Treg dysfunction leads to spontaneous production of autoantibodies to bullous pemphigoid antigens in mice

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Introduction

Bullous pemphigoid (BP) is the most common autoimmune blistering disease caused by autoantibodies to hemidesmosomal proteins; type XVII collagen (COL17 or BP180) and BP230.¹ Regulatory T cells (Tregs) are crucial for peripheral immune tolerance and maintaining cutaneous immune homeostasis.² However, it remains largely unknown whether failure of peripheral immune tolerance has an influence on the production of autoantibodies to the epidermal components.

Objective

To elucidate the target autoantigens in the skin under a condition of Treg dysfunction in mice.

Methods and Results

We analyzed sera and skin from scurfy mice 21 days after birth (P21), which lack functional Tregs due to the mutation in *Foxp3*, a master transcriptional factor of Tregs.³ Histological study, direct and indirect immunofluorescence (DIF and IIF) were performed.

Figure 1. Scurfy mice develop interface dermatitis and show linear deposition of autoantibodies at the dermal-epidermal junction.

A, A scurfy mouse and littermate control. **B**, Histopathological findings of the skin of scurfy mice. **C-E**, DIF (IgG) of the tail skin of scurfy mice (Fig 1, C), IIF of scurfy sera on the tail skin of wild-type mouse (Fig 1, D) and on 1 M NaCl-split normal murine skin (Fig 1, E). Positive and negative reactions are indicated by arrows and arrowheads, respectively. **F**, Analyses of the time course of gross appearance, histopathological findings and deposition of IgM and IgG in the skin at days 7, 9 and 12. Positive and negative reactions are indicated by arrows and arrowheads, respectively. Inflammation score is plotted along the time course. Horizontal lines indicate the mean and SEM bars. Bar=100 μ m. * P <.05, ** P <.01.

From these findings, we focused on BP antigens, BP230 and COL17. Using recombinant proteins of these antigens, we performed western blotting analyses for confirming autoantigens targeted by scurfy autoantibodies.

Figure 2. Autoantibodies in scurfy sera react with BP230.

A, Schema of murine BP230 recombinant proteins. **B-D**, Immunoblotting of scurfy sera using BP230-1 (Fig 2, B), BP230-2 (Fig 2, C) and BP230-3 (Fig 2, D) as the antigen. Positive results are indicated by arrows.

Figure 3. Autoantibodies in scurfy sera react with full-length COL17 but not with the NC14A domain of COL17.

A, Schema of full-length murine COL17 and the NC14A domain of COL17 (black box). **B** and **C**, Immunoblotting of scurfy sera using full-length COL17 (Fig 3, B) or COL17 NC14A (Fig 3, C) as the antigen. Positive results are indicated by arrows.

To investigate whether scurfy T cells are responsible for the autoantibody production, we generated *TCR β ^{-/-}*-scurfy mouse. Furthermore, we examined whether CD4⁺ T cells from scurfy mice can induce these autoantibody in recipient mice with no T cells (*TCR β ^{-/-}*).

Figure 4. Scurfy CD4⁺ T cells induce autoantibodies to COL17 and BP230-1.

A, A *TCR β ^{-/-}*-scurfy mouse. **B**, Histopathological findings of the tail skin from *TCR β ^{-/-}*-scurfy mouse. **C**, DIF of *TCR β ^{-/-}*-scurfy mouse. Negative reaction is indicated by arrowheads. **D**, A *TCR β ^{-/-}* recipient 7 weeks after the adoptive transfer of CD4⁺ T cells from scurfy mice. **E**, Histopathological findings of the ear skin from *TCR β ^{-/-}* recipients. **F**, DIF of *TCR β ^{-/-}* recipients. Positive and negative reactions are indicated by arrows and arrowheads, respectively. **G** and **H**, Immunoblotting of sera from *TCR β ^{-/-}* recipients of scurfy CD4⁺ T cells, using full-length COL17 (Fig, D) and BP230-1 (Fig, F). Positive results are indicated by arrows. Bar=100 μ m.

Conclusions

Treg dysfunction leads to the spontaneous production of autoantibodies to bullous pemphigoid antigens, BP230 and COL17 in mice.

The autoantibody production is induced by scurfy CD4⁺ T cells.

References

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Conflict of interest: All authors declare that no conflicts of interest exist.