Updates on Autoimmune Skin Bullous Diseases

Masayuki AMAGAI

Assistant Professor, Department of Dermatology, Keio University School of Medicine

Abstract: Autoimmune skin bullous diseases are a group of diseases induced by autoantibodies against adhesion molecules in the skin. The understanding of these diseases has progressed greatly through studies combining cell and molecular biological investigation of cell adhesion. A technique for producing recombinant pemphigus antigen proteins became available, and the development of the ELISA method facilitated serodiagnosis and monitoring of disease activity. Furthermore, a mouse model of pemphigus was developed through a new methodology using autoantigen knockout mice produced by gene manipulation. The pemphigus model mice produce the IgG antibodies against desmoglein3 (Dsg3) for over 6 months. This model is useful for the study of the mechanisms for antibody production and immune tolerance to peripheral antigens, as well as the evaluation of various immunosuppression therapies. In addition, monoclonal antibodies against Dsg3 were isolated from the pemphigus model mouse, and the studies using these antibodies expanded our knowledge of the molecular basis for blister formation. It is hoped that the study on autoimmune diseases of the skin will lead to elucidation of the pathophysiological mechanisms of autoimmune diseases and the development of treatment with minimal side effects.

Key words: Pemphigus; Bullous pemphigoid; ELISA; Model mouse

Introduction

Autoimmune skin bullous diseases are a group of diseases induced by autoantibodies against adhesion molecules in the skin. Typical examples of these diseases are pemphigus, which impairs adhesion between epidermal cells, and bullous pemphigoid, which impairs adhesion in the basement membrane area. The understanding of autoimmune bullous dermatoses has progressed greatly through studies combining cell and molecular biological investigation of cell adhesion. Based on the findings from these studies, an ELISA (enzyme-linked immunosorbent assay) method using recombinant antigen proteins was developed as a
diagnostic tool for clinical application. Furthermore, a mouse model of pemphigus was developed through a new methodology using auto-antigen knockout mice produced by gene manipulation.

Focusing on these topics, this article reviews the updates on autoimmune skin bullous diseases.

Pemphigus and Bullous Pemphigoid

Pemphigus is divided into two major forms, pemphigus vulgaris (PV) and pemphigus foliaceus (PF). PV is further classified into mucosal dominant type mainly affecting the oral mucosa and mucocutaneous type forming blisters and erosion not only in the oral mucosa but also in the skin. PF does not affect mucous membranes, but forms erosion and scaly erythema only in the skin.

Patients with pemphigus produce IgG autoantibodies against desmoglein (Dsg), a cadherin-type cell-cell adhesion molecule that plays important roles in the adhesion between epidermal cells. Dsg1 and Dsg3 are mainly expressed in stratified squamous epithelium including the skin and mucous membranes. Patients with mucosal dominant type of PV show anti-Dsg3 antibodies alone, while those with mucocutaneous type of PV show both anti-Dsg1 and anti-Dsg3 IgG autoantibodies. Patients with PF produce anti-Dsg1 antibodies alone. An indirect immunofluorescence (IF) assay using normal human skin as the substrate has demonstrated that the IgG autoantibodies in pemphigus react only with the cell surfaces of keratinocytes.

Bullous pemphigoid (BP) is an autoimmune disease in which the target antigens are the 230kD BP antigen (BP230, BPAG1) and 180kD BP antigen (BP180, BPAG2) found in hemidesmosomes, which are adhesion structures connecting the epidermal basal cells and the basement membrane. BP is prone to develop in aged persons. Typical clinical symptoms of BP include tense blisters on itchy urticarial erythema. Histopathologically, BP shows subepidermal blisters.

In addition to typical BP that produces skin rashes all over the body, there are various clinical subtypes such as localized BP (the type showing skin rashes in limited anatomical areas such as anterior tibial area, head, face, or neck); vesicular BP (the type showing only small vesicles); nodular BP (the type showing skin rashes resembling nodular purigo); and vegetative BP (the type showing papillomatous growth and rising of the intertriginous areas). In any type, an IF assay using healthy human skin as the substrate demonstrates that the IgG autoantibodies in pemphigoid react with the basement membrane zone.

