Marcel F. Jonkman
Editor

Autoimmune Bullous Diseases

Text and Review

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To my wife Gerrie and my children Lotte, Rutger, and Floris

Preface

'Knowledge of the history of a disease contributes materially to its understanding'. With these words Walter Lever (1909–1992) started his landmark review in 1953, in which he separated bullous pemphigoid from pemphigus [1]. The first mentioning of a blistering disease was from François Boissier de la Croix de Sauvages (1706–1767), who in 1763 introduced the term pemphigus [2]. Since then the number of autoimmune bullous diseases (AIBD) has expanded to 22 (Fig. 1). A general physician knows about one by heart: pemphigus, but might mix that up with pemphigoid.

AIBD are the most life-threatening inflammatory diseases of the skin. They are rare and comprise less than 1 % of the dermatological patients. The subject AIBD does not cover more than a single page in dermatology study guides for medical graduates. In general dermatology textbooks for postgraduate residents, AIBD comprises not more than one chapter. Although textbooks for autoimmune diseases exist, none are dedicated to AIBD. For many AIBD are

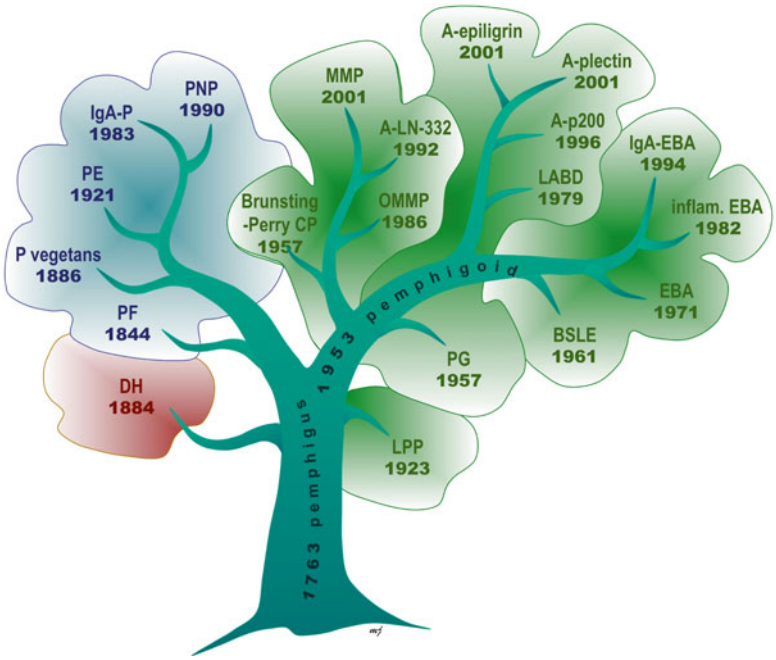

Fig. 1 Tree of autoimmune bullous diseases

confusing diseases that appeal to the medical competence of the specialist. Here we aim to provide a quick study guide for learning or look-up for the physician who practices with AIBD patients.

This book is written in direct and simple diction, strait and to the point, leaving out trivialities and not aiming for absolute completion. Tables and figures illustrate this practical guide to comprehend this 'difficult' field. At the end of each chapter, the reader is challenged to check the learned matter by multiple-choice questions.

The textbook is the effort of the senior staff and PhD's of the Center for Blistering Diseases of the University Medical Center Groningen who wrote this 'all-Groningen book'. The Center started in 1992 as national expertise center for blistering diseases in the Netherlands. The Center for Blistering Diseases has its own diagnostic immunofluorescence and immune-serological laboratory, an electron microscopic facility, and a digital image database with more than 250,000 dermatological images. The medical photographers S. Noorman and P. Toonder have taken the excellent photos of patients in this book. More than 1000 patients with AIBD have visited the Expertise Center in the last 25 years. The senior authors have gained many years of experience in clinical and laboratory diagnosis and therapy of AIBD. The 'Blister Course' in Groningen that runs annually since the year 2000 provided much of the didactic structure used in this book. I wish to thank my dedicated co-workers for sharing their knowledge to the reader and delivering that in an unobstructed way. I wish to thank Katarina Ondrekova and Jennifer Schneider for assisting in obtaining permissions and authorizations. The patients with AIBD who visited Groningen are thanked for giving us their trust and sharing their knowledge on symptoms and treatment outcome.

Groningen, The Netherlands Marcel F. Jonkman

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Contents

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Part I

Immunology and Diagnostic Tests

Basic Principles of the Immune System and Autoimmunity

1

Gilles F.H. Diercks and Philip M. Kluin

Abstract

The immune system is composed of two closely collaborative systems, an innate and an adaptive system. The innate immune system is a constitutive present system that can act rapidly to eradicate microbes. The primary cells of the innate immune system are macrophages, granulocytes, natural killer cells, and dendritic cells. The adaptive system can be divided in a humoral and cellular response. The humoral response is characterized by activation of B lymphocytes with subsequent maturation into plasma cells and production of antibodies, whereas a cellular immune response is characterized by transformation of T lymphocytes into cytotoxic T cells, capable of killing virally infected cells.

Autoreactive B and T lymphocytes can induce autoimmune diseases. Autoimmune bullous diseases are the result of type II hypersensitivity, e.g., autoantibodies are directed against cell or matrix components. In pemphigoid diseases, antibodies are directed against hemidesmosomal components, whereas pemphigus is characterized by antibodies against desmosomal proteins.

Keywords

Immune system • Autoimmunity • Pemphigus • Pemphigoid

Learning Objectives

After studying this chapter, you should know:

- The difference between the innate and adaptive immune system
- The functions of antigen presenting cells, B and T lymphocytes
- Causes of autoimmunity and types of hypersensitivity with emphasis on pemphigoid and pemphigus

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The Immune System: A Short Introduction

Two Systems: The Innate and Adaptive System

The immune system is composed of two closely collaborative systems, an innate and an adaptive system (Fig. 1.1).

<The immune system is composed of an innate and an adaptive system>

These systems are activated as the first barriers of defense, mucosa and skin, are breached. The innate immune system is a constitutive present system that can act rapidly to eradicate microbes.

<The innate system is a quick response system>

The primary cells of the innate immune system are macrophages, granulocytes, natural killer (NK) cells, and dendritic cells, but other cells like epithelial cells can also be part of it. For instance, macrophages and granulocytes are capable of phagocytosis of microorganisms by endocytosis. Pathogen-associated molecules are present on microbes and recognized by cells of the innate system by binding to Toll-like receptors. In particular these cells are effective against bacteria, whereas NK cells are used to fight viruses. They do this in an indirect way by recognizing and killing virally infected host cells. Besides the cellular response, many proteins play an important part in the innate immune system, e.g., chemokines, interleukins, interferon, and tumor necrosis factor. Binding of microbial antigens will therefore not only induce phagocytosis but also release of cytokines, which will result in an inflammatory response. Apart from these proteins, the complement system constitutes an important part of the immune system. This system can be activated directly by a microorganism itself or indirectly by binding to antibodies produced by the adaptive immune system. Eventually proteins of the complement system promote phagocytosis and inflammation.

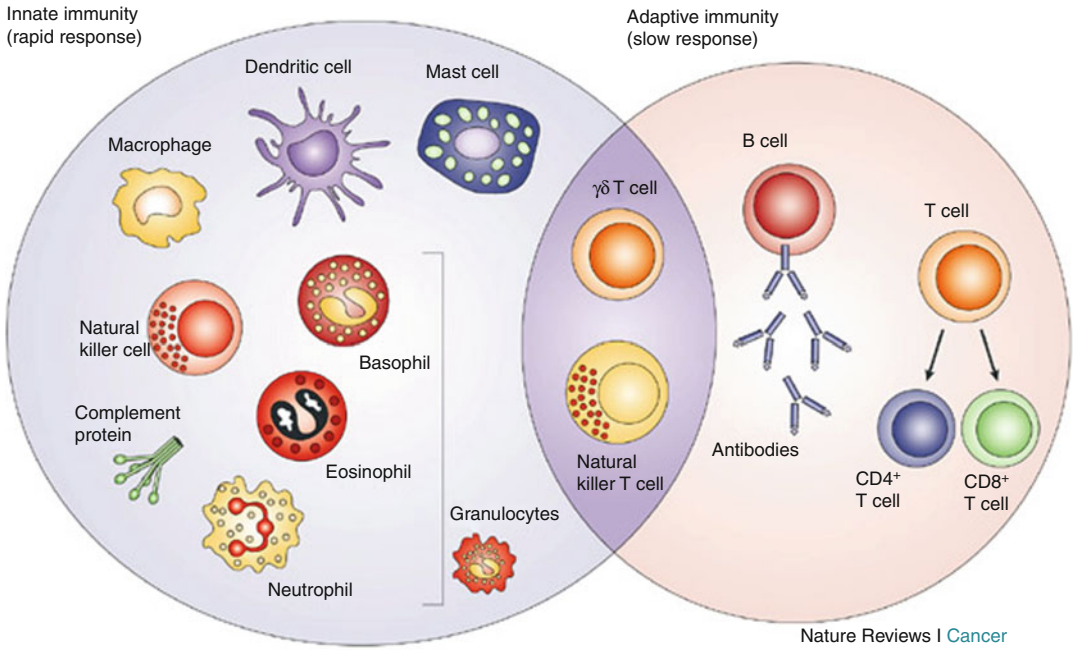

Fig. 1.1 An overview of the innate and adaptive immune system (Reprinted by permission from Macmillan Publishers Ltd: Dranoff [8], Copyright 2004)

The Adaptive System in More Detail

Next to the innate system is the adaptive immunity that can be divided in a humoral and cellular system (Fig. 1.2).

<The adaptive immune system is divided in a humoral and cellular system and is an antigen-specific system>

By definition the adaptive immunity is a "learning" system that has to be trained. In consequence the start will be slow, but once trained, the responses will also be quite fast. In contrast to the innate system, this system is antigen specific and by that more effective. An antigen as part of a microorganism or own cells, most frequently represents a protein, but it is good to know that it also can be a carbohydrate, lipid, or DNA, all being capable of inducing an antibody response.

Lymphocytes bear antigen receptors on their surface and can, on basis of these receptors, be divided into B lymphocytes and T lymphocytes. These receptors are called the B cell receptor (BCR) or cell surface immunoglobulin in B lymphocytes and the T cell receptor (TCR) in T lymphocytes.

<B and T lymphocytes are the main constituents of the adaptive immune system>

B lymphocytes originate directly from and also undergo some steps of maturation with assembly of the BCR within the bone marrow, whereas T lymphocytes start in the bone marrow but the assembly of their TCR takes place in the thymus (Fig. 1.3). After recognizing an antigen by the BCR in the peripheral lymphoid tissues such as a lymph node, B lymphocytes are activated and altered into plasma cells and large quantities of antibody are processed and secreted by these specific B cells. These antibodies have the same antigen-binding site as the BCR that first recognized the antigen. Antibodies can inactivate an antigen, e.g., a microorganism by complement binding or aid in phagocytosis of this

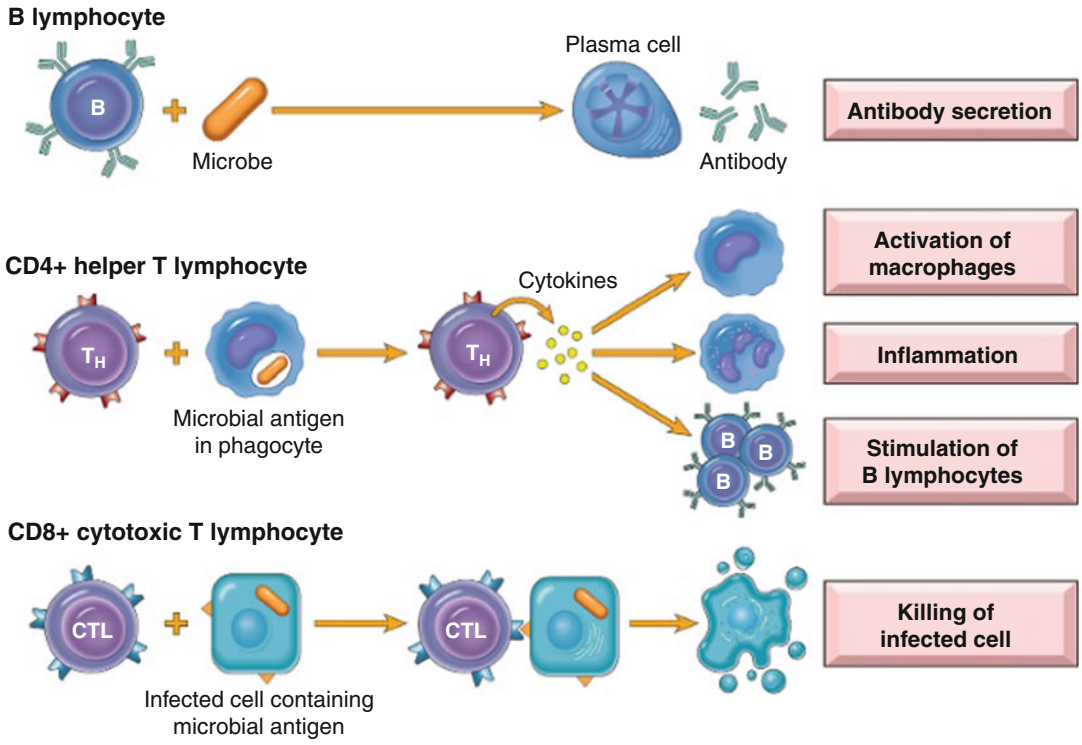

Fig. 1.2 The principle classes of lymphocytes and their functions in adaptive immunity (Reprinted with permission from: Kumar *et al.* [9], Copyright Elsevier 2010]

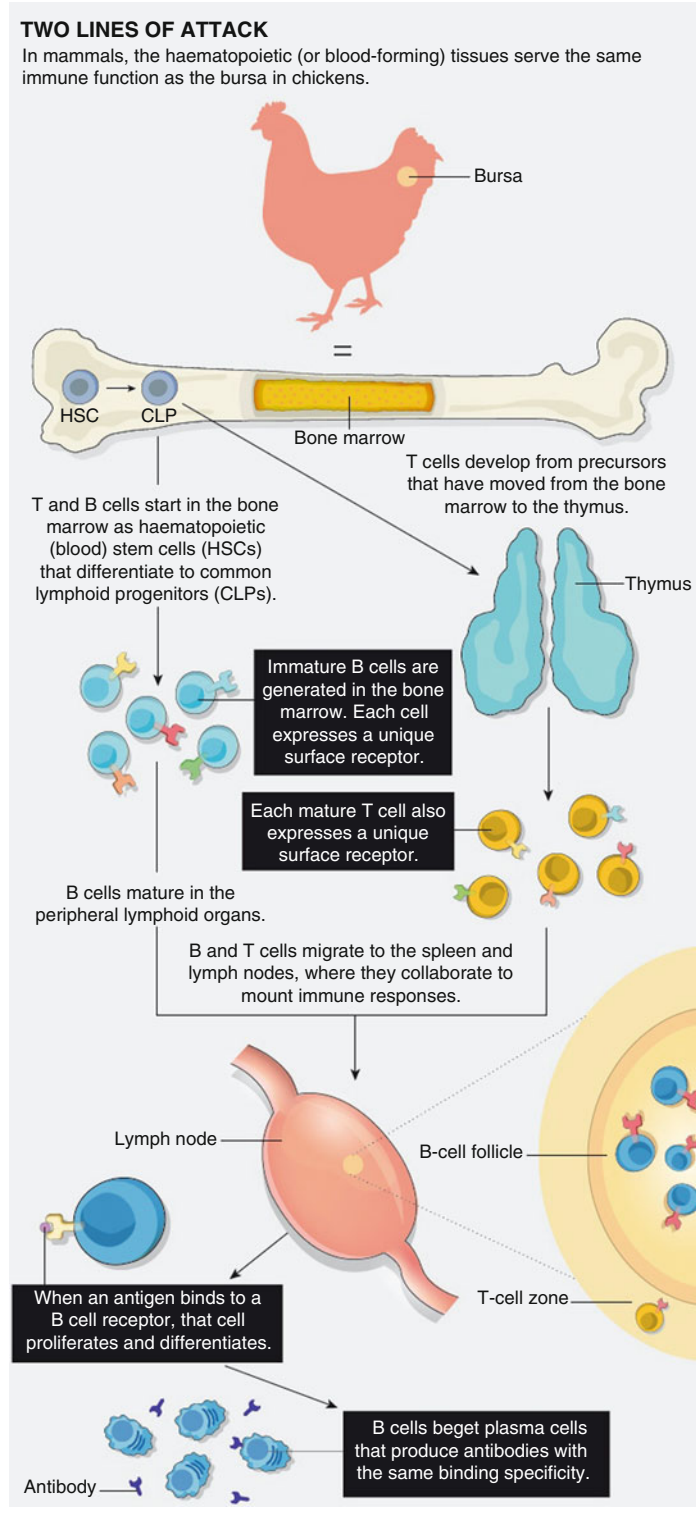

Fig. 1.3 The origin and fate of B and T lymphocytes (Reprinted by permission from Macmillan Publishers Ltd: Gitlin and Nussenzweig [10]. Copyright January 2015)

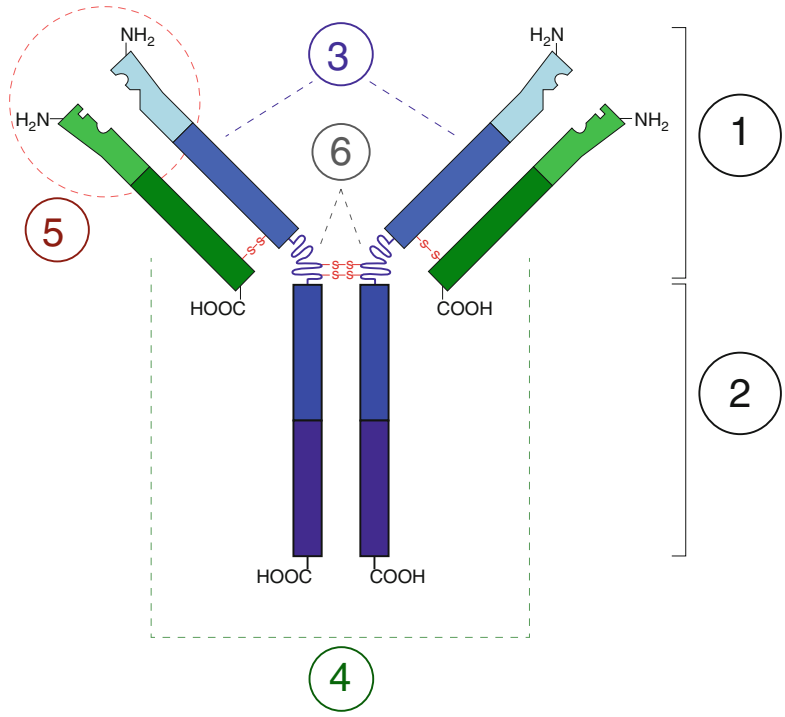

Fig. 1.4 Basic structure of an antibody (Adapted from Y_tambe: Wikipedia under a Creative Commons Attribution-Share Alike 3.0 License). *1* Fab region, *2* Fc region, *3* heavy chain, *4* light chain, *5* antigen binding site, *6* hinge regions

microorganism, the latter called opsonization. This entire process is thus called humoral immunity.

T cells are part of the cellular immunity, which basically is important in eliminating intracellular microorganisms, mainly viruses. In contrast to the BCR, the T-cell receptor can only recognize small fragments of proteins (peptide) that are presented on the surface of the infected cell by major histocompatibility complex (MHC) molecules, also called human leukocyte antigens (HLAs). Thus microorganisms first have to be degraded before they can be recognized by the system. MHC molecules are divided into class I and class II molecules. Class I molecules are present on all nucleated cells and platelets. Only a physical combination of an antigen-peptide within a specific MHC-I-class molecule can be recognized by the T cell receptor. This activation transforms this particular T cell into a cytotoxic T cell capable of killing virally infected cells by inducing apoptosis.

MHC-II-class molecules are only expressed on certain cells of the immune system, in particular dendritic cells, macrophages, and B lymphocytes, together called antigen-presenting cells. These cells can present antigen-peptides in conjunction with MHC-II molecules to T-helper cells. These antigens are derived from degraded microorganisms that are phagocytized by the antigen-presenting cells. In addition, these T-helper cells can secrete numerous cytokines, thereby inducing activation of macrophages, stimulating B cells to produce antibodies but also cytotoxic T cells to do their work. T-helper cells can therefore functionally be divided into Th1 cells, stimulating a cytotoxic T cell response; Th2 cells, involved in the humoral immune response; and different subsets of regulatory T cells, involved in controlling these processes.

<T lymphocytes can be divided in cytotoxic T cells and T-helper cells>

Importantly the interaction between the antigen-presenting cells and the T cells with interaction between HLA molecule TCRs is helped by many other receptors and ligands on these cells, generally called "costimulatory molecules."

Antibodies are produced by plasma cells, which are terminally differentiated B lymphocytes (Fig. 1.4).

<Antigen-specific antibodies are produced by plasma cells, which are terminally differentiated B lymphocytes>

Each antibody is unique and produced by a single clone of plasma cells. Antibodies are composed of an antigen-binding fragment (Fab) and a constant region (Fc), responsible for the effector function of the antibody. An antibody is made up of two identical heavy chains and two identical light chains. Both chains can be divided into a variable part, involved in antigen recognition, and a constant part. This constant part of the heavy chain divides the antibodies into five classes: IgM, IgA, IgG, IgE, and IgD. Immature B cells express IgM (sometimes in combination with IgD) class antibodies on the cell surface. However, under influence of cytokines, B cells can produce other classes of immunoglobulins, a process called isotype switching. This takes place in a specialized compartment of the lymph node, called the follicle or germinal center. In this compartment an additional process takes place, which is called affinity maturation and which means that binding of the BCR of individual B cells to the antigen is further improved. B cells with these improved receptors will more efficiently recognize the antigen after rechallenge and therefore provide a better and faster immune response, which is the idea behind the effect of boost vaccinations in all vaccination programs. While B cells that did not encounter an antigen before are called naive B cells, these improved B cells are called memory B cells.

As already mentioned, the function of free antibodies is twofold: microorganisms loaded with antibodies are phagocytized more easily because phagocytizing cells are capable of binding the Fc part of the antibodies. Besides that, once fixed to an antigen, antibodies are capable of stimulating the complement system.

<Antibodies aid in phagocytosis of microbes and stimulate the complement system>

All cells of the immune system originate from the bone marrow. The myeloid stem cells mature into granulocytes, macrophages, erythrocytes, and thrombocytes, while lymphoid stem cells differentiate to precursor B and T cells (and natural killer cells not discussed here). Maturation of B cells occurs in the bone marrow with formation of unique antigen receptors on the cell surface. In contrast, maturation of T cells takes place in a specialized organ, called the thymus. It is important that B and T cells do not react against self-antigens, since this might result in autoimmunity. Normally, these potentially autoreactive and therefore dangerous cells go into apoptosis, a process called negative selection or clonal deletion. After maturation in the bone marrow and thymus, the lymphoid cells migrate to secondary lymphoid organs, e.g., lymph nodes, spleen, and mucosa-associated lymphoid tissues.

Whereas intact microorganisms can be transported directly to be presented to the B cells in these tissues, for interaction with T cells, transport of antigens is mostly done by dendritic cells. In these peripheral lymphoid organs, both the already mentioned naïve and faster and more efficient memory B and T cells reside, which can directly be activated.

A Closer Look at the Skin

Besides having a barrier function, the skin itself is also an important immunogenic organ.

<The skin functions in the innate as well as in the adaptive immune system>

The skin possesses an innate immune response, characterized by synthesis and release of antimicrobial peptides like defensins and substance P. Next to the innate immunity, the adaptive immunity is provided by Langerhans cells, a population of dendritic cells that reside in the epidermis. These Langerhans cells can phagocytize antigens, migrate to regional lymph nodes (sometimes called veiled cells), and present the antigen to a T lymphocyte, which can result in a cellular or humoral immune response, the latter only if the antigen is also presented to B cells. Moreover, circulating macrophages, T cells, and dendritic cells, present in the dermis, provide continuous immunological surveillance.

Autoimmunity

Cells of the innate immune system recognize so-called pathogen associated molecules on microorganisms. Human cells lack these patterns on their surface, thereby preventing autoreactivity.

The adaptive immune system avoids autoreactivity by the aforementioned clonal deletion or negative selection. This result is also called *immunological tolerance*. When this tolerance is breached, autoreactive B and T cells might be formed, a process called autoimmunity.

<Autoreactive B and T lymphocytes can induce autoimmune diseases>

Immunological tolerance can be achieved by central tolerance, i.e., clonal deletion of B and T cells in the bone marrow and thymus, respectively, and peripheral tolerance. Peripheral tolerance is achieved by functional inactivation and active suppression of autoreactive mature B and T cells that have escaped clonal deletion.

<Central and peripheral tolerance prevents autoimmunity>

For complete activation of B and T cells, besides antigen-antibody binding, the already mentioned co-stimulatory signals are also necessary. These co-stimulatory signals are mostly present on cells of the innate system, i.e., macrophages and dendritic cells. Absence of these signals, e.g., in case of autoreactivity, will result in functional inactivation of the immune response. This is called anergy. Regulatory T cells (Tregs) play an important role in active suppression of the immune response by inhibitory effects on T cells, macrophages, and dendritic cells. In addition to stimulation, some of these costimulatory molecules have an opposite effect by dampening the immune interaction, a physiological process necessary to stop an immune reaction. One of these molecules is CTLA4. Interestingly, some recently developed drugs interact with these costimulatory interactions; for instance, ipilimumab, which blocks CTLA4, is presently used to improve the immune reaction against metastatic melanoma.

Unfortunately these mechanisms are not perfect and autoreactivity can still occur and might eventually result in autoimmune diseases. Several mechanisms can be responsible for breaching immunological tolerance. First, certain microorganisms can bind to the constant part of membranous IgM on the cell surface of B lymphocytes, thereby avoiding the need of co-stimulatory signals of T-helper cells. This is called a *superantigen-stimulated polyclonal lymphocytic activation*. In addition, the Epstein-Barr virus (EBV), after internalization, stimulates B cell proliferation and inhibits apoptosis by producing certain proteins, like EBNA-2 and EBNA-LP. These mechanisms result in an uncontrolled polyclonal B lymphocyte response that might produce self-reactive antibodies. Second, antigens of microorganisms might have a strong resemblance to self-antigens. This might result in a cross reaction of B and T cells against autoantigens, a process called *molecular mimicry*. Finally, exposure of the immune system to normally shielded antigens (eye, testis, brain) or exposure to newly formed antigens (neoepitopes) can result in an immune response to a self-antigen that has not previously been recognized as such. An example of a neoepitope in blistering diseases is the shed ectodomain of collagen XVII that might serve as a self-antigen.

Important modulating factors in autoimmunity are sex hormones, explaining the predominance of autoimmune diseases in women, and genetic background.

<Sex hormones and genetic background are important factors for developing autoimmune diseases>

In particular MHC genes are an important factor in developing autoimmune diseases. Associations have been found between certain MHC haplotypes and autoimmune diseases. For instance, HLA-DQβ1*0301 has been associated with various variants of pemphigoid, whereas several studies have demonstrated an association between HLA-DRB1 and pemphigus vulgaris.

Various pathophysiological mechanisms in autoimmune diseases are eventually responsible for the clinical manifestations. These hypersensitivity reactions are classified after the proposal of Gell and Coombs. In type II reactions autoantibodies are directed against cell or matrix components. Pemphigoid and pemphigus are the result of type II hypersensitivity.

<Autoimmune bullous diseases are the result of type II hypersensitivity, whereas systemic lupus erythematosus results from a type III hypersensitivity reaction>

A type III reaction is the result of deposition of antigen-antibody immune complexes in various organs, eventually resulting in tissue destruction. An example of a type III hypersensitivity reaction is systemic lupus erythematosus (SLE). Typically, SLE is more prominent in women and genetic fac-

tors contribute to the disease. SLE is characterized by the formation of IgG antibodies against nuclear antigens (ANA), in particular against double-stranded DNA (dsDNA). These circulating IgG-dsDNA complexes deposit in various organs, especially in the kidneys (glomerulonephritis), skin (facial erythema), and joints (synovitis). These immune complexes are the mediators of tissue injury, mainly by activating the complement system. As shown in Fig. 1.5, these complexes can directly be visualized by immunofluorescent techniques in a skin biopsy of the patient. Such a pattern is also called a lupus band. In fact, complement consumption and low levels of circulating complement factors C3 and C4 characterize disease activity.

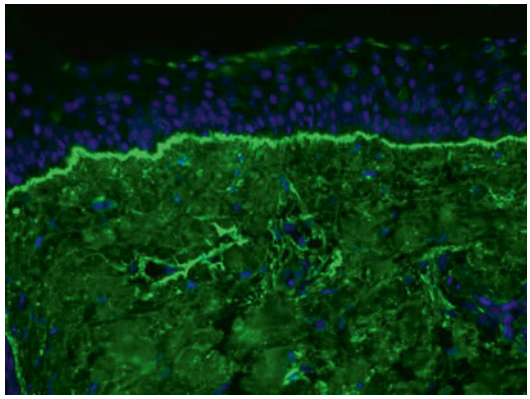

Fig. 1.5 The lupus band: deposition of immunoglobulins along the basement membrane zone

Type IV hypersensitivity or delayed-type hypersensitivity is the result of stimulation of Th1 lymphocytes that can induce tissue damage by secretion of certain cytokines. Eczema is an example of a type IV reaction.

As stated above, pemphigoid and pemphigus are the result of a type II hypersensitivity reaction. In bullous pemphigoid, autoantibodies are directed against collagen XVII (BP180) and/or

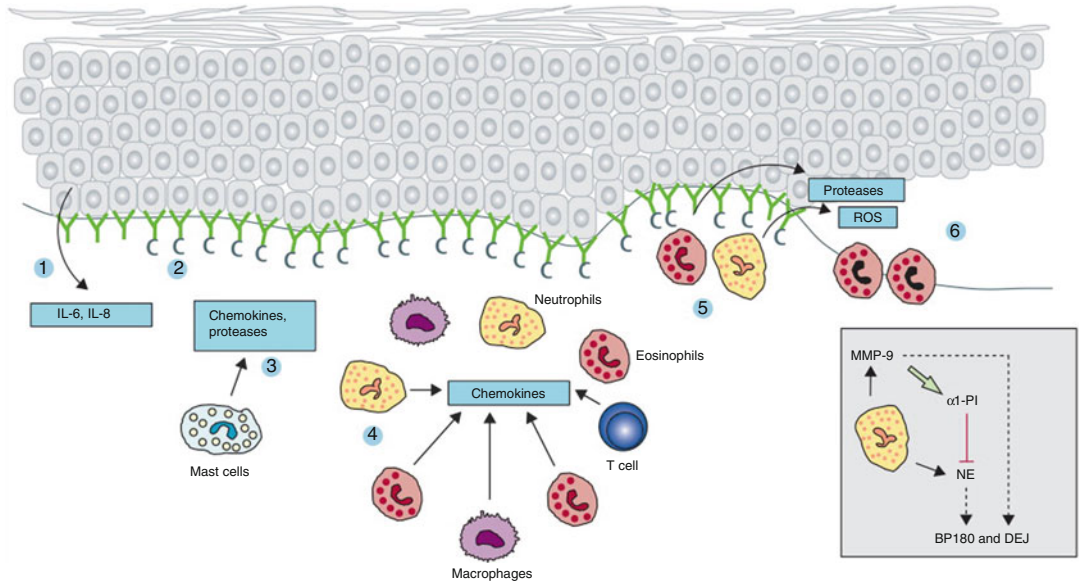

Fig. 1.6 Hypothetical sequence of events leading to blister formation in bullous pemphigoid. Binding of autoantibodies to BP180 initiates Fc receptor-independent events leading to the release of interleukin 6 (*IL-6*) and IL-8 from basal keratinocytes (*1*). Complement is activated (*2*) at the dermal–epidermal junction (*DEJ*) and mast cells degranulate (*3*). Complement activation and chemokine gradients result in the infiltration of inflammatory cells into the upper dermis (*4*). Secretion of inflammatory mediators further increases the inflammatory reaction before granulocytes at the DEJ release proteases (*insert*) and reactive oxygen species (*ROS*) (*5*) that ultimately induce dermal–epidermal splitting (*6*). As shown in the neonatal mouse model of bullous pemphigoid, matrix metalloproteinase 9 (*MMP-9*) secreted from neutrophils cleaves (*green arrow*) α1-proteinase inhibitor (*α1-PI*) to remove neutrophil elastase inhibition (*red bar*). Both MMP-9 and NE also directly degrade proteins of the DEJ including BP180 (*insert*) (Reprinted from *The Lancet*, 381, Schmidt and Zillkens [1], with permission from Elsevier)

BP230, important components of the hemidesmosome, responsible for attachment of the epidermis to the dermis.

<Pemphigoid diseases are characterized by antibodies against hemidesmosomal components>

These antibodies are mainly of the IgG class, although often in conjunction with IgA. These circulating autoantibodies react with these hemidesmosomal antigens, giving rise to a cascade of events. Binding of IgG to BP180 results in complement activation, attraction of inflammatory cells to the dermis, and release of proteases by granulocytes that ultimately induce dermal-epidermal splitting [1] (Fig. 1.6). Besides this inflammatory response, another mechanism has been proposed responsible for detachment of the epidermis from the dermis. Adhesion of antibodies to BP180 can result in internalization and endocytosis of this protein, thereby weakening the hemidesmosome (Fig. 1.7) [2]. In this case an inflammatory response is not necessary for subepidermal blistering and explains the existence of pemphigoid blisters without an inflammatory infiltrate.

Also pemphigus is caused by autoreactive antibodies, in this case directed against desmoglein 1 and 3.

<Pemphigus is characterized by antibodies against desmosomal proteins, mainly desmogleins>

Desmogleins are components of the desmosome, responsible for the attachment between keratinocytes. The exact mechanism by which these antibodies are responsible for acantholysis

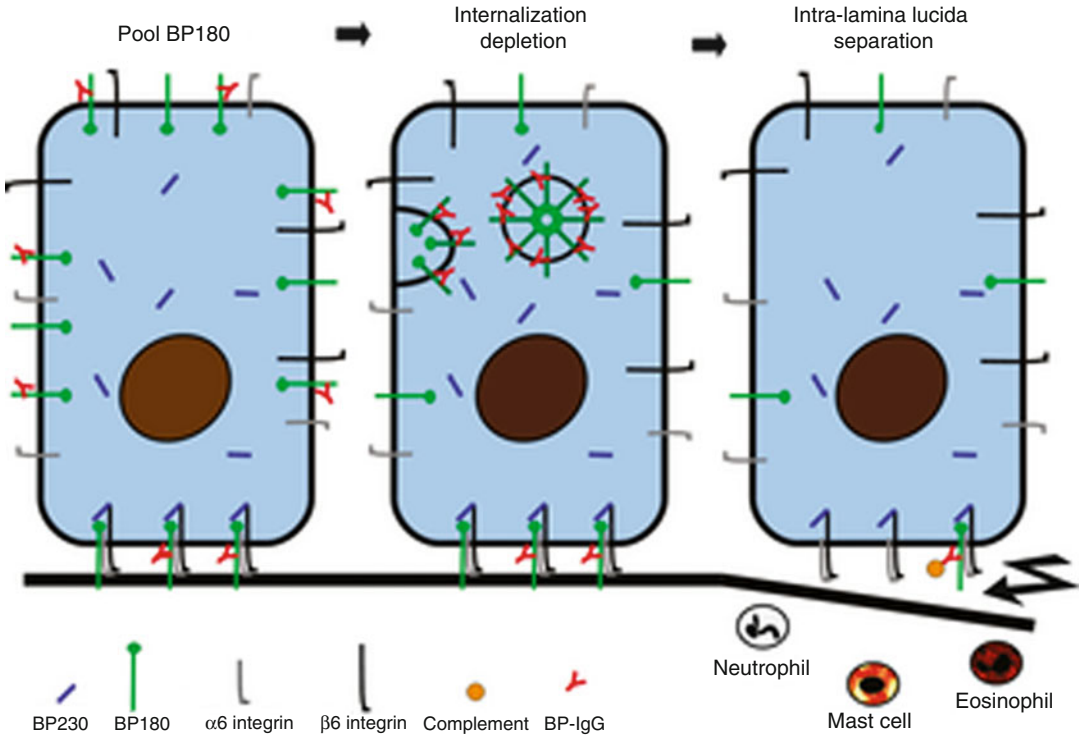

Fig. 1.7 Potential mechanisms of blistering in BP. Hemidesmosomal proteins are distributed homogeneously on the plasma membrane, and some of them compose HD at the ventral side of basal cells (*left*). HD seemed to be constantly remodeled, assembly and disassembly. Initially, autoantibodies bind to BP180, which is distributed on the plasma membrane of basal cells and lead to internalization of BP180 and depleting BP180 from the plasma membrane (*middle*). The depletion of BP180 by anti-BP180 autoantibodies may disturb the supply of BP180 and impair HD formation. Insufficient HD lacking BP180 may not have enough adhesional strength to basement membrane. Finally, intra-lamina lucida separations may be caused by mechanical stress or inflammation, such as fixation of complement and FcgR-dependent activation of neutrophils, induced via Fc fragment of pathogenic IgG (*right*) (Reprinted from: Iwata and Kitajima [2]. With permission from Wiley)

and subsequent intraepidermal blistering is not completely clear. In contrast to pemphigoid, an inflammatory response seems not to be primarily responsible. Several alternative , but not mutual exclusive, theories have been proposed. First is the steric hindrance theory, which is based on the idea that direct interference of IgG with the extracellular domain of desmoglein results in acantholysis [3]. The second theory implies that deranged cell signaling, i.e., activation of p38 MAPK [4], RhoA [5], and plakoglobin [6], interferes with desmosomal function. Finally, pemphigus IgG might influence desmosome assembly and disassembly. Binding of IgG to desmoglein could result in internalization of desmoglein by endocytosis, eventually reducing the adhesion strength between keratinocytes [7].

Review Questions

1. The innate immune system
   (a) Is an antigen-specific system
   (b) Is a quick response system
   (c) Is made up of mainly lymphocytes
2. B lymphocytes
   (a) Are efficient in killing viruses
   (b) Mature in the thymus
   (c) Differentiate into plasma cells, which produce antibodies
3. Autoimmune bullous diseases
   (a) Can be the result of a disturbed peripheral tolerance
   (b) Are an example of type III hypersensitivity
   (c) Both answers are true

Answers

1. (b)
2. (c)
3. (a)

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Suggested Further Reading

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Dermatological Examination of Bullous Diseases

2

Marcel F. Jonkman

Abstract

Physical examination in bullous diseases always comprises looking at the skin and mucous membranes. Examine the skin not only for the presence of vesicles or bullae but also for other efflorescences. Nikolsky's, Sheklakov's, and Asboe-Hansen's signs test the resilience of the skin. The mucous membranes of the mouth, nose, eyes, and genitals need to be examined systematically. Disease activity and extent of the skin and mucous membranes can be assessed using disease activity outcome measures that are validated for pemphigus, bullous pemphigoid, and mucous membrane pemphigoid.

Keywords

Nikolsky's sign • Disease activity • Autoimmune disease • Vesiculobullous disease

Introduction and Aims

Short Definition in Layman Terms

The vesicle or blister is the top efflorescence in the clinical reasoning chain for dermatological diagnosis. Finding only one single blister on the skin is sufficient to make the diagnosis bullous disease. The notion that a skin disease might be autoimmune emerges after a blister is found by physical examination. However, bullous diseases do not always present with blisters. In this chapter the skills and knowledge are outlined in the dermatological examination.

<Bullous diseases do not always present with blisters>

Learning Objectives

After reading this chapter, you will know the algorithm and definitions for the physical examination of the skin and mucous membranes for bullous diseases.

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Case Study: Part 1

A 61-year-old male presented with a widespread bullous eruption of 3 months duration. Clinically, he had numerous flaccid blisters and a few tense bullae on both inflamed and non-erythematous skin involving primarily the scalp, face, neck, and breast. Examination of the oral mucosa revealed extensive desquamative gingivitis and three erosions on the hard palate and buccal measuring up to 2 cm in diameter. Perilesional skin of an erosion exhibited a positive marginal Nikolsky's sign, the base of which was moist and exudative.

A biopsy for histopathology of the left arm revealed suprabasal blister formation with acantholysis. A biopsy for direct immunofluorescence revealed immunoglobulin G (IgG) and C5 deposition throughout the epidermis in a pattern along the cell surface. In indirect IF on monkey esophagus, circulating anti-cell surface antibodies were detected. The ELISA indices of autoantibodies for desmoglein 1 was 57 and for desmoglein 3 was >150.

Didactical Questions: Cross Section of Questions to Prime the Readers Interest

Meticulous skin examination is needed when a vesiculobullous disorder is suspected, since finding one vesicle is sufficient for making the diagnosis. However the absence of a vesicle does not exclude bullous disease, since several may come with only erythema, wheals, papules, nodules, erosions, or crusts. Vesicles are hard to identify on the mucous membranes. For instance, erosions on the gingiva may look like bright red erythema (enanthema), but the glistening surface betrays the lack of epithelium. How do we examine the integument? How can the disease activity be scored?

Facts and Figures

Definitions and Classification

The efflorescences of vesiculobullous diseases as defined by the International League of Dermatological Societies are:

- Vesicle (vesicula): A circumscribed elevation ≤1 cm in diameter that contains liquid (clear, serous, or hemorrhagic)
- Blister (bulla): A circumscribed elevation >1 cm in diameter that contains liquid (clear, serous or hemorrhagic)
- Pustule (pustula): A circumscribed lesion that contains purulent material
- Crust (crusta): Dried serum, blood, or pus on the surface of the skin
- Erosion: Loss of either a portion of or the entire epidermis

<The nomenclature of efflorescences is defined by the International League of Dermatological Societies>

The distribution of vesicles may be solitary, grouped (herpetiform), or arch-like (circinate). The content of vesicles or bullae may be clear (transudate), opaque (serous), or red-blue (hemorrhagic). If the blister cavity is hollow (air filled) within the corneal layer, then in sensu stricto it does not fulfill the definition of a bulla and might be called exfoliation or skin peeling. If the content is yellow (pustular) but yet also serous, then the transitional word vesiculo-pustule is used.

Symptoms

Burning and pain are almost invariable sensations of blisters; pruritus is particularly associated with pemphigoid diseases and dermatitis

herpetiformis. Bullous diseases may start with erythematous lesions that can be macular, papular, urticarial, or nodular before a vesicle or blister erupts. Serous vesicles may become pustular with time as secondary efflorescence. Tense bullae are characteristic of blistering diseases with subepidermal split level such as pemphigoid, whereas slack bullae that break easily are seen in bullous diseases with intraepidermal split, such as pemphigus. When the roof of the blister is lost, an erosion develops. When the liquid in the blister cavity is released, it dries out into a crust. The color of the crust depends on the nature of the blister fluid (light yellow = exudate, blue-black = blood, gold = pus). If the blister does not have an underlying erythema, it is called monomorphic such as in monomorphic pemphigoid, and pseudoporphyria.

Bullous diseases may be accompanied by itch that evokes scratching, resulting in excoriations. The lifetime of a vesicle may be extremely short by immediate scratching such as in dermatitis herpetiformis. Milia (horny pearls in the upper dermis) and scarring appear when the basement membrane is interrupted, such as in epidermolysis bullosa acquisita.

The distribution pattern of the lesions may be solitary (solitary bullous mastocytosis), grouped "en bouquet"/herpetiform (herpes simplex), circinate (linear IgA bullous disease), linear (phytophotodermatitis), or randomly (bullous pemphigoid).

Lesions may be distributed over the whole body such as in bullous pemphigoid, present in a circumscriptive area such as the head and neck in pemphigus, segmental in herpes zoster, or confined to skin folds such as in pemphigus vegetans.

The mucous membranes of body openings (eyes, nose, mouth, genitals) might be involved. Examine the eye for erythema of the upper and lower conjunctiva, synechiae of the conjunctive sac (symblepharon), corneal abnormalities (pannus), and inverted eyelashes (trichiasis). The nose (blood) crusts or erosions can be found on the septum in the nasal vestibule. White patches in the mouth cavity *may consist of blister* roof of thickening of epithelium (leukoplakia). Other efflorescences are erosions and intact vesicles or blisters. Erosions are intense red and differ from red epithelium by their glistering. Patients with bullous disease of the mucous membranes complain of pain or burning sensations of the sensitive mucosa. Ask your patient for photophobia, nasal cleaning, hoarseness, dysphagia, dysuria, and dyspareunia.

Signs

The physician may evoke signs to disclose epidermal dislodgement with the (hand gloved!) fingers.

- *Nikolsky's sign I* (normal or direct Nikolsky's sign): ability to split the epidermis on skin areas distant from the lesions of normal-appearing skin by a lateral pressure with a finger (Fig. 2.1).
- *Nikolsky's sign II* (marginal or indirect Nikolsky's sign): ability to split the epidermis of the skin far beyond the preexisting erosion, extending to a great distance on the normal-appearing skin, by pulling the remnant of a ruptured blister or rubbing at the periphery of existing lesions [1].
- *Pear sign*: old blisters that become flaccid and acquire a pear-like shape due to weight of the exudate, resembling a rubber sack filled with fluid.
- *Sheklakov's sign* (perifocal subepidermal separation): ability to extend to a limited distance a lesion in direction of the periphery by pulling the remnant of a ruptured blister, producing erosions that are limited in size, do not have a tendency to subsequent spontaneous extension, heal fast, and may show a drop of blood.
- *Pseudo-Nikolsky's sign* (epidermal peeling): ability to peel off the entire epidermis by a lateral pressure (rubbing) only on the erythematous skin areas (Fig. 2.2).
- *Asboe-Hansen's or Lutz' sign* (blister spread): ability to enlarge a blister in direction of the

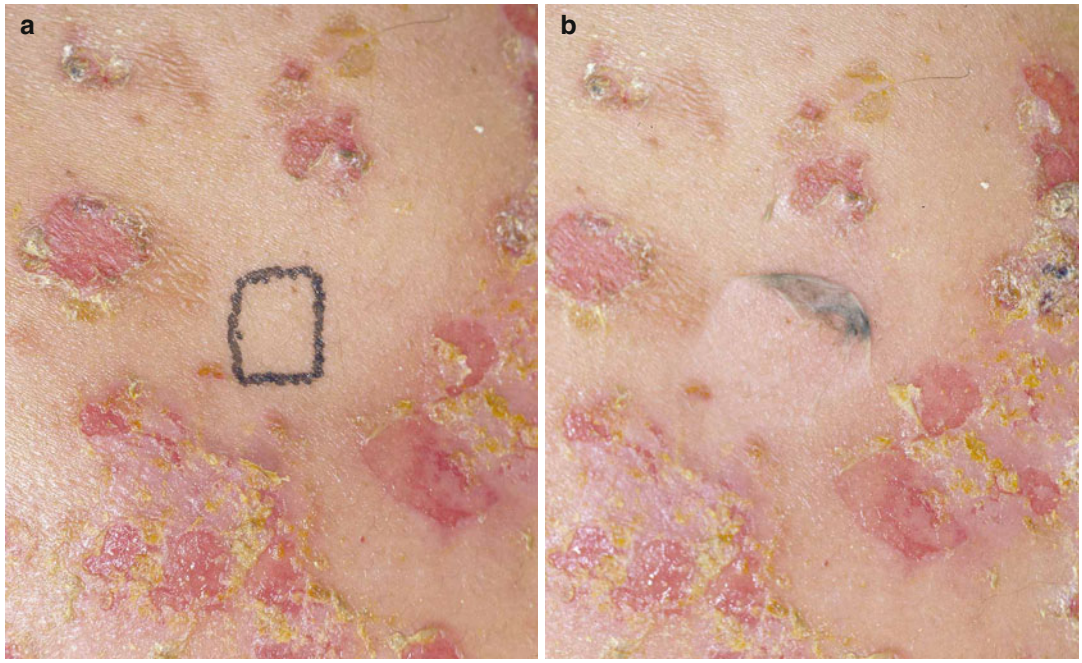

Fig. 2.1 Nikolsky's sign type I procedure in pemphigus vulgaris (**a**) before and (**b**) after pressure with a finger

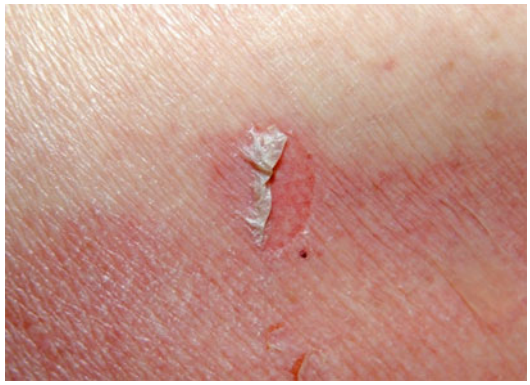

Fig. 2.2 Pseudo-Nikolsky's sign in toxic epidermal necrolysis

periphery by applying mechanical pressure on the roof of intact blister (Fig. 2.3).

<The Nikolsky's and pear signs are positive in epidermal acantholysis>

The Nikolsky's and pear signs are positive in epidermal acantholysis such as in the vulgaris, vegetans, foliaceus, erythematosus, and fogo selvagem subtypes of pemphigus and in staphylococcal scaled skin syndrome (SSSS). A *wet* Nikolsky's sign – exudative surface of the denuded skin – fits with acantholysis in the deeper epidermal layers such as in pemphigus vulgaris, whereas a *dry* Nikolsky's sign fits with acantholysis in the upper epidermis such as in pemphigus foliaceus, erythematosus, fogo selvagem, and SSSS. The Nikolsky's sign can also be evoked on the gingiva to test for oral bullous diseases [2].

Sheklakov's sign is positive in all pemphigoids and in erythema multiforme (EM), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN). Pseudo-Nikolsky's sign is positive in EM, SJS, and TEN. Asboe-Hansen's sign is positive in all bullous diseases.

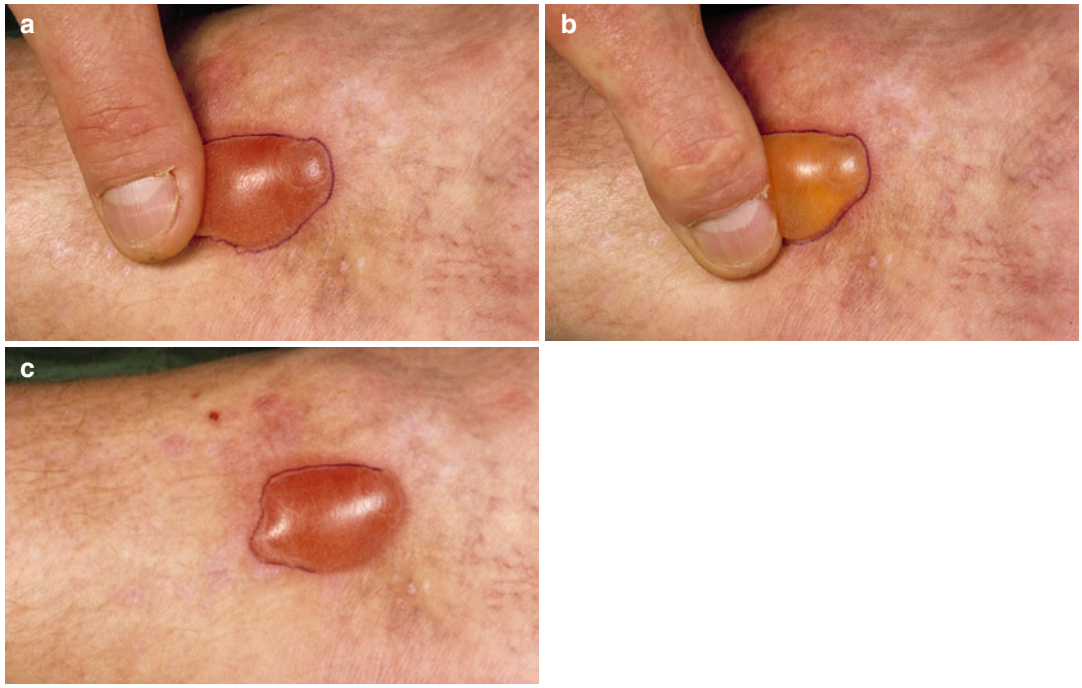

Fig. 2.3 Asboe-Hansen's (or blister spread) sign. (**a**) Draw line around the blister edge and (**b**) press with the thumb on blister. (**c**) Asboe-Hansen's sign is positive if the blister has spread beyond the line. Note the hand should have been gloved.

Definitions and Activity Scores

Pemphigus

Definitions

The consensus definitions of the clinical milestones for pemphigus are listed in Table 2.1 [3].

Pemphigus Disease Area Index (PDAI)

The activity, extent, and damage of skin and mucous membrane pemphigus can be scored with PDAI [4] (Fig. 2.4, Table 2.2).

Bullous Pemphigoid

The consensus definitions of the clinical milestones for pemphigoid are listed in [5] Fig. 2.5 and Table 2.3.

Bullous Pemphigoid Area Index (BPDAI)

The activity, extent, and damage of skin and mucous membrane pemphigus can be scored with BPDAI [5] (Fig. 2.6, Table 2.4).

Table 2.1 Pemphigus definitions [3]

Early observation points	
Baseline	The day that therapy is started by a physician
Control of disease activity (disease control, beginning of consolidation phase)	The time at which new lesions cease to form and established lesions begin to heal
Time to disease control	The time interval between baseline and control
End of the consolidation phase	The time at which no new lesions have developed for a minimum of 2 weeks, approximately 80 % of lesions have healed and when most clinicians start to taper steroids
Late observation end points	
Complete remission off therapy	Absence of new or established lesions while the patient is off all systemic therapy for at least 2 months
Complete remission on therapy	The absence of new or established lesions while the patient is receiving minimal therapy
Other definitions	
Minimal therapy	Prednisone (or the equivalent) at ≤10 mg/day and/or minimal adjuvant therapy for at least 2 months
Minimal adjuvant therapy	Half of the dose required to be defined as treatment failure
Partial remission off therapy	Presence of transient new lesions that heal within 1 week without treatment and while the patient is off all systemic therapy for at least 2 months
Partial remission on minimal therapy	The presence of transient new lesions that heal within 1 week while the patient is receiving minimal therapy, including topical steroids
Relapse/flare	Appearance of ≥3 new lesions/month that do not heal spontaneously within 1 week, or by the extension of established lesions, in a patient who has achieved disease control

Reprinted from: Murrell *et al.* [3], with permission from Elsevier

Case Study: Part 2

The patient was diagnosed with pemphigus vulgaris. At the start of therapy, the PDAI score was 23 on skin and 12 on mucous membranes.

He was successfully treated with prednisone 1 mg/kg daily tapered in 4 months and in addition 2× 1000 mg rituximab. During the induction phase, non-inflamed skin exhibited a positive direct Nikolsky's sign, beneath which was a nonexudative blister base. After 2 weeks, control of disease was reached where no new lesions anymore developed. The skin improved quicker than the mouth with a PDAI at the end of the consolidation phase of 0 and 5, respectively.

Mucous Membrane Pemphigoid Area Index (MMPDAI)

The consensus definitions of the clinical milestones for mucous membrane pemphigoid are listed [6] (Table 2.5).

The activity, extent, and damage of skin and mucous membrane pemphigus can be scored with MMPDAI [6] (Fig. 2.7, Table 2.6).

Case Study: Part 3

Patient reached complete remission while off therapy by 6 months that sustained during the total follow-up period of 18 months. The PDAI dropped to 0.

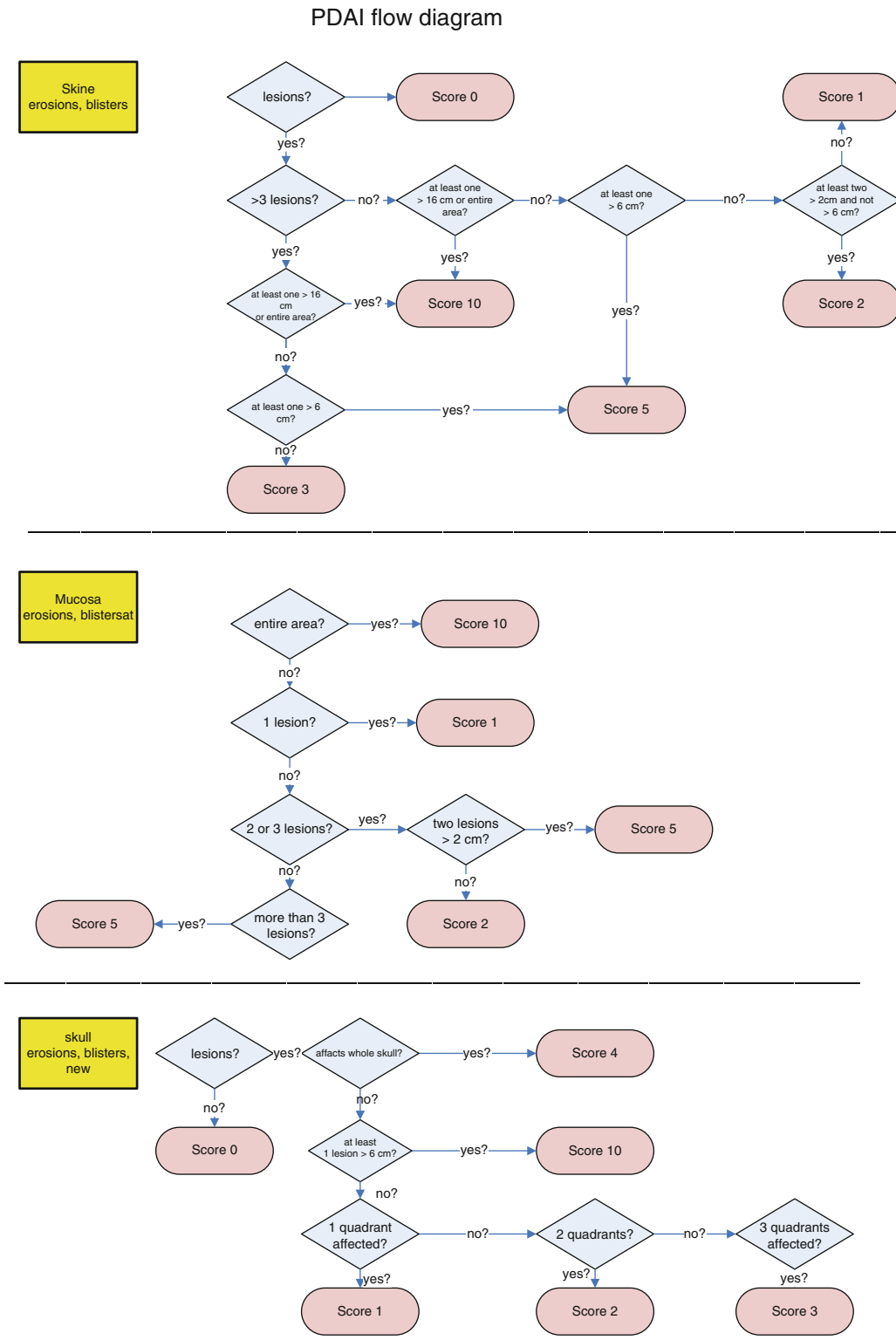

Fig. 2.4 Pemphigus disease area index (PDAI) flow diagram

Table 2.2 Pemphigus disease area index
Reprinted from Murrell *et al.* [3], with permission from Elsevier

PDAI

Name :

DOB :

Number :

Date :

Treatment phase			
	Baseline		**Complete remission on minimal therapy**
	Control of disease		**Partial remission off therapy**
	Consolidation phase		**Complete remission off therapy**
	Partial remission on minimal therapy		**Flare**

Skin	**Activity**		**Damage**
Anatomical location	**Erosions/Blisters or new erythema**		**Post-inflammatory hyperpigmentation or erythema from resolving lesion**
	0	absent	0 absent 1 present
	1	1-3 lesions, up to one lesion >2 cm in any diameter; none > 6 cm	
	2	2-3 lesions, at least two lesions > 2cm; none > 6 cm	
	3	> 3 lesions, none > 6 cm	
	5	>3 lesions; and/or at least one lesion > 6 cm	
	10	>3 lesions; and/or at least one > 16 cm or entire area	
Ears			
Nose			
Rest of the face			
Neck			
Chest			
Abdomen			
Back, buttocks			
Arms			
Hands			
Legs			
Feet			
Genitals			
Total skin scores	**/120**		**/12**

Table 2.2 (continued)

Scalp	Erosions/Blisters or new erythema		Post-inflammatory hyperpigmentation or erythema from resolving lesion
	0	absent	0 absent 1 present
	1	in 1 quadrant	
	2	in 2 quadrants	
	3	in 3 quadrants	
	4	affects whole skull	
	10	at least 1 lesion > 6 cm	
Total scalp scores	**/10**		**/1**

Total score of damage skin and scalp	**/13**

Mucous membranes	
Anatomical location	**Erosions/Blisters**
	0 absent **1** 1 lesion **2** 2-3 lesions **5** >3 lesions of 2 lesions > 2 cm **10** entire area
Eyes	
Nose	
Buccal mucosa	
Hard palate	
Soft palate	
Upper gingiva	
Lower gingiva	
Tongue	
Floor of mouth	
Labial mucosa	
Posterior pharynx	
Anogenital	
Total Mucosa Score	**/120**

Total Activity Score Skin+Scalp+Mucosa	**/250**

Total Damage Score Skin+Scalp	**/13**

[Reprinted from: Murrell DF, Dick S, Ahmed AR, Amagai M, Barnadas MA, Borradori L, et al. Consensus statement on definitions of disease, end points, and therapeutic response for pemphigus. Journal of the American Academy of Dermatology 2008;58:1043-6, with permission from Elsevier.]

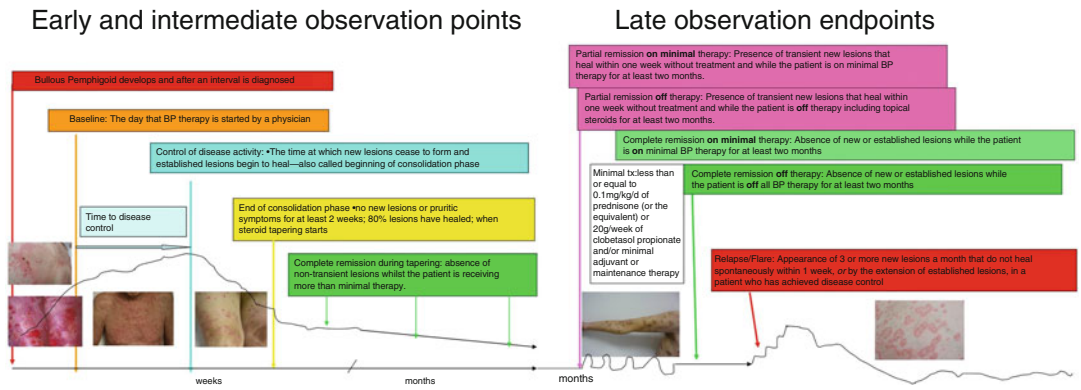

Fig. 2.5 Pictorial depiction of end points in bullous pemphigoid (Reprinted from Murrell *et al.* [5], with permission from Elsevier

Table 2.3 Definitions for bullous pemphigoid

Early observation points	
Baseline	Day that BP therapy is started by the physician
Control of disease activity	Time at which new lesions cease to form and established lesions begin to heal or pruritic symptoms start to abate
Time to control of disease activity (disease control, beginning of consolidation phase)	The time interval between baseline and control of disease activity
End of the consolidation phase	Time at which no new lesions have developed for a minimum of 2 weeks and approximately 80 % of lesions have healed and pruritic symptoms are minimal
Intermediate observation end points	
Transient lesions	New lesions that heal within 1 week or pruritus lasting <1 week and clearing without treatment
Nontransient lesions	New lesions that do not heal within 1 week or pruritus continuing >1 week with or without treatment
Complete remission during tapering	Absence of nontransient lesions while patient is receiving more than minimal therapy
Late observation end points	
Minimal therapy	≤0.1 mg/kg/day of prednisone (or equivalent) or 20 g/week of clobetasol propionate and/or minimal adjuvant or maintenance therapy
Minimal adjuvant therapy and/or maintenance therapy	Following doses or less: methotrexate 5 mg/week, azathioprine 0.7 mg/kg/day (with normal thiopurine-s-methyltransferase level), mycophenolate mofetil 500 mg/day, mycophenolic acid 360 mg/day, or dapsone 50 mg/day
Partial remission on minimal therapy	Presence of transient new lesions that heal within 1 week while patient is receiving minimal therapy for at least 2 months
Complete remission on minimal therapy	Absence of new or established lesions or pruritus while patient is receiving minimal therapy for at least 2 months
Partial remission off therapy	Presence of transient new lesions that heal within 1 week without treatment while patient is off all BP therapy for at least 2 months
Complete remission off therapy	Absence of new or established lesions or pruritus while patient is off all BP therapy for at least 2 months

Table 2.3 (continued)

Mild new activity	<3 lesions/month (blisters, eczematous lesions, or urticarial plaques) that do not heal within 1 week or extension of established lesions or pruritus once/week but less than daily in patient who has achieved disease control; these lesions have to heal within 2 weeks
Relapse/flare	Appearance of ≥ 3 new lesions/month (blisters, eczematous lesions, or urticarial plaques) or at least one large (10 cm diameter) eczematous lesion or urticarial plaques that do not heal within 1 week or extension of established lesions or daily pruritus in patient who has achieved disease control
Failure of therapy for initial control	Development of new nontransient lesions or continued extension of old lesions or failure of established lesions to begin to heal or continued pruritus despite: Clobetasol propionate 40 g/day for 4 weeks or Prednisone 0.75 mg/kg/day equivalent for minimum of 3 weeks with or without drugs used for maintenance therapy or A tetracycline on full dosing for 4 weeks or Dapsone 1.5 mg/kg/day for 4 weeks or Methotrexate 15 mg/week (if 60 kg and no major renal impairment) for 4 weeks or azathioprine 2.5 mg/kg/day for 4 weeks (if thiopurine-s-methyltransferase level is normal) or Mycophenolate mofetil 40 mg/kg/day (if normal renal function, otherwise according to age/creatinine clearance) for 4 weeks

Reprinted from Murrell *et al.* [5], with permission from Elsevier

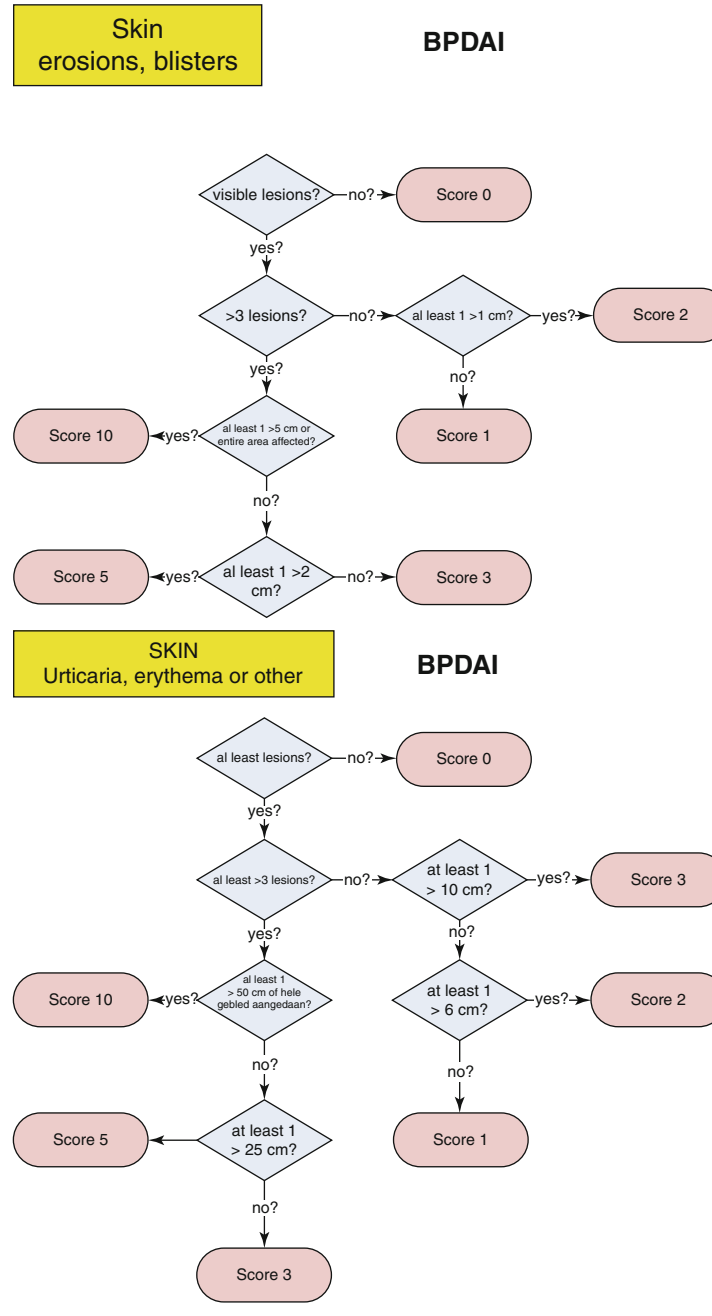

Fig. 2.6 Bullous pemphigoid area index (BPDAI) flow diagram for assessment of (**a**) skin blisters and erosions; (**b**) skin urticaria, erythema, and other lesions; and (**c**) mucous membrane blisters and erosions

Fig. 2.6 (continued)

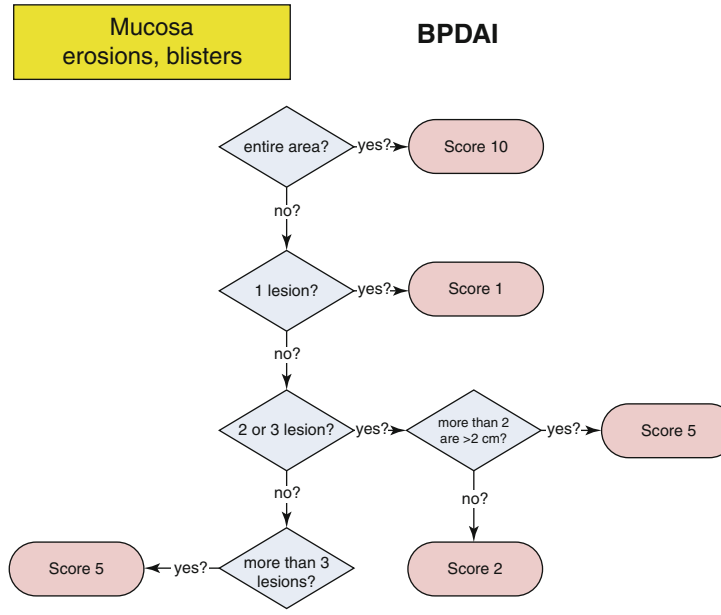

Table 2.4 Bullous pemphigoid disease area index (BPDAI)
Reprinted from Murrell *et al.* [5], with permission from Elsevier

Name :

DOB :

:

Date :

Diagnosis:			
Treatment phase:			
Number of weeks after baseline:			
Current medication:			
	Baseline		**Complete remission on minimal therapy**
	Control of disease		**Partial remission off therapy**
	Consolidation phase		**Complete remission off therapy**
	Partial remission on minimal therapy		**Flare**

[Reprinted from: Murrell DF, Daniel BS, Joly P, Borradori L, Amagai M, Hashimoto T, et al. Definitions and outcome measures for bullous pemphigoid: recommendations by an international panel of experts. J Am Acad Dermatol 2012;66:479-485, with permission from Elsevier.]

