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Establishment of a Human Allergy Model Using Human IL-3/GM-CSF–Transgenic NOG Mice

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The development of animal models that mimic human allergic responses is crucial to study the pathophysiology of disease and to generate new therapeutic methodologies. Humanized mice reconstituted with human immune systems are essential to study human immune reactions in vivo and are expected to be useful for studying human allergies. However, application of this technology to the study of human allergies has been limited, largely because of the poor development of human myeloid cells, especially granulocytes and mast cells, which are responsible for mediating allergic diseases, in conventional humanized mice. In this study, we developed a novel transgenic (Tg) strain, NOD/Shi-scid–IL-2rγnull (NOG), bearing human IL-3 and GM-CSF genes (NOG IL-3/GM–Tg). In this strain, a large number of human myeloid cells of various lineages developed after transplantation of human CD34+ hematopoietic stem cells. Notably, mature basophils and mast cells expressing FceRI were markedly increased. These humanized NOG IL-3/GM–Tg mice developed passive cutaneous anaphylaxis reactions when administered anti–4-hydroxy-3-nitrophenylacetyl IgE Abs and 4-hydroxy-3-nitrophenylacetyl. More importantly, a combination of serum from Japanese cedar pollinosis patients and cedar pollen extract also elicited strong passive cutaneous anaphylaxis responses in mice. Thus, to our knowledge, our NOG IL-3/GM–Tg mice are the first humanized mouse model to enable the study of human allergic responses in vivo and are excellent tools for preclinical studies of allergic diseases. The Journal of Immunology, 2013, 191: 000–000.

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The online version of this article contains supplemental material.

Abbreviations used in this article: BALF, bronchoalveolar lavage fluid; BM, bone marrow; CIEA, Central Institute for Experimental Animals; GVHD, graft-versus-host disease; h, human; HSC, hematopoietic stem cell; KI, knock-in; m, mouse; MCC, mast cell chymase; MCT, mast cell containing tryptase; MCTC, mast cell containing tryptase and chymase; NOG, NOD/Shi-scid–IL-2rγnull; NP, 4-hydroxy-3-nitrophenylacetyl; NSG, NOD/LtSz-scid–IL-2rγnull; PB, peripheral blood; PEA, passive cutaneous anaphylaxis; SCF, stem cell factor; Tg, transgenic; TPO, thrombopoietin.

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slightly enhanced in SGM3-Tg mice compared with non-Tg control mice following transplantation with hHSCs. Furthermore, Rongvaux et al. (31) established hTPO knock-in (KI) mice in which the mouse TPO gene locus was replaced with the corresponding human gene locus. Upon transplantation of hHSCs, this strain showed remarkable differentiation of monocytes and granulocytes. However, generation of mast cells and several granulocyte subpopulations remained insufficient. Moreover, although the efficient development of human granulocytes and mast cells was demonstrated in humanized membrane-bound SCF-Tg NSG mice (32), the function of granulocytes in humanized SCF-Tg NSG mice has not been verified, especially with respect to whether they could release cytoplasmic granules containing histamine, leukotriene, and so forth in response to stimulation.

In this study, we generated a novel NOG substrain: hIL-3 and hGM-CSF–Tg (IL-3/GM–Tg) NOG mice. Within this model, we studied the development of human myeloid cells, maturation of basophils and mast cells, and passive cutaneous anaphylaxis (PCA) reaction in response to Ag-specific hIgE and the specific Ags. Our results effectively support the usefulness of this novel platform for studying human allergies.

Materials and Methods

Ethics statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Central Institute for Experimental Animals (CIEA) and were performed in accordance with guidelines set forth by the CIEA. All experiments using human cells and serum were approved by the institutional ethical committee of the CIEA and Sagamihara National Hospital. Written informed consent was obtained from every subject enrolled in the current study.

