splenectomy for unexplained splenomegaly. The most significant adult type to be discovered in the context of unexplained splenomegaly, however, is Niemann–Pick disease Type C. This is associated with delayed, but none the less progressive, neurological dysfunction and consequently its correct identification has major clinical importance.

The microscopic abnormalities in spleens of patients with Niemann–Pick disease of Types B, C and E are similar, differing mainly in extent. Cordal macrophages appear swollen and finely vacuolated (Fig. 8.6). Varying numbers of ceroid-containing macrophages are also present (see below for the characteristics of ceroid). Small clusters of similar foamy or ceroid-filled cells may be present in addition to diffuse involvement of cordal macrophages, but nodular accumulations are less prominent than those in Gaucher's disease. Endothelial cells may be affected by the same metabolic abnormalities as the macrophages but detection of abnormal stored material in the cytoplasm of these cells by light microscopy is difficult. By electron microscopy, cytoplasmic inclusions within affected cells are a heterogeneous mixture of loosely and densely packed lipid lamellar bodies, some with solid cores (Figs. 8.7 and 8.8).

The light and electron microscopic appearances of Niemann–Pick disease in the spleen overlap considerably with those seen in ceroid histiocytoses and secondary lipid storage disorders. Consequently, definitive diagnosis can rarely be made by the histopathologist in the absence of other clinical or biochemical data.

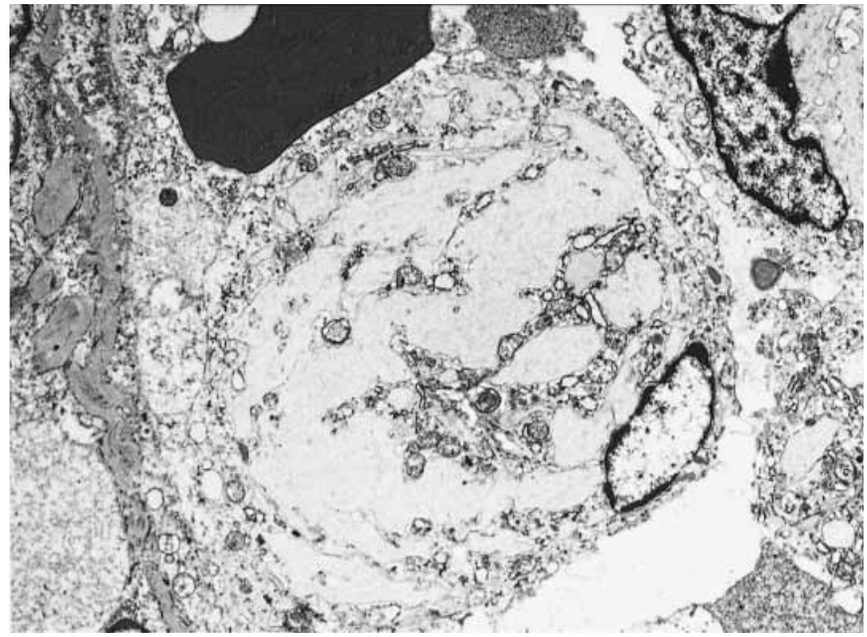
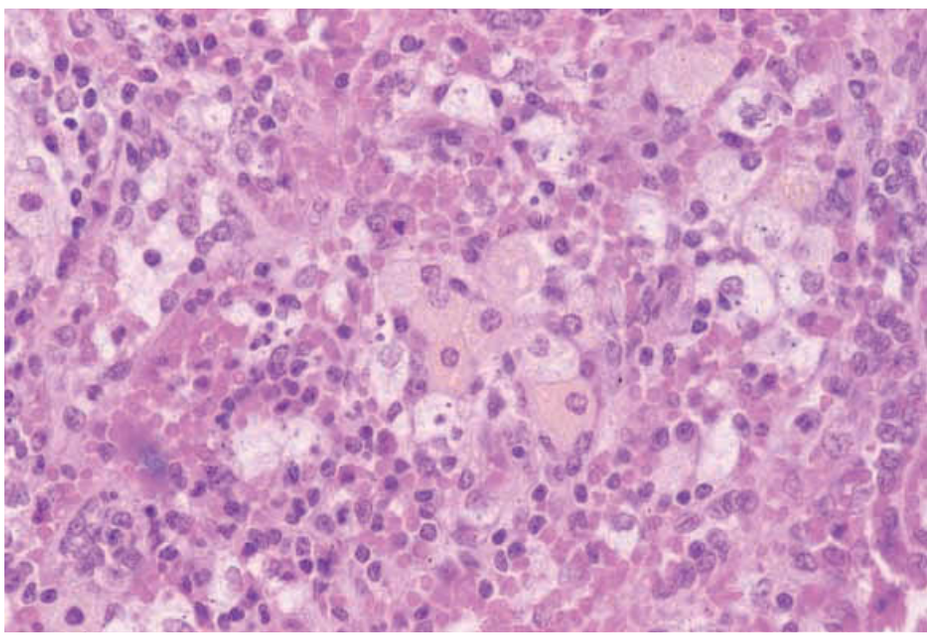


**Fig. 8.5.** Electron micrograph showing the characteristic appearance of a Gaucher's cell.

**Fig. 8.6.** Foamy cordal macrophages from the spleen of a patient with Niemann–Pick disease Type C. The cells form clusters and ribbons, but these are usually less pronounced than in Gaucher's disease. Cytoplasm within Niemann–Pick macrophages is foamy and vacuolated rather than filamentous, and deposition of ceroid imparts a yellow colour to a proportion of the cells. H&E stain.



The diagnosis of Niemann-Pick Types A and B can be confirmed by measurement of deficient lysosomal sphingomyelinase activity in peripheral white blood cells. Type C disease, however, has a different biochemical basis. It is due to an, as yet, incompletely characterized cholesterol esterification defect, demonstrable by specialized assay of cultured fibroblasts but not by peripheral blood enzymology (Morris & Carstea, 1998).

In a recent revision of terminology (see Lake, 1997), Group I Niemann–Pick disease has been defined as including all sphingomyelinase-deficient forms (including Types A and B). Type C Niemann–Pick disease and at least some cases of ceroid histiocytosis are categorized within Group II (normal sphingomyelinase activity).

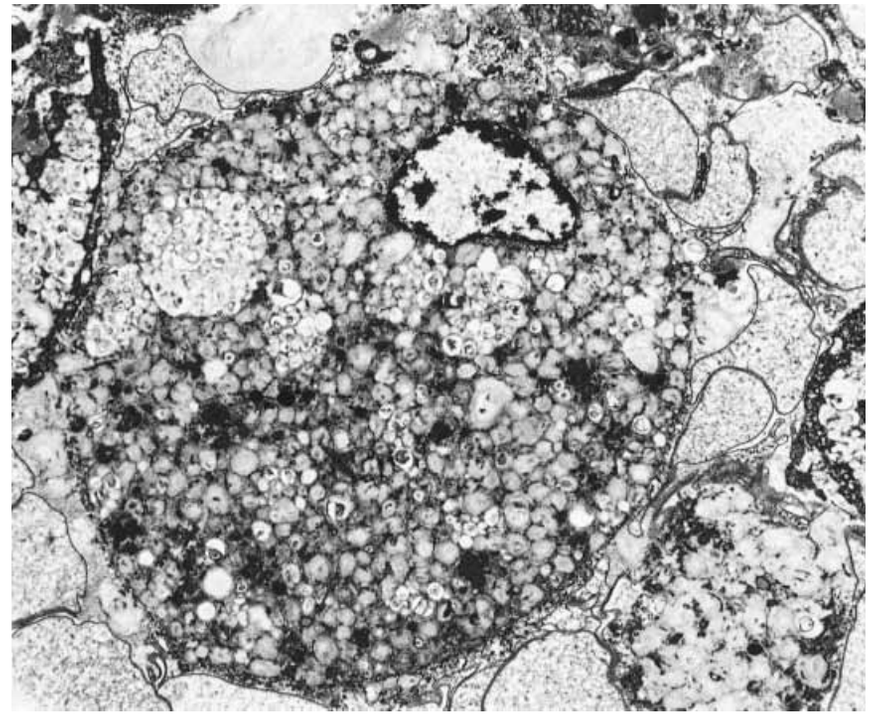
### 'Ceroid histiocytosis'

This term describes a variety of abnormalities of macrophage storage known to haematologists as sea-blue histiocyte syndromes. The terminology reflects cytoplasmic staining characteristics of the storage cells in cytological preparations using May–Grünwald Giemsa (Howard & Kesteven, 1993). These are heterogeneous disorders, many of which are of uncertain clinical significance, characterized by abnormal storage of various lipid-derived products, all resulting in similar cytological appearances. They may be primary or secondary (e.g. to high cell turnover states and some drugs). Ceroid, or lipopigment, is a substance having a complex lipid and carbohydrate composition, characterized by a yellowish, finely granular appearance in H&E-stained sections and varying degrees of PAS positivity (Fig. 8.9). In electron microscopic preparations, ceroid is dense, darkly staining material which forms a variety of intracytoplasmic inclusions, some of which resemble myelin figures.

The number of ceroid-containing cells varies widely in the red pulp of spleens

**Fig. 8.7.** Electron micrographs showing characteristic appearances of Niemann–Pick storage macrophages. Accumulation of ceroid (*b*) is reflected in an increased content of osmiophilic inclusions within the cytoplasm and hence darker staining.

(*a*)



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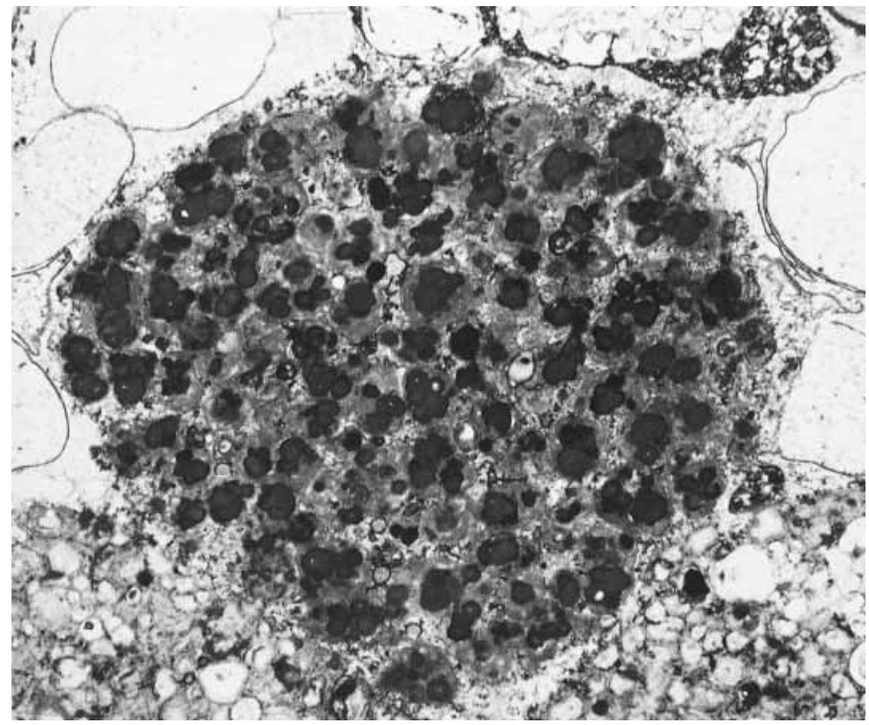

