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

Tomoko Nakahara, MD; Atsushi Takagi, MD, PhD; Jun Yamagami, MD, PhD; Koji Kamiya, MD, PhD; Yumi Aoyama, MD, PhD; Keiji Iwatsuki, MD, PhD; Shigaku Ikeda, MD, PhD

**IMPORANCE** Pemphigus vulgaris (PV) is a disease that features blistering of the skin and mucous membranes caused by autoantibodies directed against desmoglein 3 (Dsg3) and/or desmoglein 1 (Dsg1). Indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) are 2 methods that are widely used to measure Dsg3 or Dsg1 antibody titers in PV. Although the titers of these autoantibodies are generally correlated with disease activity, some patients with a high ELISA index do not have severe symptoms. We encountered a patient with PV in remission, who had a high anti-Dsg3 antibody ELISA index while the IIF result was negative.

**OBSERVATIONS** The anti-Dsg3 antibodies of our patient mainly recognized Ca2+-dependent conformational epitopes and targeted mature Dsg3 protein. We report this case focusing on the discrepancy between ELISA and IIF findings, as well as on the specific characteristics of the patient’s autoantibodies evaluated by newly developed methods.

**CONCLUSIONS AND RELEVANCE** This case emphasizes that a discrepancy between disease activity, the ELISA index for Dsg3, and/or IIF findings can occur in PV. Further research on similar patients will be required to elucidate the pathogenic mechanisms in patients with PV who have nonpathogenic antibodies and show a discrepancy between ELISA and IIF.

It is known that some patients with pemphigus vulgaris (PV) with a high enzyme-linked immunosorbent assay (ELISA) index do not necessarily have severe symptoms. The autoantibodies of such patients could display interesting characteristics, and many studies of nonpathogenic autoantibodies have been performed to understand more about the pathogenesis of PV. We encountered a patient with PV in remission who had a high ELISA index for anti-Dsg3 antibody while the indirect immunofluorescence (IIF) finding was negative, and we report this case focusing on the autoantibody profile.

**Report of a Case**

In November 2001, a woman in her 70s presented with oral erosions. Histopathological examination of biopsy specimens obtained from the oral and esophageal mucosa by her previous physician revealed epidermal blisters and acantholysis. These findings led to a diagnosis of PV, and treatment was started with prednisolone (40 mg/d). Although oral steroid therapy was continued, the patient’s blisters and erosions gradually became widespread. In August 2004, she was referred to us because of difficulty with oral intake.

At the first visit, physical examination revealed blisters and erosions all over her body, including on the oral mucosa. She had no relevant medical history. Laboratory test results revealed that the complete blood cell count and biochemical profile were within normal limits, but the serum anti-Dsg3 and anti-Dsg1 antibody indexes (determined by ELISA) were 2020 and 120, respectively. Examination of biopsy specimens of the oral mucosa again revealed epidermal blisters and suprabasal acantholysis. Direct immunofluorescence examination of the oral and esophageal mucosa demonstrated intracellular deposits of IgG and C3 in the epidermis. Indirect immunofluorescence also revealed intercellular staining of the epidermis for IgG (Figure 1A).

The patient was already taking prednisolone (35 mg/d) on admission. She was treated with betamethasone at a dose of 4 mg/d, and double filtration plasmapheresis (DFPP) was commenced. Her skin lesions improved rapidly with DFPP, which was continued until clinical remission was achieved in October 2004 after 6 sessions. Thereafter, azathioprine was added at 100 mg/d. Remission was maintained when betametha-
sone was switched to prednisolone and while prednisolone was tapered until it was ceased in October 2009. Azathioprine therapy was also stopped in December 2009.

The ELISA index for anti-Dsg3 antibody decreased when remission was achieved, but increased again in 2006 and has remained in the range of 2000 to 3000 since then (Figure 2).

In contrast, ELISA for anti-Dsg1 antibody has been negative since remission was achieved. Findings from IIF have generally been negative while the patient was in remission from 2009 to 2012 (Figure 1B and C), but a positive IIF result was detected at the onset of 2004 (Figure 1A). Indirect immunofluorescence was not only performed with normal human skin but also with guinea pig esophagus as a substrate because the latter is known to be a more sensitive substrate than the former.1-3 However, the results were the same with both substrates.

We (K.K., Y.A., and K.I.) performed an EDTA-treated ELISA of the patient’s serum sample. We devised a conformational ELISA index by subtracting the EDTA-ELISA index from the conventional ELISA index, which was considered to be a better indicator of disease activity than the conventional ELISA index.4 Although a slightly lower value was obtained compared with the conventional ELISA index, the titer was still elevated to approximately 2000 (Table 1). This result suggested that most of the patient’s antibodies were directed against Ca2+-dependent epitopes.