Generation of NOG IL-3/GM–Tg mice

Previously, we established a hIL-3 and hGM-CSF–Tg C57BL/6j-scid mouse strain in which the Tg human genes were ubiquitously expressed under control of the SRα promoter (33). This strain was backcrossed six times with NOG (formal name: NOD.Cg-prkdcscidIl2rgtmt1Sug/tm1Sug-ShiJic) mice to replace the genetic background with the aid of a marker-assisted selection protocol. Serum levels of hIL-3 and hGM-CSF were measured using human IL-3 and human GM-CSF Quantikine ELISA Kits (R&D Systems, Minneapolis, MN). The mice were maintained in the CIEA under specific pathogen–free conditions.

Human HSC transplantation

Human umbilical cord blood–derived CD34+ cells were purchased from Lonza (cat. no. 2C-101A; Basel, Switzerland). After thawing the frozen vials of cells according to the manufacturer’s protocol, cells with >90% viability were used for transplantation. NOG IL-3/GM–Tg or non-Tg mice (8–10 wk old) were irradiated with x-rays (2.5 Gy; MBR-1505SR; Hitachi Medical, Tokyo, Japan). After 24 h, 4.5–10^9 hHSCs were transplanted i.v. via the tail vein.

Flow cytometry

Bone marrow (BM), spleen, peripheral blood (PB), and bronchoalveolar lavage fluid (BALF) tissues were obtained from mice transplanted with hHSCs. Single mononuclear cell suspensions were prepared by standard methods, and RBCs were eliminated using BD Pharm Lyse (BD Biosciences, San Jose, CA). Human PB samples were obtained from healthy volunteers after acquiring their informed consent. hPBMCs were isolated by Ficoll-Hypaque (GE Healthcare, Little Chalfont, Buckinghamshire, U.K.) density centrifugation and washed with PBS. The cells were incubated with appropriate fluorochrome-conjugated Abs for 15–20 min at 4˚C. After washing with FACS buffer containing PBS with 1% FCS, the cells were resuspended in propidium iodide solution (BD Biosciences). Fluorescent signals were measured using a FACSComp multicolor flow cytometer, and the data were analyzed using FACSData software (both from BD Biosciences).

We used the following Abs: anti-hCD3–FITC and anti-mouse (m)CD45–biotin (BD Biosciences); anti-hCD19–PE (Beckman Coulter, Brea, CA); and anti-hCD45–allophycocyanin–Cy7, anti-hCD66b–FITC, anti-hLineage mixture (CD3, CD14, CD19, CD20, CD56)–FITC, anti-hCD56–PE, anti-hCD203c–PE, anti-hCD38–PE, anti-hCD34–PE–Cy7, anti-hCD3–PE–Cy7, anti-hCD5–PE–Cy7, anti-hFceRI–allophycocyanin, and anti-hCD11c–allophycocyanin (BioLegend, San Diego, CA).

Determination of human granulocytes

At 11 wk after HSC transplantation, PB was collected from the orbital vein of humanized NOG IL-3/GM–Tg and non-Tg NOG mice under anesthesia. Single-cell suspensions were prepared using BD Pharm Lyse (BD Biosciences), and hCD45+ cells were purified by eliminating mCD45+ cells using MACS. Briefly, the cells were stained with biotinylated anti-mCD45 Abs in MACS running buffer (PBS containing 2 mM EDTA and 0.1% BSA) for 15 min at 4˚C. The cells were washed with MACS buffer and subsequently labeled with anti-biotin magnetic beads (Miltenyi Biotec, Sunnyvale, CA). Labeled mCD45+ cells were eliminated using a MACS LS column (Miltenyi Biotec). The purity of hCD45+ cells in the negative fraction was typically 95–99%.

For May–Grünwald–Giemsa staining, enriched hCD45+ cells were smeared onto glass slides and air dried for 10 min. The slides were soaked in May–Grünwald solution (Muto Pure Chemicals, Tokyo, Japan) for 3 min and then washed with running water to remove excess stain. The slides were further stained with 0.5% Giemsa solution (Muto Pure Chemicals) for 15 min. After washing with running water, the slides were dried and subjected to microscopic analyses.