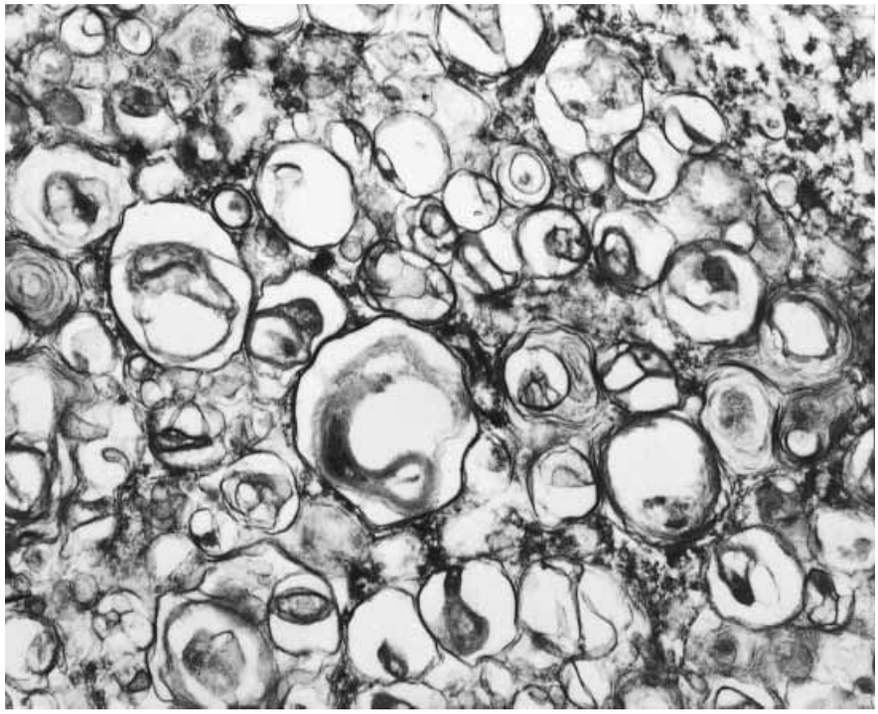

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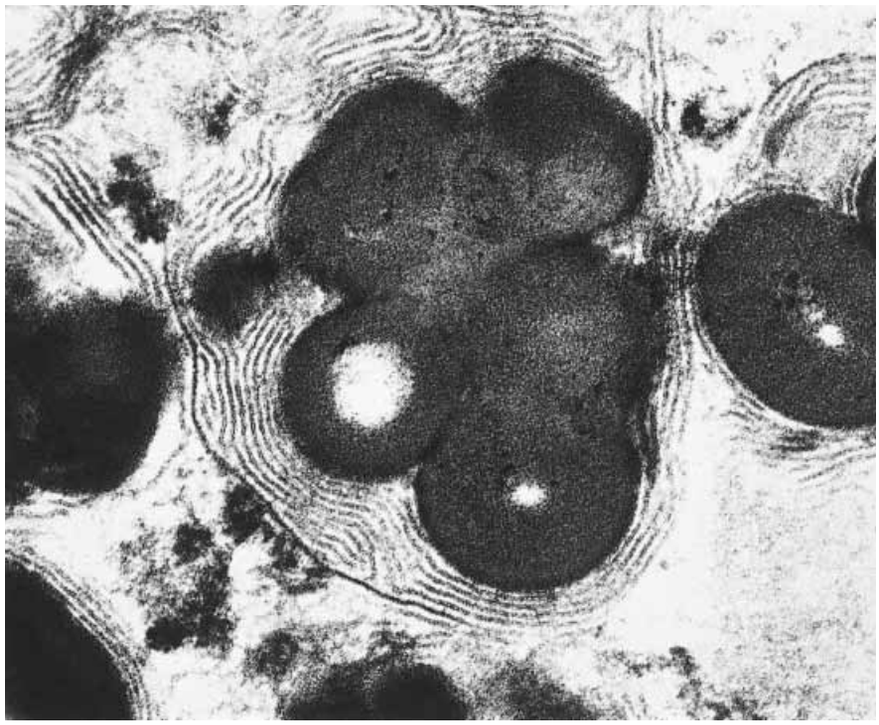
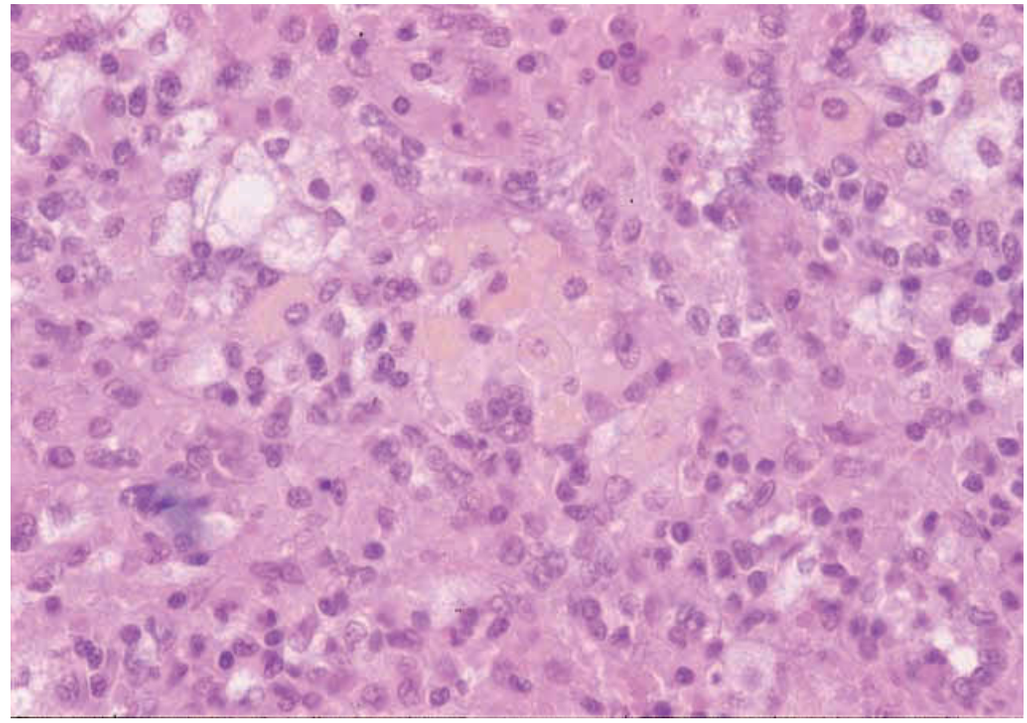
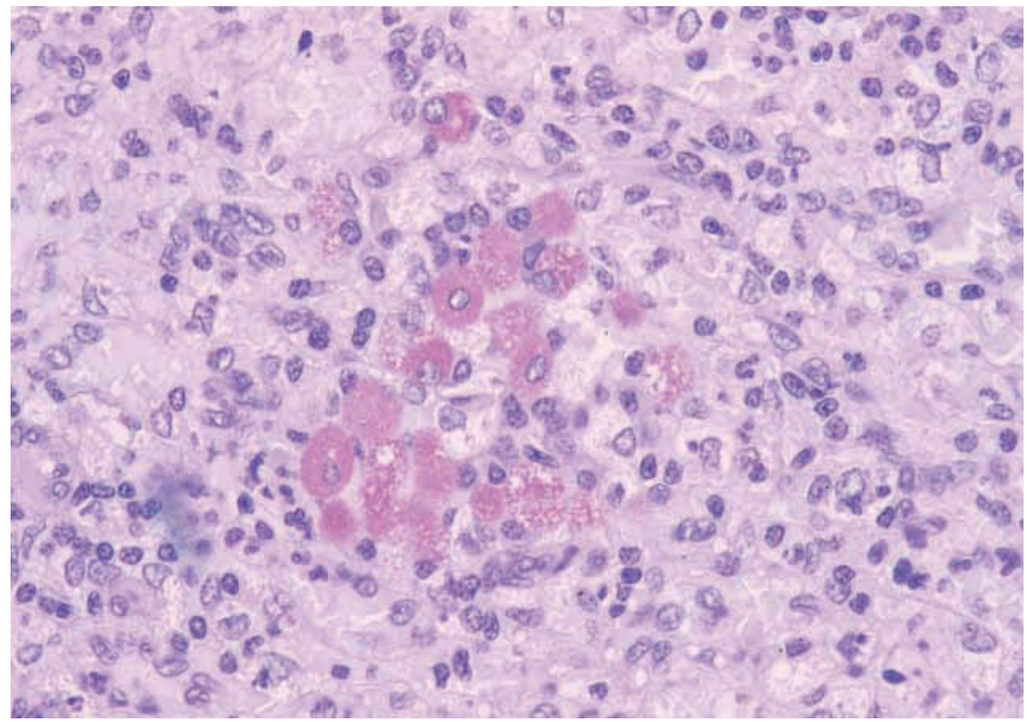


(*b*)

**Fig. 8.8.** High power electron micrographs to show the appearances of inclusions within Niemann–Pick cells. These range from loose lamellar bodies (*a*) to complex dense inclusions with concentric lamellae present peripherally (*b*).



(*a*)



(*b*)

**Fig. 8.9.** (*a*) Ceroid-containing macrophages; appearances in an H&E stained section. The presence of ceroid imparts a dull yellow colour to macrophage cytoplasm. (*b*) Ceroid-containing macrophages are variably PAS positive, in proportion to their content of lipopigment.

representing these disorders. Most of the cordal macrophages present are indistinguishable from the pale foam cells of Niemann–Pick disease; as mentioned above, accumulation of ceroid is a marker of chronicity rather than a specific correlate of one particular underlying metabolic defect.

The main importance for pathologists in recognizing the morphological features of ceroid histiocytosis is to distinguish patients with clinically insignificant or indeterminate syndromes from those who may have variants of Niemann–Pick disease. The latter obviously require additional investigations and careful follow-up.

### Secondary storage disorders

Occasional patients with idiopathic thrombocytopenic purpura (ITP) may have splenic features very similar to those of Niemann–Pick disease or ceroid histiocytosis, as discussed in Chapter 4. No data exist to confirm or exclude the possibility that this subset of ITP patients might have a *forme fruste* of a storage disorder due to inherited enzyme abormality. However, the assumption is usually made that the phagocytic function of cordal macrophages has been overwhelmed by the huge quantities of antibody-coated platelets presented to the spleen.

Helpful features suggestive of such a secondary storage disorder include lack of endothelial involvement. This is in keeping with the view that there is no inherited enzyme deficiency in these patients, only saturation of their macrophages' ability to eliminate platelet-derived lipoproteins. Also, absent or scanty ceroid deposition in foamy macrophages favours a secondary storage abnormality. As stated above, ceroid accumulation seems to correlate with chronicity and the secondary disorders develop relatively rapidly due to phagocytic overload. This contrasts with primary, inherited disorders in which normal levels of demand

for macrophage activity slowly generate enough abnormal stored product to become apparent. Lastly, tingible body macrophages in white pulp nodules are spared in secondary storage disorders, since they are not directly involved in red pulp phagocytic activity. Unfortunately, this feature may not be observed in cases in which, due to steroid treatment, germinal centres are atrophic or absent.

## Langerhans' cell histiocytosis

The term Langerhans' cell histiocytosis (LCH) describes several related but clinically distinct syndromes, characterized by unifocal or multifocal proliferation of Langerhans' cells (Cline, 1994). The normal counterparts of these cells are the Langerhans' cells of the skin and interdigitating reticulum cells of lymphoid tissues. There has been long-standing controversy regarding the reactive or neoplastic nature of Langerhans' cell proliferations. Although incidental small foci of possibly reactive LCH are not uncommon in a variety of body tissues (including the spleen), accompanying diverse pathologies, molecular genetic studies suggest that the LCH syndromes (with unifocal or multi-focal organ involvement) are neoplastic clonal proliferations of Langerhans' cells (Nezelof & Basset, 1998; Willman et al., 1994).

The spleen is involved in multifocal, rapidly progressive, multisystem LCH, which usually arises in childhood. It may also be a major or presenting site of involvement in adult LCH, usually associated with relatively indolent clinical behaviour.

Splenomegaly may be massive and red pulp is the predominant site of involvement. The cords are diffusely infiltrated by typical Langerhans' cells, accompanied by focal nodular collections of the same cells. These focal collections may be associated with peliosis-like changes in adjacent sinusoids (Fig. 8.10). As at other sites in the

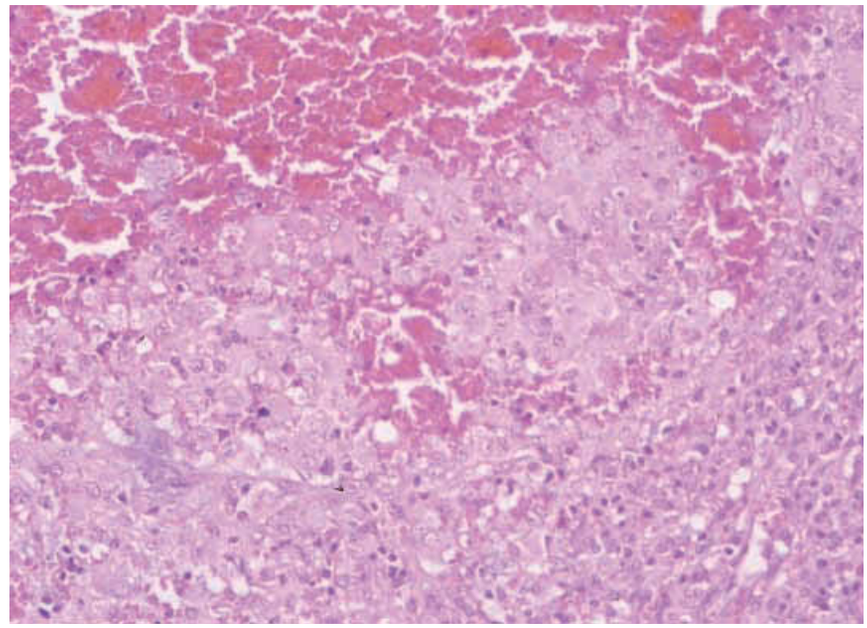


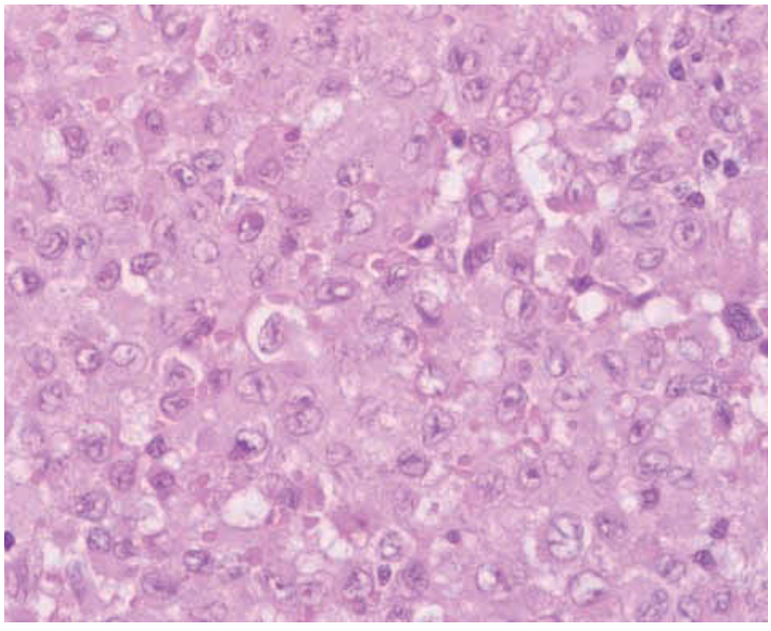
**Fig. 8.10.** Langerhans' cell histiocytosis involving splenic red pulp, giving rise to a peliosis-like blood lake. H&E stain.

body, the number of reactive eosinophils associated with the infiltrating Langerhans' cells is very variable and there may be cytological atypia within the Langerhans' cell population. White pulp structures remain intact unless the degree of splenic involvement is severe, in which case they may become progressively eroded.

The cells of LCH may resemble macrophages superficially but their characteristic elongated nuclei, often twisted and/or with a prominent linear groove, are quite distinctive (Fig. 8.11). If the appearances are in doubt, immunohistochemical staining of paraffin sections can be used to demonstrate that Langerhans' cells are CD1a and CD43 positive, strongly positive for S100 protein and only weakly CD68 positive. This pattern of reactivity is different from that of most macrophages, which are CD1a negative, strongly CD68 positive and variably reactive with antibodies directed against CD43 and S100 protein.

Ideally, the diagnosis of LCH should be confirmed by electron microscopy to demonstrate the presence of Birbeck granules within the cytoplasm of neoplastic Langerhans' cells (Fig. 8.12).

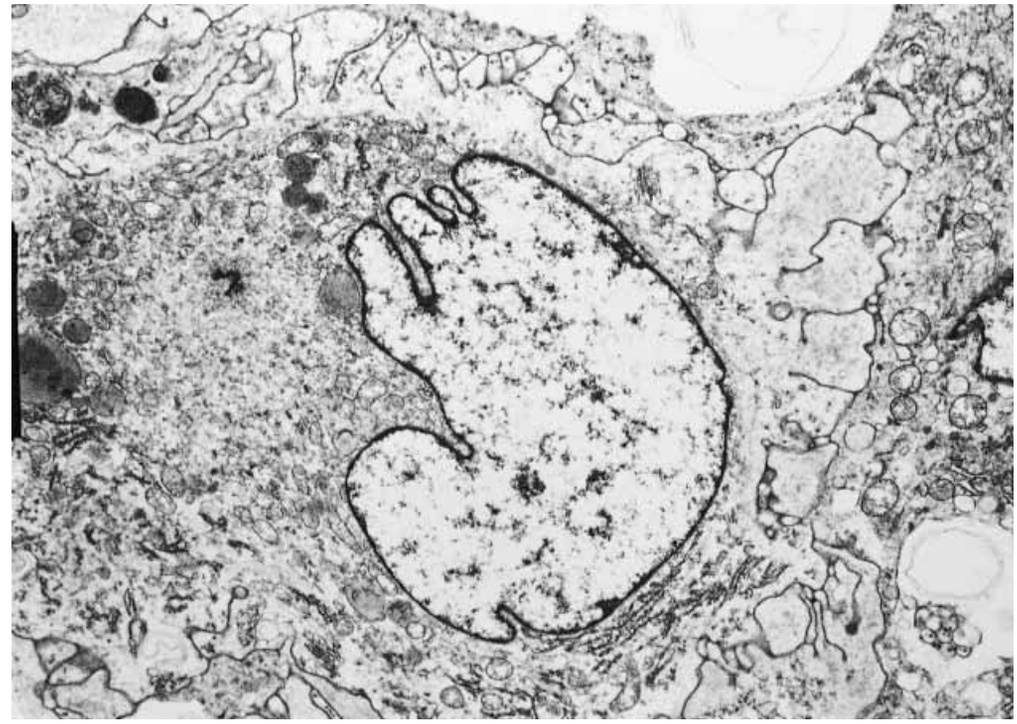
**Fig. 8.11.** High power view of the cellular infiltrate in Langerhans' cell histiocytosis. The cells have abundant cytoplasm and open nuclear chromatin. Nuclei may show longitudinal grooving and often have a complex outline. H&E stain.



