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Title	Intravenous IgG Reduces Pathogenic Autoantibodies, Serum IL-6 Levels, and Disease Severity in Experimental Bullous Pemphigoid Models
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Intravenous immunoglobulin reduces pathogenic autoantibodies, serum IL-6 levels and disease severity in experimental bullous pemphigoid models

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SUPPLEMENTARY MATERIALS AND METHODS

Mice

The COL17-humanized mice were generated as previously described (Nishie et al. 2007). Briefly, human COL17-transgenic mice (C57BL/6 background) expressing the squamous epithelium-specific K14 promoter and human COL17A1 cDNA (COL17^{m+/+,h+}) (Olasz et al. 2007) were crossed with heterozygous murine Col17^{+/-} mice (the F1 mouse had a 129/SvEv X C57BL/6 background, back-crossed with C57BL/6 over 10 generations) to produce COL17-humanized mice (COL17^{m-/-,h+}). Rag-2^{-/-} COL17-humanized mice were generated by crossing COL17-humanized mice with C57BL/6 background Rag-2^{-/-} mice (Central Institute for Experimental Animals, Kawasaki, Japan) as reported previously (Ujiie et al. 2010).

Preparation of IgG

Immunization of mice by human COL17-transgenic skin graft was performed according to the method reported previously (Olasz et al. 2007). Briefly, full thickness 1-cm² pieces of dorsal skin were removed from sacrificed human COL17-transgenic mice and

grafted onto the backs of gender-matched 6-week-old C57BL/6 wild-type mice. After the topical application of an antibiotic ointment, the graft site was covered with gauze and an elastic bandage for 14 days. The production of IgG to human COL17 was confirmed by ELISA and indirect immunofluorescence at 5 weeks after skin grafting, as described below. IgG from the skin-grafted mice (SG-IgG) was purified from the sera by affinity chromatography using the HiTrap Protein G HP (GE Healthcare Biosciences, Uppsala, Sweden). For IgG passive-transfer study, SG-IgG was prepared at 5 µg/µL. TS39-3 and BP-IgG were isolated using the same methods from the supernatant of cultured hybridoma and a patient's serum, respectively.

IF studies

DIF was performed on the skin samples using standard protocols. Briefly, mouse skins were mounted and snap-frozen into an optimal cutting temperature compound, and 5-µm cryosections were prepared. The sections were blocked with 10% BSA for 1 hour at 37 °C and incubated with antibodies such as FITC-conjugated antibodies to mouse IgG (Jackson ImmunoResearch Laboratories), Alexa Fluor 488-conjugated AffiniPure antibodies to human IgG (Jackson ImmunoResearch Laboratories) or C3 (MP Biomedicals, Solon, OH) at a 1:100 dilution. The fluorescence intensity at the DEJ was determined using ImageJ software (<http://rsbweb.nih.gov/ij/>) and dermal fluorescence for background comparison.

Immunohistochemistry

Specimens from the mice back skin were fixed in formalin and embedded in paraffin after dehydration. Antigen retrieval with pH 6.0 citrate buffer was performed on deparaffinized sections. Sections were incubated with polyclonal rabbit anti-CD3 antibody (Dako, Denmark) for 1 hour at room temperature. After washing in PBS, the sections were incubated with secondary antibodies conjugated with horseradish peroxidase for 30 minutes at room temperature. The sections were stained with 3,3'-Diaminobenzidine. To determine the CD3⁺ cell numbers, 10 back skin areas were counted.

ELISA

The titers of IgGs to the NC16A domain of human COL17 were determined by using a BP180 ELISA kit (MBL) according to the manufacturer's instructions. HRP-conjugated anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories) diluted to 1:40,000 was used as the secondary antibody. Bound antibodies were read at the optical density of 450 nm using an ELISA plate reader (Mithras, Berthold Technologies). The ELISA index value was defined by the following formula: $\text{index} = (\text{OD}_{450} \text{ of tested plasma} - \text{OD}_{450} \text{ of negative control}) / (\text{OD}_{450} \text{ of positive control} - \text{OD}_{450} \text{ of negative control}) \times 100$. The concentration of total human IgG and mouse IgG was examined by IgG

human ELISA kit (Abcam, Cambridge, UK) and IgG mouse ELISA kit (Abcam), respectively, according to the manufacturer's instructions.

