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Ultraviolet B irradiation leads to the development of experimental bullous pemphigoid targeting BPAG1e

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Antibodies in the serum of patients with bullous pemphigoid (BP) target two hemidesmosomal proteins, BP180 and BP230/bullous pemphigoid antigen (BPAG)1e [1]. In patients with BP who have only anti-BPAG1e antibodies, the autoantibodies primarily react with the C-terminal domain of BPAG1e (BPAG1e-C) [2,3]. Recently, we found that adoptive transfer of splenocytes from conditional knockout (*K5-Cre;Bpag1*$^{-/-}$) mice immunized with murine BPAG1e-C leads to BP-like phenotypes in the recipient immunodeficient mice [4].

Interleukin (IL)− 6 levels were high in both the serum and blister fluid of patients with BP in a meta-analysis [5]. Ultraviolet B (UVB) irradiation induces IL-6 production in the skin and has been considered as a trigger to develop BP [6,7]. We aimed to elucidate the importance of IL-6 induction in the development of an experimental BP model targeting BPAG1e.

We evaluated *Rag2*$^{-/-}$ immunodeficient mice that received splenocytes from *K5-Cre;Bpag1*$^{-/-}$ mice immunized with BPAG1e-C (Fig. 1a). Several weeks after splenocyte transplantation, scaling and erosions in the perioral area, ears, feet, and tail appeared in some recipient mice (Fig. 1b). Histological examination showed subepidermal blisters with hyperkeratosis and direct immunofluorescence (DIF) using anti-mouse IgG antibodies conjugated fluorescein isothiocyanate showed IgG deposition at the dermal-epidermal junction (DEJ) (Fig. 1c). Recipient mice expressing BP-like phenotypes had significantly higher serum levels of IL-6 than those without BP-like skin lesions (Fig. 1d). There were no differences in the concentration of IL-4, IL-17, or interferon (IFN)-γ (Fig. 1d).

Next, we analyzed the association between the secretion of IL-6 and disease severity at the endpoint (Fig. 1e and f). As shown in Fig. 1f, recipient mice with severe skin findings, with a clinical score 2 or 3, had significantly higher serum levels of IL-6 compared to those with mild skin changes (score 1). Furthermore, recipient mice that had severe lesions (score 2 or 3) developed the symptoms of BP-like phenotypes earlier than mice with less severe lesions (score 1) (Fig. 1f). Thus, IL-6 might play an essential role in both disease severity and early onset of BP-like skin lesions after the adoptive transfer.

Serum concentrations of IL-6 were sufficiently associated with autoantibody titers against the DEJ according to indirect immunofluorescence (Fig. 1g). Previous study revealed that murine splenic B cells cultured with human IL-6 increased IgG anti-DNA antibodies production [8], indicating that IL-6 is directly relevant to the preservation and upregulation of causative autoantibody production. Next, we collected serum from recipient *Rag2*$^{-/-}$ mice prior to the development of BP-like phenotypes to measure serum IL-6 concentrations. Whereas mice without BP-like findings continued to have low serum levels of IL-6, mice that subsequently developed BP-like phenotypes produced IL-6 at 6 days after the adoptive transfer and had high levels of IL-6 at 12 days (Fig. 1h). These results revealed that the upregulation of IL-6 secretion occurred before the appearance of BP-like skin lesions and gradually increased until the development of BP-like phenotypes. Normal human keratinocytes cultured with IgG purified from the serum of patients with BP secreted IL-6 into the medium [9]. In our studies, the source of IL-6 is obscure, but antibodies induced by BPAG1e-C-immunized splenocytes might contribute to IL-6 secretion from keratinocytes and trigger the development of BP-like phenotypes in recipient mice.

