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Letter to the Editor

Human basophils promote IgE-dependent oral allergen-induced anaphylaxis in humanized mice

Dear Editor,

Food-induced anaphylaxis (FIA) is a life-threatening immediate and systemic hypersensitivity reaction caused mainly by an IgE-mediated response to a food antigen. The systemic anaphylactic reaction causes hypothermia and affects the functions of multiple organs, including the gastrointestinal, cutaneous, respiratory, and cardiovascular systems. Mast cells (MCs) and basophils express the high-affinity receptor for IgE (FcεRI) that transmits activating signals when an IgE-antigen complex cross-links FcεRI. After the activation, MCs release pre-formed and newly synthesized mediators such as histamine and platelet-activating factor (PAF), which correlate with the severity of FIA. The role of MCs in FIA has been demonstrated using several MCs-deficient mouse models, and the absence of MCs ameliorated systemic anaphylaxis. However, these studies required the parenteral or repeated oral allergen challenge to elicit systemic anaphylaxis.[1] On the other hand, even though the basophils activation test, an in vitro assay stimulating basophils with a food allergen, helps diagnose food allergy, the role of human basophils in the effector phase of FIA is less characterized than that of MCs.[2]

To address whether human basophils are involved in the pathogenesis of FIA induced by a single oral challenge with an allergen, we established an FIA model using NOD.Cg-*prkdc*^{scid}*il2r*γ^{tm1Sug}/ShiJic (NOG)-transgenic mice ubiquitously expressing human IL-3 and GM-CSF under the control of SRα promoter (NOG-EXL) (In-Vivo Science, Tokyo, Japan). These mice were transplanted with human umbilical cord blood-derived CD34$^+$ hematopoietic stem cells (huNOG-EXL mice). In the huNOG-EXL mice, human CD34$^+$ hematopoietic stem cells give rise to differentiation into human myeloid cells, including MCs and basophils.[3] A previous report demonstrated that although passive IgE-mediated cutaneous and systemic anaphylaxis can be established by the intravenous challenge of a monovalent allergen in huNOG-EXL mice, these mice showed incomplete development of oral allergen-induced anaphylaxis.[4] We hypothesized that challenging a monovalent antigen is weak to stimulate FcεRI signaling in MCs or basophils required to elicit oral allergen-induced anaphylaxis. Therefore, we orally challenged huNOG-EXL mice with 10 mg of TNP-conjugated OVA (TNP_9-OVA) or OVA after sensitization by i.p. injection of 50 μg of anti-TNP mouse IgE (BD Bioscience, San Diego, CA). The proportion of human CD45$^+$ cells was comparable between OVA and TNP_9-OVA challenging groups (Fig. 1A). Human basophils and MCs were reconstituted in the huNOG-EXL mice as reported previously[3] (Fig. 1A). Oral challenge with TNP_9-OVA but not OVA exhibited immediate and dramatic body temperature loss in the huNOG-EXL mice, as measured by using a digital thermometer (BIO-TK8851, BiosebLab, Vitrolles, France) (Fig. 1B). These results indicated that sensitization with mouse IgE mAb followed by challenge with its specific multivalent antigen is sufficient for developing oral allergen-induced FIA in the huNOG-EXL mice.

Then we next transferred human basophils, instead of CD34$^+$ hematopoietic stem cells, derived from healthy volunteers ($n = 4$) into the NOG-EXL mice. After removing T and B cells from PBMCs by negative selection using anti-human CD4, anti-human CD8, and anti-human CD19 mAbs-conjugated MACS beads (Miltenyi Biotec, Bergisch Gladbach, Germany) in vitro, cells were incubated with 2 μg/ml of an anti-TNP mouse IgE mAb and cultured at 37 °C for 16 h. Flow cytometry analysis demonstrated that more than 80 % of mouse IgE mAb-bound cells were CD203+ basophils, while the others were CD11c + DCs (Fig. 2A). We removed free anti-TNP mouse IgE mAb from the cultured cells by washing with PBS to avoid sensitization of endogenous mouse MCs and basophils with the IgE in mice and transferred these cells (around 3×10^7 cells) from each donor into two NOG-EXL transgenic mice. Ninety minutes after the transfer, mice were orally challenged with 10 mg of TNP_9-OVA or OVA. Oral challenge with TNP_9-OVA induced a significant and immediate body temperature loss in the NOG-EXL mice compared to mice orally challenged with OVA (Fig. 2B). These results indicated that IgE-bound human basophils are sufficient for developing oral allergen-induced anaphylaxis in NOG-EXL mice.

