The role of adamalysins (ADAMs) in destruction of anchoring fibers involved in pathogenesis of selected subepidermal bullous diseases

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Abstract
Pathogenesis of autoimmune subepidermal bullous diseases, which include pemphigoid (BP) and dermatitis herpetiformis (DH), is associated with destruction of basement membrane components, leading to formation of bullous lesions. Antibodies binding to autoantigens localized in the basement membrane activate a series of immunological and enzymatic phenomena that lead to destruction of anchoring fibers. Recent data reveal the important role of certain enzymes, metalloproteinases: collagenases, stromelysins and gelatinases, as well as their tissue inhibitors in skin lesions in destruction of the basement membrane and formation of blisters. There is also evidence that adamalysins (ADAMs – a disintegrin and metalloproteinases), enzymes combining features of both adhesion molecules and proteases, may be involved in this process. Recent data and results of own preliminary studies, concerning biochemical properties of these enzymes and their high affinity to components of the basement membrane, especially collagen XVII and VII, are presented in the paper. Disturbed expression of adamalysins or lack of their inhibitors, stimulated by immune complexes present in the structures of the basement membrane, may be responsible for destruction of anchoring fibers and blister formation. Further research is necessary in order to elucidate the question whether destruction of anchoring fibers of the basement membrane in the course of bullous diseases results from overexpression of adamalysins or lack of their inhibitors, both in skin lesions and in normally appearing skin. It would also be important to discover the probable factors activating adamalysins’ expression in lesional skin and establish the possible therapeutic use of their inhibitors.

Key words: subepidermal bullous diseases, anchoring fibers, hemidesmosomes, basement membrane, adamalysins, metalloproteinases

Pathogenesis of autoimmune subepidermal bullous diseases, which include bullous pemphigoid (BP) and dermatitis herpetiformis (DH), is associated with destruction of basement membrane components, leading to formation of bullae. Basement membrane is a complex structure providing integrity of dermo-epidermal junction. Antibodies binding to autoantigens localized in the basement membrane activate a series of immunological and enzymatic phenomena that lead to destruction of anchoring fibers. Pemphigoid is a bullous dermatosis characterized by inflammatory infiltrate in the dermis and deposits of IgG immunoglobulin and C3 component of the complement alongside the basement membrane of the epidermis. Their presence may be revealed by direct immunofluorescence test (DIF), which has diagnostic importance for the disease. Ultrastructural studies confirm the presence of intensive inflammatory infiltrate at the dermo-epidermal junction, as well as destruction of hemidesmosomes and components of...
the extracellular matrix [1]. Autoantigens in BP are glycoproteins of molecular mass 180 kD (BPAG 2) and 230 kD (BPAG 1) [2].

BPAG 2, a type II transmembrane protein (collagen XVII) is the main antigen in BP. It is a key protein constituting the anchoring fibers, responsible for adhesion of epidermis to the basement membrane. A structural study revealed that extracellular fragment of this antigen, possessing COOH-terminal collagenous domain, connects the basement membrane with epidermal hemidesmosomes. NC16a fragment is thought to be the most important immunogenic part of the BPAG 2 antigen [3].

BPAG 1 belongs to the family of plakins, intracellular proteins connecting the intermediate filament of the cytoskeleton with desmosomes and hemidesmosomes. It is thought that antibodies directed against this antigen are formed secondarily to destruction of keratinocytes and release of determinants involved in autoimmune response [2].

Dermatitis herpetiformis (Duhring disease) is a dermatological syndrome of common etiology with gluten-sensitive enteropathy (GE). There are isolated reports about increased activity of tissue transglutaminase (tTG) in patients with GE, and the preferred substrate for this enzyme is gliadine, a product of gluten metabolism [4]. The most recent studies on the autoimmune pathogenesis of gluten-sensitive enteropathy revealed that deamidation reaction of the gliadine particle by tTG plays the key role in the process, leading in consequence to formation of molecules containing new epitopes [5]. It was confirmed by further studies that tissue transglutaminase is the main autoantigen of gluten-sensitive enteropathy, and IgA-EmA antibodies, routinely determined in this disease, are de facto directed against tTG [6].

There are some very interesting observations concerning the role of epidermal transglutaminase, probably an isoform of tTG, in pathogenesis of dermatitis herpetiformis. It was proved that transglutaminases catalyze calcium-dependent formation of covalent γ-glutamyl-lysine bonds [7]. Epidermal transglutaminase takes part in keratinocyte differentiation and stabilization of dermo-epidermal junction in dermal papillae. It has been also established that transglutaminase catalyses formation of cross bonds within type VII collagen, which forms the anchoring fibers [7, 8].