Cytokine and chemokine assay

Murine cytokine and chemokine levels were determined by using the 23-plex panel Bio-Plex Assay (Bio Rad), a multiplex analysis system that permits the simultaneous analysis of many kinds of murine cytokines and chemokines in a single microplate well, according to the manufacturer's instruction. The specific antibody-binding beads were incubated with each diluted sample (30~50 fold) for 1 hour, targeting cytokines and chemokines were captured, and then a biotinylated antibody for a different epitope was added to the reaction, which was then detected with streptavidin-PE. The beads were drawn single file through a flow cell where two lasers excited them. Using a dual-laser-based reader, the beads were analyzed for the detection antibody and the internal bead signature, identifying both the protein analyzed and the level of fluorescence bound to the bead.

Stimulation of HaCaT cells

HaCaT cells were grown to 80% confluence in KGM without hydrocortisone in 24-well plates. Human IgG (IVIG) at 15 mg/ml was added 2 hours prior to the stimulation with BP-IgG at 5mg/ml, and then cells were incubated for 12 hours. Culture supernatant

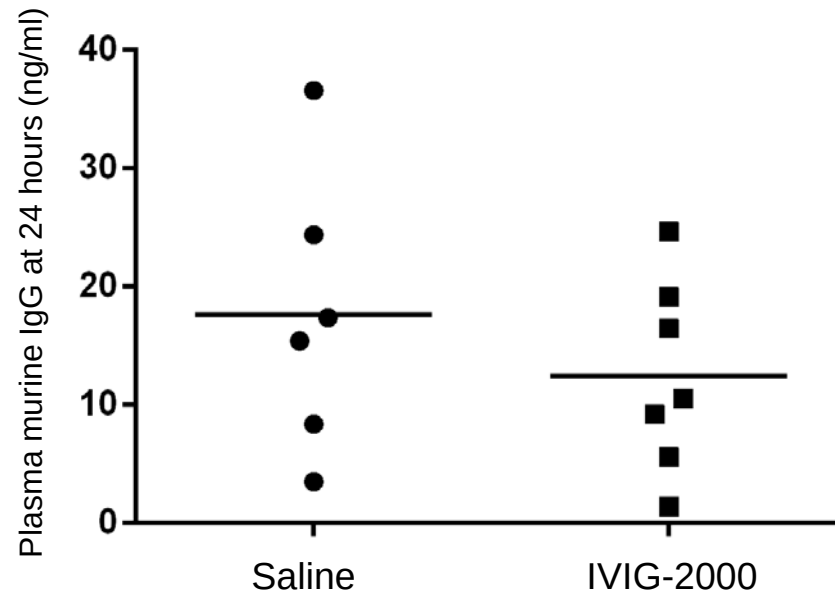
were subjected to IL-6 ELISA (eBioscience).

SUPPLEMENTARY REFERENCES

Nishie W, Sawamura D, Goto M, Ito K, Shibaki A, McMillan JR, et al. Humanization of autoantigen. *Nat Med* 2007;13:378–83.

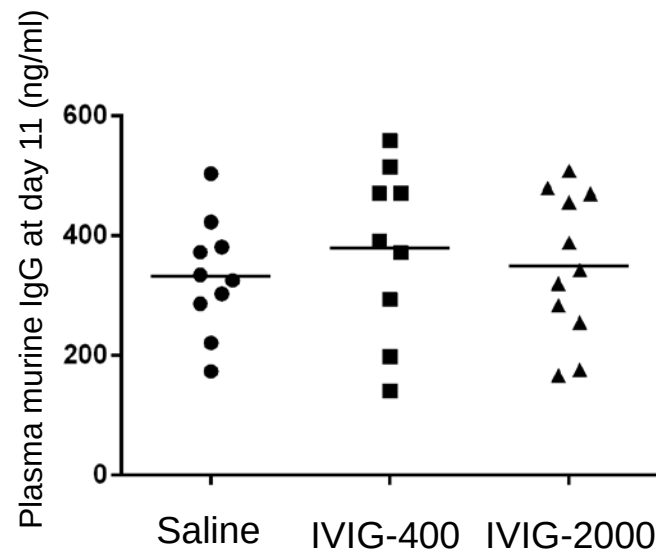
Olasz EB, Roh J, Yee CL, Arita K, Akiyama M, Shimizu H, et al. Human bullous pemphigoid antigen 2 transgenic skin elicits specific IgG in wild-type mice. *J Invest Dermatol* 2007;127:2807–17.

Ujiie H, Shibaki A, Nishie W, Sawamura D, Wang G, Tateishi Y, et al. A Novel Active Mouse Model for Bullous Pemphigoid Targeting Humanized Pathogenic Antigen. *J Immunol* 2010;184:2166–74.



Supplementary figure 1

The concentration of circulating mouse IgG at 24 hours after the passive transfer of TS39-3.



Supplementary figure 2

The concentration of circulating mouse IgG at day 11 after the adoptive transfer in the active BP model.