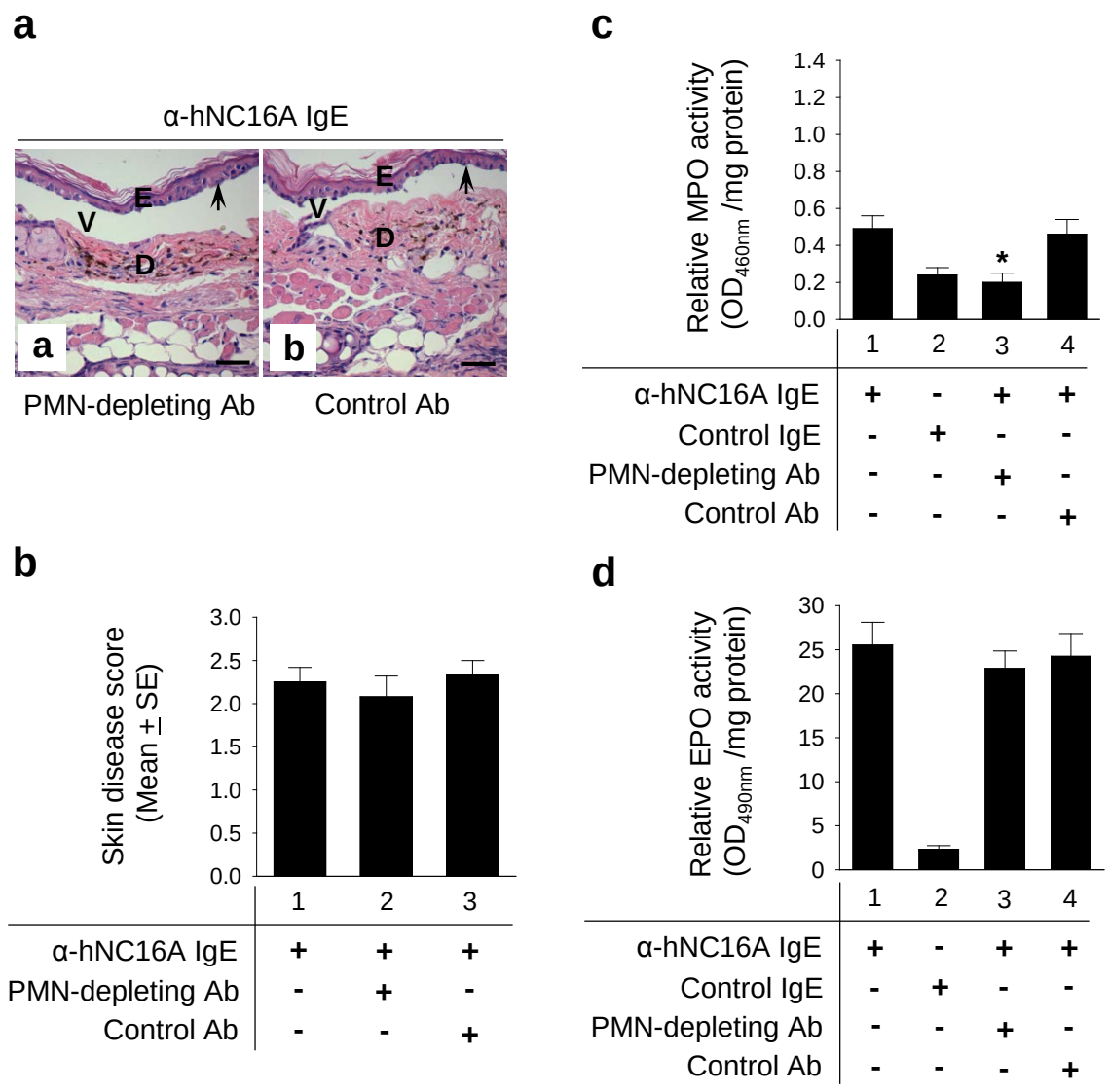
Supplementary Figure S1



Supplementary Figure S1. Anti-hNC16A IgE-induced BP in hFcεR/hNC16A mice is independent of neutrophils. Eight week old hFcεRI/hNC16A mice were pretreated with i.p. injection of PMN-depleting antibody or isotype control antibody and then injected at the ear pinna with anti-hNC16A IgE or control IgE (100 ng/g body weight). Mice were examined 48 h later after IgE injection. (**a**) H/E. Mice pretreated with PMN depleting antibody or isotype control antibody showed similar dermal-epidermal separation. (**b**) Skin disease activity. Mice treated with PMN depleting antibody (bar 2) developed the same BP disease as isotype control antibody treated mice (bar 3) and mice without any pretreatment (bar 1). (**c**) PMN infiltration. MPO activity assay showed significant difference in PMN infiltration between PMN depleting- and isotype control antibody-treated mice (bar 4 vs. 3). (**d**) Eos infiltration. EPO activity assay showed compatible Eos infiltration between PMN depleting and isotype control antibody treated mice (bar 3 vs. 4). Scale bars = 100 μm for panel a. *p<0.01, n=6 for each group.