To evaluate pathogenic autoantibodies against BPAG1e, we generated hybridoma clones using lymphocytes and splenocytes derived from recipient *Rag2*$^{-/-}$ mice with BP-like phenotypes. Even though IgG in the supernatant of the hybridoma cells bound to the DEJ in murine skin samples, BALB/c mice that received intraperitoneal injections of hybridoma cells had neither BP-like skin changes nor IgG

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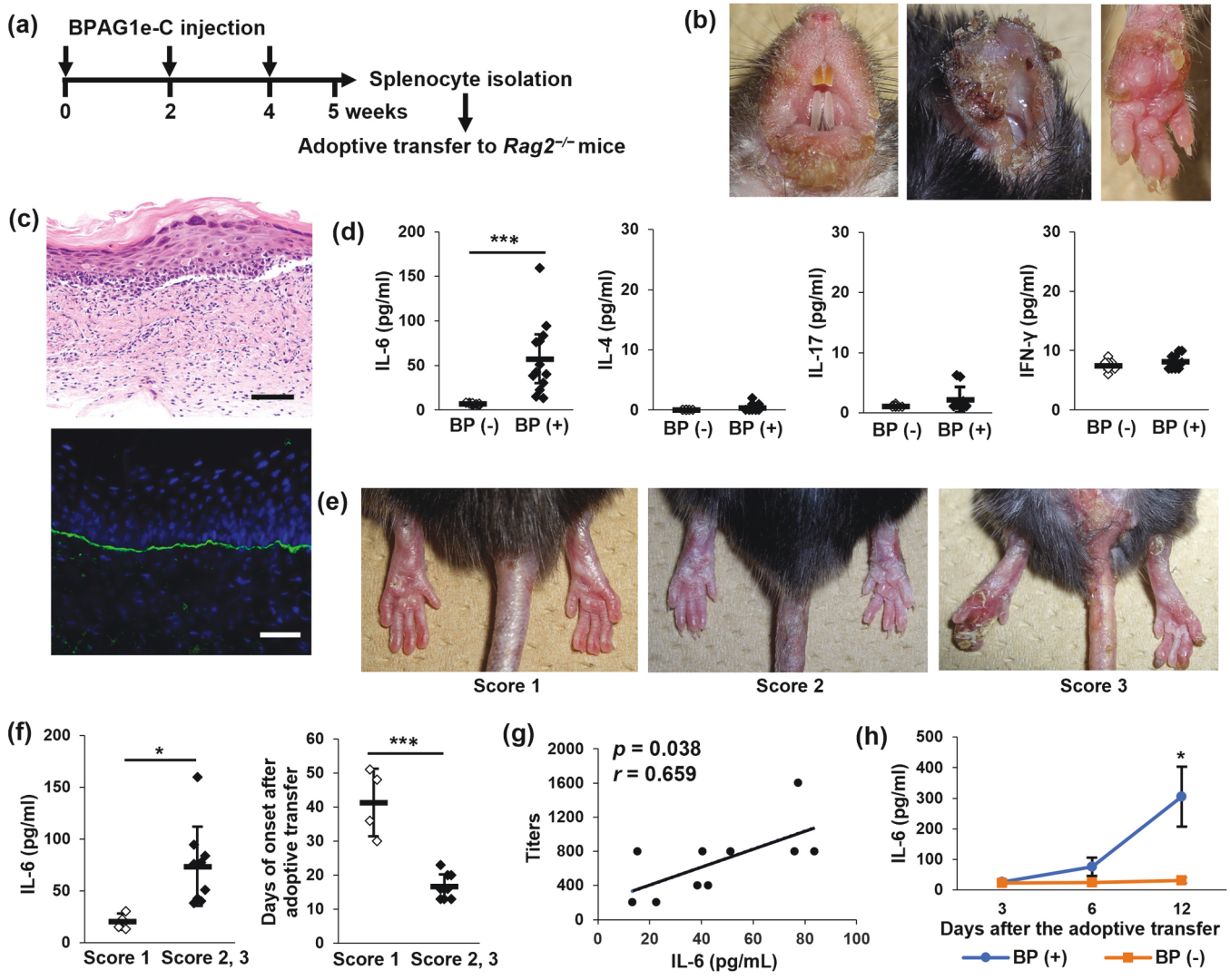

