General discussion and future perspectives

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Chapter 7

In this thesis we have investigated blistering diseases focusing on the more rare autoantigens. Identification of these antigens is not possible with the standard diagnostic test used by most laboratories i.e. serum immunofluorescence on monkey esophagus substrate. Therefore we used additional techniques as immunoblotting, recombinant antigens, salt-split skin analysis, serration pattern examination and knock-out analysis. Especially immunoblotting was useful as this enabled us to identify plectin as a minor antigen in pemphigoid (chapter 3) and the unexpected finding of low titer anti-BP180 antibodies in the T-cell mediated disease oral lichen planus (chapter 4). Also in the ‘white raven’ cases described in chapters 2 and 5 immunoblotting was crucial to their discovery. The consequent use of serration pattern analysis of the ‘linear’ BMZ immunoglobulin depositions enabled us to identify the EBA patient population that we investigated in chapter 6. The availability of the tissue and serum bank of the department that contains samples dating back to the mid-seventies of the last century provided us with the opportunity to study a large number of sera and biopsies and to calculate the frequencies of the more rare diseases.

In chapter 2 we demonstrated the importance of analysing both patient serum and skin. This ‘cold case’ patient (she visited our clinic 25 years ago) had a severe blistering disease that remained unexplained until 18 years after her death. The final diagnosis was reached through a combination of new techniques not available in those days, and with the standard immunofluorescence techniques still in use, most routine laboratories will reach the wrong conclusion even today. Serration pattern analysis appeared very crucial here – as also demonstrated in chapter 6 where the number of diagnosed cases almost tripled when compared to serum diagnostics - and it is clear that skin pathologists should have proper knowledge of the possibilities of serration pattern evaluation.

In chapter 3 we addressed plectin as a possible antigen in sAIBD. When we started this study information on plectin as an autoantigen was limited. One study was known from the literature that mentioned plectin as an antigen using a combined immunoprecipitation/immunoblot method. Furthermore we ourselves had unexpectedly found plectin in the ‘white raven’ patient described in chapter 2. We found 12 patients (frequency 4%) and using additional techniques as knock-out analysis, affinity purification and recombinant constructs we could definitively prove that plectin indeed is an antigen of pemphigoid. However from the low frequency and the observation that almost always it is present next to the main pemphigoid antigens BP230 and/or BP80 we conclude it to be a minor antigen. Epitope mapping showed that central coiled-coil rod domain was most frequently targeted by the autoantibodies, what contrasts with similar studies on other plakin family members as periplakin, envoplakin and BP230. Apparently the immunodominance of the rod domain as in plectin is not a general feature of the plakin family. Plectin is not only present in type I and type II hemidesmosomes, but also in desmosomes.1 As such it is also found in skeletal and smooth muscle tissue, and in cardiac and nerve tissue.2-4 The interesting question is if the antibodies also affect these other tissues. We did find fine dotted granular immunoglobulin depositions in arrector pili muscle but do not know if such deposition
may lead to pathology. Our study was done retrospectively and no information on this could be distilled from the patient files. Future patients however should at least be questioned on possible muscle weakness, and measurement of muscle enzymes is an interesting option to gain some insight on possible antibody induced muscle pathology.

Immunoblotting also found low titer circulating IgG to a 180 kDa protein in oral lichen planus patients. In chapter 4 we showed that this 180 kDa protein is in fact BP180, one of the major pemphigoid antigens. Lichen planus is considered a T-cell mediated disease in which cytotoxic CD8+ T-cells destroy basal keratinocytes. The antigen to which the T-cells are directed is unknown. The circulating BP180 antibodies may be a secondary humoral response resulting from exposure of epitopes to the immune system during the primary inflammatory process. Such mechanism could be cause of a condition known as lichen planus pemphigoides (LPP). LPP is characterized by both lichen planus lesions and subepidermal blisters. Circulating IgG deposits linearly along the BMZ and is directed to BP180. The targeted epitope is unique and different from the epitopes targeted by pemphigoid patients. Possibly the inflammatory changes in LP lesions may lead to some alterations within the BP180 ectodomain creating a novel antigenic site in LPP that is different from BP. Interestingly, very recent the development of anti-BP180 MMP was described in two patients with a history of oral lichen planus. What we also do not exclude is that BP180 is the actual T-cell antigen in the patients described by us. Lichen planus in that case could be the T-cell analogue of immunoglobulin mediated blistering disease. In this light it is interesting that at the 2010 ESDR meeting it was reported that desmoglein 3-specific T-cells induced interface dermatitis in an experimental mouse model.

The patient that we describe in chapter 5 had a blistering disease of the skin with widespread lesions spreading centrifugally from involved skin to skin that had not blistered before. The mucosa were not involved and the diagnosis of anti-laminin 332 pemphigoid was unexpected, as classically these patients have blistering of mucous membranes with potential scar formation. Immunoblotting on human keratinocyte medium revealed binding of IgG antibodies to proteins of 200 and 165 kDa that corresponded with the unprocessed and processed α3 chains of laminin 332. Immunoprecipitation was negative, thus the epitope is probably cryptic and different from the known epitopes on the G domain of the α3 chain of laminin 332. Use of this epitope therefore might underlie the skin complaints of this particular patient, however such hypothesis would need support by further research.

EBA is considered a relatively rare disease that can have many ‘faces’. The classic form is mechanobullous and non-inflammatory but two-third of the EBA patients in chapter 6 presented with the inflammatory form that can mimic other subepidermal bullous diseases. The antibody response can be pure IgG, pure IgA or mixed (IgG/IgA). We sought for a correlation between the class of the antibody response and the clinical phenotype. IgA appeared to be related with developing the inflammatory form. For the classical mechanobullous type we found no association with class response. Possibly the phenotype is also connected with the targeted epitope. In BP180-mediated MMP there is greater use of the carboxy-terminal epitopes than
in BP180-mediated bullous pemphigoid.\textsuperscript{9,10} For mechanobullous EBA the antigenic sites were found on the NC1 domain of type VII collagen. For inflammatory EBA the antigenic sites are still unknown. We are addressing the possibility of different epitope use in our patients currently in a collaborative study with the Universitätsklinikum Schleswig-Holstein in Lübeck. Recombinant fusion proteins of the different type VII collagen domains have been produced and the reactivity of our sera with these proteins will be tested. Another option to explore is the possible contribution of IgE antibodies. Although IgE antibodies are at a 10,000 times lower concentration in the human body they can have profound effects on blistering diseases. In bullous pemphigoid 90\% of the patients have anti-BP180 IgE antibodies that are capable of inducing eosinophil infiltration and histological blisters in engrafted human skin on SCID mice.\textsuperscript{11,12} Recently the first successful treatment of bullous pemphigoid with omalizumab, a humanized anti-IgE antibody, has been reported.\textsuperscript{13} In our EBA patients 50\% had an elevated IgE serum level compared to 16.6\% in the normal population.\textsuperscript{14} The low overall IgE titer has so far prevented the analysis of floor binding IgE antibodies by salt-split skin immunofluorescence. However seen the sensitivity of immunoblotting we hope that such analysis using the recombinant constructs might reveal the existence of anti-type VII collagen IgE. Finally the first successful EBA mouse model has just been established. Apart from the possibilities to test new therapeutic treatments, such models might also be useful to investigate the contribution of epitopes and immunoglobulin class to the disease phenotype.
REFERENCES