Although both MCs and basophils secret similar mediators such as histamine and PAF upon FcεRI crosslinking, they exhibit distinct mechanisms in mouse models of food allergy. The epicutaneous food allergen-sensitization promotes TSLP-induced IL-4 secretion by basophil that activates DC to express OX40L, leading to differentiation of type-2 helper T cells.[5] Basophil-derived IL-4 also induced MCs migration or proliferation in the effector phase of food allergy, which was crucial for diarrhea.[6] Previously, we have demonstrated that both MCs and basophils express an inhibitory immunoreceptor Allergin-1 in humans and mice.[7] We showed that basophils deficient in Allergin-1 promoted the development of FIA in a mouse model, while Allergin-1-deficient MCs exacerbated diarrhea.[7] We also showed that CV-6209, a PAF receptor antagonist, completely prevented basophils-dependent acute severe hypothermia in mice.[7] Basophils produce PAF in the anaphylaxis model,[8] and serum PAF levels correlate with the severity of anaphylaxis,[9] suggesting that PAF may be a responsible mediator in human basophil-induced FIA in the humanized mice. Taken together, these results suggest the distinct role of MCs and basophils in the pathogenesis of FIA.

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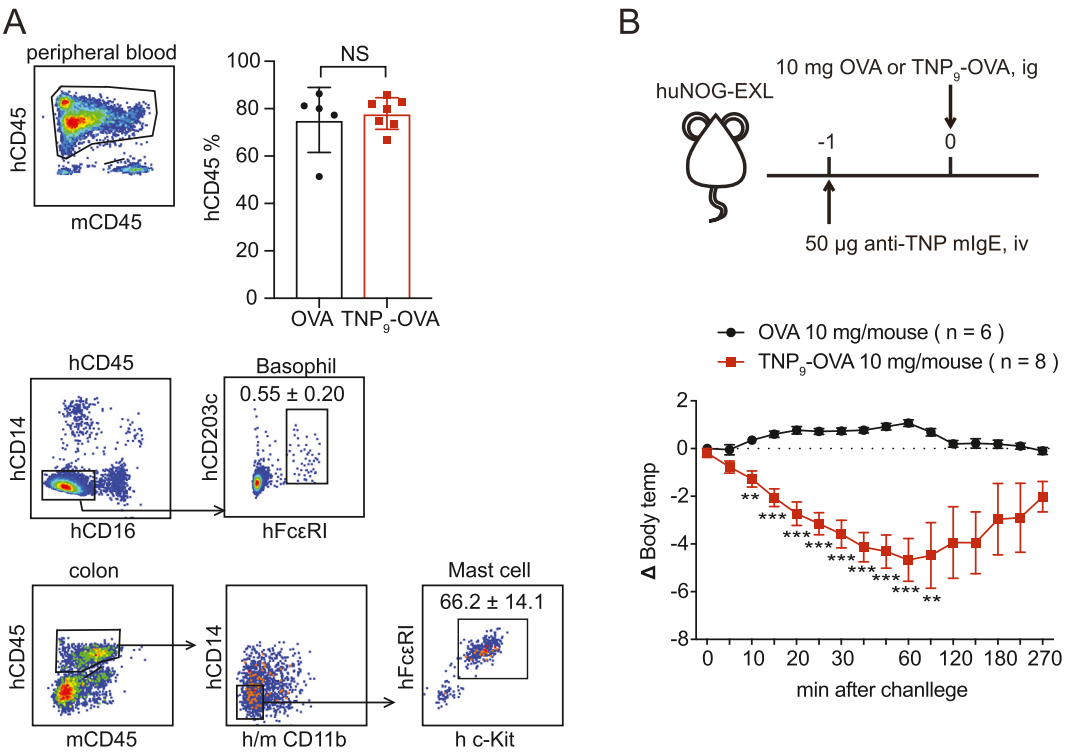

Fig. 1. Development of oral allergen-induced anaphylaxis in the huNOG-EXL mice. Human IL-3/GM-CSF transgenic NOG mice (NOG-EXL) reconstituted with human CD34+ HSCs (huNOG-EXL) were used to establish an oral allergen-induced FIA model. **(A)** Top left, a representative flow cytometric analysis of chimerism of reconstituted human CD45+ cells in the huNOG-EXL mice. Top right, mean values of chimerism of reconstituted human CD45+ cells in OVA and TNP-OVA-challenged groups. Middle, gating strategy for human basophils in the blood. A value indicates the proportion of human basophils under human CD45+ cells (n = 10). Bottom, gating strategy for human mast cells in the colon. A value indicates the proportion of human mast cells under human CD45+ cells (n = 3). Data were pooled from two independent experiments and are presented as mean ± SEM. NS, not significant between OVA and TNP_9-OVA-challenged groups by Student's *t*-test. **(B)** Top, the experimental procedure of oral allergen-induced FIA model in the huNOG-EXL mice. iv, intravenous administration; ig, intragastric administration; mIgE, mouse IgE. Bottom, body temperature was measured immediately after the oral challenge. Data were pooled from two independent experiments and are presented as mean ± SEM. ** $P < 0.01$; *** $P < 0.005$ between OVA and TNP_9-OVA-challenged groups by Student's *t*-test.

