

LETTER TO THE EDITOR

What are the “True” Pathogenic Anti-desmoglein Antibodies?

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Sir,

We read with interest the reports by Li et al. (1) and Demitsu et al. (2). The former showed that, in some populations of pemphigus vulgaris, there is a significant gap in anti-desmoglein (Dsg) 3 antibody enzyme-linked immunosorbent assay (ELISA) index as measured by conventional ELISA vs. that measured by ethylenediaminetetraacetic acid (EDTA)-treated ELISA. The latter report demonstrated that anti-Dsg1 and anti-Dsg3 antibodies in a patient with Bowen carcinoma reacted with the precursor, but not the mature, forms of Dsg1 and Dsg3. Both studies suggest that anti-Dsg1 and Dsg3 antibodies are heterogeneous and can be categorized into pathogenic and non-pathogenic. However, even these recent methods for evaluating pathogenic anti-Dsg1/3 antibodies have not completely clarified the discrepancy between the disease activity and the ELISA index.

The usefulness of anti-Dsg1/3 ELISAs and indirect immunofluorescence (IIF) for the monitoring of pemphigus, based on a possible correlation between antibody levels and disease activity, remains controversial (3, 4). A recent study showed that ELISA index values can be a valuable tool for monitoring the disease, whereas IIF titres insufficiently reflect clinical activity (5). However, an interesting report was published recently in which a patient with pemphigus vulgaris in remission had a high anti-Dsg3 antibody ELISA index, but negative IIF results (6). The case presented a discrepancy between the disease activity, the ELISA index for Dsg3 and the IIF findings. The authors tried to resolve this discrepancy by devising a conformational ELISA index in which the EDTA-ELISA index is subtracted from the conventional ELISA index, which is a better indicator of disease activity than the conventional ELISA index. However, the patient's antibodies were found to be directed against Ca²⁺-dependent epitopes even during remission, because the conformational ELISA index showed high levels even during remission. If the patient's antibodies were recognizing mainly precursor Dsg3, which is synthesized in the endoplasmic reticulum, and not recognizing mature Dsg3, then this could explain the negative findings in IIF. Using recombinant proteins, the patient's antibodies were shown to react preferentially with mature Dsg3. Thus, the mechanisms behind the inconsistency between the ELISA and IIF findings remain uncertain.

Some reports suggest that levels of the IgG₄ subclass and IgE class of anti-Dsg3 antibody are associated with

active disease and that the IgG₁ levels are associated with remission (7, 8). Nagel et al. (8) demonstrated that, in addition to IgG₄, IgE autoantibodies to Dsg3 were present in a significant number of patients with acute-onset and active pemphigus vulgaris and that tissue-bound and serum IgE autoantibodies were detected in acute-onset pemphigus vulgaris by direct and indirect IF microscopy. This concept of class switch to IgG₁ during remission is also difficult to apply in the case described by Nakahara et al. (6), because there is almost no possibility that secondary antibodies against the human immunoglobulins used in IIF contain no anti-human IgG₁ antibodies.

Here, we propose a possible explanation of the discrepancy. We recently showed that the avidity of autoantibodies differs between a disease group and healthy individuals (HI) (9). Anti-dense fine speckles 70-kDa (DFS70) antibodies are anti-nuclear antibodies that are found more frequently in patients with atopic dermatitis (AD) than in HI, although the pathogenicity of these antibodies remains obscure. By comparing conventional anti-DFS70 ELISA values and antibody-stripping ELISA values by urea, we measured the avidity of anti-DFS70 antibodies in the AD and HI groups. The avidity of the antibodies was significantly higher in the patients with AD than in the HI, even though there was no difference in the avidity of anti-diphtheria toxoid between the 2 groups. A few reports have addressed the association between autoantibody avidity and disease activity. Regarding rheumatoid arthritis, Suwannalai et al. (10) reported that higher-avidity anti-citrullinated protein antibodies were observed only in symptomatic patients, whereas low-avidity anti-citrullinated protein antibodies were observed in both healthy subjects and patients with rheumatoid arthritis. Another study showed that, in some cases of vasculitis, myeloperoxidase anti-neutrophil cytoplasmic antibody had a high affinity that correlated with disease activity, irrespective of antibody titre (11). As far as we know, there are no reports investigating the avidity of anti-Dsg1/3 antibodies.

Autoantibody-defined epitopes are often conformation-dependent. For example, centromere protein-C (CENP-C), the main target of anti-centromere antibodies, has multiple epitopes formed from the C-terminal protein (12). Affinity-purified antibody against the dimer formation of the C-terminus in a liquid phase was found to be reactive only in IIF. We also demonstrated that a synthetic compound peptide composed of

discontinuous sequences mimicked the conformation-dependent epitope found in patients with systemic lupus erythematosus (13). However, the polyclonal antibody against this peptide had weaker avidity than that of human autoantibody. The following are thought to cause affinity changes: a point mutation in an amino acid, post-transcriptional modifications and interactions with other molecules within epitopes.

