Introduction

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Chapter 1

SUBEPIDERMAL AUTOIMMUNE BULLOUS DISEASES

Blistering of skin or mucous membranes can be caused by genetic, metabolic, chemical, physical, infectious, ischemic, toxico-endogenic, and autoimmune causes. Autoimmune bullous disease is an acquired immunological reaction to self-antigens in and below the stratified epithelium. The skin comprises two compartments: the epidermis and the underlying dermis separated by an epidermal basement membrane zone (BMZ). Autoimmune bullous diseases are classically divided in those with an intra-epidermal blister, the pemphigus spectrum, and those with blister below the epidermis, the subepidermal autoimmune bullous diseases (sAIBD). In the latter autoantibodies are directed against self-antigens or autoantigens that reside in the epidermal BMZ. This thesis focuses on blisters caused by sAIBD.

The epidermal BMZ contains numerous highly specialized structures called hemidesmosomes that are composed of distinct polypeptides. At the top, the epidermal BMZ consists of the hemidesmosomal plaque followed by the plasma membrane of the basal keratinocyte. Underneath is the lamina lucida, that is electron-lucent in electron microscopy. The hemidesmosomal transmembrane molecules cross the lamina lucida and bind to laminins, in the skin the epidermis specific laminin-332. Underneath the lamina lucida is the lamina densa consisting of type IV collagen, nidogen and other structural components. Laminin-332 binds to type VII collagen. Type VII collagen is the main component of the anchoring fibrils and these are found in the sublamina densa zone, the lowest part of the epidermal BMZ. Various adhesion proteins are targeted in sAIBD (figures 1 and 2).

Hemidesmosomes (HD) contribute to the attachment of epithelial cells to the underlying basement membrane in stratified epithelia of the skin, cornea, mucous membranes of the gastrointestinal and respiratory tract and amniotic membrane. These proteins form the bridge between the cytoskeleton and extracellular matrix components. Hemidesmosomes appear in two different types: type I hemidesmosomes contain all components, whereas type II hemidesmosomes only consist of integrin α6β4, tetraspan/CD151, and plectin. Type II hemidesmosomes are found in pseudostratified and cylindrical epithelia, e.g., those lining the gastrointestinal and respiratory tract, respectively. Type I hemidesmosomes are located in basal keratinocytes of multilayered stratified squamous epithelium. Anchoring fibrils are filamentous structures that arcade below the lamina densa in the sublamina densa zone. The major component of anchoring fibrils is type VII collagen. Mutations in genes coding for the components of the dermal-epidermal anchoring complex as well as the formation of autoantibodies against these structures result in a split formation at the level of the dermal-epidermal junction.
LABORATORY DIAGNOSIS OF UNUSUAL AND USUAL VARIANTS OF sAIBD

The sAIBD can be divided in common diseases e.g. bullous pemphigoid, mucous membrane pemphigoid, linear IgA-dermatosis, pemphigoid gestationis, and less common or rare diseases e.g. anti-laminin-γ1 pemphigoid, anti-laminin-332 mucous membrane pemphigoid, epidermolysis bullosa acquisita, and lichen planus pemphigoides.

The laboratory diagnosis is made by analysing skin specimens and/or serum samples of the patients. For routine histopathological examination, a fresh vesicle or blister is biopsied at the edge of the blister (lesional), placed in formaldehyde, and processed for haematoxylin and eosin staining.\textsuperscript{12,13} Histopathology of a skin biopsy for bullous disease needs to look for type of infiltrate, structural abnormalities, and split level. The level of split allows differentiation between pemphigus and pemphigoid (sAIBD) spectrum which is intra-epidermal vs subepidermal
respectively. The infiltrate may give a hint for sAIBD, but by histopathology alone one is not able to demonstrate sAIBD.

For diagnosing sABID one needs to demonstrate depositions of immunoreactants in the epidermal BMZ by direct immunofluorescence microscopy (DIF) of perilesional skin. The biopsy must be snap frozen immediately and stored at -80 degrees or placed in saline or Michel’s medium suitable for later DIF testing. In sAIBD typically a linear deposition of IgG, IgA or complement is found along the epidermal BMZ. The linear depositions follow the BMZ in a serrated pattern. Analysis of these depositions by our group has revealed two types of serration patterns, a n-shaped and an u-shaped pattern. The sAIBD with their autoantigen located in or above the lamina densa, show a n-serrated immunofluorescence pattern, whereas the autoantigen located in the sublamina densa zone, type VII collagen, target in epidermolysis bullosa acquisita (EBA), IgA-EBA and bullous systemic lupus erythematosus (bSLE), show a u-serrated deposition pattern.