Table 2.4 (continued)

BPDAI SKIN	**ACTIVITY**		**ACTIVITY**		**DAMAGE**
Anatomical location	**Erosions/Blisters**		**Urticaria/Erythema/ Other**		**Pigmentation / Other**
	0	absent	0	absent	0 absent 1 present
	1	1-3 lesions; none > 1 cm	1	1-3 lesions; none > 6 cm	
	2	1-3 lesions, at least 1 lesion >1 cm	2	1-3 lesions, at least 1 lesion > 6 cm	
	3	>3 lesions, none >2cm	3	>3 lesions, at least 1 lesion > 10 cm	
	5	>3 lesions, and at least 1 lesion >2cm	5	>3 lesions, at least 1 lesion > 25cm	
	10	>3 lesions, and at least 1 lesion >5cm or entire area	10	>3 lesions, at least 1 lesion > 50cm or entire area	
Head					
Neck					
Chest					
Left arm					
Right arm					
Hands					
Abdomen					
Genitals					
Back/Buttocks					
Left leg					
Right leg					
Feet					
Total skin score	**/120**		**/120**		**/12**
MUCOSA	**Erosions/Blisters**				
	0	Absent			
	1	1 lesion			
	2	2-3 lesions			
	5	3>lesions, of 2 > 2 cm			
	10	Entire area			
Eyes					
Nose					
Buccal mucosa					
Hard palate					
Soft palate					
Upper gingiva					
Lower gingiva					
Tongue					
Floor of mouth					
Labial mucosa					
Posterior pharynx					
Anogenitaal					
Total score mucosa	**/120**				
Total activity score skin (blist./urtic.) + mucosa	**/360**		**Total damage score skin**		**/12**

Table 2.5 Definitions for mucous membrane pemphigoid

Early observation points	
Baseline	The day that MMP therapy is started by a physician
Control of disease	The time at which new inflammatory lesions cease to form and established lesions begin to heal
Time to control disease activity (disease control, beginning of consolidation phase)	The time interval from baseline to the control of disease activity
Control of scarring	The time needed to control scarring progression
End of the consolidation phase	The time at which no new lesions have developed for minimum of 4 weeks and approximately 80 % of inflammatory lesions have healed
Intermediate observation end points	
Transient lesions	New lesions that heal within 1 week or clear without treatment
Nontransient lesions	New lesions that do not heal within 1 week
Complete remission during tapering	The absence of nontransient lesions while the patient is receiving more than minimal therapy
Minimal therapy	Dapsone $\leq$ 1.0 mg/kg/day, $\leq$ 0.1 mg/kg/day of prednisone (or the equivalent), minocycline $\leq$ 100 mg/day, doxycycline 100 mg/day, lymecycline 300 mg/day, topical corticosteroids once a day including fluticasone propionate suspension 400 µg/once a day, colchicine 500 µg/day, Salazopyrin 1 g/day, sulfapyridine 500 mg/day, sulfamethoxypyridazine 500 mg/day, nicotinamide 500 mg/day
Minimal adjuvant therapy (and/or maintenance therapy)	The following doses or less: azathioprine (1 mg/kg/day) with normal thiopurine-s-methyltransferase level, mycophenolate mofetil 500 mg/day, mycophenolic acid 360 mg/day, methotrexate 5 mg/week, cyclosporine 1 mg/kg/day
Long-term biological therapy	Refers to therapies given intermittently, for example, when rituximab is used for MMP or IVIG monthly
Late observation end points	
Partial remission on minimal therapy	Presence of transient new lesions that heal without scarring within 1 week while patient is receiving minimal therapy for at least 2 months
Complete remission on minimal therapy	The absence of new or established lesions or pruritus while patient is receiving minimal therapy for at least 2 months
Partial remission off therapy	Presence of transient new lesions that heal within 1 week without treatment while patient is off all MMP therapy for at least 2 months
Complete remission off therapy	Absence of new or established lesions or pruritus while patient is off all MMP therapy for at least 2 months
Relapse/flare	Appearance of $\geq$3 new lesions a month (blisters, erosions) that do not heal within 1 week or extension of established lesions in patient who has achieved disease control

Reprinted from Murrell et al. [6], with permission from Elsevier
IVIG Intravenous immunoglobulin, *MMP* mucous membrane pemphigoid

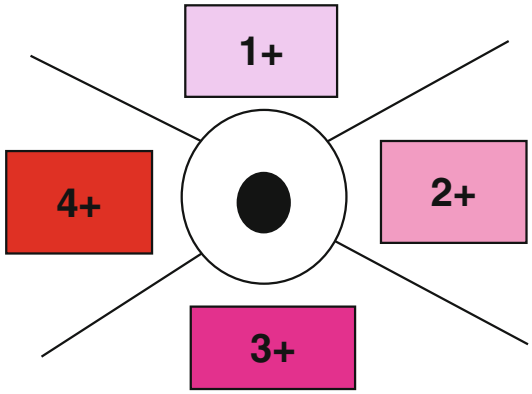

Fig. 2.7 Eye quadrants for mucous membrane pemphigoid area index (MMPDAI). Diagram to illustrate how erythema is to be scored in different quadrants of each eye for the mucosal component of the mucous membrane pemphigoid disease area index. The degree of pinkness represents how high to score this parameter

Table 2.6 Mucous membrane pemphigoid disease area index (MMPDAI)
Reprinted from Murrell *et al.* [6], with permission from Elsevier

Name :

DOB :

:

Date :

Treatment phase			
	Baseline		**Complete remission on minimal therapy**
	Control of disease		**Partial remission off therapy**
	Consolidation phase		**Complete remission off therapy**
	Partial remission on minimal therapy		**Flare**

[Reprinted from: Murrell DF, Marinovic B, Caux F, Prost C, Ahmed R, Wozniak K, et al. Definitions and outcome measures for mucous membrane pemphigoid: Recommendations of an international panel of experts. J Am Acad Dermatol 2015;72:168-174., with permission from Elsevier.]

(continued)

Table 2.6 (continued)

Skin	**Activity**		**Damage**
Anatomical location	**Erosions/Blisters or new erythema**		**Post-inflammatory hyperpigmentation or erythema from resolving lesion or scarring**
	0	absent	0 absent 1 present
	1	1-3 lesions, up to one >2 cm in any diameter, none > 6 cm	
	2	2-3 lesions, at least two > 2 cm diameter, none > 6cm	
	3	>3 lesions, none > 6 cm diameter	
	5	>3 lesions, and/or at least one >6 cm	
	10	>3 lesions, and/or at least one lesion >16 cm diameter or entire area	
Ears			
Forehead			
Rest of the face			
Neck			
Chest			
Abdomen			
Shoulders, Back			
Buttocks			
Arms & hands			
Legs& feet			
Anal			
Genitals			
Total skin scores	**/120**		**/12**

Scalp	**Erosion/Blisters/active erythema**		**Post-inflammatory hyperpigmentation or erythema from resolving lesion or scarring**
	0	absent	0 absent 1 present
	1	in 1 quadrant	
	2	in 2 quadrants	
	3	in 3 quadrants	
	4	affects whole scalp	
	10	at least 1 lesion > 6 cm	
Total scalp	**/10**		**/1**

Table 2.6 (continued)

Mucous membranes	**Activity**	**Damage**
Anatomical location	**Erosion/Blisters/active erythema**	**Post-inflammatory hyperpigmentation or erythema from resolving lesion or scarring**
Eyes (quadrants upper, lower, medial and lateral)	**0** No erythema **1** Light pink **2** Moderate pink **3** Dark pink **4** Bright red add up quadrants	0 absent 1 present
Left eye (0-16) x 0.625		
Right eye (0-16) x 0.625		
	0 absent **1** 1 lesion, or 1 quadrant eye **2** 2-3 lesions, or 2 quadrants eye **5** > 3 lesions or two lesions > 2 cm, or three quadrants eye **10** entire area, or four quadrants eye	0 absent 1 present
Nose		
Buccal mucosa		
Palate		
Upper gingiva		
Lower gingiva		
Tongue/Floor of mouth		
Labia		
Posterior pharynx		
Anus		
Genitals		
Total mucosa scores	**/120**	**/12**

Total scores skin + scalp + mucosa	**/250**	**/25**

Review Questions

1. The liquid contents of a serous vesicle is
   (a) Transparent
   (b) Opaque
   (c) Purulent
   (d) Leaking
2. Intentional epidermal detachment from within the lesion extending into normal-appearing skin is called
   (a) Nikolsky's sign I
   (b) Nikolsky's sign II
   (c) Asboe-Hansen's sign
   (d) Sheklakov's sign
3. Synonym to Nikolsky's sign I is
   (a) Marginal Nikolsky's sign
   (b) Normal Nikolsky's sign
   (c) Indirect Nikolsky's sign
   (d) Pseudo-Nikolsky's sign
4. The beginning of the consolidation phase in BP is the moment when reached
   (a) Control of disease activity
   (b) Partial remission on minimal therapy
   (c) Partial remission off therapy
   (d) Complete remission on minimal therapy
5. Minimal therapy in MMP is NOT
   (a) Dapsone 1.0 mg/kg/day
   (b) Prednisone 0.1 mg/kg/day
   (c) Doxycycline 100 mg/day
   (d) Colchicine 1 mg/day

Answers

1. (b)
2. (b)
3. (b)
4. (a)
5. (d)

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On the Web

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How to Take a Biopsy

3

Marcel F. Jonkman and Gilles F.H. Diercks

Abstract

The skin is an organ that is easy to sample for microscopy. Sections of the skin may reveal the split level of the blister, the type and distribution of invading inflammatory cells, and the presence, class, and distribution pattern of autoantibodies. For this, one needs to take a biopsy from a location with smallest change of sample error and specially fixate and the transport the specimen for the requested microscopic technique. All together, this is the playing ground of the dermatologist. Due to special transport requirements, dermatologists even take conjunctiva biopsies for immunofluorescence (IF) in case of suspicion of an autoimmune bullous disease of the eye.

Keywords

Histopathology • Immunofluorescence • Biopsy

Introduction and AIMS

Short Definition in Layman Terms

The skin is an organ that is easy to sample for microscopy. Sections of the skin may reveal the split level of the blister, the type and distribution of invading inflammatory cells, and the presence, class, and distribution pattern of autoantibodies. For this, one needs to take a biopsy from a location with smallest change of sample error and specially fixate and the transport the specimen for the requested microscopic technique. All together, this is the playing ground of the dermatologist. Due to special transport requirements, dermatologists even take conjunctiva biopsies for immunofluorescence (IF) in case of suspicion of an autoimmune bullous disease of the eye.

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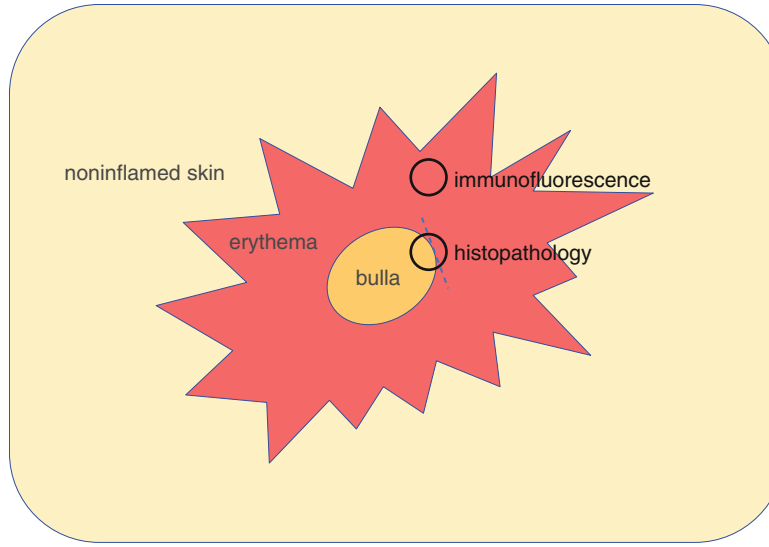

Fig. 3.1 Preferred locations of biopsies for histopathology and direct immunofluorescence microscopy is 2/3 in bulla and peribullous erythema, respectively

Learning Objectives

- Procedure of the biopsy
- Where to take a skin or mucosa biopsy in a patient suspected of an autoimmune bullous disease
- How to transport and handle a biopsy

Didactical Questions: Cross Section of Questions to Prime the Readers' Interest

What is a perilesional biopsy? What is the preferred location to biopsy for IF in nonbullous cutaneous pemphigoid? Can I biopsy for IF when the patient uses prednisolone? How is the IF biopsy transported?

Histopathology

Procedure of the Punch Biopsy

There is no rational for AIBD diagnosis to take larger samples by oval excision, than by punch biopsy. For histopathology, the punch biopsy sample should measure at least 4 mm in diameter to minimize sampling error and to provide sufficient tissue for any special staining that may be required. The procedure for performing the biopsy is explained on video [1]. Ideally, a biopsy of a very recent lesion should be sent, in formalin, to histopathology. Older lesions may yield confusing information, because there may be regeneration changes or secondary infection.

What to Biopsy

The biopsy should include two-third blister cavity and one-third peribullous skin. One may draw a line that touches the blister (tangent) for orientation before giving local anesthesia and biopsy perpendicular to the tangent (Fig. 3.1).

Transport and Handling of Biopsies

For histopathology the biopsy is submersed in a tube with 4 % formaldehyde fixative and sent at room temperature to the histopathological laboratory.

Direct Immunofluorescence

Procedure of the Biopsy

Skin

For direct immunofluorescence (DIF) microscopy of the skin, the punch biopsy sample should measure at least 4 mm in diameter. The wound is sutured at the end of the procedure.

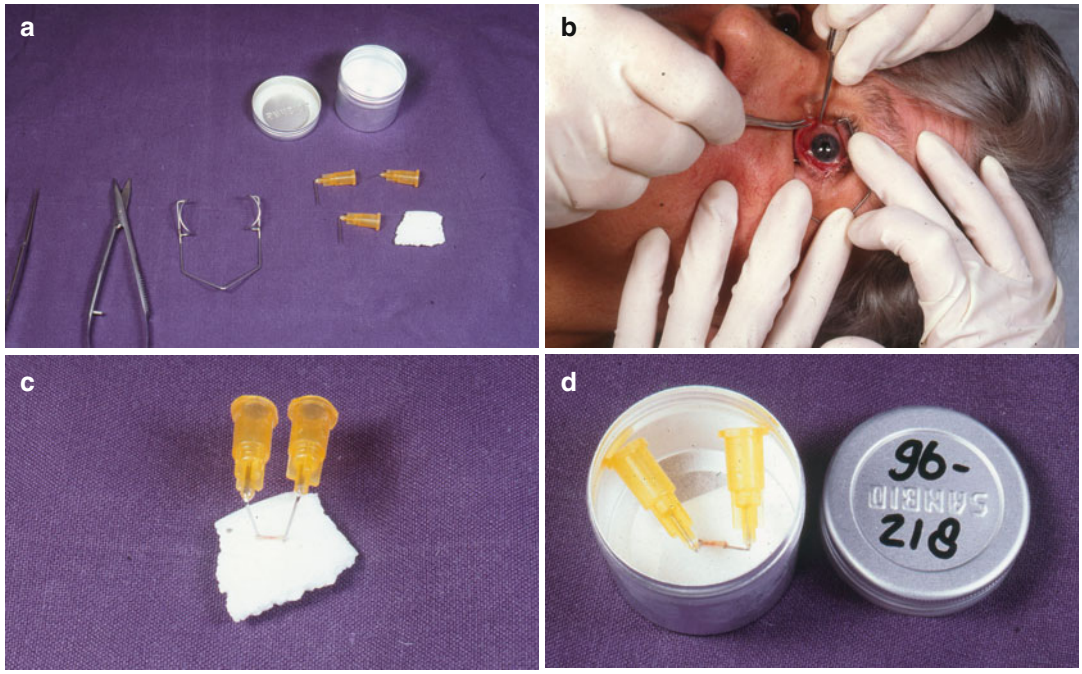

Fig. 3.2 Immunofluorescence biopsy of conjunctiva. (**a**) Micro eye tweezer, micro eye Castroviejo curved scissors, two 25 Gauge 5/8th inch orange needles bent by 90°, and aluminum vial with screwed top, (**b**) cutting of small oval piece of conjunctiva with micro scissors, (**c**) conjunctiva specimen pinned to polystyrene, (**d**) pinned specimen placed in vial

Buccal Mucosa

For DIF of the oral mucosa, the punch biopsy sample should measure at least 3 mm in diameter. The location is one-third of the distance from the corner of the mouth to the last molar to avoid the entry point of the parotis gland. The wound is not sutured at the end of the procedure. The use of a resorbable suture has no additional benefit unless hemostasis is not reached without one.

Conjunctiva

For the diagnosis of ocular AIBD DIF of the conjunctiva is the golden standard. The front of the eye is anesthetized by two droplets of oxybuprocaine 0.4 % in the conjunctiva sac. The eye is closed; anesthesia is reached after 15 s. An eye spreader is place below the upper and lower eyelids (Fig. 3.2a). The conjunctiva above the ocular bulbus is picked up with micro forceps, and an oval peace of mucous membrane with 3 mm in length is cut with a micro scissors (Fig. 3.2b). The tissue sample is placed on a piece of polystyrene (from a coffee cup) and pierced at the tip with a small needle that was bent in 90°, spread with second needle that is bent in 90°, and pierced again (Fig. 3.2c). The needles are turned to each other and the whole fits into an aluminum container (Fig. 3.2d). After screwing the cup on the container, the whole is immersed in liquid nitrogen until the boiling stops. The specimen would be lost or would curl up if it was not pinned down.

<Include 2/3rd of bulla in punch biopsy for histopathology>

What to Biopsy

For direct immunofluorescence microscopy, it is important to take an adequate skin or mucosal biopsy in order to avoid false-negative results. The consensus agrees to take a *perilesional* biopsy. The exact place of the perilesional biopsy is under debate. We prefer erythematous skin 1–2 cm adjacent to a vesicle or bulla (*lesional peribullous biopsy*), while others prefer intact non-inflamed skin beside a lesion.

<What should I biopsy for IF?> (Table 3.1)

A perilesional biopsy will increase the possibility of a positive result and one avoids a false-negative result by taking a lesional biopsy with secondary changes, e.g., erosion or ulceration of the epidermis. Moreover, lesional biopsies in pemphigoid often yield negative findings because the epidermal basement membrane is destroyed. Although in general a biopsy of clinically uninvolved skin (preferentially from the inner aspect of the upper arm) is not necessary for diagnostic purposes [2], this still might be considered, since especially in cases of pemphigoid it allows a better serration pattern analysis (see below).

There are several exceptions on this rule. In bullous systemic lupus erythematosus (SLE), apart from taking a perilesional biopsy, it is also recommended to take a biopsy of lesional skin in order to find a lupus band. In addition, it has been demonstrated that a biopsy of non-sun-exposed skin of the wrist with a positive lupus band has a predictive value of possible renal involvement. In lichen planus pemphigoides, one can decide to take a lesional and a perilesional biopsy. The lesional biopsy will typically show a lichenoid infiltrate and deposition of fibrin along the basement membrane (see below). The preferred biopsy site of clinically uninvolved skin in dermatitis herpetiformis is the extensor side of the elbow, since this is a predilection site. Finally, in nonbullous cutaneous pemphigoid, it is advised to take a lesional biopsy from a papule or perilesional biopsy from erythema..

<Always take a perilesional biopsy for IF>

Transport and Handling of Biopsies

Several options exist for handling biopsy specimens. The most widely used method is snap-frozen in liquid nitrogen. Alternatively, Michel's solution [3], which contains ammonium sulfate, N-ethyl-maleimide, potassium citrate buffer, magnesium sulfate, and distilled water, can be used, facilitating transport of biopsies from outside hospitals.

Table 3.1 Recommendation of immunofluorescence biopsy sites in autoimmune bullous diseases

	Perilesional	Uninvolved skin	Lesional
In general	✓	Inner aspect upper arm	
Exceptions			
Bullous SLE	✓	Dorsal site wrist	✓
Lichen planus pemphigoides	✓		✓
Dermatitis herpetiformis	✓	Extensor site elbow	
Nonbullous cutaneous pemphigoid	✓	✓	✓

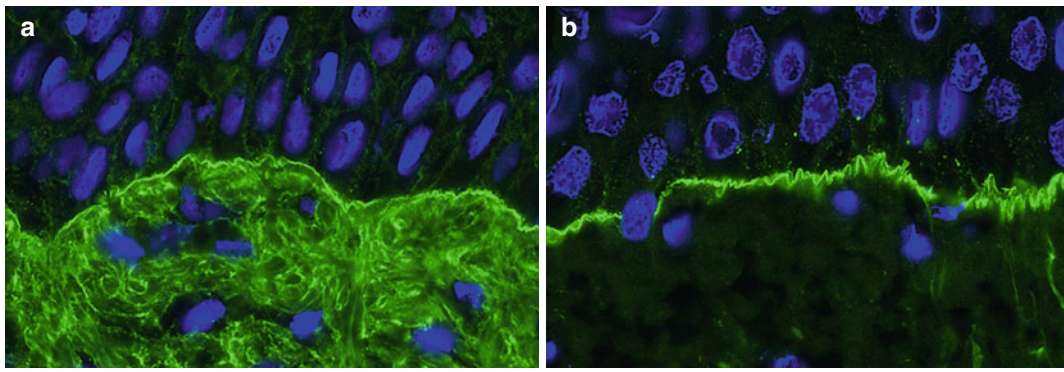

Fig. 3.3 A biopsy stored in normal saline (**b**) yields a higher signal to noise ratio than a biopsy stored in liquid nitrogen (**a**)

However, a disadvantage of both methods is the high dermal background fluorescence due to undesired specific (dermal IgG) and non-specific staining. This lowers the signal to noise ratio, which yields false-negative cases, especially in cases of pemphigoid with a weak staining of the basement membrane zone (Fig. 3.3a).

Alternatively, transport and overnight storage in normal saline can be used. Saline-stored biopsies result in a decreased background staining and an increased signal to noise ratio, eventually leading to a higher diagnostic yield [4] (Fig. 3.3b). This due to wash out of a specific bound dermal IgG.

<In general transport an IF biopsy in normal saline>

However, there are several drawbacks to this method. First, the biopsies shouldn't be kept longer than 36–48 h in saline. Longer than 48 h might washout desired immunoreactants. Second disadvantage might be the loss of epidermal in vivo antinuclear antibodies. Therefore, in cases of suspected subacute cutaneous lupus erythematosus, a snap-frozen biopsy is advisable.

After overnight storage in saline, the biopsy is snap frozen and can be stored in a −80 °C freezer or processed further for immunofluorescence microscopy.

Review Questions

1. A skin biopsy for direct immunofluorescence should preferably be taken from
   (a) Lesional bullous skin
   (b) Perilesional erythematous skin
   (c) Perilesional noninflamed skin
2. A skin biopsy for direct immunofluorescence is preferably transported in
   (a) Liquid nitrogen
   (b) Michel's medium
   (c) Saline
   (d) 4 % formaldehyde
3. A skin biopsy for direct immunofluorescence for the diagnosis of subacute lupus erythematosus is preferably transported in
   (a) Liquid nitrogen
   (b) Michel's medium
   (c) Saline
   (d) 4 % formaldehyde

Answers

1. (b)
2. (c)
3. (a)

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Direct Immunofluorescence Microscopy

4

Gilles F.H. Diercks and Hendri H. Pas

Abstract

Direct immunofluorescence plays an important role in the diagnosis of autoimmune bullous diseases. The purpose of direct immunofluorescence microscopy is to detect in vivo antibodies in patient's skin or mucosa. Direct immunofluorescence of pemphigus shows depositions of immunoglobulins and/or complement on the epithelial cell surface of keratinocytes, whereas pemphigoid shows linear deposition of immunoglobulins along the epidermal basement membrane zone. This linear deposition can be separated in an n-serrated pattern and a u-serrated pattern. An n-serrated pattern is seen in blistering diseases with binding above the lamina densa with antibodies against hemidesmosomal components, e.g., bullous pemphigoid, while a u-serrated pattern points to a sublamina densa, binding diseases caused by autoantibodies against type VII collagen, e.g., epidermolysis bullosa acquisita. Finally, dermatitis herpetiformis shows a granular IgA deposition along the epidermal basement membrane zone.

Keywords

Immunofluorescence • Pemphigus • Pemphigoid • Dermatitis herpetiformis • Porphyria

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Learning Objectives

After studying this chapter, you should know:

- The various cutaneous immunodeposition patterns in pemphigus, pemphigoid, dermatitis herpetiformis, and porphyria
- The difference between an n-serrated and u-serrated pattern in pemphigoids

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Introduction and Aims

Ever since the discovery of the presence of autoantibodies in pemphigus in 1964 by Beutner and Jordon [1], immunofluorescence microscopy has become an essential part in the diagnostics of blistering diseases. Both serum and biopsy specimens can be examined by this method. The next chapter will describe the technique of direct immunofluorescence microscopy, i.e., visualization of in vivo bound autoantibodies. After reading this chapter, the reader knows the different patterns that can be recognized in various blistering diseases.

Laboratory Preparation

The purpose of direct immunofluorescence microscopy is to detect in vivo antibodies. This is done by adding a fluorescent-labeled antibody against a human antigen, e.g., a goat antibody directed against human IgG, on a frozen section. To prepare a skin or mucosa biopsy for immunofluorescence microscopy, the following steps are recommended (Groningen protocol):

- Cut frozen sections at a thickness of 4 μm.
- Blow-dry the sections with a cold dryer for 15 min.
- Rinse the slides with PBS (NaCl 8.75 g/l, Na_2HPO_4 1.14 g/l, KH_2PO_4 0.27 g/l) for a minimum of 5 s. Wipe off excess PBS.
- Place fluorescent isothiocyanate (FITC)-conjugated antibody on the slides and incubate in a moist chamber for 30–40 min. See Table 4.1 for used antibodies.
- Rinse the slides with PBS and wash the slides subsequently for 30 min in PBS.
- Wipe off excess PBS.
- Place bisbenzimide (Hoechst 33258), which binds to double-stranded DNA and therefore provides a nuclear staining, on the slides and incubate for 5–10 min on room temperature.
- Rinse the slides with PBS and wash the slides subsequently for 30 min in PBS.
- Place a drop of PBS/glycerin (1:1) on each section and top with a coverslip.

Table 4.1 Recommended FITC-conjugated antibodies

Antibodies	Manufacturer
FITC-conjugated Goat F(ab)2 anti-human IgG	Protos 311, Protos immunoresearch, Burlingame, CA, US
FITC-conjugated Goat F(ab)2 anti-human IgA	Protos 312, Protos immunoresearch, Burlingame, CA, US
FITC-conjugated Goat F(ab)2 anti-human IgM	Protos 313, Protos immunoresearch, Burlingame, CA, US
FITC-conjugated Rabbit anti-human fibrinogen	Dako F111, Dako, Glostrup, Denmark
FITC-conjugated Rabbit anti-human C3c complement	Dako F201, Dako, Glostrup, Denmark

Immunofluorescence Patterns

Pemphigus

Pemphigus is caused by autoantibodies directed against desmosomal antigens, in particular desmoglein 1 (pemphigus foliaceus) or desmoglein 3 (mucosal pemphigus vulgaris) or desmoglein 1 and 3 (mucocutaneous pemphigus vulgaris) [2], although cases have been described with antibodies against desmocollin 1 or 3. Whatever the nature of the antibodies or the pemphigus variant, direct immunofluorescence of pemphigus shows depositions of immunoglobulins and/or complement on the epithelial cell surface (ECS) in virtually all patients [3]. This ECS deposition is in most cases throughout the entire epidermis and mucosal epithelium; therefore a subclassification cannot be made. In the majority of textbooks, this is described as a smooth pattern throughout the epidermis (Fig. 4.1a). However, in many biopsies a fine or coarse granular pattern can be observed (Fig. 4.1b, c).

<Pemphigus is characterized by ECS deposition of immunoglobulins and complement in a smooth or granular pattern> These clusters seem to be the result of clustering of IgG, Dsg3, and plakoglobin, but no other desmosomal components are involved [4]. Due to its bivalency, IgG cross-links non-junctional Dsg molecules and these cross-linked molecules then concentrate in dots. In addition to deposits throughout

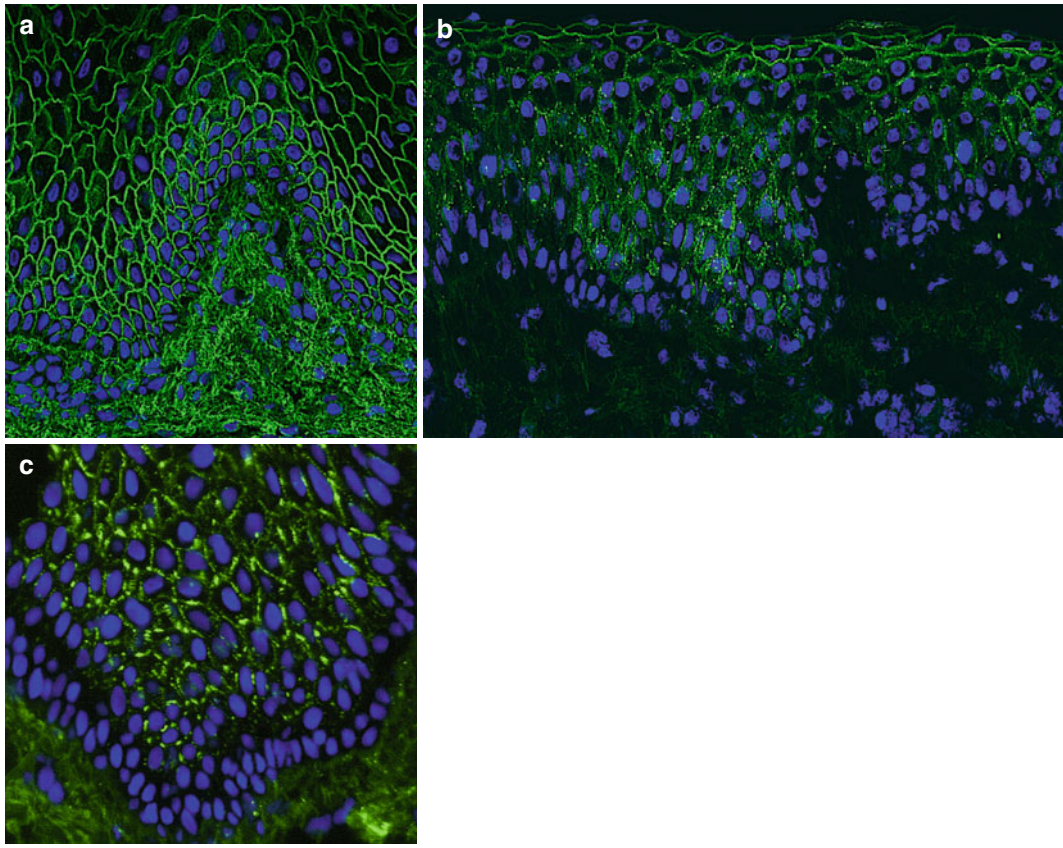

Fig. 4.1 Patterns of epithelial cell surface (ECS) staining in pemphigus: (**a**) smooth pattern, (**b**) fine granular pattern, (**c**) coarse granular pattern

the epidermis, immunoglobulins can in many cases also be observed in adnexal structures, e.g., hair follicles and sweat glands. False-positive ECS deposition can be observed in biopsies of eczema lesions. In these cases a "tram rails" pattern between the keratinocytes can be observed in contrast to the smooth or granular patterns in pemphigus (Fig. 4.2).

On top of ECS deposition, in some cases also a granular deposition of autoantibodies and complement can be found along the dermal-epidermal junction, especially in pemphigus erythematosus, now considered to be a localized form of pemphigus foliaceus (Fig. 4.3). It seems that these granules consist of IgG directed against the ectodomain of desmoglein 1, which is shed along the epidermal basement membrane.

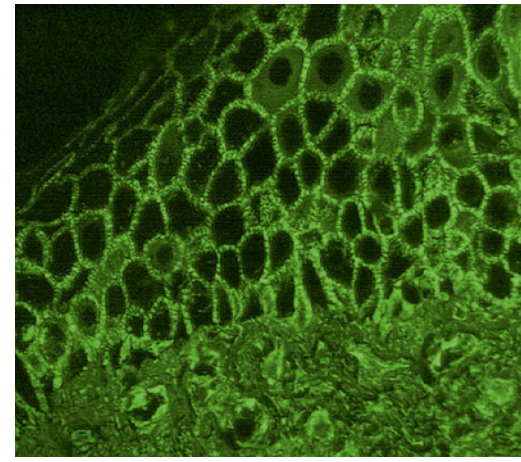

Fig. 4.2 False-positive pseudo-epithelial cell surface (ECS) staining of IgG in a tram rail pattern in eczema due to spongiotic edema

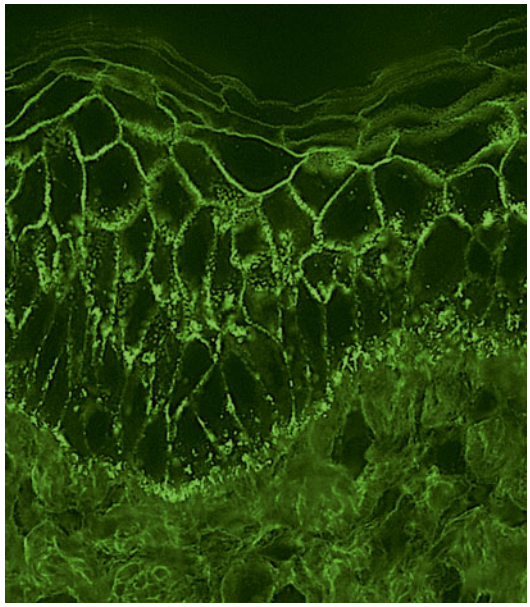

Fig. 4.3 Pemphigus erythematosus with IgG in a smooth/granular ECS deposition, and additionally a granular deposition along the epidermal basement membrane zone

In most cases of pemphigus, the ECS deposition consists of IgG with or without complement binding, although in some cases also IgA is present. However, in rare cases only IgA depositions can be found, a so-called IgA pemphigus (see Chap. 11). In general two variants of IgA pemphigus are considered, the subcorneal pustulosis type and the intraepidermal neutrophilic type. Direct immunofluorescence of the subcorneal pustulosis type shows deposits of IgA only in the upper part of the epidermis, while in the intraepidermal neutrophilic type IgA is present on the ECS throughout the entire epidermis.

Paraneoplastic pemphigus is a severe autoimmune multiorgan disease different from pemphigus vulgaris [5]. It is characterized clinically by painful stomatitis and polymorphous cutaneous manifestations in patients with underlying neoplasia. PNP comprises many antibodies; the most characteristic are periplakin and envoplakin next to desmoglein.

<Paraneoplastic pemphigus is a severe multiorgan disease with in almost all cases antibodies against envoplakin and periplakin> Direct immunofluorescence shows ECS deposits of IgG and complement throughout the epidermis consistent with other variants of pemphigus. In addition, in some cases, a linear deposition of IgG and complement can be seen, which can be attributed to additional antibodies against hemidesmosomal components (Fig. 4.4). However, in these cases the diagnosis of paraneoplastic pemphigus has to be confirmed by serology, since rare cases of coexisting pemphigus and pemphigoid are described in literature.

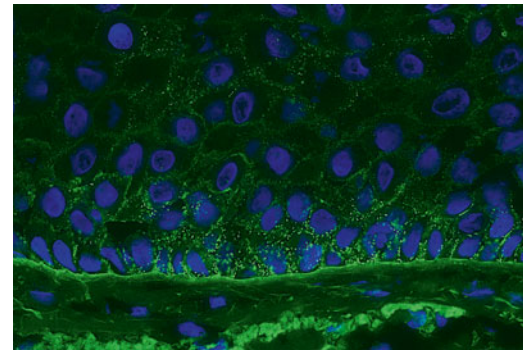

Fig. 4.4 Paraneoplastic pemphigus with IgG in a granular ECS deposition and additionally a linear deposition along the epidermal basement membrane zone

Pemphigoid

All variants of pemphigoid are characterized by a linear deposition of immunoglobulins and/or complement along the epidermal basement membrane zone [6] (Fig. 4.5a).

<Pemphigoid is characterized by a linear deposition of immunoreactants along the basement membrane> These antibodies are directed against various hemidesmosomal components and connecting molecules: (1) type XVII collagen (BP180) in bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), pemphigoid gestationis (PG), lichen planus pemphigoides (LPP), and linear IgA disease (LAD), (2) BP230 in BP, (3) laminin 332 in anti-laminin 332 pemphigoid, (4) integrin beta4 in ocular mucous membrane pemphigoid, and (5) p200 in anti-p200 pemphigoid. Moreover, in epidermolysis bullosa acquisita (EBA) and bullous SLE, antibodies against type VII collagen, present in the sublamina densa, also give rise to a linear deposition pattern.

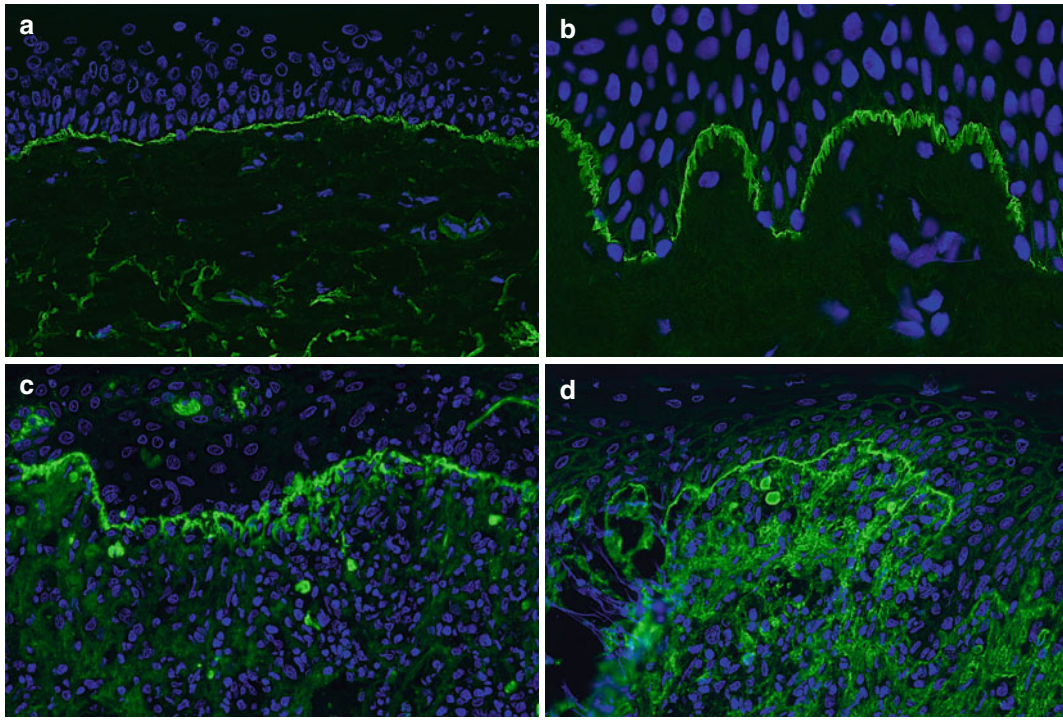

Fig. 4.5 (**a**) Linear deposition of IgG along the basement membrane in bullous pemphigoid. (**b**) Linear deposition of complement with marked gaps due to the presence of melanocytes in pemphigoid gestationis. Shaggy deposition of fibrin (**c**) and a linear deposition of IgG (**d**) along the epidermal BMZ in lichen planus pemphigoides

In case a linear deposition is observed, it is important to determine the nature of the deposits. In most variants of BP and in EBA, the deposits consist of IgG and complement. Mixed IgG/IgA depositions are commonly encountered, especially in mucosal dominant cases of pemphigoid. In addition, in some cases only IgA is present, leading to a diagnosis of LAD or IgA EBA [7, 8]. However, in mucosal-dominant pemphigoid with mixed IgA/IgG depositions, the IgG component might be very weak, which might result in a misdiagnosis of linear IgA disease. PG shows in virtually all cases a strong linear deposition of complement along the basement membrane with a weaker staining for IgG. Strikingly, in many cases of PG, interruptions in this linear deposition can be seen, caused by the presence of melanocytes (Fig. 4.5b). This can also been seen in other cases of pemphigoid but is usually less obvious. Although in a number of cases linear IgM deposition might be present in adjunct to IgG and complement, cases have been described with only linear IgM deposition. Whether these cases should be considered a variant of pemphigoid or merely a coincident finding is unknown.

In LPP, clinically characterized by blisters next to typical lichen planus lesions, in addition to a linear IgG deposition, shaggy deposition of fibrin and lichenoid infiltrate is often found (Fig. 4.5c). Furthermore, colloid bodies, ovoid or round structures consisting of keratin filaments and covered with immunoglobulins, can be found in the underlying dermis.

Bullous SLE is characterized by antibodies to type VII collagen in a patient fulfilling the ARA criteria for systemic lupus erythematosus. In bullous SLE, next to or superimposed on a linear IgG deposition, a biopsy might show a lupus band, characterized by granular deposition of immunoglobulins and complement, and the presence of epidermal in vivo antinuclear antibodies.

In most cases of pemphigoid, a linear-serrated pattern can be discerned. This serration pattern

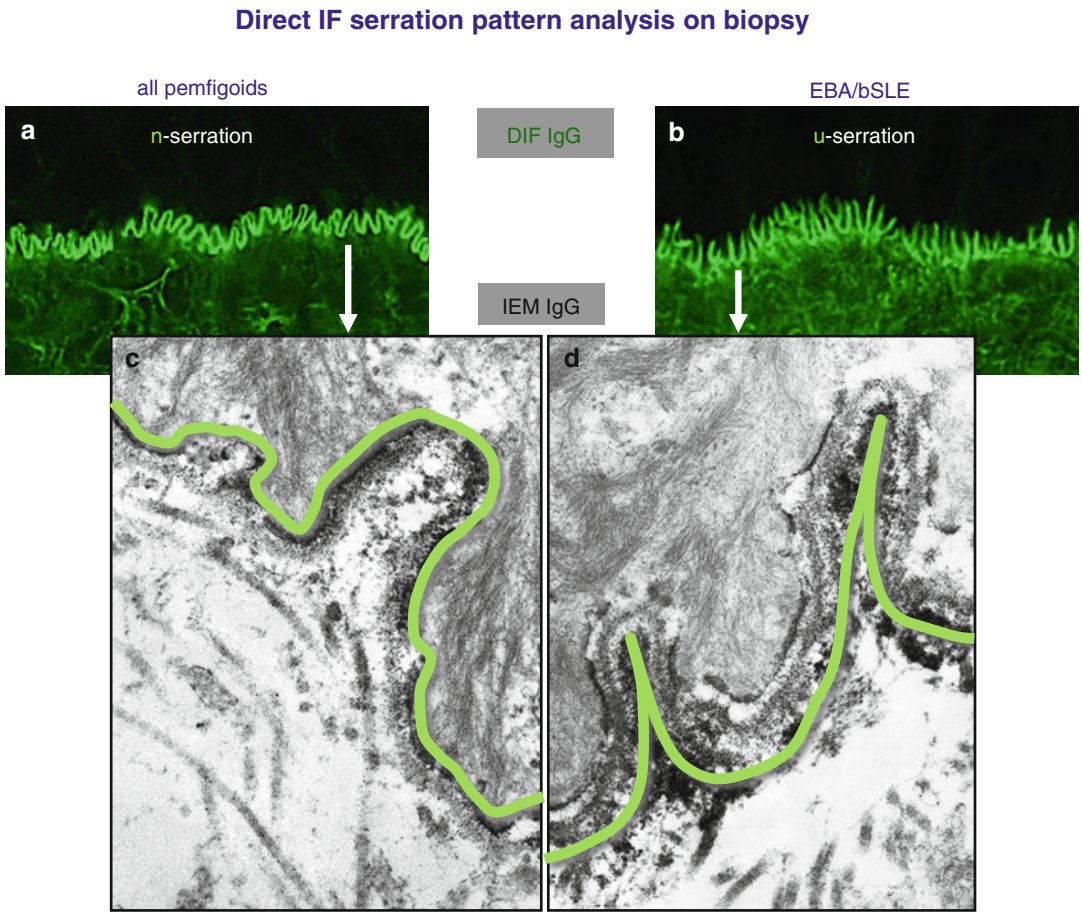

Fig. 4.6 (**a**) n-serrated pattern in bullous pemphigoid, (**b**) u-serrated pattern in epidermolysis bullosa acquisita, (**c**, **d**) immunoelectron microscopy of peroxidase-labeled IgG of perilesional skin from a patient with bullous pemphigoid (**c**) and epidermolysis bullosa acquisita (**d**). The n-serrated pattern follows the undulations of the plasma membrane, whereas the u-serrated pattern is the result of staining of anchoring fibrils between the rootlets (Copyright © 1996 American Medical Association. All rights reserved)

can be separated in an n-serrated pattern and a u-serrated pattern [9] (Fig. 4.6a, b).

<Bullous pemphigoid shows an n-serrated pattern, whereas epidermolysis bullosa acquisita shows a u-serrated pattern> The recognition of these serration patterns makes it possible to differentiate between (1) sublamina densa binding diseases caused by autoantibodies against type VII collagen, e.g., EBA and bullous SLE, and (2) blistering diseases with binding above the lamina densa with antibodies against hemidesmosomal components, e.g., BP, PG, MMP, anti-p200 pemphigoid, and anti-laminin 332 pemphigoid. This differentiation can be explained by the fact that in cases with antibodies against type VII collagen, the immunodeposits are located between the rootlets of the basal keratinocytes, leading to a u-serrated pattern (Fig. 4.6c). On the other hand, depositions above the lamina densa follow the plasma membrane in the basal cell rootlets, resulting in an n-serrated pattern (Fig. 4.6d). In some cases it is not possible to determine the serration pattern, especially in mucosal biopsies. In these cases it is wise to cut thinner sections or to take a biopsy of clinically uninvolved skin.

However, a few cases remain in which it is impossible to differentiate between an n-serrated and a u-serrated pattern. In these cases the level of

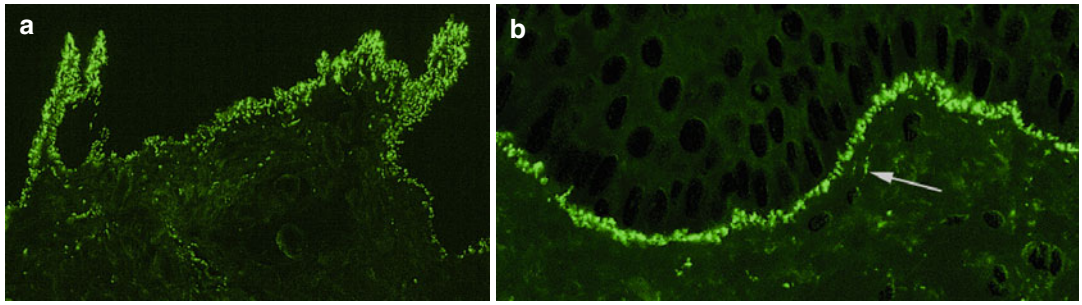

Fig. 4.7 (**a**) The granular IgA depositions in dermatitis herpetiformis are located in the dermal papillae, or (**b**) more along the dermal-epidermal junction and in superficial vessel walls (*arrows*)

the deposition of the antibodies can be determined by fluorescent overlay antigen mapping (FOAM). FOAM is a technique based on the possibility to visualize a targeted antigen relative to a topographic marker. One can use a red staining for type VII collagen, as topographic reference marker and a green staining for IgG deposits. In case of BP separate patterns of IgG deposits (green) and type VII collagen (red) can be seen with red staining on the dermal side. In contrast, EBA skin shows a pattern with overlap of green IgG deposits and red-type VII collagen staining, resulting in a yellow-orange fluorescence and lacking red staining on the dermal side. FOAM can be done using a standard immunofluorescence microscope, providing appropriate software is available. However, better results are accomplished using confocal microscopy.

Dermatitis Herpetiformis

Dermatitis herpetiformis (DH) is characterized by IgA antibodies against tissue transglutaminase, and although it has typical pruritic blisters on predilection sites, the clinical picture might resemble various variants of pemphigoid. However, direct immunofluorescence can make a clear distinction between these entities. Direct immunofluorescence of DH shows a granular deposition of IgA along the dermal-epidermal junction [10]. **<Dermatitis herpetiformis is characterized by a granular deposition of IgA along the dermal-epidermal junction>** Typically, these depositions are concentrated in the dermal papillae, although in many cases a linear granular is present (Fig. 4.7). This deposition is most probably the result of the precipitation of IgA antibodies against epidermal transglutaminase (TG3). These IgA-TG3 immune complexes can also be detected in small vessels in the papillary dermis.

Porphyria Cutanea Tarda and Pseudoporphyria

Porphyria cutanea tarda (PCT) is characterized by cell poor blisters mostly present on the dorsal sites of the hands and feet, induced by photosensitization of endogenous (porphyrins) or exogenous (e.g., NSAIDs) agents. Although this disease shows a typical clinical presentation and has a characteristic histology, the differentiation from mechanobullous EBA can be difficult. Fortunately, both entities have different immunofluorescent patterns. As described above, EBA is characterized by u-serrated linear deposition of IgG and complement along the basement membrane. PCT, on the other hand, shows a homogeneous deposition of immunoglobulins, preferably IgG, in vessel walls and in most instances a homogeneous deposition along the dermal-epidermal junction (Fig. 4.8), although also granular and fibrillar depositions have been described. **<A homogeneous deposition of particularly IgG along the dermal-epidermal junction and in vessel walls is typical in porphyria>** It has been hypothesized that the depositions in the vessel walls might reflect a reaction between physiological autoantibodies and damaged vascular endothelium. The formation of separation at the

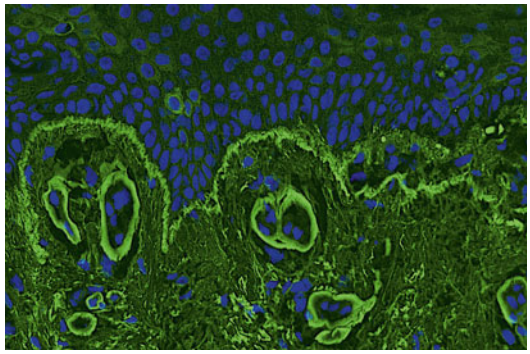

Fig. 4.8 Homogeneous deposition of IgG along the dermal-epidermal junction and in vessel walls is the hallmark of (pseudo)porphyria

lamina lucida is a secondary event caused by the release of proteolytic enzymes and destruction of laminin and type IV collagen.

Review Questions

1. Pemphigus is characterized by
   (a) A smooth epithelial surface staining
   (b) A granular epithelial surface staining
   (c) Both patterns can be observed
2. A u-serrated linear staining along the basal membrane zone can be observed in
   (a) Bullous pemphigoid
   (b) Epidermolysis bullosa acquisita
   (c) Anti-p200 pemphigoid

Answers

1. (c)
2. (b)

Acknowledgements Dr M.C. de Jong is acknowledged for providing some of the IF pictures.

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Indirect Immunofluorescence Microscopy

5

Gilles F.H. Diercks and Hendri H. Pas

Abstract

The purpose of indirect immunofluorescence microscopy is to detect circulating antibodies in patient's serum. For this purpose, an adequate substrate is necessary to visualize these antibodies. Monkey esophagus is the most widely used substrate for detecting circulating autoantibodies in patients with autoimmune bullous diseases. In all variants of pemphigus, antibodies show an epithelial cell surface pattern, resulting from present autoantibodies against the desmosomal molecules desmoglein 1 and/or 3. This pattern is also called chicken wire or honeycomb pattern. In pemphigoid, a linear deposition along the epithelial basement membrane can be observed, caused by autoantibodies against hemidesmosomes or their connecting proteins underneath.

Human salt-split skin is a valuable substrate in the diagnosis of subepidermal autoimmune bullous diseases. Important antigens in the roof of salt-split skin are type XVII collagen (BP180) and BP230, whereas laminin 332, p200, and type IV collagen are situated in the floor of the blister. This implies that bullous pemphigoid, mucous membrane pemphigoid, pemphigoid gestationis, and lichen planus pemphigoides show staining of IgG on the epidermal side of the blister. On the other hand, anti-laminin 332 pemphigoid, anti-p200 pemphigoid, epidermolysis bullosa acquisita, and bullous SLE show staining on the dermal side.

Other less used, but valuable substrates in some instances, are rat bladder and knock-out skin.

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Keywords
Immunofluorescence • Pemphigus • Pemphigoid • Dermatitis herpetiformis

Learning Objectives
After studying this chapter, you should know:

- The various substrates used in indirect immunofluorescence microscopy in autoimmune bullous diseases
- The binding patterns of autoantibodies to monkey esophagus in pemphigus, pemphigoid, and dermatitis herpetiformis
- The principle of salt-split skin and the difference between epidermal and dermal staining
- The use of rat bladder for the diagnosis of paraneoplastic pemphigus

Introduction

Indirect immunofluorescence microscopy is used to detect circulating antibodies in patient's serum. For this purpose, an adequate substrate is necessary to visualize these antibodies. In this chapter, various substrates and techniques are described to (sub)type the different variants of autoimmune bullous diseases. A summary of intraepidermal and subepidermal autoimmune bullous diseases with associated antigens and immunofluorescence patterns is described in Table 5.1.

<Indirect immunofluorescence is used to detect circulating antibodies>

Technique

To detect circulating autoantibodies with immunofluorescence microscopy, the following steps are recommended (Groningen protocol):

- Collect 5–10 ml of blood without anticoagulants.
- Dilute patient's serum: for testing on monkey esophagus, a dilution of 1:40 is recommended, for human salt-split skin 1:8 and for endomysium antibodies 1:4.
- Apply diluted serum onto a substrate for 30–40 min.
- Rinse the slides with PBS (NaCl 8.75 g/l, Na2HPO4 1.14 g/l, KH2PO4 0.27 g/l) for a minimum of 5 s. Wipe off excess PBS.
- Apply fluorescein isothiocyanate (FITC)-conjugated antibodies for 30–40 min (see Table 4.1 for the recommended IgG and IgA antibodies).
- Rinse the slides with PBS. Wipe off excess PBS.
- Rinse the slides now for 15–20 min with PBS.
- Place a drop of PBS/glycerin (1:1) on each section and top with a cover slip.

Monkey Esophagus

Monkey esophagus was the first substrate used for detecting of circulating autoantibodies in patients with autoimmune bullous diseases [1]. Other substrates that have been used are guinea pig lip, guinea pig esophagus, or monkey tongue. However, monkey esophagus seems to yield the best results [2]. Studies have shown that using two substrates, e.g., monkey esophagus and guinea pig esophagus or human skin, yields a higher sensitivity for the diagnosis of pemphigus. Moreover, due to different staining patterns on various substrates, one might differentiate pemphigus vulgaris from foliaceus. However, in daily practice, it might not be feasible to use two substrates. With commercially distributed monkey esophagus widely available, this seems to be the substrate of choice. Moreover, a reliable differentiation between pemphigus vulgaris and foliaceus is available by the introduction of specific ELISA kits for desmoglein 1 and 3.

Table 5.1 Laboratory diagnosis of autoimmune bullous diseases by direct and indirect immunofluorescence microscopy

Bullous disease	Antigen	Direct IF skin/mucosa		Indirect IF serum		
				Esophagus	*Salt-split skin*	
Intraepidermal		*ECS*	*BMZ*	*Anti-ECS*	*Anti-BMZ*	
					Epidermal	*Dermal*
Pemphigus vulgaris	Desmoglein 3 ± 1	IgG ± A, C	–	IgG	–	–
Pemphigus foliaceus	Desmoglein 1	IgG ± A, C	–	IgG	–	–
Paraneoplastic pemphigus	Plakines, desmoglein 3 ± 1, BP230, plectin, a2ML1	IgG ± A, C	IgG ± A, C	IgG	(IgG)	–
IgA-pemphigus	Desmocollin	IgA	–	IgA	–	–
Subepidermal				*Anti-BMZ*		
Bullous pemphigoid	BP230/BP180/LAD/plectin	–	IgG ± A, C n-serrated	–	IgG ± A	
Pemphigoid gestationis	BP180	–	C, (IgG) n-serrated	–	(IgG)	
Mucous membrane pemphigoid	BP230/BP180/LAD/Integrinα6β4	–	IgG ± A, C n-serrated	–	IgG ± A	
Lichen planus pemphigoides	BP180	–	IgG, C n-serrated	–	IgG	
Linear IgA disease	BP180/LAD/plectin	–	IgA n-serrated	–	IgA	
Anti-laminin 332 pemphigoid	Laminin 332	–	IgG, C n-serrated	–	–	IgG ± A
Anti-p200 pemphigoid	P200	–	IgG, C n-serrated	–	–	IgG ± A
Epidermolysis bullosa acquisita	Collagen type VII	–	IgG, IgA, C u-serrated	–	–	IgG ± A IgA
				EMA		
Dermatitis herpetiformis	Transglutaminase	–	IgA granular	IgA	–	–

IF immunofluorescence, *ECS* epithelial cell surface, *BMZ* basement membrane zone, *EMA* endomysium antibodies, *C* complement

Although studies have proposed human skin as the substrate of choice, many specimens have to be tested to find a reactive one. Moreover, sera with pemphigus autoantibodies might react with patient's own skin, but not with normal skin of other individuals, yielding false-negative results.

Monkey esophagus is normally tested with FITC-conjugated anti-human IgG (Fig. 5.1a). In addition, one can also detect IgA; however, these results have to be carefully interpreted, since anti-IgA is known to give false-*positive* results.

In practice, two main patterns can be discerned. In all variants of true pemphigus, IgG class antibodies show an epithelial cell surface (ECS) pattern (Fig. 5.1b), resulting from present autoantibodies against the desmosomal transmembrane adhesion molecules desmoglein 1 and/or 3. The ECS pattern is also called chicken wire or honeycomb pattern. The old term intercellular substance (ICS) pattern is abandoned since the immunoglobulin binding is not to a "substance" between the cells, but to the cell surface. In pemphigoid, a linear deposition along the

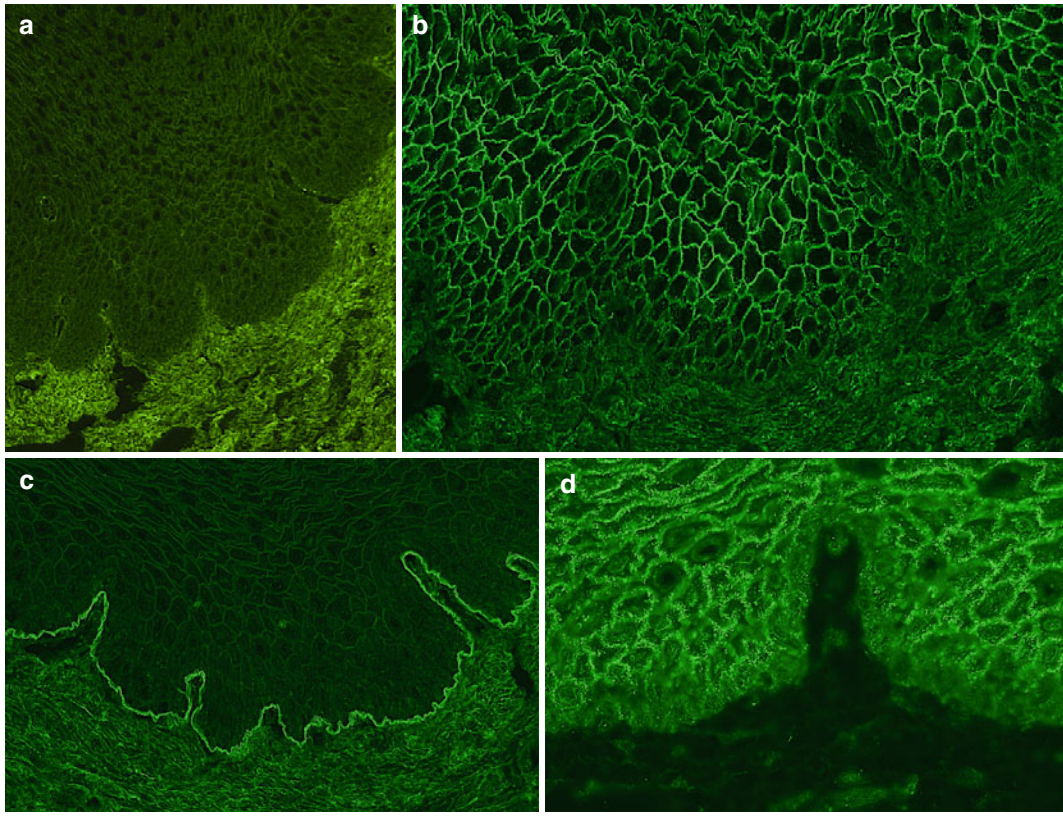

Fig. 5.1 Indirect immunofluorescence on monkey esophagus. (**a**) Control serum with negative binding, (**b**) pemphigus serum IgG binds in a smooth epithelial cell surface (ECS) pattern, (**c**) pemphigoid serum IgG binds in a linear pattern along the epithelial basement membrane zone, and (**d**) false positive ECS binding

epithelial basement membrane can be observed, caused by autoantibodies against hemidesmosomes or their connecting proteins underneath (Fig. 5.1c).

<Monkey esophagus can detect circulating antibodies against desmosomal and basal membrane zone molecules.>

Indirect immunofluorescence on monkey esophagus always shows a smooth ECS pattern (Fig. 5.1b), in contrast to the more granular pattern observed with direct immunofluorescence microscopy on skin biopsies. The sensitivity of this test for active pemphigus is up to 90 %, whereas results might be negative in quiescent cases or cases in remission. One should be aware that false-positive sera could be encountered. An important reason is the existence of blood group AB antigens on monkey epithelial cells, which might react with anti-A or anti-B antibodies present in patient's blood. This gives rise to a pseudo-ECS pattern, which at close examination reveals a coarse "barbed wire" pattern (Fig. 5.1d), instead of the sharply defined smooth pattern seen with pemphigus sera (Fig. 5.1b). Moreover, sera with pseudo-ECS pattern tend not to bind to the basal epithelial layer [3]. Absorption of A and B antibodies might resolve this problem. In addition, various other conditions might give a false-positive ECS staining, among which burn wound victims seem to be the most important.