Table I summarizes the reported heterogeneity of anti-Dsg1/3 antibodies. Further research into the avidities of anti-Dsg1/3 antibodies is required in order to

elucidate the pathogenic mechanisms of pemphigus toward establishing a measurement for “true” pathogenic anti-Dsg1/3 antibodies.

The authors declare no conflicts of interest.

Table I. *Heterogeneity of anti-desmoglein antibodies*

Conventional enzyme-linked immunoassay (ELISA) vs. conformational ELISA
Autoantibodies to precursor desmogleins vs. mature desmogleins
Autoantibody subtypes: IgG1, IgG2, IgG4, IgA and IgE

Response to the Letter to the Editor by Muro et al.

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We read with much interest the Letter to the Editor on pathogenic (true) anti-desmoglein (Dsg) antibodies by Muro et al., which commented on our article (2) and another article by Li et al. (1). We reported that anti-Dsg1 and anti-Dsg3 antibodies in a patient with Bowen carcinoma reacted with the precursor form, but not the mature form, of Dsg1 and Dsg3 (2).

It is occasionally found that the results of Dsg ELISAs and indirect immunofluorescence (IF) do not correlate with disease activity (5). In addition, individuals with no clinical features of pemphigus may show positive results in Dsg ELISA (2). In particular, we have encountered several patients with pemphigus vulgaris, who showed very high index values of Dsg3 ELISA even in the remission stage of the disease. To confirm the causes of the loss of pathogenicity of the serum autoantibodies, we considered 5 possible mechanisms, as described below, and summarized in Table I.

The first possibility is that pathogenic autoantibodies react with Ca²⁺-dependent conformational epitopes on Dsgs, because various autoantibodies have been shown

to react with such conformation-dependent epitopes (13). This possibility can be confirmed by EDTA-treated Dsg ELISA or conformational ELISA index in which the conventional ELISA index is subtracted by EDTA-treated ELISA index (14).

The second possibility is that non-pathogenic autoantibodies react with precursor fragment on immature Dsg, which is present in the endoplasmic reticulum. This possibility can be confirmed by immunoprecipitation (IP)-immunoblotting (IB) or ELISA of recombinant proteins (RPs) of immature and mature Dsg (15).

The third possibility is that pathogenicity depends on extracellular domains of Dsg. Several studies have suggested that pathogenic autoantibodies react with N-terminal domains of mature Dsg (EC1 or EC2 domain), and autoantibodies to EC3-EC5 are non-pathogenic. The best method to confirm this possibility is IP-IB of Dsg3/Dsg2 or Dsg1/Dsg2 domain swapping molecule RPs (16, 17).

The fourth possibility is that pathogenicity depends on IgG subclasses (IgG1-IgG4) (7). Alternatively, IgA

Table I. *Possible mechanisms for pathogenicity of anti-desmoglein (Dsg) antibodies along with methods to confirm each possibility*

No.	Possible mechanisms	Methods for confirmation
1	Ca ²⁺ -dependent conformation	EDTA-treated ELISA Conformational ELISA (subtraction of conventional ELISA by EDTA-ELISA)
2	Autoantibodies to precursor fragment in immature Dsgs	IB or ELISA of immature Dsg RP
3	Dependency to extracellular Dsg domains, EC1-EC5	IP-IB or ELISA of Dsg1/Dsg2 and Dsg3/Dsg2 swapping molecule RPs
4	Dependency to immunoglobulin subtypes (IgG1-IgG4, IgA and IgE)	IF, IB or ELISA using subtype-specific antibodies
5	Autoantibodies to p38 MAPK activating domain within Dsg	p38 MAPK phosphorylation study p38 MAPK inhibitor study
6	Avidity (affinity) of anti-Dsg antibodies	Autoantibody-stripping ELISA by urea treatment or inhibition by self-antigen in a liquid phase

IP: immunoprecipitation; IB: immunoblotting; RP: recombinant protein; ELISA: enzyme-linked immunoassay; EDTA: ethylenediaminetetraacetic acid; MAPK: mitogen-activated protein kinases.

or IgE, but not IgG, class of anti-Dsg antibodies may be pathogenic (8). This possibility can be confirmed by IF, IB or ELISA using antibodies specific to each immunoglobulin subtype as a second antibody.

As the fifth possibility, we speculate that pathogenic autoantibodies may react with p38 mitogen-activated protein kinases (MAPK) activating domain within the Dsg molecule, because p38 MAPK-related signal transduction has been reported to play an important role in induction of keratinocyte detachment (18). This possibility can be confirmed by detection of phosphorylated p38 MAPK or addition of p38 MAPK-inhibitor

in the study of addition of autoantibodies into cultured keratinocytes (18).

In addition, the difference in avidity (affinity) of autoantibodies suggested by Muro et al. is an interesting possibility for pathogenicity of anti-Dsg autoantibodies, which can be confirmed by autoantibodies-stripping ELISA by urea treatment or inhibition by self-antigen in a liquid phase (9).

Further research is needed in order to elucidate the reasons for non-pathogenicity of a high Dsg index in pemphigus vulgaris patients in remission.