The ultrastructural localization epidermal BMZ antigens can be determined by direct immunoelectron microscopy (DIEM) of immunolabelled semi-thin skin sections. In bullous pemphigoid (BP), mucous membrane pemphigoid (MMP) and pemphigoid gestationis (PG) the antibodies bind to the HD plaque and lamina lucida, and in EBA IgG binds in vivo in the sublamina densa zone with a “free zone” below the lamina densa. The upstanding arms of the sublamina densa zone between the rootlets of the basal keratinocytes seen at ultrastructural level in electron microscopy correlate with the u-serration (“grass”) seen at the microscopical level in DIF.

Serum analysis for circulating autoantibodies is a valuable tool in laboratory diagnosis. The classical technique is indirect immunofluorescence microscopy (IIF) performed on frozen sections of standard substrates, including human skin, monkey esophagus, and rodent or monkey bladder. A valuable addition to these substrates was salt-split human skin, skin that has been incubated in 1 M NaCl what causes a separation of epidermis and dermis at the level of the lamina lucida (figure 2).

Figure 2. Schematic overview of the hemidesmosome (right) and the focal adhesion (left). Proteins to which autoantibodies are directed in sAIBD are stained in yellow. The epidermal basement zone can be consistently split through the lamina lucida by 1.0 M NaCl incubation, separating the antigens of the epidermal and dermal compartment. [Figure courtesy of prof. dr. M.F. Jonkman, 2010]
Seperation of the epidermal and dermal compartment has two advantages, the sensitivity is increased through reduction of background fluorescence due to removal of endogenous non-specific IgG and furthermore it allows discrimination between sAIBD with epidermal or dermal binding autoantibodies due to autoantigens in either the floor of the roof of the split. More sophisticated methods include fluorescence overlay antigen mapping (FOAM) analysis and IIF on socalled 'knock-out' substrate. FOAM is an IF technique developed in our laboratory which made it possible to visualise up to three immunoreactants in different colours in a single microscopic field of the epidermal BMZ. The technique can be used to differentiate between sAIBD's that have targeted autoantigens within different levels in the BMZ. For instance, FOAM can differentiate between sAIBD with reactivity against laminin 332 and type VII collagen. IIF analysis on knock-out substrates is the analysis of serum performed on a substrate that misses the antigen of interest due to a genetic cause. We used plectin-deficient skin from a patient with epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) homozygous for a null mutation in the plectin gene (c.G13187T, p.E1914X) to prove the existence of anti-plectin autoantibodies. Also, in chapters 2 and 3 we show that affinity-purified autoantibodies to plectin bind back to human skin and muscle.

A further refinement of serum analysis in sAIBD are immunoassays that identify the targeted antigen, which are sometimes necessary for making the final diagnosis. For diagnosing sAIBD’s these include immunoblotting, immunoprecipitation and enzyme-linked immunosorvent assay (ELISA).

Immunoblot or Western blot is a method of semiquantitative determination of specific protein expression. Immunoblot allows to demonstrate reactivity to a protein in a mixture of any number of proteins while giving information about the size of the protein. For diagnostics the methods relies on the antigen-binding properties of the autoantibodies. In immunoblotting the binding properties depend on the type of detergent used, e.g. with the aggressive sodium dodecyl sulphate (SDS) proteins interactions are broken in a way that linear epitopes can be found. In contrast, conformational epitopes can instead be detected with milder detergents or other techniques like immunoprecipitation.

Immunoprecipitation (IP) is the technique of precipitating a protein complex out of solution using an antibody that specifically binds to that particular protein (antigen). The advantage of IP is that the technique can detect conformational epitopes, however some protein interactions might not be broken and can therefore not be detected. IP precipitates native protein complexes, such as the laminin-332 trimer, while immunoblotting demonstrates reactivity to individual polypeptides, such as the alpha3 laminin chain.