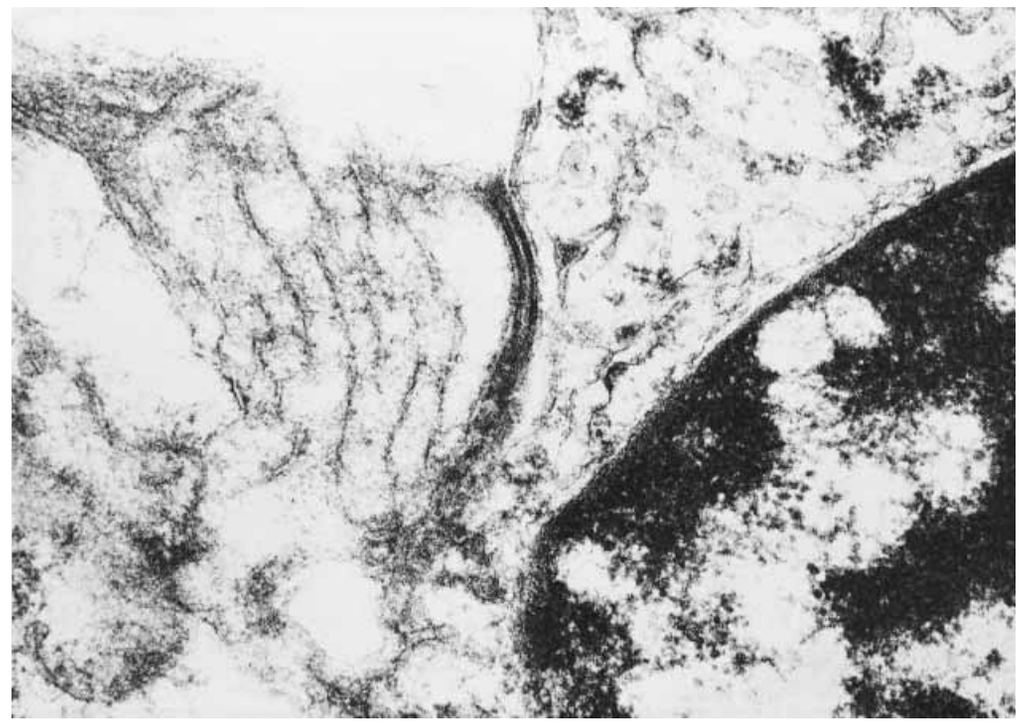
## Splenic pseudotumours and true stromal tumours

### Inflammatory pseudotumour

Splenectomy is occasionally undertaken due to rapid growth of a large tumour with clinical and radiological features suggestive of intermediate or high grade non-Hodgkin's lymphoma. The patients are usually middle-aged or older adults with few symptoms other than those directly referrable to their abdominal mass (pain, abdominal swelling, etc.). Ultrasonography or computed tomography shows a well circumscribed, nodular mass within the splenic parenchyma, usually solitary but



(*a*)



(*b*)

**Fig. 8.12.** Electron micrographs showing (*a*) the appearance of a single Langerhans' cell from LCH involving the spleen and (*b*) a Birbeck granule from the same case.

occasionally multiple. On macroscopic examination, such tumours may be 10 cm or more in diameter with a pale, whorled cut surface. Foci of haemorrhage and necrosis may be present. Microscopically they are composed of bundles of spindle-shaped myofibroblastic cells with a variable admixture of acute and chronic inflammatory cells, including macrophages and plasma cells (Fig. 8.13). The myofibroblastic proliferation is monomorphous with no significant cytological atypia and scant mitotic activity.

The true nature of these lesions, for which the somwhat unsatisfactory term inflammatory pseudo-tumour is used currently, is not known (Delsol et al. 1998) but splenectomy is curative.

### Haemangioma, lymphangioma and diffuse angiomatosis

Focal (single or multiple) and diffuse examples of haemangiomas and lymphangiomas have been described in the splenic parenchyma (Arber et al., 1997; Ruck et al., 1994; Schmid et al., 1991). They may represent small, incidental lesions or produce significant splenomegaly and pre-dispose to splenic rupture. These tumours are rare and resemble their counterparts in other organs. The vascular spaces are usually irregular and cavernous in nature, containing abundant red cells (haemangioma) or proteinaceous fluid (lymphangioma). Lymphangiomas show preferential involvement of the capsule and trabecular components of the spleen. Extensive cavernous haemangiomas are occasionally associated with anaemia, thrombocytopenia and coagulation deficits due to sequestration and/or destruction of blood components within the tumours.

Endothelial cells in most of these lesions vary in shape from flattened to cuboidal and express CD34, von Willebrand Factor and *Ulex europaeus* lectin but not CD8. This immunophenotype is indicative of origin

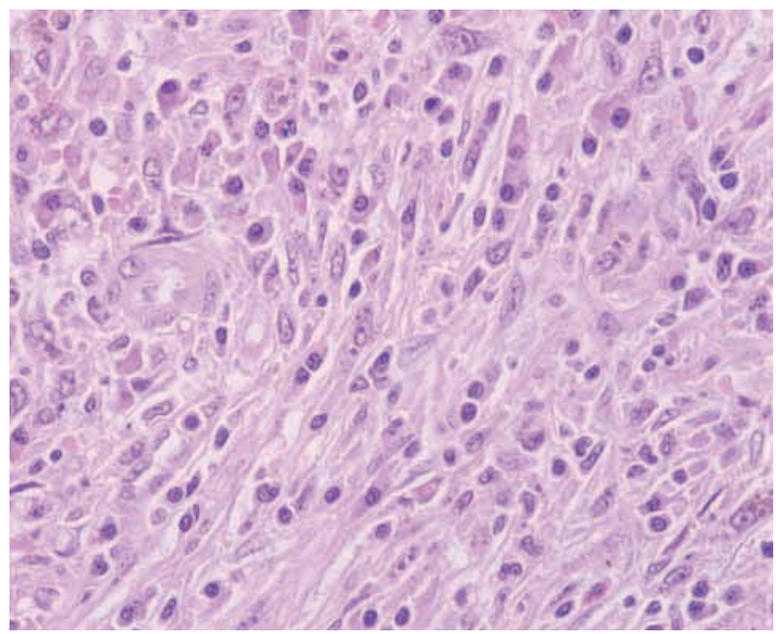
from non-sinusoidal endothelium. The most extensive examples, involving almost all of the splenic parenchyma, have been categorized as cases of diffuse angiomatosis. Origin from sinusoidal endothelium (CD8 positive) has not been specifically excluded in most reported cases of diffuse angiomatosis and there may be overlap with littoral cell angiomas.

### Littoral cell angioma, angiosarcoma and malignant spindle cell tumours

Angiomas with morphological and immunophenotypic characteristics of origin from sinusoidal endothelium are often referred to as littoral cell angiomas (Falk et al., 1991). Solitary or multiple haemorrhagic nodules are seen macroscopically within the splenic parenchyma. Histologically, these are poorly demarcated tumours, resembling clusters of closely packed, somewhat haphazard, sinusoids and merging with normal sinusoids at their periphery. In small tumours, narrow cordal structures separate some of the individual vascular channels but, with increasing size, the tumours become more solidly vascular. The endothelial cells are usually plump and cuboidal, occasionally with 'hob-nail' appearances (Fig. 8.14). They express CD8, typical of splenic sinusoidal endothelium. Papillary projections of endothelial cells may protrude into the vascular spaces. Vascular lumens also contain abundant macrophages (sometimes exhibiting erythrophagocytosis). Mild cytological atypia may be present, and the distinction between littoral cell angioma and low grade angiosarcoma can be difficult in individual cases.

Angiosarcomas of the spleen are rare, multifocal proliferations (Fig. 8.15), heterogeneous in morphology and clinical behaviour (Falk et al., 1993). In areas they may appear benign, resembling littoral cell angioma, while elsewhere they contain areas

**Fig. 8.13.** Inflammatory pseudo-tumour of the spleen showing a mixture of spindle cells and inflammatory cells, including scattered plasma cells. H&E stain.



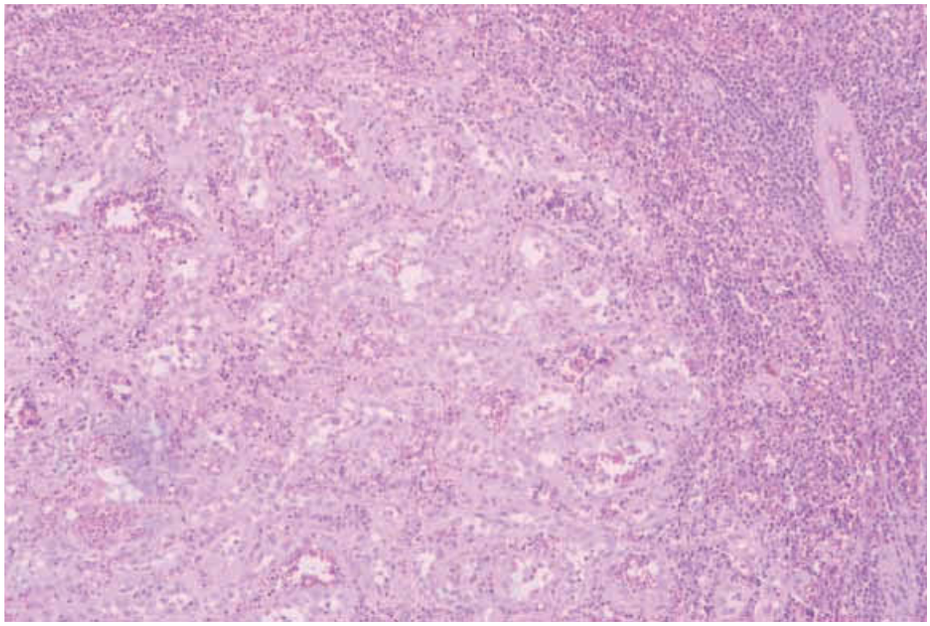


**Fig. 8.14.** Littoral cell angioma of the spleen. Vascular channels within the angioma are lined by plump endothelium having 'hobnail' appearances. H&E stain.

**Fig. 8.15.** Angiosarcoma of the spleen showing multiple, irregular, haemorrhagic nodules throughout the splenic parenchyma.

of unequivocal malignancy resembling high grade angiosarcoma at other sites (Fig. 8.16). Expression of CD8 is weak or absent in the areas which appear most malignant. However, the presence of CD8 positivity in transitional areas of low grade malignancy and benign-appearing areas in the same tumours supports the concept that these angiosarcomas arise from sinusoidal endothelium (Fig. 8.17). Unlike hepatic angiosarcomas, there is no association between the development of splenic angiosarcomas and exposure to thorium dioxide-based radiographic contrast medium (Thorotrast). The spleens in patients historically exposed to Thorotrast are small and densely fibrotic (see Chapter 9).

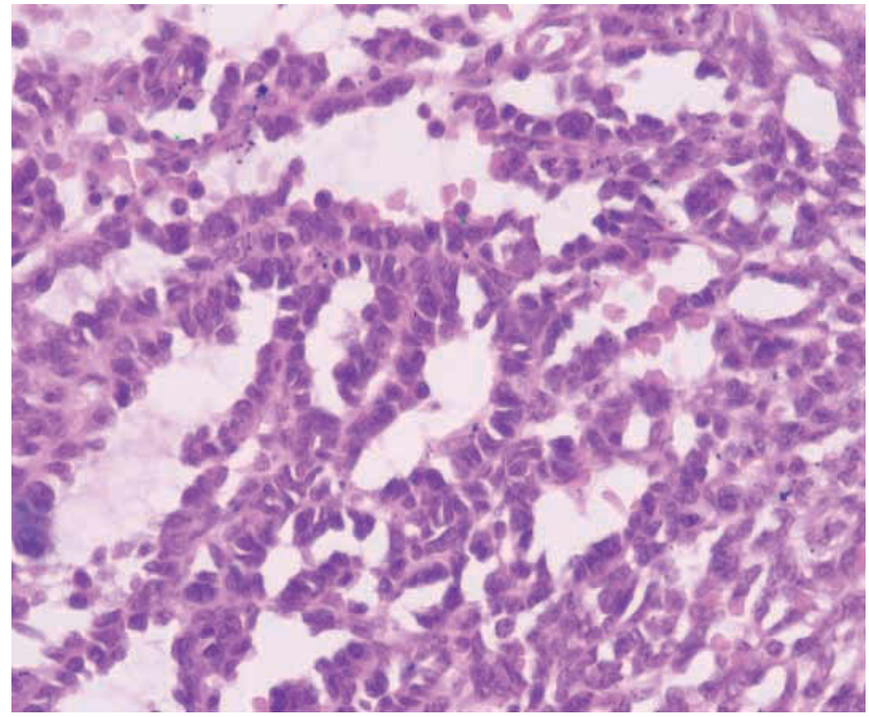
High grade angiosarcomas in the spleen usually contain areas with spindle cell morphology and little evidence of vascular differentiation. Occasional malignant neoplasms of the spleen have been described with pure spindle cell composition; these have variously been regarded as examples of malignant fibrous histiocytoma or solid angiosarcomas (Wick et al., 1982). A single case showing rhabdomyosarcomatous differentiation has been reported (Feakins & Norton, 1996). Solid malignant tumours with the characteristics of Kaposi's sarcoma have also been described in the spleen, not all of them in patients with AIDS or other immunodeficient states. In AIDS patients, the spleen may be involved as one site of disseminated Kaposi's sarcoma.

## Peliosis of the spleen

Peliosis is a rare condition, of unknown aetiology, in which ectatic sinusoids and blood-filled cysts are present throughout the organ (Garcia et al., 1982; Kohr et al., 1993). Its clinical importance lies in its association with spontaneous splenic rupture, due to a combination of spleen enlargement and fragility of the dilated, cystic sinusoids.

**Fig. 8.16.** (*a*) Relatively low-grade area within a splenic angiosarcoma showing dilated vascular spaces lined by atypical sinusoidal endothelial cells. (*b*) Area of high-grade malignancy within the same tumour, composed of malignant spindle cells forming irregular vascular channels. H&E stain.