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REFERENCES (for both papers)

- Li Z, Zhang J, Xu H, Jin P, Feng S, Wang B. Correlation of conventional and conformational anti-desmoglein antibodies with phenotypes and disease activities in patients with pemphigus vulgaris. *Acta Derm Venereol* 2015; 95: 462–465.
- Demitsu T, Yamada T, Nakamura S, Kakurai M, Dohmoto T, Kamiya K, et al. Detection of autoantibodies to precursor proteins of desmogleins in sera of a patient with Bowen carcinoma. *Acta Derm Venereol* 2014; 94: 601–603.
- Grando SA. Pemphigus autoimmunity: hypotheses and realities. *Autoimmunity* 2012; 45: 7–35.
- Patsatsi A, Kyriakou A, Giannakou A, Pavlitou-Tsiontsi A, Lambropoulos A, Sotiriadis D. Clinical significance of anti-desmoglein-1 and -3 circulating autoantibodies in pemphigus patients measured by area index and intensity score. *Acta Derm Venereol* 2014; 94: 203–206.
- Weiss D, Ristl R, Griss J, Bangert C, Foedinger D, Stingl G, Brunner PM. Autoantibody levels and clinical disease severity in patients with pemphigus: comparison of aggregated anti-desmoglein ELISA values and indirect immunofluorescence titres. *Acta Derm Venereol* 2015; 95: 559–564.
- Nakahara T, Takagi A, Yamagami J, Kamiya K, Aoyama Y, Iwatsuki K, Ikeda S. High anti-desmoglein 3 antibody ELISA index and negative indirect immunofluorescence result in a patient with pemphigus vulgaris in remission: evaluation of the antibody profile by newly developed methods. *JAMA Dermatol* 2014; 150: 1327–1330.
- Hacker MK, Janson M, Fairley JA, Lin MS. Isotypes and antigenic profiles of pemphigus foliaceus and pemphigus vulgaris autoantibodies. *Clin Immunol* 2002; 105: 64–74.
- Nagel A, Lang A, Engel D, Podstawa E, Hunzelmann N, de Pita O, et al. Clinical activity of pemphigus vulgaris relates to IgE autoantibodies against desmoglein 3. *Clin Immunol* 2010; 134: 320–330.
- Watanabe K, Muro Y, Sugiura K, Akiyama M. High-avidity IgG Autoantibodies against DFS70/LEDGF in Atopic Dermatitis. *J Clin Cell Immunol* 2013; 4: 170.
- Suwannalai P, van de Stadt LA, Radner H, Steiner G, El-Gabalawy HS, Zijde CM, et al. Avidity maturation of anti-citrullinated protein antibodies in rheumatoid arthritis. *Arthritis Rheum* 2012; 64: 1323–1328.
- Yoshida M, Sasaki M, Nakabayashi I, Akashi M, Tomiyasu T, Yoshikawa N, et al. Two types of myeloperoxidase-antineutrophil cytoplasmic autoantibodies with a high affinity and a low affinity in small vessel vasculitis. *Clin Exp Rheumatol* 2009; 27: S28–32.
- Hayashi Y, Muro Y, Kuriyama K, Tomita Y, Sugimoto K. Differences in specificities of anti-centromere sera for the monomeric and dimeric C-terminal peptides of human centromere protein C. *Int Immunol* 2000; 12: 1431–1437.
- Muro Y, Tsai WM, Houghten R, Tan EM. Synthetic compound peptide simulating antigenicity of conformation-dependent autoepitope. *J Biol Chem* 1994; 269: 18529–18534.
- Kamiya K, Aoyama Y, Shirafuji Y, Hamada T, Morizane S, Fujii K, et al. Detection of antibodies against the non-calcium-dependent epitopes of desmoglein 3 in pemphigus vulgaris and their pathogenic significance. *Br J Dermatol* 2012; 167: 252–261.
- Yamagami J, Kacir S, Ishii K, Payne AS, Siegel DL, Stanley JR. Antibodies to the desmoglein 1 precursor proprotein but not to the mature cell surface protein cloned from individuals without pemphigus. *J Immunol* 2009; 183: 5615–5621.
- Ohyama B, Nishifuji K, Chan PT, Kawaguchi A, Yamashita T, Ishii N, et al. Epitope spreading is rarely found in pemphigus vulgaris by large-scale longitudinal study using desmoglein 2-based swapped molecules. *J Invest Dermatol* 2012; 132: 1158–1168.
- Di Zenzo G, Di Lullo G, Corti D, Calabresi V, Sinistro A, Vanzetta F, et al. Pemphigus autoantibodies generated through somatic mutations target the desmoglein-3 cis-interface. *J Clin Invest* 2012; 122: 3781–3790.
- Spindler V, Rotzer V, Dehner C, Kempf B, Gliem M, Radeva M, et al. Peptide-mediated desmoglein 3 crosslinking prevents pemphigus vulgaris autoantibody-induced skin blistering. *J Clin Invest* 2013; 123: 800–811.

The corresponding author of the paper by Li et al. has been contacted but abstained from replying.