CHAPTER IX

SUMMARY AND CONCLUSIONS

During centuries bullous autoimmune diseases have remained hidden in a large group called pemphigus. The diagnostic process in dermatology is always strongly depended of the clinical description of the skin and mucous membranes. With commence of clinical dermatology in the 19th century followed by histopathology in the mid 20th century the classification of bullous diseases started.

In case of SBAD the clinical presentation and histopathology alone are not sufficient to discriminate the variety of different entities (this thesis). With the availability of immunofluorescence microscopy, immuno-electron microscopy, immunoblotting, immunoprecipitation, and enzym-linked immunosorbant assay as supportive diagnostic methods the possibilities of the diagnostic process were enhanced and tree of SBAD has extended considerably in the past 30 years (chapter 2).

This thesis focuses on the diagnostic process, in particular to the possibilities of increasing the sensitivity of immunofluorescence microscopy (IF) of SBAD.

By using "knockout" substrate, i.e skin deficient of a specific autoantigen as a substrate for indirect IF, it has been possible to differentiate between bullous pemphigoid, anti-epiligrin cicatricial pemphigoid and epidermolysis bullosa acquisita (chapters 4 and 5). This differentiation is important because the final diagnosis has a great influence on the patient's life-expectation and therapeutic possibilities.

However due to the sum of outcomes of various other diagnostic immunological laboratory techniques, use knockout substrate in IF is only necessary in a minority of cases.

Comparison of the clinical symptoms and various results of different immunohistochemical findings from the literature made it possible to describe more precisely the phenotype of IgA-mediated EBA, leading to further extension of the diagnostic tree in subepidermal autoimmune diseases (chapter 6).

By direct immunofluorescence microscopy three different staining patterns along the epidermal basement membrane zone (BMZ) were recognised in SBAD: 1) a granular pattern typically for dermatitis herpetiformis, 2) a linear pattern and 3) a mixed linear-granular pattern which was observed in other subepidermal bullous autoimmune diseases. Further differentiation was made by the determination of the immunoglobuline class of deposition, for instance exclusively IgA in linear IgA dermatosis.

Before the description of the serrated patterns differentiation between the various subepidermal bullous autoimmune diseases was mainly done by using indirect IF on 1.0 M NaCl split skin substrate. In this way these diseases were divided in two groups, which showed either epidermal (bullous pemphigoid, mucous membrane pemphigoid, linear IgA dermatosis) or dermal staining (p200 pemphigoid, anti-epiligrin cicatricial pemphigoid, epidermolysis bullosa acquisita and bullous systemic lupus erythematosus).

However further differentiation using direct IF is possibly by the recognition of the different serrated staining patterns along the epidermal BMZ. This way type VII collagen targeted autoimmune diseases such as epidermolysis bullosa acquisita and bullous SLE are recognised by the u-serrated pattern, whereas other forms of SBAD such as bullous pemphigoid, p200 and AECP have a n-serrated pattern of immunodeposition (chapter 7).

The finding of exclusively IgA deposition in the epidermal BMZ in "n-" or "u-serrated" patterns differentiates between linear IgA dermatosis and IgA-mediated epidermolysis bullosa (EBA) (chapter 6 and 7).

The diagnostic process starts with the biopsy procedure for IF. During transport and storage of biopsies loss of immunohistochemical staining and morphology should be prevented. For IF we advise the use of saline at room temperature (chapter 8). With the use of saline the transport of biopsies within clinic and sending them by mail is easier and cheaper than by using liquid nitrogen. Also compared to Michel's fixative the use of saline enhanced the diagnostic signal in direct IF. It is preferable that the saline stored biopsies are snap-frozen in the laboratory between 24 and 48 hours after the biopsy procedure.

We conclude that diagnostic IF procedure is enhanced by the use of saline as transport medium, by recognition of different serrated staining patterns along the epidermal BMZ, and by the use of specific knockout substrates. The final diagnosis is SBAD is based on the sum of clinical, immunofluorescence and serological findings (table I). Finally the results of these renewed diagnostic procedures and developments might lead to a better understanding of the pathofysiological mechanism involved in the group of bullous autoimmune diseases and hopefully to more specific therapeutic interventions.

This thesis has added a new page to the history of immunofluorescence diagnosis of SBAD. The most important findings of this thesis for routine IF are 1) the use of saline as transportmedium for IF-biopsies leading to a decrease of undesired background staining and 2) the recognition of differentiating serrated patterns in SABD seperating EBA/bullous SLE from other SBAD by direct IF alone. IF was further advanced by 3) demonstrating that AECP can be distinguished from EBA by FOAM. The armenture of IF was further extended by 4) use of "knockout" skin thereby enabling demonstration of the specific autoantigen in SBAD by indirect IF alone.