The substrates for immunoblot and immunoprecipitation are extracts of cultured keratinocytes (cell extract), SCaBER cells, cultured A431 cells, tumor cell lines, or amnion. Also extracts of epidermis, dermis, recombinant (fusion) proteins, or spent culture medium can be used as a source of antigens. Some extracts have advantages over others: extracts of SCaBER cells, cultured A431 cells, and amnion have the advantage that it contains the hemidesmosomal antigens.
and type VII collagen in sufficient amounts, while cultured keratinocyte extracts are low in type VII collagen. Dermal extract contains type VII collagen, but generates too much background in immunoblot. Immunoblot with recombinant NC1, the C-terminal part of type VII collagen, is more sensitive and ELISA with recombinant NC1, the C-terminal part of type VII collagen is known in the literature as very sensitive and specific. Enzyme-linked immunosorbent assays (ELISAs), also known as enzyme immunoassay (EIA) is a diagnostic tool used to detect the presence and titer of specific antibodies in patient's serum. Simply said, an unknown amount of antigen is affixed to a surface and then a specific antibody is applied over the surface so that it can bind to the antigen. In ELISA the antigens are present under native conditions, thus binding activity against conformational epitopes, which are epitopes in the original folded conformation of an antigen, is not lost. In contrast to immunoblot, ELISA is a quantitative method. Utilizing recombinant proteins that encompass specific regions of the BP antigens (e.g. the NC16A domain of BP180) are very specific as these domains contain various dominant epitopes to which these autoantibodies have been mapped. The usefulness of ELISA utilizing recombinant proteins of either bacterial or eukaryotic origin or affinity-purified native proteins of e.g. BP180 or laminin332 appears to be limited since 10-40% give false positive reactions in BP. Apart from initial diagnosis ELISAs can also support therapeutic treatment. Schmidt et al. first demonstrated a positive correlation between clinical disease activity and antibodies to BPAG2 as detected by enzyme-linked immunosorbent assay (ELISA). Also in pemphigus ELISA seems also a tool to monitor the disease activity. As may be clear from the above a range of assay techniques is available, each with their own specificity and sensitivity. In this thesis we have used some of these sophisticated assay techniques in addition to the common routine assays to investigate if this results in a more accurate diagnosis (and possibly a higher incidence) and to investigate the presence of possible rare or new antigens in sAIBD.

ANTIGENS AND SAIBD FORMS

Pemphigoids comprise the group of sAIBD of the skin caused by autoantibodies against the epidermal BMZ that contains hemidesmosomes and underlying extracellular matrix proteins. HD are build from at least six different proteins, i.e. plectin, bullous pemphigoid antigen 1 (BPAG1, BP230), bullous pemphigoid antigen 2 (BPAG2, BP180 or type XVII collagen), α6β4 integrin and tetraspanin/CD151 (fig. 2). Autoantibodies against BP230 and BP180 are associated with bullous pemphigoid (BP), the most common variant of pemphigoid, as well as with pemphigoid gestationis (PG), lichen planus pemphigoides (LPP) and mucous membrane pemphigoid (MMP), whereas autoantibodies against the linear IgA disease antigen (LAD-1), which is the soluble shed ectodomain of BP180, are found in linear IgA disease (LAD). The pemphigoid relevant extracellular matrix proteins comprise the soluble shed ectodomain of
Introduction

BP180 (linear IgA dermatosis antigen, LAD-1), laminin-332 named after the α3, β3, γ2 laminin chains (formerly known as epiligrin or laminin-5), laminin-γ1 (p200) and type VII collagen. Bullous pemphigoid (BP) is a chronic sAIBD of the skin that affects mainly the elderly and is characterized by large, tense bullous lesions, may start as erythematous macules and urticarial papules or plaques usually associated with severe itching. The clinicopathological features of BP were first described by Lever in 1953. BP is associated with a humoral and cellular response directed against one or both of the two self-antigens BP180 (including it’s soluble shed ectodomain LAD-1) and BP230. BP180 is a transmembrane adhesion molecule with 15 extracellular collagenous domains. Autoantibody reactivity to BP180 ectodomain is largely restricted to the NC16A region but may also be located near the carboxy-terminus. BP230 is a cytoplasmic protein involved in the anchorage of intermediate filaments to the cytoskeleton. BP230 and plectin, another protein of the hemidesmosome, both belong to the plakin family.