It was also confirmed that specific proteins: BM-40/osteotactin/SPARK and nidogen/entactin constitute the substrates for this enzyme [9]. The dominant role of transaminases in the processes of stabilization of the membrane zone, formation of extracellular matrix enclosing the cells in the network of collagen fibers [10] as well as their bonds with fibronectin was described in literature [11].

Both literature data and preliminary results of own studies on expression of selected metalloproteinasises: collagenases, stromelysins and gelatinases, as well as their tissue inhibitors in skin lesions reveal their important role in destruction of the basement membrane and formation of blisters [12]. In available literature, however, there are no data on expression and role of specific family of metalloproteinasises – adamalysins (ADAMs – a disintegrin and metalloproteinases) in this process. These enzymes combine features of both adhesion molecules and proteases.

Recent studies established biochemical properties of these enzymes and their high affinity to components of the basement membrane, especially collagen XVII and VII [13]. Obtained data created scientific basis for research aimed at establishing the role of these proteins in the pathogenesis of subepidermal bullous diseases.

Verraes et al [12] in their studies in vitro and on mouse model of pemphigoid revealed proteolysis of BPAG 2 antigen by neutrophilic elastase, secreted by inflammatory cells from the infiltrate, mostly neutrophils. These results were not confirmed by in vivo experiments, for main cells of the inflammatory infiltrate are eosinophils, which do not secrete this type of metalloproteinases.

The most recent reports suggest that collagens XVII and VII that build the anchoring fibers are proteolysed by ADAMS. This process, as well as interactions between BPAG 2 and other components of anchoring fibers and adhesion molecules, is not yet fully elucidated. It is suggested that apart from its function in preserving the integrity of dermo-epidermal junction, this antigen is also involved in signal transduction and regulation of keratinocytes’ differentiation [14].

Schmidt et al [15] observed that after binding of autoantibodies with BPAG 2, activated keratinocytes release interleukin-6, interleukin-8 and C5 component of the complement. Mastocytes and neutrophils are also activated, thus releasing specific proteinasises belonging to the family of collagenases, stromelysins and gelatinases, which digest a series of proteins constituting various structures of the basement membrane. These matrix metalloproteinases lead in consequence to formation of blisters [16]. It is suggested that binding of antibodies against NC16a fragment with the antigen results in activation of adamalysins, causing desquamation of the NC16a fragment and formation of subepidermal bulla. It seems important to confirm this theory by in vivo evaluation of these enzymes in active stage of the diseases, during remission and in healthy skin.

Studies on liquid collected from the spontaneous and artificial bullae, aimed at determination of activity of some metalloproteinases, including collagenase, revealed significantly elevated level of proteases, thus suggesting the possibly important role of these enzymes in preservation of skin integrity [17]. Metalloproteinases’ inhibitors were also present in bullous liquid, although in lower concentrations. Increasing levels of inhibitors may be responsible for decrease in tissue destruction and beginning of their repair [18]. It should seem important to confirm the association between concentrations of enzymes and their inhibitors both in bullous liquid and tissues and activity of the disease. Establishment of such correlation could be
helpful in treatment monitoring, for the titre of circulating autoantibodies of the BMZ (basement membrane zone) type, determined routinely by indirect immunofluorescence test, has no such significance. Although recently introduced immunoenzymatic tests revealing the presence of anti-NC16a antibodies increased detection level of circulating antibodies, the correlation between these antibodies and disease activity is still controversial.

Blister formation in DH is initiated by accumulation of neutrophils, attracted by CD4+ T cells secreting mainly IFN-γ, TNF-α and IL-2, in dermal papillae, forming Pierrard’s microabscesses. They are quickly transformed by oedema and inflammation into microvesicles and later bigger subepidermal blisters [19].

Histological picture of DH is characterized by inflammatory infiltrate and destruction of collagen IV and laminin in basement membrane. Inflammatory cells in DH (neutrophils) are a source of many metalloproteinases. Collagenase degrades fibers of type I, II, III, IV and X collagen, but it cannot destroy the components of basement membrane. The presence of this enzyme however is necessary for differentiation of keratinocytes. Such associations prompted research on expression of metalloproteinases in skin biopsies from patients with DH. Increased activity of collagenase-1 (MMP-1), stromielisyne-1 (MMP-3) and gelatinase was observed. These enzymes take part in blister formation through degradation of basement membrane zone elements, such as collagen VII, IV and laminin-1 [20, 21].