(*a*)



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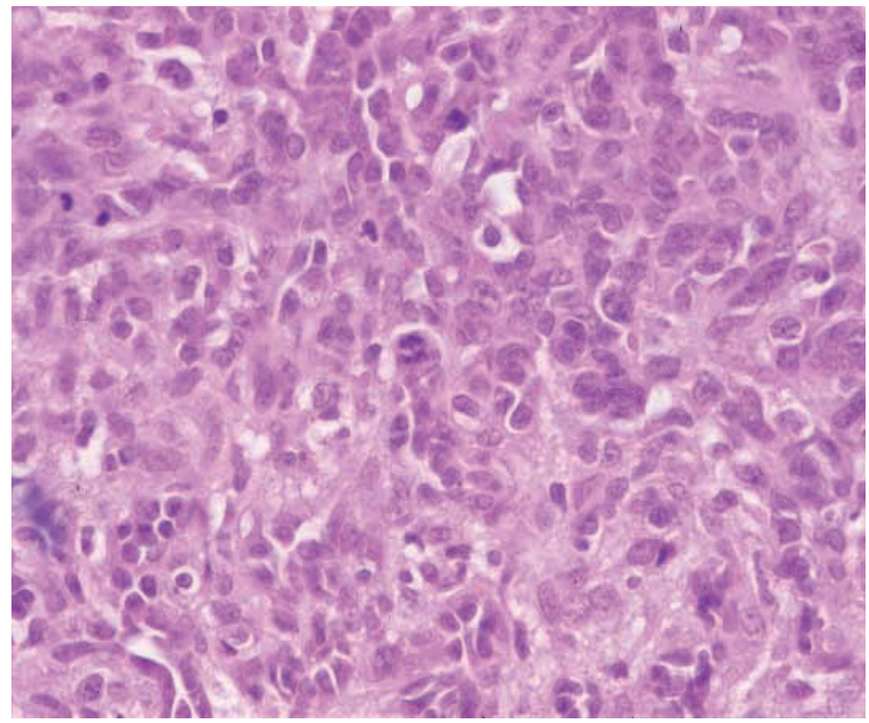


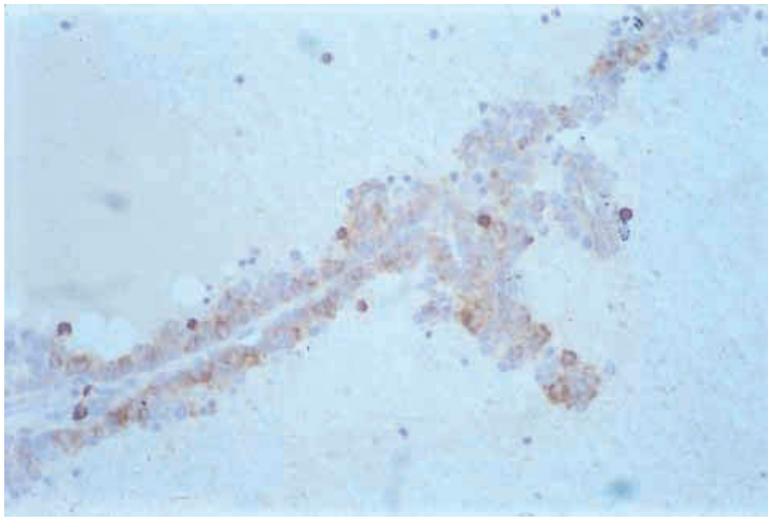


**Fig. 8.17.** Immunostaining for CD8 in low-grade components of splenic angiosarcoma supports origin of these tumours from sinusoidal endothelium. This photograph was taken from another area within the tumour illustrated in Fig. 8.16. Streptavidin–biotin complex immunoperoxidase technique.

**Fig. 8.18.** One of several fragments from a spleen removed piecemeal following spontaneous rupture due to underlying peliosis in a patient treated with oral anticoagulation.

We have also seen one case in which rupture of a peliotic spleen was precipitated by oral anti-coagulation with warfarin (Fig. 8.18).

Splenic peliosis may accompany hepatic involvement by the same process (approximately half of reported cases) or may occur in isolation. Involvement of other tissues, such as bone marrow, lymph nodes and kidneys has also been described. In one autopsy series, isolated splenic peliosis was found in 8 of 1200 consecutive post-mortem examinations (Tada et al., 1983) but most studies have suggested a lower prevalence. The condition is more common in men than in women and has been reported in association with a variety of chronic diseases including tuberculosis and HIV infection. However, splenic peliosis in individual patients has also been associated with haematological malignancies, exposure to androgenic steroids and use of oral contraceptives. Immune-complex attack directed at, or causing bystander injury to, sinusoidal endothelium has been proposed recently as a cause for peliosis (Gugger & Gebbers, 1998), but other theories include congenital malformation or varicose dilation of sinusoids and toxic necrosis of endothelium. It is not known whether the underlying mechansisms are the same as those causing focal peliosis-like changes and sinusoidal damage in spleens infiltrated by hairy cell leukaemia (see Chapter 6).

The cysts of splenic peliosis are usually less than 2 cm in diameter and are preferentially located immediately adjacent to the PALS or in the peri-follicular zone (Fig. 8.19). Here they form crescent-shaped spaces at the interface between red and white pulps, filled with blood cells, macrophages and desquamated endothelial cells. This distribution of cysts is best seen in spleens with relatively modest involvement. In more extensively involved spleens, cysts are also present throughout the red pulp, with an apparently random distribution.

Smaller cysts have a lining of sinusoidal endothelium but in larger ones this is often absent, presumed to have been destroyed as the cysts have enlarged. Individual cysts may also become obliterated by secondary thrombosis, leaving irregular fibrous scars. The white pulp is relatively normal in most cases, although it may become atrophic in spleens massively involved by peliosis.

## Splenic vascular hyalinosis

With age, many of the penicillar arteriolar branches develop irregular, eosinophilic areas of hyaline thickening (Fig. 8.20). The cause of this change is unknown and it is of no clinical significance. It is unrelated to systemic hypertension and it is not uniquely seen in older individuals, as it can occasionally be seen in perinatal specimens. The deposited material has tinctorial characteristics of fibrin or fibrinoid, reacting distinctively with trichrome stains such as Martius Scarlet Blue (MSB). It should not be misinterpreted as amyloid; it stains only weakly with Congo Red and lacks apple-green birefringence.

## Portal hypertension and the spleen

Portal hypertension may be both a cause and effect of splenic enlargement. Liver disease with portal vein obstruction (which may or may not be associated with cirrhosis) impedes splenic venous drainage and will produce alterations in the spleen over time, secondary to portal hypertension. On the other hand, a massively enlarged spleen from any cause has a greatly increased blood flow compared with normal and this alone can produce portal hypertension. Since splenic enlargement is one of the main effects of portal hypertension, the potential for a vicious spiral of splenic pathology is obvious.

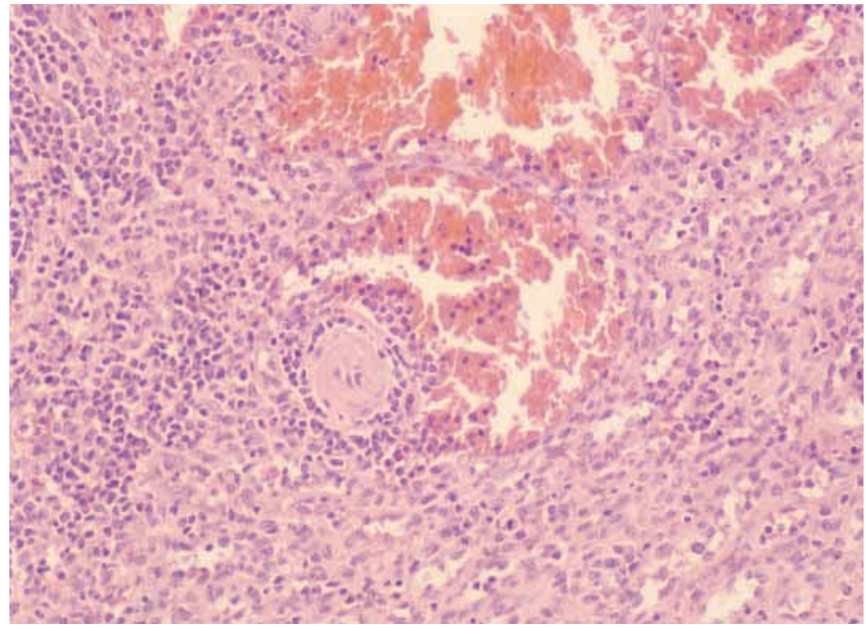
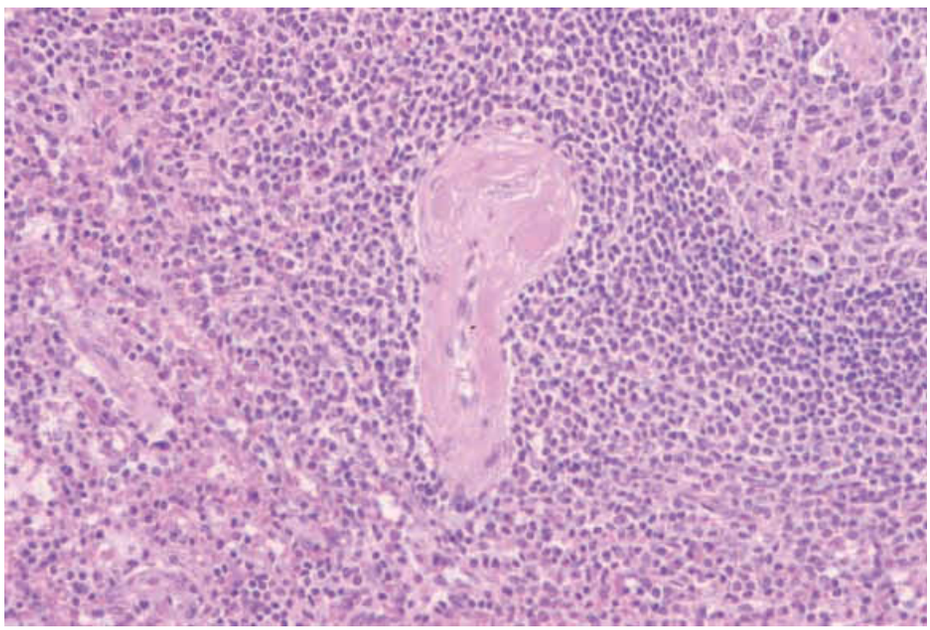


**Fig. 8.19.** Peliotic lesion developing as a crescent-shaped, blood-filled space adjacent to the PALS. H&E stain.

**Fig. 8.20.** Splenic arteriole showing irregular thickening of its wall by amorphous, eosinophilic material in splenic vascular hyalinosis. H&E stain.



The splenic appearances in portal hypertension have been termed 'fibrocongestive splenomegaly'. Sustained high pressure in the systemic venous system, as in chronic right-sided or congestive cardiac failure, tends to cause simple, passive congestion of sinusoids only and it is not clear why portal venous obstruction has more significant effects. The latter consist of progressive widening of red pulp cords and deposition of fibrous tissue within them (Fig. 8.21). Over time, the red pulp becomes progressively more pale and firm as the fibrous tissue becomes more dense. Gamna–Gandy bodies (see below) are frequently present, presumably secondary to localized areas of haemorrhagic infarction undergoing organization within the parenchyma.

## Splenic infarction

Splenic infarction (Fig. 8.22) occurs in response to systemic embolization as a result of a wide range of unrelated pathologies, most commonly thrombo-embolism from damaged cardiac valves, mural thrombi in the left-sided heart chambers or material from atherosclerotic plaques in the proximal aorta. Arterial branching within the spleen radiates from the hilum towards the capsule and is non-anastomotic. Infarcts are therefore wedge-shaped and orientated with their bases at the capsular surface. Small ones may go unnoticed by the patient but larger infarcts cause left-sided upper abdominal pain, with referral to the left shoulder tip if the adjacent diaphragmatic peritoneum becomes inflamed. Other causes of splenic infarction include red pulp occlusion by sickled red cells, massive extramedullary haemopoiesis in myeloproliferative disorders and involvement of splenic vessels by systemic vasculitides.

Initially, splenic infarcts appear haemorrhagic, but with time they become firm and

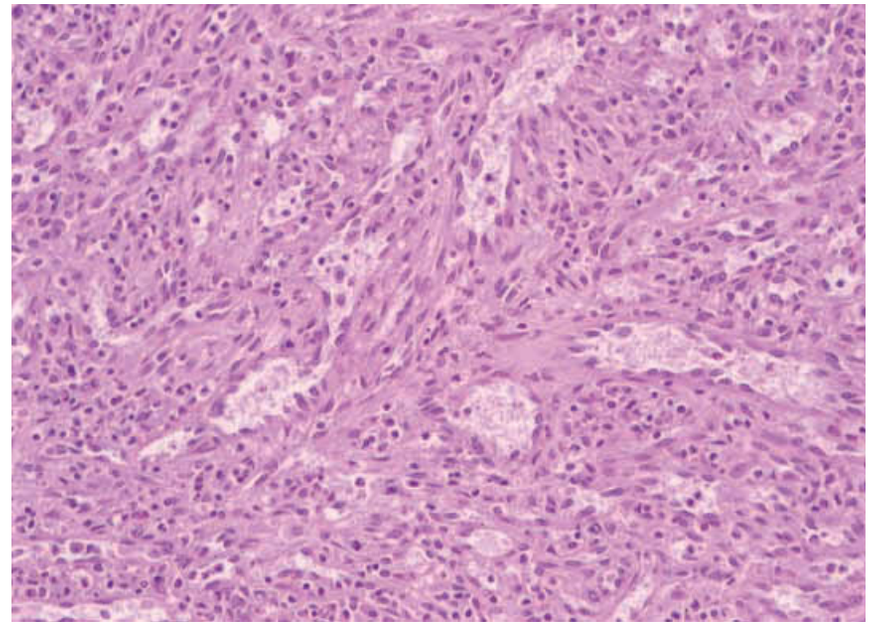


**Fig. 8.21.** Splenic red pulp from a patient with portal hypertension showing widening of cords due to accumulation of fibrous tissue. H&E stain.

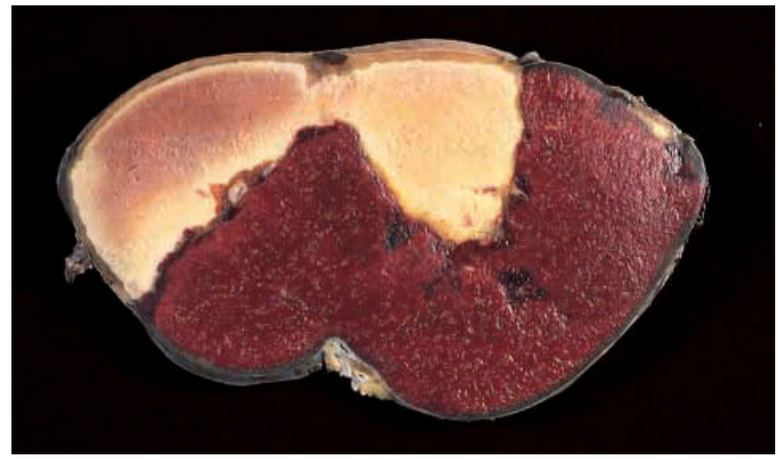


**Fig. 8.22.** Splenic infarction showing well-defined areas of yellow, necrotic tissue, sharply demarcated from the adjacent viable splenic parenchyma.