Pemphigoid gestationis (PG, formerly named herpes gestationis; HG) is a pemphigoid form that is associated with pregnancy. In pemphigoid gestationis, a transient bullous eruption occurs in pregnancy most commonly in the second or third trimester but it may be present at any stage during the pregnancy or puerperium. Through placental transfer of autoantibodies to BP180 from the mother into the neonate PG can also result in a blistering eruption of the neonate. The clinical spectrum of PG is polymorphic and may include urticarial plaques and papules, erythematous-squamous lesions and blisters. Autoantibodies in PG are mainly directed to BP180 but incidental reactions to BP230 have also been reported. Like in BP, the NC16A domain of BP180 was shown to be recognized by a large number of PG sera, in fact NC16A is more frequently recognized as an epitope as in BP.

Mucous membrane pemphigoid (MMP, formerly named cicatricial pemphigoid; CP) is a sAIBD of mucous membranes that sometimes also affects skin. Clinically, patients with MMP present with severe vesicles, erosions and crusts on mucous membranes with potential scar formation, typically affecting the oral cavity and eyes, but it also may affect the nasal, pharyngeal, laryngeal, esophageal and/or anogenital regions. Autoantibodies may react with two major regions of the BP180 extracellular domain, one is the NC16A domain (at or near the autoantibody site recognized by BP and PG) and the other is the carboxy-terminal tail. In 1992 a separate group of patients with MMP was described who had IgG autoantibodies against a set of disulfide-linked polypeptides then referred to as epiligrin. This protein, also named nicein, kalinin and BP600 at that time, is identical to laminin-332. This form of MMP is known as anti-epiligrin cicatricial pemphigoid (AECP) or with its new name anti-laminin-332 MMP. Anti-laminin-332 MMP is estimated to comprise 5% to 20% of all MMP cases. Clinical manifestations are mainly mucous membrane blistering and potential scar formation. When lesions occur on the skin, the scalp, face and/or upper trunk are predilection sites. In chapter 5 we will investigate an exceptional patient with antibodies to laminin-332 but with abnormal clinical manifestation. To date, several epithelial components in the BMZ have been identified as autoantigens in patients with MMP. These include laminin-332 (α3, β3 and γ2 subunits), lamin-311 (α3 subunit, formerly
laminin-6, BP230, BP180, and the integrin α6β4 subunits. Seen the heterogeneity of MMP in terms of targeted antigen, IIF on salt-split skin is important for diagnosis as floor staining will discriminate anti-laminin-332 pemphigoid from the other forms of MMP. As serum from EBA, bSLE and anti-laminin-γ1 patients also stain the floor, DIF analysis of skin depositions then becomes very important. All sAIBD’s which have their antigens located in or above the lamina densa, thus including anti-laminin-332 MMP and anti-laminin-γ1 (p200) pemphigoid, give a n-serrated IgG deposition pattern, thereby discriminating them from the diseases with type VII collagen as an antigen that demonstrate u-serrated IgG deposition.

Lichen planus pemphigoides (LPP) is a rare sAIBD that presents with both lichen planus (LP) and BP lesions. It is characterized by vesiculobullous lesions arising on erythematous or normal healthy skin in a patient with coexisting LP. Blisters can occur before, meanwhile or after the development of lichen planus lesions. An interesting hypothesis is that the lichenoid infiltrate in LP may lead to damage to the BMZ and basal keratinocytes, and may unmask antigens or create new antigens within the BMZ. In contrast to bullous LP, where blisters are confined to LP lesions and a consequence of basal several basal cell degeneration found in histopathology LPP blisters appear in both LP lesions and previously unaffected skin. Immunologically, linear deposits of IgG and/or C3 at the BMZ in perilesional skin can only be found in LPP. LP lesions show fibrillar fibrin at the BMZ. Autoantibodies in LPP react with the C-terminal part of the NC16A domain of BP180, although some rare cases have been described with possible 200 and 130 kD proteins. LP is a chronic inflammatory disease of skin, and oral and genital mucous membranes. Histopathology shows a prominent inflammatory band-like T-lymphocytic infiltrate with liquefactive degeneration of basal epithelial cells. It is unknown to which antigen the T cells are attracted in LP, but seen the location of the infiltrates it is not inconceivable that basal epidermal cells are involved. Seen the humoral component LPP we have investigated the sera of oral LP patients for possible autoantibodies to BP180 (chapter 4).

