NEW OBSERVATIONS IN AUTOIMMUNE BULLOUS SKIN DISEASES

Klaudia Preisz M.D.

Leader of the program: Prof. Sarolta Kárpáti M.D., Ph.D., D.Sc.
Tutor: Prof. Sarolta Kárpáti M.D., Ph.D., D.Sc.

Program of „Dermatology and venereology”

Budapest, 2005.
INTRODUCTION AND AIMS OF THE STUDY

Clinical and immunofluorescence (IF) studies in autoimmune bullous diseases are presented. Between 1998 and 2003 I evaluated 2180 direct and 4435 indirect immunofluorescence (IF) studies in the immunofluorescent laboratory of the Department of Dermatology, Venereology and Dermatooncology of Semmelweis University. I had the possibility to investigate some interesting, rare cases in details.

During these 5 years I diagnosed 108 bullous pemphigoid, 21 linear IgA dermatosis, 2 herpes gestationis, 2 epidermolysis bullosa acquisita, 2 bullous systemic lupus erythematosus, 48 pemphigus vulgaris, 8 pemphigus foliaceus, 5 IgA pemphigus, 5 paraneoplastic pemphigus, 116 dermatitis herpetiformis and 53 coeliac diseases using immunohistological studies. I identified some rare variants of autoimmune blistering diseases, like IgA pemphigus, „GVHD-type” paraneoplastic pemphigus, linear IgA dermatosis, cicatricial pemphigoid, pemphigoid nodularis. In several patients’ case the diagnosis could be established only with the application of immunofluorescent examinations.

Beside routine IF examinations, I joined to the research group of our laboratory, which published several new scientific results in autoimmune bullous diseases in the past few years: Pemphigus vulgaris antigen is localized within keratinocyte desmosomes (1993). Desmoglein1 (Dsg1) was identified as autoantigen in IgA pemphigus (2000). Ultrastructural immunogold studies proved that linear IgA dermatosis is not a single disease entity, in which the anchor fibres can be autoantigens as well (1992). Ultrastructural studies in herpes gestationis supported the involvement of hemidesmosomes in the pathogenesis of the disease, and its relationship to bullous pemphigoid (1991). In dermatitis herpetiformis (DH) the research group have shown that a special IgA antibody in the sera of the patients binds to the structures of the normal jejunum, and specific IgA deposits are present in the proximal jejunum of DH and celiac patients (1988, 1990); and identified epidermal transglutaminase as the autoantigen of dermatitis herpetiformis (2002).

The aim of my research work was studying the pathomechanism of dermatitis herpetiformis and paraneoplastic pemphigus (PNP).

I examined the immunologic phenomena of the „graft-versus-host-disease” (GVHD) like PNP cases: I was searching for new, or symptom-specific autoantibodies.

I studied small bowel biopsy samples of DH and celiac patients to compare the localization of tissue transglutaminase (TGe) and extracellular tissue-bound IgA.

I also analysed immunoglobulin, complement and epidermal transglutaminase (TGe) deposition along the vascular system of DH skin, to test the possibility of IgA immune complex precipitation within the vessel walls as a first step in the pathogenesis of skin symptoms.

PATIENTS AND METHODS

Patients

Among the 6500 IF studies I have made, I examined the vascular fluorescence in 116 untreated DH patients’ skin (4-79 years, mean age:28 years, male/female ratio 61/55) biopsy samples with usual direct IF and in 16 patients’ skin with dual immunofluorescence.

I also examined 15 patients’ (5 DH, 5 celiac disease, 5 other gastrointestinal disease) small bowel biopsy samples to compare the distribution of tissue-bound IgA and tissue transglutaminase.
Among the studied PNP patients, I had the opportunity to make new immunologic observations in one patient’s (48 year-old female) case.

**Methods**

**Direct immunofluorescent studies**

Conventional direct IF was performed on frozen sections of the patients’ skin, and in some cases on conjunctiva, small bowel, oral and genital mucous membranes. I also made dual immunofluorescence on skin and small bowel sections of DH patients: for localization of TGe, affinity purified rabbit antisera raised against recombinant TGe proenzyme were used. Binding of mouse monoclonal TGc was detected by a mix of monoclonal, labelled mouse antibodies. The sections were analysed by conventional IF and confocal laser scanning microscopy.

**Indirect immunofluorescent studies**

For indirect IF, most often sections of monkey oesophagus, in some cases rabbit and guinea-pig oesophagus, normal human skin, rat bladder and rat lung were incubated with serum samples from the patient and control subjects and with the appropriate, labelled secondary antibodies (IgG, IgA, in special cases with IgG1, IgG4, IgA1). To analyse diseases in the pemphigoid group I used salt-split skin indirect immunofluorescent examination: we incubated normal human skin separated in the lamina lucida via incubation in 1.0 M NaCl solution with serum samples of the patient and labelled secondary antibodies.

**Immunofluorescence on COS7 cells transfected with human desmocollin 1, 2 and 3 (made in collaboration)**

Transient transfection of COS7 cells using lipofectamin reagent was carried out according to the manufacturer’s recommendations. The construct of human desmocollin1 (Dsc1) and Dsc2, containing the entire coding sequence was subcloned into the eukaryotic expression vector pcDNAI/Amp. cDNA of human Dsc3 was subcloned into the eukaryotic expression vector, pcDL-Sra296. Unfixed COS7 cells (transiently transfected with Dsc1, 2, or 3) were incubated with the PNP patients’ sera or specific antibodies diluted in the same buffer and subsequently with secondary labelled antibodies.