Early studies have shown that indirect immunofluorescence microscopy on monkey esophagus has a sensitivity for bullous pemphigoid of around 60–80 % with a high specificity [4]. In

this respect, it is important to notice that active disease activity is more likely to give a positive result, while inactive cases are mostly negative. Although numbers vary, most studies on mucous membrane pemphigoid show a much lower sensitivity, as low as 10–20 % [5] probably due to lower concentration of circulating antibodies. The sensitivity of indirect immunofluorescence is also low in epidermolysis bullosa acquisita (EBA) [6].

In addition to the pemphigus and pemphigoid staining patterns, other binding patterns can be encountered. Cytoplasmic staining of epithelial basal cells has been associated with drug-induced skin reactions (Fig. 5.2). However, sensitivity and specificity seem to be low. These antibodies might among others also be demonstrated in burn victims and after bone marrow transplantation, but also in pemphigus and pemphigoid patients. Antinuclear antibodies can be observed in monkey esophagus, although monkey esophagus is not the substrate of choice for assessing antinuclear antibodies. One pattern that might be of some importance is a stratified epithelium-specific antinuclear antibody that is directed against a 70-kd antigen and is characterized by a fine speckled nuclear staining. This antibody can be found in chronic ulcerative stomatitis, a condition closely related to erosive lichen planus [7].

An important use of monkey esophagus is detection of IgA anti-endomysium antibody in patients with celiac disease or dermatitis herpetiformis and will detect IgA antibodies directed against the endomysium, a connective tissue layer that surrounds individual muscle fibers (Fig. 5.3). This layer contains transglutaminase, the primary autoantigen in celiac disease and dermatitis herpetiformis [8].

<IgA binding to the endomysium of smooth muscle cells is indicative of celiac disease and dermatitis herpetiformis.>

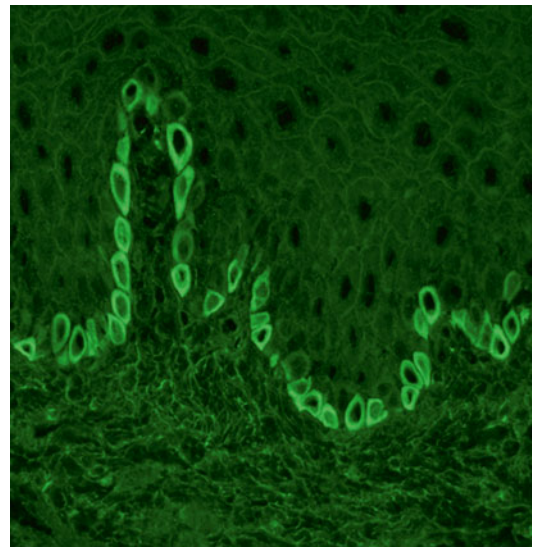

Fig. 5.2 Basal cell cytoplasmic staining by indirect immunofluorescence on monkey esophagus

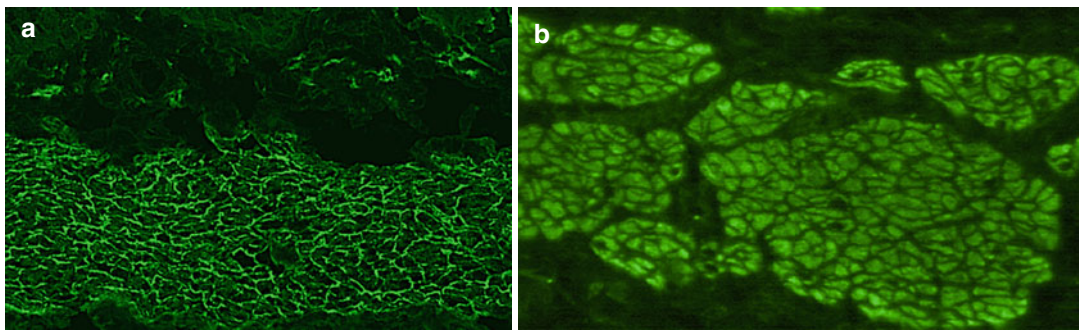

Fig. 5.3 IgA antibodies directed against (**a**) the endomysium of smooth muscle fibers in dermatitis herpetiformis and (**b**) a negative control

Human Salt-Split Skin

In the 1980s, the first studies using human salt-split skin for the diagnosis of subepidermal autoimmune bullous diseases were performed [9], and this technique proved to be a valuable asset to monkey esophagus.

<Salt-split skin is the substrate of choice for detecting antibodies in pemphigoid disease.>

Normal human skin is incubated for 48–72 h in 1.0 mol sodium chloride, which produces a reproducible split in the lamina lucida, separating epidermal- and dermal-located pemphigoid antigens. Important antigens in the roof of salt-split skin are type XVII collagen (BP180) and BP230, whereas laminin 332, p200, and type IV collagen are situated in the floor of the blister (Fig. 5.4a). This implies that bullous pemphigoid, mucous membrane pemphigoid, pemphigoid gestationis, and lichen planus pemphigoides show staining of IgG on the epidermal side of the blister (Fig. 5.4b). On the other hand, anti-laminin 332 pemphigoid, anti-p200 pemphigoid, epidermolysis bullosa acquisita, and bullous SLE show staining on the dermal side (Fig. 5.4c).

<Bullous pemphigoid shows an epidermal staining on salt-split skin, whereas EBA shows a dermal staining.>

In various cases also expression of IgA in addition to IgG can be demonstrated. Solely IgA deposition can be seen in linear IgA disease and IgA epidermolysis bullosa acquisita on the epidermal and dermal side, respectively.

The sensitivity of salt-split skin in comparison with monkey esophagus for pemphigoid sera is comparable to that of monkey esophagus with a sensitivity between 70 and 80 %. For EBA, the sensitivity is around 40–50 % [6]. Specificity of salt-split skin is high for all types of autoimmune bullous diseases and ranges between 97 and 100 %.

Rat Bladder

Paraneoplastic pemphigus is an autoimmune bullous disease associated with an underlying neoplasm and with antibodies directed against various antigens, of which envoplakin and periplakin are considered the most important, but also includes desmoglein 1 and 3, desmoplakins, and plectin.

Indirect immunofluorescence on monkey esophagus might show a smooth ECS pattern and therefore does not differentiate between pemphigus and paraneoplastic pemphigus. However, the transitional epithelium of rat bladder is rich in envoplakin and periplakin, but is devoid of desmoglein 1 and 3. In case of paraneoplastic pemphigus, rat bladder shows an ECS staining, whereas pemphigus will be negative (Fig. 5.5).

Rat bladder testing for paraneoplastic pemphigus has a sensitivity of about 74 %, but a high specificity [10]. With respect to the low sensitivity, additional testing using immunoblot or immunoprecipitation raises the sensitivity to 100 %.

Knock-Out Skin

In addition to the abovementioned more or less routine techniques for serological detection of autoantibodies, more advanced immunofluorescence techniques can be used to further characterize present autoantibodies.

One of these techniques is using knock-out skin, i.e., skin of patients with inherited forms of epidermolysis bullosa (EB) completely lacking certain molecules at the dermoepidermal junction [11]. Patients with severe recessive dystrophic EB are devoid of type VII collagen, whereas patients with junctional EB, Herlitz-type lack laminin 332. Using skin of these patients as a substrate makes it possible to differentiate between EBA and anti-laminin 332 pemphigoid, diseases that both are characterized by dermal staining on salt-split skin. EBA will show absence of staining on type VII collagen-deficient skin, but in contrast will show a linear staining along the BMZ in laminin 332-deficient skin (Fig. 5.6). Anti-laminin 332 pemphigoid will show opposite results. A dermal staining on salt-split skin and a positive staining on both deficient skin substrates suggest an anti-p200 pemphigoid.

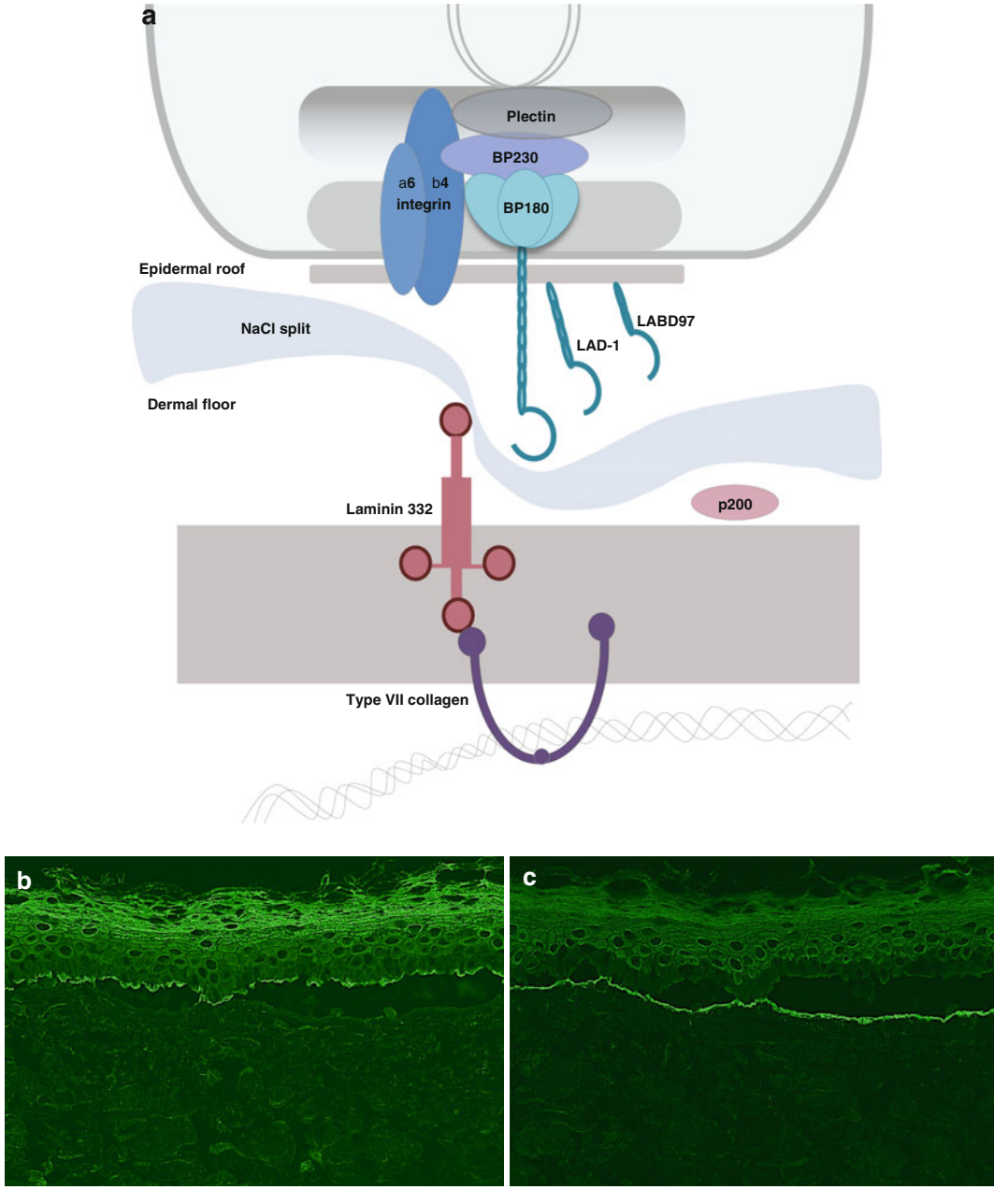

Fig. 5.4 (**a**) Diagram of basement membrane zone in salt-split skin. Indirect IF in bullous pemphigoid with antibodies to BP180 shows (**b**) an epidermal staining; epidermolysis bullosa acquisita with antibodies against type VII collagen shows a (**c**) dermal staining

A major disadvantage of this technique is the need of a sufficient concentration of circulating antibodies, which might be low, particularly in EBA. In these cases, one needs a skin biopsy to determine the serration pattern or eventual use of fluorescent overlay antigen mapping by direct immunofluorescence (see previous chapter) to differentiate between these diseases.

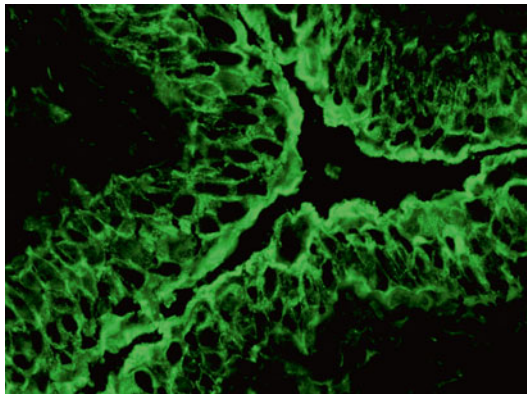

Fig. 5.5 Positive indirect IF on rat bladder with IgG staining in ECS pattern in paraneoplastic pemphigus

Review Questions

1. Using monkey esophagus as a substrate, pemphigus is characterized by:
   (a) A linear pattern along the basement membrane
   (b) An epithelial cell surface staining in a chicken wire pattern
   (c) Both patterns can be observed
2. A dermal staining in salt-split skin can be observed in:
   (a) Anti-laminin 332 pemphigoid
   (b) Anti-p200 pemphigoid
   (c) Epidermolysis bullosa acquisita
   (d) All of the abovementioned variants

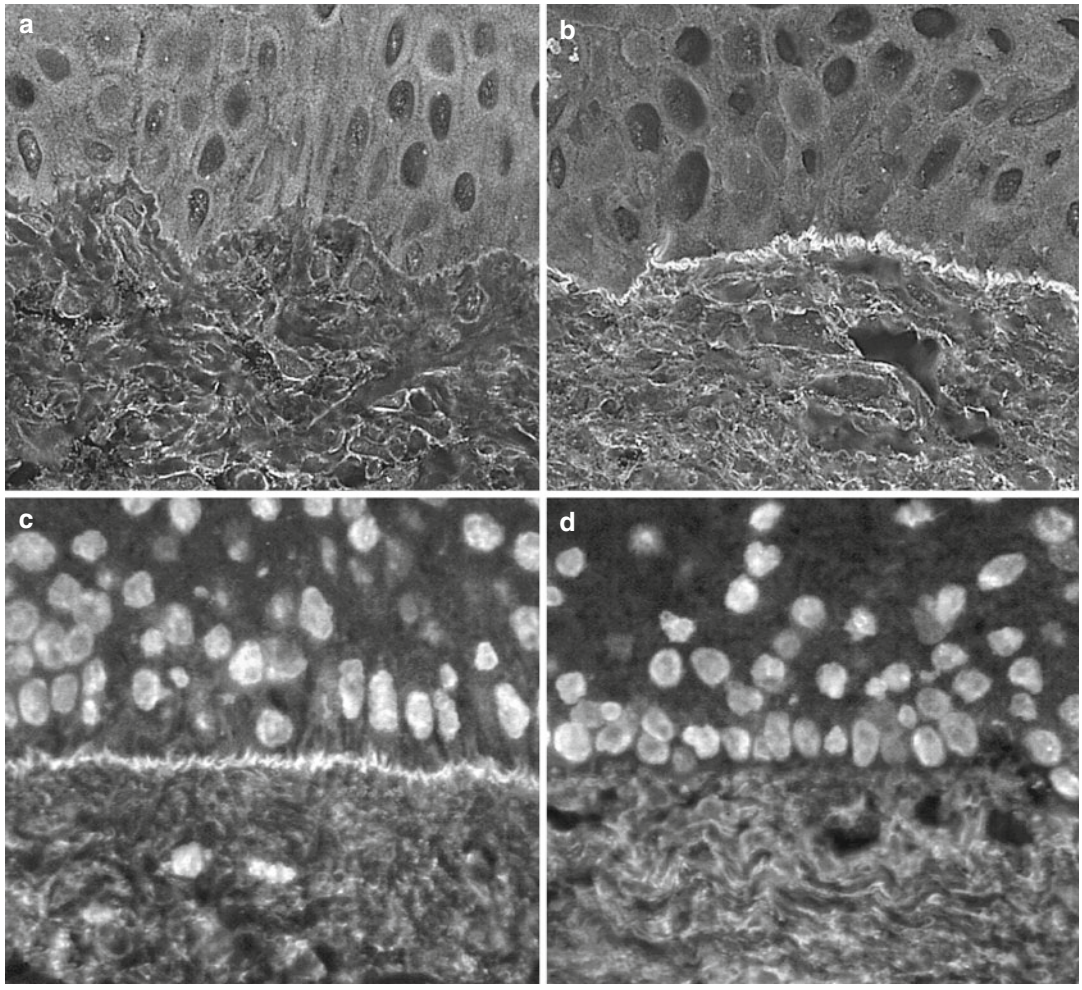

Fig. 5.6 Staining for serum IgG on knock-out skin substrates lacking either (**a**, **c**) laminin 332 or (**b**, **d**) type VII collagen. Anti-laminin 332 pemphigoid shows absent IgG binding in (**a**) laminin 332-deficient skin, while a positive binding in (**b**) type VII collagen-deficient skin. EBA shows a reverse binding pattern (**c**, **d**) (Reprinted from Vodegel *et al.* [11], copyright 2003, with permission from Elsevier)

3. The substrate of choice for testing for paraneoplastic pemphigus is:
   (a) Monkey esophagus
   (b) Rat bladder
   (c) Salt-split skin

Answers

1. (b)
2. (d)
3. (b)

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Immunoassays

6

Hendri H. Pas

Abstract

Immunoassays are helpful serological techniques for the laboratory diagnosis of autoimmune bullous diseases (AIBD) and for monitoring disease activity of individual patients. The three main immunoassays are immunoblotting, immunoprecipitation, and ELISA. All three make use of the ability of the autoimmune IgG to bind to the target antigen. Immunoblotting is used to visualize the apparent molecular mass of the antigen, thereby enabling its identification. The same goes for immunoprecipitation that, although being a more laborious technique than immunoblotting, has the advantage that it recognizes more epitopes on the autoantigen than immunoblotting. Whereas immunoblotting and immunoprecipitation are qualitative assays, ELISA is a rapid and easy quantitative technique. ELISA therefore enables monitoring of the antibody titer during the disease course.

Keywords

Antigen • Epitope • Immunoglobulin • Immunoblot • Immunoprecipitation • ELISA • Autoimmune disease • Pemphigus • Pemphigoid

Introduction and AIMS

Pemphigus and pemphigoid are autoimmune bullous diseases that are characterized by autoantibodies to epithelial proteins. Immunoblot, immunoprecipitation, and ELISA are laboratory techniques that can visualize to which epithelial protein(s) the autoantibodies are directed. ELISA furthermore can quantify the autoantibody titer. In this chapter we will briefly outline how these techniques work and how results should be interpreted.

Learning Objectives

After reading this chapter you should be able to:

- Understand the principle of immunoassays
- Interpret the results of immunoassays

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- Decide if immunoassays could be helpful in managing your patient
- Choose which immunoassays to perform for individual patients

Immunoblotting

Immunoblotting is a qualitative test to identify which autoantigen(s) is involved in a particular AIBD patient. Briefly, denatured skin proteins are separated and sorted on molecular mass by polyacrylamide gel electrophoresis (PAGE) and then transferred onto membrane filters to facilitate further incubation and washing steps. The filters are overlaid with patient serum and after washing bound IgG is stained. The antigens then become visible as purple bands and are identified by apparent molecular weight (Fig. 6.1) [1]. Skin proteins are obtained by extracting cultured human keratinocytes or human skin with the harsh soap sodium dodecyl sulfate (SDS). SDS has the ability to completely dissolve protein complexes including large structures as hemidesmosomes and desmosomes that contain major pemphigus and pemphigoid autoantigens. SDS is a negatively charged molecule and binds to proteins in an assumed SDS/protein ratio of 1.4. This destroys the native conformation of proteins that unroll and take on a linear shape. As all proteins become negatively charged with an even distribution of charge per unit mass, they are fractionized according to size during electrophoresis.

<Immunoblotting is a qualitative test to identify the targeted autoantigen>

SDS also has a disadvantage as it destroys conformational epitopes. An epitope is the part of the antigen that is recognized by the antibody, and they have an average size of around 15 amino acids [2]. Epitopes are divided in two categories: linear epitopes that consist of a continuous stretch of amino acids and are thus determined by the primary structure and conformational epitopes formed by separate stretches of amino acids that lie close together in the native conformation of the protein and which are thus determined by the tertiary structure (Fig. 6.2). It is this last category of epitopes that is destroyed by the SDS and is missed in immunoblotting. For this reason immunoblotting is not suited for diagnosing pemphigus vulgaris (PV) or pemphigus foliaceus (PF) as the pathogenic epitopes of the autoantigens desmogleins 1 and 3 are largely conformational epitopes. In case of paraneoplastic

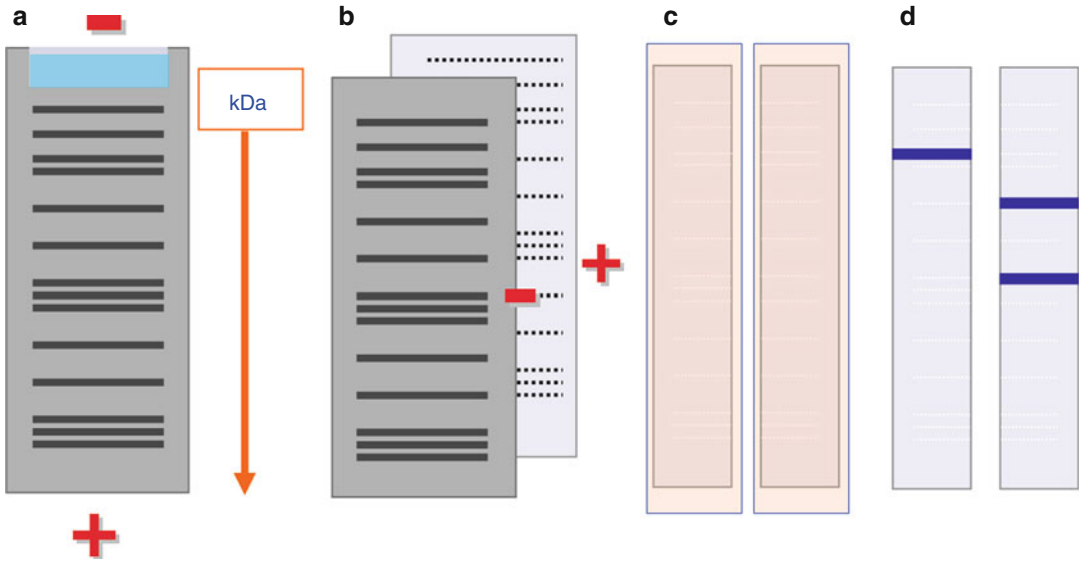

Fig. 6.1 Principle of immunoblotting. (**a**) Molecules in a skin protein extract are separated by gel electrophoresis. (**b**) The protein pattern is electrophoretically transferred to a membrane filter that facilitates further handling. (**c**) The filter is immersed in diluted patient serum. (**d**) Bound IgG is visualized by staining

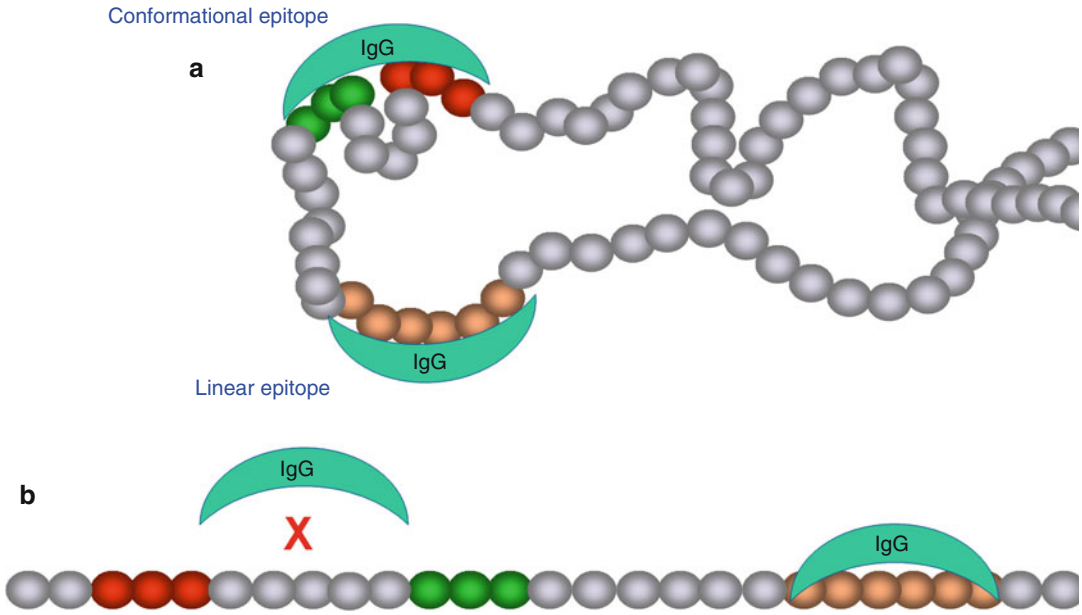

Fig. 6.2 Two classes of epitopes. (**a**) A native protein with a conformational epitope (*red*, *green*) is formed by two different parts of the molecule, while a linear epitope is formed by a continuous stretch of amino acids (orange). (**b**) A denatured protein loses its native conformation. Therefore, IgG cannot bind anymore to the conformational epitope as it is destroyed, while the linear epitope is still available

pemphigus (PNP) however, it is a good option as here immunoblotting has a reported 89 % sensitivity and 100 % specificity for detecting the simultaneous presence of antibodies to envoplakin and periplakin that is specific for PNP [3]. For identification of autoantibodies to pemphigoid antigens, immunoblotting has a varying sensitivity and is not the first choice when alternatives are available. For type VII collagen and BP230, ELISAs can now be commercially obtained. For laminin 332 50 % sensitivity was reported [4]. Diagnosis of anti-p200 pemphigoid seems to have high sensitivity, but the quality of the dermal extract, which requires a sophisticated extraction procedure, is important, and the assay is therefore only performed in a few highly specialized laboratories. Although plectin antibodies were found by immunoblotting in 4 % of all pemphigoid patients, the sensitivity is not known. Immunoblotting has additional value for detecting antibodies to BP180 which is the dominant antigen of the pemphigoid group. An ELISA for BP180 is available but can only detect antibodies to a small stretch of BP180 named NC16A that is reported to contain the major immunodominant

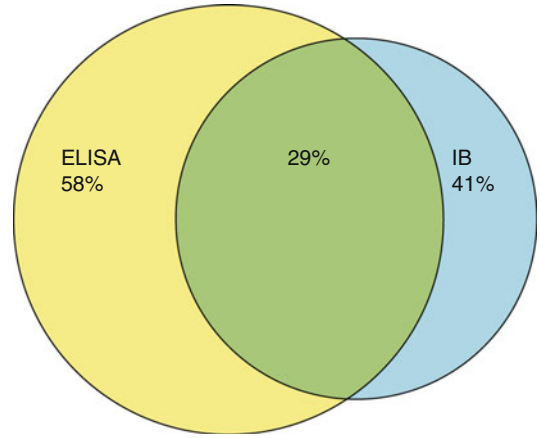

Fig. 6.3 Venn diagram showing detection of anti-BP180 IgG antibodies in 357 patients with bullous or mucous membrane pemphigoid. When combined, anti-BP180 IgG was found in 70 % of the cases

epitopes. In contrast in immunoblotting the full-length BP180 molecule is available. When ELISA and immunoblotting are compared, immunoblotting detects 12 % of the tested cases additional to ELISA (Fig. 6.3). Conversely immunoblotting misses half the cases found by ELISA indicating

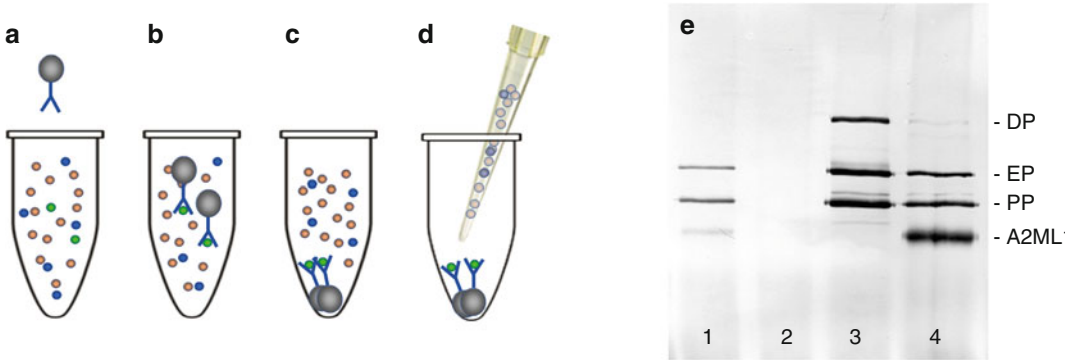

Fig. 6.4 Principle of nonradioactive immunoprecipitation. (**a**) Patient IgG bound to beads is added to a protein extract. (**b**) The IgG on the beads bind the disease-causing antigen(s). (**c**) The beads are spun down by centrifugation. (**d**) The supernatant containing the other skin proteins is removed. (**e**) The antigen(s) is visualized by gel electrophoresis followed by immunoblotting. Here sera were analyzed for IgG to PNP antigens. Lane 1 PNP patient, lane 2 PV patient, lane 3 PNP patient, lane 4 PNP patient. *DP* desmoplakin, *EP* envoplakin, *PP* periplakin, *A2ML1* alpha-2-macroglobulin-like 1

that also here loss of conformational epitopes plays a role.

Immunoprecipitation

Immunoprecipitation has played an important role in identification of the autoantigens involved in AIBD, the last one being alpha-2-macroglobulin-like 1 protein in PNP [5]. However as it is a labor intensive technique, it is expensive and therefore not much used in routine diagnostics of AIBD.

The advantage of immunoprecipitation over immunoblotting is that the protein extracts for immunoprecipitation are prepared with soft soaps that do not denature proteins and the conformational epitopes therefore remain intact. Classical immunoprecipitation is performed with radioactive labeled protein extracts. The patient serum is first incubated with protein G-coupled beads. Protein G is a molecule that specifically binds IgG from the serum. After washing the beads are added to the radioactive extract where the patient IgG will bind to the autoantigen(s) in question. The beads are then removed by centrifugation and washed, and the IgG and the radioactive antigen(s) are eluted in SDS-PAGE sample buffer. The sample is then separated and sorted on molecular size by polyacrylamide gel electrophoresis. Next the radioactive bands are visualized by fluorography and as in immunoblot indentified on basis of molecular mass. Also unknown antigens can be identified by analyzing the radioactive band with advanced mass spectrometry methods. As working with radiochemicals is subject to strict regulations, it is an easier option to perform nonradioactive immunoprecipitation that is a combination of immunoprecipitation and immunoblotting. The procedure is largely the same but with nonradioactive substrates. After immunoprecipitation and gel electrophoresis, the gel is blotted, and the filter is incubated with a cocktail of antibodies that are specific for the antigens in question. After washing the blot can is stained to visualize which antigens have been precipitated from the extract (Fig. 6.4).

<Immunoprecipitation has a higher sensitivity than immunoblot>

ELISA

Enzyme-linked immunosorbent assay (ELISA) is a technique that enables to measure the autoantibody response to a single autoantigen in a quantitative manner. ELISAs are commercially available and easy to perform, so can be introduced in every diagnostic lab. As all serological assays ELISA is based on the binding of patient IgG to the autoantigen. Principle of ELISA is that a small plastic well (200 µl volume) on a plate

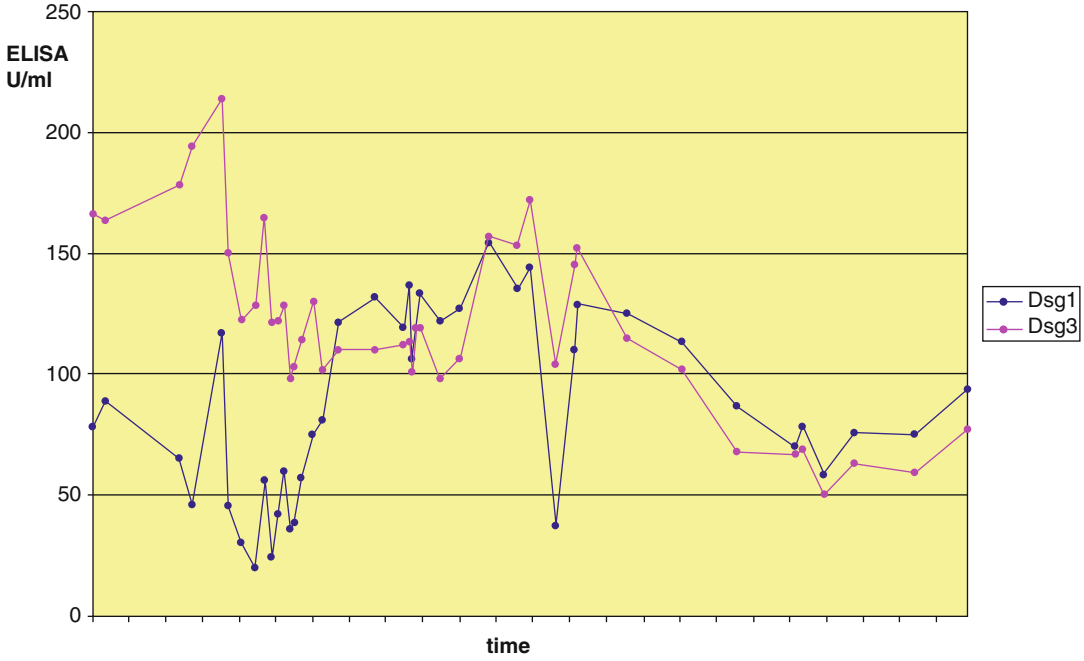

Fig. 6.5 Titer monitoring in pemphigus by ELISA to Dsg1 and Dsg3. The patient was followed for 8 years

is coated with a single antigen. The coated molecules are recombinantly produced and consist of the whole or particular part(s) of an antigen. The antigen is not denatured before coating and therefore contains both linear and conformational epitopes. Serum is brought into the coated well, and if IgG to the antigen is present, it will become bound. Next an antihuman IgG to which a special enzyme is conjugated is brought into the well. This will bind to the IgG, and the more IgG is bound to the well, the more of the enzyme will be bound. After washing the unbound IgG, a substrate is brought in the well that can be converted by the enzyme into a colored product. The more patient IgG is bound, the more color will be produced, and the amount of color is thus an indication of the amount of specific autoimmune IgG in the serum of the patient. This enables serological disease monitoring (Fig. 6.5). In January 2015 six different ELISAs were commercially available to, respectively, the pemphigus antigens desmoglein 1, desmoglein 3, and envoplakin and to the pemphigoid antigens BP230, BP180, and type VII collagen. Of these the ELISAs to desmogleins 1 and 3 are most widely used as it enables discriminating between pemphigus vulgaris and pemphigus foliaceus. Pemphigus foliaceus has antibodies to desmoglein 1 but not to desmoglein 3, while mucosal dominant pemphigus vulgaris has IgG to desmoglein 3 only and mucocutaneous pemphigus vulgaris to both desmogleins 1 and 3 [6]. The sensitivity to detect pemphigus is 89 % by ELISA, which is slightly better than 86 % by indirect immunofluorescence microscopy on monkey esophagus in our hands. Being quantitative these ELISAs are well suited to follow antibody titers (expressed as relative arbitrary units). The change in titer values from the desmoglein 1 ELISA fairly well corresponds to the activity of skin disease. However the results of the desmoglein 3 ELISA should be interpreted with more caution [7]. For about two-thirds of the patients, there is a correlation with mucosal involvement but for the other third ELISAs may stay unchanged despite clinical improvement. Evidence is building that this is due to the presence of nonpathogenic antibodies to desmoglein 3 [8]. ELISA however cannot discriminate between pathogenic and nonpathogenic antibodies.

<ELISA is a quantitative assay for monitoring disease activity>

The envoplakin ELISA has been developed to diagnose paraneoplastic pemphigus. Its sensitivity is estimated to be 63 % and lower than immunoblotting [3]. The ELISA to type VII collagen was found to have 45 % sensitivity due to approximately half of the patients having a very low undetectable serum titer [9]. For patients that have an ELISA-detectable serum titer, the type VII collagen ELISA values correspond well with disease activity. Above we already discussed the BP180 ELISA. This ELISA contains a small recombinant fragment NC16A, approximately 6.5 % of the entire extracellular domain of BP180, but contains the immunodominant domains. Exact figures of its sensitivity are not known, but based on the comparison with immunoblot results, it can be estimated to be in the order of 70 %. The BP230 ELISA is less sensitive, and its diagnostic added value has to be found only 5 % [10].

Review Questions

1. Which assay is quantitative?
   (a) Immunoblot
   (b) ELISA
   (c) Immunoprecipitation
   (d) All three
2. The size of an epitope is on average
   (a) 15 amino acids
   (b) 50 amino acids
   (c) 150 amino acids
3. ELISA values ……..parallel disease activity
   (a) Always
   (b) Most times
   (c) Seldom
4. You have treated a patient with rituximab. What assay would you request to evaluate your therapy?
   (a) Immunoblot
   (b) ELISA
   (c) Immunoprecipitation
   (d) All three

Answers

1. (b)
2. (a)
3. (b)
4. (b)

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Part II

Pemphigus

Structure of Desmosomes

7

Ena Sokol

Abstract

Desmosomes are cell-cell junctions that connect neighboring cells to their intermediate filament networks that serve to provide mechanical strength to the tissue. Major proteins that compose desmosomes are desmogleins, desmocollins, plakoglobin, plakophilins, and desmoplakin. Isoforms of desmosomal proteins have different distribution in tissues, some of them being more ubiquitous and others being expressed only in specific tissues. The importance of desmosomal proteins for sustaining epithelial architecture is demonstrated in genetic, autoimmune, and infectious human blistering diseases.

Keywords

Cell-cell junctions • Desmosome • Desmoglein • Desmocollin • Plakoglobin • Plakophilin • Desmoplakin

Introduction and Aim

Importance of desmosomes for the architecture and mechanical strength of epithelial tissue is demonstrated in several blistering diseases. Perturbation of a desmosomal structure by autoantibodies, toxins, or gene mutations can disrupt the architecture and strength of the skin and mucous membranes. Therefore proper function of all the proteins that are part of the complex desmosomal structure is important for tissue integrity. In this chapter we aim to explain the components of a desmosome and their organization into an adhesive structure.

Learning Objectives

After studying this chapter, you should be able to:

- Know the structure of a desmosome.
- Know desmosomal proteins and their isoforms.
- Learn the distribution of isoforms of desmosomal proteins.

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Desmosomes: Cell-Cell Adhesion Structures

Cell junctions are specialized structures that interconnect neighboring cells to each other (cell-cell junctions) or cells to the matrix (cell-matrix junctions). They can serve as strong sealing points involved in tissue barrier (tight junctions), or as mechanical cell attachment (adherens junctions, desmosomes, and hemidesmosomes), or as communication channels (gap junctions).

Desmosomes (desmo, bond; soma, body) are cell-cell junctions that interconnect intermediate filament networks of neighboring cells and provide strong mechanical strength [1, 2]. They are abundant in stratified epithelium, such as epidermis and epithelium of mucosa, and in heart muscle, but are also present in simple epithelium and in non-epithelial cells like meningeal cells of the arachnoid and the follicular dendritic cells of lymph follicles [3].

Desmosomes can be easily recognized by electron microscopy by their extracellular core domain (ECD) and two opposite dense plaques. Within each plaque two zones can be distinguished: the outer dense plaque (ODP) and the inner dense plaque (IDP) (Fig. 7.1a, b). Proteins that make distinctive zones of desmosomes are transmembrane proteins which belong to cadherin family and cytoplasmic plaque proteins which belong to armadillo and plakin family of proteins. Desmosomal cadherins are desmogleins and desmocollins; armadillo proteins in desmosomes comprise plakoglobin and plakophilins, while the main plakin protein in desmosomes is desmoplakin [1–3]. Other members of plakin family such as plectin, periplakin, and envoplakin are also found in desmosomes [3]. Extracellular domains of desmosomal cadherins compose ECD where they mediate adhesion, while their intracellular domains together with plakoglobin and plakophilins make ODP. IDP comprises desmoplakin that couples to the intermediate filament network (Fig. 7.1c). Prestructure of a complete desmosome is the so-called half desmosome which is made of a desmosomal plaque and desmosomal cadherins at one side not connected to its opposite part [4]. Half desmosomes and desmosomes should be distinguished from hemidesmosomes which are cell-matrix junctions and are explained in Chap. 13.

Autoimmune bullous diseases with antibodies against certain desmosomal proteins demonstrate the importance of desmosomes for tissue integrity.

<Desmosomes are cell-cell junctions that provide strong intercellular adhesion>

Desmosomal Proteins and Their Isoforms

Desmosomal cadherins are calcium-dependent glycoproteins and are desmogleins (Dsgs) and desmocollins (Dscs). They require calcium for binding to their opposites. When stable desmosomes become calcium independant they form a midline in the ECD and are called hyperadhesive. In conditions without calcium, half desmosomes will still be formed, but will be soon internalized, and complete desmosomal structure will not be achieved [4]. In humans there are four types of Dsgs and three types of Dscs which are differently distributed. All Dsc isoforms have two forms: form "a" and shorter form "b," which are results of alternative splicing (Fig. 7.1d). Both Dsgs and Dscs have extracellular part consisting of four cadherin repeats (EC1–EC4) and a fifth domain termed extracellular anchor (EA), as well as transmembrane domain (TM) located in the plasma membrane and intercellular part starting with an intracellular anchor (IA) (Fig. 7.1d). The rest of the intercellular part differs where intercellular cadherin-like sequence (ICS) that binds plakoglobin is present in Dsc a form and Dsgs. Dsgs have additional intracytoplasmic regions: intercellular proline-rich linker (IPL), variable number of repeat unit domain (RUD), and glycine-rich desmoglein terminal domain (DTD) [1–3].

Plakoglobin also termed as γ-catenin is an armadillo protein that localizes both to desmosomes and to adherens junctions. Plakoglobin contains twelve armadillo repeats flanked by distinct amino- and carboxy-terminal domain. In

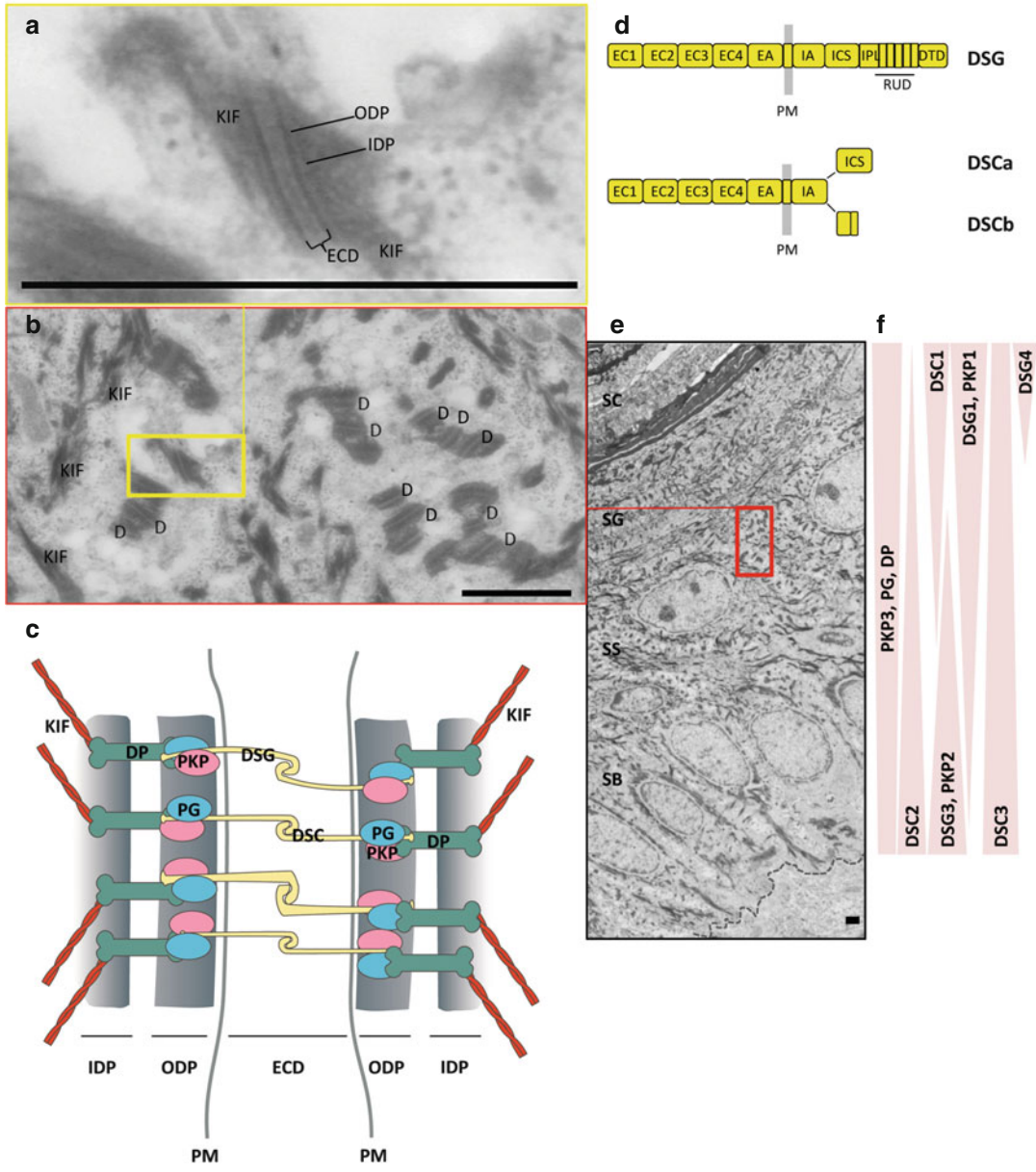

Fig. 7.1 Desmosomal structure, structure of desmosomal cadherins, and expression of desmosomal proteins in human epidermis. (**a**) Magnified desmosome and desmosomal distinctive zones. (**b**) Magnified region of epidermis with multiple desmosomes. *Yellow box* is magnified in panel **a**. (**c**) Schematic presentation of a complete desmosome. Desmosomal proteins are presented in different shapes and colors. Note the organization of desmosomal proteins in ECD, ODP, and IDP. (**d**) Structure of desmosomal cadherins: desmoglein and desmocollin "a" and "b" form. Desmogleins differ in number of RUDs. (**e**) Human skin and epidermal layers. Dotted line presents border between dermis and epidermis. *Red box* is magnified in panel **b**. (**f**) Expression of isoforms of desmosomal proteins through epidermal layers that are shown in panel **e**. *Dsg* desmoglein, *Dsc* desmocollin, *Pg* plakoglobin, *Pkp* plakophilin, *Dp* desmoplakin, *SB* stratum basale, *SS* stratum spinosum, *SG* stratum granulosum, *SC* stratum corneum, *D* desmosome, *KIF* keratin intermediate filaments, *ECD* extracellular core domain, *ODP* outer dense plaque, *IDP* inner dense plaque, *PM* plasma membrane, *EC* extracellular domain, *EA* extracellular anchor, *IA* intracellular anchor, *IPL* intercellular proline-rich linker, *RUDs* repeat unit domains, *DTD* desmoglein terminal domain. Panels **a**, **b**, and **e** are electron microscopy images that were taken from a nanotomy dataset of normal human skin (Reprinted with permission from Sokol *et al.* [7]). Scale bar 1 μm

desmosomes plakoglobin binds to the cytoplasmatic tail of desmosomal cadherins, and it is reported that it binds desmoplakin [1–3].

Plakophilins (Pkps) are armadillo proteins that contain nine armadillo repeats with an insert between that bends the whole structure. There are three isoforms of Pkps. Pkps can bind all other desmosomal components, and it is shown that they can bind intermediate filaments or enhance interactions in the desmosomal plaque [1–3].

Desmoplakin belongs to plakin family proteins, and it is a key linker between the desmosomal plaque and intermediate filaments. Desmoplakin has two isoforms in which globular amino- and carboxy-parts are connected with central α-helical coiled-coil rod domain. Amino-terminal domain contains binding sites for plakoglobin and Pkp, while carboxy-terminal domain contains binding site for intermediate filaments [1–3].

Periplakin, envoplakin, and plectin are also found in desmosomes, but it is not clear how important they are for the structure and function [3].

Isoforms of desmosomal proteins are differently distributed in human tissues [5]. All desmosomes bearing tissues express plakoglobin and desmoplakin. Dsg2, Dsc2, and Pkp2 are mostly found in simple epithelia. Dsg1 and Dsg3 and Dsc1 and Dsc3 are specific for stratified epithelia. Expression of isoforms of desmosomal proteins in the epidermis is cell layer dependent, and it is shown in Fig. 7.1e, f. Expression of Dsg1 decreases from upper toward lower epidermal layers, while Dsg3 is present in the basal and suprabasal layers. Dsg4 is found in the upper layers of the epidermis and in the hair follicle [6]. Different expressions of isoforms of desmosomal proteins explain localization of lesions in certain autoimmune bullous diseases.

<Desmosomal proteins are desmogleins, desmocollins, plakoglobin, plakophilins, and desmoplakin>

Review Questions

1. *Which cytoskeletal filaments desmosomes interconnect*?
   (a) Microtubules
   (b) Intermediate filaments
   (c) Actin filaments
   (d) All of the above
2. *Which desmosomal proteins require calcium molecules for their activation and binding to their opposites*?
   (a) Plakophilins
   (b) Plakoglobin
   (c) Desmogleins and desmocollins
   (d) Desmogleins and plakoglobin
3. *What is the expression pattern of Dsg1 and Dsg3 in the epidermis*?
   (a) Dsg1 and Dsg3 are expressed in all layers of the epidermis.
   (b) Dsg1 and Dsg3 are expressed in the basal and granular layer of the epidermis.
   (c) Dsg3 is expressed in the basal and suprabasal layers, while Dsg1 expression decreases from the upper to the lower layers of the epidermis.
   (d) Dsg1 expression decreases from the basal to the upper layers of the epidermis, while Dsg3 expression decreases from the upper to the lower layers of the epidermis.

Answers

1. (b)
2. (c)
3. (c)

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Additional Reading

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Pemphigus Vulgaris

8

Gerda van der Wier and Marcel F. Jonkman

Abstract

Pemphigus is a group of chronic mucocutaneous blistering diseases caused by autoantibodies directed against the desmosomal cadherins desmoglein 1 and/or desmoglein 3 (Table 8.1). Pemphigus can be divided into two major forms, pemphigus foliaceus (PF) and pemphigus vulgaris (PV). The mucosal dominant form of PV is characterised by blistering of the mucous membranes and antibodies directed against desmoglein 3. Patients with mucocutaneous PV have blistering of both the mucous membranes and the skin, and the autoantibodies are directed against desmogleins 1 and 3. The diagnosis is based on histopathological examination, immunofluorescence microscopy and enzyme-linked immunosorbent assays (ELISA). Treatment of pemphigus vulgaris comprises systemic corticosteroids, together with adjuvant immunosuppressive drugs and/or rituximab.

Keywords

Pemphigus • Desmosome • Immunoglobulin • Desmoglein • Steric hindrance • Compensation hypothesis

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Introduction and AIMS

Short Definition in Layman Terms

Pemphigus vulgaris (PV) is a chronic autoimmune bullous disease that affects the mucous membranes only (mucosal dominant PV) or both the mucous membranes and the skin (mucocutaneous PV) (Fig. 8.1).

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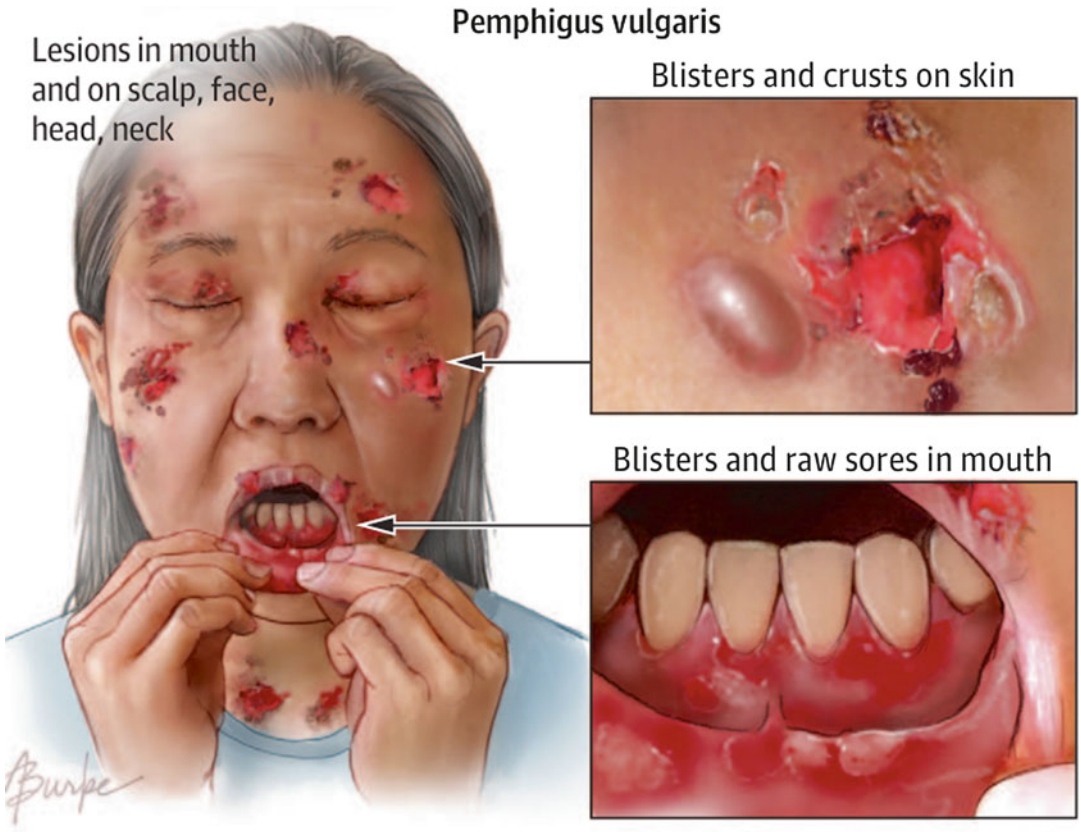

Fig. 8.1 Typical features of patient with pemphigus vulgaris (Copyright © 2014 American Medical Association. All rights reserved [1])

Learning Objectives

After reading this chapter you will know how to recognise a patient with pemphigus vulgaris, which specific tests and diagnostics should be done to confirm your diagnosis, and how to treat a patient with pemphigus vulgaris.

Case Study: Part 1

A 58-year-old female had erosions and hyperpigmentation on the skin of the neck, the armpits, the legs and the mammary folds since 8 months. She also has painful erosions of the oral mucosa. Nikolsky's signs I and II were positive. She was known with hypertension for which she was treated with a calcium antagonist and a beta blocker.

Didactical Questions: Cross Section of Questions to Prime the Readers' Interest

Why do some patients with pemphigus vulgaris have only mucous membranes affected, while others have both skin and mucous membranes affected? What is the histopathology of PV? How do autoantibodies cause acantholysis in the skin and mucous membranes?

Facts and Figures

Definitions and Classification

The term pemphigus is derived from the Greek word *pemphix*, which means blister. Pemphigus is a group of chronic mucocutaneous blistering

Table 8.1 IF findings and clinical symptoms of subtypes of pemphigus

	Target antigens	DIF	Clinical symptoms
Pemphigus vulgaris, mucosal dominant	Dsg3	ECS IgG, IgA ± C3c	Painful erosions of the oral mucosa
Pemphigus vulgaris, mucocutaneous	Dsg1, Dsg3	ECS IgG, IgA ± C3c	Painful blisters and erosions of the oral mucosa and skin
Pemphigus vegetans, Hallopeau type	Dsg3	ECS IgG, IgA ± C3c	Pustules accumulate in body folds and around orifices, easily secondarily infected
Pemphigus vegetans, Neumann type	Dsg3	ECS IgG ± C3c	Papillomas accumulate in the body and around orifices, easily secondarily infected
Pemphigus foliaceus	Dsg1	ECS IgG ± C3c	Crusted plaques with multiple layers of scaling, which easily erodes at the scalp, temples, periorbicular area, neck, upper chest and back
Endemic pemphigus	Dsg1	ECS IgG ± C3c	Localised form (forme fruste) and generalised form (bullous invasion, keratotic, hyperpigmented, pemphigus herpetiformis and exfoliative erythroderma)
Pemphigus erythematosus	Dsg1	ECS/BMZ IgG ± C3c	Lupus-like butterfly rash and seborrhoeic distribution. Evoked by UV light
Pemphigus herpetiformis	Dsg1, Dsg3	ECS IgG, IgA ± C3c	Grouped (herpetiform) distribution of itching erythematous vesicular/bullous/papular lesions, often in an annular-shaped pattern. Nikolsky's sign is negative
IgA pemphigus, subcorneal pustular dermatosis type	Dsg1	ECS IgA ± C3c	Erythematous skin lesions with tiny superficial circinate pustules, desquamation from the edges surfacing the entire body, particularly in the intertriginous areas
IgA pemphigus, intraepidermal neutrophilic IgA dermatosis type	Unknown	ECS IgA ± C3c	Annular erythematous plaques with circinate pustules and crusts that spread outwards and heal inwards in a sunflower-like appearance
Drug-induced pemphigus	Dsg1, Dsg3,	ECS IgG, IgA ± C3c	Prodromal stage with pruritus and nonspecific lesions preceding the genuine pemphigus lesions, mimicking all variants of pemphigus
Paraneoplastic pemphigus	Envoplakin, periplakin, desmoplakin, BP230, plectin, A2ML1, Dsg1, Dsg3	ECS/BMZ IgG, IgA ± C3c	Painful severe oral stomatitis, with haemorrhagic crusts. Flaccid to tense blisters at the face, trunk and extremities. Generalised lichenoid erythema. Sporadically shortness of breath. Underlying neoplasm

Dsg1 desmoglein 1, *Dsg3* desmoglein 3, *BMZ* basement membrane zone pattern, *ECS* epithelial cell surface pattern

diseases caused by autoantibodies directed against the desmosomal cadherins desmoglein 1 (Dsg1) and/or desmoglein 3 (Dsg3) (Table 8.1). Pemphigus can be divided into two major forms, based on the level of the blister in the epidermis. The superficial forms of pemphigus are grouped under pemphigus foliaceus, the deep forms under pemphigus vulgaris (mucosal dominant

pemphigus vulgaris and mucocutaneous pemphigus vulgaris) and its variant pemphigus vegetans.

<Blistering in pemphigus is caused by autoantibodies directed against desmoglein 1 and/or 3.>

Epidemiology

Pemphigus is rare and its incidence has been estimated to about 0.2 cases per 100,000 per year in Central Europe. The incidence of the different subtypes of pemphigus varies from 0.076 in Finland to 0.67 in Tunisia. Countries with high incidence of pemphigus are Bulgaria, Greece and the Mediterranean region of Turkey. Pemphigus vulgaris (PV) is the most common subtype comprising 83.1 % of all cases in Southern Turkey [2]. The mean age of onset of the disease is approximately 40–50 years of age. There is a slight female predominance.

Pathogenesis

In 1964 Beutner and Jordan observed circulating antibodies directed against the cell surface of keratinocytes in the sera of patients with PV [3]. Later it was demonstrated that autoantibodies in pemphigus are pathogenic and induce blister formation in skin organ culture systems and in neonatal mice. In 1982 Stanley *et al.* characterised the PV antigen at the molecular level by immunoprecipitation using cultured keratinocyte extracts as a substrate. All the PV sera identified a glycosylated 130 kDa glycoprotein [4]. In 1991 Amagai *et al.* isolated a cDNA clone for the PV antigen by immunoscreening a human keratinocytes expression library with autoantibodies prepared from the sera of patients with PV [5]. Analysis of the deduced amino acid sequences of the cDNA clones revealed the nature of pemphigus antigens being desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3). Both antigens are member of the cadherin family of calcium-dependant homodimeric 'cadherins' that are located in epithelial cell-cell contacts such as adherens junctions and desmosomes.

Desmoglein Compensation Hypothesis

The desmoglein compensation hypothesis explains why the skin or mucous membranes are affected in various forms of pemphigus. This theory states that Dsg1 and Dsg3 can compensate for each other and prevent acantholysis when autoantibodies bind to either molecule (Fig. 8.2) [6]. In the skin, Dsg1 is expressed throughout the whole epidermis, but more intense in the superficial layers, whereas Dsg3 is confined to the basal and suprabasal layers. Antibodies to Dsg1 therefore cause blisters in the superficial epidermis since in this area Dsg3 is not present to compensate for the loss of Dsg1. The result is PF, which clinically only affects the skin.

In the mucosa, Dsg3 is expressed throughout the whole epithelium, whereas Dsg1 is confined to the superficial layers. Antibodies to Dsg3 therefore cause blisters deep in the mucosa, since in this area Dsg1 is not present to compensate for the loss of Dsg3. The skin remains unaffected, because Dsg1 is present throughout the epidermis and compensates for loss of Dsg3. The result is mucosal dominant PV.

If both Dsg1 and Dsg3 are targeted by antibodies, no compensation is possible. The level of blistering is suprabasal, since 'melting' of desmosomes starts in both the skin and mucosa in the lower epithelium at the entry point of IgG. The result is mucocutaneous PV.

<The desmoglein compensation hypothesis explains the localisation and the level of the blister in pemphigus>

The exact cellular mechanism by which pemphigus IgG induces acantholysis has been a subject of debate since the discovery of pemphigus autoantibodies by Beutner and Jordan. Since then acantholysis has been explained by several theories: (1) steric hindrance, (2) deranged cell signalling and (3) impairment of desmosome assembly and increased desmosome disassembly.

Steric Hindrance

The steric hindrance theory is based on the idea that there is direct interference of pemphigus IgG with the amino-terminal extracellular domain of desmogleins, which form the trans-adhesive interface between keratinocytes. This would lead to a

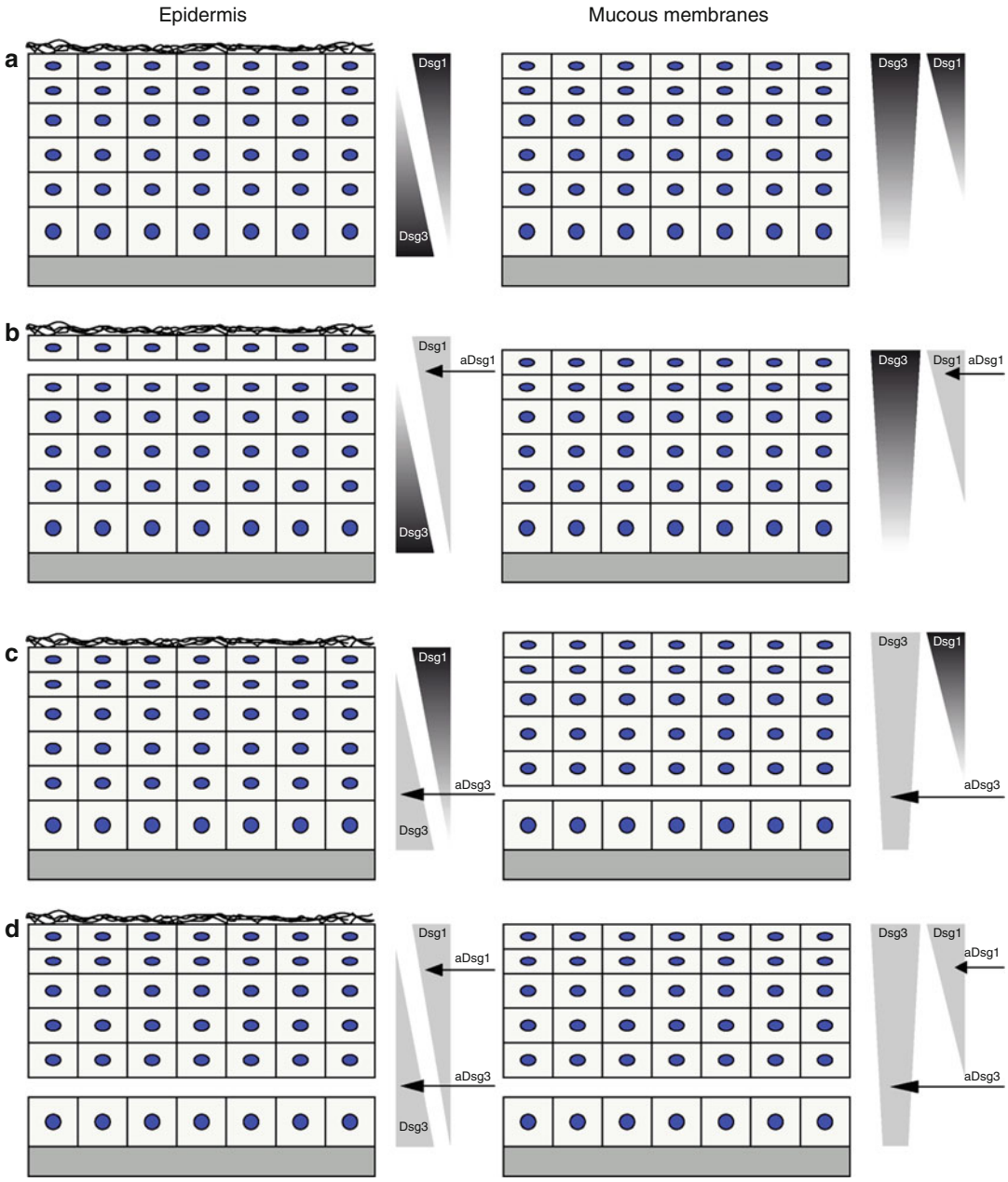

Fig. 8.2 Desmoglein compensation hypothesis. (**a**) Normal distribution of desmoglein (Dsg) 1 and Dsg3 in the epidermis and mucous membrane. (**b**) In pemphigus foliaceus, IgG directed against Dsg1 causes subcorneal blistering in the skin because in the lower layers Dsg3 compensates for the loss of function of Dsg1. In the mucosa however anti-Dsg1 antibodies do not cause blistering, because there is sufficient Dsg3 present throughout all the layers to compensate for Dsg1. (**c**) In mucosal dominant pemphigus vulgaris (PV), IgG directed against Dsg3 does not cause blistering of the skin because Dsg1 compensates for the loss of function of Dsg3. However, there is suprabasal blistering of the mucous membranes because there is no sufficient Dsg1 present to compensate for Dsg3. (**d**) In mucocutaneous PV antibodies directed against both Dsg1 and Dsg3 cause blistering of the skin and the mucous membranes

lengthwise splitting of the desmosomes which has indeed been observed by electron microscopy in pemphigus patients and mouse models.

Cell Signalling

Signalling pathways that play a role in the pathogenesis of pemphigus involve complicated interactions between p38 mitogen-activated protein kinase (p38MAPK), RhoA, protein kinase C (PKC), epidermal growth factor receptor (EGFR), plakoglobin and c-Myc [7].