Due to reports that collagen VII and enactin are the probable substrates for adamalysins, determination of their expression in the skin of patients with dermatitis herpetiformis should be of utmost importance. The most recent studies stress the role of the epidermal isoform of tTG in the process. Levels of antibodies directed against this enzyme can be high in sera of patients with Duhring disease. Sardy et al [22] proved that deposits of immune complexes in dermal papillae, recognized as pathognomonic for DH, contain epidermal isoform of transglutaminase.

It is still controversial whether skin lesions develop after activation of inflammatory processes by certain critical mass of deposits in the dermis, or another mediator of inflammation, such as IL-1 or TNF-α, is also necessary [23]. It was demonstrated that one of adamalysins, ADAM 17, possessing the features of collagen-degrading protease, activates TNF-α molecule by “desquamating” it from cell surface. It should be interesting to confirm elevated expression of both TNF-α and ADAM 17 in skin lesions of patients with subepidermal bullous diseases [24].

Airola et al [25], on the other hand, demonstrated the increased expression of collagenase only in basal layer keratinocytes, where it is inhibited by laminin and activated by presence of type I and IV collagen. Destruction of laminin, underlying formation of blisters in dermatitis herpetiformis is a sufficient signal for increased production of collagenase. It seems, however, that other group of proteases – adamalysins – may be responsible for initiating destruction of laminin and collagen within anchoring fibers.

Increased acitivity of stromielisyne-1 was found in most skin biopsies from patients with DH. This enzyme degrades basement membrane proteins and activates tissue procollagenase. Expression of matrilysine, another collagenase-activating metalloproteinase involved in proteolysis of basement membrane proteins, such as entactin and type IV collagen, was not observed [26]. Collagenase and elastase, enzymes which are probably released by neutrophils, were also found in bullous liquid from patients with DH [18]. Studies with flow chromatography revealed that the majority of enzymes are secreted by inflammatory cells forming the infiltrate [27]. It seems that activation of these families of metalloproteinases is secondary to the inflammatory process. Primary destruction of fibers caused by deposits of immunoglobulins may thus depend on adamalysins.

Metalloproteinases are secreted mainly by inflammatory cells. Paracrine/autocrine effects of various cytokines on cells in inflammatory infiltrate and skin cells may lead to imbalance between metalloproteinases and their inhibitors.
causing disturbances in architecture of extracellular matrix [28]. Decreased level of tissue inhibitors of matrix metalloproteinases (TIMPs) may also lead to degradation and remodeling of extracellular matrix [29].

Presented results of studies suggest that research on the role of adamalysins in pathogenesis of subepidermal bullous diseases should be especially interesting. They are present on cell surface, acting both as adhesion molecules and proteases. Their characteristic transmembrane and cytoplasmatic domains, similar in structure to EGF, suggest the possible role of these enzymes in adhesion and interaction between individual cells as well as between cells and components of extracellular matrix [30].

It has been proved that ADAMs are involved in normal development of the skin, catalyzing the process of removing amino peptides in the processing of procollagens to collagens. The presence of specialized domains and double role of ADAMs in cellular migration, differentiation and adhesion influences contents and changes of extracellular matrix and may also cause abnormal protein degradation in pathological processes [31].

Secretion of adamalysins in inactive form provides their quick response to environmental changes and pathogenic factors. In vitro studies demonstrated that activity of ADAMs, similarly to other MMPs, is stimulated by IL-1, thus confirming the possibility of their induction by an inflammatory process [32].

Many proteins, including cytokines and growth factors with their receptors, as well as adhesion molecules, are synthesized as transmembrane structures, proteolytically desquamated from cell surfaces. One of adamalysins, ADAM 17, releases from cellular surface L-selectin, TNF-α and its receptor as well as receptor for IL-6 [33]. Last year studies showed that also collagen XVII, i.e. BPAG 2, may serve as a substrate for ADAM 17, ADAM 9 and ADAM 10, synthesized in inactive form by keratinocytes [14].

Disturbed expression of adamalysins or lack of their inhibitors, stimulated by immune complexes present in the structures of the basement membrane, may be responsible for destruction of anchoring fibers and blister formation. There is an urgent need for further research aimed at elucidation of the question whether destruction of anchoring fibers, and thus the basement membrane, in the course of bullous diseases results from overexpression of adamalysins or lack of their inhibitors, both in skin lesions and in normally appearing skin. It would also be important to discover the probable factors activating adamalisyne expression of adamalysins in lesional skin and establish the possible therapeutic use of their inhibitors.

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