SUPPLEMENTAL MATERIALS

MATERIALS AND METHODS

Mice

The humanized hNC16A mice were generated as described (Liu et al., 2008). The humanized hFcεRI mice were generated by the replacement of the Fcer1a mouse locus with the syntenic human FCER1A locus (see Figure 3a). Briefly, Homologous recombination is used to generate an approximately 9.5 kb deletion of mouse DNA from chromosome 1 extending from 173,219,342 to 173,228,893 (GRCM38-mm10). This includes the promoter and all exons and introns of the mouse gene. The deleted region is repopulated with the corresponding 44 kb human locus extending from human chromosome 1 159,268,197 to 159,311,999 (GRCh38-hg38). The larger size of the human locus reflect the more complex 5' structure of this gene. The human FCERI is driven under its own promoter elements. The genetic manipulations leave a nonfunctional mutant loxP site at the junction between the Olfr gene and the human segment of DNA and a FRT site (yellow triangle) at the second junction between human and mouse DNA. Probe A indicates the location of the DNA probe to verify the humanization of the Fcer1a locus. hFcεRI/hNC16A mice were generated by crossing hNC16A with hFcεRI mice. Expression of hFcεRI on affinity purified eosinophils was confirmed by using a two-step stain with human IgE incubation followed by FITC-labeled anti-human IgE antibody (Thermofisher Scientific, cat#H15701). Eosinophil-deficient ΔdblGATA mice (Yu *et al.*, 2002) were obtained from the Jackson Laboratories (Bar Harbor, ME) and crossed with hFcεRI/hNC16A mice to generate eosinophil-deficient hFcεRI/hNC16A mice.

Clinical disease scoring

The extent of cutaneous disease (clinical disease activity) in anti-BP180 antibody-injected neonatal mice was scored as follows: "(-)", no detectable skin disease; "1+", mild erythematous reaction with no evidence of the "epidermal detachment" sign; "2+", intense erythema and "epidermal detachment" sign involving 10-50% of the epidermis in localized areas; and "3+", intense erythema with frank "epidermal detachment" sign involving more than 50% of the epidermis. The disease activity in adult mice was scored based on dermal-epidermal separation by H/E: "(-)", no detectable dermal-epidermal separation; "1+", mild inflammatory reaction and <25% epidermal detachment; "2+", intense inflammatory reaction and epidermal detachment involving 25% to 50% of the epidermis; and "3+", intense inflammatory reaction with frank "epidermal detachment" sign involving more than 50% of the epidermis.

Immunoblotting

Recombinant hNC16A was subjected to SDS-PAGE. Following transfer to nitrocellulose, the membrane was probed with anti-hNC16A IgG, anti-hNC16A IgE, or control IgE and bound antibody was detected with anti-human IgG (Southern Biotechnology, cat#2040-05) or anti-human IgE (Sigma-Aldrich, cat#A9667) HRP conjugated secondary antibodies.

Indirect and direct immunofluorescence

For indirect immunofluorescence, anti-hNC16A IgG and IgE (1μg/ml) were incubated with skin sections of hNC16A mice. Binding of anti-hNC16A IgG and IgE to the BMZ was detected by FITC-conjugated anti-human IgG and IgE antibodies (ThermoFisher Scientific, cat# 62-8411, cat# H15801). For direct immunofluorescence, BMZ bound human IgG or IgE was detected using commercially available FITC-conjugated anti-human IgG and anti-human IgE Fc antibodies (Thermo Fisher Scientific cat# H15701). Eosinophils were detected using rat anti-mouse MPB

monoclonal antibody (provided by Dr. J. Lee, Mayo Clinic Arizona), followed by Alexa Fluor 488-conjugated goat anti-rat antibody (Life Technologies, cat#a11006).

Eosinophil activation in vitro

Eosinophil superoxide production (oxidative burst) *in vitro* was measured by reduction of cytochrome *c* as described with some minor modification (Kobayashi *et al.*, 2003). Briefly, freshly isolated eosinophils were resuspended in HBSS with 0.03% gelatin, 10mM HEPES and 200mM cytochrome *c* (Sigma-Aldrich, St. Louis, MO) at $5x10^5$ cells/ml; 100µl of cell suspension was dispensed into each well of 96-well flat-bottom microplate. The reactions were initiated at 37˚C with 100µl of PMA (Calbiochem, La Jolla, CA) at 0-20ng/ml. Absorbance at 550nm was read every 10min for 2h in a microplate reader (Molecular Devices, Sunnyvale, CA). EPO release by eosinophils (eosinophil degranulation) in response to challenge with PAF was determined using a peroxidase assay as described (Adamko et al., 2004; Dyer et al., 2010). Briefly, freshly isolated eosinophils were resuspended in RPMI 1640, without phenol red, at $5x10^5$ cells/ml; 100µl of cell suspension was dispensed into each well of 96-well flat-bottom microplate. Cells were incubated at 37˚C, 5% CO2 for 30min in the absence or presence of PAF (0-10µM), 100µl *o*-phenylenediamine (OPD) reagent (800µl 5mM OPD in 4ml 1M Tris, pH8, 5.2ml H_2O), and 1.25µl 30% H_2O_2). The reaction was terminated by the addition of 100µl 4M H_2SO_4 to each well and read at 492nm. The baseline EPO content was cells without treatment, while the total EPO content was cells lysed in 0.2% SDS. Eosinophil degranulation are expressed as the percentage of total EPO [(absorbance of stimulated sample – untreated control)/total EPO content X 100].

SUPPLEMENTAL REFERENCES

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