Unusual variants of subepidermal autoimmune bullous diseases
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Summary

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Subepidermal acquired immunobullous diseases (sAIBD) are characterized by circulating and/or tissue bound autoantibodies directed to proteins of the epidermal basement membrane zone. There are several types of sAIBD, of which some are common and others are more rare. Among the common pemphigoids we find bullous pemphigoid, anti-BP180 mucous membrane pemphigoid and linear IgA dermatosis, whereas the rare are formed by anti-laminin 332 mucous membrane pemphigoid, anti-laminin γ1 pemphigoid, and epidermolysis bullosa acquisita. Common or rare are thus in fact defined by the frequency numbers. The calculation of frequency numbers however depends on the ability to recognize specific diseases, and thus on the discriminating power of the used diagnostic laboratory methods. This thesis is largely dedicated to studying rare sAIBD variants. In chapter 1 we overview the sAIBD, their frequencies, and the laboratory techniques used to diagnose them. We furthermore discuss differences in immunoglobulin class response and epitope use by the different pathologic antibodies. Epidermolysis bullosa acquisita (EBA) is a sAIBD with autoantibodies against type VII collagen. Two clinical variants of EBA are recognized: the classic type with trauma-induced blisters with milia and dystrophic nails and the inflammatory type with acute blisters on erythematous base resembling BP or less commonly MMP. In chapter 2 we studied an exceptional rare case of EBA of the inflammatory type, with conflicting diagnostic tests. The diagnosis EBA for this patient was originally founded on electron microscopy that revealed the split level to be in the sublamina densa zone and later confirmed by the u-serrated pattern of the BMZ immunoglobulin deposition. In contrast, salt-split skin analysis revealed the presence of circulating IgA antibodies that stained the roof instead of the floor of the blister. By immunoblotting and ‘knock-out’ immunofluorescence analysis we could demonstrate these IgA antibodies to be directed to plectin. That we did not find circulating antibodies to type VII collagen was not that strange, as we will see in chapter 6. This case illustrates that analysis of serum alone might lead to the wrong diagnosis and advocates for proper analysis of both skin and serum for sAIBD patients. Hemidesmosomal proteins are most frequently targeted in sAIBD. Well-characterized autoantigens are the intracellular plaque protein BP230, the transmembrane BP180 and its shed ectodomain LAD-1. In chapter 3 we describe a fourth hemidesmosomal protein that is autoantigen in sAIBD. This protein is plectin that is a member of the plakin protein family, similar to BP230, and is found in both hemidesmosomes and desmosomes. In a cohort of two hundred and eighty-two sAIBD patients we found 11 patients with anti-plectin antibodies by immunoblotting, what gives a relative frequency of 3.9 % among sAIBD. Further proof of the anti-plectin nature of these antibodies were provided by affinity-purified antibodies that bound back to normal human skin in a pattern typical for plectin, i.e. to the epidermal basement membrane zone as well as to keratinocytes in the epidermis, and to myocytes, and by the absence of binding to plectin-deficient skin of a patient with epidermolysis bullosa simplex with muscular dystrophy. The epitopes were mapped by immunoblotting using recombinantly produced overlapping plectin domains from the N-terminal actin binding domain to the COOH-end of the rod domain. The central coiled-coil rod domain appeared to be the immunodominant hotspot.
Summary

in 10 of 11 sera. Furthermore we found that most patients with anti-plectin antibodies also had antibodies to other pemphigoid antigens, and we concluded that plectin is a minor antigen in sAIBD. Reviewing the patient files did not reveal a different clinical phenotype compared to patients without anti-plectin autoantibodies.

Lichen planus is a chronic inflammatory disease of skin, and oral and genital mucous membranes. The histopathology of oral lichen planus (OLP) is quite uniform showing a prominent inflammatory band-like lymphocytic infiltrate with liquefactive degeneration of basal epithelial cells. In our diagnostic laboratory, sera from patients suffering from inflammatory lesions of the oral mucosa are routinely screened for pemphigus and pemphigoid antibodies. We then noticed that some patients with proven OLP had weak IgG reactions to a 180-kDa protein by immunoblot, which could be BP180. In chapter 4 we further studied this phenomenon in a larger series of patients. In sera of forty-seven consecutive patients with defined OLP immunoblotting with normal keratinocytes extract showed for eight (17%) sera a reaction with the 180 kDa band. To demonstrate that this was BP180 we performed further immunoblot experiments using cell extracts of BP180-deficient keratinocytes and of recombinantly produced full-length BP180 and overlapping NC16A domain fragments. The reaction to the 180 kDa protein was lost on BP180-deficient keratinocyte extracts. Five of the eight sera reacted with recombinant full-length BP180 and two also reacted with the NC16A domain. The antibody titers were low as all sera were negative by indirect immunofluorescence on monkey esophagus substrate, and all but one negative on salt-split skin substrate. Direct immunofluorescence of corresponding skin biopsies did not show any IgG deposition along the basement zone. Clinically and histologically no differences were seen between the eight OLP patients who had anti-BP180 antibodies and OLP patients who did not have these antibodies. We suggest that the development of autoantibodies might be a secondary, humoral, response to BP180 due to the chronic inflammatory process caused by the primary cellular response. Such a process is known as epitope spreading. Whether BP180 is also a pathogenic T-cell antigen in OLP needs to be solved.

Mucous membrane pemphigoid encompasses a group of chronic subepithelial autoimmune bullous diseases where predominantly the mucous membranes become affected with the potential of scar formation. The autoimmune targets are heterogeneous being BP180 and laminin 332 formerly known as epiligrin. When laminin 332 is the antigen the disease is called anti-laminin 332 pemphigoid or anti-epiligrin pemphigoid. In chapter 5 we describe a unique case of anti-laminin 332 pemphigoid with skin blistering but no involvement of the mucous membranes. Immunoblotting on human keratinocyte extracellular matrix using the patient’s serum revealed binding of IgG antibodies to bands of 200 and 165 kD corresponding to the unprocessed and processed α3 chains of laminin 332. No other antigens were identified by the patient’s serum. DIF showed a marked reduction of IgG deposition between the skin in front of and behind the wave of blisters, what led us to hypothesize that the direction of blister spread is propelled by the difference in IgG load along the epidermal BMZ.
The clinical phenotype of EBA can be divided in mechanobullous and inflammatory. In chapter 6 we investigated if a correlation exists between the isotype of in vivo antibody deposition and the clinical phenotype. We performed a prospective cohort study and for the first time included patients based on the serration pattern of the immunoglobulin BMZ deposition. Among 364 sAIBD patients we found 20 (5.5%) cases of EBA/BSLE. When for this same cohort we would have included on basis of serological reactivity then only 7 (1.9%) cases would have been found. This demonstrates that roughly two in every three EBA patients have no detectable serum titre, would have been missed by serum analysis alone and underlines the importance of serration pattern analysis of immunodepositions in skin biopsies. Then using serological criteria we included an additional 18 retrospective cases of EBA /BSLE using stored samples of our tissue/serum bank. Twenty-two (63%) of these 38 cases had the inflammatory subtype that mimics other types of subepidermal autoimmune bullous diseases. We sought for possible correlation of antibody class and clinical phenotype. In the group with a pure IgG response the mechanobullous phenotype dominated (67%), while vice versa in the group with a pure IgA response the inflammatory phenotype dominated (91%). No correlation of antibody class or phenotype was found with mucous membrane involvement.

All studies were performed in the Center for Blistering Diseases in Groningen with exception of the OLP study that was in part performed in the IPM BIOTECH in Hamburg.