Assembly and Disassembly

PV IgG leads to depletion of non-junctional Dsg3 by endocytosis. Eventually, assembly of desmosomes fails due to shortage of non-junctional Dsg3 building blocks. Besides binding of PV IgG to non-junctional Dsg3, it might also be possible that PV IgG binds to junctional Dsg3 in the core domain of desmosomes. This leads to disassembly of Dsg3 from the desmosomes and possible internalisation into endosomes.

Diagnosis Paths

History and Physical Examination

Almost all patients with pemphigus vulgaris have painful erosions of the oral mucosa. More than half of the patients also develop blisters and erosions on the skin (mucocutaneous PV). In mucosal dominant PV, there are only oral lesions present (Fig. 8.3).

The disease often starts on the mucous membranes in the oral cavity leading to erosions. The most common sites are the gingiva, buccal mucosa and tongue. The erosions extend peripherally and may spread to involve the pharynx and larynx with difficulty in eating and drinking and hoarseness of the voice. The lesions do not scar and therefore are benign. Blood crusts may be present on the nasal septum. Other mucosal surfaces include the conjunctiva, oesophagus, vagina, urethra and rectum.

After weeks to months, the disease progresses with lesions appearing on the skin. The predilection sites on the skin are facial temples, the scalp

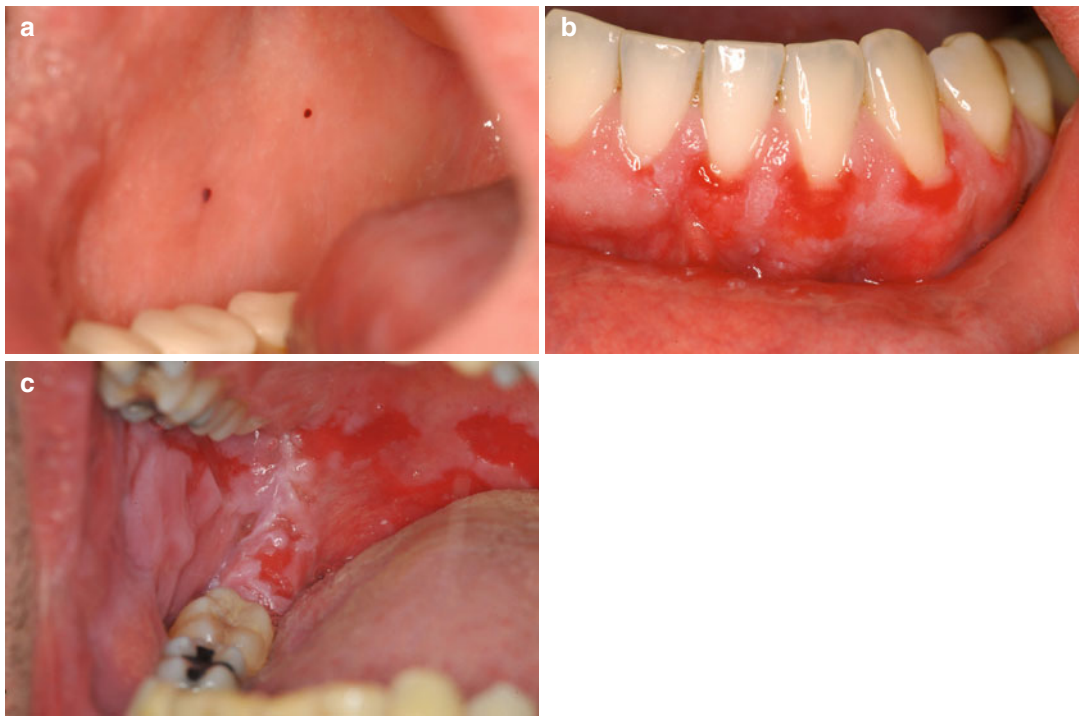

Fig. 8.3 Mucosal dominant pemphigus vulgaris. Early phase shows (**a**) haemorrhagic vesicles on the buccal mucosa and (**b**) desquamative gingivitis. Late phase shows (**c**) whitish blister roofs (like bacon) and erosions on the buccal mucosa and soft palate

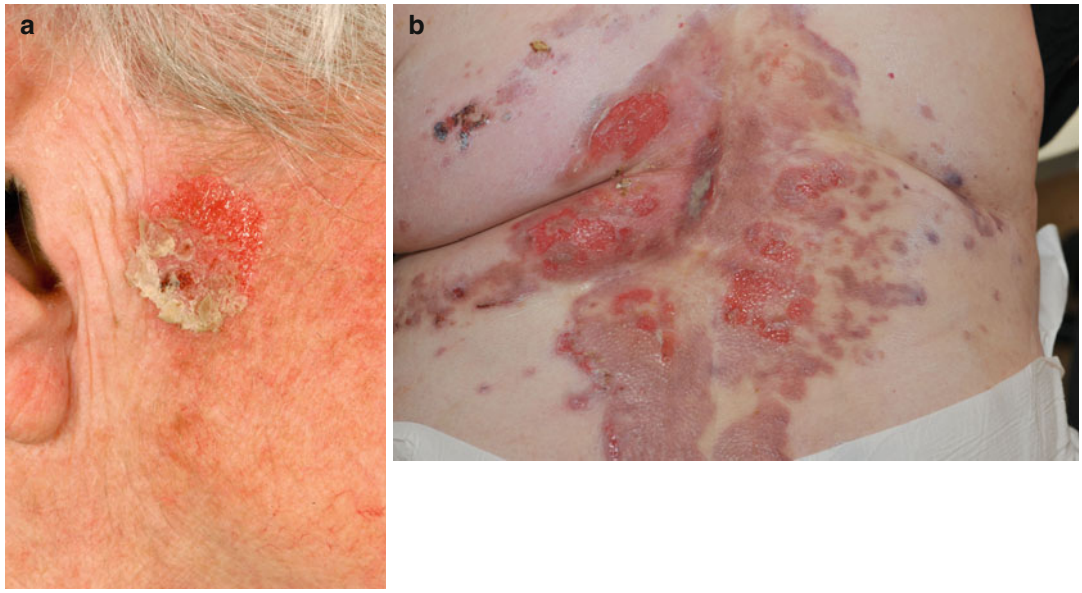

Fig. 8.4 Mucocutaneous pemphigus vulgaris. (**a**) Positive Nikolsky's sign type II on a crust of the temple. (**b**) Symmetrical erosions in dusky erythema on the back

and upper chest (Fig. 8.4). The first lesion of the skin is a blister that is filled with a clear fluid, on a normal or erythematous skin, which breaks easily resulting in a painful erosion. The fluid within the blisters may become haemorrhagic, turbid or even seropurulent. The erosions enlarge to form large denuded areas, which become crusted. Crusts are piled up into vegetating plaques due to reblistering of regenerated epithelium underneath. The cutaneous barrier loss may lead to complications as infections or metabolic disturbances. Before systemic corticosteroids became available, about 75 % of patients who developed PV died within a year.

A characteristic feature of all forms of active and severe pemphigus is the Nikolsky's sign, produced when lateral pressure is applied adjacent to a lesion leading to separation of the epidermis. The lack of cohesion of the skin may also be demonstrated with the bulla-spread phenomenon (Asboe-Hansen sign).

Lesions of PV generally heal with crusts followed by re-epithelialisation. There is no scarring, although postinflammatory hyperpigmentation may persist for months in patients with Fitzpatrick skin types IV and V. Mild forms of the disease may regress spontaneously. Most patients with pemphigus vulgaris eventually enter a phase of complete remission in which they can be maintained lesion-free with minimum doses of corticosteroids (i.e. prednisolone <10 mg) or without therapy. As medications are tapered, flares in disease activity with development of new lesions and itching are not uncommon.

Pemphigus vegetans is a subtype of PV in which lesions accumulate in body folds (axillae, submammary and groin) and around orifices (lips, anus). The lesions consist of pustules (Hallopeau type) (Fig. 8.5a) or papillomas (Neumann type) (Fig. 8.5b) or a combination of both (Fig. 8.5c). The affected skin is easily secondarily infected, which explains the foul smelling. As mentioned before, vegetating plaques are common in PV (Fig. 8.6), but the presence of sterile pustules or papillomas in the forementioned regions make it pemphigus vegetans.

General Diagnostics

The initial histopathological finding in pemphigus is intercellular widening between keratinocytes in the epidermis, accompanied by invasion of

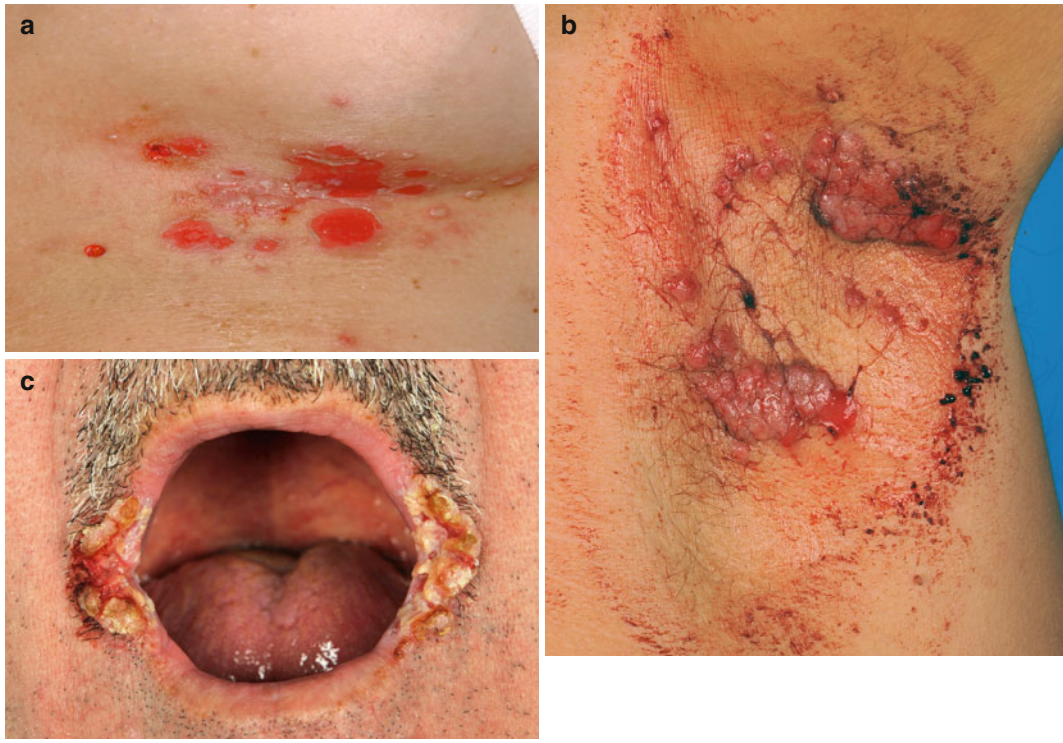

Fig. 8.5 Pemphigus vegetans. (**a**) Hallopeau type with pustules in the body folds. (**b**) Neumann type with papillomas in the axilla. (**c**) Combined Hallopeau and Neumann type with pustules and papilloma in the corners of the mouth (angular stomatitis)

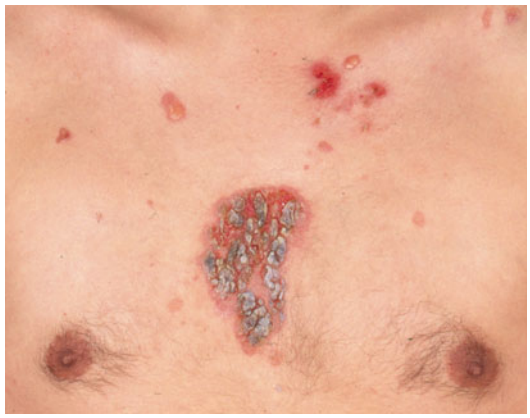

Fig. 8.6 Pemphigus vulgaris: a common vegetating plaque

eosinophilic granulocytes (*eosinophilic spongiosis*). Characteristic for PV is an intraepidermal blister usually just above the basal layer due to loss of cell-cell contact (*suprabasilar acantholysis*) (Fig. 8.7). A few rounded-up acantholytic keratinocytes (*acanthocytes*) as well as clusters of detached epidermal cells float in the blister cavity. The basal cells loose lateral desmosomal contact with adjacent keratinocytes, but remain attached to the basement membrane via hemidesmosomes, thus giving the appearance of a row of tombstones. The acantholytic process may also involve the hair follicles.

<Pemphigus is microscopically characterised by acantholysis>

Specific Diagnostics

Immunological Tests

All forms of pemphigus are associated with the presence of skin-bound and circulating antibodies against epithelial cell surface antigens.

Direct Immunofluorescence

Tissue-bound intercellular antibodies are present in lesions and adjacent healthy skin in virtually all patients with pemphigus as detected by direct immunofluorescence microscopy (IF). They are

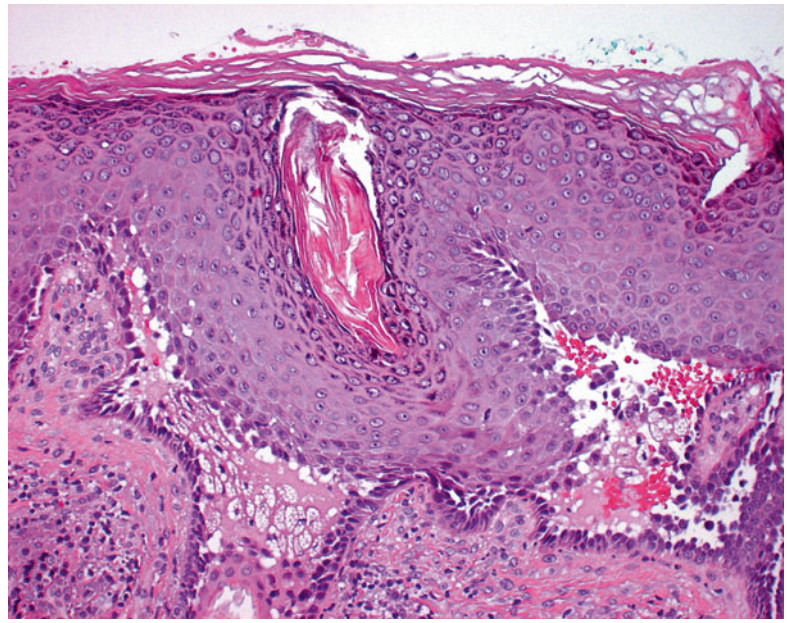

Fig. 8.7 Histopathology of pemphigus vulgaris. Suprabasal acantholysis with basal cells lining the blister floor like 'a row of tombstones' (H&E)

usually IgG, but IgM and IgA with or without complement may also be deposited. See Chap. 4 for more on direct IF in pemphigus.

Indirect Immunofluorescence

Circulating epithelial cell surface (ECS) antibodies in the serum are detectable in up to 89 % of patients by ELISA and/or indirect IF. There is a correlation between the titre of desmoglein 1 antibodies and skin activity of the disease. Serum monitoring of antibody titres may be useful in guiding therapy, since a rise in their titre usually precedes a recurrence in disease activity, while they usually decrease with successful treatment and disappear in patients in remission.

ELISA

Enzyme-linked immunosorbent assays (ELISA) are available to detect antibodies directed against Dsg1 and Dsg3 (see Chap. 6). The presence of antibodies directed against Dsg3 is associated with mucosal PV, whereas antibodies directed against Dsg1 are associated with PF. Both types of antibodies are present in mucocutaneous PV. ELISA kits are available with the ectodomain of desmoglein produced in mammalian cells (MBL, Nagoya, Japan and EUROIMMUN, Luebeck, Germany). The latter has the advantage of containing the mature protein only and not the propeptide as well. It is thought that pathogenic antibodies are directed against conformational epitopes only, and these epitopes are present in the mature desmogleins, while nonpathogenic antibodies recognise both mature and propeptide isoforms, correlating with binding of nonconformational epitopes.

Case Study: Part 2

Histopathology of lesional skin sampled from the edge of a blister shows suprabasal acantholysis. Direct immunofluorescence staining of lesional peribullous skin and of healthy skin showed ECS deposition of IgG and C3c. Indirect immunofluorescence on monkey oesophagus is positive for IgG ECS antibodies. ELISA demonstrated a Dsg1 index of 138 and a Dsg3 index of 100. A diagnosis was made of mucocutaneous pemphigus.

Treatment Tricks

Initial Treatment and Escalator

Systemic Corticosteroids

The treatment of pemphigus was symptomatic until the introduction of corticosteroids in the

1950s. The majority of patients in the presteroid era usually died from overwhelming sepsis within 1 year after disease onset. Systemic corticosteroids are still the mainstay of therapy for this disease. Their use has transformed an almost invariably fatal disease into one whose mortality is less than 6 %. Side effects of systemic steroids are numerous and may include infection, diabetes, osteoporosis, myopathy, gastrointestinal bleeding, cataracts or central nervous system toxicity.

<Systemic corticosteroids are the mainstay of therapy for pemphigus>

Immunosuppressive Agents

Immunosuppressive agents are commonly used in combination with systemic corticosteroids in order to increase efficacy and may have a steroid-sparing effect, thereby allowing reduced maintenance doses and less side effects of systemic corticosteroids. The most commonly used adjuvants are azathioprine (2–3 mg/kg), mycophenolate mofetil (2000 mg/day), mycophenolic acid (1440 mg/day), cyclophosphamide (≤2 mg/kg), methotrexate (10–15 mg/week) and dapsone.

High-Dose Human Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) neutralises autoantibodies by several mechanisms including anti-idiotypic antibodies, interference with the cytokine network, modulation of B- and T-cell functions, inhibition of complement and cytokine production and blocking activation and upregulation of inhibitory Fc receptors. A major advantage if IVIG is compared with other treatment options is its excellent safety profile. Adverse events are generally mild, and reported side effects include headache, fever, chills, myalgia, flushing, hypotension, tachycardia and gastrointestinal symptoms. The standard dose is 2 g/kg/month in 2–4 gifts. The costs of IVIG medication are as high as $10.000/month. Low dose IVIG (0.2 mg/kg/month) may be effective in selected cases [8].

Plasmapheresis and Immunoadsorption

Rapid removal of circulating autoantibodies can be achieved by plasmapheresis (exchanging plasma by fresh-frozen plasma or human albumin) or by immunoadsorption (only removing immunoglobulin). In the past years immunoadsorption replaced plasmapheresis in the treatment of pemphigus. Immunoadsorption allows the processing of the two- to threefold plasma volume per treatment session and is associated with a lower rate of adverse events like infections and allergic reactions [9].

Rituximab

Rituximab is a chimeric murine-human monoclonal anti-CD20 antibody, originally developed for the treatment of B-cell malignancies. CD20 is an antigen expressed on the surface of pre-B and mature B cells. Rituximab binds to transmembrane CD20, reduces circulating B cells and prevents their maturation into all antibody-secreting plasma cells, not just those making pathogenic antibodies. It may become first line treatment in pemphigus with a chance of complete cure. The consensus dosage of rituximab in pemphigus is a cycle of 2×1000 mg with a 2-week interval. The cycle is repeated after 6 and 12 months. To prevent infusion reactions the protocol lists premedication with prednisolone 25 mg i.v., clemastine 2 mg i.v. and paracetamol 1000 mg p.o.

Follow-Up and Tapering

Treatment should be started with predniso(lo)ne in a dosage of 1.0–1.5 mg/kg/day in combination with azathioprine. Taper predniso(lo)ne by 25 % reduction in biweekly steps (at <20 mg more slowly!). A rule of the thumb quick tapering schedule is 80–60–40–30–25–20–15–12.5–10–7.5–5–2.5–0 mg in steps of 2 weeks. Raise dose by two steps when new lesions occur, or continue dose if tapering is not possible. Consider immunosuppressive adjuvants such as

azathioprine, mycophenolate mofetil, mycophenolic acid, cyclophosphamide or methotrexate for steroid sparing. One should be aware of the side effects of use of systemic steroids such as infection, diabetes, osteoporosis, myopathy, gastrointestinal bleeding, cataracts or central nervous system toxicity. When patients are treated with rituximab and use two or more immunosuppressive agents besides this, then treatment to prevent *Pneumocystis jiroveci* pneumonia and herpes pneumonia should be started with co-trimoxazole 480 mg/day and valacyclovir 500 mg/day during the 3 months following the rituximab infusion.

Case Study: Part 3

Treatment was started with prednisolone 1 mg/kg and azathioprine 50 mg and raised in 2 weeks to 3 mg/kg. The dosage of prednisolone was reduced with 5 mg/week until 35 mg/day. The erosions of the oral mucosa healed, but the erosions of the skin continued to develop. As second-line treatment, the patient was given rituximab. The dosage of prednisolone was then further reduced with 5 mg/week and from 15 mg, with 2.5 mg/week until 0. A few weeks later skin lesions healed and Dsg1 indices dropped to normal. Monotherapy with azathioprine 100 mg/day was continued. After 1.5 years the patient was still in complete remission, and the treatment with azathioprine was stopped.

Review Questions

1. What is the most common location of mucocutaneous pemphigus vulgaris?
   (a) Temples
   (b) Feet
   (c) Genitals
2. The most important risk factor for pemphigus is
   (a) Hair colour
   (b) Country of birth
   (c) Profession
3. Patients with mucosal dominant PV have antibodies directed against
   (a) Desmoglein 1
   (b) Desmoglein 3
   (c) Desmogleins 1 and 3
4. First-line treatment of pemphigus is
   (a) Superpotent topical corticosteroids
   (b) Systemic corticosteroids
   (c) Azathioprine
   (d) Rituximab

Answers

1. (a)
2. (b)
3. (b)
4. (b)

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Pemphigus Foliaceus

9

Laura de Sena Nogueira Maehara
and Marcel F. Jonkman

Abstract

Pemphigus foliaceus (PF) is a group of autoimmune skin blistering diseases comprising four subtypes: Cazenave, endemic, erythematosus, and herpetiformis. The common characteristic is the frequent presence of pathogenic anti-desmoglein 1 antibodies leading to bullae in the skin. Other characteristics – clinical, histological, pathogenesis, and therapy – vary and are specified in this chapter.

Pemphigus foliaceus Cazenave first described by Cazenave is the classical form of PF. The elemental dermatological lesions are erythematous plaques with pastry puff squames predominantly affecting seborrheic areas. The histopathological hallmark is acantholysis in the upper part of the epidermis (subcorneal or intraspinous). The disease is caused by autoantibodies to desmoglein 1 on the epithelial cell surface. Therapy is based on systemic corticosteroids and immunosuppressive adjuvants. Rituximab and other anti-CD20 biologics targeting B lymphocytes have changed the prognosis of the disease from chronic relapsing to long-term remissions.

Endemic pemphigus foliaceus is a variant of PF that is endemic under peasants in rural Brazil, Indian tribes and gold miners in Colombia, and under women in Tunisia. The histopathology, immunology, and therapy are similar to PF Cazenave.

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Keywords
Desmosome • Immunoglobulin • Autoimmune disease • Vesiculobullous disease • Pemphigus

Introduction and Aims

Short Definition in Layman Terms

Pemphigus foliaceus is an autoimmune bullous disease that affects the skin in middle-aged patients. Pemphigus foliaceus causes red spots with crusts and scale on the skin (Fig. 9.1). Foliaceus means "squamous-like fallen leaves." The spots commonly start on the scalp and may spread over the entire body (Fig. 9.2). Scars do not develop. The disease cannot spread to other people (it is not contagious). The disease is caused by autoantibodies directed to the adhesion molecule desmoglein 1 on the surface of skin cells. Treatment involves finding ways to "calm down" the body's immune system. Prednisone, an oral form of steroid, is usually the first treatment used. Other drugs that take over the effects of steroids on the immune system are sometimes used to allow earlier discontinuation of prednisone treatment. Newer agents are CD20 antibodies such as rituximab, which may provide months of disease relief and reduce the need for prednisone.

Learning Objectives
After reading this chapter you should be able to recognize PF, understand the pathogenesis, choose complementary diagnostic procedures, and suggest a plan for therapy.

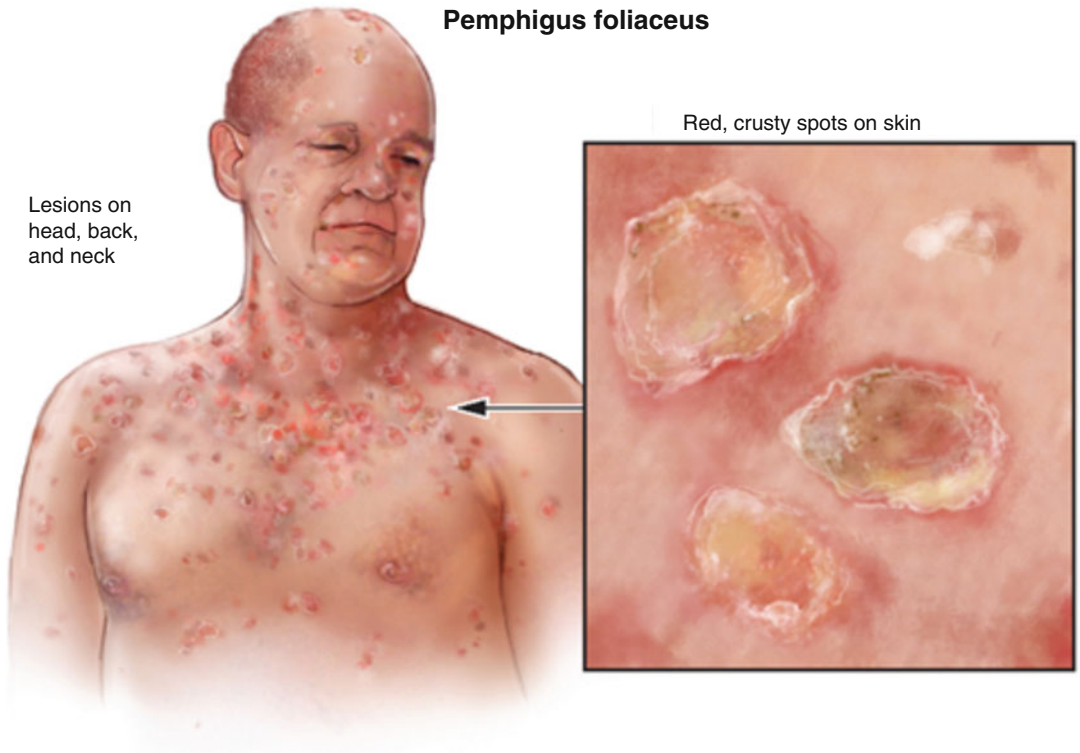

Fig. 9.1 Pemphigus foliaceus with typical distribution of crusted scaly erythematous plaques (Copyright © 2014 American Medical Association. All rights reserved [1])

Case Study: Part 1
A 42-year-old man presented with crusted plaques and erosions since one month on his face, trunk, arms, and legs. He thinks they start as bubbles that become wounds. The lesions are painful and itch slightly. After being questioned, he recalls having desquamation of the scalp for 1 year.

Didactical Questions: Cross Section of Questions to Prime the Reader's Interest

What is the differential diagnosis? Which are the diagnostic tests that should be performed today? Would you prescribe any therapy? What other questions should be asked before prescription?

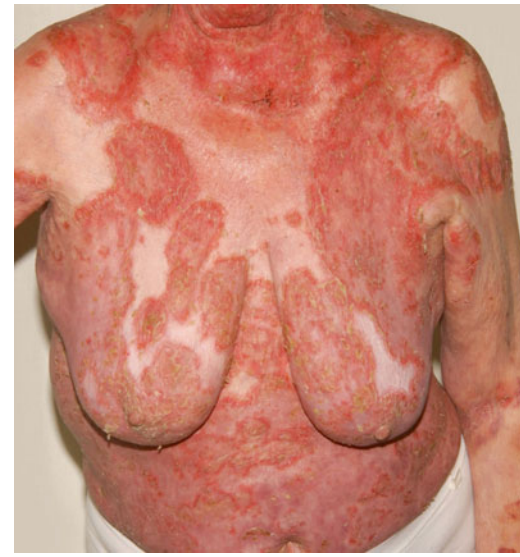

Fig. 9.2 Pemphigus foliaceus: circinate crusted scales and erosions on polycyclic erythema on the trunk

Facts and Figures

Definitions and Classification

Pemphigus foliaceus Cazenave is the classical form of PF that is defined by acantholysis in the upper epidermis that leads to crusted, scaling plaques (Fig. 9.3) and erosions and contrariwise to pemphigus vulgaris has no mucosal involvement. The disease is caused by autoantibodies against desmoglein 1 on the epithelial cell surface.

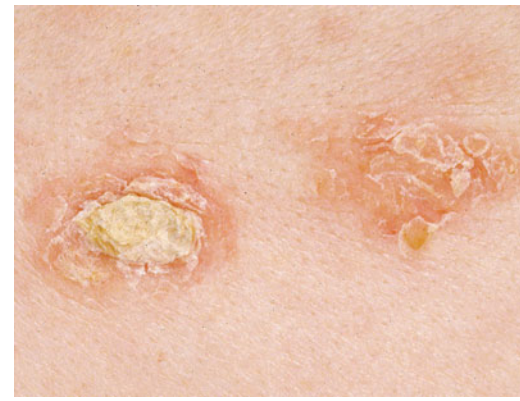

Fig. 9.3 Pemphigus foliaceus: crusted scales resembling pastry puff or in French *milles-feuilles*

Epidemiology

The age of onset of PF is in the fifth decade. Men and women are equally affected. PF comprises about 17 % of all cases with pemphigus. The incidence of PF in non-endemic areas is approximately 0.04 new cases per 100,000 per year.

Pathogenesis

Pathogenic desmoglein 1 (Dsg1) autoantibodies bind the cell membrane and induce loss of cell-cell contact (*acantholysis*) [2]. The compensation theory states that a subcorneal cleft is formed due to the absence of compensatory desmoglein 3 (Dsg3) in the upper part of the epidermis. Likewise, mucosal lesions are not present in PF because of the presence of Dsg3 in the entire mucosal epithelium, which compensates for the loss of Dsg1.

Diagnosis Paths

History and Physical Examination

The patient is in good general condition and may complain of itch or pain that may be localized on

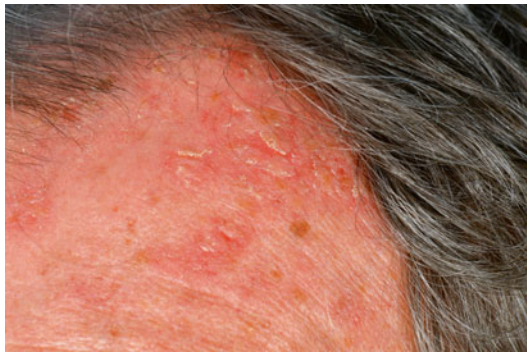

Fig. 9.4 Pemphigus foliaceus: erosions and crusted scales on the forehead mimicking actinic keratosis

the scalp or temples for years. Scaly erythema in the scalp may be misdiagnosed for seborrheic dermatitis or actinic keratosis (Fig. 9.4). A clue to diagnosis is a positive Nikolsky's sign II. Typical is the crusted plaque with multiple layers of scaling (Fig. 9.3), which easily erodes. The diseases may spread over the trunk and extremities. Predilection sites are the scalp, temples, periorbicular area, neck, upper chest, and back. The oral cavity is not affected. The extent of the disease may reach erythroderma in severe cases.

General Diagnostics

After the suspicion of pemphigus, hematological, oncologic, endocrine, cardiovascular, and infectious medical history are needed to screen for risk factors of oral corticosteroid treatment and evolving complications of immunosuppressive therapy. Any medication in use should be listed, in order to detect possible triggers (see Chap. 12, Drug-Induced Pemphigus) and future interactions of drugs.

Specific Diagnostics

The diagnosis of PF is established by histopathological examination of a skin biopsy demonstrating a subcorneal split. In the lower epidermis epidermal cell-cell widening not commencing to acantholysis is visible. Direct immunofluorescence of peribullous lesional skin demonstrates patient's IgG in an epithelial cell surface (ECS) pattern. Likewise, patient's serum may contain circulating IgG capable of binding monkey esophagus in an ECS pattern. Autoantibodies specific to Dsg1 are demonstrable by ELISA.

Case Study: Part 2
The patient was healthy and was not receiving any medication. Two skin biopsies were taken (1) from the edge of a bulla, not being erosive, for histopathology and (2) from peribullous erythematous skin for direct immunofluorescence microscopy. Evidence was found for a subcorneal blister and for ECS IgG depositions in a coarse granular pattern, respectively. Indirect immunofluorescence microscopy on monkey esophagus showed ECS IgG in a smooth pattern. The ELISA index for antibodies to Dsg1 was >150 and to Dsg3 0. A diagnosis of PF was made.

Treatment Tricks

Initial Treatment and Escalator

Systemic corticosteroid is the first line of therapy of PF. It works within days directly on the skin and therefore not only on the immune system taking into account that circulating IgG has a half-life of 20 days. Since PF is less painful and severe than PV, a dose of prednisolone is recommended as low as 0.5 mg/kg. Escalation in refractory cases is possible up to 1.5 mg/kg.

Follow-Up and Tapering

Since the skin lesions in PF clear more slowly than in PV, one can expect long-term corticosteroid therapy. Tapering of corticosteroids should be started when no new lesions had developed for a minimum of two weeks, and approximately 80 % of lesions had healed, that is, at the end of consolidation phase with 25 % of the dose, until 20 mg/

day, when tapering should be performed more slowly. In responsive cases the tapering schedule is 40-35-30-25-20-15-12.5-10-7.5-5-2.5-0 mg in steps of 2 weeks. Take two steps back if new lesions develop. Start again if disease is not controlled. Tapering on alternating days to avoid repression of the adrenal-hypothalamic axis is not recommended, since the skin will flare up on reduced days.

First-line adjuvant therapy for corticosteroid sparing is azathioprine 2–3 mg/kg, mycophenolate mofetil 2000 mg, and mycophenolic acid 1440 mg. Alternatives are dapsone <150 mg, cyclophosphamide 2 mg/kg, and methotrexate <25 mg/week.

Anti-CD20 monoclonal antibody such as rituximab 2x1000 mg intravenously is officially the second-line therapy, but may replace first-line adjuvants. Repeat the infusions at 6 and 12 months. If more than two immunosuppressive agents are combined (i.e., prednisolone, rituximab, and azathioprine), then prophylaxis against fungal, bacterial, and viral infections is recommended. In patients with refractory disease, intravenous immunoglobulin 2 g/kg/month in four gifts might be considered.

Case Study: Part 3

Therapy was started with prednisolone 0.5 mg/kg. Two weeks later, the patient had improvement of 50 % of the lesions and did not present new lesions. After two weeks, tapering was possible. In addition azathioprine was given at a dose of 3 mg/kg. After 16 weeks while reaching a dose of 10 mg prednisolone, no improvement was seen. The prednisolone dose was raised to 15 mg (two steps back). No further tapering was possible. Since the patient was dependent on more than minimal (>10 mg) corticosteroid therapy, it was decided to start rituximab 2 x 1000 mg. Because azathioprine was continued, prophylaxis against opportunistic infections was added. Four months later the patient reached complete remission, while prednisolone had been tapered to zero. Azathioprine was continued at minimal dose of 1 mg/kg for 1 year after stopping prednisolone.

Endemic Pemphigus

Introduction and Aims

Endemic pemphigus foliaceus, also referred as *fogo selvagem* ("wildfire"), is a subtype of PF first mentioned as *pemphigus brasiliensis* in the medical lexicon in 1763 by François Boissier de la Croix de Sauvages. In endemic PF the same pathogenic antibodies to Dsg1 were demonstrated as in PF Cazenave. *Fogo selvagem* patients are young rural workers, children, or relatives living in endemic areas in Brazil. In Colombia, two groups of endemic patients have been described: (1) Indian tribes in the southern areas of the Amazonian and Orinoquian forest regions and (2) endemic PF in gold-mining regions of El Bagre [3]. Patients with the new variant present not only antibodies to Dsg1 but also to other adhesion molecules. In Tunisia, endemic areas are also rural, and patients are mostly women. The use of traditional cosmetics was suggested as a risk factor [4]. Recently, a small focus of endemic PF was noticed in Tanzania [5].

Facts and Figures

Endemic PF in Brazil was reported in deforested rural areas, close to rivers, affecting workers and their relatives, including young children. After blackfly bites were shown to increase the risk [6], many studies have been performed to link the fly and other hematophagous insects to the disease. The theory is that insect's saliva, through molecular mimicry, triggers the synthesis of IgE, IgM and IgG1 to Dsg1, with later class switch to pathogenic IgG4. Nonpathogenic IgG1 binds extracellular domain 5 (EC5) of Dsg1, whereas pathogenic IgG4 binds EC1 and EC2 of Dsg1. The risk factor for the disease would be some specific HLA alleles (DRB1*0404, DRB1*1402, and DRB1*1406). Although endemic PF is common in Brazil, a recent report showed that pemphigus vulgaris, and not endemic PF, is more frequent in one endemic area in São Paulo [7].

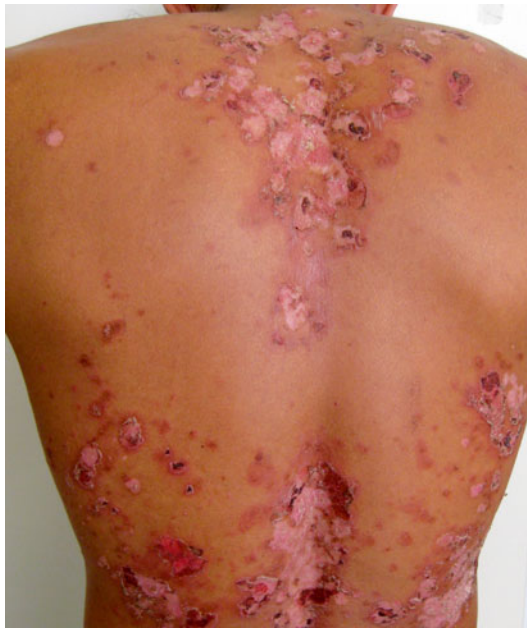

Fig. 9.5 *Fogo selvagem*: erosions and circinate crusted scales symmetrical on the back in young male

Diagnosis Paths

Endemic PF patients present with crusted plaques similar to PF (Fig. 9.5). Clinical presentations include localized form (forme fruste) and generalized forms (bullous invasion; keratotic; hyperpigmented; pemphigus herpetiformis; and exfoliative erythroderma). For histological and immunological exams, refer to PF.

Treatment Tricks

Patients diagnosed with endemic PF are treated similarly to those with PF.

Pemphigus Herpetiformis

Introduction

The diagnostic criteria of pemphigus herpetiformis (PH) were first reported by Jabłońska *et al.* in 1975 [8]. Before the use of immunofluorescence, the clinical presentation was named dermatitis herpetiformis with acantholysis. The skin disease is remarkable itchy, which is uncommon

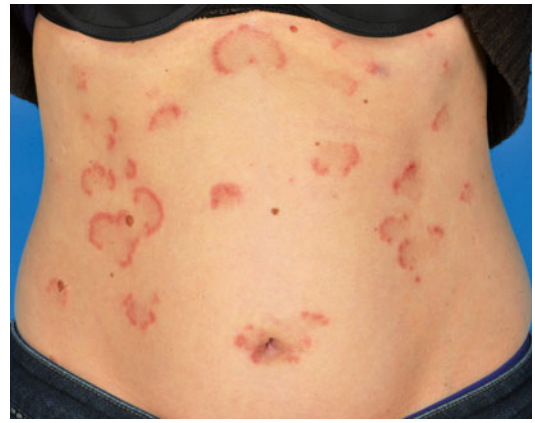

Fig. 9.6 Pemphigus herpetiformis: arciform erythematous papules and beginning vesiculation on the trunk

for pemphigus. Moreover, Nikolsky's sign is negative.

Facts and Figures

Pemphigus herpetiformis (PH) is a variant of pemphigus with arciform (Fig. 9.6) and annular lesions and severe itch that resembles clinically dermatitis herpetiformis; however, all immunological findings fit with pemphigus. The main autoantigen is desmoglein 1, and for that reason PH is called a variant of pemphigus foliaceus. In a minority of the cases, one may find autoantibodies against desmoglein 3 and also find suprabasilar acantholysis agreeing with a variant of pemphigus vulgaris. PH can be the initial presentation and later evolve to classic non-endemic pemphigus foliaceus, *fogo selvagem*, and pemphigus vulgaris [9].

<Pemphigus herpetiformis (PH) is variant of pemphigus with annular lesions and itch that resembles clinically dermatitis herpetiformis>

Case Study: Part 1

A 70-year-old female was hospitalized because of a very itchy dermatosis. Dermatological examination showed erythematous macules that became confluent to large symmetrical areas. Many crusts and some vesicles were present. Nikolsky's sign was negative.

Diagnosis Paths

The diagnosis is based on the criteria listed in table. 9.1.

The differential diagnosis of PH includes dermatitis herpetiformis, pemphigus foliaceus, pemphigus vulgaris, bullous pemphigoid, IgA pemphigus, and linear IgA disease.

Treatment Tricks

PH is considered to be less life threatening than other types of pemphigus. It usually responds

Table. 9.1 Suggested diagnostic criteria for pemphigus herpetiformis

Characteristic appearances	Mandatory
Grouped (herpetiform) distribution of itching erythematous vesicular/bullous/papular lesions, often in an annular-shaped pattern	*Yes*
Eosinophilic/neutrophilic spongiosis/intraepidermal pustules with or without acantholysis	No
Skin-bound epithelial cell surface IgG and/or C3	*Yes*
Circulating epithelial cell surface IgG[a]	No
Detection of circulating IgG autoantibodies against desmoglein 1 and/or 3 and desmocollin 1 and/or 3[a]	No

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[a]At least one of the two criteria (positive indirect immunofluorescence microscopy or detection of specific autoantibodies) should be fulfilled if direct immunofluorescence microscopy is not available

Case Study: Part 2

Histopathology showed intraepidermal pustules with neutrophilic granulocytes and some acanthocytes (Fig. 9.7). Direct immunofluorescence detected tissue-bound epithelial cell surface IgG and IgA in rough desmo-pattern in the lower 2/3 of the epidermis. Indirect immunofluorescence on monkey esophagus was positive for circulating epithelial cell surface IgG and IgA. ELISA revealed IgG and IgA antibodies against desmoglein 1. A diagnosis was made of pemphigus herpetiformis. Additional workup revealed the presence of heart failure and normal iron anemia.

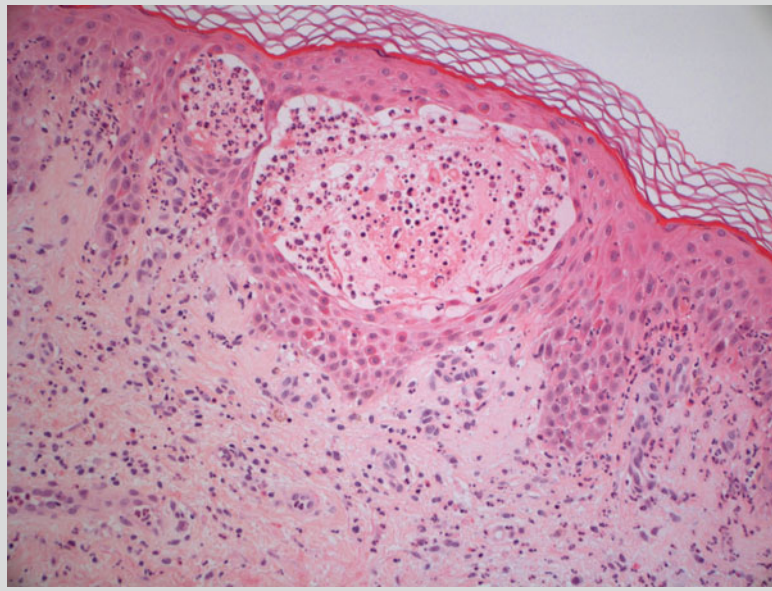

Fig. 9.7 Histopathology of pemphigus herpetiformis. Two intraepidermal pustules filled with neutrophilic granulocytes

well to monotherapy with dapsone, which is considered the drug of first choice.

Case Study: Part 3
Because of anemia and heart failure, dapsone was relatively contraindicated. Treatment consisted of minocycline 200 mg and topical whole-body ultrapotent corticosteroid. Itch was treated by hydroxyzine 10 mg bd. Captopril was changed to losartan, since it might induce pemphigus. The skin lesions resolved, and the patient was discharged from hospitalization after 4 weeks. Complete remission was maintained while on minocycline 100 mg bd and topical lesional corticosteroids. Prednisolone could be avoided.

Pemphigus Erythematosus

Introduction

Pemphigus erythematosus (PE) was first described in 1926 by Senear and Usher [11] as a condition with a lupus-like butterfly rash or severe seborrheic dermatitis, which they suggested was a combination of pemphigus vulgaris and lupus erythematosus (LE). When insights into the differences between pemphigus vulgaris and PF crystallized, PE was not classified with pemphigus vulgaris but considered an early form of PF. When immunofluorescence became a diagnostic tool, the association with LE revived. Chorzelski *et al.* [12] described a "lupus-band" deposition in sun-exposed skin areas of patients with PE, together with antinuclear antibodies (ANAs) as in LE. Later it came clear that the gross findings in patients with PE do not meet the criteria for systemic LE as published by the American College of Rheumatology.

<Pemphigus erythematosus is not related to lupus erythematosus>

Facts and Figures

The nature of the "lupus-band phenomenon" in PE was disclosed by Oktarina *et al.* [13]. The granular BMZ depositions located below the lamina densa consist of IgG, complement, and the shed desmoglein 1 ectodomain. It was hypothesized that shedding of the Dsg1 ectodomain was the result of UV-induced apoptosis. Patients with PE are often erroneously treated by UV phototherapy for a presumed psoriasis [13].

<Pemphigus erythematosus is a localized form of pemphigus foliaceus often elicited by UV exposure>

Diagnosis Paths

The diagnosis is based on the criteria listed in Table. 9.2. The differential diagnosis of PE includes pemphigus foliaceus, acute cutaneous lupus erythematosus, and psoriasis.

<Immunofluorescence of pemphigus erythematosus shows a pseudolupus band that consists of IgG, complement, and desmoglein 1 ectodomain>

Case Study: Part 1 [13]

An 80-year-old woman was admitted to our hospital with a 3-year history of generalized progressive erythemato-squamous skin lesions with pustules and flaccid blisters. This had been diagnosed elsewhere as psoriasis pustulosa complicated by secondary infection with *Staphylococcus aureus*. The patient had received several therapies including methotrexate, systemic erythromycin, acitretin, and ciclosporine. Due to methotrexate-related hepatotoxicity and insufficient effectivity of the other therapies, the patient switched over to a twice-weekly regimen of psoralen-UVA (PUVA) therapy with 40 mg methoxsalen. During PUVA therapy, the skin lesions worsened, and therapy was stopped after 3 weeks. Physical examination revealed suberythroderma, consisting of confluent and scattered red macules with scales and purulent crusts. In the face a malar distribution was present (Fig. 9.8). Multiple erosions and flaccid blisters were seen and Nikolsky's sign was positive. The mucous membranes were not involved.

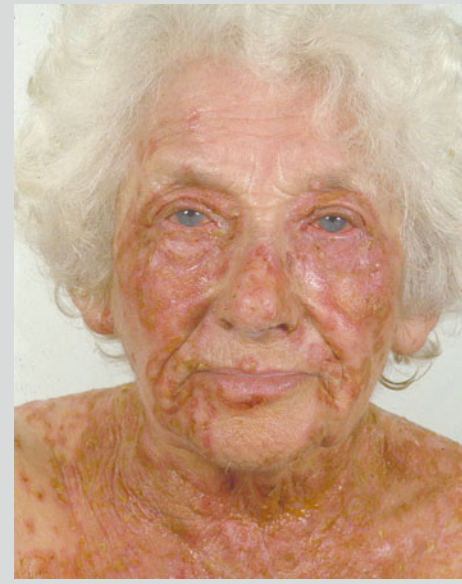

Fig. 9.8 Pemphigus erythematosus: typical facial butterfly eruption (Copyright © 2012 American Medical Association. All rights reserved)

Table. 9.2 Suggested diagnostic criteria for pemphigus erythematosus

Characteristic appearances	Mandatory
Malar erythemato-squamous plaques and vesicles in a "butterfly" pattern	*Yes*
Recent UV exposure	No
Subcorneal blister	No
DIF: IgG and/or C3 depositions at epithelial cell surface	*Yes*
DIF: granular IgG and C3 depositions at epithelial basement membrane zone	*Yes*
IIF: Circulating epithelial cell surface IgG	No
ELISA: detection of circulating IgG autoantibodies against desmoglein 1	No
Absence of raised ANA titer	*Yes*

Treatment Tricks

The treatment of PE is similar to that of PF. Protection to UV light should be advised.

Review Questions

1. Which localization of lesions is more likely to be present in a pemphigus foliaceus patient?
   (a) Abdomen
   (b) Feet
   (c) Upper trunk
2. The most prevalent area for endemic pemphigus is
   (a) Rural
   (b) Urban
   (c) None

Case Study: Part 2

Histopathology revealed subcorneal blisters. Direct immunofluorescence microscopy showed intraepidermal epithelial cell surface depositions of IgG and C3c and in addition coarse granular depositions of IgG and C3c that colocated to the shed desmoglein 1 ectodomain in the lower epidermal basement membrane zone (Fig. 9.9). Indirect immunofluorescence on monkey esophagus showed ECS IgG antibodies with a titer of >1:320, and retrospective ELISA analysis demonstrated anti-Dsg1 antibodies. Blood tests were negative for antinuclear, anti-ENA, anti-dsDNA, anti-SSA, anti-smooth muscle, and anti-striated muscle antibodies. A diagnosis of pemphigus erythematosus was made.

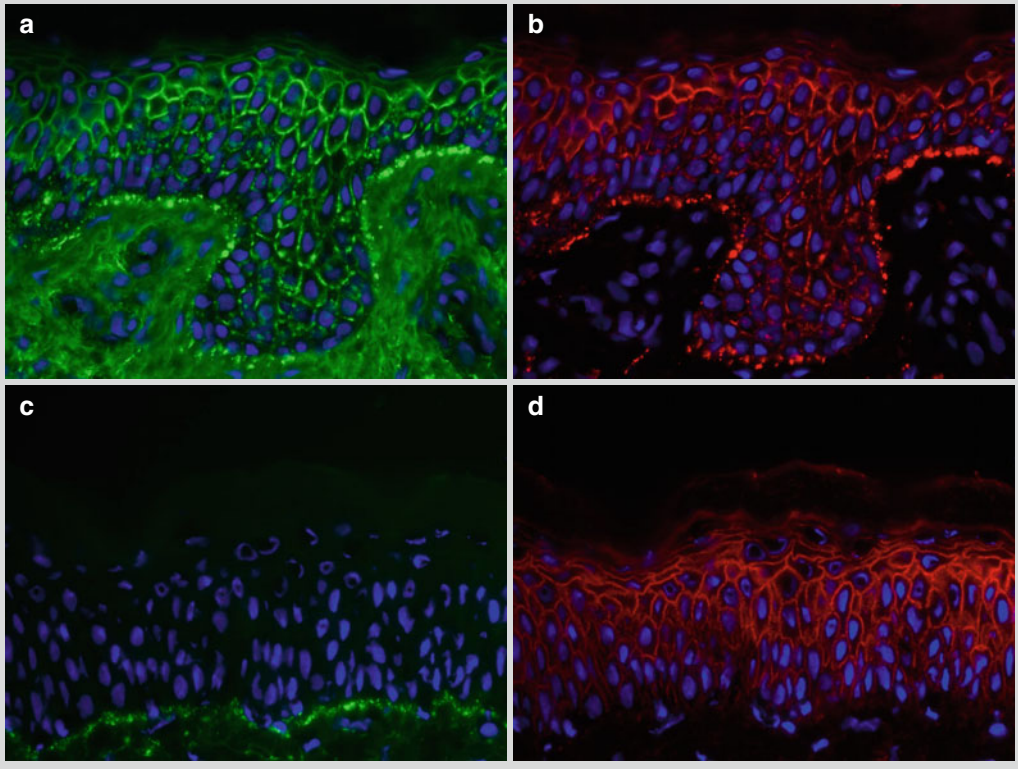

Fig. 9.9 *Direct i*mmunofluorescence of pemphigus erythematosus (PE) reveals granular depositions of IgG (*green* in **a**) along the epidermal basement membrane zone (EBMZ) that co-localize with the Dsg1 ectodomain (*red* in **b**). In the skin from a patient with systemic lupus erythematosus (SLE), a lupus band is detected of granular IgG along the EBMZ (*green* in **c**), without Dsg1 ectodomain precipitations (*red* in **d**). Note that the epithelial cell surface shows clusters of IgG and Dsg1 in PE (**a**, **b**), whereas negative IgG and smooth Dsg1 along the epithelial cell surface in SLE (**c**, **d**)

Case Study: Part 3

The patient received multidisciplinary care. Geriatric doctor was consulted for drug therapy advice because of previous hepatotoxicity. Specialized nurses provided dressings for painful erosions. The patient was kept in a dark room. Therapy was started with prednisolone 0.5 mg/kg (20 mg). Three weeks later, the patient had improvement of 80 % of the lesions and did not present new lesions since 2 weeks, so tapering was possible. Adjuvant therapy was not necessary, and after 12 weeks the patient was in complete remission on minimal therapy (5 mg prednisolone). Six months later, the patient was in complete remission off therapy. In 2 years of follow-up, the lady was in partial remission off therapy and died 5 years later for other medical reasons.

3. First-line treatment of pemphigus foliaceus is
   (a) Dapsone
   (b) Cyclophosphamide
   (c) Systemic corticosteroids

Answers

1. (c)
2. (a)
3. (c)

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Further Reading

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On the Web

International Pemphigus & Pemphigoid Foundation http://www.pemphigus.org/.

JAMA Dermatology Patient Page, Pemphigus http://archderm.jamanetwork.com/article.aspx?articleid=1879985.

Paraneoplastic Pemphigus

10

Angelique M. Poot, Gilles F.H. Diercks, Hendri H. Pas, and Marcel F. Jonkman

Abstract

Paraneoplastic pemphigus is a rare but severe autoimmune disease characterized by severe stomatitis and a variety of cutaneous manifestations in association with an underlying neoplasia. Pulmonary involvement may also occur. The pathogenesis involves the production of autoantibodies against desmogleins, plakins, and the protease inhibitor alpha-2-macroglobulin-like 1, but T-cell-mediated autoimmunity is also thought to play a role. Diagnosis usually relies on the demonstration of a specific subset of circulating autoantibodies in patient serum, although in a small subset of patients, these autoantibodies might be absent. Due to its rarity, there are no set of guidelines for treatment. The general approach includes a variety of immunosuppressive agents and treatment of the underlying neoplasia. Despite treatment, paraneoplastic pemphigus has high mortality rates, often due to sepsis, respiratory failure, or progression of the underlying malignancy.

Keywords

Autoimmune disease • Pemphigus • Paraneoplastic • Plakins • Alpha-2-macroglobulin-like 1 • Stomatitis • Neoplasia • Paraneoplastic autoimmune multiorgan syndrome

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Introduction and Aims

Short Definition in Layman's Terms

Paraneoplastic pemphigus (PNP) is an autoimmune disease, with severe blistering of the lips and oral mucosa, and occurs in the presence of an underlying neoplasm.

Learning Objectives
After reading this chapter you will:

1. Be able to recognize the spectrum of clinical manifestations of paraneoplastic pemphigus
2. Know which neoplasms are most often associated with paraneoplastic pemphigus
3. Know the tools and pitfalls in the diagnostic approach of paraneoplastic pemphigus

Case Study: Part 1
A 69-year-old female with hemorrhagic crusts covering her lips and painful erosions on the buccal mucosa presented at the emergency department. Erythematous macules and erosions were seen on her trunk and extremities. In addition, bullae were present on palms and soles. The patient mentioned having lost 10 kg in the last 6 months.

Didactical Questions

The manifestations of paraneoplastic pemphigus may be clinically indistinguishable from those of other blistering diseases.

How can we differentiate between paraneoplastic pemphigus and other clinically similar diseases? And why is this differentiation important?

Facts and Figures

Definitions and Classification

PNP is characterized by a painful oral stomatitis, a variety of skin manifestations, and a complex autoimmune response. It occurs in the presence of an underlying neoplasm, of which it may be the first sign in 10–30 % of cases. PNP is sometimes be referred to as paraneoplastic autoimmune multiorgan syndrome (PAMS), because next to the mucous membranes and the skin, other organs such as the lungs may be affected and because the histological hallmark for pemphigus, i.e., intraepidermal acantholysis, is not always present in PNP [1, 2].

<The clinical hallmark of PNP is a painful stomatitis>

Epidemiology

To date, around 500 PNP cases have been described worldwide, since 1990. It comprises 3–5 % of all pemphigus cases. The underlying neoplasm is most often lymphoproliferative in nature, such as non-Hodgkin's lymphoma, thymomas, and leukemia. Sarcomas and other solid malignancies may also be found. In addition, benign lymphoproliferative diseases may be underlying, such as Castleman's disease, which is most prevalent in young adults and children with PNP [1].

<The underlying neoplasm in PNP is most often lymphoproliferative in nature>

Pathogenesis

The autoantibody response in PNP is directed against multiple antigens found in the skin and mucosa, including the proteins of the plakin family (such as envoplakin, periplakin, desmoplakin, and BP230), the protease inhibitor alpha-2-macroglobulin-like 1 protein (A2ML1), and the desmosomal cadherin desmoglein 3 and less often desmoglein 1. Plakins and cadherins are involved in cell-cell or cell-matrix adhesion. The source of these autoantibodies and their exact role in the pathogenesis of PNP are not yet fully understood. Neoplastic cells may produce these autoantibodies themselves or may stimulate B cells to do so. The autoantibodies are thought to induce blisters of the mucosa and skin, via acantholysis or other means. Cellular immunity also

plays a role in PNP. The variety of clinical manifestations of PNP is attributed to the balance between the cellular and humoral response. A cellular autoimmune reaction produces more lichenoid clinical features, whereas the humoral autoimmune reaction leads to more pemphigus and pemphigoid-like clinical manifestations [2].

<The balance between the humoral and cellular autoimmune response determines the type of cutaneous manifestations in PNP>

Diagnosis Paths

History and Physical Examination

PNP usually affects adults, with an average age of onset being 60 years. Rarely children may also be affected.

The most characteristic clinical feature of PNP is a painful severe oral stomatitis, with hemorrhagic crusts and erosions of the intraoral mucosa, extending to include the vermilion border of the lips. (Fig.10.1b, 10.2a) Conjunctival and genital mucosa may also be involved. Cutaneous manifestations range from flaccid to tense blisters as seen in pemphigus vulgaris (Fig. 10.1a) and bullous pemphigoid, painful erythema and skin detachment as seen in toxic epidermal necrolysis, targetoid lesions as seen in erythema multiforme, and lichenoid papules and plaques as seen in lichen planus (Fig. 10.2), or the variable manifestations of graft versus host disease, but may also be absent in a subset of patients. The distribution typically involves the face, trunk, and extremities but may also include palms and soles, which distinguishes it from the classical pemphigus variants. A subset of patients, ranging from 8 to 93 %, may develop shortness of breath or even respiratory failure, due to bronchiolitis obliterans [3, 4].

<A subset of PNP patients develop bronchiolitis obliterans>

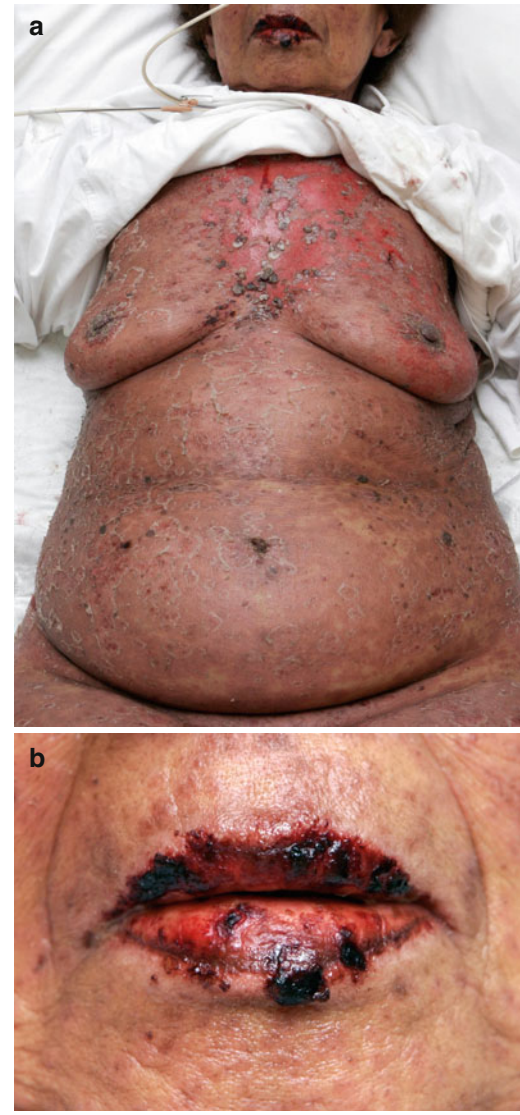

Fig. 10.1 Paraneoplastic pemphigus in a female with pemphigus phenotype showing (**a**) confluent polycyclic erythema and collaret scales on the trunk with erosions and vegetating plaques on the breast, and (**b**) stomatitis with hemorrhagic crusts on the lips

Diagnostics

Diagnosis of PNP is based on three main features (Table. 10.1). The demonstration of antibodies to both envoplakin and periplakin is most sensitive and specific. Immunoblotting, immunoprecipitation, and indirect immunofluorescence on rat bladder urothelium (Fig. 5.5) are suitable tools to detect these antibodies [5]. Direct immunofluorescence of patient skin may also be used but is not very sensitive and specific for PNP (Fig. 4.4).

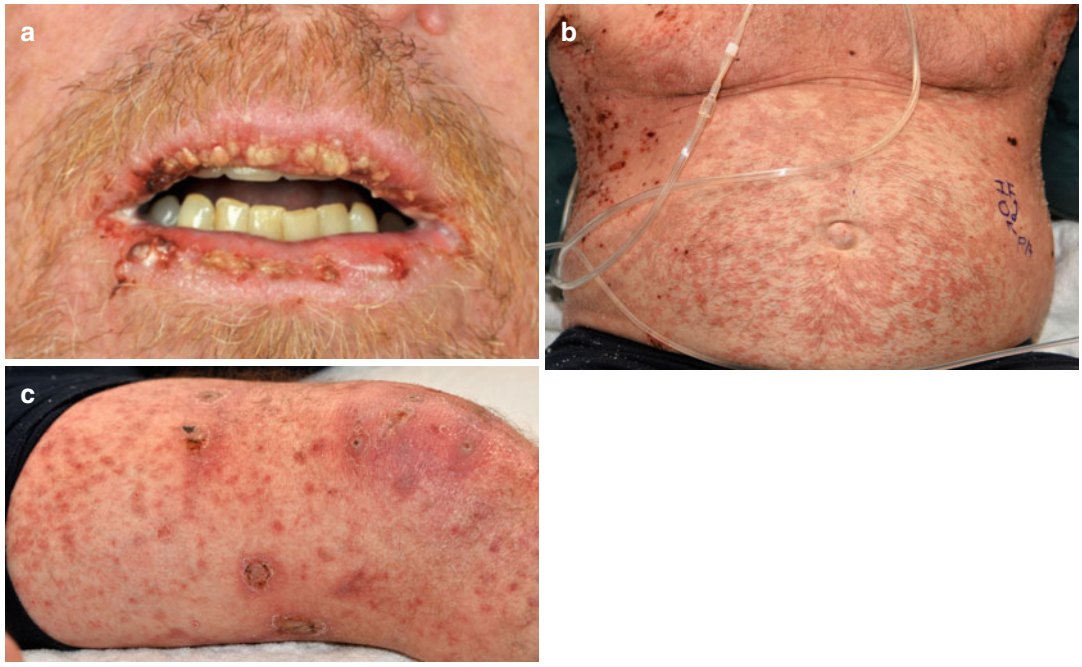

Fig. 10.2 Paraneoplastic pemphigus in a male with lichenoid phenotype showing (**a**) stomatitis with erosions and crusts on the lips, (**b**) Fine erythematosquamous plaques on the trunk, and erosions in the flanks. (**c**) On the upper leg, lichenoid papules and plaques are discernable

Table 10.1 Diagnostic criteria for paraneoplastic pemphigus

#	Criterium
1	Presence of severe stomatitis (cheilitis)
2	The presence of an underlying neoplasm
3	The demonstration of antibodies to both envoplakin and periplakin and /or A2ML1 in the serum of patients

<The diagnosis of PNP is confirmed by the demonstration of antibodies to both envoplakin and periplakin and /or A2ML1 in patient serum>

In a small subset of PNP patients, often with lichenoid skin lesions, no circulating antibodies are detected, probably because the cellular autoimmune response and not the humoral dominates in these patients with "lichenoid PNP."

Histological features of PNP vary, including intraepidermal acantholysis, subepidermal blistering, interface dermatitis, and keratinocyte apoptosis and necrosis. Therefore, histology alone is not sufficient to confirm the diagnosis of PNP [1, 2].

<A small subset of PNP patients are seronegative>

Case Study: Part 2

Drug history was negative, ruling out toxic epidermal necrolysis. Serology showed negative immunoblot results but a positive IgG staining of the rat bladder urothelium by indirect immunofluorescence. The diagnosis PNP was made. Further imaging studies revealed multiple abdominal masses, which were cytologically diagnosed as non-Hodgkin's lymphoma.

Treatment and Prognosis

Treatment of PNP is comparable to that of pemphigus vulgaris. In addition, the underlying neoplasm must be treated. Despite treatment, mortality rates are high, with a 1-year survival

rate of 49 %. Bronchiolitis obliterans impairs the prognosis. Deaths are mainly due to infections, respiratory failure, and progression of the underlying malignancy [3]. Patients with resectable tumor such as Castleman's disease have the best prognosis and mostly survive.

Case Study: Part 3

The patient was started on R-CHOP chemotherapy (rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisolone) but after 1 week developed an *S. aureus* sepsis and respiratory failure. Three weeks later she died of multiorgan failure.