Fig. 1. IL-6 secretion is upregulated in mice with experimental BP targeting BPAG1e. (a) Schematic diagram of the experiments for splenocyte isolation. *K5-Cre;Bpag1*$^{-/-}$ mice were primed with a subcutaneous injection of BPAG1e-C protein. At 2 weeks and 4 weeks, mice were boosted with a subcutaneous injection of BPAG1e-C protein. To gain splenocytes, mice were sacrificed 5 weeks after the first immunization. (b) Representative images of the perioral area, ear, and foot at 21 days after splenocyte transplantation. (c) Representative histological images with HE staining and DIF on the sole at 21 days after splenocyte transplantation. Original magnification, 200 × . Scale bar = 50 μm. (d) When the score became 3 or when there were no additional skin lesions for 1 week, we measured serum concentrations of various cytokines with ELISA. Mice with experimental BP targeting BPAG1e had high serum levels of IL-6. Data were obtained from three or four independent experiments (n = 10 per group). (e) Evaluation of experimental BP mice: score 1, scaling and slight erosion; score 2, erosions and crusting; score 3, severe erosions, ulcers, and crusting, or death. (f) Mice with experimental BP that had high levels of IL-6 had severe clinical symptoms. Relationship between the period of phenotype development and clinical severity. (g) Relationship between IL-6 concentrations and titers of autoantibodies against BPAG1e-C protein. (h) Upregulation of IL-6 secretion prior to the development of BP-like phenotypes. Data were obtained from three or four independent experiments (n = 10 per group). Data in Figs. 1d, 1 f, and 1 h are shown as means ± SD. * $P < 0.05$; * * $P < 0.001$, Student's *t*-test. BP, bullous pemphigoid; BPAG, bullous pemphigoid antigen; DIF, direct immunofluorescence; ELISA, enzyme-linked immunosorbent assay; HE, hematoxylin and eosin; IL, interleukin.

deposition at the DEJ on DIF microscopy (data not shown). Since previous studies demonstrated the importance of IL-6 overexpression prior to the development of BP-like phenotypes, we focused on the induction of IL-6 in BALB/c mice that received hybridoma cells.

First, we exposed the shaved dorsum of BALB/c mice to 300 mJ/cm^2 of UVB and confirmed that IL-6 levels in the serum immediately elevated after UVB exposure and peaked at 24 h (Fig. 2a). Next, at 3 days after injection of either hybridoma cells or SP2/0-Ag14 control cells, we exposed recipient BALB/c mice to UVB and observed them at 3, 6, and 9 days after exposure (Fig. 2b). At 3 days after UVB exposure, there were no visible differences between the two groups (Fig. 2c). On day 6, mice that received hybridoma cells had extensive erythema with a thickened crust and scaling. The control mice that received SP2/0-Ag14 cells had mild erythema and scaling. At 9 days after UVB irradiation, the control mice had almost a normal appearance. However, the mice that received hybridoma cells had delayed erythematous lesions and erosions. On days 6 and 9, the mice that received hybridoma cells had significantly higher clinical scores than the control mice (Fig. 2d). On the other hand, non-UVB-irradiated mice that received hybridoma cells showed neither erythema nor scaling (Fig. 2c).

In the mice that received hybridoma cells, histological examination revealed subepidermal blisters with acanthosis and DIF showed IgG deposition at the DEJ on the dorsal skin (Fig. 2e). The skin on the abdominal region, which was not exposed to UVB, did not have subepidermal blister formation or IgG deposition at the DEJ (Fig. 2e). Furthermore, the mice that received SP2/0-Ag14 myeloma cells did not have histological findings consistent with BP-like phenotypes (Fig. 2e). UVB irradiation induced IL-6 expression in