We (K.K., Y.A., and K.I.) evaluated the effect of PV-IgG purified from this patient on cell-cell adhesion of DJM-1 cells by the previously reported method.4 The patient’s PV-IgG did not cause a decrease of cell-cell adhesion, suggesting that her autoantibodies were probably nonpathogenic.

We (J.Y.) analyzed the patient’s serum antibodies to determine whether most of them recognized precursor Dsg3 (preDsg3) molecules. Recombinant proteins of precursor and mature Dsg3 (matDsg3) using Chinese hamster ovary cells as previously reported.5 The optical density obtained by ELISA targeting matDsg3 was similar to that for preDsg3 (Table 2). After adsorption of matDsg3, the ELISA for preDsg3 became negative. In contrast, adsorption of preDsg3 did not result in a negative ELISA result for matDsg3, although it reduced the optical density to some extent. These results suggested that most of the anti-Dsg3 antibodies of our patient recognized matDsg3, while some recognized both preDsg3 and matDsg3, and there were few or none recognizing preDsg3 alone. Immunoprecipitation of the patient’s IgG targeting preDsg3 and matDsg3 also confirmed that.

We (K.K., Y.A., and K.I.) performed an EDTA-treated ELISA of the patient’s serum sample. We devised a conformational ELISA index by subtracting the EDTA-ELISA index from the conventional ELISA index, which was considered to be a better indicator of disease activity than the conventional ELISA index.4 Although a slightly lower value was obtained compared with the conventional ELISA index, the titer was still elevated to approximately 2000 (Table 1). This result suggested that most of the patient’s antibodies were directed against Ca2+-dependent epitopes.

We (K.K., Y.A., and K.I.) evaluated the effect of PV-IgG purified from this patient on cell-cell adhesion of DJM-1 cells by the previously reported method.4 The patient’s PV-IgG did not cause a decrease of cell-cell adhesion, suggesting that her autoantibodies were probably nonpathogenic.

We (J.Y.) analyzed the patient’s serum antibodies to determine whether most of them recognized precursor Dsg3 (preDsg3) molecules. Recombinant proteins of precursor and mature Dsg3 (matDsg3) using Chinese hamster ovary cells as previously reported.5 The optical density obtained by ELISA targeting matDsg3 was similar to that for preDsg3 (Table 2). After adsorption of matDsg3, the ELISA for preDsg3 became negative. In contrast, adsorption of preDsg3 did not result in a negative ELISA result for matDsg3, although it reduced the optical density to some extent. These results suggested that most of the anti-Dsg3 antibodies of our patient recognized matDsg3, while some recognized both preDsg3 and matDsg3, and there were few or none recognizing preDsg3 alone. Immunoprecipitation of the patient’s IgG targeting preDsg3 and matDsg3 also confirmed that.

We (K.K., Y.A., and K.I.) performed an EDTA-treated ELISA of the patient’s serum sample. We devised a conformational ELISA index by subtracting the EDTA-ELISA index from the conventional ELISA index, which was considered to be a better indicator of disease activity than the conventional ELISA index.4 Although a slightly lower value was obtained compared with the conventional ELISA index, the titer was still elevated to approximately 2000 (Table 1). This result suggested that most of the patient’s antibodies were directed against Ca2+-dependent epitopes.

We (K.K., Y.A., and K.I.) evaluated the effect of PV-IgG purified from this patient on cell-cell adhesion of DJM-1 cells by the previously reported method.4 The patient’s PV-IgG did not cause a decrease of cell-cell adhesion, suggesting that her autoantibodies were probably nonpathogenic.

We (J.Y.) analyzed the patient’s serum antibodies to determine whether most of them recognized precursor Dsg3 (preDsg3) molecules. Recombinant proteins of precursor and mature Dsg3 (matDsg3) using Chinese hamster ovary cells as previously reported.5 The optical density obtained by ELISA targeting matDsg3 was similar to that for preDsg3 (Table 2). After adsorption of matDsg3, the ELISA for preDsg3 became negative. In contrast, adsorption of preDsg3 did not result in a negative ELISA result for matDsg3, although it reduced the optical density to some extent. These results suggested that most of the anti-Dsg3 antibodies of our patient recognized matDsg3, while some recognized both preDsg3 and matDsg3, and there were few or none recognizing preDsg3 alone. Immunoprecipitation of the patient’s IgG targeting preDsg3 and matDsg3 also confirmed that.

We (K.K., Y.A., and K.I.) performed an EDTA-treated ELISA of the patient’s serum sample. We devised a conformational ELISA index by subtracting the EDTA-ELISA index from the conventional ELISA index, which was considered to be a better indicator of disease activity than the conventional ELISA index.4 Although a slightly lower value was obtained compared with the conventional ELISA index, the titer was still elevated to approximately 2000 (Table 1). This result suggested that most of the patient’s antibodies were directed against Ca2+-dependent epitopes.