In vitro stimulation of human basophils and mast cells

At 18 wk after HSC transplantation, BM mononuclear cells from NOG IL-3/GM–Tg mice and hPBMCs were stimulated with 1 μg/ml imonomycin (Sigma-Aldrich, St. Louis, MO) for 30 min at 37˚C in a 5% CO2 incubator. After stimulation, these cells were stained with allophycocyanin-Cy7–anti-hCD45, FITC–anti-hCD63 (Beckman Coulter), PE–anti-hCD203, and allophycocyanin–anti-hFceRI mAbs and were analyzed by flow cytometer.

Immunohistochemistry

For histological analyses, the lungs, spleen, stomach, and skin from humanized NOG IL-3/GM–Tg and non-Tg mice were fixed with 4% paraformaldehyde (Wako, Osaka, Japan) and embedded in paraffin. The samples were then serially sectioned to a thickness of 3 μm using a microtome and placed on silane-coated slides (Muto Pure Chemicals). After deparaffinization, sections were incubated overnight at 4˚C with anti-human mast cell chymase (MCC) Abs (clone CC1; Leica Microsystems, Tokyo, Japan) and anti-human FcεRI Ab (clone CRA-1) (34, 35) and then incubated with a peroxidase-labeled polymer-conjugated anti-mouse Ab (Histofine Simple Stain Max-PO; Nichirei Biosciences, Tokyo, Japan) for 30 min at room temperature. For color development, the sections were incubated with a 0.02% solution of the substrate 3,3’-diaminobenzidine (Dako, Carpinteria, CA) and 0.006% H2O2. Immunostained sections were counterstained with hematoxylin (Sakura Finetech, Tokyo, Japan) for visualization of nuclei. For immunoﬂuorescence staining, 4-μm-thick frozen sections of tissues were fixed in 99% ethanol for 30 min and incubated with 10% normal goat serum (Nichirei Biosciences) for 30 min. An anti-human mast cell tryptase mAb (clone 10D11; Leica Microsystems) or an anti-human MCC mAb was fluorescently labeled with either Alexa Fluor 488 or Alexa Fluor 546 using a Zenon Mouse IgG2b or IgG1 Kit (Life Technologies, Carlsbad CA), according to the manufacturer’s instructions, and we used these mAbs for double staining of the sections. After 2-h incubation at room temperature, the sections were washed ﬁve times with PBS and mounted with Immunoselect Antifading Mounting Medium DAPI (Dianova, Hamburg, Germany). The specimens were visualized by ﬂuorescence microscopy (Axio Imager M1; Carl Zeiss Microscopy, Tokyo Japan).

PCA reactions

An anti-4-hydroxy-3-nitrophenylacetyl (NP) human/mouse chimeric IgE Ab (Serotec, Oxford, U.K.), which is composed of murine V region and human C region, was injected intradermally at six locations (0.5 μg/ location) on the dorsal side of humanized NOG IL-3/GM–Tg, non-Tg, and nonhumanized NOG mice. The same amount of hIgE Ab (Abcam, Tokyo, Japan) was injected into the ventral side of the mice as negative controls. Twenty-four hours after sensitization, the mice were injected i.v. with 500 μg NP-conjugated BSA (Biosearch Technologies, Novato, CA) with Evans blue dye toTrivia of the dye was injected i.v. 2 h after observing the blue staining of the reverse-side sites on the opposite side of the injection sites. For pollinosis PCA reaction, Japanese cedar pollen–specific IgE titer in the serum was determined by CAP-RAST. Serum obtained from Japanese cedar pollinosis patients were recruited from the outpatient clinic of Sagamihara National Hospital. Japanese cedar pollen–specific IgE titer in the serum was determined by CAP-RAST. Serum obtained from Japanese cedar pollinosis patients were recruited from the outpatient clinic of Sagamihara National Hospital. Japanese cedar.