Serodiagnosis with ELISA Using Recombinant Antigen Proteins

ELISA can be applied to any antigen, provided that highly purified antigen has been obtained. A major advantage of this assay is the ability to measure antibody titers, because the assay quantifies the reactivity of autoantibodies by means of a color reaction, and the result is read on a spectrophotometer. Inter-assay and inter-facility comparisons of assay results can be achieved by the use of the ELISA score (index value) calculated from the comparison with the reactivity of a standard specimen.

As a tool for serodiagnosis of pemphigus, an ELISA method using recombinant Dsg1 and Dsg3 as coated antigens has been developed and put into clinical use.¹⁻⁴ The recombinant proteins used in this method are produced in baculovirus expression system using cultured insect cells, and have been confirmed to have proper 3-dimensional structures. Serodiagnosis is made as described below, based on the combination of ELISA result using Dsg1 and Dsg3 (Fig. 1).⁶⁻⁷

If the tested serum is positive for Dsg3 and negative for Dsg1, the diagnosis is mucosal dominant type of PV. If the serum is positive
The Dsg1 and Dsg3 ELISA has been covered by the health insurance system and the results of basic studies have been widely utilized in daily clinical practice.

A Novel Mouse Model for Pemphigus

Why are autoantibodies attacking the self components formed in autoimmune diseases? We have not clearly answered this question as yet even after the turn of the century. The treatment for autoimmune diseases is mainly based on steroids, immunosuppressants, and plasmapheresis. These therapies suppress the immune system in general, and thus have the problem of causing severe side effects. The study of the mechanisms for autoantibody production and the development of new antigen-specific immunosuppressive therapies will require animal models simulating diseases in humans.

Conventionally, mouse models of autoimmune diseases have been produced by forced immune reactions using repeated immunization of various wild-type mice with antigen proteins in various immune adjuvants. While several models have been produced by this method, the success of model preparation strongly depends on empirical factors, because for both Dsg3 and Dsg1, the diagnosis is mucocutaneous type of PV. If the serum is negative for Dsg3 and positive for Dsg1, the diagnosis is PF. It should be noted that serodiagnosis is not the final diagnosis. Final diagnosis should be made based on the clinical as well as histopathological findings.

For serodiagnosis of bullous pemphigoid, an ELISA method using recombinant protein of NC16a domain, where the main epitope of BP180 is located, was developed.6,7 Because the ELISA method quantitatively measures antibody titers, it is useful for monitoring disease progression.1,2,8 ELISA scores can be used as a guide in the determination of a steroid tapering schedule. In plasmapheresis, clearance rate can be calculated from the ELISA score of the serum before treatment and that of the waste fluid, and this provides an index for objective evaluation of the antibody removal rate. In patients showing repeated remissions and aggravations, an increase in the ELISA score sometimes precedes an aggravation and aids early treatment.

The measurement in ELISA is based on an enzyme reaction. When the patient has a very high antibody titer, this means that the serum dilution factor must be increased more than usual 100 fold and the result of ELISA must be converted to the true antigen titer.8

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immune responses vary depending on mouse strains and the type of adjuvants. The autoimmune response of these model mice is often transient, and few achieve a persistent autoimmune state similar to actual diseases.

In addition, organ-specific autoimmune diseases in humans usually involve antigen-specific disruption of autoimmune tolerance, and other immune functions remain in normal conditions. However, the conventional method using forced immunity induces the activation of the immune system in general, and often results in great differences from actual diseases, such as the manifestation of systemic inflammatory reactions.

A new method for producing mouse models of organ-specific autoimmune diseases has been invented recently. In autoantigen knockout mice, the immune system in the process of development does not encounter an autoantigen that is missing in such mice. Therefore, the lymphocytes that are reactive to this autoantigen are not removed or inactivated in these mice, and the immune tolerance to the knocked out gene product is not established.