Linear IgA dermatosis (LAD) is an acquired sAIBD and a pemphigoid disease with exclusively IgA deposition along the epidermal BMZ. Clinical manifestations in LAD are heterogenous and may mimic other bullous diseases but mainly consist of vesiculobullous lesions affecting the skin and mucosal surfaces. Lesions may appear as a string of pearl-like or in a “crown of jewels” configuration. Initially, a 97-kDa protein was reported as target antigen in LAD, later the linear IgA antigen 1 (LAD-1) as a 120 kDa protein was found in medium of cultured keratinocytes. These proteins were identified as fragments of the shed BP180 ectodomain. Also BP180, BP230, and type VII collagen have been reported as targeted antigens. IgA to type VII collagen is also known as IgA-EBA or LAD-II.

Anti-laminin-γ1 pemphigoid (formerly known as anti-p200 pemphigoid) is a recently discovered subtype of pemphigoid in which autoantigens are directed to the laminin gamma 1 chain that is located in the lower lamina lucida. Clinically, most reported cases present with tense blisters and urticarial eruptions, symptoms that closely resemble BP.
Introduction

Epidermolysis bullosa acquisita (EBA) is a sAIBD characterized by autoreactivity to type VII collagen in the anchoring fibrils and thereby the sole autoantigen located in the sublamina densa zone. Type VII collagen is composed of three identical α-chains. Each α chain consists of a central collagenous domain flanked by a 145-kDa non-collagenous amino-terminal domain (NC1) and a 30 kDa carboxy-terminal domain (NC2). EBA is a heterogeneous disease that may be characterized by either an inflammatory or a mechanobullous clinical phenotype. Bullous systemic lupus erythematosus (bSLE) is a bullous variant of systemic lupus erythematosus with autoantibodies against type VII collagen. BSLE is clinically characterized by a widespread and often transient, non-scarring vesiculo-bullous eruption, dramatically responding to dapsone. This inflammatory type of bSLE is in sensu stricto bSLE. BSLE can segregate into different phenotypes: an inflammatory one, which is more frequent, and a classical EBA-like phenotype, this is classic-EBA in SLE.

EBA can mimic other sAIBD diseases. The classic phenotype, first described by Roenigk et al. in 1971 resembles the “classic dermolytic” form of inherited epidermolysis bullosa. Clinically the classic, mechanobullous phenotype mimics dystrophic epidermolysis bullosa hereditaria, while mild cases resemble acral blistering porphyria cutanea tarda, that heal with atrophic scarring, milia and hypo-or hyperpigmentation. Exclusive involvement of the scalp, neck and shoulders leading to scarring, resembles Brunsting-Perry pemphigoid. The inflammatory phenotype, first described by Gammon in 1984, mimics BP or MMP, clinically presenting with widespread vesicles or bullae involving intertriginous and flexural areas without milia formation or scarring. It may also present with grouped circinar vesicles and arciform erythema and then resemble LAD. DIF of perilesional skin of EBA/bSLE shows an u-serrated pattern of depositions in upstanding arms (“grass”) of the sublamina densa zone between the rootlets of the basal keratinocytes.

In chapter 6 we will investigate if the use of the serration pattern recognition by DIF increases the yield of EBA/bSLE diagnosis within the spectrum of sAIBD, and how the clinical phenotype varies among this subset.