**Immunoblotting (made in collaboration)**

Human epidermis was homogenized, separated proteins were electrophoretically transferred to a nitrocellulose sheet. Strips of the blotted sheet were treated with PNP patients’ or normal sera, and subsequently incubated with peroxidase-conjugated anti-human IgG rabbit antiserum. The reaction was visualized with 4-chlor-1-naphtol in the presence of hydrogen peroxide.

Beside normal human epidermis we also used rat lung extracts and the recombinant protein of BP180 NC16a for immunoblotting PNP patient’s sera.

**ELISA (made in collaboration)**

Previously constructed baculovirus transfer vectors containing the entire extracellular domains of Dsg3, Dsg1 and Dsc1,2,3, (and the constant region of human IgG1) were cotransfected into cultured insect Sf9 cells. Microtiter wells, coated with the proper antigens, were incubated with PNP patients’ sera, and with peroxidase-conjugated rabbit anti-human IgG. Color development was achieved with tetramethylbenzidine, polyethylene glycol 4000, and hydrogen peroxide.
RESULTS

NEW OBSERVATIONS IN PARANEOPLASTIC PEMPHIGUS

1. Identification of new autoantigens: Dsc2, Dsc3, BP180

   On immunoblotting, serum IgG of our PNP patient reacted with the 210-kDa envoplakin and the 190-kDa periplakin on human epidermal extracts, as well as with the recombinant BP180 NC16a domain. ELISA assays detected anti-Dsg3 antibodies of both IgG and IgA classes, and IgG antibodies against Dsc3. Indirect IF on transfected COS7 cells proved, however, that the patients’s serum contained both IgA and IgG antibodies to Dsc3 and IgG antibodies to Dsc2.

2. Detection of circulating antibodies against pulmonary tissue

   Indirect IF on rat pulmonary tissue revealed binding of circulating IgG (1:320) and IgA (1:80) to the bronchial epithelium. Immunoblotting studies on rat lung extracts remained negative.

NEW OBSERVATIONS IN DERMATITIS HERPETIFORMIS

1. Results of immunofluorescent studies in the small bowel

   All examined patients with DH (5/5) and CD (5/5) had the specific IgA deposition in the small bowel mucosa. In the control group none of the patients (5/5) had IgA deposition in the small bowel. The distribution of TGc was the same in all patients’ (DH, CD and control group) biopsy samples. The binding of monoclonal anti TGc antibody and the deposition of IgA gave similar patterns in the IF studies.

   With double labeling and confocal microscopy I could observe colocalization of the extracellular tissue-bound IgA and TGc in all patients with GSE (DH and CD).

2. Analysis of vascular fluorescence in the skin of DH patients

   In 74 (64%) of 116 patients studied with direct IF, a significant blood vessel staining accompanied the DH-specific IgA fluorescence. Combined vascular IgA and C3 positivity was detected in 39, a combination of IgA, C3 and IgM in 5, and IgA alone in 18 skin samples. Vascular IgM deposits alone were observed in 12 patients. No specific signal with antihuman IgG was detected. In 68 of 74 cases (92%), the fluorescence involved only the small vessels of the papillary dermis. In 6 patients (8%), also subpapillary IgA and C3 vascular deposition was present.

   Dual immunofluorescence, performed in 16 DH skin clearly demonstrated the colocalization of TGc and IgA both in the vascular walls and within the papillary peri-and intervacular DH bodies, in all sections of the 16 patients. Contrary, only partial colocalization of C3 and TGc was detected, some vessels were positive only for TGc without C3 signal. TGc and IgM did not colocalize in the studied samples. TGc did not colocalize either with the immunoglobulin or with the complement precipitates of the dermis.
CONCLUSIONS

PARANEOPLASTIC PEMPHIGUS

1. My results support, that the pathomechanism of PNP is complex, PNP is not a single disease entity. Beside the known, diagnostic autoantigens several other desmosomal and basement membrane components can play a pathogenic role.

2. Desmogleins and desmocollins represent the major adhesive component of the desmosomes. Similar to the Dsg autoantibodies inducing acantholysis in PNP patients, a pathogenic role of anti-Dsc antibodies can be supposed.

3. Anti-BP180 antibodies can be detected not only in the diseases of the pemphigoid group. Autoantibodies against BP180, one of the major components of the dermoepidermal junction, can explain (beside „cross-reactive” anti-desmoplakin antibodies) why dual staining: immunoglobulin and complement deposition not only on the surface of the keratinocytes, but also along the basement membrane developed in our PNP patient’s case.

4. The presence of IgA and IgG type circulating antibodies against pulmonary tissue suggest that autoantibodies from this patient were able to bind to the respiratory epithelium, and this is one likely mechanism of the severe lung damage.

DERMATITIS HERPETIFORMIS

1. Tissue-bound IgA, a rather specific marker of GSE colocalizes with the distribution of TGc in DH and CD patients, and corresponds to the deposition of endomysium antibodies in the small bowel.

2. After finishing this work, our examinations on a bigger patient group confirmed these results, so a new immunohistological method will be available in the routine diagnostic of gluten-sensitive enteropathy.

3. The frequent occurrence of IgA and C3 vascular fluorescence mostly in the superficial, small vessels of DH patients’ skin supports possible immune complex precipitation along the microvascular system and confirms its significance in the pathomechanism of skin symptoms.

4. Colocalization of IgA and TGc in the vessel walls further supports the significance of TGc (and not TGc) in the development of DH skin symptoms.