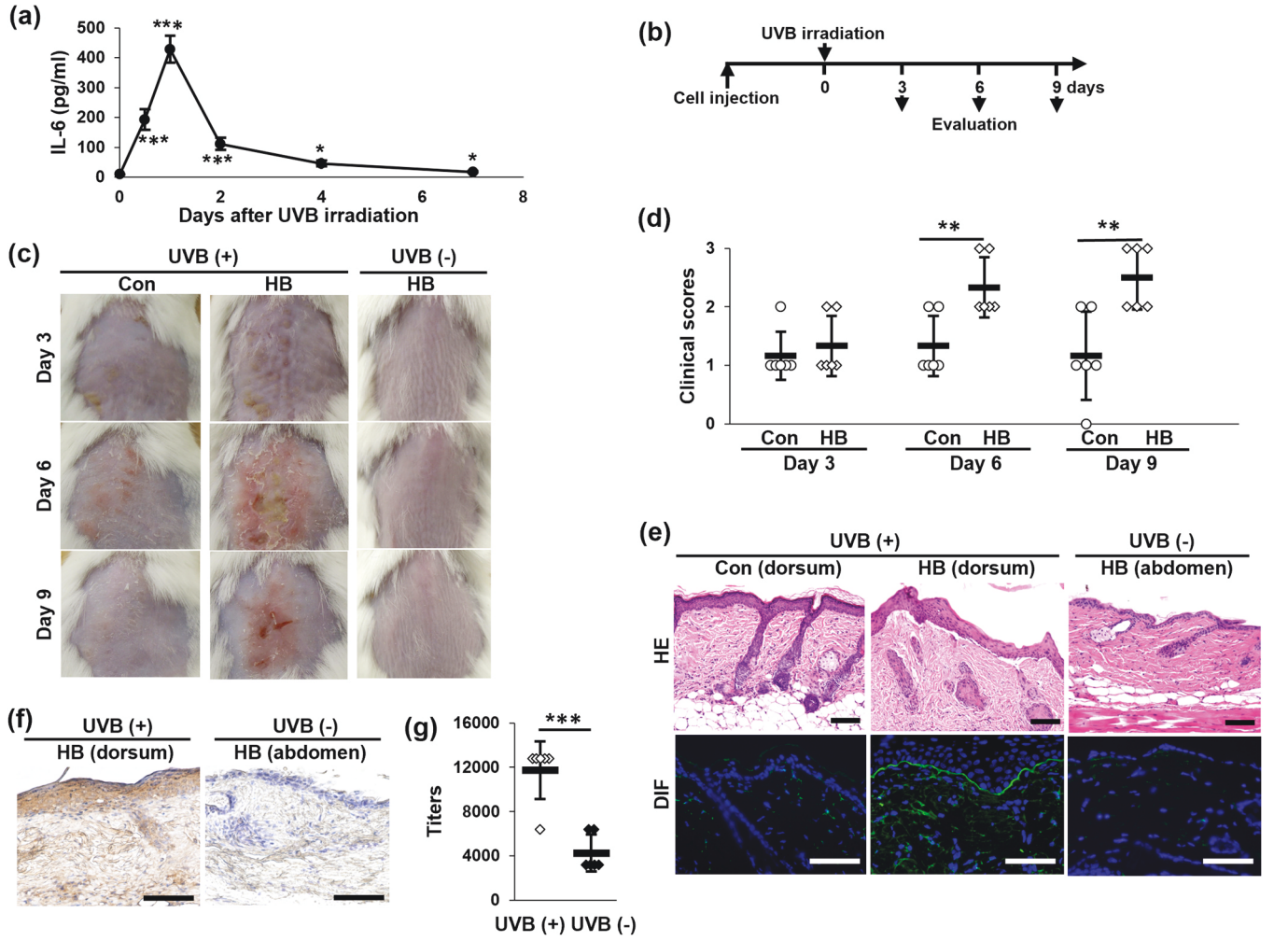

Fig. 2. UVB exposure triggers autoimmune reactions via autoantibodies against murine BPAG1e. (a) Upregulation of IL-6 secretion with UVB exposure in BALB/c mice. (b) Schematic representation of the experimental paradigm. BALB/c mice were intraperitoneally injected with either hybridoma cells (HB) or SP2/0-Ag14 control cells (Con). At 3 days after injection, mice were exposed to UVB (300 mJ/cm^2) on the shaved dorsum. Clinical findings were evaluated every 3 days. (c) Representative images of clinical findings after UVB exposure. (d) On days 6 and 9, clinical scores of mice that received hybridoma cells were significantly higher than those of control mice. (e) Representative histological images with HE staining and DIF on the dorsum and abdomen at 9 days after UVB exposure. (f) Representative immunohistochemical images with anti-IL-6 antibody on the dorsum and abdomen at 9 days after UVB exposure. (g) On day 9, UVB irradiation induced higher titers of autoantibodies against BPAG1e-C protein in the hybridoma cell-transferred mice compared to those in the non-UVB-exposed mice. Data were obtained from two independent experiments (n = 6 per group). Data in Figs. 2a, 2d, and 2 g are shown as means ± SD. Scale bar = 50 μm. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, Student's *t*-test. BPAG, bullous pemphigoid antigen; Con, control cells; DIF, direct immunofluorescence; HB, hybridoma cells; HE, hematoxylin and eosin; IL, interleukin; UVB, ultraviolet B.

keratinocytes and lymphocytes of hybridoma cell-received mice (Fig. 2 f), suggesting that UVB might be a trigger for BP by targeting BPAG1e via IL-6 upregulation. On the other hand, UVB irradiation increased antibody titers in mice that received hybridoma cells (Fig. 2g). The non-exposed abdominal skin showed no IgG deposition at the DEJ (Fig. 2e), indicating that UVB-induced antigen exposure might be necessary for anti-BPAG1e antibodies to bind to the DEJ.

