Low Strength of Correlation between the Intensity of Neutrophil Elastase Expression in Lesional Skin and the Level of Serum IgA Antibodies to Epidermal Transglutaminase in Dermatitis Herpetiformis

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Abstract: Whereas it has been shown that neutrophil elastase (NE) is a crucial enzyme degrading the dermal-epidermal junction (DEJ) in bullous pemphigoid (BP), experimental studies on the role of NE in dermatitis herpetiformis (DH), a disease in which, as in BP, an intra-*lamina lucida* blister is formed, are scanty. The aim of this study was to analyse whether there is a correlation between levels of serum IgA antibodies to the epidermal transglutaminase (TG3), an enzyme believed to be the autoantigen of DH, and expression of NE in lesional skin in DH. A series of 21 consecutive patients with DH was studied. The levels of IgA antibodies to TG3 in sera were calculated with ELISA. The expressions of NE were examined with immunohistochemical technique in sections of lesional skin using a mouse monoclonal antibody to human NE. The digital microscopic image analysis with the appropriate software was then used to measure intensities of NE expression. The correlation between the intensity of NE expression in lesional skin and the level of serum IgA antibodies to TG3 in DH was of low strength. Thus, it is speculated that in DH the engagement of IgA autoantibodies to the enzyme, TG3, on cutaneous neutrophils might not be a principal stimulus to releasing NE, the enzyme known to degrade DEJ in subepidermal blistering diseases with autoimmunity to DEJ structural proteins.

REPORT

The plausible pathophysiology of dermatitis herpetifomis (DH) is that the Fc region of IgA autoantibody to epidermal transglutaminase (TG3), an enzyme believed to be the most disease-specific autoantigen of DH, [1] activates neutrophils by engaging their CD89 receptor which leads to the release of neutrophil elastase (NE), NE-mediated destruction of the dermal-epidermal junction (DEJ) and the formation of an intra-*lamina lucida* blister. Whereas it was shown that NE is crucial DEJ-degrading enzyme in bullous pemphigoid (BP), [2-3] experimental studies on the role of NE in DH are scanty. Early data suggested that in blister fluids in patients with DH an activity of elastase, probably NE, can be detected [4-5]. The aim of this study was to analyse whether there is a correlation between levels of serum IgA antibodies to TG3 and expression of NE in lesional skin in DH.

NE deposits and IgA antibodies to TG3 were evaluated in a series of 21 consecutive dapsone-untreated patients with DH, regardless whether they had concomitant intestinal problems or not. The diagnosis of DH in each case was made when whichever pattern of possible 7 diagnostic patterns of granular IgA deposition was detected with direct immunofluorescence of nonlesional skin [6-8]. The levels of IgA antibodies to TG3 in sera were calculated with ELISA in AU/ml (manufacturer's cut-off value 18AU/ml) (Immundiagnostic, Germany). The expressions of NE were examined with immunohistochemical technique in sections of lesional skin showing histological features of DH, namely neutrophil-rich infiltrate at the tips of dermal papillae, using a mouse monoclonal antibody to human NE (Dako, Denmark) and LSAB+ system+ HRP visualisation kit (Dako, Denmark). The digital microscopic image analysis with the appropriate software [9] was then used to measure intensities of NE expression in the area of the visibly strongest immunohistochemical reaction under the magnification x200 in percentages of expression [10]. The results of NE expression analysis in a representative DH patient are shown on Figs. (**1-4**). The appropriate immunohistochemical procedure controls were performed. Statistical analysis was done using Spearman's rank correlation coefficient.

The strength of correlation between the intensity of NE expression in lesional skin and the level of serum IgA antibodies to TG3 in DH (Spearman's r=0.2822; P-value<0.05) (Fig. **5**) was low and should not be regarded as a moderate relationship [11]. Eleven DH cases had the level of serum IgA antibodies to TG3 above the manufacturer's cut-off value (52.4%); range of levels was 4.79-176.52 AU/ml. The low percentage of DH cases with elevated levels of serum IgA antibodies to TG3 in this study should not be considered unusual since in a recently published investigation only 52% of DH patients were shown to have those levels elevated [12]. It is known that dapsone impairs the functions of neutrophils, but our results were not influenced by this drug as none of patients was treated with dapsone prior to the establishment of the diagnosis of DH. The reliable information on the use of gluten-free diet by patients studied was unfortunately unavailable to us. It is conceivable that the strict gluten-free diet should normalize serum levels of IgA antibodies

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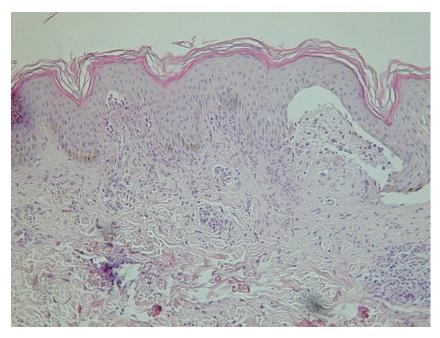E-mail: dmoch@sylaba.poznan.pl