Utilizing this fact, the splenocytes (T and B cells) of immunized autoantigen knockout mice are transferred into mice that express the autoantigen. To prevent the rejection of transplanted splenic cells, this procedure is conducted in a Rag2 knockout mouse, which is an immunodeficient mouse lacking mature T and B cells. The transferred T and B cells derived from the autoantigen knockout mouse encounter the autoantigen in the recipient mouse, and a persistent autoimmune response is expected to take place.

Based on this principle, a mouse model of pemphigus was produced using Dsg3 knockout mice (Fig. 2). When Dsg3 knockout mice without immune tolerance to Dsg3 were immunized with rDsg3, this procedure easily induced antibodies that could bind to Dsg3 in vivo. Next, the splenocytes from the immunized Dsg3 knockout mice were adoptively transferred into Rag2 knockout mice expressing Dsg3. The production of the IgG antibodies against Dsg3 was detected in the blood of the recipient mice 4 to 7 days after transfer. The antibody titer reached a peak after 21 days, and persistent antibody production was observed for over 6 months. The antibodies did not show reactivity with Dsg1, and were specific to Dsg3.

Deposition of mouse IgG was observed in the cell membranes of Dsg3-expressing stratified squamous epithelium in the skin, oral mucosa, and esophagus of the recipient mice. In addition, impairment of cell adhesion was seen in the epidermis and mucous epithelium, as well as blister formation just above the basal
cell layer, which is a histopathological change characteristic of PV. Extensive erosions in the oral cavity caused inhibition of food intake, and body weight loss was observed from 7 days after the adoptive transfer, when antibody production became evident. Some recipient mice showed crusted erosions in areas usually scratched by the mice, such as the area around the nose.

Based on these findings, the mice produced here were considered a model showing characteristic clinical, histopathological, and immunological features of pemphigus. Among the types of pemphigus, these mice were considered a model of mucosal dominant type of PV, because they produced only anti-Dsg3 IgG antibodies and the main symptoms were observed in mucous membranes.

**Isolation of Monoclonal Antibodies Inducing Pemphigus**

Do all IgG autoantibodies that can bind to Dsg3 in vivo equally induce blisters, or do different antibodies have different pathogenic activity to induce blisters? The answer to this question is important for understanding the mechanism of blister formation in pemphigus and the reason why severity varies among cases. Taking advantage of the fact that the pemphigus model mice produced antibodies with pathogenic activity, we isolated several types of anti-Dsg3 IgG monoclonal antibodies and analyzed the relation between their pathogenic activity and the conformational epitopes recognized by the antibodies.\(^\text{10}\) We obtained 9 monoclonal antibodies (AK mAb) that were reactive with Dsg3.

When the hybridoma cells of each AK mAb were inoculated into the intraperitoneal cavity of mice, only the hybridoma of AK23 mAb induced the phenotype of PV in mice. Next, we analyzed the conformational epitopes of these mAbs. Dsg1/Dsg3 chimeric molecules and point mutated molecules were used for this analysis. AK23 mAb was found to occur in V3, K7, P8, and D59 considered to comprise the adhesive interface formed between Dsg3 molecules. The other mAb types that did not induce blister formation were found to recognize functionally unimportant parts that are not directly related to the interaction between Dsg3 molecules.

These results indicated that not all autoantibodies recognizing Dsg3 have equal pathogenic activity, and autoantibodies recognizing different sites on the Dsg3 molecule have different degrees of pathogenicity. A possibility was suggested that the difference in severity might be explained by the different epitopes of autoantibodies in different cases.

In the future, the use of pemphigus model mice is expected to facilitate the elucidation of autoantibody production mechanisms and the development of new disease-specific therapies. In addition, the method using autoantigen knockout mice demonstrated in this study can be applied widely to other autoimmune diseases.

**Conclusion**

The pathogenetic mechanism of autoimmune diseases has not been clarified as yet. The dermatoses discussed in this paper are examples of those few diseases that can be explained in terms of molecular biology. It is hoped that the findings in dermatoses will open a path for true understanding of the pathophysiological mechanisms of autoimmune diseases.

**REFERENCES**