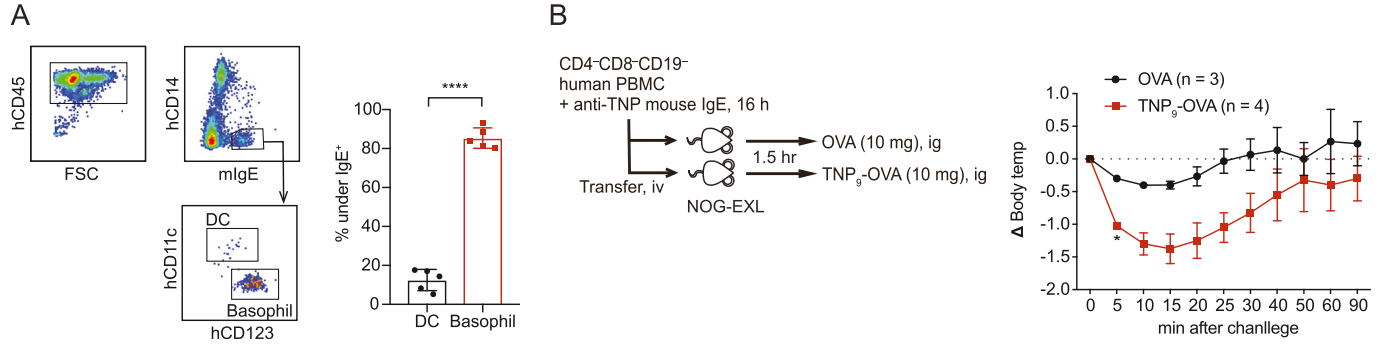

Fig. 2. Human basophils promoted oral allergen-induced anaphylaxis in the NOG-EXL mice. **(A)** Left, flow cytometric analysis of IgE-bound cells in CD4+, CD8+, and CD19+ cells-removed PBMCs after incubation with anti-TNP IgE antibody for 16 h. Right, mean proportions of IgE-bound basophils and DCs. Data are pooled from 5 independent experiments and are presented as mean ± SEM. **** $P < 0.001$ between DC and basophil by Student's *t*-test. **(B)** Left, experimental procedure. Anti-TNP IgE-bound CD4+CD8+CD19+ cells-removed PBMCs from each donor were i.v. transferred to two NOG-EXL mice, and then mice were challenged with TNP_9-conjugated OVA or OVA. Right, body temperature was measured immediately after the oral challenge. Data are pooled from 4 independent experiments and are presented as mean ± SEM. *$P < 0.001$ between OVA and TNP_9-OVA-challenged groups by Student's *t*-test.

Previously, NOD-SCID IL2Rgamma null (NSG) mice transferred with PBMC from a peanut allergy patient and sensitized with an intraperitoneal injection of peanut extract exhibited oral allergen-induced anaphylaxis.[10] However, reconstitution of NSG mice with human PBMC containing T cells leads to xenograft reactions, resulting in organ infiltration with human immune cells. Therefore, we reconstituted the NOG-EXL mice with human T cells and B cells-removed PBMCs. One of the still remaining limitations of this study was that we transferred IgE-sensitized T and B cell-removed human PBMC but not purified basophils into the mice due to the limited number of basophils in the human PBMCs. Unlike rodents, several human blood cells, including monocytes/macrophages and DCs, in addition to MCs and basophils, constitutively express FcεRI that can bind human and murine IgE. Indeed, we detected IgE-bound DCs in the transferred cells after culture with IgE (Fig. 2A). However, since IgE-bound human DCs may function as anti-inflammation rather than pro-inflammation,[11] our results showed that transferred IgE-bound human basophils, but not DCs, elicited the FIA

in the NOG-EXL mice. This finding contributes to a better understanding of the pathogenesis of FIA.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2023.11.007.

Conflict of interest

The authors have no conflict of interest to declare.

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