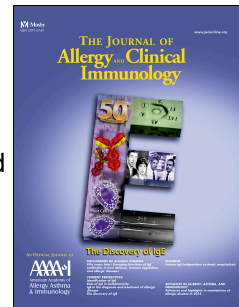


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Regulatory T cell deficiency and autoimmune skin disease: beyond scurfy mouse and IPEX

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Regulatory T cells (Treg), which specifically express forkhead box P3 (Foxp3) as the master regulatory gene, are actively engaged in the maintenance of peripheral immune tolerance and prevention of autoimmunity^{1,2}. Scurfy mice and patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome have mutations in the Foxp3 gene and consequently lack functional Treg, developing a variety of autoimmune reactions in various tissues including the skin³.

Autoimmune bullous skin disease is classified into pemphigus and pemphigoid diseases, which have several distinct disease subtypes⁴. Bullous pemphigoid (BP), the most common type of pemphigoid disease, develops clinically tense blister on the skin

and immunologically IgG autoantibodies to cutaneous basement membrane zone (BMZ)⁴.

IgG anti-BMZ antibodies in BP react with two major BP antigens, BP180 (also called type XVII collagen) and BP230, both of which are components of hemidesmosome⁵⁻⁷. BP180 is a transmembrane protein with many collagenous and non-collagenous (NC) domains in the extracellular region, while BP230 is an intracytoplasmic protein with N- and C-terminal globular domains and central rod domain (Fig. 1). While precise mechanism of autoantibody production in BP is still obscure, the report by Muramatsu et al.⁸ in this issue of JACI sheds a new light on the mechanism.

In this study, the authors attempted to characterize the autoantigens in the skin, particularly cutaneous BMZ components, recognized by autoantibodies in the sera from scurfy mice and IPEX syndrome patients. In the sera of scurfy mice, they detected autoantibodies to both BP180 and BP230, particularly the regions other than NC14A domain in murine BP180 and the central rod domain of murine BP230. No reactivity was found to murine desmoglein 3, which is the desmosomal component protein and is

targeted in pemphigus vulgaris, another type of autoimmune bullous skin disease.

Passive transfer of scurfy IgG to neonatal wild-type mice failed in induction of blister formation in the skin, indicating that the antibodies to BP180 and BP230 in scurfy mouse were non-pathogenic. Interestingly, *Tcrb*^{-/-} scurfy mice, which lack $\alpha\beta$ T cells but possess B cells, showed neither the eczematous skin lesions nor IgG deposition to cutaneous BMZ, which were found in scurfy mice. The authors further demonstrated that adoptive transfer of CD4⁺ T cells from scurfy mice into *Tcrb/Tcrd* double knockout mice was able to induce all the eczematous skin lesions, IgG deposition to cutaneous BMZ and production of antibodies to BP230 and BP180 in the recipients. In addition, scurfy mice deficient in the expression of signal transducer and activator of transcription (Stat6) showed reduced production of autoantibodies.

They also detected IgG autoantibodies to human BP230 and BP180 in the sera of IPEX syndrome patients who possessed loss-of-function mutations in the *Foxp3* gene as in scurfy mice. These results collectively indicate that Treg deficiency/dysregulation causes the production of autoantibodies to BP antigens in both scurfy mice and IPEX syndrome patients by activating autoreactive CD4⁺ Th2 or Tfh cells, or both. These

results also suggest that Treg might be involved in the production of autoantibodies and the development of skin lesions in certain BP patients.

The key points for the cellular immunity-related findings in this study are summarized in the graphical abstract in the original paper by Matsumura et al.⁸ In addition, we also illustrate how Treg dysfunction leads to abnormal skin conditions in scurfy mice or IPEX syndrome, as well as a possible application of this disease process to the development of blister formation in BP (Fig. 1A). However, the presence of Treg dysfunction and the following disease process in BP patients should be confirmed in the future studies.

In addition, the results of the experiments for the humoral autoimmunity in this study are also very interesting; i.e., the reactivity profiles of autoantibodies in both scurfy mice and IPEX patients are quite different from the profiles of pathogenic and immunogenic domains reported in previous studies of BP⁵⁻⁷.