Fig. (1). Neutrophil-rich infiltration at the tips of dermal papillae in a patient with DH (H+E staining) (magnification, x200).
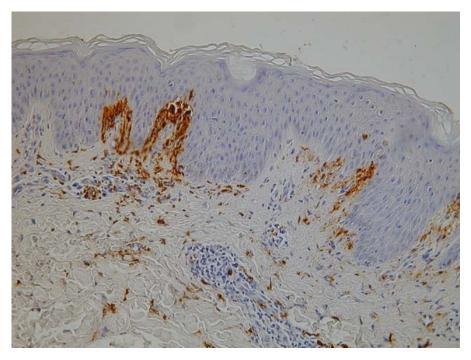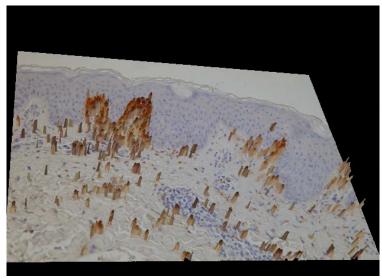

Fig. (2). NE deposits in immunohistochemistry in lesional skin in a patient with DH (magnification, x200).

not only to tissue transglutaminase (TG2) but also to TG3, so our results could be influenced by a dietary factor. Still, all our patients had cutaneous rash severe enough to prompt them to seek dermatological attention, so we believe that their dietary habits had low impact on their skin disease. Therefore, we feel that levels of IgA antibodies to TG3 obtained in our study truly reflect the severity of their skin disease, irrespectively of either intestinal or dietary considerations.

One might even speculate, on the basis of our study, that in DH the engagement of IgA autoantibodies to the enzyme, TG3, on cutaneous neutrophils might not be a principal stimulus to releasing NE, an enzyme known to degrade DEJ in subepidermal blistering diseases with autoimmunity to DEJ structural proteins [2]. This hypothesis should be experimentally verified, especially as we have previously shown that the expressions of NE in lesional skin in patients with DH and BP are not statistically different [10]. Still, there is a possibility that this stimulus is provided not by pooled IgA autoantibodies to TG3, but specifically by IgA1 or even IgA2 subclass autoantibodies to TG3. As 2 splicing isoforms of human TG3 have recently been identified, [13] it is currently unclear whether the IgA autoantibodies to those TG3 isoforms might differ in neutrophil activating capacities, hence whether there might be unequal NE releasing in

Fig. (3). Intensity of NE deposits processed with digital microscopic image analysis superimposed on NE deposits immunohistochemistry in lesional skin in a patient with DH.
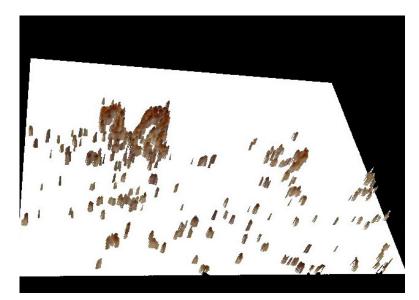

Fig. (4). Intensity of NE deposits processed with digital microscopic image analysis in lesional skin in a patient with DH.
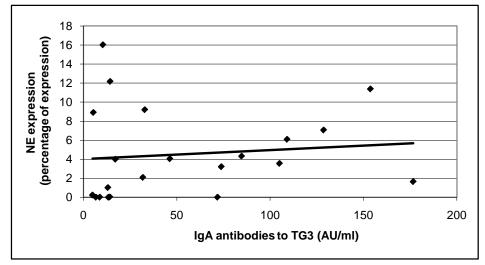

Fig. (5). Low strength of correlation between the intensity of NE expression in lesional skin and the level of serum IgA antibodies to TG3 in DH (Spearman's r=0.2822; P-value <0.05).

response to the production of IgA autoantibodies to various TG3 isoforms. Alternatively, IgA autoantibodies to TG3 might activate neutrophils to releasing matrix metalloproteinases. Matrix metalloproteinases in turn might more significantly contribute to the DEJ damage in DH than in BP [14]. Yet another possibility is that other autoantigen-autoantibody systems act on cutaneous neutrophils such as IgA/IgA1 autoantibodies to TG2, the autoantigen supposedly more celiac disease-specific than DH-specific. Finally, it is conceivable that the main contribution of neutrophils to the DH cutaneous pathophysiology might not be connected with release of DEJ-degrading enzymes. In addition to releasing them, neutrophils in DH might be primarily involved in antigen-specific (TG3 and/or TG2-driven) autoimmune response, as it has been shown that a subpopulation of human neutrophils possesses a T cell receptor (TCR)-based variable immunoreceptor [15]. It seems though that the TG3 involvement in DH pathogenesis might not be as profound as it has initially been suggested.

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