This is the first report demonstrating UVB-induced blister formation in the presence of anti-BPAG1e antibodies. These results support the possibility that UVB exposure helps trigger the induction of IL-6 and the binding between autoantibodies and BPAG1e, leading to the development of subepidermal blistering, as in BP.

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CRediT authorship contribution statement

Kazuhito Kogawa: Conceptualization, Methodology, Investigation, Writing – original draft. **Yasushi Matsuzaki:** Methodology, Investigation, Formal analysis, Writing – reviews & editing. **Atsuko Kimura:** Software. **Satoko Minakawa:** Data Curation. **Hajime Nakano:** Validation. **Koichi Ito:** Resources. **Hiroshi Kijima:** Investigation. **Daisuke Sawamura:** Supervision, Writing – reviews & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdermsci.2022.12.001.

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References

[1] P. Bernard, L. Didierjean, F. Denis, J.H. Saurat, J.M. Bonnetblanc, Heterogeneous bullous pemphigoid antibodies: Detection and characterization by immunoblotting when absent by indirect immunofluorescence, J. Invest. Dermatol. 92 (1989) 171–174.

[2] T. Hamada, Y. Nagata, M. Tomita, W. Salmhofer, T. Hashimoto, Bullous pemphigoid sera react specifically with various domains of BP230, most frequently with C-terminal domain, by immunoblot analyses using bacterial recombinant proteins covering the entire molecule, Exp. Dermatol. 10 (2001) 256–263.

[3] T. Hayakawa, K. Teye, T. Hachiya, R. Uehara, M. Hashiguchi, T. Kawakami, et al., Clinical and immunological profiles of anti-BP230-type bullous pemphigoid: Restriction of epitopes to the C-terminal domain of BP230, shown by novel ELISAs of BP230-domain specific recombinant proteins, Eur. J. Dermatol. 26 (2016) 155–163.

[4] E. Makita, Y. Matsuzaki, T. Fukui, A. Matsui, S. Minakawa, H. Nakano, et al., Autoantibodies to BPAG1e trigger experimental bullous pemphigoid in mice, J. Invest. Dermatol. 141 (2021) 1167–1176. e3.

[5] E.H. Kowalski, D. Kneibner, K. Kridin, K.T. Amber, Serum and blister fluid levels of cytokines and chemokines in pemphigus and bullous pemphigoid, Autoimmun. Rev. 18 (2019) 526–534.

[6] H. Narumi, H. Nakano, Y. Matsuzaki, D. Sawamura, K. Hanada, Immunohistochemical analysis of in vivo UVB-induced secretion of IL-1α and IL-6 in keratinocytes, Mol. Med. Rep. 4 (2011) 611–614.

[7] J. Ceryn, M. Skibińska, P. Barasińska, M. Noweta, J. Narbutt, A. Lesiak, UVB-induced bullous pemphigoid in a patient with psoriasis, Post. Dermatol. Alergol. 39 (2022) 228–230.

[8] M. Mihara, Y. Ohsugi, Possible role of IL-6 in pathogenesis of immune complex-mediated glomerulonephritis in NZB/W FL mice: Induction of IGG class anti-DNA autoantibody production, Int. Arch. Allergy Appl. Immunol. 93 (1990) 89–92.

[9] E. Schmidt, S. Reimer, N. Kruse, S. Jainta, E.B. Bröcker, M.P. Marinkovich, G.J. Giudice, D. Zillikens, Autoantibodies to BP180 associated with bullous pemphigoid release interleukin-6 and interleukin-8 from cultured human keratinocytes, J. Invest. Dermatol. 115 (2000) 842–848.

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