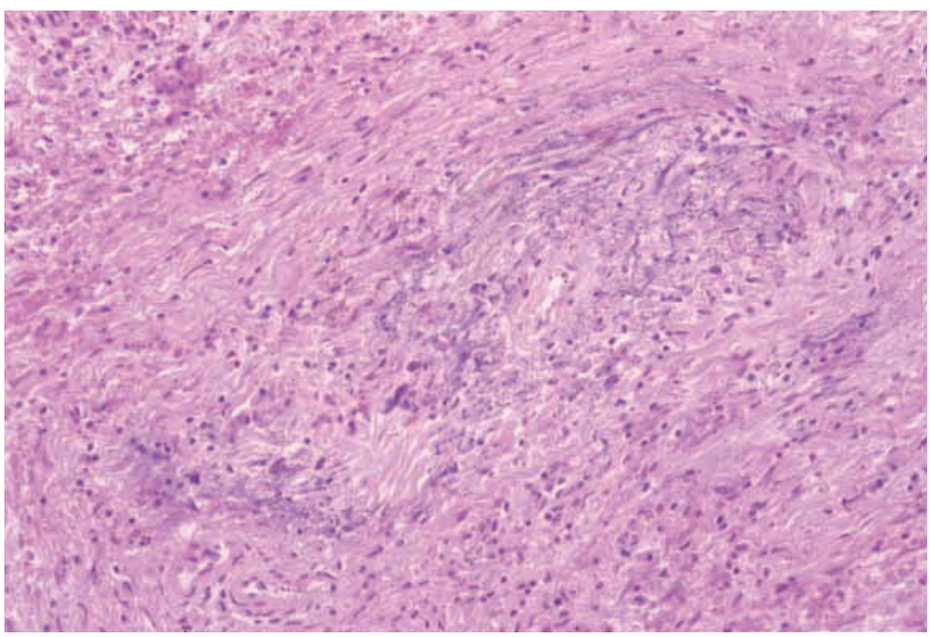
deep yellow in colour. They are usually sterile but may develop into chronic abscess cavities in the presence of septicaemia or following embolization by infected material, as in bacterial endocarditis.

## Gamna–Gandy bodies

These are fibrous patches within the parenchyma of the spleen, often visible macroscopically as yellow or brown flecks, preferentially situated in or near connective tissue trabeculae. They may have a hard, gritty consistency. They contain abundant haemosiderin and other crystalline material of uncertain origin. Iron, calcium and other material is deposited within the lesion in linear or serpiginous structures, sometimes obviously within a blood vessel wall or appearing to represent the shadowy outline of an obliterated vessel (Fig. 8.23). Gamna–Gandy bodies sometimes contain abundant macrophages, including epithelioid cells and foreign body-type giant cells (Fig. 8.24).

It is thought that these curious structures arise due to intra-parenchymal haemorrhage from diverse causes. They therefore occur in patients with sustained portal hypertension and in association with infarcts or foci of parenchymal trauma. However, they are frequent incidental findings and, in many instances, the precise cause of any preceding parenchymal haemorrhage is unclear.

**Fig. 8.23.** Gamna–Gandy body showing irregular fibrosis with deposition of crystalline and amorphous basophilic material, possibly associated with an obliterated blood vessel. H&E stain.



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Chapter 9

# Metastases and miscellaneous conditions

## Appearances and primary origins of metastatic cancers involving the spleen

Metastases originating from a wide variety of primary sites have been described in the spleen, sometimes as part of widespread dissemination but often solitary. Compared with the frequency of lymph node spread from carcinomas and melanomas, splenic involvement by metastasis is very rare. This may reflect paucity or absence of afferent lymphatics (see Chapter 2) as a route of entry for metastasizing cells into the spleen. Alternatively, the spleen may offer an unfavourable environment for the growth of tumour micro-emboli. In some cases, splenic involvement by carcinoma may represent direct invasion from metastasis within one or more hilar lymph nodes rather than true metastatic origin within the spleen. Nevertheless, some genuine intrasplenic metastatic growths do occur with no spatial proximity to hilar structures or evidence of trans-capsular spread of tumour into the spleen from an origin in adjacent tissues.

Most metastatic non-lymphoid tumours in the spleen, regardless of their origin, produce localized masses within the tissue (Fig. 9.1), detected by ultrasonography or radiology, or because they cause palpable splenic enlargement. These lesions are often solitary and cystic, mimicking a variety of benign cysts which occur in the spleen (Fig. 9.2). They may be extensively necrotic. In

**Fig. 9.1.** Solitary metastasis within the spleen from a primary squamous cell carcinoma of bronchus.

such cases, it may be difficult to confirm the malignant nature of the lesion if few or no remaining tumour cells are viable. Extensive sampling of the walls of such cavitating lesions is required, to detect tiny residual foci of tumour and to exclude pathology such as an infective lesion which may have developed in a patient immunocompromised due to chemotherapy or radiotherapy. The spleen would be an unusual but not impossible site in which to find a solitary tuberculous or mycotic abscess. Necrotic lymphoma should also be considered in the differential diagnosis and immunohistochemistry may be valuable, selecting target antigens which are relatively resistant to degradation during necrosis, such as cytokeratins and CD20.

Usually the context of an intrasplenic metastasis is that of a patient known to have a previous malignancy, often treated many years previously and with no other evidence of recurrent disease. In cases where lesions have been monitored before surgical intervention, growth has generally been slow and at least partly controlled by salvage chemotherapy regimes or irradiation.

Instances are sufficiently rare to make specific associations unreliable, but metastatic endometrial adenocarcinoma appears over-represented in published case reports. We have also seen metastatic colorectal adenocarcinoma, breast carcinoma and malignant teratoma (with differentiated and undifferentiated components, including focal choriocarcinoma).

**Fig. 9.2.** Cystic metastatic deposit, one of two present in this particular spleen, present as part of disseminated malignant teratoma, undifferentiated, originating within the testis.

## Hamartomas

Splenic hamartomas are uncommon, typically incidental, lesions found in spleens removed for unrelated reasons. Sometimes splenectomy is performed because an intrasplenic mass has been detected. They are well-circumscribed lesions macroscopically, usually solitary, which may resemble haem-

angiomas (Fig. 9.3). However, microscopically they are seen to contain red pulp cords and sinusoids in haphazard array (Fig. 9.4). Organized white pulp structures are not found, although scattered lymphocytes may be present, with other inflammatory cells, throughout the stroma.

Macroscopically similar nodules in which abundant, reactive lymphoid follicles predominate and compress any intervening red pulp structures are probably examples of localized reactive lymphoid hyperplasia (Burke & Osborne, 1983) rather than splenic hamartomas.

## Splenic cysts and false cysts

A variety of lesions may present in the spleen as cysts, including metastatic tumour deposits and nodules of lymphoma. Central necrosis with subsequent liquefaction, often induced by treatment, is the usual mechanism by which solid nodules of Hodgkin's and non-Hodgkin's lymphoma become cystic. Some metastases are similarly necrotic, while others become cystic by virtue of accumulation of mucin or fluid within viable tumour nodules. Angiomas, granulomatous lesions, abscesses and infarcts may all occasionally undergo cystic change.

Epithelial inclusion cysts occur in the spleen and their origin is mysterious. They are usually detected in childhood, either incidentally or because of splenomegaly. They are usually unilocular and may reach a size of many centimetres in diameter. Their lining consists of trabeculated, pearly white tissue and their wall is often densely fibrotic, sometimes calcified. Microscopically, the lining epithelium is usually stratified squamous in type, with areas of thinning due to pressure atrophy. Skin adnexal structures are absent, but foci of glandular or transitional epithelial differentiation may be found. The degree of

**Fig. 9.3.** Splenic hamartoma forming a large, well-defined, round mass, sharply demarcated from adjacent normal splenic parenchyma.

epithelial atrophy may be extreme in some cases, making identification of the lining difficult. It has been suggested that epithelial cysts in the spleen represent metaplasia from embryonic remnants of capsular mesothelium, entrapped within the parenchyma as fusion of rudimentary splenic foci occurs (Burrig, 1988). Origin from inclusions of other embryonal tissues cannot be ruled out, nor mesothelial implantation as a result of capsular trauma.

Cystic lesions in the spleen are occasionally found, which genuinely have no epithelial lining. Some examples of these are also multilocular. Many have heavily calcified walls with abundant haemosiderin deposition. A history of previous trauma may be obtained and these lesions are considered to represent false cysts formed as a result of parenchymal injury without rupture of the splenic capsule.

Parasitic cysts may also occur in the spleen, most notably hydatid cysts due to the larvae of *Echinococcus* tapeworms. The appearances of such cysts are equivalent to those of their more common counterparts in the liver and rupture carries the same hazards (Sparks et al., 1976).

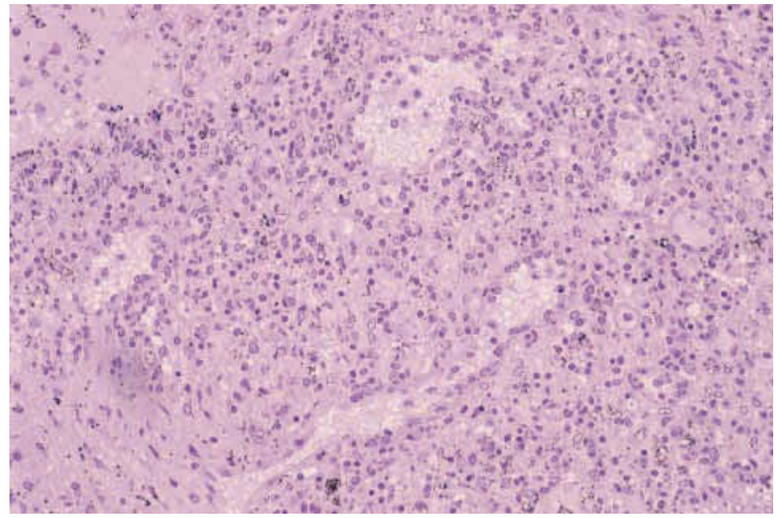

**Fig. 9.4.** Histological appearances of splenic hamartoma. The lesion consists of haphazard splenic cords and sinusoids, with no white pulp elements present. H&E stain.

## Amyloidosis

Systemic amyloidosis frequently involves the vasculature of the spleen, as it does the vessels of other organs. Red and white pulp vessels may be affected, with amyloid deposits being identifiable within their walls. Such deposits should not be confused with splenic vascular hyalinosis (see Chapter 8). Splenomegaly with hyposplenism occasionally results from extensive involvement, with generalized red pulp ('lardaceous spleen') or white pulp ('sago spleen') patterns of involvement. Very rarely, localized amyloid tumour formation may occur without generalized involvement of the rest of the organ.

Macroscopic and microscopic patterns of splenic involvement by amyloidosis do not correlate with the type of amyloid present. Amyloid L (AL), derived from immunoglobulin light chains, and amyloid A (AA), derived from serum amyloid protein A, are the most common types. In extensive white pulp involvement, nodules of amyloid replace the normal follicles, which may appear virtually acellular in the most severe cases. Diffuse red pulp involvement varies from thickening of sinusoidal walls to solid, homogeneous replacement with only widely spaced, compressed sinusoidal lumens remaining. Typical Congo Red staining with apple green birefringence in polarized light is found in all patterns of involvement. Immunostaining can be used, as in other tissues involved by amyloidosis, to discriminate between AL and AA types.

Accompanying lymphoma may be evident in some cases of AL and should be sought assiduously if not apparent. Chronic inflammatory processes are likely to underlie AA. Splenic involvement by either type may be isolated or one component of widespread amyloidosis.

## Congenital anomalies of splenic size, number or anatomical position

At post-mortem examination of fetuses, stillborn infants or neonates who fail to survive due to the circulatory complications of left-sided cardiac isomerism, the presence of bilateral spleens is common. Equivalent cases with right-sided isomerism are usually asplenic. In some fetuses and babies, however, the isomerism affects only thoracic organs and the spleen is normal.

Multiple, small spleens occupying a normal position may be found at post-mortem examination of fetuses and preterm infants dying from diverse causes. This occurrence reflects the multi-focal origin of

the spleen, with failure or delay of fusion occurring in some individuals.

Splenic ectopia also occurs in a small number of individuals, most commonly associated with splenic–gonadal fusion. In this condition, a portion of the rudimentary spleen descends with the left gonad. The resultant ectopic spleen tissue may be continuous with the normally situated spleen or, more usually, may become completely separated. It may then subsequently be found during surgery for correction of an incompletely descended testis or (more rarely) ovary. Alternatively, the ectopic splenic tissue may continue to grow at the site of a normally descended gonad and require surgery due to the presence of a painful mass.

Ectopic splenic tissue within the tail of the pancreas has also been described, characteristically associated with trisomy 13.

Further details of these and other congenital abnormalities involving the spleen can be found in specialized textbooks of perinatal pathology (e.g. Wigglesworth & Singer, 1998).

## Splenunculi and splenosis

Splenunculi (small, accessory spleens) are a common incidental finding (Fig. 9.5), of no particular significance except that they may expand post-splenectomy and account for occasional cases of failure of splenectomy to control hypersplenism. They form as a consequence of the normal, multi-focal development of the spleen, with failure of fusion of one or more of the contributory foci. Their small size relative to the main spleen represents their limited blood supply and consequent differential growth.

The term ‘splenosis’ describes the presence of multiple nodules of splenic tissue within the abdomen resulting from disruption of the splenic capsule due to trauma or during surgery. Splenic parenchyma dis-

**Fig. 9.5.** Splenunculus found incidentally at the hilum of a spleen removed for ITP.

placed from the interior of the spleen may survive within the peritoneal cavity and develop sufficient blood supply to grow and function. Occasionally, foci of splenosis are found at the external aspect of the capsule of intact spleens removed with no preceding history of injury or abdominal surgery, presumably representing subclinical capsular injury which has resolved spontaneously.

As with splenunculi, unsuspected deposits of splenotic tissue may account for persistent splenic activity in patients who have had their spleens removed to relieve hypersplenic symptoms.

## Splenic atrophy and fibrosis

The spleen declines in weight in extreme old age, but there is no objective evidence to support the widely held belief that there is a progressive shrinkage of the organ during older adult life. Moreover, splenic white pulp in elderly individuals remains capable of remarkable stimulation. This can be seen in some cases of ITP or AIHA occurring in elderly patients and even in spleens removed incidentally during surgery for intra-abdominal tumours.

Atrophy does occur in immunocompromised patients, as described earlier, first recognized as reduction in the number and size of white pulp nodules accompanied, if T cells are depleted, by increased numbers of penicillar arterioles which have no surrounding PALS. Red pulp atrophy is less easy to detect but increased density of connective tissue trabeculae is a useful clue. This reflects loss of volume of the intervening cords and sinusoids. At early stages of red pulp atrophy, however, subtle reductions in red pulp volume are probably not detectable in individual patients. Nor is weighing the spleen an infallible indicator, since passive congestion due to cardiac disease may mask a loss in the true parenchymal weight.

For reasons which are unknown, splenic atrophy occurs in patients with coeliac disease. Less often, it has been found in chronic inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. Whether or not such atrophy is a consequence of malabsorption or underlying immunological disturbance remains speculative.