We (K.K., Y.A., and K.I.) evaluated the effect of PV-IgG purified from this patient on cell-cell adhesion of DJM-1 cells by the previously reported method.4 The patient’s PV-IgG did not cause a decrease of cell-cell adhesion, suggesting that her autoantibodies were probably nonpathogenic.

We (J.Y.) analyzed the patient’s serum antibodies to determine whether most of them recognized precursor Dsg3 (preDsg3) molecules. Recombinant proteins of precursor and mature Dsg3 (matDsg3) using Chinese hamster ovary cells as previously reported.5 The optical density obtained by ELISA targeting matDsg3 was similar to that for preDsg3 (Table 2). After adsorption of matDsg3, the ELISA for preDsg3 became negative. In contrast, adsorption of preDsg3 did not result in a negative ELISA result for matDsg3, although it reduced the optical density to some extent. These results suggested that most of the anti-Dsg3 antibodies of our patient recognized matDsg3, while some recognized both preDsg3 and matDsg3, and there were few or none recognizing preDsg3 alone. Immunoprecipitation of the patient’s IgG targeting preDsg3 and matDsg3 also confirmed that.
Moreover, Kamiya et al. reported that the EDTA-mAbs either only bind to preDsg or bind with both preDsg and matDsg, whereas nonpathogenic pathogenic mAbs from patients with PV or mice preferentially bind to epitopes on matDsg. A dissociation study was correlated with the pathogenicity of antibodies who show a discrepancy between ELISA and IIF results.

The results of the analysis on the patient’s serum sample emphasize that a discrepancy between disease activity, the ELISA index for Dsg3, and/or IIF findings can occur in PV. What is the antibody profile that underlies this discrepancy?

Our patient remained in remission for approximately 11 years, including more than 6 years without any treatment. Despite being in remission, she persistently had a high ELISA index for anti-Dsg3 antibody, although the IIF result was negative. The positive IIF result at the onset of PV contains some possibilities. The result at the onset is consistent with the blistering lesions that began on the oral mucosa. It suggests that the anti-Dsg3 antibodies at the onset were pathogenic, but they changed to nonpathogenic antibodies during the course, which was not detected with IIF. Another possibility would be that the presence of anti-Dsg1 antibodies in the early period of the disease mainly attributed to the positive IIF result because the IIF result was negative after anti-Dsg1 antibodies became undetectable in the remission phase. This suggests that the negative IIF result during remission was associated with the profile of our patient’s anti-Dsg3 antibodies. The present case emphasizes that a discrepancy between disease activity, the ELISA index for Dsg3, and/or IIF findings can occur in PV. What is the antibody profile that underlies this discrepancy?

Previous studies have shown that pathogenic monoclonal anti-Dsg antibodies (mAbs) predominantly recognize Ca²⁺-dependent conformational epitopes. It was also reported that pathogenic mAbs from patients with PV or mice preferentially bind to epitopes on matDsg, whereas nonpathogenic mAbs either only bind to preDsg or bind with both preDsg and matDsg. Moreover, Kamiya et al. reported that the EDTA-treated ELISA revealed a decrease of anti-Dsg3 antibodies targeting Ca²⁺-dependent epitopes in the inactive phase of PV.

We initially predicted that the autoantibodies of our patient would mainly recognize non-Ca²⁺-dependent epitopes and would mainly bind to preDsg3 because antibodies targeting preDsg3 synthesized in the endoplasmic reticulum might be negative by IIF. However, we unexpectedly found evidence that most of the antibodies in our patient recognized Ca²⁺-dependent conformational epitopes and targeted matDsg3. This is consistent with a previous report that the antibodies of patients with PV predominantly recognize epitopes at the N-terminus of Dsg throughout the course of the disease.

There have been some previous reports of similar patients with a high ELISA index and negative IIF result. In patients with rheumatoid arthritis taking thiol compounds, autoantibodies targeting nonconformational epitopes of either Dsg3 or Dsg3 have been detected by ELISA without blistering skin lesions, while the IIF result remained negative. The authors mentioned that the discrepancy was possibly related to a difference of peptide structure between the recombinant Dsg used for the ELISA and native human Dsg in epidermal cryosections, and these patients never developed features of PV. In addition, Li et al. reported that epitope spread occurs in patients with endemic pemphigus foliaceus during remission and relapse. Serum obtained from some patients during remission predominantly reacted with EC5, and the IIF result was negative despite the ELISA result being positive. Therefore, they hypothesized that the EC5 domain may be sequestered in vivo because of its proximity to the cell membrane, which may prevent it from binding to circulating antibodies.