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pollinosis patients (carrier polymer radioallergosorbent score = 5) was diluted 2-fold in saline and injected intradermally at two locations on the dorsal side of the mice. After 24 h, these mice were injected i.v. with 100 μl the cedar pollen extract (200 JAU/ml; Torii Pharmaceutical, Tokyo, Japan) with 0.5% Evans blue dye. After 30 min, dorsal or ventral skins from these mice were isolated and weighed. Evans blue dye was extracted from the skin by incubation in 5 ml formamide at 60˚C overnight and quantified by OD at 620 nm in a spectrometer (Bio-Rad, Tokyo, Japan).

**Statistical analysis**
Mean values and SD were calculated using Microsoft Excel (Microsoft, Redmond, WA). Student t tests were used to identify significant differences.

**Results**

*Generation of NOG IL-3/GM–Tg mice*

We generated NOG IL-3/GM–Tg mice by speed congenic techniques using a marker-assisted selection protocol (36) to change the genetic background of the mice from C57BL/6J to NOD. IL-3/GM–Tg mice, in which all of the microsatellite markers were replaced by the NOD haplotype at N6, were further crossed onto the NOG background. We confirmed the expression of the hIL-3 and hGM-CSF transgenes in the N3, N5, and N7 generations (Table I). For hHSC transfer, we used NOG IL-3/GM–Tg mice backcrossed more than seven times.

*Human hematopoiesis in hu-HSC NOG IL-3/GM–Tg mice*

To investigate the effects of hIL-3 and hGM-CSF on hematopoiesis in humanized mice, we transplanted hCD34+ HSCs into NOG IL-3/GM–Tg and non-Tg mice. The frequencies of hCD45+ cells and various lineages of human leukocytes in PB and various tissues

<table>
<thead>
<tr>
<th>Generation</th>
<th>NOD Marker (%)</th>
<th>hIL-3 (pg/ml)</th>
<th>hGM-CSF (pg/ml)</th>
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<td>51.4 ± 40.5</td>
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<td>N7</td>
<td>100</td>
<td>82.04 ± 40.24</td>
<td>35.03 ± 11.6</td>
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ND, Not determined.

**FIGURE 1.** Human hematopoiesis in NOG IL-3/GM–Tg mice. (A) Schematic view of the reconstitution of human immune systems in NOG IL-3/GM–Tg mice. NOG IL-3/GM–Tg mice and non-Tg littermates (8–10 wk old) were transplanted with 4–5 × 10⁴ hCD34+ HSCs 1 d after x-ray irradiation (2.5 Gy). Human leukocytes were analyzed at the indicated time points. (B) Kinetics of hCD45+ cell engraftment. The frequency of human leukocytes in PB was analyzed by flow cytometry at the indicated time points over 12 wk. Data are mean ± SD (n = 10 mice/group). (C and D) Frequencies of engrafted multilineage hCD19+, hCD3+, hCD56+, hCD33+, hCD66b+, and hCD14+ human leukocytes in the hCD45+ cell population were analyzed by flow cytometry. *p < 0.05, **p < 0.005, ***p < 0.0005, ****p < 0.00005.
from these mice were analyzed at different time points (Fig. 1A). The chimerism of hCD45+ cells in PB was significantly higher in hu-HSC NOG IL-3/GM–Tg mice than in non-Tg NOG mice throughout the analysis period (Fig. 1B, 1C). For the myeloid lineage, hu-HSC NOG IL-3/GM–Tg mice had significantly higher frequencies of total hCD33+ myeloid cells, hCD66b+ granulocytes, and hCD14+ monocytes than did non-Tg mice (Fig. 1C, 1D). In particular, the increase in hCD66b+ granulocytes was dramatic compared with the low differentiation of this population in conventional NOG mice. For lymphoid cells, the increase in the hCD3+ T cell population was more evident in Tg mice than in non-Tg mice at 12 wk post-HSC transplantation, resulting in a lower frequency of hCD19+ B cells in Tg mice than in non-Tg mice; the development of hCD36+ NK cells was not influenced (Fig. 1C, 1D). Human leukocytes, other than lymphoid and myeloid lineage cells, were scarce in hCD45+ cells in PB of Tg and non-Tg mice (Supplemental Fig. 1A).