We summarize the key points for the humoral immunity-related findings in this study in Figure 1B. Antibodies to murine BP180 NC14A domain (corresponding to human BP180 NC16A domain) is well known to be able to reproduce blister formation

in passive autoantibody transfer mouse models, and one study suggested the BP230 C-terminal domain as a putative pathogenic region. In BP patients, the majority of patient sera react with BP180 NC16A domain⁵ and with N- and C-terminal domains of BP230 as the most immunogenic domains⁶. In addition, patient sera from other pemphigoid diseases preferentially react with specific domains of BP180. For example, autoantibodies from linear IgA bullous dermatosis patients react with 15th collagenous domain and its surrounding region⁷, while autoantibodies from dipeptidyl peptidase 4 inhibitor-related BP patients recognize the non-NC16A central domain, and autoantibodies from patients with anti-BP180-type mucous membrane pemphigoid react with the C-terminal domain of BP180 (Fig. 1B).

In contrast, Muramatsu et al. showed that autoantibodies in the sera of both scurfy mice and IPEX patients reacted with non-NC14A and NC16A domains of BP180, respectively. The most predominant antigenic site of anti-BP230 antibodies in scurfy mice was the central rod domain of BP230, and this reactivity was also seen in one IPEX patient (Fig. 1B).

Taken together, autoantibodies to BP antigens in scurfy mice appear to be

non-pathogenic, although eczematous skin lesions seemed to be associated with antibodies to non-NC16A domain of BP180 in IPEX syndrome. These distinct epitopes and pathogenicity of autoantibodies to both BP230 and BP180 between scurfy mouse/IPEX syndrome patients and BP patients are intriguing and need to be examined in the future studies.

Another intriguing issue is that both scurfy mice and IPEX patients developed eczematous skin lesions but not blistering skin lesions, which are characteristic clinical features in BP. This suggests that the Treg dysfunction may allow autoreactive CD4⁺ T cells to directly attack the cutaneous BMZ and induce the eczematous skin lesions. Also interesting is the finding that an IPEX patient with a gain-of-function mutation in Foxp3 gene showed psoriasiform skin lesions without autoantibody production, while other IPEX patients produced antibodies to BP antigens with or without eczematous skin diseases. Considering the major role of Th17 cells in development of psoriasiform skin lesions in psoriasis, another inflammatory skin disease, this result may clinically indicate that different types of Foxp3 mutation potentially skew the inflammatory outcome to some specific phenotypes involving Th17, Th1 or Th2 cells, as suggested by

different phenotypes in knock-in mouse models of Foxp3 mutations⁹.

As another perspective, because this study showed that scurfy sera reacted most frequently with central rod domain of BP230, and because Hayakawa et al. identified a subset of BP patients reactive solely with BP230¹⁰, the pathogenic role of anti-BP230 autoantibodies in BP should also be examined.

As discussed above, the findings in this study provide a number of new insights into the pathogenetic mechanisms of BP. Further characterization of BP antigen-specific T cells and elucidation of the Th2/Tfh-stimulated autoantibody production, in addition to analyses of the roles of other immunological factors (e.g., roles of infiltrating eosinophils) and genetic factors (e.g., contribution of HLA haplotypes), will facilitate our understanding of how BP and other autoimmune diseases develop and how Treg are involved in the autoimmune processes.

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Figure legend

Fig. 1: Illustrations for cellular and humoral immunity-related findings in the study by Muramatsu et al.

(A) Schematic presentations how Treg dysfunction leads to abnormal skin conditions in scurfy or IPEX syndrome, as well as a possible disease process in the development of blister formation in BP. The issues, which should be confirmed in the future studies, are indicated by “?”.

(B) Schematic presentations of the protein structures of human BP180 and BP230 and the differences in immune reactivity. Beneath the schematic structures of both BP180 and BP230, the positive domains are shown for (1) pathogenicity in passive transfer mouse disease models, (2) autoantibodies in bullous pemphigoid (BP) patients, (3) autoantibodies in scurfy mouse sera, and (4) autoantibodies in IPEX patient sera. Shaded rectangles indicate positive domains. Positive rates for both scurfy mice and IPEX patients in this study are shown within rectangles.

Treg: regulatory T cell. Th2: type 2 helper T cell. Tfh: follicular helper T cell. Th17: type 17 T cell. Abs: antibodies. NC16A domain: Non-collagenous (NC) 16A domain of

176 BP180 (corresponding to NC14A domain in murine BP180. C15 domain: 15th
177 collagenous domain of BP180. DPP4: dipeptidyl peptidase 4. LABD: linear IgA bullous
178 dermatosis. MMP: mucous membrane pemphigoid.