Review Questions

1. PNP patients are characterized clinically by:
   (a) A severe stomatitis
   (b) The combination of flaccid and tense blisters
   (c) lichenoid plaques
2. Which of the following results confirm the diagnosis PNP?
   (a) Serum IgG binding to monkey esophagus
   (b) A dual ECS and BMZ IgG deposition pattern in patient skin
   (c) Serum IgG binding to rat bladder urothelium
   (d) Positive anti-desmoglein 3 IgG serum antibodies by ELISA
   (e) Serum IgG binding to the roof of salt-split skin
3. Theoretically, which subset of PNP patients is more likely to have negative serology?
   (a) Patients with flaccid intraepidermal blisters
   (b) Patients with tense, subepidermal blisters
   (c) Patients with lichenoid plaques, showing interface dermatitis in histology
4. Which autoantibodies are most sensitive and specific for PNP?
   (a) Antibodies to both envoplakin and periplakin
   (b) Antibodies to BP230
   (c) Antibodies to desmoglein 3
   (d) Antibodies to A2ML1

Answers

1. (a)
2. (c)
3. (c)
4. (a)

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IgA Pemphigus

11

Barbara Horváth and Marcel F. Jonkman

Abstract

IgA pemphigus (IGAP) is a rare distinct variant of pemphigus characterized by tissue-bound and circulating autoantibodies exclusively from the IgA class against desmosomal and non-desmosomal proteins of the epidermis. Based on the clinics, histology, direct immunofluorescence, and autoantibody profile, it is classified in two types: the subcorneal pustulosis dermatosis (SPD)-type and the intraepidermal neutrophilic IgA dermatosis (IEN)-type. The first-line therapy is dapsone.

Keywords

IgA pemphigus (IGAP) • Immunoglobulin A (IgA) • Desmosomes • Pustular disease

Introduction and Aims

Short Definition in Layman's Terms

IgA pemphigus is a distinct form of pemphigus characterized by tissue-bound and circulating IgA autoantibodies against desmosomal and non-desmosomal epithelial cell surface antigens.

<IgA pemphigus is a rare disease mediated by IgA autoantibodies against epithelial cell surface antigens>

Learning Objectives

After reading this chapter, you will be able to diagnose and differentiate pustular dermatoses and to recognize the classic clinics of IgA pemphigus. You will be able to perform and interpret the immunological tests and to make a treatment algorithm.

Didactical Questions; Cross Section of Questions to Prime the Readers' Interest

How can you diagnose a sterile pustular dermatosis? What would you see in the histopathological section? How can you make the difference between autoimmune and

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Case Study: Part 1

A 77-year-old male patient presented with widespread annular erythematous plaques with tiny pustules at the periphery on the trunk and extremities (Fig. 11.1). There was erythema, edema, and desquamation on the palms and footpads. The body folds like armpits and groin were not affected. Patients had malaise, but no fever was detected. Previously, there were no changes in medication. Medical history was negative for atopic disease and psoriasis. No drug allergy was previously documented.

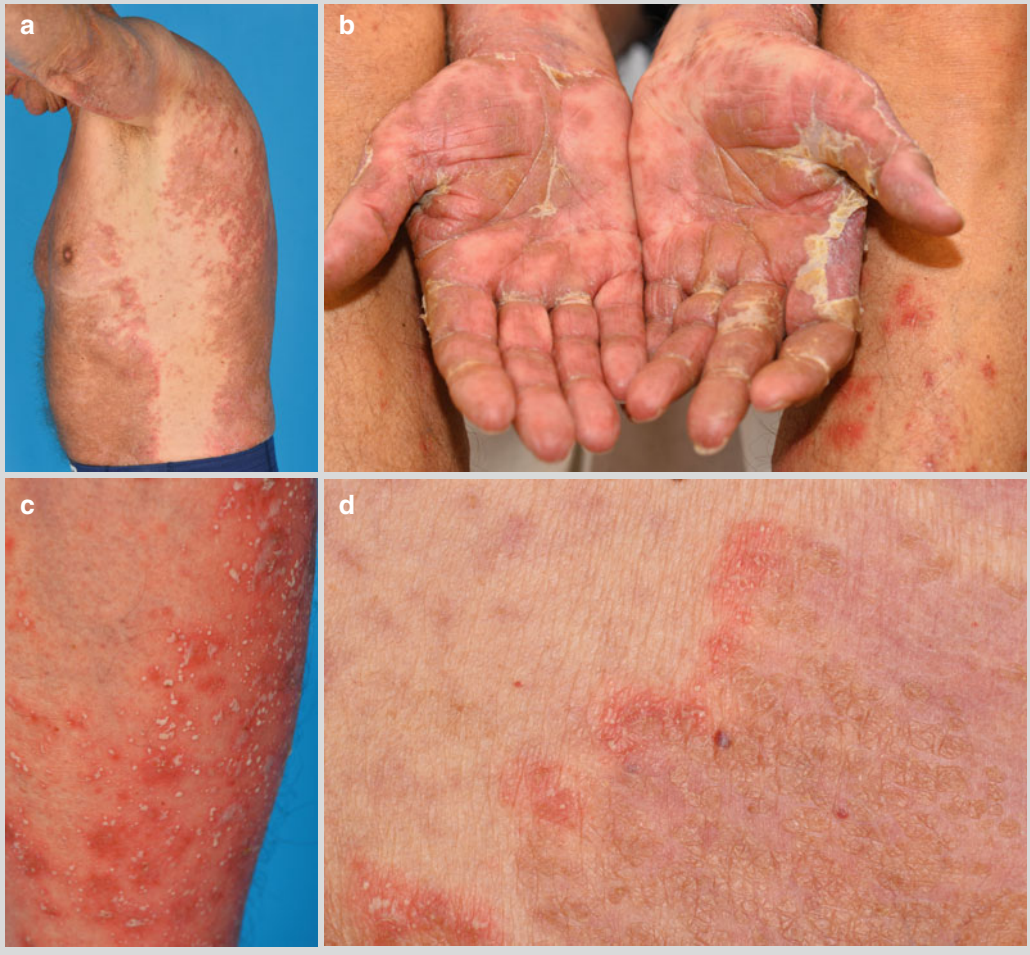

Fig. 11.1 IgA pemphigus in a 77-year-old male with erythematous plaques with miliary to lenticular pustules over (**a**) the trunk and (**c**) extremities including the (**b**) palms and soles. (**d**) In detail, the pustules are distributed on the advancing edge (circinate) of the erythematous plaques

autoinflammatory diseases? In this section, the focus is on the clinical differential diagnostics and workup of patients with extensive pustular dermatosis.

Facts and Figures

Definitions and Classification

IgA pemphigus (IGAP) is an autoimmune bullous disease characterized by tissue-bound and circulating autoantibodies exclusively from the IgA class against desmosomal and non-desmosomal epithelial cell surface proteins of the epidermis [1]. Based on the clinics, histology, and the autoantibody pattern, it is divided into two different forms: the *subcorneal pustulosis dermatosis* (SPD) *type* and the *intraepidermal neutrophilic IgA dermatosis* IEN-type [2]. However, there are still cases of atypical IGAP which cannot be classified into these two forms and better are named IGAP spectrum. Moreover, in classic subcorneal pustular dermatosis or Sneddon-Wilkinson's disease, no autoantibodies are detected in the skin or serum.

<Sneddon-Wilkinson's disease is similar to IgA pemphigus SPD type but without detectable IgA depositions in the skin>

Epidemiology

IGAP has several synonyms such as intraepidermal neutrophilic IgA dermatosis, intercellular IgA dermatosis, IgA pemphigus foliaceus, intraepidermal IgA pustulosis, and IgA herpetiform pemphigus. The disease is relatively new; only about 70 patients are described in the literature [1]. Because of its rarity and newly discovery, the age and race distribution are not very well investigated. Based on limited cases, there is a slight female predominance with an age distribution of 1 month to 92 years, with an average of 48 years [3]. It seems widely distributed as several cases are reported from all over the world.

Pathogenesis

In the SDP-type IGAP, the IgA autoantibodies target the desmosomal cadherin desmocollin 1 (Dsc1) which is expressed in the upper part of the epidermis [4]. The autoantigen of the IEN-type IGAP is still unclear, as some studies report reactivity against desmoglein 1 and desmoglein 3, desmocollins 1–3, as well as other, still unknown, non-desmosomal proteins on the epithelial cell surface [5].

Using immunoelectron microscopy, gold particles are mostly seen in the extracellular spaces between keratinocytes at desmosomes in SPD-type IGAP. In contrast, in IEN-type, the gold particles are mainly in the intercellular spaces in non-desmosomal areas [5].

Once IgA is bound to the keratinocyte surface, neutrophils accumulate in the epidermis leading to intraepidermal blister, later pustule formation. However, the exact pathomechanism is still unknown.

<In SDP-type IGAP, the IgA autoantibodies target desmocollin 1, whereas the autoantigen in IEN-type IGAP is still unclear>

Diagnosis Paths

History and Physical Examination

Onset of IgA pemphigus is subacute [1], first small fragile vesicles appear but soon they transform to pustules. The lesions spread centrifugal and form annular plaques with collarette-like scaling. The SPD-type is undistinguishable from the classic SPD; there are erythematous skin

lesions with *tiny superficial* circinate pustules and later desquamation from the edges surfacing the entire body, particularly in the intertriginous areas. In contrast, the IEN-type is characterized by annular erythematous plaques with circinate pustules and crusts that spread outward and heal inward, giving the lesions a so-called *sunflower-like appearance*. Mucous membranes are almost always spared [1].

General Diagnostics

Routine histopathology in the SPD-type IGAP shows infiltration of neutrophils in the epidermis and upper dermis with subcorneal pustules, and acantholysis can be seen in a minority of cases. The IEN-type is characterized by blisters filled with neutrophils in the middle layers of the epidermis, and acantholysis is sparse or absent. Sometimes eosinophils are seen in the intraepidermal pustules [6].

Associations of diseases such as IgA gammopathy, diffuse large B-cell lymphoma, and lung cancer have been reported.

Specific Diagnostics

By direct immunofluorescence, the SPD-type IGAP shows IgA depositions on cell surfaces in the uppermost layers of the epidermis. Conversely, in the IEN-type, the IgA depositions are distributed over all layers of the epidermis [7].

The circulating IgA antibodies are detectable only about 50 % of the cases on indirect immunofluorescence. Using normal human skin sections, autoantibodies react with the upper part of the epidermis in the SPD-type, whereas with the whole epidermis in the IEN-type [2].

Standard immunoblotting technique can be disappointing, as no consequent reactivity can be seen, maybe due to the conformation-sensitive epitopes in IGAP. Only some cases with anti-Dsg3 showed reactivity in immunoblot [2]. ELISA testing for IgA to desmoglein 1 and desmoglein 3 is not standard [2]. The most useful assay to detect IgA antibodies targeting conformation-dependent epitopes on desmocollin 1 is using cDNA-transfected COS-7 cells [4]. However, this technique is available only in specific laboratories.

Case Study: Part 2

Routine laboratory examination showed leukocytosis (WBC: 16.9 10^9/ml) with neutrophilia (15.46 10^9/ml) and elevated ESR (71 mm per hour) and CRP (177 IU/ml). Common bacterial swab of the pustule and blood showed no microorganism. Histopathology revealed intra- and subcorneal neutrophil accumulations (pustules) in the epidermis without the presence of eosinophil granulocytes. Direct immunofluorescence microscopy showed fine granular ECS depositions of IgA (2+) in the upper epidermal layers (Fig. 11.2). On indirect immunofluorescence, no circulating autoantibodies either of IgA or IgG class were detected on monkey esophagus. Further serological examinations on salt-split skin, Western blot, and desmoglein 1 and desmoglein 3 ELISA were all negative for both IgA and IgG.

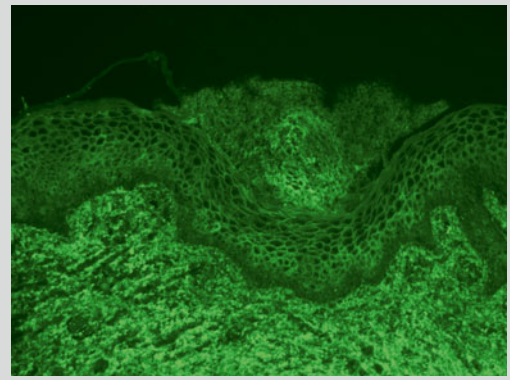

Fig. 11.2 Direct immunofluorescence of skin biopsy reveals epithelial cell surface (ECM) depositions of IgA in the epidermis. Note the pustule in the center due to subcorneal accumulation of neutrophils

Treatment Tricks

Initial Treatment and Escalator

Due to its rarity, treatment protocols are missing. Treatment algorithm is adapted and from pemphigus and from the neutrophilic dermatoses.

The first-line therapy is dapsone (25–125 mg/day) because it suppresses several functions of neutrophils [8]. If dapsone is contraindicated or not effective, retinoids are the drugs of choice. Previously etretinate was given with success; nowadays, several successes are reported by acitretin [9] or isotretinoin.

Topical or systemic corticosteroids are also used. There are single case reports which describe positive effect of adalimumab and mycophenolate mofetil, colchicine, tetracycline, sulfamethoxazole/trimethoprim, methotrexate, and cyclosporine. Surprisingly, positive effect of UVA photochemotherapy (PUVA) is observed [10].

<The first-line therapy is dapsone because it suppresses several functions of neutrophils>

Case Study: Part 3

After excluding glucose-6-phosphate dehydrogenase (G6PD) deficiency, patient received dapsone orally. The initial dose was 50 mg per day, which was increased up to 75 mg daily after 1 week under blood controls. Unfortunately soon after, patient developed dyspnea and acral cyanosis. Blood examination showed a slightly elevated methemoglobin within the normal range and elevated sulfhemoglobin, and patient still had good hemoglobin levels, but the reticulocytes were low (not compensating hemolysis). After tapering and stopping dapsone, the cyanosis improved, but patient was not able to restart dapsone because of the return of acrocyanosis and dyspnea. In the next step, patients received topical clobetasol ointment daily with acceptable result.

Follow-Up and Tapering

IGAP seems to be recalcitrant disease, so frequently combined therapy is needed [10].

Review Questions

1. Which is not a subtype of IGAP?
   (a) Subcorneal pustulosis dermatosis type
   (b) Intraepidermal neutrophilic IgA dermatosis type
   (c) Sneddon-Wilkinson's disease
2. Which form of IGAP is characterized by erythematous skin lesions with tiny superficial pustules, particularly in the intertriginous areas?
   (a) SPD-type
   (b) IEN-type
   (c) Both types
3. Which form of IGAP is characterized by the so-called sunflower-like appearance?
   (a) SPD-type
   (b) IEN-type
   (c) Both types
4. First-line treatment of IGAP is?
   (a) Dapsone
   (b) Systemic corticosteroids
   (c) Azathioprine
5. Which medication is the 2nd choice?
   (a) Super potent topical corticosteroids
   (b) Retinoids
   (c) Azathioprine

Answers

1. (c)
2. (a)
3. (b)
4. (a)
5. (a)

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Drug-Induced Pemphigus

12

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Abstract

Drug-induced pemphigus can be induced or triggered by drugs, most often penicillamine and other thiol-associated drugs. The time from first intake of the drug to onset of the reaction is often relatively long compared to other cutaneous adverse drug reactions. Clinically, it can present as pemphigus foliaceus (most often), pemphigus erythematosus, pemphigus herpetiformis, or pemphigus vulgaris. Opposing to idiopathic pemphigus, pruritus, a prodromal stage, and absence of mucosal involvement are frequent, while laboratory findings are similar. The pathogenesis is not completely known, but probably includes endogenous and exogenous factors. Withdrawal of the culprit drug is mandatory, although the disease may not subside if caused by nonthiol drugs.

Keywords

Vesiculobullous disease • Autoimmune disease • Adverse drug reaction • Pemphigus • Drug induced • Drug triggered • Penicillamine • Thiol-associated drugs

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Short Introduction in Layman Terms

Pemphigus can be induced or triggered by drugs. In drug-induced pemphigus (DIP), the autoimmune disease was not present before exposure to the putative drug, whereas in drug-triggered pemphigus (DTP), the autoimmune process was already programmed and only facilitated by the drug. Contrary to the latency time in most other cutaneous adverse drug reactions, latency between start of new medication and onset of the reaction can be up to several months. Timely

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withdrawal of the culprit drug will often result in full resolution in DIP, whereas in DTP, this is generally not the case.

Learning Objectives
After reading this chapter, you should be aware that

- Some drugs can induce or trigger pemphigus: in every patient with pemphigus, in particular in new cases, a meticulous drug history should be taken to identify and withdraw potential culprits to achieve a potential remission.
- Clinical features in DIP are rather different from idiopathic pemphigus: itching and absence of mucosal involvement can be important clues for the differentiation (cave pemphigus herpetiformis).
- Different subtypes of pemphigus seem to be provoked by different types of drugs, often with a different prognosis.

Case Study: Part 1
A 57-year-old woman presented with pruritic, painful erosions and crusts on the upper trunk since 2 weeks. She denied fever and the use of new medication. Careful history learned that captopril had been prescribed for hypertension since 6 months. Moreover, penicillin i.v. had been used for 10 days for erysipelas, 2 weeks before the onset of the lesions on the trunk.

Facts and Figures

To date, more than 60 drugs have been associated with pemphigus that can be classified in three different groups (Table. 12.1): (1) thiol-associated drugs (drugs containing a thiol (-SH) group or a disulfide bond that releases SH groups or "masked thiols": nonthiol drugs containing sulfur that metabolizes to an active thiol group), (2) phenol drugs, and (3) nonthiol nonphenol drugs [1–5].

Table 12.1 Drugs involved in inducing or triggering pemphigus, grouped according to their chemical structure

Thiol-associated drugs
Penicillamine
Captopril
Penicillins and its derivatives (aminopenicillins)
Cephalosporins[a]
Piroxicam
Gold sodium thiomalate
Thiamazole
Bucillamine
Thiopronin
Pyritinol[a]
5-thiopyridoxine[a]
Phenols (drugs containing a phenol ring)
Cephalosporins[a]
Aspirin
Rifampicin
Levodopa
Heroin
Pentachlorophenol
Phenobarbital
Pyritinol[a]
5-thiopyridoxine[a]
Nonthiol, nonphenol drugs
ACE inhibitors other than captopril
Most NSAIDs
Nifedipine
Biological modifiers of the immune response[b]
Glibenclamide
Psoralens
Others

ACE angiotensin-converting enzyme, *NSAID* nonsteroidal anti-inflammatory drug
[a]Some of these are both thiol and phenol drugs
[b]Including rituximab, interferon-α, interleukin-2, vaccines

Penicillamine and other thiol-associated drugs are most frequent inducers; it is estimated that up to 7 % of patients treated with penicillamine for at least 6 months will acquire pemphigus [1]. Lesions may appear from days to several months after drug initiation; thiol drugs have a longer time latency (>300 days), compared to nonthiol drugs (~ 128 days). Besides, some cases of "contact pemphigus" have been described to topical application of, e.g., ophthalmic drops and cutaneous ointments (e.g., imiquimod, cantharidin) [3].

Subtypes of DIP comprise pemphigus foliaceus (PF, most cases), pemphigus erythematosus (PE), pemphigus herpetiformis (PH, few cases), and pemphigus vulgaris (PV). Contrary to idiopathic pemphigus, DIP is often associated with pruritus and has a prodromal stage with nonspecific lesions preceding the genuine pemphigus lesions. Full-blown DIP often shows scaling and crusting (PF, Fig. 12.1), seborrheic lesions with a butterfly distribution predominantly on the face (PE), or small vesicles with crusted erosions grouped to annular or gyrate lesions (PH) [1].

Features of PV are most often seen in DTP in users of nonthiol drugs, while PF or PE are more common in DIP caused by thiol-associated drugs. Mucosal involvement is mainly restricted to the PV subtype and otherwise rare. DIP caused by thiol drugs will often subside after drug withdrawal, in contrast to pemphigus due to nonthiol drugs [1]. In the majority of DIP and DTP cases, tissue-bound antibodies and less often (low titer) circulating antibodies are in accordance with idiopathic pemphigus, making differentiation difficult [2].

Although more than 200 case reports of DIP have been published, DIP is rare condition and affects men and women equally. However, penicillins are regularly prescribed and probably often overlooked as a culprit, indicating that pemphigus might be more often drug related than previously substantiated.

Pathogenesis is not completely known, but probably comprises endogenous (e.g., genetic) and exogenous factors (e.g., drugs). Immunologic acantholysis may start with biochemical events resulting in neoantigen formation and autoantibody production. Thiol-associated drugs and immune modulators could also directly interfere with the immune system resulting in release of forbidden B cell clones. Moreover, autoantibodies could be mediated by enzymes promoting plasminogen activators. Phenol drugs may cause cytokine release, promoting acantholysis and effecting regulation and synthesis of complement and proteases. The nonthiol, nonphenol drugs, may promote immune acantholysis in several ways: by overexpression of target antigens, overactivation of the immune system, amplification of the local immune response, and release of plasminogen activators [2, 4].

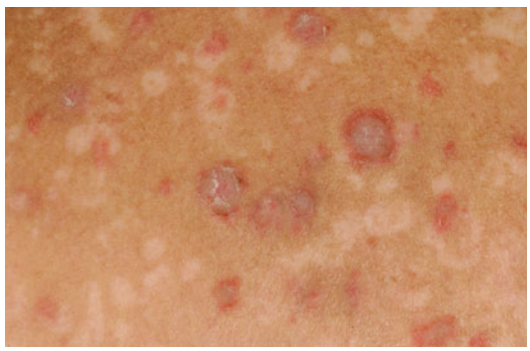

Fig. 12.1 Drug-induced pemphigus foliaceus in a female who received penicillamine for seronegative rheumatoid arthritis

Diagnosis Paths

Every new case of pemphigus and flare-ups should be thoroughly investigated for a potential drug relation. Cases of DIP may present with nonspecific manifestations before lesions occur, e.g., pharyngitis. Pruritus and absence of mucosal involvement are important hints for DIP. History includes a meticulous history, in particular, of last year's drug use, nonspecific prodromal skin lesions, and pruritus, followed by a thorough dermatological examination of skin and mucosae. Histopathology may reveal eosinophilic spongiosis, epithelial necrosis, and variability of the epidermal splitting level, even in a single biopsy, and rather dense dermal infiltrates [2].

Case Study: Part 2

The patient had pruritus, scaling, and small erosions on the face and upper body, while mucosal involvement was absent. Histology revealed cleavage of the epidermis at several levels and dermal mixed infiltrates containing many eosinophils. DIF identified intercellular epidermal staining, mainly confined to the upper layers. The ELISA test detected antibodies to desmoglein 1.

Treatment Tricks

Suspension of the suspected culprit drug is mandatory. Drug withdrawal will lead to remission in approximately 50 % of cases of DIP caused by thiol-associated drugs, opposing to only 15 % in those due to nonthiol drugs, and/or may reduce the need for therapeutic intervention.

Case Study: Part 3

Captopril was withdrawn, while penicillin had already been stopped a few days earlier. Prednisolone 0.5 mg/kg resulted in remission within a few weeks. The preferred diagnosis was DIP, caused by captopril and/or penicillin. The patient was informed about the diagnosis, possible causes, need for a careful follow-up, and advice to avoid certain drugs, especially those with "thiol groups" (see Table. 12.1).

Review Questions

1. Choose the correct statement about drug-induced pemphigus:
   (a) In drug-induced pemphigus (DIP), the autoimmune disease was not present before the drug exposure.
   (b) In drug-triggered pemphigus (DTP), the autoimmune disease was not present before the drug exposure.
   (c) In drug-triggered pemphigus (DTP), the autoimmune process will be stopped after suspension of the culprit drug.
2. In DIP, lesions may appear from days to several months after drug initiation. Which drug is more likely to induce pemphigus with a longer time latency?
   (a) Enalapril
   (b) Penicillamine
   (c) None
3. Drug withdrawal will lead to remission of pemphigus in approximately:
   (a) 50% of cases due to nonthiol drugs
   (b) 50% of cases of DIP caused by thiol drugs
   (c) None of above

Answers

1. (a)
2. (b)
3. (b)

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On the Web

Litt's Drug Eruption & Reaction Database. http://www.drugeruptiondata.com.

Part III

Pemphigoids

Structure of Hemidesmosomes and the Epidermal Basement Membrane Zone

13

Iana Turcan and Marcel F. Jonkman

Abstract

Hemidesmosomes are complex, multiprotein structures that mediate the attachment of epithelial cells to the underlying basement membrane. While providing mechanical attachment, these adhesion units are extremely dynamic. They play a significant role in signaling pathways involved in the various important cell functions, such as differentiation, wound healing, and survival. Structurally, hemidesmosomes contain the following molecules: plectin (over 500 kDa protein), BP230 (230 kDa antigen, also known as BPAG1), integrin α6β4, and BP180 (180 kDa protein, also known as BPAG2 or type XVII collagen, and CD151 (protein of tetraspan superfamily). The epidermal basement membrane zone can be viewed as a thin sheet of matrix underlying the basal epithelial cells. It consists of lamina lucida and lamina densa, mainly containing laminin and type IV collagen networks. Type VII collagen which enters into the composition of semicircular anchoring fibrils provide the attachment to the papillary dermis underneath the lamina densa of the basement membrane. When molecules in hemidesmosomes or in the basement membrane zone become target of autoantibodies, a particular acquired subepidermal autoimmune bullous disease (sAIBD) will develop.

Keywords

Hemidesmosome • Dermal-epidermal junction • Basement membrane zone • Autoimmune disease • Pemphigoid

Introduction and Aims

Learning Objectives

The role of hemidesmosomes and basement membrane in maintaining tissue organization and integrity is demonstrated in several sAIBDs.

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Table 13.1 Targeted molecules and their corresponding autoimmune disease at the site of hemidesmosomes and basement membrane zone

Location	Molecule	Autoimmune bullous disease
Hemidesmosome	Plectin	Anti-plectin pemphigoid Paraneoplastic pemphigus
	BP180 BP230	Bullous pemphigoid Nonbullous cutaneous pemphigoid Brunsting-Perry pemphigoid Lichen planus pemphigoid Pemphigoid gestationis Linear IgA disease Mucous membrane pemphigoid
	LAD-1, LABD-97	Linear IgA disease
	Integrin α6β4	Mucous membrane pemphigoid
Basement membrane	Laminin 332 Laminin 311 (α3 chain)	Mucous membrane pemphigoid
	Type VII collagen	Epidermolysis bullosa acquisita
	p200/laminin γ1	Anti-p200 pemphigoid

In this chapter, our aim is to explain the structural complexity and function of hemidesmosomal and basement membrane zone proteins, their relationship to each other and list the sAIBDs that involve them (see Table. 13.1).

Facts and Figures

Hemidesmosomes

Hemidesmosomes (HDs) are specialized complexes that provide attachment of the intermediate filament network in epithelial cells to the underlying basement membrane in the skin; mucous membranes of the cornea, oral cavity, pharynx, larynx, esophagus, genitals, and in the amnion. The name originates from its appearance as half of a desmosome, a cell-cell anchoring complex (see Chap. 7). HDs have a tripartite electron-dense plaque structure including inner hemidesmosomal plaque, outer hemidesmosomal plaque, and the subbasal dense plaque (Fig.13.1).

<Hemidesmosomes connect intermediate filaments to basement membrane matrix>

Subsequent is a succinct description of the most relevant constituents of HDs.

Plectin is a protein of the plakin family with a molecular mass over 500 kDa. This polypeptide consists of a central coiled-coil rod domain flanked by the globular N-terminal head domain and a C-terminal tail domain at each end, respectively. The N-terminus provides binding sites for integrin β4, BP180, and actin filaments, while the C-terminus connects to intermediate keratin filaments. Furthermore, plectin plays a role in attaching intermediate keratin filaments through association with BP230 [1]. Plectin has many isoforms with a long common rod domain, which are distributed in specific tissues such as stratified squamous epithelia, heart, skeletal muscle, and nerve tissue. Plectin 1a is the dominant isoform in hemidesmosomes. This protein may become target for autoimmunity. Although a rare event, anti-plectin antibodies have been identified in sera from bullous pemphigoid (BP) patients [2]. Plectin has also been implicated as an autoantigen in paraneoplastic pemphigus (PNP).

Similar to plectin, BP230 is a member of the plakin protein family. Also known as BPAG1, this molecule was the first discovered antigen to be targeted in bullous pemphigoid (BP). Structurally, BP230 is composed by a central coiled-coil rod domain flanked by N- and C-termini at each end, respectively. The N-terminus plays an important function in integrating BP230 into the HD and has BP180 and integrinβ4 as ligands; the C-terminus connects to intermediate keratin filaments [3]. Through alternative splicing, the DST gene encoding BP230 generates tissue-specific isoforms

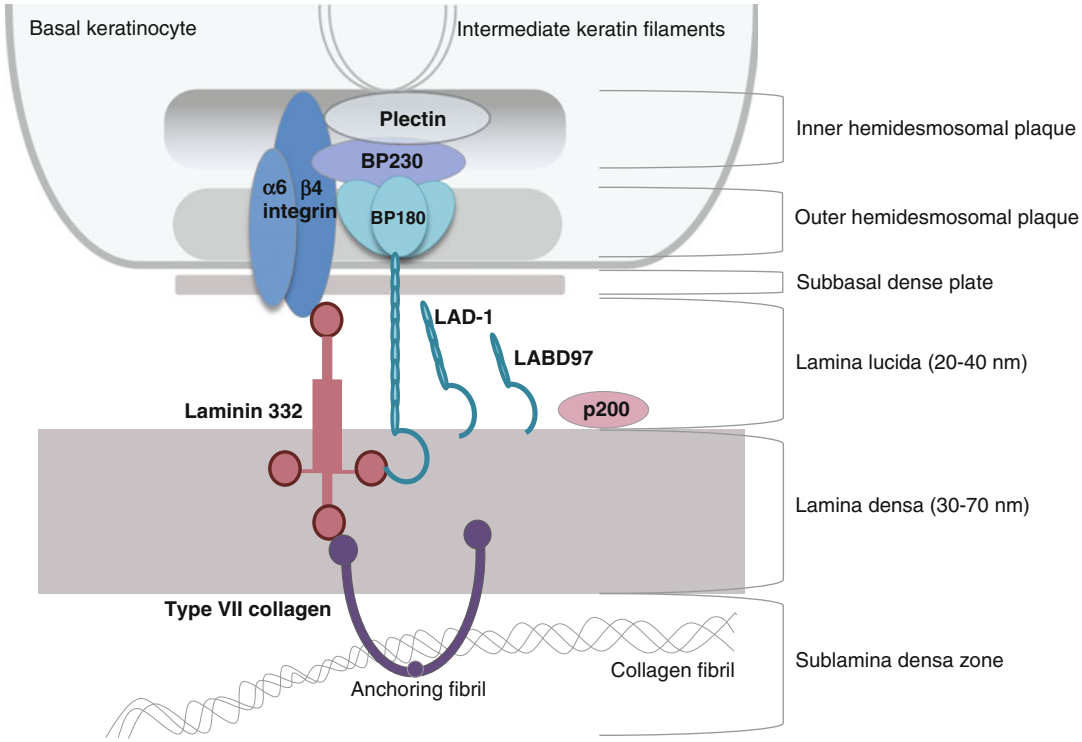

Fig. 13.1 Schematic representation of the hemidesmosome and dermal-epidermal junction including all molecules known to be targeted in autoimmune bullous diseases

expressed in the skin, central nervous system, and muscles, respectively [4]. BP230 has been involved as an autoantigen in several sAIBDs including BP, MMP, Brunsting-Perry pemphigoid, pemphigoid gestationis (PG), lichen planes pemphigoides (LPP), and linear IgA disease (LAD).

180 kDa bullous pemphigoid antigen or BP180, also known as BPAG1 or type XVII collagen, is a transmembrane hemidesmosomal glycoprotein. The N-terminal is noncollagenous and located intracellular, while the extracellular domain has a triple-helical shape containing collagenous repeats, hence the term type XVII collagen. Intracellularly, BP180 interacts with integrin α6β4 and plectin and aids the integration of BP230 into the HD. The extracellular domain crosses lamina lucida into the lamina densa where it binds laminin 332 [5]. BP180 is expressed in the skin, mucosa, central nerve tissue, teeth, placenta, and umbilical cord. Specific autoimmunity targeting this antigen leads to a spectrum of subepidermal autoimmune disorders such as BP, mucous membrane pemphigoid (MMP), Brunsting-Perry pemphigoid, PG, LPP, and LAD. Notably, the ectodomain of BP180, by means of stepwise proteolytic cleavage, generates the 120-kDa (LAD-1) and 97-kDa (LABD-97) antigens. These shed ectodomains are deposited in the lamina lucida and may become target of IgA autoantibodies in LAD.

Integrin α6β4 is a transmembrane molecule at the heart of the HDs. The integrin β4 subunit has a large intracellular domain which interacts with the intracellular domain of BP180 and links intermediate keratin filaments through plectin and BP230. The extracellular domains of the integrin α6 and integrin β4 subunits bind to laminin 332 in the extracellular matrix [6]. Integrin α6β4 is expressed in stratified squamous and transitional epithelia such as the skin, mucous membranes, gastrointestinal tract, and urinary tract. Both α6 and β4 integrin subunits have been suggested as autoantigens in MMP in some studies; the evidence may benefit from more validation.

Epidermal Basement Membrane Zone

The epidermal basement membrane provides architectural linkage and a functional continuity between epidermis and the underlying dermis. Another important task is the maintenance of a barrier for unrestricted passage of chemical or pathological agents into the body or water and electrolytes out of the body. Basement membrane is too small to be visualized with light microscopy and can be identified only by electron microscopy. It contains an electron-lucent 20–40 nm thick layer named lamina lucida and a 30–70 nm thick electron-dense layer named lamina densa. This division is, nevertheless, a tissue preparation and dehydration artifact resulting from the retraction of plasma membrane and thus exposure of lamina lucida [5]. The structural composition of basement membrane involves supramolecular aggregates that include laminin isoforms, type IV collagen, type VII collagen, perlecan, and nidogen [7].

<Basement membrane zone interfaces epithelial and dermal compartment>

Following is a succinct description of the most relevant constituents of the basement membrane.

Laminins represent a family of heterotrimeric molecules consisting of three different chains, α, β, and γ, which assemble into cross-shaped polypeptide. It is found in stratified squamous, transition, and simple epithelia [8]. Laminin 332 is a major component of the epidermal basement membrane and by binding integrin establishes a firm linkage to the underlying matrix. An additional function is mediation of keratinocyte migration [9]. Laminin 332 may become a target antigen in MMP. Also laminin γ1 chain has been involved in some cases of anti-laminin γ1/anti p-200 pemphigoid.

p200 is a 200 kDa polypeptide in the lower lamina lucida, whose exact identity has not yet been fully clarified. The associated sAIBD is anti-p200 pemphigoid.

Type VII collagen is the main, if not the sole, component of anchoring fibrils in the sublamina densa zone. Anchoring fibrils have a semicircular shape and link the lamina densa to the papillary dermis underneath. Structurally, it consists of three identical α-chains which organize into a triple-helical collagenous structure flanked by globular N-terminus (NC1) and C-terminus (NC2). This molecule is expressed in the basement membrane zone of the skin, cornea, oral cavity, pharynx, larynx, genital mucosa, esophagus, and chorioamnion [10]. Autoantibodies targeting type VII collagen are associated with epidermolysis bullosa acquisita (EBA).

Type IV collagen provides an architectural scaffold for other macromolecules by forming a network of interactions. Up to date, no autoimmune cutaneous disease was found to be associated with this molecule. Nevertheless, autoantibodies against alpha3 chain of type IV collagen in the basement membrane of the lungs and kidney were detected in Goodpasture syndrome.

Review Questions

1. Which protein is a structural component of the hemidesmosome?
   (a) Integrin α6β4
   (b) Type IV collagen
   (c) Laminin 332
   (d) Type VII collagen
2. Which protein is a structural component of the lamina densa?
   (a) BP230
   (b) Laminin 332
   (c) Integrin α6β4
   (d) BP180
3. BP180 and BP230 proteins are associate with the following sAIBDs:
   (a) BP, MMP, Brunsting-Perry pemphigoid, PG, LPP, EBA
   (b) BP, MMP, Brunsting-Perry pemphigoid, PG, LPP, LAD
   (c) BP, MMP, LPP, PG, p-200 pemphigoid, LAD

Answers

1. (a)
2. (b)
3. (b)

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https://en.wikipedia.org/wiki/Hemidesmosome.
https://en.wikipedia.org/wiki/Collagen,_type_XVII,_alpha_1.
https://en.wikipedia.org/wiki/Basement_membrane.

Cutaneous Pemphigoid

14

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Abstract

Pemphigoid diseases are a heterogeneous group of subepidermal autoimmune bullous diseases (sAIBDs) that are characterized by autoantibodies against different structural proteins of hemidesmosomes in the epidermal basement membrane zone (EBMZ) (Table. 14.1). Cutaneous pemphigoid includes many subtypes, such as bullous pemphigoid (BP), nonbullous cutaneous pemphigoid (NBCP), Brunsting-Perry cicatricial pemphigoid, lichen planus pemphigoides (LPP), pemphigoid gestationis (PG), anti-p200 pemphigoid, and anti-plectin pemphigoid. Classification of sAIBD subtypes is mainly based on target antigens and/or clinical manifestations. Pathogenesis of cutaneous pemphigoid is mediated by predominantly IgG autoantibodies against different structural proteins in the EBMZ. Diagnosis is based on a combination of clinical criteria, a linear n-serrated deposition pattern along the EBMZ in direct immunofluorescence microscopy and serology. BP is the most common sAIBD and most frequently affects elderly. The incidence of BP increased substantially in the past decades. The clinical manifestations of pemphigoid diseases are heterogeneous. The typical presentation of BP is a severe pruritus with predominantly cutaneous lesions consisting of tense blisters or vesicles, erythema, and urticarial plaques. In NBCP, blistering is completely absent, while pruritus is severe, and papules, plaques, and excoriations may be present. Mucosal lesions develop in 10–20 % of patients. Nikolsky's sign is negative in pemphigoid. Recommended therapy consists of whole-body application of superpotent topical corticosteroids or oral corticosteroids.

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Keywords
Hemidesmosome • Immunoglobulin • Basement membrane zone • Autoimmune disease • Vesiculobullous disease • Pemphigoid

Bullous Pemphigoid

Introduction and Aims

Short Definition in Layman Terms

Bullous pemphigoid (BP) is the most common subtype of sAIBDs. BP mainly affects elderly and is clinically characterized by severe itch with tense blisters, erythema, or urticarial plaques (Fig. 14.1). BP is mediated by an immune response against two proteins in the hemidesmosomes that are important for maintaining the integrity of the skin. Dysfunction may lead to subepidermal blistering. In BP mainly the skin is affected, but involvement of mucous membranes may occur. Treatment of BP is based on suppression of the immune system, with corticosteroid creams applied to the skin or oral drugs.

<BP is the most common autoimmune bullous disease mainly affecting elderly>

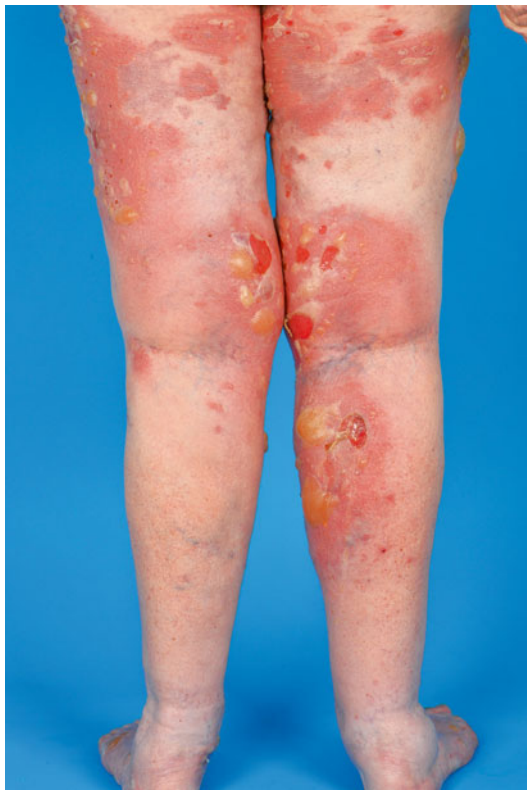

Fig. 14.1 Infiltrated urticarial plaques with tense blisters on predilection sites of BP: the flexural surfaces of the legs and the thighs. Multiple ruptured blisters leave eroded areas

Learning Objectives

After reading this chapter, you should be able to recognize the typical clinical presentation of BP, know the target antigens, and the hallmarks in histopathology and immunofluorescence microscopy. You should also be aware of the diagnostic algorithm and the treatment options in BP.

Case Study: Part 1
An 83-year-old woman with severe itch for several months is treated by her general practitioner with various ointments. Later on, she also develops erythematous papules and urticarial plaques on her back and extremities, with also some vesicles. Diagnosed as urticaria, she was treated with topical corticosteroids and oral antihistamines, without improvement. The dermatologist noted multiple tense blisters on erythematous skin and erosions on the flexor aspects of the extremities at physical examination. Nikolsky's sign was negative, and mucous membranes were unaffected.

Didactical Questions: Cross Section of Questions to Prime the Readers' Interest

Which diagnostic steps are essential when a blistering disease is suspected? What are the

Table 14.1 Target antigens, IF findings, and clinical symptoms of subtypes of sAIBDs

Disease type	Target antigens	IF findings		Clinical symptoms
		DIF	IIF SSS	
Bullous pemphigoid	BP180 BP230	n-serrated EBMZ IgG ± IgA, IgE, C3c	Epidermal	Pruritus, urticaria, tense blisters without predominant mucosal involvement
Nonbullous cutaneous pemphigoid	BP180 BP230	n-serrated EBMZ IgG ± IgE, C3c	Epidermal	Pruritus, eczematous lesions, urticarial plaques, erythematous papules or nodules
Brunsting-Perry pemphigoid	BP180 LAD-1	n-serrated EBMZ IgG ± C3c	Epidermal	Erosions and blisters confined to the head, face, neck, and upper trunk leaving atrophic scars
Lichen planus pemphigoides	BP180 BP230	n-serrated EBMZ IgG ± C3c	Epidermal	Tense blisters independent of the lichenoid plaques and papules of lichen planus
Pemphigoid gestationis	BP180 BP230	n-serrated EBMZ C3c ± IgG	Epidermal	Intense pruritic urticarial rash, papules, and tense blisters starting around umbilicus and then spread over the body
Linear IgA disease	BP180 LAD-1, LABD-97	n-serrated EBMZ IgA ± IgG	Epidermal	Tense blisters and erosions in "string of pearls," without predominant mucosal involvement
Anti-plectin pemphigoid	Plectin	n-serrated EBMZ IgG ± C3c	Epidermal	Pruritus, urticaria, tense blisters without predominant mucosal involvement
Anti-p200 pemphigoid	p200	n-serrated EBMZ IgG ± C3c	Dermal	Pruritus, tense bullae, vesicles, urticarial plaques, predominantly on the extremities and trunk
Epidermolysis bullosa acquisita	Type VII collagen	u-serrated EBMZ IgG ± IgA	Dermal	Mechanobullous variant: acral blistering that heals with scarring and milia Inflammatory variant: widespread vesicles and blisters, without scarring or milia

EBMZ epidermal basement membrane zone, *IF* immunofluorescence microscopy, *DIF* direct IF, *IIF SSS* indirect IF salt-split skin, *IgG/IgA/IgE* immunoglobulin G/A/E, *C3c* complement C3

similarities and differences between pemphigus and pemphigoid? Can you make the differentiation on clinical symptoms alone? How do you make the diagnosis of BP and what is the first-choice treatment?

Facts and Figures

Definitions and Classification

Pemphigoid: the etymology of the word pemphigoid is "form of a blister" (*pemphix*, blister and *eidos*, form in Greek). Therefore, bullous pemphigoid is a pleonasm. In 1953, Walter F. Lever differentiated pemphigoid diseases from pemphigus, based on histopathology and clinical presentation. He described intraepidermal separation and loss of cell adherence between keratinocytes (acantholysis) in pemphigus and introduced the term pemphigoid for diseases with subepidermal splitting [1]. The classification of sAIBDs is based on different clinical symptoms, target antigens, and autoantibody isotypes (Table 14.1).

<BP is characterized by subepidermal blister formation>

Epidemiology

BP most frequently affects elderly, with onset of disease usually after the age of 70 years old. Incidences have been described from 1.21 to 2.17 per 100,000 persons per year. Moreover, the incidence rises substantially with age, up to 15–33 per 100,000 per year in people older than 80 years. The incidence of BP in Europe has more than doubled in the last decade, which might be related to both the increasing age of the

general population, multidrug use, and the availability and quality of diagnostics. BP rarely occurs in infancy and childhood. BP has been associated with a high morbidity and a considerable 1-year mortality rate ranging from 20 to 40 %. Most important risk factors for poor outcome are high age, widespread disease, a low Karnofsky score, and high doses of oral corticosteroids [1].

Pathogenesis

BP is characterized by the presence of IgG autoantibodies against components of hemidesmosomes in the EBMZ, which maintain the dermoepidermal integrity. Binding of autoantibodies to the antigens initiates a complex process, leading to separation of the epidermis and the dermis with subepidermal blister formation. Additionally, deposits of IgA, IgE, and complement may also be found along the EBMZ. Autoantibodies in BP patients target two hemidesmosomal proteins: BP180 and BP230. BP180 is a 180-kDa transmembrane protein in the lamina lucida and lamina densa. Most BP patients have autoantibodies against the extracellular part of the 16th non-collagenous domain (NC16A) of BP180 (immunodominant region). BP230 is a 230-kDa intracellular component of the hemidesmosomal plaque. However, the pathogenic relevance of autoantibodies against BP230 is not clear yet. Isoforms of both BP180 and BP230 are also expressed in the central nervous system, which might explain the association between BP and neurological diseases, such as dementia, Parkinson's disease, and stroke in up to half of patients with BP [1].

<Main target antigens in BP are BP180 and BP230>

Diagnosis Paths

History and Physical Examination

BP typically presents with severe pruritus, localized or generalized tense blisters, and erythema or urticarial plaques (Fig. 14.2). Nikolsky's sign is negative. Predilection sites are the trunk, abdomen, and flexural aspects of the extremities. Blisters may arise on both healthy and erythematous skin, often have a transparent or serous exudate, and can persist for several days. Ruptured blisters leave erosions and crusts, but do not heal with scarring. Mucosal involvement is seen in 10–20 % of BP patients, mostly the oral mucosa. A pitfall can be the number of patients that only have pruritus and excoriated, eczematous, or urticarial lesions preceding the development of blisters, or persistent nonbullous cutaneous pemphigoid [2]. A detailed medical history should be obtained, including a medication history with recent drug intake. Furthermore, the extent of BP should be assessed, for example, with the BP Disease Area Index (BPDAI, see Chap. 2).

<Main clinical symptoms of BP are tense blisters, urticarial plaques, and itch>

General Diagnostics

Which diagnostic steps are essential when a blistering disease sAIBD is suspected? Both a skin biopsy of a recent, intact bulla for routine histopathological analysis and a lesional peribullous skin biopsy for DIF are needed.

The diagnosis of BP is based on a combination of criteria comprising clinical features, histopathological findings, specific direct IF findings, and serology (Table. 14.2). A complete blood count often shows peripheral eosinophilia. Histopathology of a bullous lesion shows subepidermal splitting and an inflammatory infiltrate composed of mainly eosinophils and neutrophils. However, in absence of blistering, the histopathology may be nonspecific and be limited to eosinophilic spongiosis or an eosinophilic infiltrate in the upper dermis. Direct immunofluorescence microscopy reveals a linear n-serrated immunodeposition of IgG and/or complement C3 along the EBMZ. Occasionally, other Ig subclasses can be found, such as IgA, IgM, and IgE. Direct immunofluorescence microscopy is the gold standard in autoimmune bullous diseases, AIBDs combined with immune serological tests to reach a high sensitivity.

<BP shows an IgG/C3c n-serrated pattern along the EBMZ in direct IF>

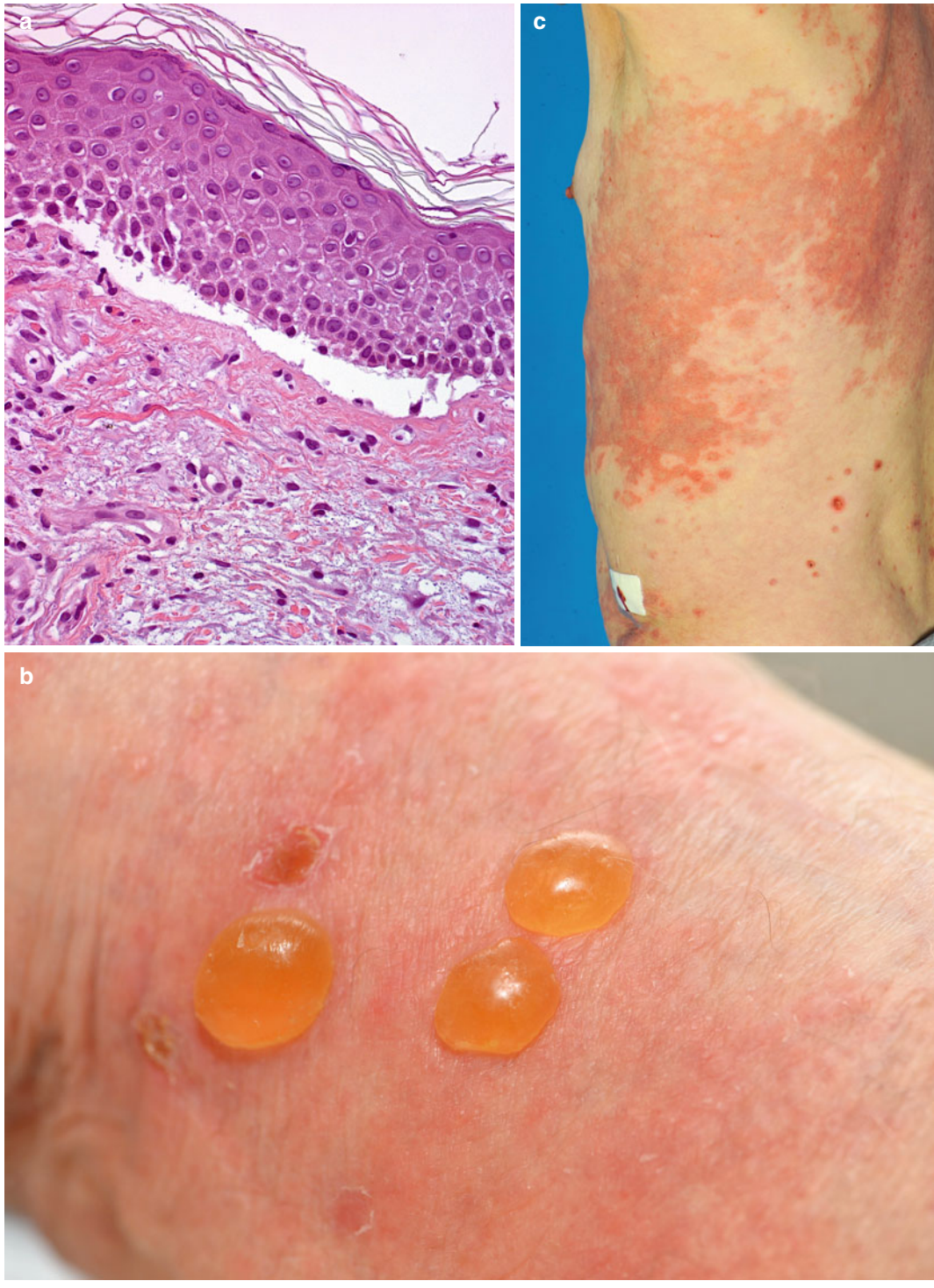

Fig. 14.2 Hallmarks of BP: (**a**) Histopathology of H&E section of lesional skin biopsy with subepidermal blister formation with eosinophils and a dermal inflammatory eosinophilic infiltrate (magnification 400×), (**b**) tense bullae on inflamed, erythematous skin, (**c**) confluent infiltrated urticarial plaques on the trunk

Table 14.2 Clues to diagnosis of BP

Clinical clues for diagnosis of BP	Diagnostic clues for diagnosis of BP
Elderly with severe pruritus	Peripheral eosinophilia
Eczematous lesions, papules, or nodules	Subepidermal splitting
Urticarial plaques	Dermal inflammatory infiltrate of eosinophils
Localized or generalized tense blisters	DIF IgG/C3c along EBMZ n-serrated pattern
Mucosal lesions	IIF on monkey esophagus IgG+
Polypharmacy	IF SSS IgG epidermal binding
Nikolsky's sign negative	NC16A ELISA positive
Good response to oral corticosteroids	Immunoblot BP180 positive

Specific Diagnostics

Diagnosis of BP can be confirmed by taking blood samples to perform indirect IF (IIF) studies or ELISA, but the choice of serological test can depend on availability, costs, and local expertise. IIF on monkey esophagus is being used to detect circulating autoantibodies. Furthermore, IIF on 1.0 M NaCl-split skin (SSS) substrate shows binding of autoantibodies to the epidermal side (roof) of the artificial split and is associated with a high positive predictive value in typical BP patients. Combining the IIF SSS technique (epidermal or dermal binding) with serration pattern analysis (n-serrated versus u-serrated) allows to differentiate between different subtypes of pemphigoid and EBA (see Chap. 4). BP180 NC16A ELISA can detect circulating antibodies against BP180. In case of negative results, additional BP230 ELISA can be performed. Immunoblot can be used to test the patient's serum reactivity to BP180, BP230, and/or other rare targeted antigens.

Case Study: Part 2

Histopathology of a lesional biopsy of an intact blister showed a subepidermal blister with a dense inflammatory infiltrate of eosinophils. A lesional peribullous skin biopsy for direct IF showed linear depositions of IgG 3+, IgA 1+, and C3c 3+ in an n-serrated pattern along the EMBZ. Serological testing by indirect IF on monkey esophagus showed anti-EMBZ IgG antibodies, IIF SSS was positive for IgG on the epidermal side of the salt-split skin. BP180 NC16A and BP230 ELISA IgG indexes were 51 (positive) and 7 (negative), respectively. Immunoblot was positive for BP180 and BP230 IgG. The diagnosis was made of BP, which initially presented only with pruritus.

Treatment Tricks

Initial Treatment and Therapeutic Ladder

BP can have a clinical course that may last from several months to years. The high age of BP patients and the possible presence of comorbidities can make the treatment management more difficult. Recommended first-line therapy for mild, moderate, and severe disease is superpotent topical steroids (clobetasol propionate cream) 30–40 g/day applied daily over the whole body, including blisters, erosions, and healthy skin, but sparing the face [3]. Whole-body application of superpotent topical corticosteroids is considered to be effective and safe and has a lower cumulative dose of corticosteroids and less side effects compared to oral corticosteroids. Patients with localized BP can be treated with superpotent topical corticosteroids applied to lesional skin only. Oral corticosteroids (prednisone 0,5 mg/kg/day) are often used in treatment of moderate to severe BP and may be accompanied by adjunctive superpotent topical corticosteroids and/or immunosuppressive agents, such as azathioprine, mycophenolate mofetil, mycophenolic acid, and methotrexate. Systemic anti-inflammatory antibiotics (tetracyclines) are commonly

used as alternative or adjunctive treatment, potentially combined with nicotinamide/niacinamide. In refractory cases of BP, intravenous immunoglobulin (IVIG) and anti-CD20 monoclonal antibody (rituximab) may be considered.

<Whole-body application of superpotent topical steroids is first-choice therapy in BP>

Follow-Up and Tapering

BP can last for several years and has the tendency to relapse. Serum levels of anti-BP180 NC16A IgG antibodies by ELISA correspond with disease severity and activity and can be used as a biomarker and to identify patients with a high risk of relapse. Current evidence suggests to continue initial topical treatment until 15 days after disease control, when no new lesions arise and lesions begin to heal. Then treatment should be reduced by a tapering schedule, with daily treatment in the first month, every 2 days in the second month, two times a week in the third month, and once a week starting in the fourth month. Doses of oral corticosteroids should be gradually tapered, based on clinical course and when available on serum levels of anti-BP180 NC16A IgG antibodies by ELISA [3].

Case Study: Part 3

First-line therapy with whole-body application of superpotent topical corticosteroids (40 g/day) improved her complaints, but appeared to be insufficient. Therefore, the patient received adjunctive treatment with azathioprine 50 mg/day, later on 100 mg/day. Pruritus and the frequency of blistering reduced; after 2 months, the patient reached complete remission and azathioprine was stopped. Complaints of itch returned at the end of the tapering schedule of topical corticosteroids. Therefore, treatment was restarted with 40 g whole-body superpotent topical corticosteroids twice a week and following the tapering schedule until long-term complete remission.

Nonbullous Cutaneous Pemphigoid

Short Definition in Layman Terms

Nonbullous cutaneous pemphigoid (NBCP) is the subset of patients with immunopathological findings of BP and pruritus, but no blister development. It is of importance to be aware of this subtype of pemphigoid. These patients are mainly elderly, presenting with pruritus on primary normal, noninflamed skin (no skin lesions) or with nonbullous skin lesions, and are often misdiagnosed as xerosis, drug reactions, dermatitis, renal or liver impairment or scabies.

Definitions and Classification

In 1953, Walter F. Lever added the pleonasm "bullous" to the name pemphigoid in an attempt to separate it from mucous membrane pemphigoid. We now know that BP is not always bullous. In the literature, there is no unanimity on how to name the subset of patients with pemphigoid without blistering. The coined terms include pruritic nonbullous pemphigoid, nonpruritic pemphigoid, pemphigoid nodularis, papular pemphigoid, prurigo-nodularis-like pemphigoid, nonbullous BP, prodromal BP, and BP incipiens. Because these patients have pemphigoid of the skin without blistering, we classify this subtype as NBCP [2].

Epidemiology

Of all patients presenting with BP, approximately 20 % show no blistering. The majority of the patients with NBCP is above 70 years of age. NBCP is probably underdiagnosed in elderly with chronic itch, because clinicians do not think of pemphigoid as the underlying cause of chronic itch in elderly, in absence of blistering.

<Approximately 20 % of patients with BP do not show blistering>

Pathogenesis

IgG is the predominant autoantibody in BP patients; however, the role of IgE autoantibodies has also been reported before in BP to be pathogenic [4]. This insight is of interest for NBCP. Elderly individuals with pruritic dermatosis often present with elevated IgE serum levels. These elevated serum levels were also described in up to 70 % of the BP patients. In previous reports was shown that the IgE was bound to the pathogenetic NC16A domain of BP180. These observations suggest a critical role of IgE autoantibodies in the pathogenesis of BP and may be a marker for the intensity of the pruritic symptoms. Furthermore, complement activation has been reported to play an important role in the formation of blisters in BP. Therefore, BP and NBCP might differ in the way of complement activation.

Clinical Symptoms

The clinical presentation of NBCP is heterogeneous and may mimic other inflammatory diseases as mentioned before. Patients can present with pruritus, eczematous eruptions consistering of, urticarial plaques, and erythematous papules or nodules (Fig. 14.3). In some patients, pruritus on primary normal, noninflamed skin is the only symptom.

<Think of NBCP in elderly with therapeutic refractory itch>

Diagnosis Paths

The gold standard for NBCP is a positive DIF with linear, n-serrated, IgG, and/or C3c along the EBMZ. In the absence of blisters, we recommend a biopsy for DIF in NBCP from lesional skin, preferentially a papule. When DIF is negative, diagnosis can be made by IIF on SSS (epidermal binding) combined with BP180 NC16A ELISA or immunoblot. One must be aware that patients with only a single positive result by BP180 NC16A or BP230 ELISA cannot be considered as having BP or NBCP, as detection of circulating IgG autoantibodies against BP180 or BP230 is a common finding in elderly with nonbullous pruritic disorders. The relevance of these autoantibodies in patients without clinical symptoms of BP is not clear yet.

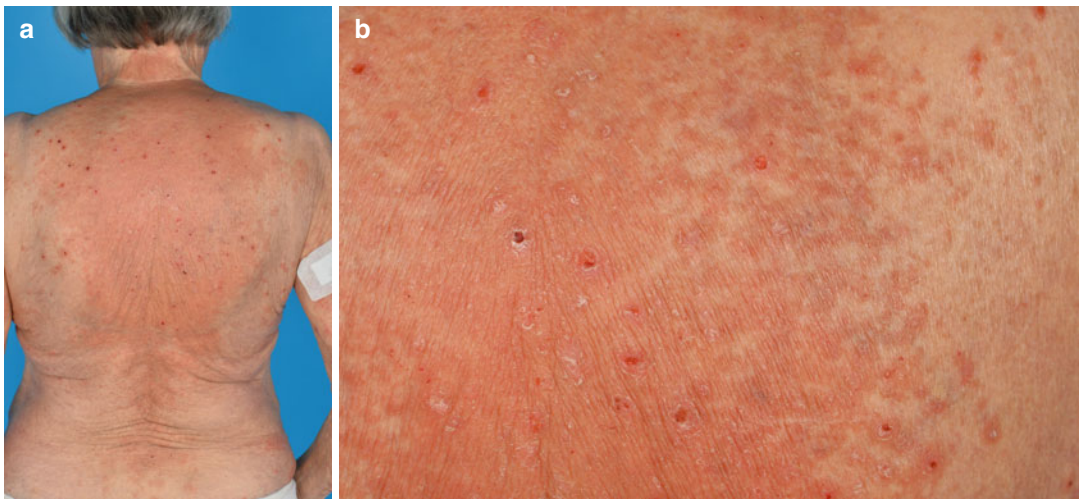

Fig. 14.3 An elderly NBCP patient with pruritic, excoriated eczematous lesions on the back (**a**), and in detail (**b**); DIF showed linear IgG along the BMZ in an n-serrated pattern

Treatment Tricks

Treatment of this intense pruritic condition is essential. The fist-line therapy for NBCP is the same as in BP: whole-body application of superpotent topical corticosteroids. If nonresponding, systemic treatment with low-dose methotrexate is the next step. In some cases, low-dose oral corticosteroid is sufficient.

Brunsting-Perry Cicatricial Pemphigoid

Short Definition in Layman Terms

Brunsting-Perry cicatricial pemphigoid is a form of local pemphigoid confined to the head and neck area and leading to scarring. It may be difficult to recognize, because of the rarity and resemblance with other diseases like epidermolysis bullosa acquisita, erosive pustular dermatosis of the scalp, chronic infection, squamous cell carcinoma or folliculitus decalvans. A skin biopsy for DIF must be performed for a correct diagnosis.

Facts and Figures

In 1957, Brunsting and Perry described a rare local form of cicatricial pemphigoid patients who presented with itchy erosions with blisters that heal with scarring at the site of the scalp, face, and neck [5]. Circulating IgG autoantibodies target BP180 and LAD-1. Subepidermal split formation occurs in most cases at the level of the lamina lucida. The targeted C-terminal domain of BP180 that is located in the lamina densa, might be responsible for the scarring phenotype. The average age at onset of symptoms is 58 years and the male/female ratio is 2:1.

Clinical Symptoms

Brunsting-Perry cicatricial pemphigoid clinically presents with erosions and blisters of the head, neck, and shoulder area that heal with scarring and milia (Fig. 14.4). The scarring of the scalp will develop in permanent alopecia. Mucosal involvement is rarely seen. In the minority of the patients (<5 %), Brunsting-Perry cicatricial pemphigoid develops in a OMMP with scarring of the conjunctiva.

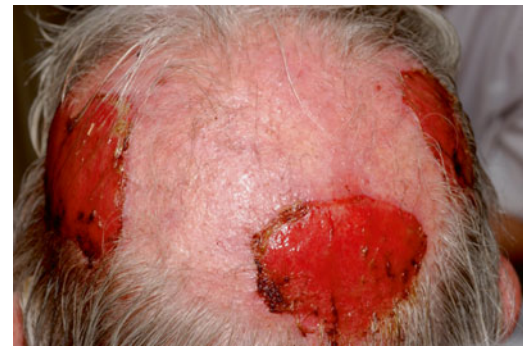

Fig. 14.4 Sharply bordered erosions on the scalp with scarring alopecia in a patient with Brunsting-Perry pemphigoid

<Brunsting-Perry cicatricial pemphigoid is localized on the head, neck, and shoulders>

Diagnosis Paths

Histopathological biopsy of the border of an erosion of the scalp shows subepidermal blistering with lymphocytes, neutrophils, and eosinophils and the presence of extensive scarring in the dermis, with loss of hair follicles. DIF on perilesional skin shows linear deposits of IgG and C3 in the n-serrated pattern along the EBMZ. DIF of normal healthy skin of the upper arm may also show deposits of IgG and C3c. The serological tests are usually negative.

Treatment Tricks

The disease is responding well to oral corticosteroids (prednison 0.5–0.75 mg/kg/day) in combination with immunosuppressive agents like azathioprine (2–3 mg/kg/day). For painful erosions, using a wound dressing with a silicon layer is useful.

Lichen Planus Pemphigoides

Introduction and AIMS

Lichen planus pemphigoides (LPP) is a rare subepidermal autoimmune bullous disease (sAIBD) characterized by a combination of clinical, histological, and immunological features of both lichen planus (LP) and BP. In the bullous form of LP, blistering is restricted to LP lesions; however, in LPP, blisters appear also on normal appearing skin.

Facts and Figures

The term lichen planus pemphigoides or "lichen ruber pemphigoides" was first used by Kaposi in 1892, describing a dermatosis with lichen planus lesions with additional blistering. The pathogenesis of LPP is not completely understood yet; LPP is associated with an autoimmune response directed mostly against the NC16A domain of BP180. A suggested theory is that LP lesions damage the basal keratinocytes and expose the BP180 antigens, leading to a secondary autoimmune response with autoantibodies to the EBMZ [1]. The mean age of onset is usually younger (50–60 years) than in BP.

Diagnosis Paths

LPP clinically presents with a lichenoid eruption of papules and plaques preceding bullous lesions on both LP lesions and previously normal skin. LPP predominantly affects the extremities and tends to be less severe than BP. Histopathology shows typical findings of LP in papular lesions and subepidermal blistering in biopsies of bullous lesions. The diagnosis of LPP is confirmed by detection of IgG autoantibodies or C3c directed against the EBMZ by DIF of a perilesional biopsy, and detection of circulating IgG autoantibodies against BP180 NC16A, and enables to distinguish LPP from bullous LP.

<In LPP, blisters may arise on LP lesions and previously normal skin>

Treatment Tricks

Simultaneous treatment of LP lesions and bullous lesions is needed to avoid an ongoing stimulation of the autoimmune process at the EBMZ. Treatment follows algorithms as for LP and BP [1]. The prognosis is good, with a reported low rate of recurrence of blistering.

Pemphigoid Gestationis

Short Definition in Layman Terms

Pemphigoid gestationis (PG) is a pregnancy-associated subtype of pemphigoid which manifests in the second or third trimester of pregnancy. Sporadically, this disease presents within 4 weeks after birth.

<PG usually manifests in second and third trimester of pregnancy>

Facts and Figures

Holmes and Black suggested in 1982 to name the disease PG instead of herpes gestationis (HP), because of the correlation of the clinical spectrum and immunological findings with pemphigoid diseases. PG is characterized by autoreactivity to the NC16A domain of BP180.

Epidemiology

The annual incidence of PG is 1:50.000 pregnancies. No difference in phenotype is seen in both Caucasians and Afro-Americans. PG can arise at any moment in childbearing age.