Most other causes of splenic atrophy reflect ischaemia and/or iatrogenic intervention. Repeated episodes of infarction, e.g. due to sickling of red cells in sickle cell disease or showers of thrombo-emboli in bacterial endocarditis will eventually lead to atrophy. In sickle cell disease this is usually severe by teenage, as discussed in Chapter 4. Spontaneous resolution of splenomegaly, and occasionally true splenic atrophy, may follow infarction of enlarged spleens in chronic myeloproliferative disorders.

Irradiation, intentional or otherwise, causes splenic atrophy. This is, at least in part, due to vascular changes and subsequent ischaemia. Typical endothelial degenerative changes and 'radiation fibroblasts' may be seen in areas of splenic fibrosis induced by therapeutic irradiation, undertaken to shrink a massively enlarged spleen or treat localized lymphomatous deposits. Severe white pulp depletion and fibrous obliteration of the red pulp follow heavy doses of irradiation.

An example of unintentional splenic irradiation is that which occurred in patients in whom Thorotrast, a colloidal suspension of thorium dioxide, was used for angiography in the 1930s and 1940s. Thorium dioxide is an alpha-particle emittter, which is taken up avidly by macrophages and hence concentrated in the splenic cords (as well as bone marrow and liver). It has an extemely long half-life and, over many years, will auto-irradiate tissues surrounding the macrophages in which it remains stored. In the liver and bone marrow, this irradiation has been associated with development of angio-

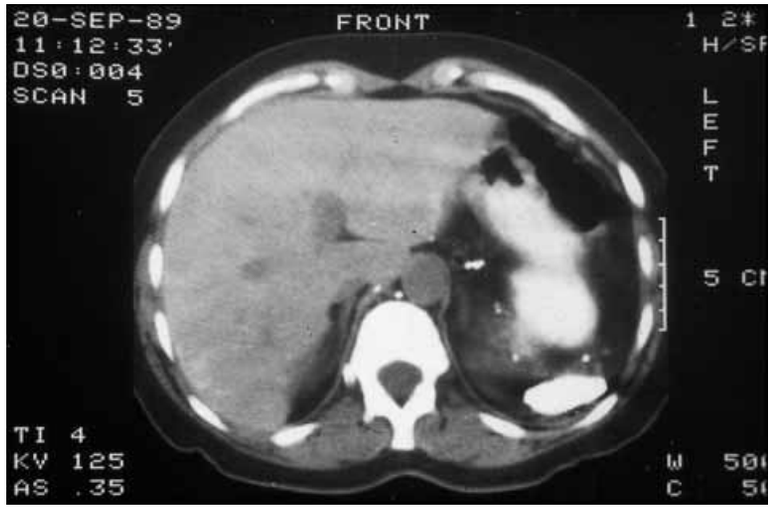


**Fig. 9.6.** Abdominal computed tomography (CT) scan image from a patient with Thorotrast-induced hepatic angiosarcoma. Note the contracted, densely radio-opaque spleen. Calcification of the severely atrophic, fibrotic spleen has resulted from auto-irradiation following thorium accumulation in cordal macrophages.

sarcomas (and also epithelial malignancies in the liver) 15 to 25 years following exposure. In the spleen, however, it has not proved carcinogenic but rather has induced dense fibrosis (Manning et al., 1983), ultimately reducing the organ to a hard nubbin of fibrous tissue, frequently heavily calcified (Fig. 9.6).

## Peri-splenitis ('sugar-icing' of the spleen)

This curious pathological finding, very commonly noted as an incidental observation at post-mortem examination, remains unexplained in pathogenetic terms. It takes the form of pearly white thickening of the splenic capsule to form irregular plaques or, occasionally, a dense rind on the surface of the organ. The convex surface is usually more severely affected than the concave, medial aspect of the spleen. That it is not simply a long-term outcome of loculated intra-peritoneal infection (i.e. a variant of subdiaphragmatic abscess) is suggested by the absence of any similar pathology occurring in relation to the upper surface of the liver. In fact, the appearances of organized, subdiaphragmatic abscesses are quite different, with dense adhesions between the liver capsule and the under-surface of the diaphragm.

Perhaps the position of the lateral surface of the spleen, immediately against the inner aspect of the lower ribs, predisposes to capsular trauma. This might occur with, or without, clinically evident injury to the left flank, leading to minor parenchymal disruption as a result of subcapsular haemorrhage or ischaemia. The microscopic appearances are generally those of bland fibrosis but there are sometimes features reminiscent of those in Gamna–Gandy bodies, with irregular deposits of iron and crystalline material present (Fig. 9.7). In the absence of any specific evidence relating to

the precise mechanism(s) underlying peri-splenitis, it is possible to speculate that the condition may have pathogenetic features in common with Gamna–Gandy body formation.

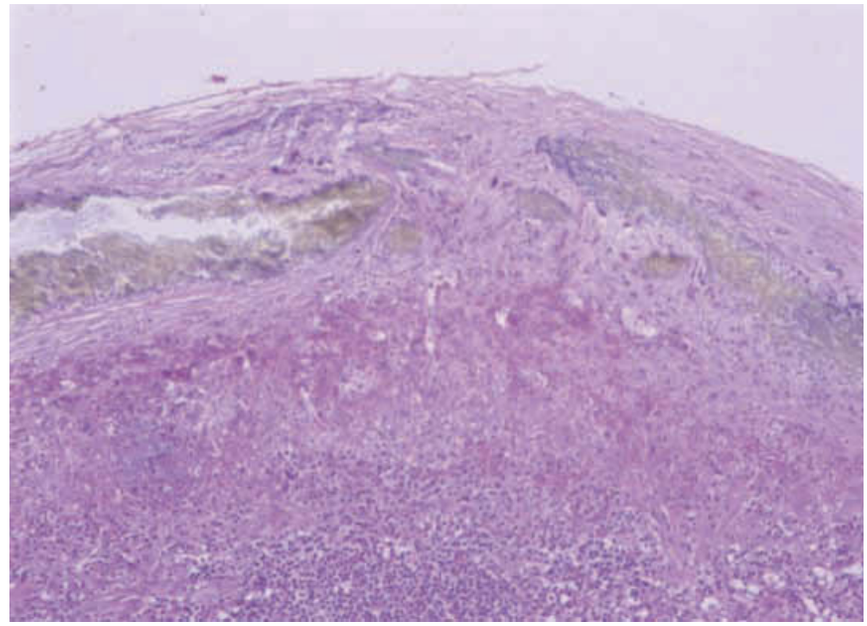

**Fig. 9.7.** Peri-splenitis showing fibrous thickening of the spleen capsule with deposition of crystalline and amorphous material similar to that seen in Gamna–Gandy body formation. H&E stain.

## Autolysis in splenic tissue

Autolysis of the spleen occurs rapidly post-mortem and may render the tissue uninterpretable histologically. Similar disintegration of histological features, although usually less severe, occurs in splenectomy specimens inadequately penetrated by fixative. In septicaemic patients, autolysis is accelerated and the spleen may already be semi-liquid by the time of post-mortem examination. White pulp features survive better than red pulp, so that suspected lymphomas may be differentiated from white pulp hyperplasia if post-mortem examination is undertaken within a day or two after death. However, the cytology of individual lymphoid cells, particularly those of the various low grade non-Hodgkin's lymphomas, is poorly preserved and precise subclassification may be difficult. Large cell lymphomas and Hodgkin's disease infiltrates often retain sufficient detail for accurate diagnosis.

Red pulp may appear congested, but otherwise featureless, in H&E stained sections due to autolysis. Reticulin staining is valuable to demonstrate the surviving cordal scaffold. Vascular pathology may remain detectable by trichrome and by Congo Red staining, etc., as appropriate.

Extramedullary haemopoietic cells, if present, survive relatively well. Erythroid precursors remain distinctive, with their dense nuclei and organization into intra-sinusoidal nests. Megakaryocytes become rather shrunken and hyperchromatic but are still easily recognizable. Granulopoietic cells are more difficult to recognize in partly autolysed tissue; they lose their granularity

and may simulate either macrophages or plasma cells. As discussed in Chapter 7, granulopoiesis is not usually a significant component of incidental EMH, and the presence of early granulocyte precursors in more than very small numbers is suggestive of an underlying myeloproliferative condition. In autolysed tissue, localization of candidate granulopoietic cells at the periphery of white pulp nodules or the margins of connective tissue trabeculae is a clue to their true identity. If the white pulp appears atrophic, however, these features must be interpreted with caution, since unexpectedly large lymphoid cells may occur in some patterns of evolving or abnormal lymphoid reactions, e.g. in response to EBV infection or in immunocompromised individuals.

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Chapter 10

# Summary: some key points in splenic differential diagnosis

## Introduction

A few topics within the extensive range of splenic pathology recur as particular diagnostic problems for many histopathologists. In the following sections, we have attempted to provide an approach to assist in reaching a correct diagnosis in some of these problem areas. The specific topic of incidental and traumatic changes in spleens removed for surgical access or following trauma has been covered in detail in Chapter 3. Here we now summarize analytical approaches for the differential diagnosis of low-grade lymphomas, granulomatous lesions and extramedullary haemopoiesis in the spleen.

## Reactive spleen or neoplastic lymphoid proliferation?

Systematic analysis of each splenic compartment will sort out most cases and can be aided by immunohistochemistry. For further details of individual types of lymphoma and their characteristic immunophenotypes, see Chapter 6. Reference to the inventory of normal spleen immunohistology in Chapter 1 may be helpful and an additional review of splenic lymphomas can be found in a recent monograph of extranodal lymphomas (Isaacson & Norton, 1994).

### Germinal centres (GC)

*Checklist:*

Are GC present or absent?

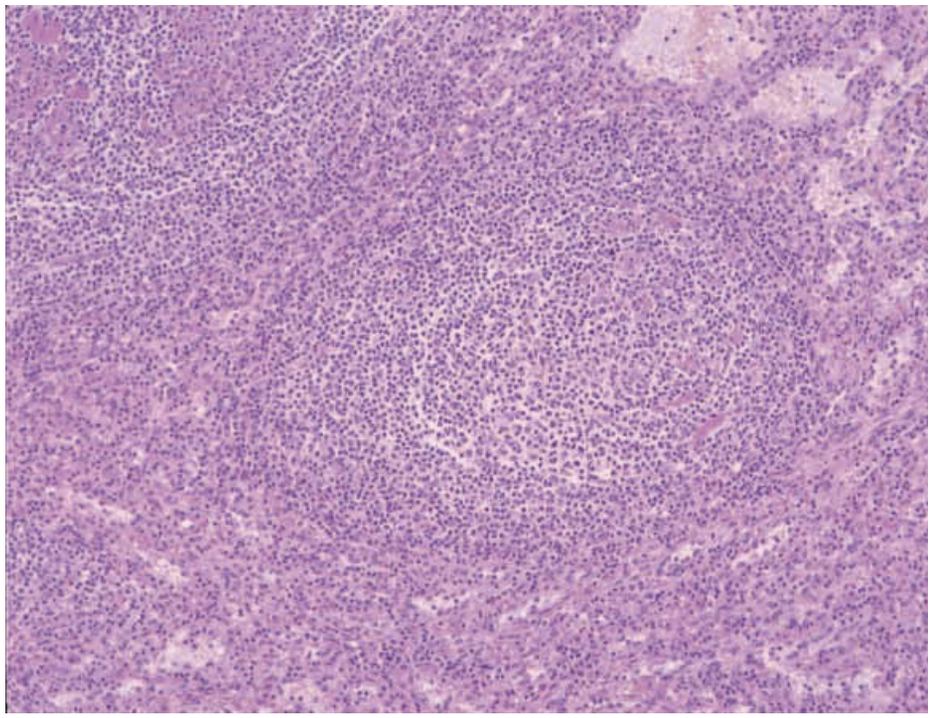
If present – are they enlarged, involuting or atrophic?

If prominent – what is their cellular composition?
is there accompanying red pulp involvement?

The size of GC present is usually fairly uniform throughout the spleen, so that only rarely does the degree of GC activity appear to vary between different areas; this applies to neoplastic as well as to reactive GC. Occasionally, focal lymphoid hyperplasia may occur (Burke & Osbourne, 1983) but given the much more common appearances of generalized white pulp prominence, reactive enlarged GC are always accompanied by well-defined mantles and less consistently by expanded marginal zones also. As in a lymph node, a mixture of centrocytes and centroblasts is present, with tingible body macrophages and organization of the GC into dark (centroblast-rich) and light (centrocyte-rich) zones.

Lack of definition of the boundary between GC and mantle zones, together with centrocyte predominance within GC and lack of red pulp involvement are the hallmarks of low-grade follicle centre cell lymphoma (FCCL) in the spleen (Fig. 10.1). At this site, FCCL rarely has a diffuse component, although the disease within involved hilar or distant lymph nodes in the same patient may do so; the compartmentalization of white and red pulps in the spleen seems to dictate follicularity and the cells of FCCL, except in unusual cases with leukaemic overspill, show little propensity to enter red pulp. In advanced cases with massive splenic involvement, nodular red pulp satellites may be found.

When red pulp involvement is evident in addition to white pulp expansion, chronic lymphocytic leukaemia (CLL), mantle cell



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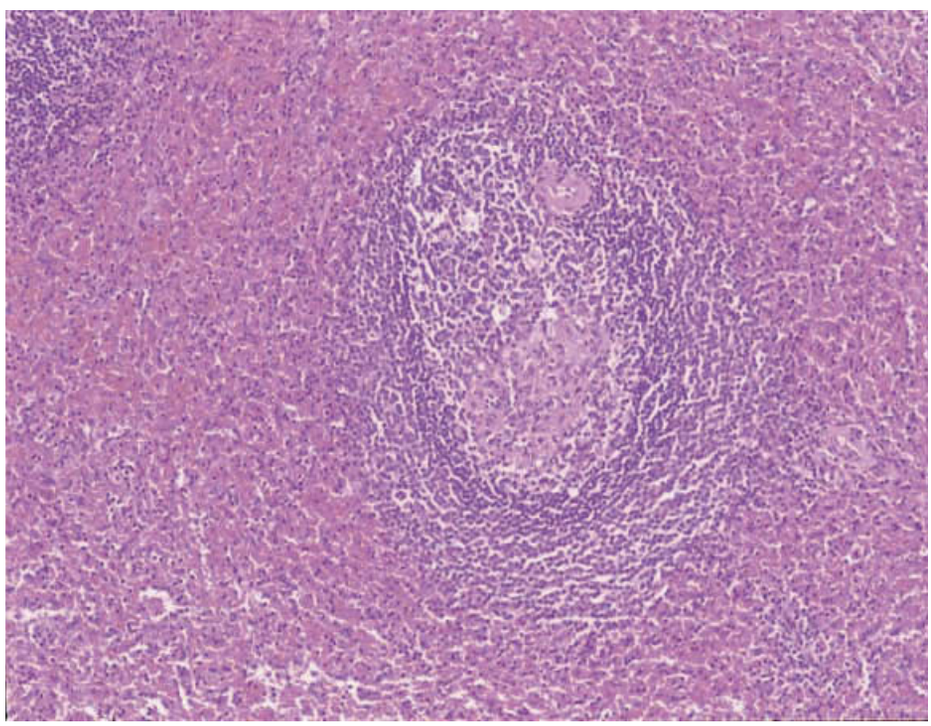


(*b*)

**Fig. 10.1.** (*a*) Nodule of FCCL within the spleen and (*b*) reactive splenic white pulp follicle. Note the centrocyte predominance of FCCL, the poor definition of its mantle zone, and the absence of tingible body macrophages compared with the reactive follicle. H&E stain.

lymphoma (MCL), splenic marginal zone lymphoma (SMZL/SLVL) or lymphoplasmacytoid lymphoma (LPL) are much more likely diagnoses than FCCL. Check all available haematological data, particularly any record of peripheral blood lymphocytosis, plasma paraprotein and/or bone marrow involvement. Nodules of white pulp may be prominent, but GC are usually atrophic or involuting in these lymphoproliferative disorders. They sometimes show colonization by cells typical of the underlying lymphoma (see below) and consequently appear spuriously enlarged.