**EPIDEMIOLOGY OF SAI BD**

BP has an annual incidence ranging from 2.6 cases per million population in the Arabian Gulf to resp. 13 and 14 cases per million population in the north-west of Germany and the north-east of Scotland, with a rapid increase after the age of 60 years, it is a disease of the elderly. The 1-year survival in the US compared to Europe was item of discussion several years ago, since the frequencies of mortality-rate were resp. 11% in the US compared to 29-41% in Europe. Differences in age and general condition beyond different hospital care systems are plausible for the huge difference in numbers. Often, also MMP occurs in the elderly. Childhood LAD occurs at mean age of 4.5 years. EBA has been reported in both children and adults and may
occur at any age. As one of the more rare sAIBD in Western Europe, EBA has a reported annual incidence of approximately 0.25 per million.\textsuperscript{115} The hemidesmosomal proteins are far more frequently targeted in sAIBD than the proteins in the (sub)lamina densa zone. In the pemphigoid group (BP, MMP, PG, LAD and LPP) incidences per million reach from 2.6 to 14 in BP,\textsuperscript{101-106} 1 in MMP,\textsuperscript{115} 20 per million pregnancies in PG\textsuperscript{116,117} to incidences that are less well known as in LAD (ca 4 to 6 per million)\textsuperscript{118-120} while the incidence of LPP is estimated 2 to 3 per million.\textsuperscript{102} In contrast to LPP, lichen planus is a far more common disease and reaches incidences from 0.22\% to 2\% of the adult population.\textsuperscript{121,122} For the more common sAIBD, techniques such as ELISA and immunoblot are well developed to detect the antigens. But what if other rare autoantigens are present? Are the current techniques sufficient to reach the diagnosis or do techniques need to be adjusted or newly developed? How to find rare diseases or common diseases with additional rare antigens? In this thesis we hope to find answers to these questions.

**IMMUNOLOGY AND EPITOPE MAPPING**

The immune system protects us against all kinds of potential pathogens as bacteria, parasites and viruses. Unfortunately, the system does not function without faults and sometimes becomes activated against our own body. In autoimmune diseases an immunological reaction is present against self-molecules. The immune system consists of a non-specific innate and a specific adaptive immune system. The innate immune system provides immediate defense, while the acquired ‘adaptive’ immune system takes time to respond. Both immune systems contain humoral mechanisms of defensive strategy against pathogens and cellular components. Antigen and antibody interact due to complementarity in shape and fit together precisely in a “lock and key” manner. In autoimmunity the adaptive immune system plays a major role.

**Isotypes**

Immunoglobulins (antibodies, Ig’s) have different structural variants (isotypes or classes) based on different heavy chain domains. In human beings there are five major types of heavy chains giving five classes of Ig, named IgG, IgA, IgD, IgE and IgM. IgG is the most abundant Ig, particularly in the extravascular fluids where it neutralizes toxins and combats microorganisms by activating complement. IgA is present as a dimer linked to a secretory component and exists mainly in mucous membranes and in secreted fluids as tears, saliva and breast milk. In the mucous secretions it is the major Ig. IgM provides an early defense as it is the first Ig produced and therefore acts before sufficient IgG is present. Immunoglobulin D is a cell surface receptor largely present on the lymphocyte and functions together with IgM as the antigen receptor on naive B-cells. Immunoglobulin E triggers inflammatory reactions, it binds to the Fc-receptor on
mast cells and when it binds an antigen this leads to local recruitment of antimicrobial agents through degranulation of the mast cells and release of inflammatory mediators. Autoimmune responses may be limited to one single class but may also exist of more classes. Different classes may exert different clinical manifestations. For example when IgA forms the major or only immune response this could lead to a higher degree of involvement of the mucosa.

All classes, with the exception of IgD, have been found as autoantibodies in sAIBD. Interaction between IgG, IgA or IgE and Fc receptors on binding antigen-associated antibody can lead to immunoregulatory reactions trigger effects such as phagocytosis, antibody-dependent cellular toxicity and acute inflammatory responses.

More than 90% of BP patient sera contain IgG autoantibodies that bind the BP180 and/or BP230 protein. IgA and IgE autoantibodies are also frequently found in 22-89% of BP sera. Patients with MMP often have additional IgA autoantibodies directed against the ectodomain of BP180. Recently, circulating IgA and IgE autoantibodies in anti-laminin 332 MMP have been described as has been known for BP for several years.

EBA was first described in 1971 as a IgG-mediated disease resembling dystrophic epidermolysis bullosa. In 1984 Gammon for the first time described a pemphigoid-like EBA patient. An IgA-mediated subtype of EBA was then described in 1994 by the group of Zambruno, and reviewed by Vodegel et al. In chapter 6 we investigate if the different immune responses to type VII collagen are connected with different clinical phenotypes.