It is known that a combination of mAbs targeting different epitopes of Dsg3 shows stronger pathogenicity than a single mAb. Yamagami et al. characterized Dsg-specific mAbs from patients with pemphigus and reported that specific amino acids in the heavy-chain complementarity-determining region 3 (HCDR3) are critical for pathogenicity, although antibody binding can be uncoupled from pathogenicity. In their study, replacement of a specific amino acid abolished pathogenicity and also decreased the IIF titer in some cases, although the IIF result did not become negative.

It is also possible that a point mutation of an antigenic epitope or antibody abolished pathogenicity in the present patient and affected the IIF response, but the actual reason for the discrepancy between ELISA and IIF findings remains uncertain at present. It seems that only the result of the cell dissociation study was correlated with the pathogenicity of anti-Dsg3 antibodies in our patient.

Discussion

The results of the analysis on the patient’s serum sample emphasize that a discrepancy between disease activity, the ELISA index for Dsg3, and/IIF findings can occur in PV. Further research into other cases like our patient will be required to elucidate the mechanisms operating in patients with PV with nonpathogenic antibodies who show a discrepancy between ELISA and IIF results.

Conclusions

The results of the analysis on the patient’s serum sample emphasize that a discrepancy between disease activity, the ELISA index for Dsg3, and/or IIF findings can occur in PV. Further research into other cases like our patient will be required to elucidate the mechanisms operating in patients with PV with nonpathogenic antibodies who show a discrepancy between ELISA and IIF results.
Role of the Sponsor: The Ministry Health, Labor, and Welfare had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: Takatoshi Kuhara, DVM, PhD, Atopy Research Center, Juntendo University Graduate School of Medicine, provided assistance for indirect immunofluorescence with guinea pig esophagus. No financial compensation was provided.

REFERENCES

NOTABLE NOTES

World War One
The Department of Masks for Facial Disfigurements

Faisal R. Ali, MA, BM, BCH, MRCP; Alexander E. T. Finlayson, BMedSci, MRCP; James Fox, MA, PhD

The First World War devastated the political, economic, and social landscape of the early 20th century. Equally pervasive, ruinous consequences were evidenced by the many thousands of soldiers who sustained disfiguring facial injuries during battle. Born in Keswick, Francis Derwent Wood (1871-1926) crafted a career as a sculptor, first as a pupil at the National Art Training School and later during a series of apprenticeships with the great artists of the time.1 In 1914, Wood joined the Royal Army Military Corps initially as an orderly, but he soon took charge of the splint unit in the Third London General Hospital, Wandsworth. Wood resolved to exercise his prowess as a sculptor to reconstruct faces for men and women who had suffered devastat- ing facial injuries, superseding the contemporary practice of crudely attaching gelatin and rubber parts.

Wood describes his endeavors in his seminal Lancet paper of 1917:2 My work begins where the work of the surgeon is completed. When the surgeon has done all he can to restore functions, to heal wounds, to support flabby tissues by bone-grafting, to cover areas by skin-grafting, I endeavour by means of the skill I happen to possess as a sculptor to make a man’s face as near as possible to what it looked like before he was wounded. As they were in life so I try to reproduce them, beautiful or ugly, the one desideratum is to make them natural.

Working with a team of professional artists in the Department of Masks for Facial Disfigurements, Wood exercised the pedantry of a societal portrait artist, driven by the desire to create a mask replicating the patient’s original facial contours. After surgical wounds had fully healed, during several sittings a number of precise casts and countercasts were fashioned. Using the injured portions of the face and series of preinjury photographs as reference points, Wood and his team would create a highly accurate mask in silvered copper that rested comfortably on the face. Each mask was completed by painting with oil while being worn by the owner to match its owner’s skin complexion as closely as possible. The results, published in The Lancet, appear remarkable.

Although the department remained open only until 1918, during its brief lifetime, several hundred masks were produced. Wood’s scheme is a rare example of the mimetic capacities of an artist serving functional, nonartistic ends. Despite advances in surgery rendering such masks obsolete, the early 20th century sculptor recognized what dermatologists and aestheticians have qualified more recently: the profound psychological sequelae of abnormalities of the skin and need for mimicry of features as close as possible to nature. These principles hold as true today as they did a century ago.

Author Affiliations: Dermatology Centre, Salford Royal NHS Foundation Trust, University of Manchester; Manchester Academic Health Science Centre, Manchester, England (Ali); Department of Primary Care, University of Oxford, Oxford, England (Finlayson); Department of History of Art, University of Cambridge, Cambridge, England (Fox).

Corresponding Author: Faisal R. Ali, MA, BM, BCH, MRCP, Dermatology Centre, University of Manchester, Salford Royal NHS Foundation Trust, Manchester M6 8HD, England (faisal.ali01@cantab.net).