### Mantle zones

*Checklist:*

- Does the white pulp contain solid nodules of mantle-type cells?
- If so, are GC absent or present but atrophic?
- If GC are present, how well defined are the inner and outer borders of mantle zones?
- Is there lymphocytosis in the red pulp?

Mantle zones surround active germinal centres of reactive white pulp nodules. These have a very clearly demarcated inner boundary with the GC and a less distinct outer boundary with the marginal zone. When GC are absent, white pulp nodules appear to be composed almost exclusively of mantle cells – monotonous small lymphocytes with no blast cells evident. Neoplastic mantle cells/zones are usually present in the context of either MCL or CLL. They may be identical to reactive mantle cells (small, lymphocytic cells), resemble centrocytes or even mimic lymphoblasts (so-called 'blastoid variant' of MCL). Proliferation characteristics may help – minimal in reactive, low in CLL, low to moderate in MCL variants composed of lymphocyte-like or centrocyte-like cells and high in MCL blastoid variant; cells expressing Ki67 antigen are para-immunoblasts in CLL and not morphologically blastic at all in most cases of MCL.

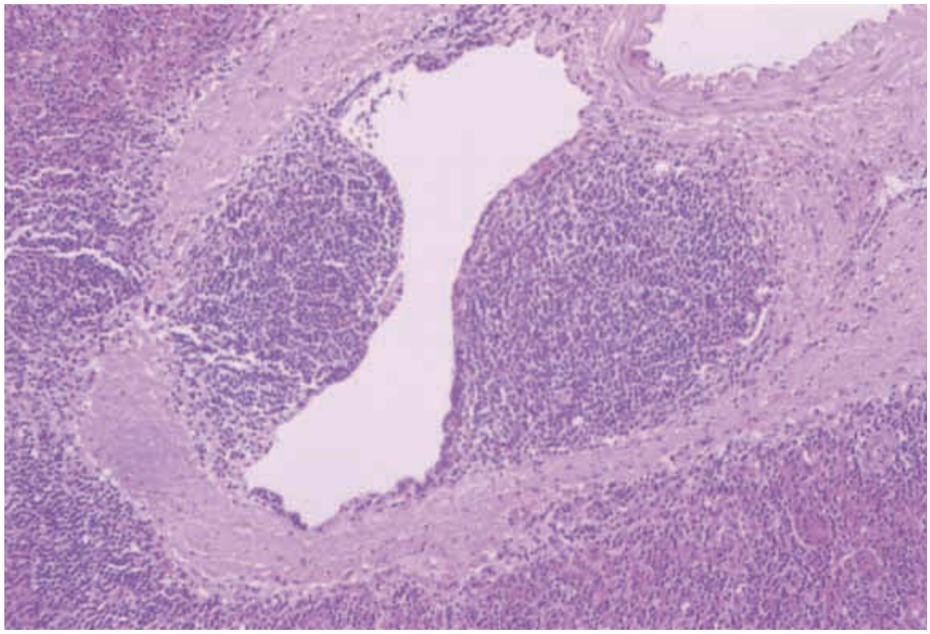


**Fig. 10.2.** Trabecular infiltration by sheets of small lymphocytes in a case of B-CLL involving the spleen. H&E stain.

In MCL, mantle zones are expanded around atrophic/absent GC, marginal zone differentiation is not usually prominent and there is diffuse spillover of identical cells into red pulp. The latter feature is a correlate of the strong tendency for MCL to have a significant leukaemic component. Cyclin D1 positivity is virtually diagnostic.

Chronic lymphocytic leukaemia also probably originates from cells within the mantle zone. Usually no GC, or only very atrophic ones, are present. White pulp nodules may vary in size from small, inactive-appearing to large. When large, they often have irregular margins blending with diffuse and nodular infiltrates in the red pulp. Infiltration of larger vessel walls within connective tissue trabeculae is often prominent (Fig. 10.2) and marginal zone differentiation can be pronounced in occasional cases. The hallmark of CLL is the presence of para-immunoblasts, which commonly aggregate to form proliferation centres. Proliferation centres viewed at low magnification are often multiple within a nodule or are clearly visible within red pulp nodular infiltrates, features which help distinguish them from true GC.

Lymphoplasmacytoid lymphoma resembles CLL but with plasmacytic differentiation. Proliferation centres are usually absent and this diagnosis should not be made if they are prominent (diagnose CLL with plasmacytic differentiation instead). Look for Dutcher bodies and for accompanying reactive mast cells which are features of LPL.

## Marginal zones

*Checklist:*

Are marginal zones present or absent?

If present – are they expanded?
are they accompanied by enlarged or involuting/atrophic GC?

are they clearly demarcated from the adjacent red pulp?

Is there an accompanying red pulp lymphocytosis?

Are there satellite nodules ± epithelioid granulomas in red pulp?

Marginal zone differentiation may accompany CLL, MCL or FCCL as well as being a feature of SMZL/SLVL. So, concentrate on cytology in the centres of white pulp nodules and/or in areas of red pulp involvement to make differential diagnoses.

### Peri-arteriolar lymphoid sheaths (PALS)

*Checklist:*

Is the PALS evident or not?

If present – is it reduced or expanded?
what is its cellular composition?

The PALS forms a cellular cuff around smaller connective tissue trabeculae and the penicillar arterioles which emerge from these. By contrast, the largest trabeculae, containing obvious large veins and arteries, are frequently bare. The PALS is usually at least 3–4 cells in depth, expanding in areas of white pulp nodule formation. It may appear slightly 'moth-eaten' compared with the compact, monotonous mantle-type lymphocytes which make up unstimulated white pulp nodules, reflecting the presence of interdigitating reticulum cells and scattered large lymphoid cells. When there is a reduced CD4-positive T cell population, as in AIDS, the PALS is less prominent (absent in severe cases) and the underlying stroma may appear fibrotic.

Since the PALS is the normal CD4-positive T cell compartment of the spleen, T cell lymphomas and leukaemias expressing CD4 preferentially localize here. These are usually cytologically atypical tumours composed of irregular blast cells; the main exception is T cell prolymphocytic leukae-

mia (T-PLL) which consists of monotonous, generally small prolymphocytes and, in keeping with its leukaemic presentation, shows extensive, diffuse spillover into the red pulp.

## Red pulp

*Checklist:*

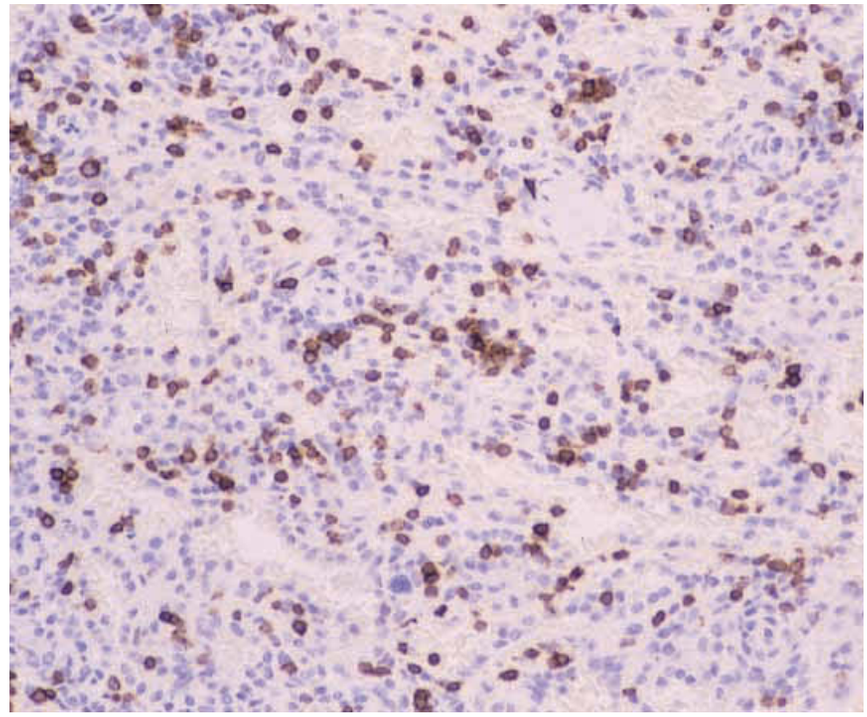
Is there any lymphocytosis?

If so – is it diffuse or nodular?
is it cordal, peri-capillary or intra-sinusoidal?
what is its cellular composition?

Remember that red pulp is a compartment within the spleen normally occupied by certain subpopulations of lymphoid cells as well as having its major function in phagocytosis. The red pulp is home to most of the spleen's CD8-positive T cells and plasma cells, which are normally present in small numbers, widely dispersed. Other lymphocytes may accumulate in the red pulp in reactive and neoplastic conditions. Peripheral T cell lymphomas of CD8-positive cells show preferential, usually diffuse involvement of the red pulp without expansion of the PALS. Large granular lymphocyte leukaemia infiltrates the red pulp insidiously and may be undetectable without immunostaining to demonstrate a T or NK cell infiltrate (Fig. 10.3). Hepatosplenic ($\gamma\delta$) T cell lymphoma characteristically involves the intrasinusoidal compartment and infiltration may be quite subtle. The cells are considerably larger than sinusoidal endothelial cells, the latter being either flat and inapparent or cuboidal, closely packed around the sinusoidal margin. Apart from such rarities, infiltration of the red pulp by monotonous medium-sized or large blast cells should raise suspicion of acute myeloid leukaemia. Involvement of the spleen by large B cell lymphoma, whether primary or part of disseminated disease, is usually represented by

**Fig. 10.3.** CD3 immunostaining of splenic red pulp to illustrate (*a*) normal appearances and (*b*) greatly increased T cell numbers in a case of T-cell large granular lymphocytic leukaemia. Streptavidin–biotin complex immunoperoxidase technique.

(*a*)



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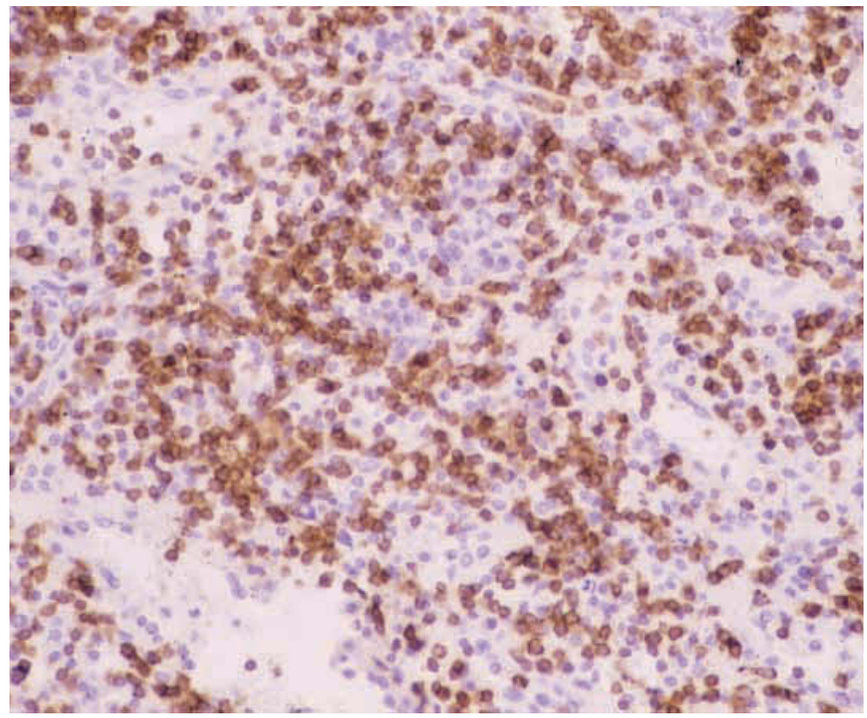


solid tumour mass(es) not preferentially confined to either red or white pulp.

Reactive and neoplastic red pulp lymphocytoses are difficult to distinguish unless the neoplasm is composed of recognizably atypical cells or cells with characteristic cytology, as in hairy cell leukaemia. Interpret the cytology of red pulp lymphoid infiltrates carefully alongside white pulp changes, which may be more discriminatory. Reactive infiltrates are generally diffuse but plasma cells and B lymphocytes may cluster around red pulp capillaries, making these unusually prominent. Such capillaries, cut in cross-section, should not be mistaken for satellite nodules of SMZL/SLVL; they are composed of small lymphocytes and/or mature plasma cells rather than the heterogeneous mixture typical of SMZL/SLVL (which recapitulates the mixed population in reactive marginal zones). Immunophenotyping may help, especially if plasma cells are prominent to permit accurate determination of kappa and lambda light chain expression. Very florid nodular or clumped lymphocytosis in the red pulp is always suspicious of non-Hodgkin's lymphoma.

Do not forget the basic principle that extensive and/or diffuse red pulp involvement by lymphoma generally goes with leukaemic behaviour. Characteristically FCL does not involve red pulp diffusely and shows, at most, scattered nodular foci in this compartment.

## Hilar lymph nodes

*Checklist:*

Is hilar lymphadenopathy present?

If so, do the lymph nodes show features of any type of NHL?

Splenic hilar lymphadenopathy is rare as an accompaniment to straightforward reactive stimulation of splenic white pulp. Reactive lymph node features are well known and the

reader is referred elsewhere for details (Ferry & Harris, 1997). CLL in hilar lymph nodes shows typical diffuse and/or nodular infiltration with proliferation centres. MCL also demonstrates diffuse or nodular infiltration, composed of cytologically monotonous centrocyte-like or lymphocyte-like cells and often accompanied by large, eosinophilic macrophages; sometimes an obvious mantle zone distribution is evident around pre-existing follicles. In FCCL, hilar lymph node involvement is typically distinctly follicular, with absent or compressed mantles.

Do not expect to see marginal zone differentiation in any non-Hodgkin's lymphoma in hilar lymph nodes. Because this feature is absent or poorly developed here, SMZL/SLVL is less distinctive at these sites than within the spleen (unlike most of the other low-grade lymphomas under consideration in its differential diagnosis); it looks like MCL but with more cytological heterogeneity and without accompanying eosinophilic macrophages.