Concomitant antigens
Patients with sAIBD can have more than one targeted antigen. Well known examples are the concomitant antigens BP180 and BP230 which are frequently both targeted in BP while these antigens are closely located in the hemidesmosome. Other close targeted antigens are laminin-332 and type VII collagen, which are described in the literature in one particular patient. Also, subepidermal bullous disease has been described with autoantibodies to both the p200 autoantigen and the alpha3 chain of laminin-332. An example of intermolecular spreading is a patient in which autoantibodies are both targeted to the hemidesmosome and to type VII collagen. The phenomenon of “epitope spreading” has often been used as an explanation why different antigens are targeted. Epitope spreading is the development of immune responses to endogenous epitopes secondary to the release of self antigens during a chronic autoimmune or inflammatory response.

Epitopes
The part of the hypervariable regions on the antibody that actually contacts the antigen is termed the paratope, and the part of the antigen which is in contact with the paratope is called the epitope or immunodominant site. Each B-cell synthesizes an immunoglobulin specific to only one epitope. The recognized epitope determines where on the protein the antibody binds.
The question if recognition of a specific epitope leads to specific disease features is ongoing. The best studied antigen in this respect is BP180. In bullous pemphigoid the immunodominant epitope of BP180 is NC16A, which consists of five subdomains, named MCW-0 to MCW-4 (or NC16A subdomain 1 to 5). In BP three regions (NC16A subdomain 1 to 3) on NC16A are targeted. Other parts of BP180 that contain epitopes are the intracellular domain where IgE autoantibodies are reported to bind. In PG also the NC16A domain is targeted, but here domains 2 and 3 react. The immunodominant epitopes in LPP are located in NC16A4 subdomain. Heterogenous binding with autoantibodies to BP180 NC16A was demonstrated in MMP patients. Another major epitope in MMP is located in the extracellular carboxyl-terminal domain of BP180. This means that in MMP, IgG is deposited in the lamina lucida and the lamina densa. Involvement of the lamina densa may explain the susceptibility to scar formation in MMP. Pure oral pemphigoid (OP), presenting with exclusive oral lesions, was recently studied by epitope mapping. OP patients without scarring lesions showed preferentially autoantibodies which targeted the BP180 extracellular domain. For BP230, other major autoantigen in BP, epitope mapping demonstrated multiple antigenic reactive sites located predominantly within the B and C subdomains of the COOH-terminus of BP230. In EBA there are four major epitopes along the N-terminal part of type VII collagen (NC-1) that are targeted by IgG autoantibodies in mechanobullous EBA patients (figure 3). For inflammatory EBA, although several case reports have suggested that the triple helical domain of type VII collagen is the major targeted domain, this is rather unusual. The major target of autoantibodies in this subgroup might, as well as in mechanobullous EBA, be the NC-1 domain. For IgA EBA the immunodominant site is still unknown.

To study effects of epitope use of animal models could be useful. Well characterized antibodies can be injected to study their clinical effect. Such studies are also interesting in relation to drug development.

**Figure 3.** Schematic overview of type VII collagen with on the amino-terminal part of the protein (left) three chains of the non-collagenous-1 domain (consisting of the cartilage matrix protein, nine fibronectin-III repeats and a subdomain resembling von Willebrand factor A) connected to one central collagenous domain and to another three chains of the non-collagenous-2 carboxyl terminal part (right).

**Aim of the study**

In these study we report in depth on the clinico-immuno-histopathological phenotype of unusual or rare variants of subepidermal autoimmune bullous diseases In chapter 2 we focus on an unresolved “cold” case with bullous disease with confusing immunofluorescent findings.
The next chapters of this thesis focus on rare autoantibodies in the more common diseases. In chapter 3 we study plectin as autoantigen in pemphigoid. In chapter 4 we investigate sera of oral lichen planus patients for a serological association with pemphigoid. In chapter 5 we will focus on a case with anti-laminin 332 autoantibodies but with a completely aberrant clinical presentation.

In chapter 6 we analyse the variance of epidermolysis bullosa acquisita phenotypes and the immunoglobulin class responses and investigate if these are correlated to the clinical phenotype. This study was performed in our national bullous diseases reference centre at the department of Dermatology of the UMCG. The centre hosts a large biobank of sera and biopsies that date back to the seventies. As part of the standard diagnostic procedure for patients suspected of sAIBD, biopsies and serum are taken from each case referred to us. After diagnostic procedures the samples were stored in our biobank at -80C. The large number of samples in this collection enabled us to study the "white ravens".
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Chapter 1


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Introduction


Chapter 1