Pathogenesis

The pathogenesis of PG is not fully known. It is believed that PG is caused by loss of protection of the fetoplacental unit against allogeneic recognition by the mother. In normal pregnancy, there is no expression of MHC II antigens on the tro-

phoblast. This is a mechanism that protects the fetus against recognition by the maternal immune system. Within PG patients, however, there is an aberrant expression of MHC class II molecules in the placenta. Hereby, BP180, which occurs in the placenta, is presented to the maternal immune system. An immune response occurs with the formation of autoantibodies against BP180, after which a cross-reaction occurs in the skin with BP180 [6].

Clinical Symptoms

PG presents with pruritic urticarial plaques, vesicles, and tense blisters starting around the umbilicus, followed by expansion over the trunk and the distal extremities (Fig. 14.5). Remission is usually seen within 6 months. In the minority of the patients (<5 %), PG persists and converts into BP. Recurrence of PG occurs in more than 90 % of the additional pregnancies. Exacerbation may occur prior to menses or after starting oral anticonception. Because of placental insufficiency, there is a risk of growth retardation and premature delivery of the fetus. There is no increased risk of stillbirth or spontaneous abortion. In 10 % of the neonates, a transient form of BP is seen. Neonatal disease has a mild course with remission within days to weeks [6].

<Inform the patient about the possibility of recurrence of PG in following pregnancies>

Diagnosis Paths

Histopathology shows subepidermal blistering with eosinophilic infiltrate. Final diagnosis can be made by DIF showing C3c and IgG depositions in an n-serrated pattern along the EBMZ. IgG1 and IgG3 having strong complement-binding proper-

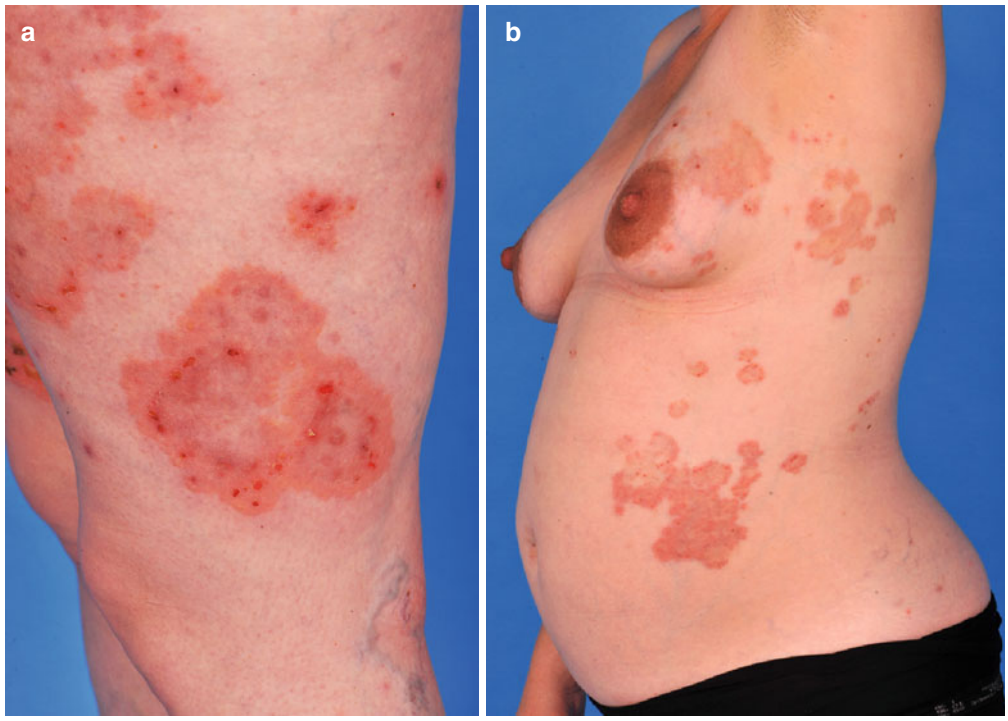

Fig. 14.5 Pemphigoid gestationis in a woman in the 24th week of gestation with pruritic eruption of circinate vesicles on urticarial plaques (**a**, **b**). (Reprinted with permission from *Ned Tijdschr Geneeskd*. 2009;153:B36)

ties cause the presence of C3c. IIF performed on 1 M NaCl-split skin substrate shows binding of antibodies to the epidermal side (roof) of the split and immunoblot analysis reveals reactivity to the 180-kDa antigen.

Treatment Tricks

The first-line therapy for PG is (super) potent topical corticosteroids in combination with oral H1-receptor antagonist. Oral corticosteroids can be introduced at an initial dose of 0.25–0.5 mg/kg/day when (super) potent topical corticosteroids are not sufficient enough. Cooperation with the gynecologist is recommended.

Anti-p200 Pemphigoid

Introduction and Aims

Anti-p200 pemphigoid is a recently defined, rare sAIBD characterized by autoantibodies against a 200-kDa protein (p200) of the EBMZ. The molecular identity of the pathogenic autoantigen has yet to be defined. Anti-p200 pemphigoid is probably often misdiagnosed and classified as BP or inflammatory EBA, because of low availability of diagnostic assays and expertise.

Facts and Figures

Originally described in 1996 by Zillikens *et al.* as a novel sAIBD with autoantibodies against an unknown 200-kDa component of the EBMZ, the disease was consequently termed anti-p200 pemphigoid [7]. Since then, it was renamed to anti-laminin γ1 pemphigoid as a new entity in sAIBD. Serum samples of 90 % of anti-p200 patients appeared to recognize the glycoprotein laminin γ1, mainly the C-terminus region. However, ex vivo and in vivo studies were unable to show pathogenic activity of laminin γ1 [8].

<The autoantigen in anti-p200 pemphigoid is a 200-kDa protein in the lower EBMZ>

Clinical Symptoms

The clinical presentation of anti-p200 pemphigoid is heterogeneous and may mimic BP, LAD, and inflammatory EBA. Most patients present with pruritus and tense bullae, vesicles, and erythematous or urticarial plaques, predominantly on the extremities and trunk. When monomorphic blistering occurs solitary on hands and feet, it may resemble dyshidrosiform pemphigoid (Fig. 14.6). In approximately 10–20 % of patients, mucous membranes are involved. Lesions normally heal without scarring. Patients tend to be younger than in BP. An association with psoriasis was seen in about 30 % of reported cases, mostly in Japanese patients [8].

Diagnosis Paths

Anti-p200 pemphigoid is characterized by subepidermal blistering with a mainly neutrophilic inflammatory infiltrate, in contrast to a typical eosinophilic infiltrate in BP. However, histopathology alone cannot differentiate anti-p200 pemphigoid from other sAIBD. DIF of a perilesional biopsy shows linear deposits of IgG and/or IgA and complement C3 along the EBMZ in an n-serrated pattern. Using serration pattern analysis, anti-p200 pemphigoid can be differentiated from EBA with a u-serrated pattern along the EBMZ. Autoantibodies in anti-p200 pemphigoid bind to the lower lamina lucida; therefore, IIF on salt-split skin reveals binding of circulating autoantibodies along the dermal side of the artificial split. This method allows differentiating anti-p200 pemphigoid from BP, but not from anti-LN-332 MMP and/or EBA. In order to distinguish from these antigens, IIF analysis on knockout skin (see Chap. 5) with sera from anti-p200 pemphigoid patients shows positive reactivity with skin from patients with junctional EB completely lacking expression of LN-332 and with skin from patients with recessive dystrophic EB completely lacking expression of type VII collagen. Moreover, clinical presentation of anti-p200 pemphigoid and anti-LN-332 MMP differs substantially.

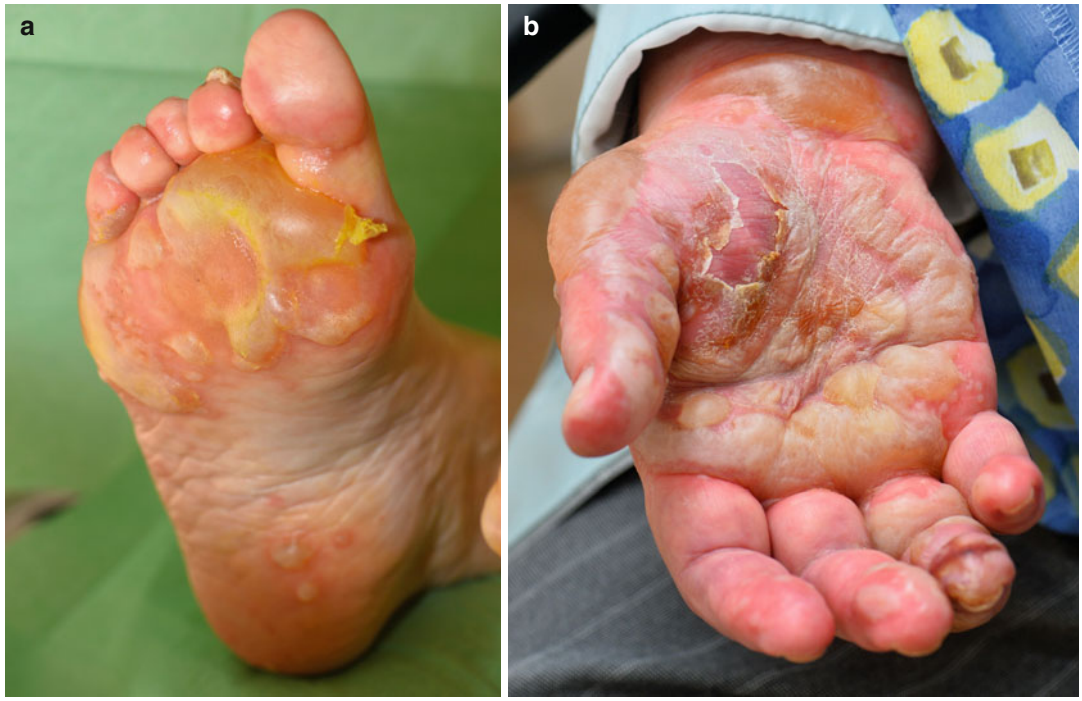

Fig. 14.6 Anti-p200 pemphigoid. Resembling dyshidrosiform pemphigoid with multiple tense blisters on (**a**) the right foot and (**b**) the palm of the right hand

Immunoblotting of dermal extracts shows positive reactivity of anti-p200 pemphigoid patients' serum samples with a 200-kDA protein band.

<Anti-p200 pemphigoid is characterized by a mainly neutrophilic infiltrate in histology, IgG n-serrated pattern along EBMZ in DIF, and dermal binding in IIF SSS>

Treatment Tricks

Treatment of anti-p200 pemphigoid follows the same guidelines as for BP. The clinical course is usually less severe than in BP and patients tend to respond more rapidly to treatment. First-choice treatment in mild to moderate disease is superpotent topical steroids (clobetasol propionate cream). In severe disease, oral corticosteroids (prednisolone 0,5 mg/kg/day) can be used, with adjunctive immunosuppressive therapy used frequently, such as dapsone or azathioprine.

Anti-Plectin Pemphigoid

Anti-plectin pemphigoid is a very rare subtype of pemphigoid characterized by autoantibodies against plectin. This protein is a member of the plakin family in the hemidesmosome (see Chap. 13) and can be detected by immunoblot analysis. All reported patients with anti-plectin autoantibodies demonstrated concomitant antibodies against other pemphigoid antigens. Most often, reactivity is also seen against BP180, BP230, and/or LAD-1. The central coiled-coil rod domain of the plectin molecule appears to be the immunodominant region [9]. A possible explanation of the presence of anti-plectin antibodies could be the epitope-spreading phenomenon, a secondary autoimmune response to other antigens in hemidesmosomes during a chronic autoimmune process in the EBMZ. Because of this reactivity against multiple antigens, the direct pathogenicity and clinical manifestations of anti-plectin antibod-

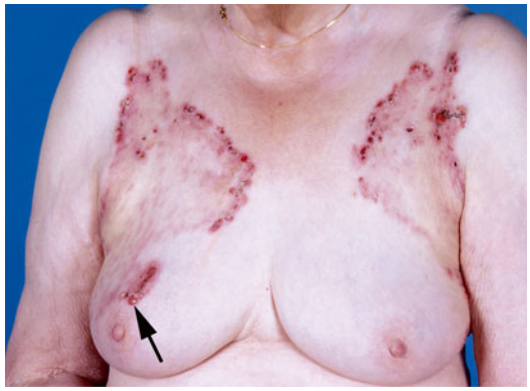

Fig. 14.7 Anti-plectin pemphigoid in an elderly female with arciform erythema and circinate distribution of bullae (*arrow*) and crusts on the chest

ies cannot be determined; most patients show clinical manifestations resembling bullous pemphigoid (Fig. 14.7) [9].

Review Questions

1. What are the three main clinical symptoms of BP?
   (a) Eczema, urticaria, and tense blisters
   (b) Pruritus, urticaria, and tense blisters
   (c) Pruritus, nodules, and tense blisters
   (d) Papules, nodules, and tense blisters
2. First-line treatment of mild and severe BP is
   (a) Superpotent topical corticosteroids whole-body application
   (b) Oral corticosteroids
   (c) Azathioprine
   (d) Dapsone
3. Nonbullous cutaneous pemphigoid may mimic:
   (a) Dry skin (xerosis cutis)
   (b) Scabies
   (c) Atopic dermatitis
   (d) All of mentioned above
4. Patients with Brunsting-Perry cicatricial pemphigoid present with:
   (a) Predominantly mucosal involvement
   (b) Tense blisters predominantly on the extremities and trunk
   (c) Erosions and blisters at the head, neck, and shoulder area
   (d) Itch, urticaria, and flat blisters
5. LPP is characterized by:
   (a) Autoantibodies targeting collagen VII
   (b) Blisters on both LP lesions and previously normal skin
   (c) Blisters solitary on LP lesions
   (d) Vesicles and tense blisters starting around the umbilicus
6. Which statement about PG is correct?
   (a) PG manifests in the first trimester of pregnancy.
   (b) The BP180 C-terminal domain is the target antigen.
   (c) Exacerbation can occur before menstruation or after starting oral anticonception
   (d) There is an increased risk of stillbirth
7. Diagnosis of anti-p200 pemphigoid is confirmed by:
   (a) Histopathology with a neutrophilic inflammatory infiltrate
   (b) IIF on knockout skin or immunoblot with dermal extract
   (c) N-serrated anti-EBMZ immunodepositions in DIF
   (d) Dermal binding in IIF SSS

Answers

1. (b)
2. (a)
3. (d)
4. (c)
5. (b)
6. (c)
7. (b)

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Further Reading

Schmidt E, Zillikens D. Pemphigoid diseases. Lancet. 2013;381:320–32.

On the Web

N-versus-u-test: practice serration pattern analysis http://www.nversusu.umcg.nl.

Mucous Membrane Pemphigoid

15

Jorrit B. Terra and Joost M. Meijer

Abstract

Mucous membrane pemphigoid (MMP) is the subgroup of pemphigoid which affects mucous membranes. Several subtypes are classified based on clinical symptoms and target antigens, such as ocular mucous membrane pemphigoid (OMMP), localized vulvar pemphigoid (LVP), and anti-laminin 332 MMP (anti-LN-332 MMP). Autoantibodies are directed against various structural proteins in the epidermal basement membrane zone (EBMZ), with the 180-kD antigen (BP180) as the main target antigen. Other antigens, such as BP230, α6β4 integrin, and laminin 332, can also be targeted by autoantibodies. Clinically, MMP is characterized by erosions and blistering of the oral mucosa (85 %), conjunctiva (65 %), and, less frequently, the nose (20–40 %), esophagus (5–15 %), pharynx (20 %), larynx (5–10 %), and genitals (20 %). Clinical severity is highly variable in the different subtypes of MMP. Progressive scar formation is a severe complication in active disease in OMMP and anti-LN-332 MMP, resulting in blindness or upper airway obstruction when not treated accurately. Previously, the term cicatricial pemphigoid was used synonymously for MMP. However, at present, the term refers to the rare clinical phenotype with scarring skin lesions. Patient's and doctor's delay is frequently seen in MMP. For an accurate diagnosis, direct immunofluorescence microscopy (DIF) and detection of circulating autoantibodies in serum are mandatory. Management and prognosis of MMP depends on the severity and extent of the disease and involves a stepwise approach with first-choice treatment with oral corticosteroids (CS), often used in combination with adjuvant immunosuppressive drugs to reduce the adverse effects caused by long-term CS use.

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Keywords
Hemidesmosome • Immunoglobulin • Basement membrane zone • Autoimmune disease • Desquamative gingivitis • Pemphigoid • Mucous membrane

Learning Objectives
After reading this chapter, you should be able to recognize the different phenotypes of MMP and know the target antigens and level of splitting in the epidermis-dermis in MMP. You should also know the diagnostic algorithm in MMP and should be able to practice general treatment strategies.

Case Study: Part 1
A 62-year-old man presents with desquamative gingivitis of the oral mucosa, diagnosed as oral lichen planus for several years (Fig. 15.1a). No other mucosal surfaces are affected, but the patient does complain of itch. His oral lesions were treated with superpotent topical corticosteroids that did not relieve the symptoms. Because of failure of treatment, he was referred to our clinic.

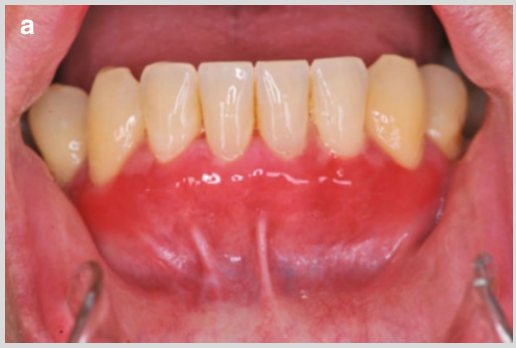

Fig. 15.1a Clinical manifestations of a patient with MMP limited to oral mucosa with desquamative gingivitis and blisters located on the attached gingival mucosa proximal of the lateral incisors

Mucous Membrane Pemphigoid

Short Definition in Layman's Terms

Mucous membrane pemphigoid (MMP) is the nomenclature for the whole group of patients with pemphigoid affecting the mucous membranes (Table 15.1). Circulating autoantibodies target components of the EBMZ. In MMP, the oral mucosa is mostly affected (85 %), but all mucous membranes can be involved. In a minority of patients also, the skin is affected. It is not clear yet whether MMP with lesions limited to the oral mucosa is only a stage of MMP or a distinct clinical entity. Patients clinically present with erosions, erythema or small blisters on mucous membranes, or in case of limited oral lesions with desquamative gingivitis (Fig. 15.1b). The intake of nutrition or fluids can be reduced because of pain. MMP with exclusively oral lesions is frequently unrecognized in the early inflammatory stage and often misdiagnosed as oral lichen planus, oral aphthosis, or other inflammatory oral diseases.

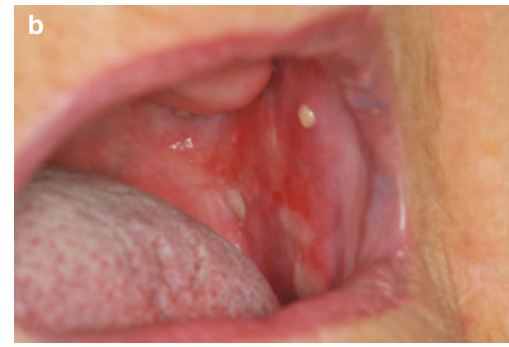

Fig. 15.1b Another patient with MMP with blistering on the buccal mucosa

Table 15.1 Target antigens, IF findings, and clinical symptoms in subtypes of mucous membrane pemphigoid

Disease type	Target antigens	IF Findings		Clinical symptoms
		DIF	IIF SSS	
MMP	BP180, BP230, α6β4 integrin	n-serrated EBMZ IgG ± IgA, C3c	Epidermal	Erosions and blisters of the oral, nasal, eyes, pharyngeal, laryngeal, esophagus and anogenital mucosa
Ocular MMP	BP180	n-serrated EBMZ IgG ± IgA	Epidermal	Dry eye, conjunctivitis, trichiasis, fornix shortening, symblepharon, ankyloblepharon
Localized vulvar pemphigoid	BP180	n-serrated EBMZ IgG ± IgA, C3c	Epidermal	Vulvar itching, burning sensation, pain, dyspareunia and dysuria
Anti-laminin-332 MMP	Laminin-332	n-serrated EBMZ IgG ± C3c	Dermal	Erosions and ulcerations of the supraglottic area, nose, eyes, oesophagus and anogenital region, aphonia, dyspnoe

MMP mucous membrane pemphigoid, *EBMZ* epidermal basement membrane zone, *IF* immunofluorescence microscopy, *DIF* direct IF, *IIF SSS* indirect IF salt-split skin, *IgG/IgA* immunoglobulin G/A, *C3c* complement C3

Didactical Questions: Cross Section of Questions to Prime the Readers Interest

How can you clinically differentiate between oral lichen planus and oral MMP? On which three criteria is the diagnosis MMP based? MMP can resemble pemphigus vulgaris oris; name the clinical differences between these two diseases. A mucosal biopsy for direct immunofluorescence (DIF) will confirm the diagnosis of MMP. What is the additional value of also taking a biopsy for DIF of normal healthy skin?

Facts and Figures

MMP is a subepidermal autoimmune bullous disease (sAIBD) with predominantly mucosal involvement and is characterized by autoreactivity mostly to BP180. BP180 is a 180-kDa transmembrane glycoprotein that ultrastructurally spans the lamina lucida and curves back from the lamina densa into the lamina lucida. In MMP autoantibodies predominantly recognize the C-terminal epitopes of BP180. NC16A is the second immunodominant domain. In addition, autoantibodies may target BP230. Autoreactivity to the α6 and β4 integrin subunits has been associated with oral and ocular MMP, respectively. The main autoantibody isotype is IgG, predominantly of the IgG1 and IgG4 subclass, but deposits of IgA and complement C3 may be found [1, 2]. The incidence of MMP as a group has been estimated at 1.3–2.0 per million per year in France and Germany, respectively. MMP often occurs earlier in life than BP, with age of onset commonly observed between 60 and 70 years [2]. Women are affected almost two times more often than men. MMP is rare in children. No racial differences have been seen.

Diagnosis Paths

History and Physical Examination

Patients with MMP clinically present with erosive or erythematous patches and small blisters of mucosa consisting of nonkeratinized stratified squamous epithelium. Oral lesions occur predominantly on gingival and palatal mucosae and less often on the tongue or buccal mucosa (Fig. 15.1a). Other mucosae can be affected, such as the conjunctiva (65 %) and, less frequently, the nose (20–40 %), esophagus (5–15 %), pharynx (20 %), larynx (5–10 %), and genitals (20 %). MMP patients often present with complaints of bleeding, pain, dysphagia, and erosions or blister of the mucosa. Blisters of the mucosa are frequently seen but often rupture rather quickly as a result of mechanical and traumatic forces. The majority of MMP patients with lesions limited to oral mucosa have gingival lesions resulting in the so-called desquamative gingivitis. The gingival erythema may be confused with nonspecific gingivitis as part of chronic periodontal disease. The intake of nutrition or fluids can be reduced

because of pain. In other forms of MMP, lesions tend to heal with scar formation; however, in oral lesions, reepithelialization without scarring may occur. Furthermore, the extent of (milder forms of) MMP should be assessed, for example, with the MMP Disease Area Index (MMPDAI, see Chap. 2).

<Desquamative gingivitis in MMP may be seen as nonspecific gingivitis>

Diagnostics

MMP should be differentiated from other diseases with involvement of the (oral) mucosa, such as (erosive) oral lichen planus, pemphigus vulgaris, erythema multiforme, oral aphthosis, and dermatitis herpetiformis. Diagnosis of MMP is based on clinical presentation with oral mucosal lesions and DIF of intact buccal mucosa that shows a linear deposition of IgG and/or complement C3 and IgA along the EBMZ. Serration pattern analysis is often not possible on mucosal biopsies. Therefore, an additional biopsy of healthy skin (e.g., on medial side of upper arm) may be required. IIF performed on 1 M NaCl-split skin substrate shows binding of autoantibodies on the epidermal side of the artificial split. The titer of circulating autoantibodies in serum is frequently low and often not detectable. Immunoblot is of additional value in diagnostics of MMP. The IF findings of MMP are identical to BP, and the distinction should be made based on clinical symptoms.

<An additional biopsy of healthy skin is required for DIF serration pattern analysis in MMP>

Case Study: Part 2

Mucosal biopsy for DIF showed IgG 1+ and complement C3 1+ along the EBMZ, and the serration pattern was undeterminable in this mucosal biopsy. Indirect IF on monkey esophagus and salt-split skin was negative for IgG and IgA. Immunoblot showed positive staining for BP180 IgG but was negative for BP230. BP180 NC16A index was 39 (positive). The diagnosis of MMP was made, based on clinical symptoms of affected oral mucosa and a positive DIF.

Treatment Tricks

Initial Treatment and Treatment Ladder

Mild lesions of the oral mucosa can be treated effectively with moderate to superpotent topical corticosteroids, or alternatively tetracyclines combined with nicotinamide. Another treatment option in more severe lesions is dapsone (25–200 mg/day), or systemic CS (0.5 mg/kg/day) with azathioprine (100–150 mg/day) [1]. Refractory cases may require high-dose systemic CS, cyclophosphamide, intravenous immunoglobulin (IVIG), or anti-CD20 antibody rituximab.

Follow-Up and Tapering

It has been suggested that MMP with lesions limited to the oral mucosa has a better prognosis compared to other subtypes of MMP. However, the clinical symptoms are highly variable, and the number of reports in literature regarding follow-up and treatment is limited. An otolaryngologist should examine patients with nasal or laryngeal symptoms, whereas an oral and maxillofacial surgeon is expert on oral lesions.

Case Study: Part 3

Treatment was started with dapsone 50 mg/day and, after a G6PD deficiency was excluded, increased to 100 mg/day. Because of increasing fatigueness and loss of appetite, treatment was switched to cyclophosphamide up to 2 mg/kg/day. Unfortunately, the disease was not controlled. The patient therefore received an intravenous cycle of anti-CD20 antibody 2×1000 mg which reduced the blister frequency and subjective complaints.

Ocular Mucous Membrane Pemphigoid

Short Definition in Layman's Terms

Ocular mucous membrane pemphigoid (OMMP), previously named ocular cicatricial pemphigoid

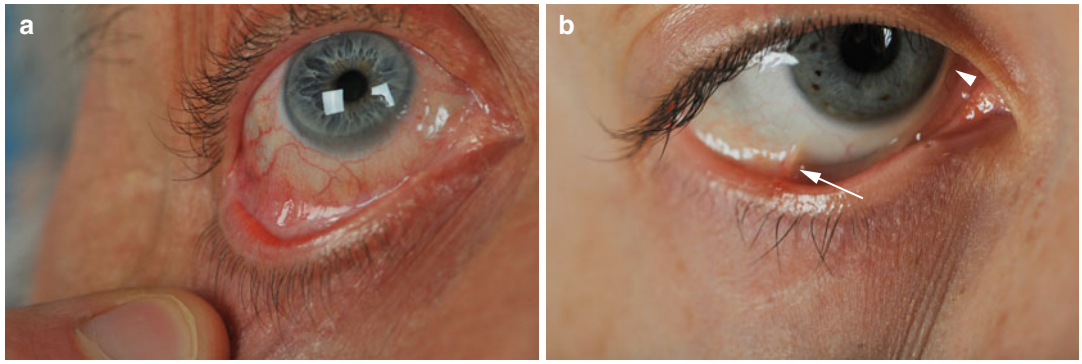

Fig. 15.2 Four clinical clues of ocular MMP: (**a**) conjunctivitis and fornix shortening, (**b**) symblepharon (*arrow*), and trichiasis (*arrowhead*)

(OCP), is defined as MMP with lesions of the conjunctiva. If the lesions are confined to the eyes, then the term is *pure* OMMP. Clinical severity is variable and can range from burning sensation of the eyes to scarring resulting in blindness. Early recognition of OMMP is of utmost importance because of the scarring potential phenotype.

Clinical Symptoms

OMMP usually starts unilaterally with a recurrent inflammatory process resulting in clinical features of dry eye, conjunctivitis, trichiasis, fornix shortening, symblepharon, and ankyloblepharon formation (Fig. 15.2). In the final stage of the disease, pannus occurs, total keratinization of the entire ocular surface, resulting in blindness when not treated accurately. In most cases, the disease is bilateral within 2 years.

Diagnosis Paths

The target antigen in OMMP is the 180-kDa antigen. In patients with OMMP, DIF (conjunctiva) is frequently the only positive assay and shows IgG and/or IgA in linear n-serrated pattern along the epidermal BMZ. These biopsies can be performed by the dermatologist or ophthalmologist. With high suspicion of OMMP and negative DIF conjunctiva, DIF of oral mucosa and IIF are recommended for diagnosis. IIF performed on 1 M NaCl-split skin substrate shows binding of antibodies to the epidermal site (roof) of the blister and by immunoblot analysis reveals immunoglobulin binding to the 180-kDa antigen.

Treatment Tricks

Mild OMMP is treated with dapsone, mycophenolate mofetil, or mycophenolic acid. Blepharitis should be treated with eyelid hygiene and topical tetracycline cream. In rapidly progressive OMMP with impending blindness, dexamethasone pulse therapy or systemic CS (1.0 mg/kg/day) in combination with cyclophosphamide should be the first choice of treatment. Consultation of the ophthalmologist is needed to evaluate the effect of treatment with slit-lamp examination. In refractory cases of OMMP, the anti-CD20 antibody rituximab, a single cycle of two infusions of 1000 mg, or intravenous immunoglobulin (IVIG) may induce remission. Surgical intervention like eyelash ablation or amniotic membrane transplantation can be performed when OMMP is in clinical remission.

<In rapidly progressive OMMP, aggressive therapy is needed to prevent cicatrization>

Localized Vulvar Pemphigoid

Short Definition in Layman's Terms

Localized vulvar pemphigoid (LVP) is a rare subtype of pemphigoid with solitary lesions in the

genital region. Findings at vulvar inspection can be very similar to lichen sclerosus and lichen planus. Full examination of the skin, mouth, eyes, and nasal mucosa is essential for adequate diagnosis.

Definitions and Classification

In classic MMP, woman can present with erosions and blisters at any mucosal surfaces. LVP is defined as pemphigoid limited to the cornified epithelium (skin) of the vulva and perineum. LVP can present at two different episodes in life, (i) in childhood, around 10 years, called juvenile or childhood LVP (Fig. 15.3), and (ii) at postmenopausal age, called adult LVP. Because of the similarity with lichen sclerosus and lichen planus, doctor's delay is frequently seen. On occasion, the disease is erroneously confused with sexual abuse.

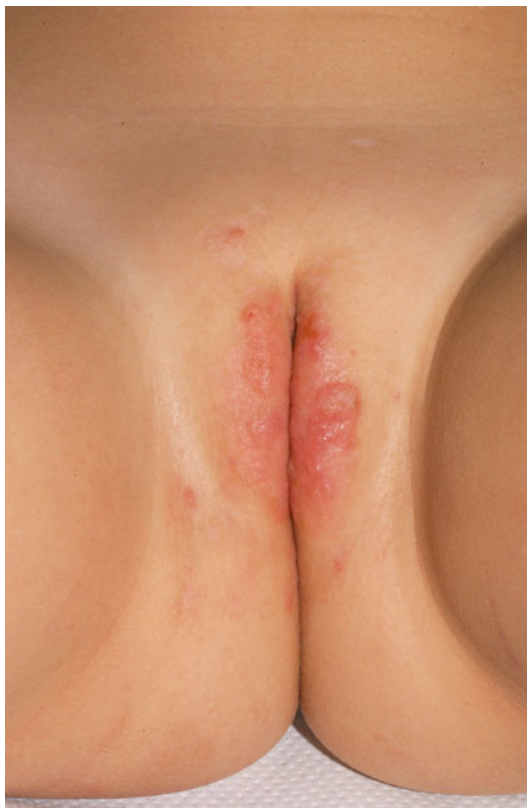

Fig. 15.3 A young girl with juvenile LVP, presenting with vulvar erosions

Clinical Symptoms

Patients may complain of vulvar itch, burning sensation, pain, dysuria, and, in adults, dyspareunia. Upon inspection of the vulvar erosions and ulceration with structural architectural changes (scarring), labial fusion and clitoral burial can be seen.

<LVP clinically resembles lichen sclerosus and lichen planus>

Diagnosis Paths

Histopathology in the early phase shows similarities with lichen sclerosus like subepidermal edema. At a latter phase, a subepidermal blister underneath with an infiltrate existing from lymphocytes eosinophils and/or neutrophils, with or without fibrosis, is seen. DIF shows IgG, IgA, and C3c depositions in the n-serrated pattern along the epidermal BMZ. IIF on monkey esophagus is often negative because the circulating autoantibodies usually have a low titer. IIF performed on 1 M NaCl-split skin substrate shows binding of antibodies to the epidermal site (roof) of the blister.

Treatment Tricks

Topical tetracycline cream is the first-line therapy. Superpotent topical corticosteroids (TC) can be used after failure of treatment. Dapsone is the treatment of choice when systemic treatment is needed.

Anti-laminin 332 Mucous Membrane Pemphigoid

Introduction

Short Definition in Layman's Terms

Anti-laminin 332 MMP (anti-LN-332 MMP) is a rare subtype of MMP that is difficult to distinguish from other forms of MMP at first sight. It is known for the scarring phenotype with airway

obstruction due to pharyngeal and laryngeal involvement or loss of vision because of subconjunctival fibrosis and cicatrization. Furthermore, patients have an increased relative risk for malignancy, especially adenocarcinoma [3]. Because of this clinical aggressive behavior, it is important to diagnose patients in an early phase of the disease.

Definitions and Classification

Domloge-Hultsch *et al.* were the first to describe an sAIBD with autoantibodies that bind epiligrin: anti-epiligrin cicatricial pemphigoid [4]. Epiligrin appeared to be a mixture of laminin 5, now named laminin 332 (LN-332), laminin-6 (LN-311), and laminin-7 (LN-321). LN-332 is a heterotrimeric protein consisting of α3, β3, and γ2 laminin subunits. Approximately 5–20 % of all MMP patients show circulating IgG autoantibodies against LN-332 [3].

<Anti-laminin 332 MMP is previously known as anti-epiligrin cicatricial pemphigoid>

Pathogenesis

Anti-LN-332 MMP is a form of MMP with circulating autoantibodies targeting LN-332. This protein is present in the lamina lucida of the basement membrane zone of keratinizing and nonkeratinizing stratified squamous epithelia and connects hemidesmosomes to anchoring fibrils by interlinking integrins α6β4 and BP180 to type VII collagen. In most patients, the IgG autoantibodies predominantly target the laminin α3 subunit, although IgG autoantibodies

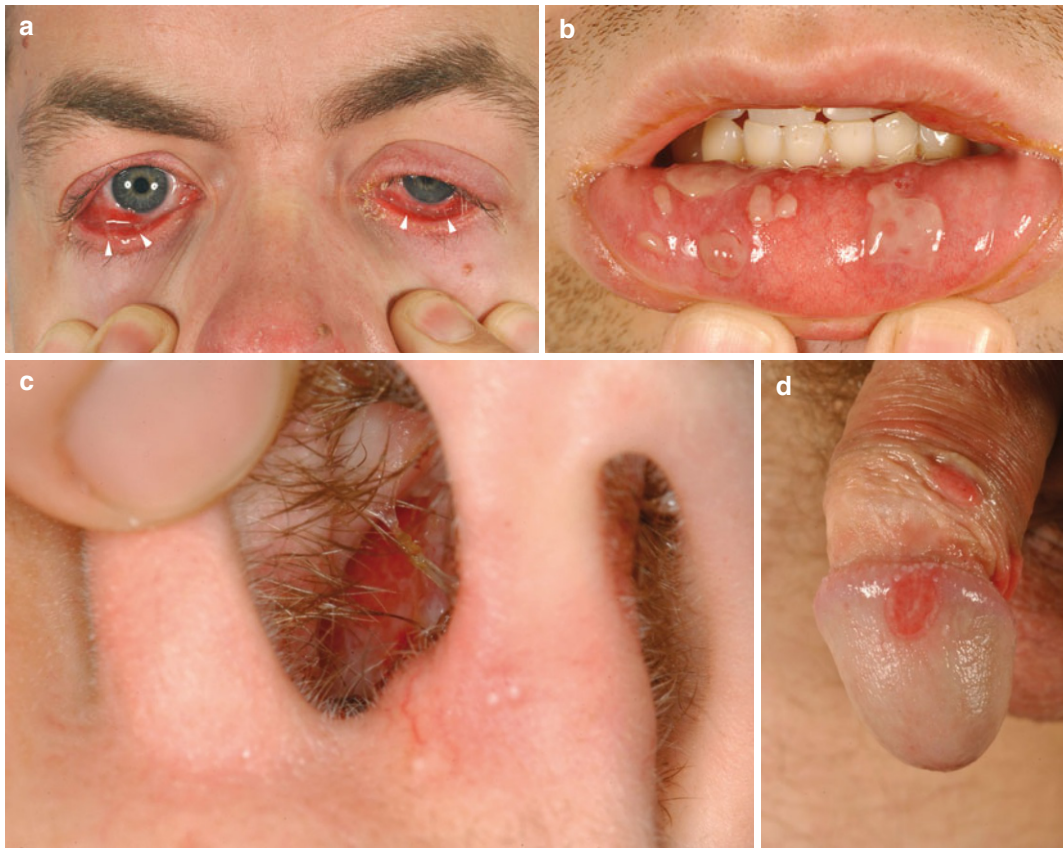

Fig. 15.4 Clinical features in a patient with anti-LN-332 MMP. (**a**) Conjunctivitis with symblepharon (*arrowheads*) and edema of the upper eyelid, (**b**) extensive blistering of the oral mucosa, (**c**) erosions on nasal mucosa, and (**d**) genital ulcers (Reprinted from Terra *et al.* [5] with permission from Wiley)

targeting the β3 or γ2 subunits have also been described [3].

Clinical Symptoms

Anti-LN-332 MMP mimics other forms of MMP and presents with involvement of the mucosal surfaces of the mouth, eyes, nasopharynx, oropharynx, larynx, and anogenital region (Fig. 15.4). In most patients, the skin is also involved but usually less severe. In some cases, the pharyngeal and laryngeal are the only regions involved (Fig. 15.5). Patients may present with aphonia (loss of voice) due to edema, erosions, and ulcerations of the supraglottic area. This is followed by scarring of the larynx, and acute upper airway obstruction due to initial laryngeal edema may occur, necessitating tracheotomy. In these patients, a doctor's delay is frequently seen because of ignorance of this autoimmune bullous disease.

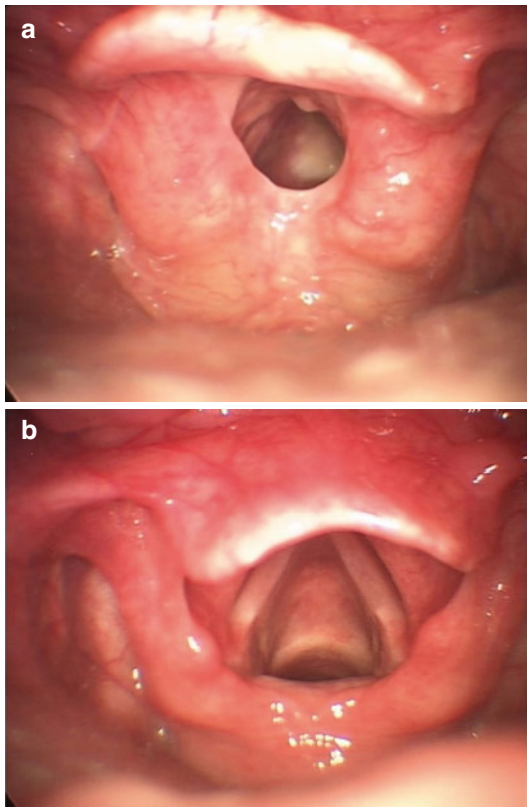

Fig. 15.5 Laryngeal cicatrization of the aryepiglottic folds with supraglottic stenosis (**a**), compared with healthy control (**b**). The ventral side is shown at the top. (Reprinted from Terra *et al.* [5] with permission from Wiley)

Diagnosis Paths

We developed a new algorithm (Table 15.2) to diagnose anti-LN-332 MMP based on the combination of clinical symptoms, state-of-the-art laboratory diagnostics, and multidisciplinary cooperation [5]. Patients with anti-LN-332 MMP should be screened for neoplasia. This occurs in approximately 20% of the patients, mostly adenocarcinoma.

<Because of the increased risk for malignancy, patients with anti-LN-332 MMP should be thoroughly oncologically screened>

Treatment Tricks

Patients with anti-LN-332 MMP must be treated promptly and adequately to achieve control of disease and to delay progression. A multidisciplinary approach is a necessity when multiple mucosal sites are affected. An intense standard cooperation with the ophthalmologist and otolaryngologist for these patients is needed.

Treatment used is always a combination of oral corticosteroids (prednisolone) and a steroid-sparing adjuvant. For acute crisis management, dexamethasone pulse therapy can be started. Dapsone and cyclophosphamide are preferred choice when ocular involvement is present. Other immunosuppressant drugs given include mycophenolate mofetil, mycophenolic acid, intravenous immunoglobulins, and rituximab.

Review Questions

1. What is the main target antigen in MMP?
   (a) BP180
   (b) BP230
   (c) LN332
   (d) Collagen VII
2. Rapidly progressive OMMP with impending blindness should be treated with:
   (a) Dapsone
   (b) Azathioprine
   (c) Cyclophosphamide
   (d) Mycophenolic acid

Table 15.2 Diagnostic criteria for anti-laminin 332 (anti-LN-332) mucous membrane pemphigoid

#	Criterium[a]
Major criteria	
1.	Subepithelial erosions or blisters on mucous membranes frequently associated with scarring phenotype
2.	Immunodepositions along the EBMZ in an n-serrated pattern by DIF
3.	IgG bound to the dermal side of 1.0 M NaCl-split human skin by IIF
Minor criteria	
1.	Anti-LN α3, β3, or γ2 IgG binding by immunoblot analysis on keratinocyte cell extract
2.	IgG reactivity to native LN-332 by ELISA
3.	Serum immunoprecipitation of LN-332 trimer
4.	Negative IIF on LN-332 knockout skin, while positive IIF on type VII collagen knockout skin
5.	IgG deposits in EBMZ overlay LN-332 by FOAM

EBMZ epidermal basement membrane zone, *DIF* direct IF, *IIF* indirect IF, *IgG* immunoglobuline G, *FOAM* fluorescence overlay antigen mapping

[a]To diagnose anti-LN-332 MMP, at least three major criteria or two major criteria and one minor criterion must be obtained

3. Specific diagnostics for anti-LN-332 MMP are:
   (a) DIF IgG u-serrated and SSS dermal binding
   (b) DIF IgG u-serrated and SSS epidermal binding
   (c) DIF IgG n-serrated and SSS dermal binding
   (d) DIF IgG n-serrated and SSS epidermal binding
4. First-line therapy of juvenile LVP consists of:
   (a) Topical superpotent corticosteroids
   (b) Topical tetracycline cream
   (c) Oral corticosteroids (0.5 mg/kg/day)
   (d) Dapsone
5. Which statement about MMP is incorrect?
   (a) Oral mucosa is affected in the majority of the patients.
   (b) Serration pattern analysis is often not possible on mucosal biopsies.
   (c) The titer of circulating autoantibodies in serum is frequently low and often not detectable.
   (d) Patients have an increased relative risk for malignancy, especially adenocarcinoma.

Answers

1. (a)
2. (c)
3. (c)
4. (b)
5. (d)

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Epidermolysis Bullosa Acquisita

16

Marcel F. Jonkman

Abstract

Epidermolysis bullosa acquisita (EBA) is a form of pemphigoid which may come with scarring that looks like dystrophic epidermolysis bullosa hereditaria. This subtype with scarring is named mechanobullous EBA, because blisters are evoked by sudden mechanical trauma, such as hitting the back of the hand to the edge of a table. The other subtype with erythematous lesions without scarring is named inflammatory EBA and may look like bullous pemphigoid. The mucous membranes can be involved in both subtypes. The pathogenesis is mediated by IgG or IgA against type VII collagen, which is the component of anchoring fibrils below the lamina densa. Diagnosis is confirmed by detecting u-serrated linear pattern of immune depositions with direct immunofluorescence microscopy. The pathogenesis of both clinical subtypes is unknown and is not related to binding of a particular epitope of the autoantigen. EBA is associated with systemic lupus erythematosus and colitis ulcerosa. The disease is relative refractory to treatment.

Keywords

Hemidesmosome • Immunoglobuline • Basement membrane • Autoimmune disease • Vesiculobullous disease • Pemphigoid

Introduction and AIMS

Short Definition in Layman Terms

Epidermolysis bullosa acquisita (EBA) means acquired, not inherited, bullous loosening of the skin. The disease is evoked by antibodies against a component of the skin that attaches the upper part like Velcro to the bottom part. The original cause of this autoimmune disease is unknown.

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Patients with EBA complain of skin blisters after minor bumping or of red spots that come spontaneously. There is no cure, but the disease can be kept quit with medical drugs that alter immunity.

Learning Objectives
After reading this chapter, you know the clinical forms of EBA, understand the pathogenesis, and are aware how to make the diagnosis. You also can make a treatment proposition that fits with the disease subtype and the patient needs.

Case Study: Part 1
A 36-year-old woman attracted blisters on her feet after jogging. Later she spontaneously developed also blisters on the shoulders and abdomen. The lesions were painful and itchy. At physical examination tense blisters on normal skin on both sites of the hands and feet and on the extensor surface of the elbows were seen.

Didactical Questions: Cross Section of Questions to Prime the Readers' Interest

Why is EBA included in the pemphigoid spectrum? What determines the clinical subtype? What is the blister level considering that the immune deposits are so low in the basement membrane zone? How do you make the diagnosis? And what options do we have for this treatment refractory disease?

Facts and Figures

Definitions and Classification

Epidermolysis bullosa acquisita (EBA) is a subepidermal autoimmune bullous disease (sAIBD) characterized by autoreactivity to type VII collagen located in the anchoring fibrils in the epidermal basement membrane zone (EBMZ) [1]. EBA is a clinically heterogeneous disease that may be characterized by either a mechanobullous or an inflammatory phenotype (Fig. 16.1).

Epidemiology

The estimated EBA annual incidence in central European countries is about 0.25 new cases/million/year. The frequency of EBA among patients with sAIBD is 5.5 %. EBA may occur at any age and occurs in both children and adults. A gender preference of females exists of 2.2. The ratio of the mechanobullous and inflammatory phenotypes of EBA is 1:2 [2].

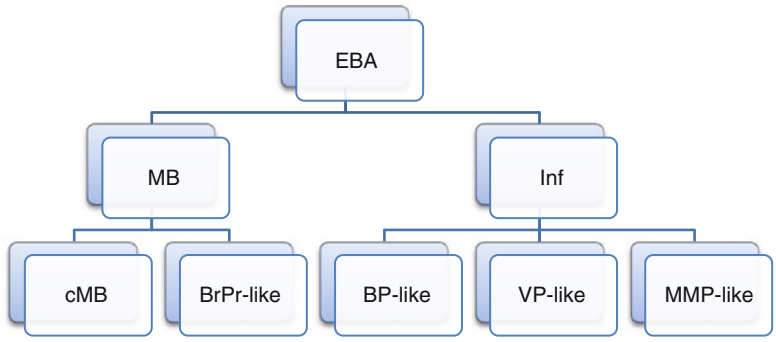

Fig. 16.1 Clinical phenotypes of epidermolysis bullosa acquisita. *EBA* epidermolysis bullosa acquisita, *MB* mechanobullous, *Inf* inflammatory, *cMB* classic mechanobullous, *BrPr-like* Brunsting-Perry-like, *BP-like* bullous pemphigoid-like, *VP-like* vesicular pemphigoid-like, *MMP-like* mucous membrane pemphigoid-like

Pathogenesis

The main autoantibody isotype in EBA is IgG, predominantly IgG1 and IgG4 subclass, but deposits of IgA and complement may also be found along the epidermal BMZ. Most EBA patients' sera react with epitopes located within the noncollagenous (NC)-1 domain of human type VII collagen (Fig. 16.2). Binding of patient autoantibodies to the collagenous or the NC-2 domain is rarely observed. No correlation is detected between antibody specificity to type VII collagen subdomains and clinical phenotype (mechanobullous/inflammatory).

Split formation is dependent on activation of neutrophils through the Fc domain of immunoglobulin. Split formation occurs in most cases at the level of the lamina lucida, and not at the level of the anchoring fibrils in the sublamina densa zone. These split formations within the lamina lucida most likely represent the intra-lamina lucida-separating effects of leukocyte-derived proteolytic enzymes, when such cells are chemoattracted to the dermoepidermal junction by bound immunoreactants.

Diagnosis Paths

History and Physical Examination

The classic mechanobullous phenotype mimics dystrophic epidermolysis bullosa hereditaria, while mild cases look like acral blistering of porphyria cutanea tarda that heal with atrophic scarring, milia, and hypo- or hyperpigmentation (Fig. 16.3). Scalp, neck, and shoulder involvement occurs in 20 % and leads to extensive nonhealing erosions with scarring, reminiscent of Brunsting-Perry pemphigoid (Fig. 16.4).

The inflammatory phenotype presents with widespread vesicles and bullae involving intertriginous and flexural areas that heal with no or few milia without scars (Fig. 16.5). This phenotype comprises a bullous pemphigoid-like presentation with a widespread inflammatory vesiculobullous eruption involving the trunk and extremities or skinfolds and a presentation with predominantly mucosal involvement reminiscent of mucous membrane pemphigoid with scars on the mucosal surfaces (Fig. 16.6). EBA is associated with SLE and inflammatory bowel disease.

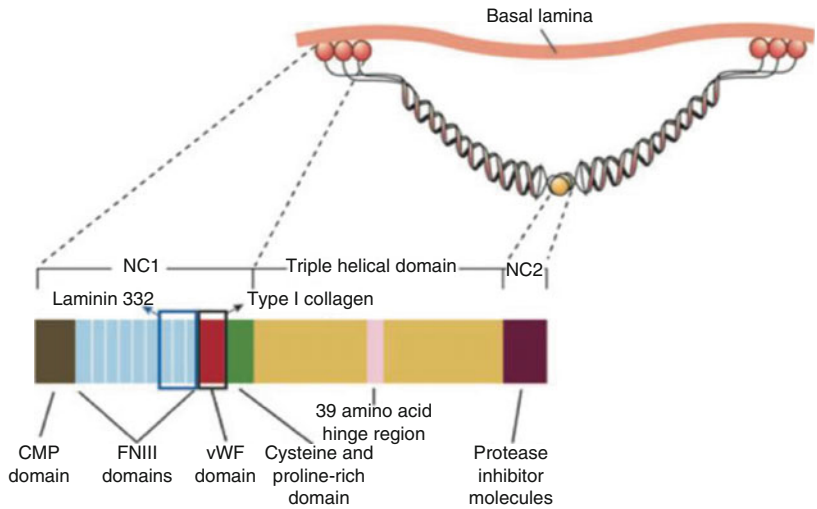

Fig. 16.2 Diagram of type VII collagen shows the immunodominant NC-1 domain. *N* aminoterminal, *C* carboxyterminal, *CMP* cartilage matrix protein, *FNIII* fibronectin type III-like repeats, *VWFA* a domain of von Willebrand factor, *NC-1* noncollagenous aminoterminal domain, *NC-2* noncollagenous carboxy-terminal domain (Reprinted from Kim and Kim [6]. © 2013 European Academy of Dermatology and Venereology, with permission from Wiley)

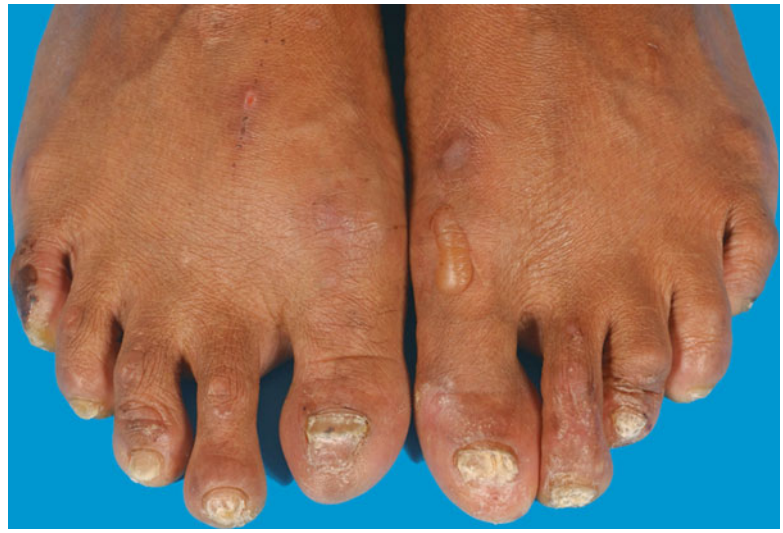

Fig. 16.3 Mechanobullous EBA in a young female showing bullae and crusts and nail dystrophy on the feet

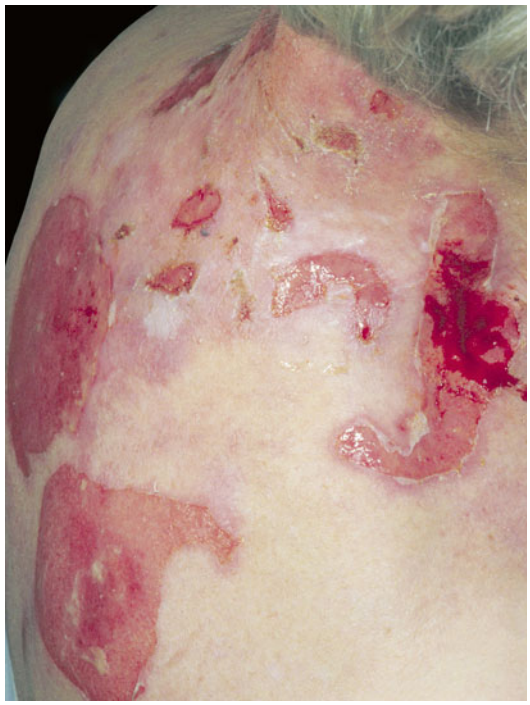

Fig. 16.4 Brunsting-Perry-like mechanobullous EBA in a female with affected neck and scalp

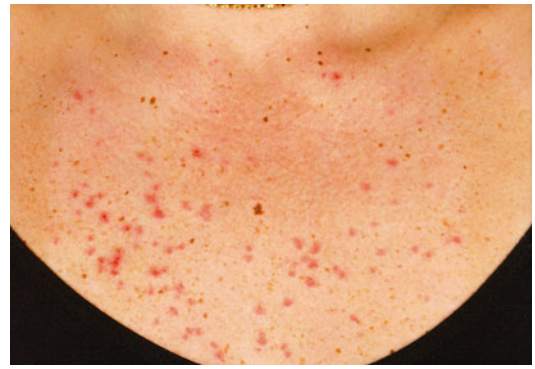

Fig. 16.5 Inflammatory EBA in a 37-year-old female showing lenticular erythematous papules with erosive top on the breast

General Diagnostics

Transition from mechanobullous to inflammatory phenotype or vice versa is sometimes found. This may account for a temporary flare of widespread inflammatory bullae that was noticed in the mechanobullous phenotype during an exacerbation in a minority of patients. Vice versa, development of extensive milia formation and scarring were not seen any of the inflammatory phenotyped patients.

Specific Diagnostics

Diagnosis can be made by indirect immunofluorescence microscopy (IIF) performed on 1 M NaCl-split skin (SSS) substrate showing binding of antibodies to the dermal site (floor) of the

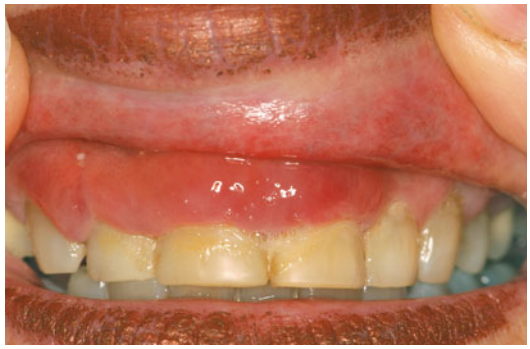

Fig. 16.6 Mucous membrane pemphigoid-like EBA in a female with hypertrophic gingiva

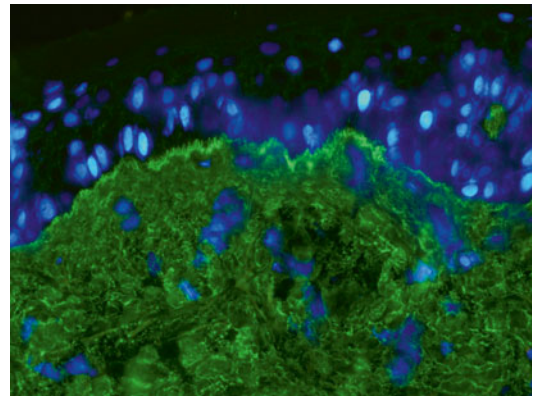

Fig. 16.7 U-serrated linear immunodeposition (IgG) along the EBMZ diagnostic for EBA or BSLE

blister and by immunoblot analysis revealing immunoglobulin binding to the 290 kDa antigen. The newly developed type VII collagen ELISA has a sensitivity of 45 %. Combining SSS and ELISA reaches a sensitivity of 50 %. This means that half of the patients with EBA are seronegative [3].

In serological negative cases, direct immunofluorescence (DIF) on sodium chloride-separated skin biopsy might reveal the diagnosis. More simply, the diagnosis can be reached through serration pattern analysis by DIF, revealing linear u-serrated immunodepositions along the epidermal BMZ, which proofs for the diagnosis of EBA [4]. DIF serration pattern analysis by n-versus-u can be learned on the website (Fig. 16.7).

Case Study: Part 2

Skin biopsy for direct IF revealed linear depositions of IgG 4+, IgA 3+, and C3c 1+ in a u-serrated pattern along the epidermal basement membrane zone. Examination of the serum by indirect IF was positive for IgG 2+ and IgA 1+ in the floor of salt-split skin. Indirect IF on knockout skin was negative on type VII collagen-deficient skin, whereas positive on laminin 332-deficient skin. The type VII collagen ELISA index of IgG was 137 (positive). ANA titer was 1:160 and DNA Farr <3 IU/ml. A diagnosis was made of epidermolysis bullosa acquisita, mechanobullous type.

Treatment Tricks

Initial Treatment and Escalator

EBA is a chronic disease that is often refractory to many treatment modalities. The first-line therapy for EBA is a combination of low-dose corticosteroids, colchicine, or dapsone. Colchicine is often used as a first-line drug because of its low incidence of serious side effects at a dose of 0.5–1 mg/day [5]. Dapsone is prescribed at a dose of 25 mg/day that is gradually increased to 100 mg/day. As corticosteroid one can choose prednisolone 30 mg/day or methylprednisolone 8–16 mg/day. Other immunosuppressive agents may be considered: mycophenolate mofetil, azathioprine, methotrexate, and cyclophosphamide. In refractory cases of mechanobullous EBA, intravenous immunoglobulin (IVIG) in a dose of 2 g/kg/month in 4–5 regular infusions might reduce the blister frequency. Recently a new drug has become available: anti-CD20 antibody rituximab. This biologic drug is effective in some intractable cases of EBA at a single cycle of two infusions of 1000 mg or four infusions of 375 mg per square of height in meters [6].

Follow-Up and Tapering

The median time to remission of patients with EBA is 9 months. The long-term prognosis of patients with EBA has proven excellent, and most live a normal life on low-dose medication [6].

Case Study: Part 3

First-line therapy with prednisolone 30 mg and azathioprine 150 mg was insufficient. Subsequently, she received for 1 year human intravenous immunoglobulin 2 g/kg/month in three gifts per month in home setting. The blister frequency then reduced. Minimal therapy consisted the last 6 years of prednisolone 7.5 mg and azathioprine 150 mg.

Review Questions

1. In which domain are the immunodominant epitopes of type VII collagen?
   (a) NC1
   (b) Collagenous
   (c) NC2
2. The most frequent clinical phenotype of EBA is
   (a) Mechanobullous
   (b) Inflammatory
   (c) (a) and (b) are equal frequent
3. What is the split level of the blisters in EBA?
   (a) Basal cells
   (b) Lamina lucida
   (c) Lamina densa
   (d) Sublamina densa
4. First-line treatment of EBA are
   (a) High-dose corticosteroids
   (b) Colchicine and dapsone
   (c) Rituximab infusions
5. Which disease or syndrome is associated with EBA?
   (a) Atopic syndrome
   (b) Neoplasia
   (c) Inflammatory bowel disease

Answers

1. (a)
2. (b)
3. (b)
4. (b)
5. (c)

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Bullous Systemic Lupus Erythematosus

17

Marcel F. Jonkman

Abstract

Bullous systemic lupus erythematosus (BSLE) is a confusing concept. With BSLE type I, an autoimmune response to type VII collagen is mend, leading to epidermolysis bullosa acquisita in patients with systemic lupus erythematosus (SLE). If no autoimmune reaction to type VII collagen is demonstrable, BSLE type II is mend, which consists of an acute generalized hemorrhagic vesiculobullous eruption in SLE. Vesicular eruptions may also occur rarely in subacute cutaneous lupus erythematosus due to severe inflammatory reaction with subepidermal clefting, which in extreme cases may resemble erythema multiforme (Rowell syndrome) or toxic epidermal necrolysis.

Keywords

Lupus erythematosus • Epidermolysis bullosa acquisita • Type VII collagen

Introduction and AIMS

Short Definition in Layman Terms

Bullous systemic lupus erythematosus (SLE) is a condition when blisters occur in patients with SLE. Mostly it is an autoimmune disease against type VII collagen and as such resembles epidermolysis bullosa acquisita (EBA). Conversely, blistering may be caused by an undefined acute, severe inflammatory skin reaction in SLE.

Learning Objectives

After reading this chapter, you understand the clinical and immune-histological presentations, differential diagnosis, and treatment options of vesiculobullous eruptions in bullous systemic lupus erythematosus (BSLE).

Case Study: Part 1

A black female, 22 years old, had an acute generalized vesiculobullous eruption that was

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controlled with systemic corticosteroids. One year before, a vascular hemiplegia was reversed with aspirin. Two years after the skin eruption, she started having blisters on the extremities after bashing her skin. Raynaud's phenomenon was present. Blood tests revealed leucopenia, ANA 1:640, Coombs, nRNP, SSA, and lupus anticoagulant all positive. A diagnose of SLE was made that was controlled with prednisolone 7.5 mg.

Table 17.1 Diagnostic criteria for BSLE (including types I and II)

1. A diagnosis of SLE based upon American Rheumatism Association (ARA) criteria
2. Vesicles and bullae arising upon but not limited to sun-exposed skin
3. Histopathology compatible with dermatitis herpetiformis
4. Negative or positive indirect IF for circulating BMZ antibodies using separated human skin as substrate
5. DIF of lesional and non-lesional skin revealing linear or granular IgG and/or IgM and often IgA at the BMZ. Immunoelectron microscopy would demonstrate the immune reactants below the basal lamina

Adapted from Camisa and Sharma [1] and revised Camisa and Grimwood [2]
BSLE bullous systemic lupus erythematosus, *BMZ* basement membrane zone

Didactical Questions: Cross Section of Questions to Prime the Readers' Interest

What is the direct IF of skin biopsy in BSLE? What autoantigen is involved in BSLE?

Facts and Figures

Definitions and Classification

The diagnostic criteria for BSLE are listed in Table 17.1. Two immunologically distinct subtypes of BSLE are recognized: type I with and type II without anti-type VII collagen antibodies (Table 17.2).

Table 17.2 Classification of blistering in lupus erythematosus

Type	Presentation
BSLE, type I	Bulla with milia and scarring or only with erythematous macules
BSLE, type II	Acute generalized blistering with systemic symptoms
Bullous CLE	Circinate vesicles on annular plaques
TEN-like CLE	Acute or subacute diffuse dusky erythema with positive pseudo-Nikolsky's and Asboe-Hansen's signs, with initial photodistribution, and no identification of culprit drug
TEN in SLE	Acute erythema with positive pseudo-Nikolsky's and Asboe-Hansen's signs and identification of culprit drug

BSLE bullous systemic lupus erythematosus, *ANA* antinuclear antibodies, *CLE* cutaneous lupus erythematosus, *SLE* systemic lupus erythematosus, *TEN* toxic epidermal necrolysis

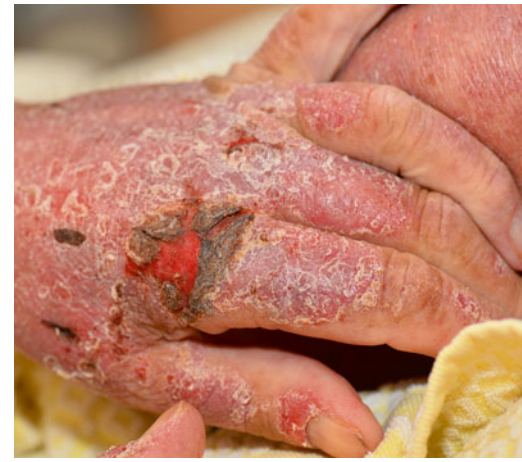

Fig. 17.1 Bullous lesion in erythemato-squamous plaques due to subacute cutaneous lupus erythematosus in a patient with SLE

Also blistering due to severe local inflammatory response occurs in subacute cutaneous lupus erythematosus (SCLE) with or without SLE (Fig. 17.1).

Epidemiology

BSLE typically affects young adults and starts in the second or third decade of life.

Pathogenesis

BSLE type I is caused by autoantibodies against type VII collagen. The pathogenesis is similar to that of epidermolysis bullosa acquisita (EBA) (see Chap. 16). The typical histopathology is a subepidermal vesicle and neutrophilic microabscesses at the papillary tips, indistinguishable from dermatitis herpetiformis.

BSLE type II is caused by a severe vacuolar alteration of the dermoepidermal junction, dermal edema, and sometimes leukocytoclastic vasculitis. Bullous cutaneous LE is caused by apoptotic epidermal changes that when fulminant may resemble toxic epidermal necrolysis.

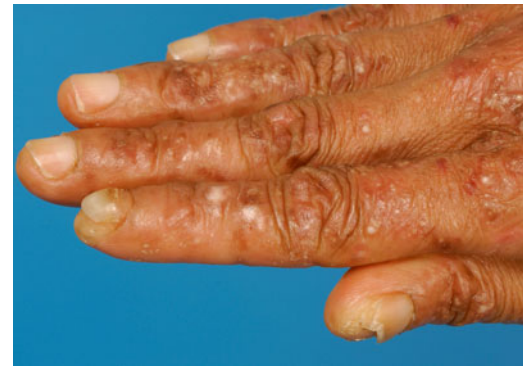

Fig. 17.2 Bullous systemic lupus erythematosus, type I, shows monomorphic blisters, skin atrophy, milia, and nail dystrophy of the hand dorsum

Diagnosis Paths

History and Physical Examination

The clinical presentation of bullous SLE is generally that of acute-onset, generalized blistering eruption in SLE patients. Patients with BSLE type II may present with fever. Sun exposure may elicit the condition.