### And finally – if no white or red pulp structures are discernible in the spleen

When this is the case, the entire spleen has usually been infiltrated by a monotonous population of small-to-medium-sized lymphoid cells and the diagnosis is hairy cell leukaemia (HCL). Prolonged search of multiple sections in HCL may eventually reveal a few residual, widely spaced (usually atrophic) white pulp nodules.

There is practically nothing else which destroys the underlying white and red pulp architecture in the way that HCL does. This picture should not be mistaken for red pulp hyperplasia or a myeloid proliferation. Even the most extensive, apparently destructive, myeloid leukaemic infiltrate does not efface white pulp structures totally. Moreover, even in badly fixed or autolysed spleens the basic underlying scaffold of white pulp structures

remains, despite rapid effacement of red pulp features. Other diagnostic features of HCL are the presence of peliotic cysts, blood lakes and areas of interstitial haemorrhage, with complete disruption of the normal reticulin pattern of red pulp cords and sinusoids (Fig. 10.4).

## Granulomas and cordal macrophage disorders

### Are there defined granulomas or dispersed/diffuse sheets of macrophages?

Most conditions with localized granulomas are equivalent to granulomatous processes in other tissues, including lymph nodes, and will be very familiar to histopathologists. Diffuse processes may represent storage diseases, including that occasionally associated with ITP, infections such as *M. avium-intracellulare* (MAI) or leishmaniasias, or not truly be macrophage infiltrates at all, as in Langerhans' cell histiocytosis (see below).

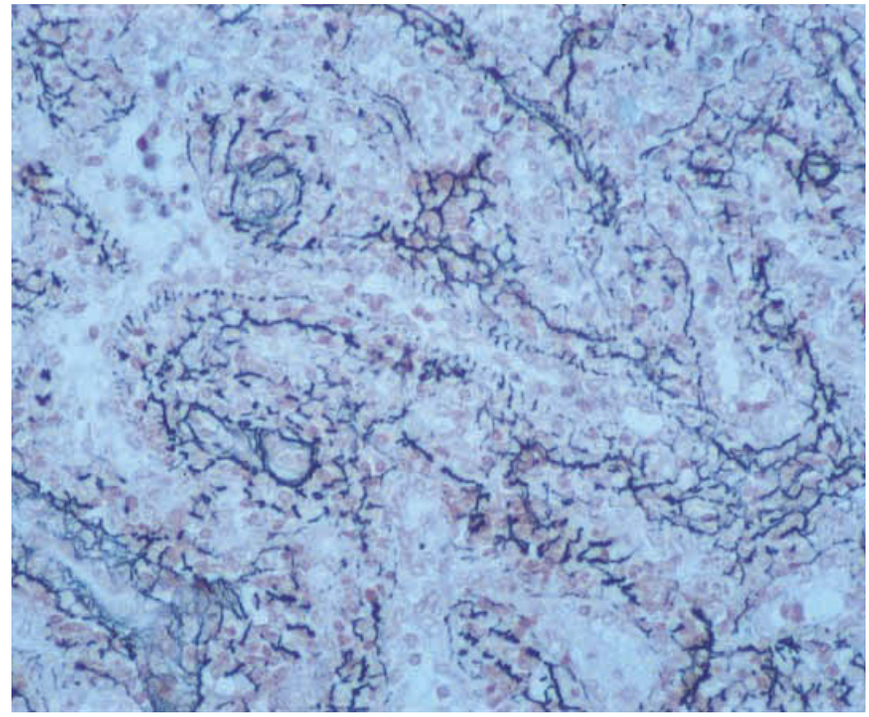
### Is red pulp, white pulp or both involved?

Incidental oleogranulomas typically occupy the interface between red and white pulp and their macrophages contain variously sized lipid droplets. Granulomas in the spleens of patients with Hodgkin's disease typically involve the PALS.Other focal granulomas are inconsistent in distribution between compartments; tuberculous and sarcoid-like granulomas are usually found in the red pulp unless involvement of the spleen is massive. Small epithelioid granulomas associated with SMZL/SLVL are characteristically the focus of red pulp satellites of lymphoma and other features of the lymphoma usually predominate.

All of the storage disorders, and Langerhans' cell histiocytosis, involve red pulp diffusely, although vaguely nodular

**Fig. 10.4.** Reticulin staining of the splenic red pulp in (*a*) normal spleen and (*b*) hairy cell leukaemia. Gordon and Sweet's silver stain for reticulin.

(*a*)



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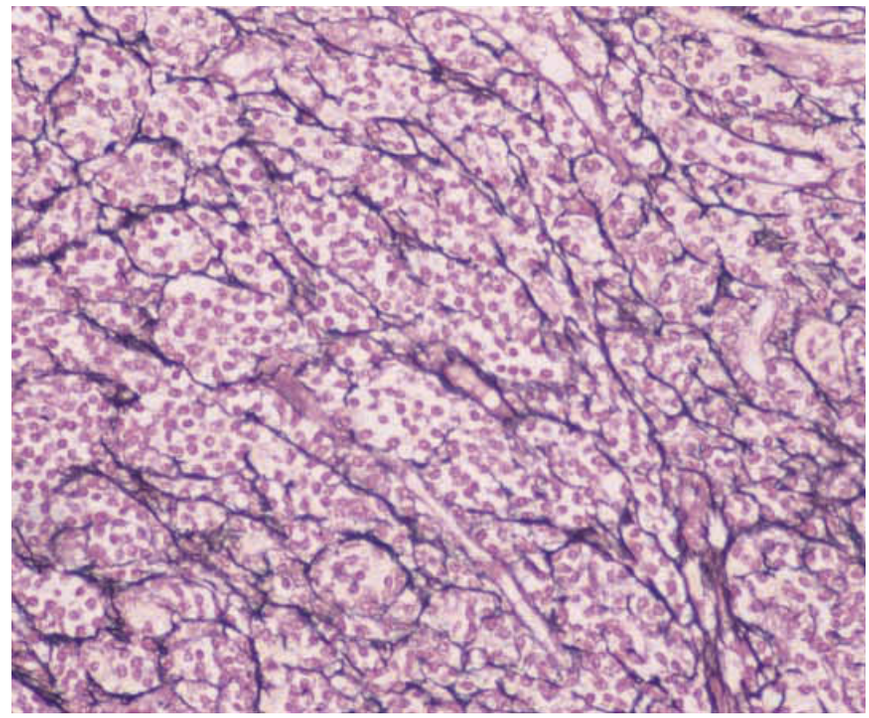

collections may also be evident, particularly at early stages when the degree of splenic involvement is relatively mild. White pulp tingible body macrophages, if present, may share the abnormal cytological features of red pulp macrophages in primary storage disorders but are usually spared in secondary storage disorders.

### What are the cytological characteristics of the macrophages present?

*Checklist:*

If focal, do they have characteristics to suggest TB, sarcoidosis, etc.?

If diffuse, are there characteristic features of Gaucher's or Niemann–Pick disease?

Most focal granulomatous lesions are recognizable and can be investigated along identical lines to those in other tissues. It should be remembered that epithelioid granulomas are common in patients with a variety of immunodeficiencies and are usually of unknown cause, as discussed in Chapter 5. Epithelioid granulomas are also the usual type found in the spleens of patients with Hodgkin's disease; it should be remembered that these occur independent of splenic involvement by neoplasm (see Chapter 6). Oleogranulomas are recognizable by virtue of their position and content of lipid vacuoles, as described above.

Diffuse red pulp macrophage abnormalities pose a greater challenge for interpretation. Infective causes such as MAI or leishmania should always be borne in mind, if only to be excluded.

Leishmaniasis is one of the most common opportunistic infections in AIDS patients in areas such as Southern Europe. Gaucher's cells and Langerhans' cells have distinctive cytological features. The other storage disorders all contain foamy macrophages with varying degrees of ceroid accumulation (see Chapter 8). All of the storage disorders tend to show areas of aggregation

into loose clusters as well as diffuse involvement. Very marked ceroid deposition (imparting a yellow colour with H&E and intense PAS positivity) is a marker of long-term excessive lipid breakdown product storage and suggests a primary disorder such as Niemann–Pick disease. Secondary storage disorders contain few ceroid-rich cells. Electron microscopy may not be helpful in distinguishing between the underlying causes of abnormal ceroid accumulation and biochemical investigations are necessary to achieve firm diagnoses in patients with possible Niemann–Pick disease.

## Extramedullary haemopoiesis – is it significant or not?

By significant, we mean EMH representing neoplastic myeloproliferation. Incidental, non-neoplastic, EMH is a frequent finding in splenectomy specimens and may cause concern if not recognized as such.

### Check the clinical information available

If a haematological diagnosis of myeloproliferative or myelodysplastic disease has already been made, expect to find significant EMH. Significant EMH is rare in patients with no haematological features to suggest underlying bone marrow abnormality.

### Know when to expect incidental EMH

Any spleen with associated hypersplenism is likely to contain foci of incidental EMH. This means potentially that any enlarged spleen may do so. However, if the spleen is massively enlarged, without obvious pathology (e.g. lymphomatous infiltration) to account for it, any EMH found must be regarded as potentially significant; incidental EMH does not itself cause marked spleen enlargement.

### Check the cytology and distribution of the EMH

Incidental EMH is usually minor in quantity, scattered in distribution and predominantly erythroid in differentiation. Single, scattered megakaryocytes do not on their own constitute EMH at all, since small numbers of such cells circulate normally and pass through the spleen as they do so. Erythroid cells can be highlighted by Giemsa staining (blue–black cytoplasm in early precursors) or immunostaining for glycophorins. They form clusters with normal maturation from large, central early forms to peripheral, small late normoblasts. Such clusters are usually intra-sinusoidal within the red pulp.

The presence of early granulocytic precursors is highly suspicious of significant EMH. They are found in the red pulp, around trabeculae and larger blood vessels. They may often be seen very clearly at the interface between red and white pulps. Their presence can be demonstrated by immunostaining for muramidase, which emphasizes their relatively large size and oval shape (Fig. 10.5). They have large, round nuclei with an open chromatin pattern, easily distinguishable from the lobulated nuclei of mature polymorphs.

In significant EMH, erythropoiesis is frequently dysplastic, resembling megaloblastic erythroid differentiation. Late, compact normoblasts are reduced in number and primitive-appearing, early forms predominate. If this cytological pattern is seen, a diagnosis of incidental EMH is extremely unlikely (unless the patient is truly folate or vitamin B12 deficient!).

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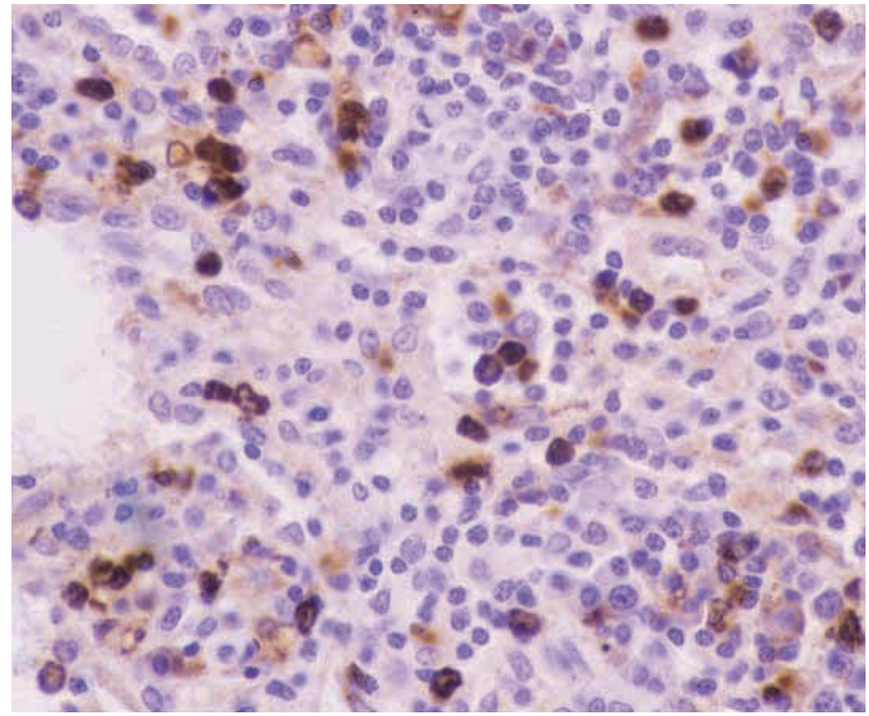

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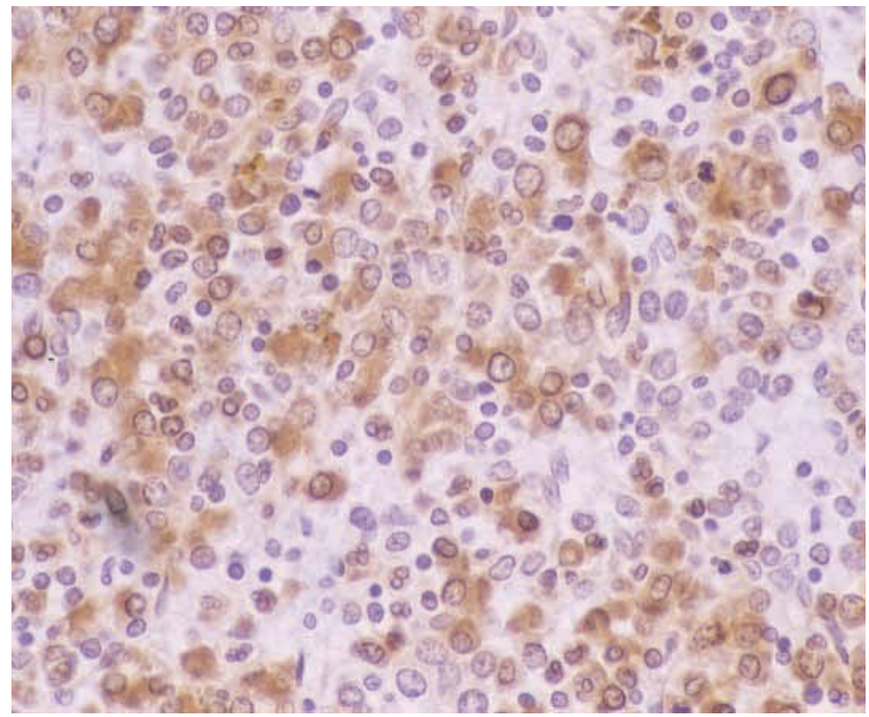

(*b*)

**Fig. 10.5.** Splenic red pulp immunostained to demonstrate muramidase expression by maturing granulocytes. (*a*) Incidental EMH in a spleen removed to control hypersplenism in a patient with underlying AIHA. (*b*) Significant EMH in a spleen removed because of splenomegaly caused by a chronic myeloproliferative disorder (subtype not specified; so-called 'cellular phase of myelofibrosis'). Note the relatively low numbers of muramidase positive cells in (*a*) compared with (*b*), reflecting the occurrence of significant granulopoiesis within the spleen in pathological, rather than simply incidental, EMH. Streptavidin–biotin complex immunoperoxidase technique using a polyclonal antibody reactive with muramidase.

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Note: page numbers in *italics* refer to figures and tables.