General Diagnostics

Patients with BSLE exhibit features of SLE including malar erythema, cutaneous lupus erythematosus, oral erosions, and photosensitivity. Serology reveals positive ANA, with Sm and dsDNA antibodies. Direct IF of unaffected skin might reveal a so-called lupus band, granular or homogenous depositions of IgM, IgG, IgA, or complement along the BMZ. In case of SCLE, SSA antibodies can be detected, which are visible by direct IF of patient's skin as in vivo ANA.

At dermatological examination, one may find a clinical presentation similar to chronic mechanobullous EBA (Figs. 17.2 and 17.3). This condition always fits BSLE type I [3].

The clinical presentation may also look like inflammatory EBA, although more acute, generalized, with multiple vesicles on erythematous patches and with malar erythema. This condition may fit BSLE types I or II. In case of cutaneous LE in patients with SLE, the presentation may be acute (and not distinguishable from BSLE type II) or subacute similar to vesicular SCLE. Mucous membranes may be involved. If the skin detaches in large sheets, it may look like erythema multiforme (formerly known as Rowell's syndrome) or toxic epidermolytic necrolysis (TEN).

Specific Diagnostics

The distinction between BSLE type I or type II is made by IF of skin and serum (Table 17.3) [4].

ANA is not a specific finding, since it may be present in skin or serum in all SLE patients. Epidermal in vivo ANA is typically found in lesional and non-lesional SCLE skin (Fig. 17.4). A diagnosis of TEN is made by histopathology of erythematous skin showing transepidermal necrosis.

Case Study: Part 2

At age of 31 years, she was referred to us. Dermatological examination is shown in Figs. 17.2 and 17.3. Direct IF is shown in Fig. 17.4. By indirect IF no binding of IgG and IgA was found to human split skin, and immunoblot was negative for IgG and IgA. A diagnosis was made of BSLE type I based on the linear, u-serrated, pattern of IgG and C3c deposition.

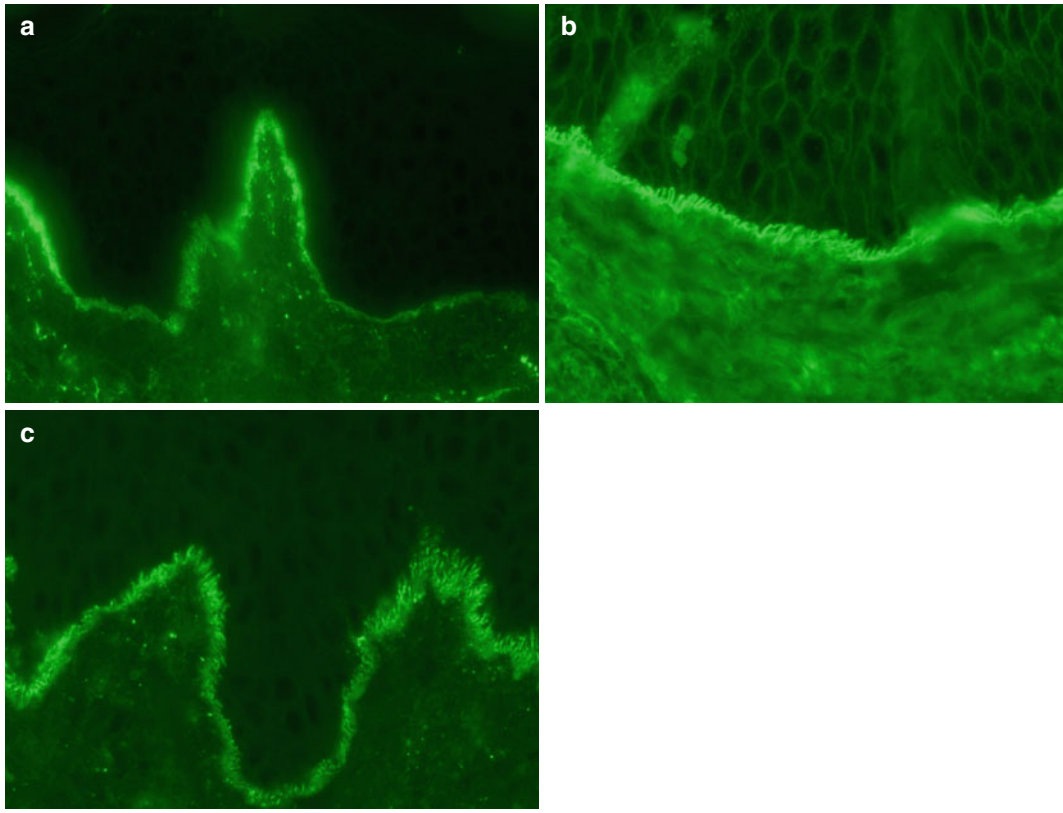

Fig. 17.3 DIF of sun-exposed healthy skin (dorsum hand) in BSLE reveals fibrin in the high papillary blood vessels. Along the BMZ exists a broad homogenous and granular (**a**) IgM (2+) deposition and a linear deposition in u-serrated pattern of (**b**) IgG (3+) and (**c**) C3c (+)

Table 17.3 Typing of blistering in lupus erythematosus by immunofluorescence analysis

Type	DIF	IIF split human skin
BSLE, type I (a) EBA, mechanobullous (b) EBA, inflammatory	Linear, u-serrated, immunodepositions along BMZ	Dermal binding
BSLE, type II	Granular/homogeneous immunodepositions along BMZ	ANA
Bullous SCLE	Epidermal in vivo ANA	Negative
TEN-like (S)CLE	Epidermal in vivo ANA	Negative
TEN in SLE	Granular/homogeneous immunodepositions along BMZ	ANA

DIF direct immunofluorescence microscopy, *IIF* indirect immunofluorescence microscopy, *BSLE* bullous systemic lupus erythematosus (LE), *ANA* antinuclear antibodies, *SCLE* subacute LE, *SLE* systemic LE, *TEN* toxic epidermal necrolysis, *BMZ* epidermal basement membrane zone

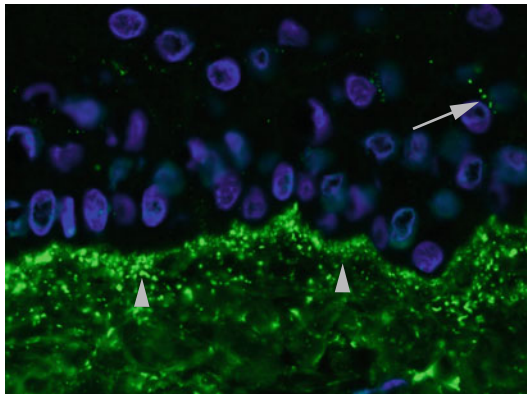

Fig. 17.4 DIF of bullous subacute cutaneous lupus erythematosus in a patient with SLE reveals granular deposition of IgG along the BMZ (*arrowheads*) and sparse intracellular antibodies (*arrow*) to epidermal cells (in vivo ANA)

Treatment Tricks

Initial Treatment and Escalator

The acute forms of BSLE may respond dramatically to dapsone. For acute cutaneous LE, systemic corticosteroids might be necessary.

Chronic forms of BSLE may better respond to systemic corticosteroids with an additional immunosuppressive agent, such as azathioprine (1–2 mg/kg) or mycophenolate mofetil (2 g). Rituximab is an option in EBA/BSLE, although it is not labelled for SLE.

Follow-Up and Tapering

Many patients with chronic BSLE need long-term low-dose prednisolone (<10 mg). Continuation of an immunosuppressive agent is therefore advised. First-line therapy for subacute and chronic cutaneous LE is topical potent corticosteroids and hydroxychloroquine (2 × 200 mg). Colchicine is a therapeutic option for treatment of neutrophil-mediated bullous diseases and may be used in chronic mechanobullous BSLE type I.

Case Study: Part 3
The recurrent blistering improved by continuous daily medication of prednisolone 7.5 mg, azathioprine 50 mg, and hydroxychloroquine 200 mg.

Review Questions

1. What is the most common location of acute BSLE?
   (a) Temples
   (b) Upper trunk
   (c) Hands and feet
   (d) Genitals
2. The immunodominant domain of type VII collagen in BSLE is the
   (a) NC-1 domain
   (b) Collagenous domain
   (c) NC-2 domain
3. A diagnosis of BSLE type II is made in a patient with SLE if indirect IF is negative, and direct IF shows the depositions at the BMZ in the following pattern
   (a) Linear, n-serrated
   (b) Homogenous
   (c) Granular and linear, u-serrated
   (d) None
4. First-line treatment of dermatitis-herpetiformis-like acute BSLE is
   (a) Superpotent topical corticosteroids
   (b) Systemic corticosteroids
   (c) Azathioprine
   (d) Dapsone

Answers

1. (b) Upper trunk. The hand and feet are commonly affected in chronic BSLE.
2. (a)
3. (b)
4. (d)

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Linear IgA Disease

18

Barbara Horváth and Marcel F. Jonkman

Abstract

Linear IgA disease (LAD) is a group of heterogeneous autoimmune subepidermal bullous diseases characterized by exclusively IgA autoantibodies targeting a component of the epidermal basement membrane zone. The lamina lucida-type LAD, also known as chronic bullous dermatosis of childhood, is a rare disease yet the most common autoimmune bullous disease among children with a peak incidence at 4–5 years of age. The major target antigens in lamina lucida-type LAD are 120 kDa LAD-1 antigen and its carboxyterminal proteolysed form 97 kDa LAD antigen 1 (LABD97). Both proteins are produced by cleavage of the extracellular domain of BP180, one of the main structural components of the hemidesmosome. The autoantigen targeted by IgA in the sublamina densa-type LAD, also known as IgA epidermolysis bullosa acquisita, is type VII collagen. In adults, LAD may also be drug induced.

Keywords

Linear IgA disease (LAD) • Immunoglobuline A (IgA) • Chronic bullous dermatosis of childhood (CBDC) • Hemidesmosomes • Basement membrane zone • Dapsone

Introduction and AIMS

Short Definition in Layman Terms

Linear IgA disease (LAD) is an itchy blistering skin disease with grouped vesicles on erythematous patches caused by linear IgA depositions in the epidermal basement membrane zone (EBMZ).

<LAD is characterized by exclusively IgA autoantibodies targeting components of EBMZ>

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Learning Objectives
After reading this chapter, you will be familiar with the clinical presentation of LAD and you will be able to setup a diagnostic algorithm and to propose a therapeutic plan.

Case Study: Part 1
A 44-year-old female presented with itchy erythematous plaques with tense blisters, erosions, and crusts at the periphery, on the head, trunk, and extremities. She developed erosions at the vaginal mucosa, but other mucosal surfaces were spared. Previous medical history reported primary Sjögren's syndrome and idiopathic hyperhidrosis. There was no medication previously administered.

Didactical Questions: Cross Section of Questions to Prime the Readers' Interest

How can you diagnose LAD? What would you see in the histopathological section? How can you make the difference between LAD and other autoimmune bullous diseases? What is the drug of choice for LAD?

Facts and Figures

Definitions and Classification

LAD is a heterogeneous group of subepidermal autoimmune bullous diseases characterized by autoantibodies exclusively from the IgA class targeting different antigens of the EBMZ. According to age LAD can be divided in juvenile and adult forms, which differ slightly in their clinical presentations but share common immunopathological features.

According to the splitting, there are two different forms of LAD. In the lamina lucida-type LAD, the two most common antigens are the 120 kDa molecular mass LAD-1 antigen and the 97 kDa molecular mass LAD antigen 1 (LABD97) with BP180 being recognized by a minor subset. Laminin 332 and p200 are rare autoantigens in lamina lucida-type LAD [1]. In the sublamina densa-type LAD, also called IgA epidermolysis bullosa acquisita (EBA), the splitting level is deeper and the target antigen is type VII collagen (Chap. 16).

<The LAD autoantigens are LAD-1, LABD97, BP180, laminin 332, p200, and type VII collagen>

Epidemiology

LAD is a rare disease with an estimated incidence rate of 0.23–0.67/million/year depending of the geographical region [2]. The distribution in the population is biphasic affecting primarily young children at age of 4–5 years and adults in the fifth decade. The childhood form seems to be chronic but self-limiting with disease duration of 1–5 years [3]. The adult form is more chronic and recalcitrant to different treatments.

Although LAD is mostly idiopathic, there are some known triggers such as drugs (mostly reported vancomycin), malignancies, UV light, and internal diseases (ulcerative colitis, collagen diseases). For drug-induced LAD, we refer to Chap. 19.

<LAD or chronic bullous dermatosis of childhood is the most common autoimmune bullous disease in children>

Pathogenesis

The two most common LAD antigens are LAD-1 and LABD97 (Fig. 18.1). Both are cleaved from the extracellular domain of BP180 by ADAM9 and -10 and plasmin, respectively. The first cleavage is just in the NC16A domain of the BP180 (Fig. 18.1), and this explains that only 20 % of the sera of LAD patients react with the NC16A domain [1]. IgA is a chemoattractant of neutrophilic granulocytes, which leads to blister formation. In an animal model with passive transfer of murine IgA, monoclonal antibodies against LAD autoantigens in an SCID mouse resulted in subepidermal vesicle formation with neutrophil influx [4].

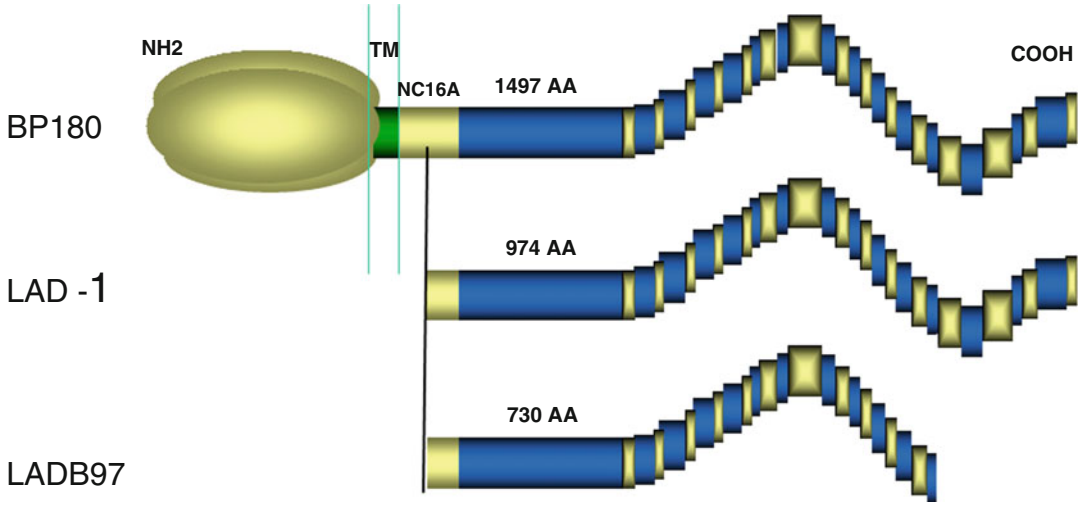

Fig. 18.1 Diagram of BP180 and shed derivates of its extracellular domain: LAD-1 and LABD97 proteins. BP180 is a transmembrane protein of the hemidesmosome containing an intracellular (N-terminus), transmembrane, and extracellular (C-terminus) domain. The extracellular domain consists 15 collagenous domains (C1-15) at the C-terminus end and a noncollagenous NC16A domain downstream from the transmembrane domain (TM). LABD97 is a proteolytic product of the extracellular domain containing 1209 amino acids and the N-terminus is just within the domain 3 of the NC16A. The N-terminus of LAD-1 seems to be near the N-terminus of LABD97 but the C-terminus is the same as in the full BP180 protein (1497 amino acid)

Diagnosis Paths

History and Physical Examination

According to the biphasic population distribution, LAD has different phenotypes in adults and in children; the latter was previously called chronic bullous dermatosis of childhood (CBDC). In adults some cases resemble dermatitis herpetiformis (DH) with pruritic papulovesicular eruption on the extensor surfaces. The unique presentations of LAD are tense circinate vesicles and blisters on urticarial plaques on the trunk and limbs. The blistering in LAD is more grouped peripheral (circinate) to the plaques, unlike BP where blistering is more to the spread over the urticarial plaque. The circinate configuration forms a ring, a "crown of jewels" (Fig. 18.2) or more serpiginous, a "string of pearls" (Fig. 18.3b) [5]. In children the predilection sites are legs, lower arms, and genitals (Fig. 18.3) or localized and exclusively on the lower eyelid (Fig. 18.4). Mucous membrane involvement occurs up to 80 % of cases.

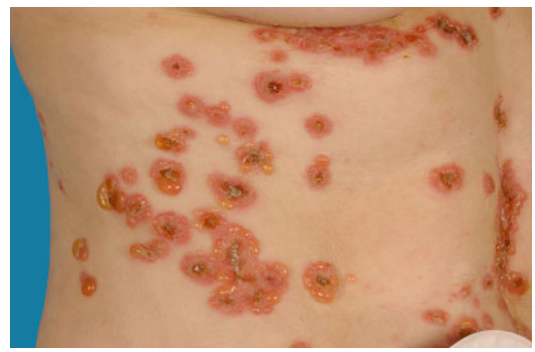

Fig. 18.2 Linear IgA disease in an adult presenting circinate grouped vesicles and bullae ("crown of jewels") on the abdomen

Moreover, drug-induced cases are more atypical in the clinics with more severe course, especially cases mimicking toxic epidermal necrolysis (TEN) [6]. Careful medical history according to medication in the last weeks is mandatory.

Several cases report associations with other diseases as hematological malignancies (Hodgkin's disease and B-cell lymphomas), different solid cancers (esophagus and bladder), and other autoimmune diseases as SLE, multiple sclerosis, dermato-

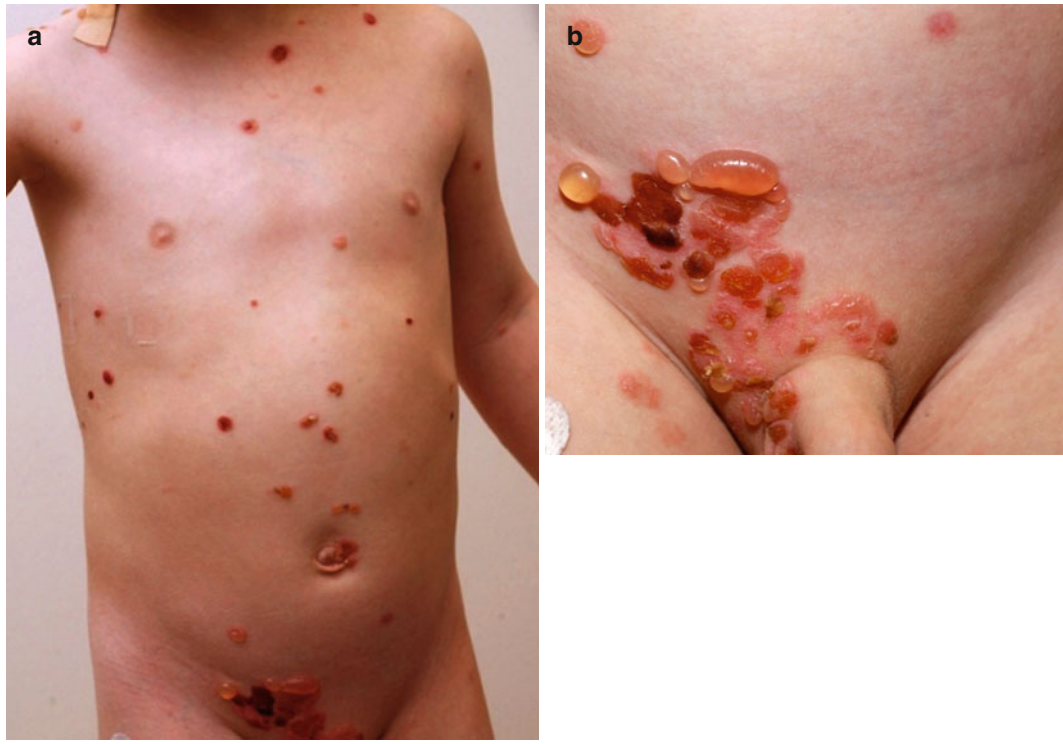

Fig. 18.3 (**a**) Juvenile linear IgA disease in a young boy presenting with serous bullae and hemorrhagic crusts on the trunk, and (**b**) a serpiginous configuration in pubic and genital area ("string of pearls")

Fig. 18.4 Solitary vesicle on lower eyelid in a boy with localized linear IgA disease

myositis, rheumatoid arthritis, Sjögren's syndrome, and Crohn's disease. Whether these are true associations or coincidences needs confirmation [5].

<In juvenile LAD, the skin lesions are located on the legs, lower arms, and perineum in a configuration known as a "crown of jewels">

Another presentation with grouped vesicles in circinate configuration and arciform erythema resembles linear IgA disease and is called IgA epidermolysis bullosa acquisita (IgA-EBA) with exclusively IgA deposits along the epidermal BMZ. IgA-EBA patients had widespread or localized vesicles, mostly without larger bullae formation (Fig. 18.5). Mucosal involvement is present in five out of eight patients with vesicular pemphigoid-like IgA-EBA but scarring of mucosal surfaces is absent.

General Diagnostics

Histological examination of perilesional and lesional skin shows subepidermal blister formation with predominantly neutrophil infiltrate in the papillary dermis with occasionally some eosinophils or mononuclear cells.

Specific Diagnostics

The gold standard for the diagnostics of LAD is the linear deposition of IgA along the EBMZ by

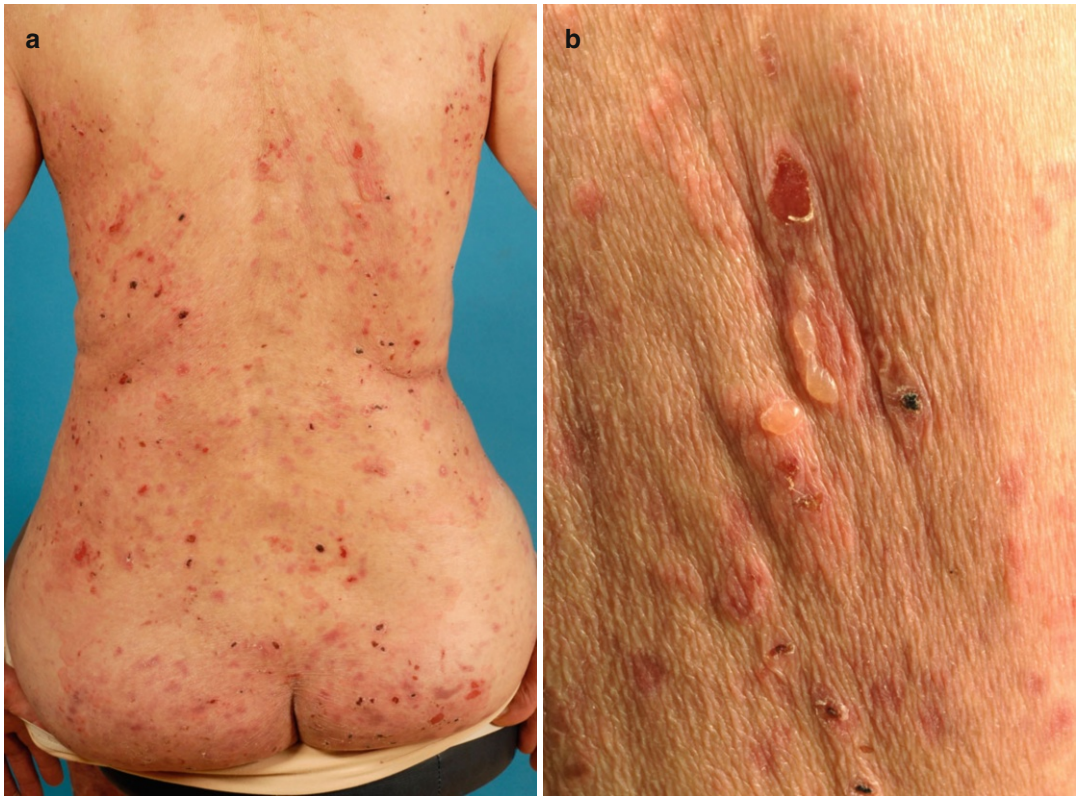

Fig. 18.5 Vesicular sublamina densa-type linear IgA disease (IgA-EBA) showing (**a**) multiple excoriated papules and macules on the trunk (**b**) with some small vesicles

direct immunofluorescence (DIF) (Fig. 18.6). The autoantibodies are mainly from IgA1 class [7]. Indirect immunofluorescence (IIF) on monkey esophagus fails to detect autoantibodies in most cases due to the low circulating autoantibody titers. Using salt-split skin can raise the sensitivity of the serology, where the majority of the patients show epidermal bindings (lamina lucida-type LAD) and in a minority of the cases is the signal on the dermal side of the split (sublamina densa-type LAD). However some cases show a mixed pattern. Interestingly in drug-induced LAD, circulating IgA against the EBMZ on salt-split skin is mostly not detectable [8]. Western blotting seems to be more sensitive to detect circulating IgA autoantibodies against LABD97 and LAD-1.

It should be mentioned here that in minority of the cases, also IgG may be seen by DIF parallel to IgA, although in less intensity. These cases should be considered rather as overlap syndromes and designated mixed IgG/IgA bullous pemphigoid.

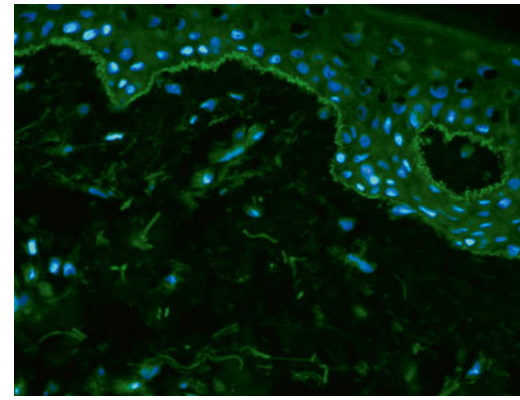

Fig. 18.6 Direct immunofluorescence in lamina lucida-type linear IgA disease shows linear, n-serrated, deposition of IgA along the EBMZ

<The linear deposition of IgA along the EBMZ in the lamina lucida-type of LAD has an n-serrated pattern (Fig. 18.6), whereas a u-serrated pattern in the sublamina densa-type LAD>

Case Study: Part 2
Routine histopathology taken from the border of a blister showed subepidermal blister filled mostly with neutrophils. DIF reveals exclusively IgA deposits in a linear n-serrated pattern along the EBMZ. IIF showed no binding of serum IgA to salt-split skin.

Treatment Tricks

Initial Treatment and Escalator

Dapsone is the first-line treatment. Before starting, glucose-6-phosphate dehydrogenase (G6-PD) deficiency should be excluded. Starting dose is 0.5 mg/kg daily slowly rising up to maximum 2.5–3.0 mg/kg until itch and blistering are controlled. The average dose to control the disease is about 100 mg daily; sometimes higher doses are needed, but hemolysis is obligate above doses of 100 mg [9]. The mechanism of dapsone to inhibit neutrophil chemotaxis on the site on IgA deposition is not well understood yet. It is known that dapsone inhibits neutrophil lysosomal activity and myeloperoxidase-mediated iodination; however it does not have any effect on antibody or complement deposition [4]. Further it was shown that dapsone inhibits neutrophil adherence to EBMZ antibodies on a dose-dependent manner, which covers the pharmacological range of serum dapsone levels [4].

Alternatives are sulfonamides (sulfapyridine in a dose of 15–60 mg/kg/day) alone or in combination with dapsone. Combination of these two drugs has cumulative efficacy without additive toxicity [10]. In partial effect both can be combined with topical or oral corticosteroids [9]. There are several other treatment options reported as mycophenolate mofetil, mycophenolic acid, colchicine, cyclosporine, methotrexate, HIVIG, cotrimoxazole, different antibiotics, and immunoadsorption, most of them single or small case series [9].

<First-line treatment for LAD is dapsone>

Follow-Up and Tapering

Patients on dapsone therapy should be carefully monitored for hemolysis and methemoglobinemia. Read more in Chap. 20. In cases with intolerance consider extreme low dapsone doses such as 12.5 mg daily (1/8 of tablet).

Case Study: Part 3
Patient was treated with dapsone climbing up to 200 mg daily without achieving remission. Later adalimumab and rituximab were tried in an off-label setup without success. Systemic high-dose corticosteroids and colchicine could not also book any success. Importantly patient developed several side effects from these systemic medications. From the chronic high-dose dapsone usage, she developed serious methemoglobinemia up to 21 % MetHb in the peripheral arterial blood. Patient should receive several times methylene blue intervention to treat methemoglobinemia. Under adalimumab treatment, patient developed an interstitial pneumonitis, which was considered as a side effect of the TNF-alpha blocker; however the underlying Sjögren's disease could not be excluded. As a side effect of high-dose steroid, patient developed Cushing syndrome with weight gain and diabetes. At the end patient was treated with mycophenolic acid in a dose of 360 mg QID, and she achieved at least partial remission.

Review Questions

1. What is the most common location of LAD in childhood?
   (a) Perineum
   (b) Head
   (c) Pals and soles
2. The most important characteristics on DIF in LAD is
   (a) IgA deposition in epithelial cell surface pattern
   (b) Linear IgA deposition along the EBMZ

(c) Granular IgA deposition along the EBMZ
(d) Granular IgA deposition in dermal vessels

3. Mucosal involvement occurs in …% of the patients with LAD
   (a) 10%
   (b) 30%
   (c) 80%
   (d) 100%
4. First-line treatment of LAD is
   (a) Superpotent topical corticosteroids
   (b) Systemic corticosteroids
   (c) Dapsone
   (d) Rituximab

Answers

1. (a)
2. (b)
3. (c)
4. (c)

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On the Web

DermNet NZ: http://www.dermnetnz.org/immune/linear-iga.html.

Drug-Induced Pemphigoid and Linear IgA Disease

19

Sylvia H. Kardaun and Joost M. Meijer

Abstract

Drug-induced pemphigoid and drug-induced linear IgA disease can be difficult to differentiate from bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), and linear IgA disease (LAD) because of only minor differences in clinical, histopathologic, and immunopathologic features. However, differentiation can be of major importance because of a different approach, treatment, and outcome. Diagnosis is mainly based on the time relation between start of the suspected drug(s) and onset of the lesions. Drug-induced BP and drug-induced LAD tend to be self-limiting after withdrawal of the culprit drug.

Keywords

Autoimmune disease • Vesiculobullous disease • Adverse drug reaction • Drug induced • Drug triggered • Bullous pemphigoid • Linear IgA disease • Diuretics • Vancomycin

Short Introduction in Layman Terms

Drug-induced pemphigoid (BP) and drug-induced linear IgA disease (LAD), clinically quite similar to BP, respectively LAD, are clinical variants that are caused or triggered by drugs. Key to diagnosis is awareness of the possibility of a drug etiology, followed by a thorough anamnesis, including an elaborate medication history. The cause-effect relationship of drug-induced dermatoses, including drug-induced BP and drug-induced LAD, is based on the time relation between start of the suspected drug(s) and onset of the lesions. Although drug algorithms, e.g., the Naranjo score or the recently revised imputability method, can be used to discriminate between spontaneous and drug-induced cases, these are unfortunately most often not conclusive. Moreover, rechallenge with the suspected culprit drug is often not ethical. In some cases a culprit can be found by (stepwise) dechallenge. After discontinuation of the culprit drug, drug-induced

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BP and drug-induced LAD most often tend to heal rapidly.

Drug-Induced Pemphigoid

Drug-induced BP is difficult to differentiate from idiopathic BP because of only subtle differences in clinical presentation and histopathological findings. No more than 15 % of BP is associated with inducing factors, including drugs [1]. Two types of drug-induced BP have been described: an acute, self-limiting eruption, which responds rapidly after withdrawal of the causative drug (*drug-induced BP proper*), and a chronic eruption resembling BP, but with a more severe and persistent clinical course (*drug-triggered BP*) [2]. Because rechallenge with the suspected drug can generally be regarded unethical due to the severity of the reaction, a drug-induced etiology will often not be established.

Facts and Figures

More than 50 different drugs have been associated with BP over the years. Most common is furosemide, followed by loop diuretics, angiotensin-converting enzyme inhibitors, penicillins, neuroleptics, antidiabetics, and antiarrhythmics [2, 3]. Drugs associated with BP are shown in Table 19.1 [4].

Drugs may act as a trigger in patients with a genetic susceptibility, either by deregulating the immune response or by acting as haptens and changing the antigenic properties of proteins in the epidermal basement membrane zone (EBMZ) [1].

Table 19.1 Drugs associated with BP

Amoxicillin	Nadolol
Ampicillin	Omeprazole
Anti-influenza vaccine	Penicillamine
Arsenic	Penicillin
Azapropazone	Phenacetin
Captopril	Placental extracts
Clonidine	Potassium iodide
Dactinomycin (actinomycin D)	Practolol
Enalapril	Psoralens
Flupenthixol	Risperidone
Furosemide	Salicylazosulfapyridine
Gold thiosulfate	Sulfonamide
Ibuprofen	Thiopronin
Interleukin-2	Tiobutarit
Mefenamic acid	Tolbutamide
Methyldopa	

Contact Pemphigoid

In addition, external use of a number of preparations on the skin or mucous membranes has been documented to provoke cases of either BP or MMP, which are referred to in Table 19.2 [4].

Diagnosis Paths

Features to suspect a drug-induced etiology are a young age at onset, tense bullae on healthy-appearing skin (monomorphic blisters) (Fig. 19.1), erythema multiforme-like lesions, and in rare cases a positive Nikolsky's sign. Furthermore, a history of recently started drugs and the clinical course may point to a relation with drugs. Drug-induced BP may arise up to 3 months after drug initiation.

The histopathological findings in drug-induced BP are mostly similar to BP and may additionally include intraepidermal vesicles and/or necrotic keratinocytes. The blister cavity and the dermis may contain numerous eosinophils and neutrophils. The findings in direct (DIF) and indirect immunofluorescence (IIF) microscopy are generally in accordance with BP. Laboratory studies may show blood eosinophilia [1, 2].

Table 19.2 Drugs associated with contact BP

Bullous pemphigoid	Cicatricial pemphigoid
Anthralin	Epinephrine
Benzyl benzoate (30 %)	Idoxuridine
Coal tar	Pilocarpine
5-Fluorouracil	Timolol
Iodophor adhesive band	

Case Study: Part 1

A 47-year-old man complained of tense blisters, erosions, and itching on both upper legs, 6 days after start of flucloxacillin. Physical examination revealed localized tense blisters on normally appearing skin on the upper legs (Fig. 19.1). Mucous membranes were not involved.

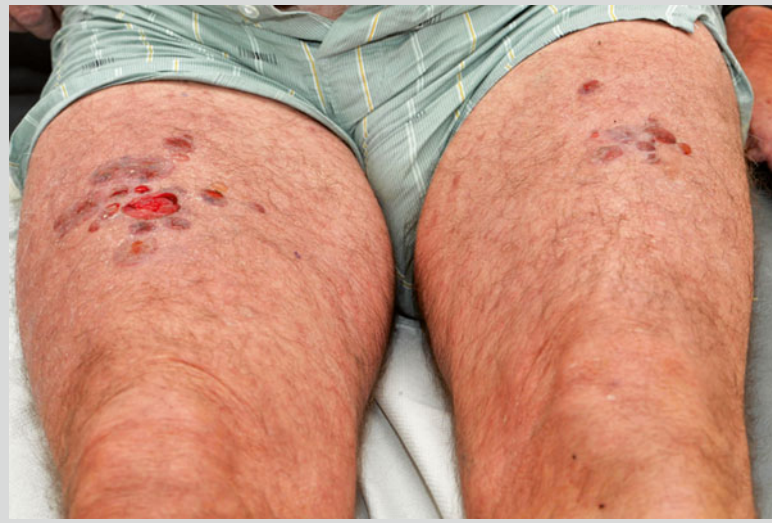

Fig. 19.1 Localized tense bullae and erosions on normal appearing skin on both upper legs

Case Study: Part 2

Histopathology showed a subepidermal blister with eosinophilic spongiosis and a dermal infiltrate with numerous eosinophils. DIF revealed linear n-serrated deposition of IgG (1+) and C3c (2+) along the EBMZ. IIF on monkey esophagus showed positive IgG along the EMBZ and IIF on salt-split skin showed positive IgG (3+) epidermal binding. Further laboratory investigations revealed a slight peripheral blood eosinophilia (0.58×10^9/L).

Case Study: Part 3

Laboratory investigations confirmed BP. An association with flucloxacillin was suspected. After withdrawal of flucloxacillin and therapy with daily lesional very potent topical corticosteroids (clobetasol 0.05 % cream) and zinc oxide oil, the lesions healed within four weeks, without relapse. The time relation with flucloxacillin, the quick response after its withdrawal and the presentation with bullae on normal appearing skin made drug-induced BP the preferred diagnosis.

Treatment Tricks

After withdrawal of the culprit, most patients respond rapidly to treatment, generally without relapses, indicating that drug-induced BP should be considered. Treatment follows the guidelines for BP, after which complete remission is most often achieved within 6 weeks.

Drug-Induced Linear IgA Disease

Most reported cases of LAD have been classified as idiopathic. Drug-induced LAD, first described in 1981, tends to be more atypical and severe than LAD with significantly more frequent large erosions, sometimes complicated by secondary

infection and a positive Nikolsky's sign, and may clinically resemble toxic epidermal necrolysis (TEN) [5].

Facts and Figures

Various drugs have been associated with LAD, of which vancomycin has been reported most frequently (46 % of cases). Other drugs include antihypertensives (captopril), antibiotics (trimethoprim-sulfamethoxazole), and non-steroidal anti-inflammatory drugs (naproxen) [5, 6]. Drugs associated with LAD are shown in Table 19.3 [7]. The onset of symptoms ranges from 5 to 26 days (median 10 days) after drug initiation and symptoms usually resolve within four weeks after withdrawal [5].

Diagnosis Paths

The diagnosis of drug-induced LAD is, similar to idiopathic LAD, based on clinical presentation, histopathology, detection of linear deposition of IgA along the basement membrane zone by DIF, and detection of circulating IgA antibodies by IIF, with in addition start of suspected medication. Histopathology and DIF are essential to confirm LAD and to differentiate from TEN. Clinically, drug-induced LAD tends to be more severe than LAD. Histopathologically no significant difference has been demonstrated between both entities, although focal necrotic keratinocytes arranged near the basal membrane are more frequent in drug-induced LAD. The rate of positive IIF reactivity in drug-induced LAD is rather low, probably due to the heterogeneity of target antigens [5].

Table 19.3 Drugs associated with LAD

Acetaminophen	Imipenem
Amiodarone	Iodine
Amoxicillin	Ketoprofen
Ampicillin	Lithium carbonate
Atorvastatin	Naproxen
Captopril	Penicillin G
Cefamandole	Phenytoin
Ceftriaxone	Piroxicam
Diclofenac	Somatostatin
Furosemide	Sulfamethoxazole-trimethoprim
Gemcitabine	Vancomycin
Glibenclamide	Verapamil
Interferon-γ/ interleukin-2	Vigabatrin

Treatment Tricks

The optimal therapeutic option would be withdrawal of the suspected culprit (dechallenge) without any further treatment, followed by rechallenge to confirm diagnosis. However, this remains complicated due to ethical problems, especially in more severe reactions. Otherwise, there is a good response to treatment similar to LAD. Recommended first-line treatment after withdrawal of the culprit includes moisturizing ointments and/or topical corticosteroids [5].

Review Questions

1 Which clinical symptoms could differentiate drug-induced BP?
   (a) Tense bullae on healthy-appearing skin (monomorphic blisters)
   (b) Young age of onset
   (c) Erythema multiforme-like lesions
   (d) All of mentioned above
2. Which drug is most often associated with drug-induced BP?
   (a) Vancomycin
   (b) Enalapril
   (c) Furosemide
   (d) Ibuprofen
3 Which drug is most often associated with drug-induced LAD?
   (a) Vancomycin
   (b) Captopril
   (c) Naproxen
   (d) Trimethoprim-sulfamethoxazole

4. Onset of symptoms in drug-induced LAD ranges from:
   (a) <3 days
   (b) 5–26 days
   (c) 1–2 months
   (d) >6 months

Answers

1. (d)
2. (c)
3. (a)
4. (b)

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Part IV

Other Inflammatory Bullous Diseases

Dermatitis Herpetiformis

20

Barbara Horváth and Marcel F. Jonkman

Abstract

Dermatitis herpetiformis (DH) is the specific skin manifestation of celiac disease (CD) caused by the digestion of gluten in HLA-DQ2 or HLA-DQ8 individuals. DH is characterized by intensely pruritic polymorphic papulovesicular eruption on the extensor surfaces of the body. Both diseases are characterized by circulating IgA autoantibodies against tissue transglutaminase (tTG), which bind to the smooth muscle layer of the monkey esophagus causing the so-called EMA positivity. In addition, DH patients have another autoantibody population targeting the epidermal transglutaminase (eTG), the autoantigen of DH, which protein is highly homologous to tTG.

Keywords

Dermatitis herpetiformis (DH) • Celiac disease • Gluten-sensitive disease (GSD) • Tissue transglutaminase (tTG) • Epidermal transglutaminase (eTG) • Immunoglobulin A (IgA) • Dapsone

Introduction and Aims

Short Definition in Layman Terms

Dermatitis herpetiformis is the skin manifestation of celiac disease. The trigger of both diseases is known; ingestion of gluten in certain HLA phenotypes (HLA-DQ8 or HLA-DQ2) leads to an autoimmune reaction characterized by IgA autoantibodies against tissue transglutaminase (tTG) and later in DH patient against the epidermal transglutaminase (eTG).

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<Dermatitis herpetiformis (DH) is the specific skin manifestation of celiac disease (CD) caused by the digestion of gluten in HLA-DQ2 or HLA-DQ8 individuals>

Learning Objectives

After reading this chapter, you will be able to differentiate dermatitis herpetiformis from other pruritic dermatoses and to recognize the classic clinics of DH. You will be able to perform and interpret the immunological tests, to make a treatment algorithm, and to manage patients with DH.

Case Study: Part 1

A 41-year-old male patient presented with itchy papules, blisters on knees, elbows, shoulders, and lower back for 3 years. He had no mucosal involvement, nor digestive tract symptoms. He was non-atopic, and family history reported no skin disease or other problems. There was no medicine administered before.

Didactical Questions; Cross Section of Questions to Prime the Readers' Interest

How can you diagnose DH? What would you see in the histopathological section? How can you make the difference between other autoimmune and immunological diseases? In this section the focus is on the clinical differential diagnostics and workup of patients suspected for DH.

Facts and Figures

Definitions and Classification

Dermatitis herpetiformis is the cutaneous manifestation of celiac disease; both diseases are the different phenotype of gluten-sensitive disease (GSD). This means that in both diseases gluten is the trigger that in certain susceptible HLA phenotypes provokes autoimmune reaction. An autoantibody population against tTG characterizes CD. As gluten sensitivity is obligate to develop in DH, also DH patients have tTG autoantibodies, however, with low affinity. Moreover, patients with DH develop another autoantibody population against the eTG [1].

Epidemiology

Due to its genetic background, DH has a different prevalence geographically. DH is most common in patients with North-European origin. Studies report a prevalence of 1.2–39.2 per 100,000 people, with an incidence range of 0.4–2.6 per 100,000 people per year. DH is rare in the Asian population and even more rare in African Americans. Familial cases were reported. Male-to-female ratio is 1.5:1–2:1. Interestingly an opposite female predominance is known in CD. The onset of the disease is variable, mostly in the fourth decade, but childhood and geriatric cases are not rare. The childhood onset is more reported in the Mediterranean area [2].

<DH is most common in patients with North-European origin>

Pathogenesis

There is a strong genetic predisposition for DH. In patients with HLA-DQ2 and/or HLA-DQ8 phenotype, an autoimmune reaction develops after the ingestion of gluten (Fig. 20.1). First, tTG modifies gliadin, the alcohol-soluble fraction of gluten to an antigen, which binds to the HLA-DQ2/HLA-DQ8 molecule to evoke cellular and humoral (anti-gliadin antibodies) immune reactions. Moreover, the tTG-bound gliadin serves also as a strong antigen producing excessive autoantibody against the enzyme complex (tTG antibodies). These humoral and cellular immune reactions lead to inflammation and damage of the gut mucosa, resembling changes seen in CD [2].

The subclinical gluten sensitivity is obligate to develop DH. eTG and tTG share common epitopes within the enzymatically active domain. It is hypothesized that epitope spreading is the suspected mechanism after the development of the new autoantibody population targeting the eTG. This is supported by the fact that children have mainly CD with high levels of anti-tTG and low levels of anti-

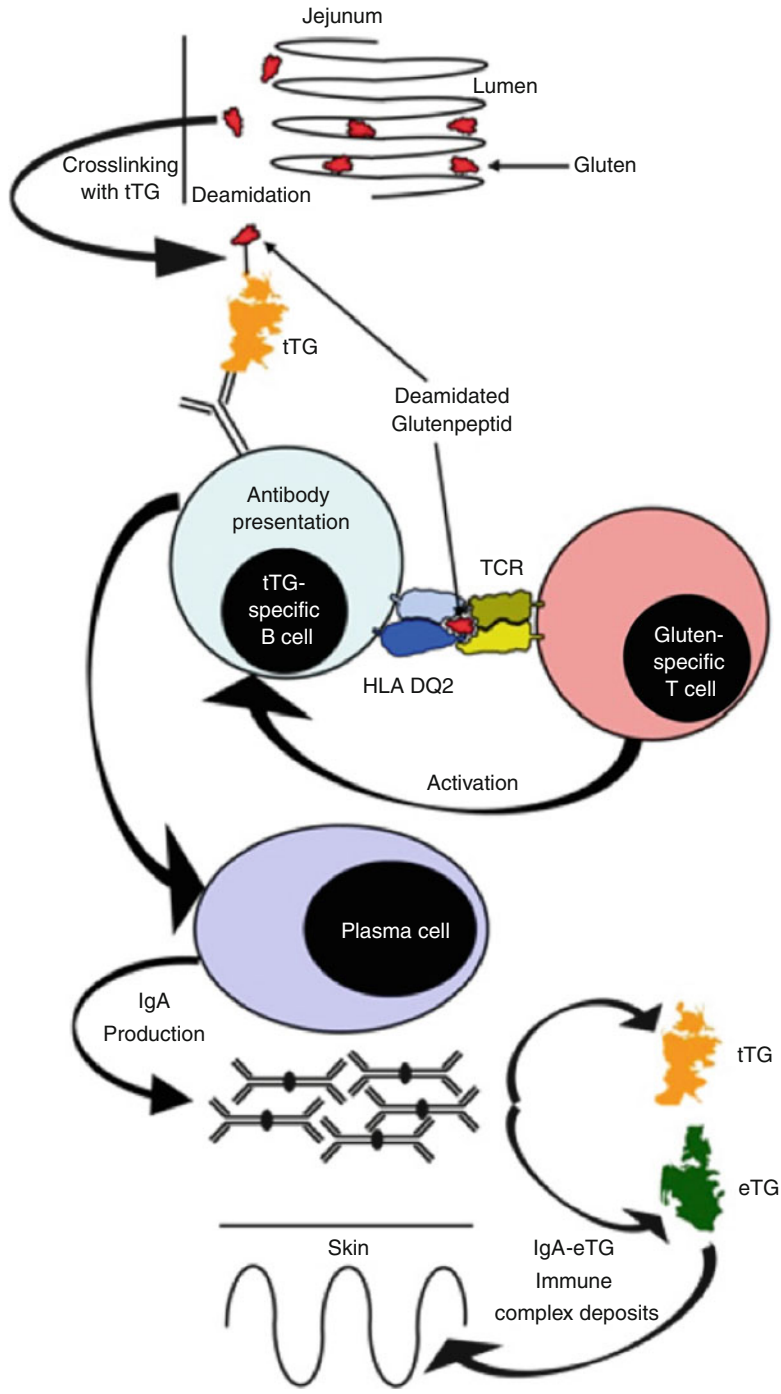

Fig. 20.1 Diagram of the hypothetical pathogenesis of DH. Incomplete metabolized gluten peptides are deaminated in the gut and covalently cross-linked with tTG. The gluten peptide tTG complex induces an anti-tTG and anti-eTG autoimmune response by gluten peptide-specific T cells, which activate tTG-specific B cells. The subsequently produced IgA autoantibodies, which cross-react with eTG, are deposited as eTG-immune complexes in the dermis. *TCR* T-cell receptor (Reprinted from Sárdy and Tietze [9] with kind permission from Springer Science and Business Media)

eTG compared with adults. Moreover, CD mainly develops in childhood, whereas DH is the disease of adults, suggesting that the epitopes spreading need time to take place [2].

The circulating IgA autoantibodies against eTG in DH target epidermal transglutaminase, a protein that plays a role in the formation of the cornified envelop of the epidermis. In DH skin eTG is co-localized with IgA at the BMZ in the papilla tips supporting that eTG is the autoantigen of DH [1]. However, it remains to be elucidated whether there are true circulating IgA-eTG immune complexes in DH, since deposits of IgA and eTG in the dermal vessel

are seen frequently (Fig. 20.2) [3], clinically corresponding with the digital purpura in DH (Fig. 20.3) [4].

<In DH skin eTG is co-localized with IgA at the EBMZ in the papillary tips supporting that eTG is the autoantigen of DH>

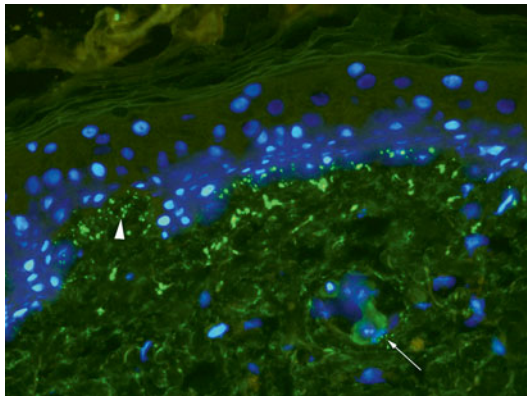

Fig. 20.2 DIF of DH shows granular IgA depositions below the basement membrane zone (*arrowhead*) and in the walls of dermal blood vessels (*arrow*)

Diagnosis Paths

History and Physical Examination

The classic clinical presentation of DH is a very itchy polymorphous skin eruption comprising erythema, urticarial plaques, papules, vesicles, excoriations, and purpura sometimes in herpetiform configuration. The lesions are distributed typically on the extensor surfaces of the body, such as the knees, elbows (Fig. 20.4), and shoulders, in the so-called vertical distribution (Fig. 20.5). Large blisters are rarely seen. The disease has a fluctuating course driven mostly

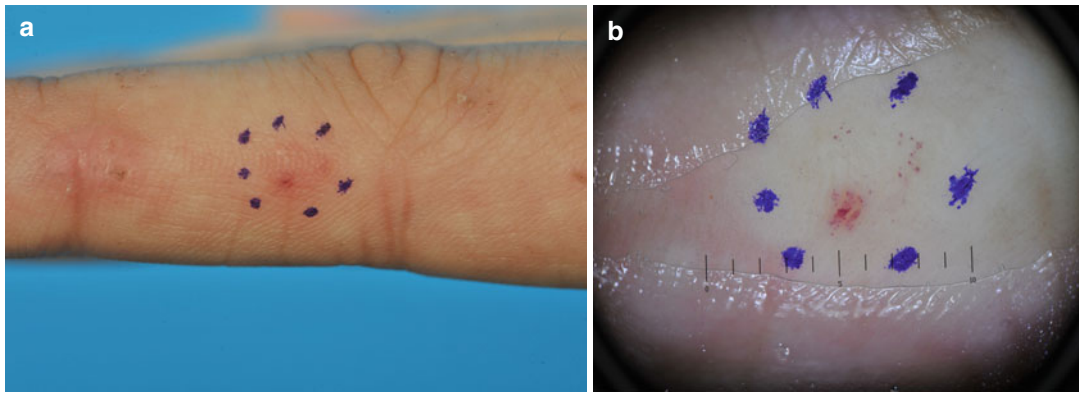

Fig. 20.3 (**a**) Digital purpura in dermatitis herpetiformis. (**b**) Dermoscopy reveals coagulated capillaries

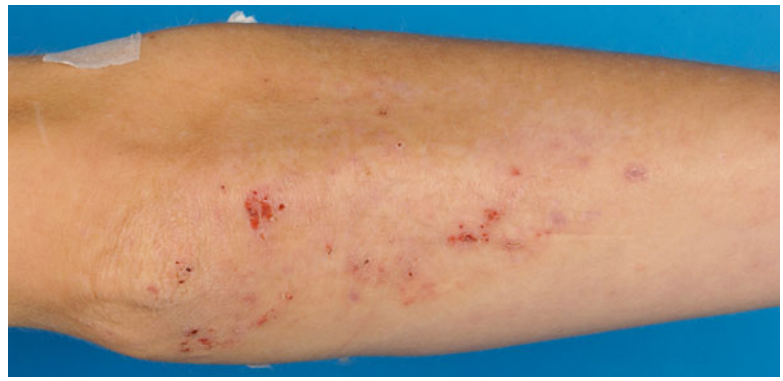

Fig. 20.4 The extensor surface of the elbow is a predilection place in DH and the preferable side for IF biopsy

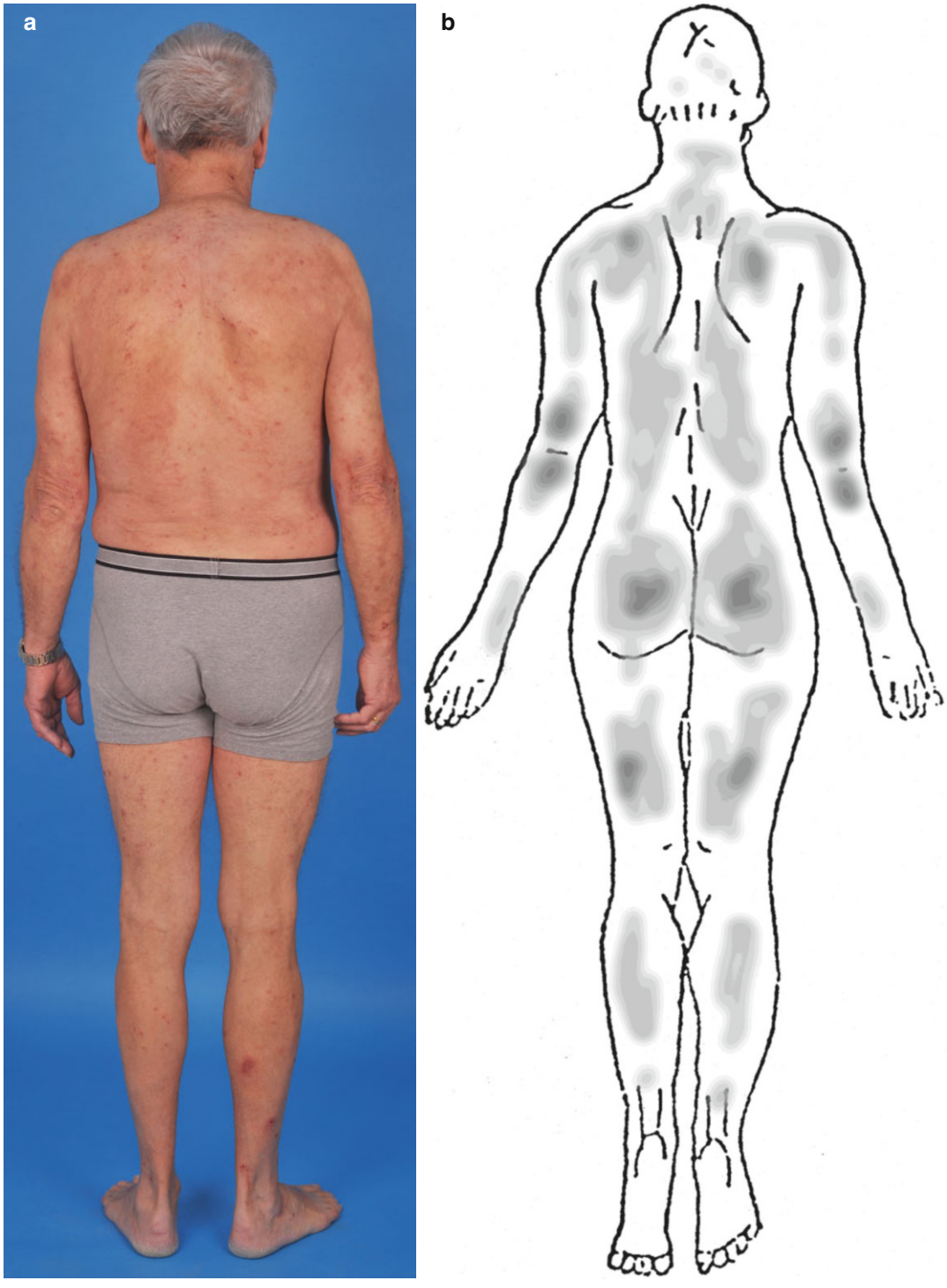

Fig. 20.5 The vertical distribution (Vertikal Korrespondenz) of skin lesions at the backside of the body typical for DH (**a**, **b**)

but not always by gluten ingestion and improves under UV light (seasonal flare-ups). There are known associations with autoimmune thyroid disease and other autoimmune diseases [4].

General Diagnostics

The histological picture is unique in DH. Routine histology in DH shows infiltration of neutrophils at the dermoepidermal junction just above the papilla tips, called microabscesses (Fig. 20.6).

Specific Diagnostics

On direct immunofluorescence (DIF), granular deposits of IgA at the dermal papilla tips or along the BMZ are seen (Figs. 4.9 and 20.2), mostly representing the location of the neutrophils on the routine histology (Fig. 20.6). By indirect immunofluorescence (IIF) on monkey esophagus IgA binding is seen on the smooth muscle layer corresponding with the endomysium (EMA positivity, Fig. 5.3a). The major antigen of EMA is tTG [4]. In the blood IgA anti-tTG is positive. However, patients with IgA deficiency can also develop CD as well as DH resulting in autoantibodies from the IgG class. Therefore, simultaneous measurement of IgA levels is mandatory for diagnostics.

<DIF shows granular IgA deposits along the BMZ. On monkey esophagus EMA positivity is seen representing autoantibodies against tTG>

Case Study: Part 2

Routine laboratory examination showed no abnormality. On histopathological examination, subepidermal blisters forming with neutrophil microabscesses at the dermal papilla tips were seen. On direct immunofluorescence granular deposits of IgA (2+) and complement (C3C 1+) were present at the BMZ. By indirect immunofluorescence on monkey, esophagus anti-EMA positivity (1+) was seen.

Treatment Tricks

Initial treatment and Escalator

Gluten-free diet (GFD) is the first choice of treatment (Table 20.1). Every patient with DH should be informed about this; however, consistent adherence on GFD is difficult. A patient with symptoms of celiac disease should be referred to

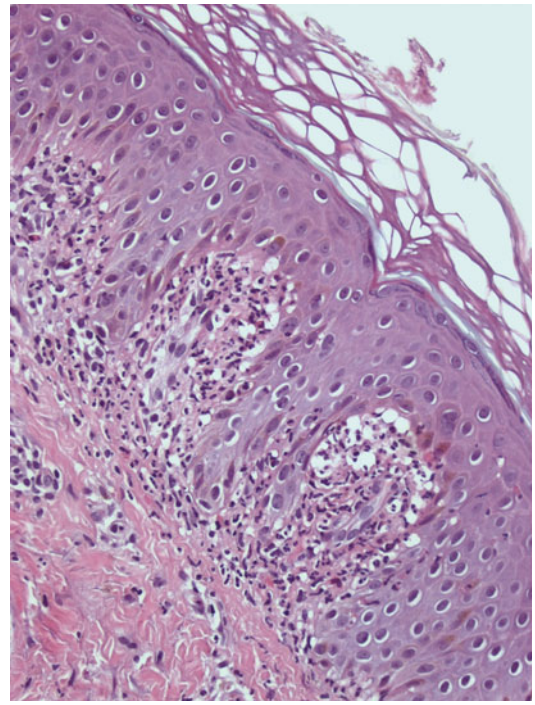

Fig. 20.6 Histopathology of DH shows neutrophil microabscesses in the dermal papillae

Table 20.1 Gluten-free diet

Foods to avoid	Foods allowed
Grains and starches	Grains and starches
Wheat	Tapioca
Kamut	Soybean
Rye	Potato
Barley	Buckwheat
Oats	Quinoa
Many cereals	Rice
	Corn
	Coconut flour
	Almond meal flour

http://www.celiaccentral.org

a gastroenterologist. A patient without gut symptoms can be managed primary by a dermatologist. Since most patients with DH have mild CD, denying of GFD is acceptable as long as they are carefully monitored for signs of malabsorption, anemia, vitamin B12 deficiency, osteoporosis, and thyroid dysfunction.

Patients with severe CD may develop enteropathy-associated T-cell lymphoma (EATL). Since patients with DH – per definition – do not develop severe CD, they do not develop EATL and therefore do not have to be warned for the possibility of developing intestinal lymphoma.

Unfortunately, the effect of GFD takes time. The skin rash disappears in months after the cessation of gluten, and gluten rechallenge causes flare-up of the disease within days.

To achieve quick improvement of itch, patients need dapsone as medication at the beginning of the therapy. Dapsone, a member of the sulfonamide antibiotics, is the first-line medical treatment for DH. Dapsone inhibits the diapedesis of neutrophils, and migration to the EBMZ bounds IgA in a dose-dependent manner [5]. Dapsone can be started with 50 mg/day. Administration of dapsone reduces the itch promptly within 48 h. Glucose-6-phosphate dehydrogenase deficiency should be excluded within the first week of treatment and prior to raising the dosage of dapsone.

Follow-Up and Tapering

Hemolysis is obligate during dapsone treatment and is dose dependent. It is compensated by reticulocytosis. During dapsone treatment the patient should be carefully monitored for excessive hemolysis: drop in hemoglobin concentration with 1 g/dL, insufficient elevation of reticulocytes, lactate dehydrogenase above 225 U/L, and total bilirubin above 17 µmol/mL. For methemoglobinemia arterial concentrations of metHb should be measured regularly. Agranulocytosis and dapsone hypersensitivity syndrome are the most feared idiosyncratic side effects of dapsone. When remission is achieved, dapsone can be tapered to the minimal effective dose. If the patient constantly adheres to GFD, the dosage of dapsone can be further reduced or even stopped (see box below).

Case Study: Part 3

Patient received dapsone orally. The initial dose was 50 mg/day, which was increased up to 75 mg daily after 1 week under blood controls, and excluding glucose-6-phosphate dehydrogenase (G6PD).

Pharmacology of Dapsone [6]

Oral availability is as high as 86 %, so it is administered exclusively orally. During its metabolisms in the liver, two metabolites are produced: via acetylation, the nontoxic metabolites acetyl and diacetyl dapsone, and via N-hydroxylation, the potentially toxic hydroxylamine. The latter reaction occurs by the cytochrome P450 enzyme complex (CYP450), so medicines inducting this enzyme can increase the amount of toxic metabolites [6]. Table 20.2 shows the medicines influencing the CYP450.

Table 20.2 Medications inducting and inhibiting cytochrome P450 (CYP450) [7]

Inhibitors of CYP450	Inductors of CYP450
Diltiazem	Glucocorticosteroids
Itraconazole	Rifampicin
Ketoconazole	Carbamazepine
Metronidazole	Phenobarbital
Omeprazole	Phenytoin
Paroxetine	
Fluoxetine	
Amitriptyline	
Cimetidine	
Haloperidol	
Erythromycin	
Clarithromycin	
Ritonavir	

The side effects of dapsone are either dose dependent or dose independent.

- Dose-dependent side effects:
  - *Methemoglobinemia*. The development of methemoglobinemia is the direct effect of the toxic metabolite hydroxylamine, which transforms the hemoglobin to metHB, which binds and release less oxygen. Clinical symptoms of methemoglobinemia are due to the lack of oxygen in the tissues (Table 20.3).
  - *Hemolysis*. It is an indirect effect of hydroxylamine and related on oxygen-free radicals. Moreover, addition of cimetidine can reduce the concentration of hydroxylamine. Interestingly G6PD-deficient patients are less susceptible to methemoglobinemia and more susceptible to hemolysis due to decrease in NAPDH formation in the erythrocytes.
- Dose-independent side effects:
  - *Agranulocytosis* is idiosyncratic, thought that hydroxylamine binds in the bone marrow to the myeloid precursor cell to inhibit their maturation. Typically fever with neutropenia appears 1–3 months after the first dose of dapsone.
  - *Dapsone hypersensitivity syndrome* (*DHS*) with fever, rash, and organomegaly (lymphadenopathy, hepatosplenomegaly) with elevated erythrocyte sedimentation rate and liver enzymes. The interval is much shorter 1 up to 6 weeks after the first dose.

Table 20.3 Clinical symptoms of methemoglobinemia [8]

% of total hemoglobin[a]	Symptoms
<10 %	None
10–20 %	Cyanotic discoloration of the skin
20–30 %	Anxiety, headache, tachycardia, lightheadedness
30–50 %	Fatigue, confusion, dizziness, tachypnea, tachycardia
50–70 %	Coma, seizures, arrhythmias, acidosis
>70 %	Death

[a]Assume hemoglobin = 15 g/dL. Patients with lower hemoglobin concentrations may experience more severe symptoms for a given percentage of metHb level

Review Questions

1. What is the trigger of DH?
   (a) Gluten
   (b) Gliadin
   (c) Reticulin
   (d) Drugs
2. Which HLA loci are associated with DH and CD?
   (a) HLA-DQ8
   (b) HLA-B51
   (c) HLA-DQ2
   (d) (a) + (c) are correct
3. Which are the typically involved areas on the body in DH?
   (a) Backside of the body
   (b) Mucosal surfaces
   (c) Palms and soles
   (d) Front side of the body
4. First-line medication of DH is:
   (a) Systemic corticosteroids
   (b) Gluten-free diet
   (c) Doxycycline
   (d) Dapsone
5. Which is allowed in the gluten-free diet?
   (a) Wheat
   (b) Potato
   (c) Rye
   (d) Barley

Answers

1. (a)
2. (d)
3. (a)
4. (d)
5. (b)

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Stevens–Johnson Syndrome/Toxic Epidermal Necrolysis and Erythema Exsudativum Multiforme

21

Sylvia H. Kardaun

Abstract

In the past, definitions and even the name giving of erythema exsudativum multiforme, Stevens–Johnson syndrome and toxic epidermal necrolysis have been confusing. However, after a consensus classification, which separates erythema exsudativum multiforme from Stevens–Johnson syndrome and toxic epidermal necrolysis, it is now generally accepted that Stevens–Johnson syndrome and toxic epidermal necrolysis are variants within a continuous spectrum of severe, potentially fatal, mucocutaneous adverse drug reactions, whereas erythema exsudativum multiforme is a distinct, generally more mild entity with different clinical signs, mainly precipitated by infections, e.g. herpes simplex virus.

Keywords

Keratinocyte • Epidermis • Skin • Mucosae • Apoptosis • Epidermal necrosis • Adverse drug reactions • Stevens–Johnson syndrome • Toxic epidermal necrolysis • Erythema exsudativum multiforme

Introduction and Aims

Short Introduction in Layman Terms

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe, mainly drug-induced reactions with widespread skin and mucous membrane involvement, characterised by massive epidermal necrosis. Erythema exsudativum multiforme (EEM) presents an acute, most often acrofacial eruption characterised by target lesions. Although generally relatively mild and self-limiting, EEM can be recurrent and is most often triggered by infections, especially viruses.

Learning Objectives

After reading this chapter, you will be able to distinguish SJS/TEN from EEM and other (autoimmune) blistering diseases. You understand that

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SJS/TEN is most often caused by drugs and presents a spectrum that can be divided in three subtypes, predominantly based on the percentage of the detached and detachable body surface area (BSA). Moreover, you will know that EEM, although not an infection by itself, is most often caused by various infections, with herpes simplex virus (HSV) as the most important agent, while drugs are rarely the cause.

Didactical Questions: Cross Sections to Prime the Readers' Interest

What are typical and atypical targets and what is their importance? Can EEM evolve to SJS or TEN? Which drugs are notorious for inducing SJS/TEN and should patients avoid all of these after having experienced SJS/TEN? What are long-lasting sequelae of SJS/TEN? What special care should be taken for patients, suffering from eye involvement?

Case Study: Part 1

A 46-year-old man complained of a painful throat and subfebrile temperature starting 2 weeks after neurosurgery. Because 2 days later a skin rash and stinging eyes develop, he decides to consult his GP. What is your differential diagnosis? What info do you need to come to a diagnosis?

Facts and Figures

Definitions and Classification

SJS/TEN are severe, potentially fatal, mucocutaneous adverse drug reactions, characterised by massive epidermal necrosis. EEM has been reported under a variety of labels and eponyms and up to now is still surrounded by confusion. It can be divided into EEM minus, characterised by the sudden onset of red papules, some of which develop into "target" or "iris" lesions, and EEM majus, showing in addition haemorrhagic mucosal involvement as can be seen in SJS/TEN. In particular EEM majus and SJS are still often erroneously used as synonyms. EEM is an entity different from SJS/TEN with a different aetiology. In 1993, consensus was reached on case definition, classification and nosology, recognising five categories varying from EEM majus to TEN (Table 21.1) [1].

This classification is based on three clinical criteria: the morphology of the individual lesions,

Table 21.1 Differences between erythema exsudativum multiforme majus (EEM majus), Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and SJS/TEN overlap syndrome [1]

Clinical entity	EEM majus	SJS	SJS/TEN overlap	TEN[a]
Primary lesions	Raised typical or atypical target lesions	Flat atypical target lesions, erythematous/purpuric maculae	Flat atypical target lesions, ill-defined erythematous/purpuric maculae	Ill-defined (dusky) erythema and maculae, flat atypical target lesions
Distribution	Mainly acrofacial	Isolated lesions, partly confluent on the face and trunk	Isolated lesions, partly confluent on the face and trunk	Isolated lesions, partly confluent on the face, trunk and elsewhere
Intensity		+	++	+++
Mucosae	Involved	Involved	Involved	Involved
Systemic symptoms	Minimal/absent	Usual	Always	Always
Detached body surface area (BSA)	<10 %	<10 %	10–30 %	>30 %[a]

+ mild, ++ moderate, +++ severe

[a]*NB* including TEN with large confluent erythema without discrete lesions with a detached BSA ≥10 %

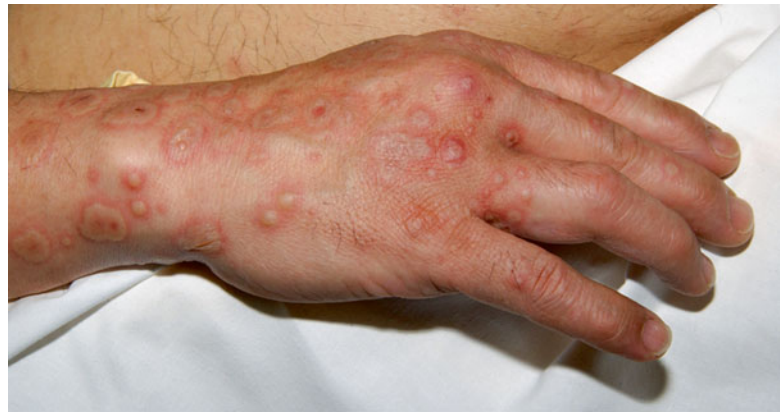

Fig. 21.1 Typical target lesions in EEM minus showing three well-defined colour zones and borders

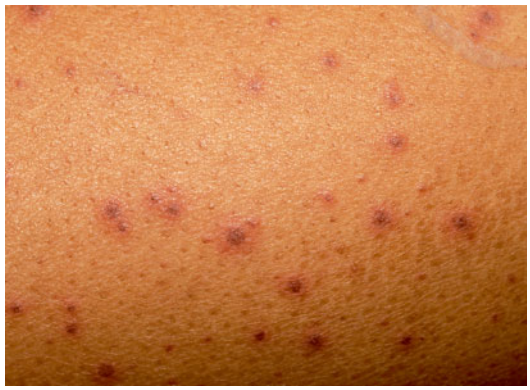

Fig. 21.2 Flat atypical target lesions in SJS with poorly defined borders and two colour zones

their distribution and the maximal extent of epidermal detachment. Typical target lesions have regular round and well-defined borders with at least three different concentric zones: a purpuric central disc with or without a blister, a raised oedematous, pale intermediate ring and an erythematous outer ring (bull's eye or iris lesion) (Fig. 21.1). By contrast, atypical target lesions, which can be raised or flat, have an appearance reminiscent of targets but present with only two zones and/or poorly defined borders, while the centre can also be vesicular or bullous (Fig. 21.2). Detachment can present as blistering or erosions. Within this classification, EEM majus is regarded a different entity, whereas SJS and TEN are considered to represent two ends of a spectrum of a single disease in which TEN is the maximal variant, mainly differing by the extent of skin detachment, but based on similar pathogenesis, risk factors and causality. Moreover, SJS can progress into TEN.

EEM majus is characterised by mainly acrofacial typical or raised atypical targets and epidermal detachment <10 % of the BSA. In the spectrum of SJS/TEN on the other hand, skin lesions are widespread with blisters arising on erythematous or purpuric macules and/or flat atypical targets. In EEM, lesions usually appear symmetrically on the distal extremities and may progress proximally, while in SJS/TEN, the reaction often starts on the upper trunk and face. Mucous membrane involvement, present in both SJS/TEN and EEM majus, tends to be more severe in SJS/TEN. EEM majus differs from SJS/TEN by occurrence in younger males, more frequent recurrences, less fever, milder mucosal lesions and lack of risk factors associated with SJS/TEN [2]. Where EEM is mainly associated with infections, SJS/TEN is most often drug induced.

Stevens–Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN)

Facts and Figures

The onset of SJS/TEN is abrupt. Prodromes, usually starting as flu-like symptoms such as high fever, sore throat, anorexia and malaise, are often followed by erosive stomatitis and eye involvement. Next, burning, painful and often ill-defined

erythematous and/or purpuric maculae (spots) and flat, atypical target lesions occur. Maculae most often start in a symmetrical distribution on the face, neck and upper trunk, extending distally with a tendency to rapid coalescence. Generally within hours, extensive mucocutaneous blistering and detachment on an erythematous base develop, sometimes for up to 10 days. Blisters are flaccid and can become confluent, while large sheets of epidermis slough off, leaving an exposed, weeping dermis and large areas of detachment. At gentle pressure, blisters can often be moved laterally due to detachment (positive Asboe–Hansen sign). Also pressure on erythematous skin may cause detachment (pseudo-Nikolsky's sign). Target lesions in SJS/TEN reminiscent of the target lesions in EEM, however, are flat and atypical.

In SJS, maculae (sometimes with a purpuric aspect), atypical target lesions, blisters and areas of detachment are most often prominent on the upper chest. Although boundaries are rather artificial, total detached and detachable skin at the maximum stage in SJS is <10 % of the BSA, between 10 and 30 % in SJS/TEN overlap and over 30 % in TEN (Figs. 21.3, 21.4 and 21.5).

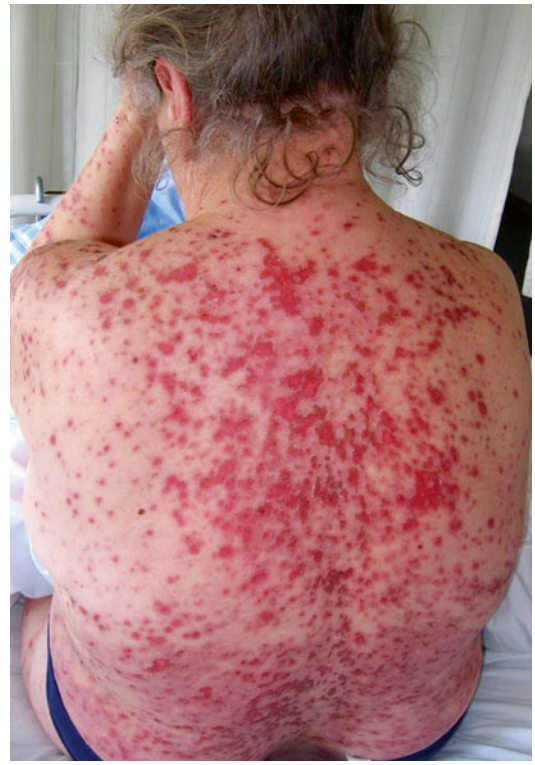

Fig. 21.3 SJS showing widespread small erythematous macules. Mainly on the central part of the thorax, the lesions are partly confluent with small areas of detachment

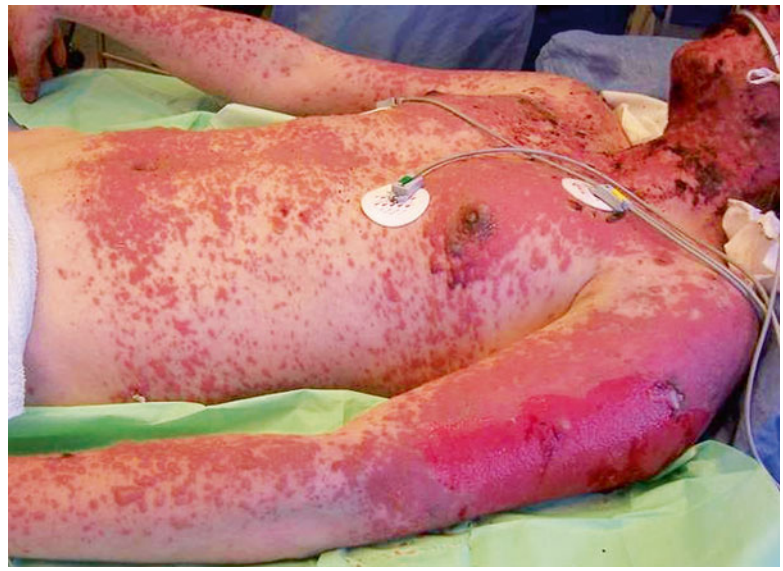

Fig. 21.4 SJS/TEN overlap with widespread maculopapular lesions on the face, neck, arms and thorax and haemorrhagic mucosal involvement. Lesions are confluent on the neck, arms and central thorax and in addition show bullae and extensive erosive areas

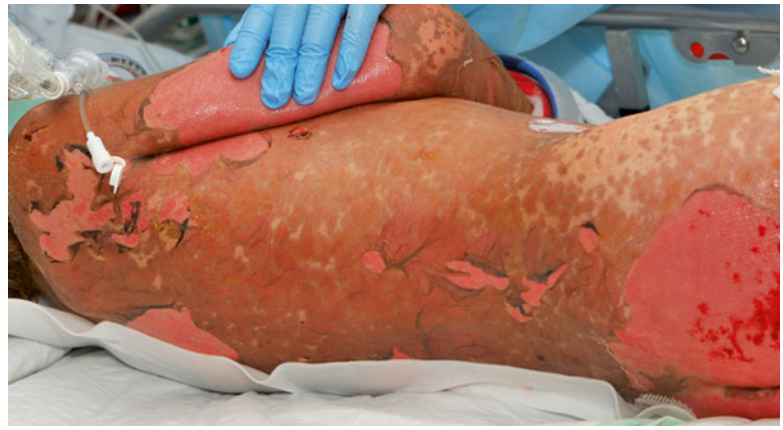

Fig. 21.5 TEN with large areas of necrotic epidermis and large sheets of sloughed-off moist, erosive erythematous skin

Generally multiple mucosal membranes, including oral, ocular, nasal, urethral, vaginal, anal, tracheobronchial and gastrointestinal mucosae, are affected in SJS/TEN, with haemorrhagic blistering and erosions. Visceral involvement may occur; anaemia and lymphopenia are frequent, while neutropenia often predicts bad prognosis. Pneumonitis or even acute respiratory distress syndrome may occur. Complete healing, especially in TEN, can last 3–6 weeks, while especially erosions on the back, buttocks and mucosae may take longer.

SJS/TEN presents a severe, life-threatening disease. The mortality rate, mainly caused by sepsis or multiorgan failure, is on average about 25 %, ranging from 12 % in SJS to 46 % in TEN [3]. More than 50 % of patients surviving suffer from long-term sequelae of which the frequent ocular complications, not rarely leading to impaired vision and even blindness, are most feared. Other sequelae include lung function impairment, symblepharon, conjunctival synechiae, dry eyes, entropion, ingrowing eyelashes, cutaneous scarring, altered pigmentation, eruptive nevi, persistent erosions/strictures of the mucous membranes and nail dystrophy.

Epidemiology

The incidence of SJS is estimated at 1.2–6.0 per million per year and that of TEN at 0.4–1.2 per million per year in Europeans. The mean age for SJS/TEN ranks between 48.2 years and 53.4 years (range 1–98), with a slight female preponderance in TEN [2]. In HIV the incidence is approximately 1000-fold higher than in the general population.

Pathogenesis and Aetiology

Although pathogenesis is not yet fully elucidated, several mechanisms have been postulated. Nowadays, it is believed that SJS/TEN is a process in which an inappropriate immune activation is triggered in response to certain drugs or their toxic metabolites. Massive keratinocyte apoptosis is the main feature, and cytotoxic T cells (CTLs) are the main effector cells. CTLs can activate the caspase cascade, including apoptosis either through Fas–Fas ligand binding or the perforin/granzyme B pathway, responsible for keratinocyte death in SJS/TEN [4]. Blister T cells from patients exert drug-specific cytotoxic activity against both autologous B lymphocyte cell lines and keratinocytes and are mediated by granzyme B. The discrepancy between the paucity of the infiltration of immune cells in the skin of patients with SJS/TEN and the overwhelming keratinocyte apoptosis has led to the search for cytotoxic proteins and/or cytokines that may "amplify" the extent of keratinocyte apoptosis that CTLs alone could induce upon cell–cell contact. Recent findings suggest that especially granulysin, a powerful pro-inflammatory cytotoxic protein released from CTLs and natural killer cells, also turns on extensive keratinocyte apoptosis [4].

A genetic predisposition is also of importance. An association between HLA, SJS/TEN and ethnic background has been emphasised: HLA-B*1502 is, for instance, strongly associated with the use of carbamazepine in SJS/TEN patients of Southeast Asian ancestry, especially in Han Chinese [4].

Aetiology

SJS/TEN nearly always represents an idiosyncratic reaction to medication. Although about 200 drugs have been reported to cause SJS/TEN, only a limited number of drugs are responsible for a large part of the reactions. In absolute case numbers, allopurinol is the most common cause of SJS/TEN in Europe. The highest risk occurs during the first 2 months of treatment with a sharp drop of incidence thereafter. However, although some drugs have a high relative risk compared to other drugs, the actual risk remains low. Drugs identified to have a significantly raised risk are allopurinol, carbamazepine, phenytoin, phenobarbital, lamotrigine, sulphamethoxazole–trimethoprim and other sulphonamide antibiotics such as sulphasalazine, NSAID's of the oxicam type, nevirapine and sertraline [5]. However, there are some cases with an infectious origin (e.g. mycoplasmal pneumonia in SJS) or without any obvious identifiable cause.

Confounding nondrug risk factors are HIV, other infections, recent cancer, recent radiotherapy and collagen vascular disease, while the association with corticosteroid use is controversial [5].

Diagnosis Paths

History and Physical Examination

Most important is the acute onset of extensive painful mucocutaneous blistering with the typical clinical presentation, often preceded by a prodromal stage and systemic symptoms. At suspicion of SJS/TEN, an accurate medication history is essential to detect a possible association, with special attention to drugs, introduced 4–28 days before onset of the reaction.

General Diagnostics

Diagnosis mainly relies on the clinical picture, confirmed by histopathology (clinicopathological correlation). Typical clinical signs initially include painful erythematous and livid macules on the skin on which a positive pseudo-Nikolsky's sign (Fig. 2.2) can be induced. This is often followed by blistering and epidermal detachment within hours. Mucosal involvement develops shortly before or simultaneously with skin signs in almost all cases.

Workup of immediate cryosections or conventional formalin-fixed sections of the skin, preferentially taken from a blister edge, should confirm diagnosis. Histopathology of SJS, SJS/TEN overlap and TEN essentially shows the same picture, featuring widespread keratinocyte apoptosis scattered throughout the epidermis with subepidermal blistering secondary to extensive presence of necrotic keratinocytes, resulting in (almost) full-thickness epidermal necrosis. The dermis may show slight perivascular lymphocytic infiltrates (Fig. 21.6).

Specific Diagnostics

To distinguish SJS from SJS/TEN overlap or TEN, the total maximum detached BSA is the predominant discriminating factor (Fig. 21.3, 21.4 and 21.5).

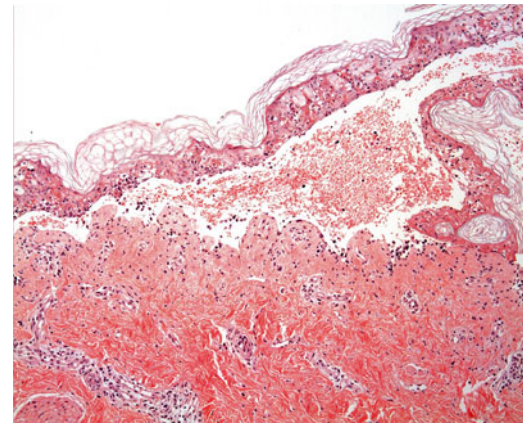

Fig. 21.6 Histopathology of SJS/TEN showing many apoptotic cells resulting in almost total necrotic epidermis and subepidermal splitting. The dermis shows very sparse lymphocytic infiltrates

Table 21.2 SCORTEN criteria and mortality

Independent prognosis factors	Weight
Age ≥40 years	1 point
Malignancy present	1 point
Percentage body surface area ≥10 %	1 point
Heart rate >120	1 point
Serum urea ≥10 mmol/l	1 point
Serum glucose ≥14 mmol/l	1 point
Serum bicarbonate ≤20 mmol/l	1 point
Total score	*Mortality* (%)
0–1 points	3.2
2 points	12.2
3 points	35.3
4 points	58.3
≥5 points	90.0

The main differential diagnoses of SJS/TEN are acute generalised exanthematous pustulosis (AGEP), generalised bullous fixed drug eruption (GBFDE), staphylococcal scalded skin syndrome (SSSS) and autoimmune bullous diseases, including linear IgA disease and paraneoplastic pemphigus, but also pemphigus vulgaris and bullous pemphigoid. Differentiation of AGEP and SSSS can be made by histopathology, while autoimmune bullous diseases can be ruled out by direct immune fluorescence investigations. Differentiation of GBFDE is difficult and can be made on subtle differences in the clinical presentation.

Within the first 3 days of admission, SCORTEN, a severity-of-illness score for TEN predicting prognosis, should be performed (Table 21.2) [6]. Although in vivo or in vitro testing may confirm the suspected culprit drug, the sensitivity of these tests is rather limited in SJS/TEN.

Case Study: Part 2

History reveals that carbamazepine was taken since 2 weeks. Two days later body temperature is 38.9° C. The skin eruption has meanwhile extended and is very painful, with many erythematous papular lesions mainly on the upper torso, face, arms and legs, some with blistering. Severe conjunctivitis is observed, while lips, mouth and genital area show extensive blistering and erosions. Pseudo-Nikolsky's sign is positive (Fig. 21.7). What is your differential diagnosis now?

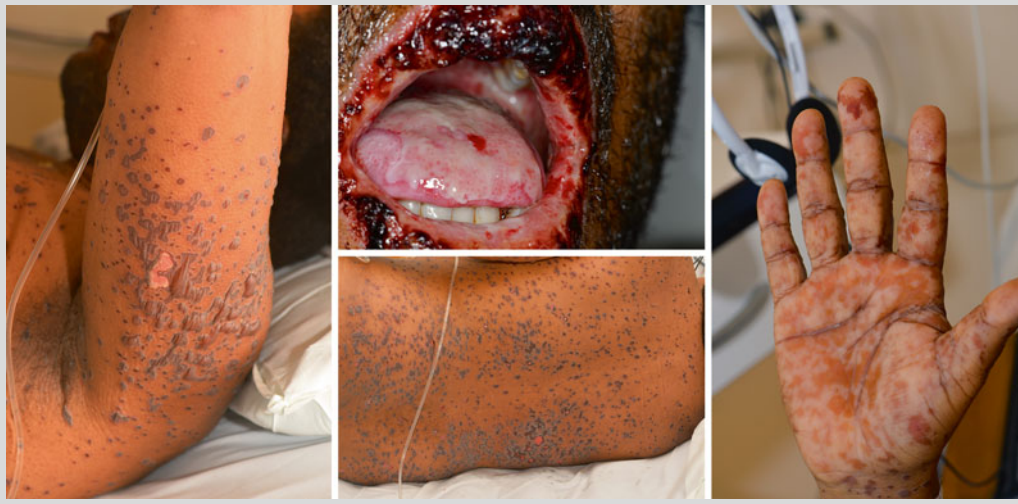

Fig 21.7 Describe what you observe. What is your diagnosis?

Treatment Tricks

Initial Treatment and Escalator

Treatment requires specific expertise and facilities: early admission to a center of expertise reduces the risk of infection, mortality and length of hospitalisation. Management in the acute stage should be multidisciplinary and includes supportive care and evaluation of the severity and prognosis of disease by means of SCORTEN. With a score of ≥3 or when ≥20 % of the BSA is detached or detachable, transfer to an intensive care unit should be considered. Restoring the barrier function of skin and mucosae as quickly as possible and in the meantime preventing negative effects of its loss is of eminent importance [7]. Because of massive loss of body temperature and fluid, the patient is preferentially treated on an "air-fluidised" bed in a temperature- and moisture-regulated room with, for aseptic reasons, a laminar downflow stream. To protect patients from infection, nursing has to be barrier protected.

First line of treatment is cessation of the suspected culprit. For drugs with short half-lives, prompt withdrawal on the first day of blistering/erosions has a positive effect on the outcome and lowers mortality.

Apart from withdrawal of the culprit and intensive supportive care, various options for systemic treatment have been suggested. However, results are variable and generally accepted guidelines are still lacking. A short course of high-dosed pulse therapy, 1.5 mg/kg bodyweight dexamethasone on three consecutive days, early in the process, might positively influence the immune-mediated cascade leading to apoptosis [7]. The supposed rationale that intravenous immunoglobulins (IVIG) inhibit activation of the death receptor by Fas-inhibiting antibodies is questioned and the reported results are inconsistent. Recently, a favourable outcome, although statistically not significant, was reported for treatment with cyclosporin, orally 3 mg/kg/day for 10 days, tapered over 1 month [8].

Follow-Up and Tapering

Intensive monitoring includes vital parameters, laboratory investigations (blood count, electrolytes, renal and liver function, blood gases, bicarbonate, glucose, blood culture, urine analysis, etc.), mucocutaneous cultures and BSA involvement.

The hypercatabolic state and mucosal involvement induced by SJS/TEN often demand nutritional correction by nasogastric feeding. A critical element of supportive care is the management of fluid and electrolyte requirements. Hyponatremia, hypokalaemia or hypophosphatemia, which quite frequently do occur, necessitate appropriate early and aggressive replacement therapy.

Blisters should be treated conservatively because blistered skin acts as a natural biological dressing, likely favouring reepithelialisation. Removing only the epidermis that is curled up is preferred over debridement, which is still often performed in burn units. Extensive wound care includes emollients (petrolatum gauzes), local antiseptics and nonadhesive hydrocolloid dressings. Central lines should be avoided while antibiotics are only given if needed. Pain and anxiety control are essential; systemic corticosteroids should be avoided late in the process [7, 9].

Because of the combined involvement of skin, eyes and other mucous membranes, interdisciplinary follow-up and treatment of sequelae are recommended. Special attention should be given to the prevention of ocular complications. Daily examination by an ophthalmologist can help to diminish the risk for permanent visual loss due to corneal scarring or neovascularisation. Eye drops, saline, topical steroids or antibiotics should be installed every 2 hrs; developing synechiae should be disrupted. In case of corneal defects, amniotic membranes to cover the ocular surface decrease pain, preserve visual acuity and protect against scarring [9]. Scleral contact lenses may promote corneal healing. Prolonged ophthalmologic follow-up is recommended because corneal involvement may progress for months.

Survivors should not be reexposed to the suspected or related causative drug(s). Drugs of the same therapeutic/pharmacological subgroup can be used if needed, provided they are structurally different from the causative drug.

Case Study: Part 3
Histology of the edge of a blister reveals nearly full-thickness epidermal necrosis, subepidermal splitting and sparse dermal lymphocytic infiltrates. Together with the clinical picture, the diagnosis fits within the spectrum of SJS/TEN. The total detached BSA that will ultimately be reached determines the final diagnosis. Carbamazepine is immediately stopped, and treatment is started with dexamethasone pulse therapy 1.5 mg/kg for 3 days intravenously. Patient is nursed barrier protected in a laminar downflow room on an "air-fluidised" bed and intensively monitored including SCORTEN and vital parameters and extensive wound care.

Erythema Exsudativum Multiforme (EEM)

Facts and Figures

Definitions and Classification

EEM is an acute, often symmetrical, polymorphous eruption, with a diversity of lesions: erythema, papules, vesiculo-bullae, nodules and purpura. It may present with only few lesions but can also be rather extensive. Characteristic is the acrofacial distribution, which may spread centripetal. Most lesions develop within 24–72 h as small nummular erythematous lesions, which may become papular. Some may become livid and bullae or purpura may develop in the centre, creating the so-called target or iris lesions. Target lesions in EEM are raised and can be typical (Fig. 21.1) and/or atypical.

EEM varies from mild (EEM minus, the most common form) with symmetrical distributed, most often mildly itching classical "target lesions" to a more severe form (EEM majus). The difference is based on the presence and severity of mucosal and systemic symptoms (e.g. fever and malaise), which are absent or minor in the minus and more pronounced in the majus form.

In EEM majus 50 % has influenza-like prodromes with a classic time course, usually starting 1–14 days before lesions appear, while prodromal symptoms are usually absent or mild in EEM minus. Lesions evolve over 1–2 weeks. Mucosal involvement often presents with clearly haemorrhagic crustae and erosions on the lips, mouth, eyes and/or genitals.

Resolution normally results within 2–3 weeks; EEM majus may have a more protracted course: mucosal lesions generally heal without sequelae, and clearing may take 3–6 weeks. Skin lesions may heal with hyper- and/or hypopigmentation, and scarring is usually absent. Most patients have an uncomplicated course, with exception of the immunocompromised and those with secondary bacterial infections. Although generally self-limiting, recurrences are common and are most often preceded by or occur simultaneously with an overt or subclinical HSV infection.

Mortality in EEM minus is virtually absent and about 1 % in EEM majus; sepsis secondary to loss of the cutaneous barrier is the principal cause [2].

Epidemiology

The exact incidence rate of EEM is not known, but is estimated at somewhere between 0.01 and 1 % of the population. EEM is predominantly observed in young adults with a peak incidence in the second and third decades of life and is rare during early childhood and in adults, older than 50 years [10]. EEM has a slight male preponderance, but no racial bias.

Pathogenesis

Pathophysiology of EEM is still not fully understood. Most likely it is a distinct skin-directed immune reaction, triggered by a variety of stimuli, in particular viral, bacterial or fungal infections (about 90 %) or chemical products in certain "predisposed" individuals. However, to date no clearly genetic predisposition for EEM has been defined. Of note, several physical agents such as trauma, cold and ultraviolet radiation have been described as triggers for outbreaks of EEM related to infectious agents, drugs or systemic disease.

HSV is clearly most commonly associated with EEM, followed by *Mycoplasma pneumoniae*. More rarely, EEM has been associated with drugs or systemic disease. In the majority of children and adults, the disease is precipitated by HSV types 1 and 2 in persons with a normal immunity to HSV, but who possibly have difficulty in clearing the virus. HSV suppression and prophylaxis with antiviral therapy (e.g. valacyclovir) have been shown to prevent recurrent EEM [10].

Diagnosis Paths

History and Physical Examination

Diagnosis relies on the clinical picture: an acute eruption in an adolescent or young adult simultaneously suffering from herpes or just recovering from it or having a history of recurrent, similar attacks. Characteristic is the presence of target lesions and the acral predilection on the back of the hands and feet (sometimes palmoplantar) and extensor sites of the elbows, knees, neck, face, mouth, eyes and genitals. History should document recent constitutional symptoms, previous or current HSV, *Mycoplasma pneumoniae*, other infections and all use of medication, in particular started in the previous 2 months.

General Diagnostics

Besides SJS/TEN, several other diseases may be considered including urticaria, (urticarial) vasculitis, toxic/viral exanthema, annular/gyrated erythemas and M. Sweet, while herpes, stomatitis, aphthosis and SJS should be considered in case of mucosal involvement. The possibility of SJS, GBFDE, exanthematous drug eruption or urticaria should be strongly considered if the presumed aetiology is drug induced. The most important differential diagnosis however is urticaria, especially in its early acute stage. The main difference is that in EEM the centre of the lesions may show a darker, dull, purple aspect, and the presence of blisters, erosions or crusts versus normal skin in urticaria. Moreover, EEM lesions are not transitory and appear during a short period of only few days, while oedema is not a prominent feature.

Specific Diagnostics

Histopathology typically reveals an acute interface dermatitis with apoptotic epidermal keratinocytes, especially at the interface, sometimes resulting in more widespread epidermal necrosis and in addition a moderate lymphocytic, sometimes mixed superficial perivascular infiltrate. The clinicopathological correlation in the differential diagnoses is of importance. In urticaria histopathology shows some perivascular mixed infiltrates, while an interface dermatitis or apoptotic epidermal cells, characteristic for EEM, are lacking.

Treatment Tricks

Most often, EEM is self-limiting and requires no treatment. However, it is essential to identify and treat the eliciting factor.

Otherwise, treatment is usually symptomatic, including oral antihistamines, analgesics, local skin and mucosal care. Liquid antiseptics, such as 0.05 % chlorhexidine, help to prevent superinfection. Patients feeling ill and having extensive lesions can be treated with corticosteroid creams against pruritus and anti-inflammatory drugs and/or lidocaine for pain killing. For oral lesions antiseptics can be useful, as are local corticosteroids and/or pain-killing preparations. For eye involvement an ophtalmologist might be consulted to prevent infection and scarring. Topical treatment, including genital lesions, may be performed with gauze dressings or a hydrocolloid. For more severe cases, meticulous wound care is needed. Infections should be appropriately treated after cultures and/or serologic tests have been performed. Suppression of HSV can prevent HSV-associated recurrent EEM, but antiviral treatment after the eruption of EEM has started is without effect on its course. Although systemic corticosteroids are often given in severe cases, their beneficial use has not been evidenced, and their use should be restricted to the very early stage of the disease.

Review Questions

1. Which drug is most often associated with SJS/TEN?
   (a) Allopurinol
   (b) Penicillin and its derivates
   (c) NSAIDs
   (d) Quinolones
2. The following clinical symptoms differentiate SJS from EEM majus:
   (a) Typical target lesions
   (b) Detached and detachable BSA > 10 %
   (c) Fever
   (d) All of mentioned above
3. Regular observed long-lasting sequelae in SJS/TEN are:
   (a) Impaired vision
   (b) Disturbed liver function
   (c) Disturbed kidney function
   (d) Cutaneous scarring
4. SCORTEN indicates:
   (a) The severity of SJS/TEN
   (b) The total detached and detachable BSA
   (c) The prognosis in TEN
   (d) The severity and prognosis in EEM/SJS/TEN
5. Regarding medication in SJS/TEN:
   (a) SJS and TEN can be elicited by identical medication.
   (b) In SJS/TEN the half-life of a culprit medication that has been withdrawn is decisive for its course.
   (c) In SJS/TEN all medication should be stopped.
   (d) A relatively limited number of drugs has been associated with SJS/TEN.

Answers

1. (a)
2. (a)
3. (a)
4. (c)
5. (a)

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Porphyria Cutanea Tarda and Pseudoporphyria

22

Marjolein S. Bruijn and Jorrit B. Terra

Abstract

Porphyria cutanea tarda (PCT) is a metabolic disorder caused by genetic or acquired partial deficiencies in the heme biosynthesis pathway. This results in accumulation of photoactive porphyrins, mainly uroporphyrins. Pseudoporphyria is a disorder caused by abnormal drug metabolites that accumulate as photoactive substances. PCT and pseudoporphyria clinically present with symptoms including photosensitivity, skin fragility, bullae, erosions, crusts, and scarring. Cutaneous symptoms exclusively involve sun-exposed skin.

Diagnosis is based on a combination of clinical and immune-histopathological features. The level of uroporphyrin in urine is raised in PCT, whereas it is normal in pseudoporphyria.

Therapy consists of sun protection. Triggering factors like alcoholism, estrogen therapy, and hepatitis C should be eliminated in patients with PCT. Phlebotomy and low-dose chloroquine are therapeutic options. In pseudoporphyria the culprit drug should be stopped.

Keywords

Porphyria cutanea tarda • Pseudoporphyria • Vesicular bullous disease • Sun exposure

Introduction and Aims

Short Definition in Layman Terms

Porphyria cutanea tarda (PCT) is a metabolic disorder in which porphyrins accumulate in the skin. Skin fragility and blistering arise after UVA light exposition. Pseudoporphyria mimics PCT, but not due to underlying porphyrin disorder, but due to abnormal drug metabolism.

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Learning Objectives

After reading this chapter, you should be able to recognize the typical clinical presentation of PCT and know the triggering factors for PCT and pseudoporphyria.

Case Study

A 39-year-old woman presented with complaints of skin fragility and bullae on the dorsum of the hands (Fig. 22.1) and bullae and hemorrhagic crusts and erosions on her face and neck. She noticed hypertrichosis on her face (Fig. 22.2). The skin lesions gave burning sensation, especially after sun exposure. Mucosae were not involved.

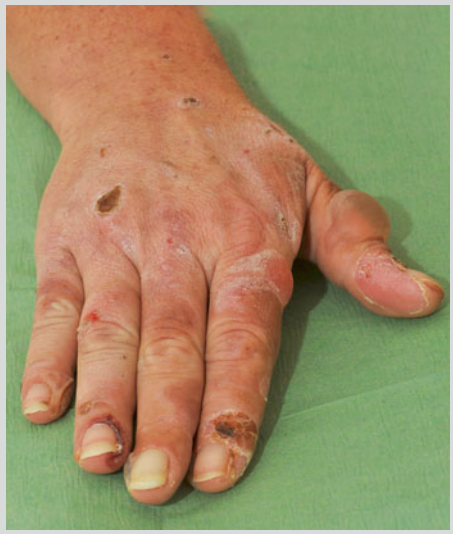

Fig. 22.1 PCT with bullae, hemorrhagic crusts, and erosions on the dorsum of the hand

Fig. 22.2 PCT with lanugo-like hypertrichosis on the face

Didactical Questions: Cross Section of Questions to Prime the Readers Interest

What could have made you think of PCT, and what is your strategy to confirm diagnosis? What do you see in the histopathology and direct immunofluorescence (DIF) when you take a biopsy of a fresh blister? What are the risk factors for PCT and pseudoporphyria?

Facts and Figures

Definitions and Classification

PCT is a bullous photosensitivity disorder. Partial deficiencies in the heme biosynthesis pathway lead to accumulation of porphyrins. PCT can be subdivided in an acquired and a familial form. The familial form is not very penetrant, and therefore, triggering factors are crucial to create clinical findings.

Pseudoporphyria mimics PCT. The clinical, histological, and immunofluorescent characteristics are similar, but there are no accompanying biochemical porphyrin abnormalities.

Epidemiology

PCT is worldwide the most common porphyria and typically presents in the fourth decade of life. PCT overall affects males and females equally, with some studies suggesting male predominance in the acquired form [1]. The epidemiology of pseudoporphyria differs depending on the etiologic agent.

Pathogenesis

PCT is the result of inhibition of uroporphyrinogen decarboxylase (UROD) in the liver, which causes accumulation of mainly uroporphyrins. This inhibition is established in the presence of iron, reactive oxygen species, and activation of cytochrome P450 [2]. Alcoholism, estrogens, iron overload, and liver-related dis-

eases like hepatitis C and hemochromatosis are therefore risk factors. HIV is also reported as a predisposing factor for PCT [2]. Uroporphyrins diffuse into the dermal-epidermal junction where they interact with UVA light, of approximately 400 nm wavelength radiant energy [2, 3]. As a result, reactive oxygen species are formed which produce the main symptoms of PCT.

The precise pathophysiologic mechanism of pseudoporphyria is not fully understood yet. Pseudoporphyria is a photosensitive disorder caused by abnormal drug metabolites that act as photoactive substances. Medication particularly nonsteroidal anti-inflammatory drugs (NSAIDs) like naproxen can cause pseudoporphyria. Other suspected medications are antibiotics, diuretics, and retinoids [4]. Other causes of pseudoporphyria are excessive exposure to UVA light by tanning beds and chronic renal failure or dialysis (Table 22.1).

Table 22.1 Causes of pseudoporphyria [4]

Ultraviolet light
UVA tanning beds
PUVA
Excessive sun exposure
NSAIDs
Naproxen
Nabumetone
Oxaprozin
Ketoprofen
Mefenamic acid
Diflunisal
Chronic renal failure/ dialysis
Antibiotics
Nalidixic acid
Tetracycline
Diuretics
Chlorthalidone
Bumetanide
Furosemide
Hydrochlorothiazide/ triamterene
Retinoids
Isotretinoin
Etretinate
Miscellaneous
Cyclosporin
5-Fluoroucil
Carisoprodol/ aspirin
Pyridoxine
Amiodarone
Flutamide
Dapsone
Coca-Cola

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Diagnosis Paths

History and Physical Examination

Cutaneous findings exclusively involve sun-exposed areas of the body like the dorsum of the hands, forearms, upper chest, and face. Patients complain of photosensitivity and skin fragility. Cutaneous findings include hemorrhagic crusts, vesicles, bullae, and superficial scars or milia. The bullae are tense and filled with clear fluid (Fig. 22.1). Additional hypo- and hyperpigmentation, hypertrichosis, sclerodermoid plaques, scarring alopecia, onycholysis, and dystrophic nails may be present. Patients, particularly females, may have lanugo-like hypertrichosis on the periorbital and temporal regions of the face (Fig. 22.2). Although most clinical features in PCT and pseudoporphyria are similar, features as hypertrichosis, hyperpigmentation, and sclerodermoid plaques are rarely present in pseudoporphyria [4]. The clinical presentation of PCT and pseudoporphyria is subacute, and the relationship with sun exposure may therefore be missed (Fig. 22.3).

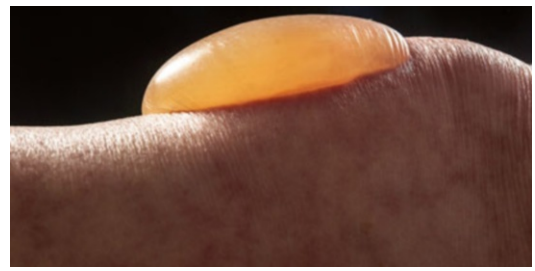

Fig. 22.3 Pseudoporphyria in a paraplegic wheelchair patient showing a tense monomorphic bulla filled with clear fluid on the sun-exposed knee. The culprit drug was tolterodine [5]

General Diagnostics

The diagnosis of PCT is made with urine test demonstrating increased uroporphyrin levels, but also increased levels of precursors like penta-, hexa-, and hepta-carboxylated porphyrins, and coproporphyrins can be detected in the urine. Also plasma and fecal porphyrin levels are increased. Screening of urine on uroporphyrins is however sufficient for PCT diagnosis. Almost all patients have elevated serum iron and ferritin levels. A biopsy of a fresh blister for histology shows a subepidermal cell-poor blister with "festooning" of dermal papillae and PAS-positive glycoproteins at the basement membrane zone and around the blood vessels. DIF shows immunoglobulin, mainly IgG, and complement deposition at the basement membrane zone and around the blood vessels (Fig. 4.10).

Specific Diagnostics

Exclude risk factors like hemochromatosis, hepatitis C, and HIV infections.

Treatment Tricks

Initial Treatment and Escalator

Sunlight avoidance is the first step in the treatment of PCT and pseudoporphyria. Besides sun protection, treatment entails discontinuation of suspected risk factors. Other measures include iron depletion by regular phlebotomy or low-dose chloroquine. Chloroquine is capable of binding porphyrins, forming water-soluble complexes readily excreted in the urine [3]. Higher doses of chloroquine can cause liver toxicity and should be avoided. In pseudoporphyria the culprit drug should be stopped for at least 3 months before evaluation.

Follow-Up and Tapering

Once a biochemical remission in PCT is instigated, there is a high chance of relapse even if the underlying cause has been treated. Additionally potential development of hepatocellular carcinoma is possible, and therefore, follow-up is advised.

Review Questions

1. How can you differentiate between PCT and mechanobullous EBA?
   (a) Clinical symptoms
   (b) Histological findings
   (c) DIF serration pattern analysis
2. What is the main treatment of PCT?
   (a) Phlebotomy
   (b) Low-dose chloroquine
   (c) Sun protection
3. What is the most frequent cause of pseudoporphyria?
   (a) Tetracycline
   (b) Naproxen
   (c) Excessive use of Coca-Cola

Answers

1. (c)
2. (c)
3. (b)

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Rare Diseases Clinical Research Network NHI: https://www.rarediseasesnetwork.org/porphyrias/patients/PCT/

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Bullous Dermatitis Artefacta

23

Marcel F. Jonkman

Abstract

Bullous dermatitis artefacta is a psychodermatologic factitious disorder in patients who mimic skin disease by inflicting themselves with blisters. The diagnosis is often apparent at first visit. The diagnosis should never be immediately revealed to the patient. Instead a serious, yet limited, workup is advised while developing a trustful patient-doctor relation. The strategy is to give the patient the impression you know it was self-inflicted but leaving an escape for clearance by trivial causes imagined by the patient such as avoiding drinking coffee (narrow escape). In refractory cases, when confrontation becomes unavoidable, dual approach by dermatologists and psychiatrists is necessary.

Keywords

Factitious disorder • Psychodermatology • Self-injury • Munchausen syndrome

Introduction and Aims

Short Definition in Layman Terms

Bullous dermatitis artefacta is a mental abnormality in patients who mimic skin disease by inflicting themselves with blisters. The diagnosis is often immediately apparent to the doctor at first visit. The patient should be approached in such a way that the he is not losing face. Premature confrontation or embarrassing accusations should be avoided. Treatment strategy is narrow escape: the patient is almost confronted while he gets the chance to opt out by avoiding any scapegoat (coffee) or taking any rescue (vitamin C) that cleared the skin. In difficult cases the patient has to be confronted with the diagnosis by a psychiatrist.

<Despite the spot diagnosis, DA needs a serious workup.>

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Learning Objectives

After reading this chapter, you will understand the clinical presentation, histopathology, differential diagnosis, and treatment approaches of bullous and vesiculobullous eruptions in bullous dermatitis artefacta (DA).

Case Study: Part 1

A mother with child consulted the Center for Blistering Diseases after visiting three dermatologists before in the last year because of episodes of erosions in the face of the 12-year-old daughter. All dermatologists came to a prompt diagnosis of dermatitis artefacta and ask the girl if she did it herself. She denied. The episodes persisted.

At dermatological examination, I saw a shy but cooperative girl with linear erosions in the face with erythematous border. The mother was receptive for advice.

A skin biopsy for direct IF was negative.

Didactical Questions: Cross Section of Questions to Prime the Readers' Interest

What is the presentation of DA? What is the approach to avoid frustration of the doctor?

Facts and Figures

Definitions and Classification

Bullous dermatitis artefacta (DA) is a cutaneous factitious disorder in which the patient intentionally evokes blisters or erosions but denies self-infliction (Fig. 23.1). The synonym bullous pathomimia [1] is not used anymore, since not all patients are fully aware of their self-inflicting behavior that mimics bullous disease (Table 23.1). In automutilation (non-suicidal non-hidden self-injury, DSM-5), the patient also purposely wounds its own skin but in contrast to DA admits self-infliction, such as cutting with a knife that does not mimic other skin disease. Neurotic excoriations are due to excessive compulsory scratching because of perceived itch.

Epidemiology

The patient with bullous DA is predominantly a teenage female. One of the parents, mostly the

Table 23.1 Diagnostic criteria for factitious disorders [3]

#	Criteria
1	Intentional production of physical or psychological signs or symptoms
2	Motivation for the behavior is to assume the sick role
3	Absence of external incentives for the behavior (e.g., economic gain, avoiding legal responsibility, or improving physical well-being, as in malingering)

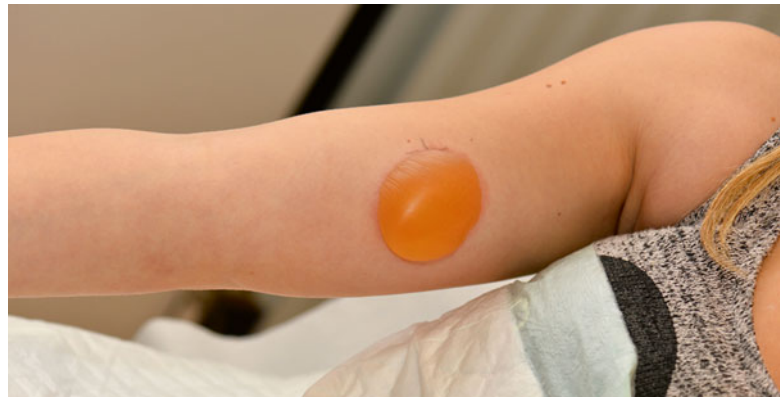

Fig. 23.1 Solitary monomorphic bulla on the arm of a teenager with bullous dermatitis artefacta. The level of blistering was subepidermal and probably thermally or by suction induced

mother, is present at first consultation. The prognosis improves with younger patient and shorter history of DA.

Pathogenesis

The patient keeps the secret of self-infliction or shares it with a relative ("folie à deux") or is the victim of a parent (Munchausen-by-proxy syndrome). The loneliness of the secret is compensated by the attention that the skin disease evokes in others. The patient may also not be fully conscious of the self-harm by dissociation. The psychopathology of this behavior is associated with borderline personality disorder, multiple personality disorder, posttraumatic stress syndrome, anorexia, and bulimia. Simply said: the patient dies for attention, but shows indifference for pain ("la belle indifference").

Diagnosis Paths

History and Physical Examination

Bullous DA is a spot diagnosis (Fig. 23.2): the physician often immediately recognizes the bizarre pattern of the skin lesions and considers artifacts. New lesions have developed "spontaneously" days before the first visit. The medical history is hollow with no timeline or evolution pattern. The patient appeals the competence of the doctor by questioning how these lesions suddenly can develop (Table 23.2).

General Diagnostics

Despite the spot diagnosis, DA needs a serious workup. This is important for a trustful patient-physician relation but may also prevent to step into the pitfall of missing DA-like autoimmune bullous disease. I remember the case of a 58-year-old female with a 10-year history of crusted erosions on arms and neck that healed with scars. She had visited three dermatologists, a rheumatologist and a psychologist who all presumed the diagnosis bullous DA. Taking a DIF biopsy turned out to be linear IgA disease. Treatment

Table 23.2 Signs of bullous dermatitis artefacta

Type	Example
Bizarre or regular distribution of lesions	Bullae at regular distance (like wallpaper), symmetrical, on arms
Does not fit in known disease	Solitary blister without primary erythema
Medical shopping	Visited several dermatologists including a rheumatologist
"La belle indifference"	Looking untouched while presenting with several painful erosions
Improves under zinc oxide plaster	Healed lesions on lower legs, except at edge of the plaster

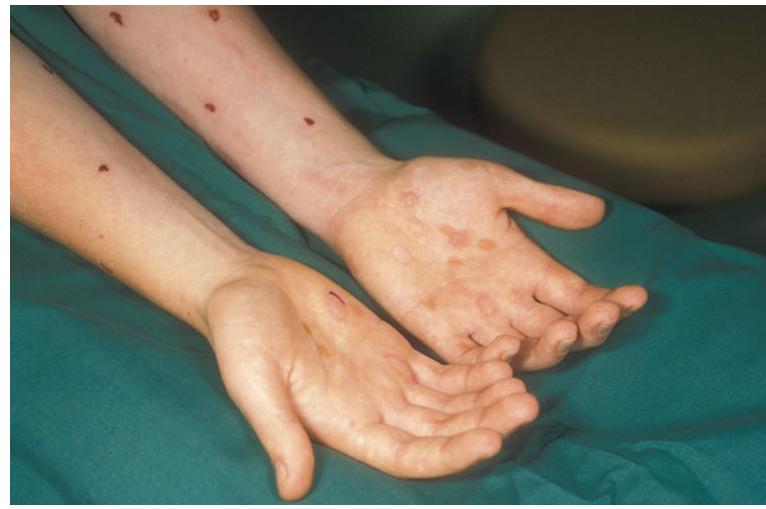

Fig. 23.2 Typical presentation of bullous dermatitis artefacta presenting with crusted erosions over the lower arm and linear bullae in the palm of the hand. The even distribution pattern resembles wallpaper

with dapsone cleared the lesions within weeks and saved her marriage.

The most important differential diagnosis of bullous DA is porphyria cutanea tarda and pseudoporphyria (Chap. 22). Therefore, take a biopsy, examine urine for uroporphyrins, and check history for culprit drugs. Limit the investigations and visits, however, since that keeps the doctor to remain expert in the eyes of the patient.

The physician should take all efforts at the first visit to develop a trustful relation with the patient. Show genuine personal interest and ask the patient questions about social setting (home, school, sports). Address the accompanying person separately in the conversation. Do not let the patient lose face in anyway.

Specific Diagnostics

Histopathology of the edge of a blister may reveal the factitious nature. The level of blistering depends on the type of trauma (Table 23.3).

Table 23.3 Level of blistering in artificial bullae

Level of blistering	Trauma
Intracorneal	Plucking
Subcorneal of granular	Rubbing
Intrabasal or intraspinal	Electric
Subepidermal	Suction, thermal, acids
Deep cutaneous	Alkalines

The weakest spot (locus minoris resistentiae) in the skin may divert due to skin disease. For instance, repeated friction by handling a gardening tool (Fig. 23.3) results in a *physiological* friction blister in the granular layer (interface between living and dead epidermis, Figs. 23.4 and 23.5). However, in patients with hereditary epidermolysis bullosa, the *pathological* friction blister is intrabasal or subepidermal at the site of the affected adhesion molecule.

Case Study: Part 2
My spot diagnosis was dermatitis artefacta. The patient was sent away with the nurse for drinking thee, and I took the opportunity to confront the mother with the diagnosis in an empathetic yet definite way. She initially could not believe my conclusion. The patient and her mother agreed to keep a skin diary.

Treatment Tricks

Narrow Escape

Tell your patient which diagnoses have been excluded with certainty. Take skin complains seriously and treat symptomatically. Build a safe environment.

Fig. 23.3 Physiological friction blister in normal individual due to repeated trauma with shovel during gardening

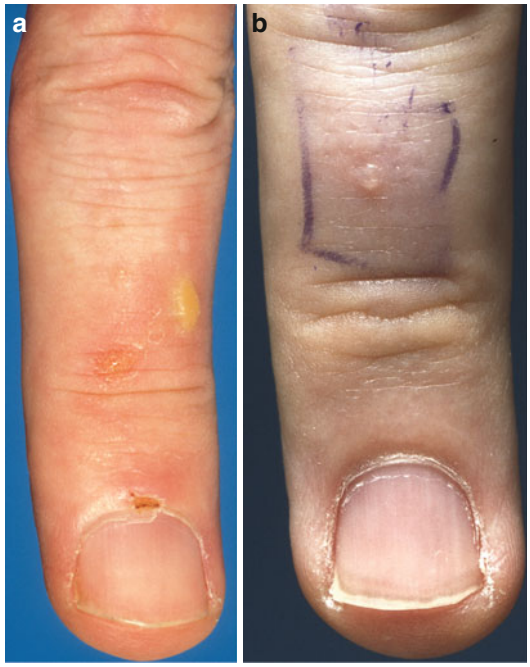

Fig. 23.4 (**a**) Vesicle on the digit in a patient with factitious disorder. (**b**) Patient in (**a**) was able to induce a vesicle in 30 s on the digit of his doctor by friction with his thumbnail (Reprinted with permission from *Ned Tijdschr Geneeskd*. 2000;144(31):1465–9)

First step in treatment is "narrow escape" thus avoiding loss of face of the patient [2]. Create a narrow escape by giving the patient the feeling you know that it self-inflicted, but never directly question it, nor accuse the patient. For instance, at first visit I told a patient during physical examination that I have seen this before, and it remarkably looks like a burn blister. At the end of the consultation, I promise the patient to tell what it is at next visit after finishing all examinations. In the meanwhile I ask the patient to keep a diary of new blisters. Keeping a diary provides extra attention. At second visit the lesions may have cleared. I have heard because the patient stopped drinking coffee, took vitamin C, or confessed to his/her mother when brought to bed. Show happiness and agree with the conclusion of the patient. If the problem persists, then introduce the psychiatrist.

<The first-line management of DA is narrow escape, and the second is confrontation.>

Dual Approach

DA that is not responding to narrow escape is generally managed by dual (or holistic) approach by dermatologist as the skin expert and the psychiatrist/psychologist for mental exploration. Offer psychological help by explaining that such chronic skin disorder will have serious impact on the patient's mental well-being. The psychiatrist may be introduced after the second visit or be present from the start in special clinics for psychodermatology.

The aim is not to elicit a confession. The patient sins against the ground rule that he or she is dedicated to be cured. The dual approach also protects the dermatologist from incompetent feelings, elicited by patient demands when relapse occurs. Aggressive emotions in a physician may lead to aim of unmasking the patient. Be conscious of this countertransference.

At some stage, in refractory cases, there is no other option than to confront the patient with the self-inflicted nature of the skin problem. This should be done without moral judgment preferable by or in the presence of the psychiatrist. If the patient-doctor relation developed in trust, the patient will not walk away and let her lesions be treated symptomatically. After all, they also deserve compassion as sufferers of a chronic skin disorder.

Case Study: Part 3

The parents supported her in keeping a skin diary. They noticed repeated rubbing of the face. One night before bed, the daughter confessed to her mother that she was nervous at school before math and then rubbed her face. The mother suggested her child to take a different doll to school every week, and every time she felt nervous, she would cuddle the doll instead of rubbing her face. Complete remission was reached! The doll was the narrow escape introduced by the mother. Other scholars now also took a doll to school to desensitize themselves when nervous. As follow-up, I advised consultation by a pediatric psychiatrist to screen for anxiety disorders in her child.

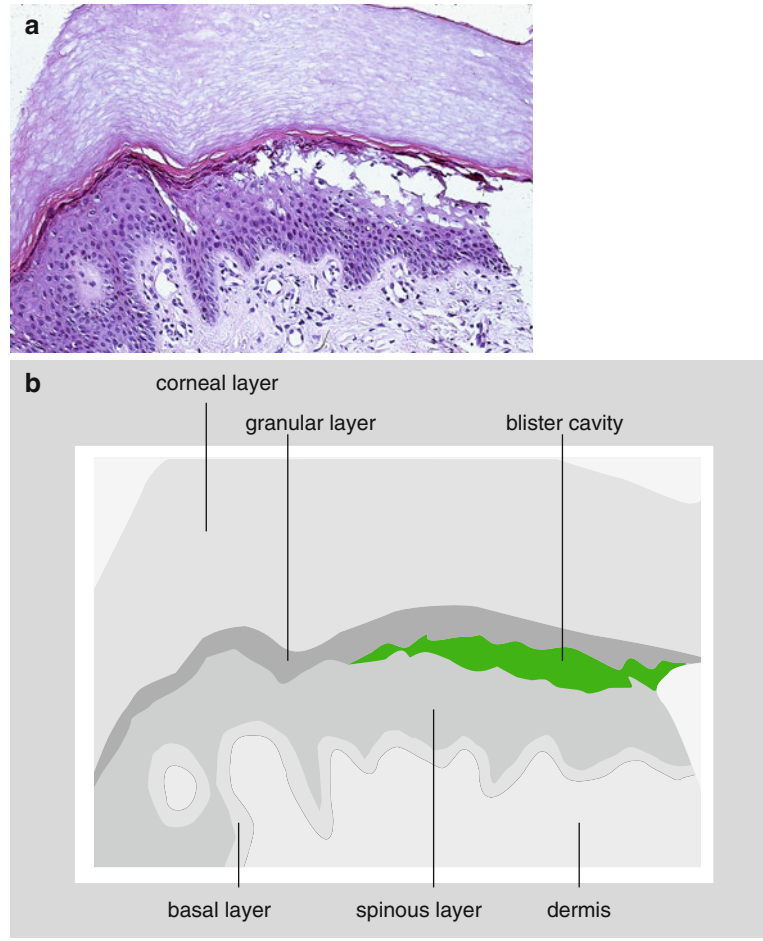

Fig. 23.5 (**a**) Histopathology of *physiological* friction blister in patient with dermatitis artefacta reveals split level beneath granular layer. (**b**) Diagram depicting (**a**) (Reprinted with permission from *Ned Tijdschr Geneeskd*. 2000;144(31):1465–9)

Review Questions

1. What is most typical of the distribution of DA lesions?
   (a) Multiple
   (b) Asymmetrical
   (c) Trunk
   (d) Regular
2. The blister level of a thermal blister is:
   (a) Subgranular layer
   (b) Spinous
   (c) Intrabasal
   (d) Subepidermal
3. First approach to a patient with DA is:
   (a) Confrontation
   (b) Supportive empathy and serious investigation
   (c) Narrow escape
   (d) Referral to psychiatrist
4. What examples are NOT a narrow escape?
   (a) Starting a food supplement
   (b) Stopping certain food
   (c) Zinc plaster
   (d) Placebo

Answers

1. (d)
2. (d)
3. (b)
4. (c)

References

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2. van Rijssen A, Molier L, Vrijlandt AJ, Jonkman MF. Bullous dermatitis self-induced. Ned Tijdschr Geneeskd. 2000;144:1465–9.
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Additional Reading

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On the Web

Merck Manuals: http://www.merckmanuals.com/professional/psychiatric_disorders/somatic_symptom_and_related_disorders/factitious_disorder_imposed_on_self.html.

NYU Langone: http://www.med.nyu.edu/content?ChunkIID=165426.

Psycho-dermatology, British Association of Dermatology: http://www.bad.org.uk/healthcare-professionals/clinical-services/service-standards/psycho-dermatology.

Wikipedia: http://en.wikipedia.org/wiki/Factitious_disorder.

Part V

Resources

Patient Support Groups and International Centers for AIBD

24

Joost M. Meijer and Marcel F. Jonkman

Abstract

Patients and their families should be informed about disease, prognosis, available treatment options, process of clinical follow-up, and possible adverse events or complications. Patients should also be informed about the existence of local or national patient support groups or patients' associations. These associations contribute to promote knowledge of the disease and improve patients' access to information, healthcare, and social services, and they can help in referring patients to centers of expertise for AIBD.

Keywords

Autoimmune bullous diseases • Patient support groups • Centers of expertise • Biologics

Patients and their families must be informed about the disease, the prognosis, available treatment options, and possible adverse events or complications. The process of clinical follow-up and monitoring of disease activity and treatment should be explained carefully in advance. In rare diseases such as AIBD, clinical research may lack relevance for patients and does not always focus on unmet needs and priorities identified by patients and clinicians. Future research agendas with input from patients and clinicians may lead to improvement of relevant research questions, such as curative long-term therapies and limitation of therapy-related adverse events and complications.

Anti-CD20 Biologics

Changes in treatment options for AIBD are being expected with approval of anti-CD20 monoclonal antibody rituximab and intravenous immunoglobulin (IVIG) and with the development of next-generation anti-CD20 monoclonal antibodies. Clinical trials focus on efficacy and safety of anti-CD20 biologics in pemphigus, whereas

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case reports are being published on treatment of pemphigoid. Moreover, these relative expensive treatment options may become available in more countries when patents expire and biosimilars are reaching the market. The current use of anti-CD20 biologics as second-line treatment in refractory cases of AIBD may shift to first-line treatment.

Patients should also be informed about the existence of local or national patient support groups or patients' associations. These associations contribute to promote knowledge of the disease and improve patients' access to information, healthcare, and social services, and they can help in referring patients to referral centers for AIBD (Table 24.1). The dermatologists in Europe work together in the task force AIBD of the European Academy of Dermatology and Venereology and in Japan in the Pemphigus Study Group.

Table 24.1 National patient support groups and centers of expertise for AIBD

Country	Patient support group	National centers of expertise
Australia	Australasian Blistering Diseases Foundation: http://blisters.org.au	Department of Dermatology at St. George Hospital, University of New South Wales, Sydney
Austria		Department of Dermatology, Division of Immunology, Allergy, and Infectious Diseases, Medical University of Vienna, Vienna
Belgium		Department of Dermatology, University of Gent, Gent
Brazil		Department of Dermatology, University of Sao Paulo, Sao Paolo
Bulgaria		Department of Dermatology, Alexander's University Hospital, Sofia
Canada	Canadian Pemphigus and Pemphigoid Foundation: http://www.dermatology.ca/wp-content/uploads/2012/01/CPPF-brochure-EN.pdf	Division of Dermatology, Sunnybrook Health Sciences Center, Toronto
Croatia		Department of Dermatology, School of Medicine University of Zagreb, Zagreb
Czech Republic		Department of Dermatology, Masaryk University, Brno
Denmark		Department of Dermato-Venereology, Aarhus University Hospital, Aarhus
Egypt		Department of Dermatology, Cairo University, Cairo
Finland	Ihoyhdistys: ihoydistys@gmail.com	Department of Dermatology, Tampere University Hospital, Tampere
France	Association Pemphigus Pemphigoïde, France: www.pemphigus.asso.fr	Groupe Bulle, Department of Dermatology, Rouen University Hospital, Rouen Groupe Hospitalier Henri-Mondor, Créteil
Germany	Pemphigus und Pemphigoid Selbsthilfegruppe: www.pemphigus-pemphigoid-selbsthilfe.de	Department of Dermatology, University of Lübeck, Lübeck Department of Dermatology and Allergology, University Hospital, Philipps University Marburg, Marburg Department of Dermatology and Allergology, Ludwig Maximilian University, Munich
Greece		Department of Dermatology, Aristotle University of Thessaloniki, Thessaloniki
India		Department of Dermatology, Venereology and Leprology, Postgraduate Institute of Medical Education and Research, Chandigarh Department of Dermatology and Venereology, All India Institute of Medical Sciences, New Delhi

Table 24.1 (continued)

Country	Patient support group	National centers of expertise
Indonesia		Department of Dermatology, Gadjah Mada University, Yogyakarta
Iran		Autoimmune Bullous Diseases Research Center, Department of Dermatology, Tehran University of Medical Sciences, Tehran
Italy	Associazione Nazionale Pemfigo-Pemfigoide: www.pemfigo.it	Istituto Dermopatico dell'Immacolata, IRCCS, Rome
Israel		Department of Dermatology, Tel Aviv University, Tel Aviv
Hungary		Department of Dermatology, Semmelweis University, Budapest
Japan	http://hp.kanshin-hiroba.jp/tenpou-ruitenpousou/pc/	Department of Dermatology, Keio University School of Medicine, Tokyo Kurume University Institute of Cutaneous Cell Biology, Kurume, Fukuoka
South Korea		Department of Dermatology and Cutaneous Biology Research Institute, Yonsei University College of Medicine, Gangnam Severance Hospital, Seoul
Lebanon		Department of Dermatology, American University of Beirut Medical Center, Riad El Solh/Beirut
Morocco		Service de Dermatologie, CHU Ibn Sina, Université Med V, Souissi, Rabat
Nepal	Blistering Disease Foundation of Nepal	Civil Service Hospital, Naya Baneshwor, Kathmandu
the Netherlands	Netwerk Nederland voor Pemphigus en Pemphigoïd www.pemphigus.nl	Center for Blistering Diseases, University Medical Center Groningen, Groningen
Poland		Department of Dermatology, Medical University of Warsaw, Warsaw
Serbia		Clinic of Dermatovenereology, Clinical Center of Serbia, Belgrade
Spain	Asociación Española de Pénfigo, Penfigoide y Otras Enfermedades Vesiculoampollosas: info@aeppeva.org	Department of Dermatology, University Hospital Clínic de Barcelona, Barcelona
Switzerland		Department of Dermatology, University of Bern, Inselspital, Bern
Tanzania		Regional Dermatology Training Center at Kilimanjaro Christian Medical University College, Moshi
Tunisia		Department of Dermatology, Charles Nicolle Hospital Tunis, Tunis
Turkey	www.turkdermatoloji.org.tr	Department of Dermatology, Akdeniz University, Antalya Department of Dermatology, Karadeniz Technical University, Trabzon
United Kingdom	http://pemfriends.co.uk http://www.pemphigus.org.uk	Department of Dermatology, Churchill Hospital, Oxford St. John's Institute of Dermatology, Guy's and St. Thomas' Hospital NHS Trust, London

(continued)

Table 24.1 (continued)

Country	Patient support group	National centers of expertise
USA	International Pemphigus Pemphigoid Foundation: www.pemphigus.org	Department of Dermatology, University at Buffalo, Buffalo, NY Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, NC Department of Dermatology, Duke University Medical Center, Durham, NC Department of Dermatology, School of Medicine, University of Utah, Salt Lake City, UT Department of Dermatology, University of Iowa, Iowa City, IA Department of Dermatology, University of Texas Southwestern Medical Center, Dallas, TX Department of Dermatology, University of Pennsylvania, Philadelphia, PA Department of Dermatology, Stanford University, Stanford, CA St. Joseph Mercy Health System, Department of Dermatology, Ann Arbor Department of Dermatology, St. Joseph Mercy Ann Arbor Hospital, Ann Arbor, MI Department of Dermatology, University of California, Irvine, CA Laboratory for Investigative Dermatology, Rockefeller University, New York, NY Division of Dermatology and Cutaneous Sciences, Center for Investigative Dermatology, Michigan State University, East Lansing, MI

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