We next investigated other organs. The engraftment of human HSCs, defined as hCD34+hCD382 cells in hCD45+ lineage2 cells, in the BM showed 4- and 3-fold increases in frequency and cell number, respectively, in Tg mice compared with non-Tg mice (Fig. 2A, 2B). Spleens were markedly enlarged in hu-HSC NOG IL-3/GM–Tg mice (Supplemental Fig. 1B). Consistent with this finding, weight and total splenocyte numbers were ~3-fold higher in Tg mice than in non-Tg mice (Supplemental Fig. 1C, 1D). Although the percentage of CD45+ cells did not differ between Tg and non-Tg mice, the absolute number of hCD45+ cells increased in Tg mice, reflecting an increase in total cell number (Fig. 2C, 2D). As in the PB, the frequency and absolute number of hCD33+ myeloid cells in the spleen were increased in hu-HSC–Tg mice, and expansion of hCD66b+ granulocytes was especially remarkable (Fig. 2D, 2E). An increase in the hCD33+hCD11c+ myeloid dendritic cell population was also confirmed (Fig. 2D, 2E). In addition, the number of cells in BALF samples was increased 4-

![FIGURE 2. Analysis of the BM and spleen in NOG IL-3/GM–Tg mice. BM cells were isolated from the tibiae of hu-HSC NOG IL-3/GM–Tg and non-Tg mice and stained with anti-hCD34, anti-hCD38, and anti-hCD45 Abs and anti-lineage markers to identify hHSCs (hCD34+hCD382 cells in the hCD45+ lineage2). A representative HSC staining pattern (A) and the mean frequency and cell numbers [(B), n = 5/group] are shown. (C–E) After the preparation of splenocytes from hu-HSC NOG–Tg and non-Tg mice at 20–22 wk postreconstitution, the total number of cells was counted, and the cells were stained with various Abs to facilitate analysis by flow cytometry. (C) Frequency (upper panel) and number (lower panel) of hCD45+ human leukocytes. Data are the mean ± SD (n = 5 mice/group). (D and E) Differentiation of human myeloid and lymphoid cells in hu-HSC NOG IL-3/GM–Tg mice. After staining with Abs specific for myeloid (hCD33, hCD66b, and hCD11c) and lymphoid (hCD19 and hCD3) markers, the cells were analyzed by flow cytometry. (D) Representative plots. (E) Frequency (upper panels) and absolute cell number (lower panels) for each hCD45+ cell subpopulation. Absolute cell number was calculated by multiplying the frequency by the total cell number. *p < 0.05, **p < 0.005, ***p < 0.0005, ****p < 0.00005.](http://www.jimmunol.org/)
fold in hu-HSC NOG IL-3/GM–Tg mice (Supplemental Fig. 1D), whereas there was no significant difference in BM cell numbers (Supplemental Fig. 1D). Overall, the numbers of myeloid cells were increased in BM and BALF samples from hu-HSC NOG IL-3/GM–Tg mice compared with non-Tg mice in terms of both frequency and absolute cell number (Fig. 2E, Supplemental Fig. 1E, 1F). The frequency of B cells was lower in spleen and BM from hu-HSC NOG IL-3/GM–Tg mice than in non-Tg mice (Fig. 2E, Supplemental Fig. 1E). Nevertheless, the number of B cells was higher in spleen and BALF tissues from hu-HSC NOG IL-3/GM–Tg mice compared with non-Tg mice (Fig. 2E, Supplemental Fig. 1F). The increase in the total number of hCD45+ cells may compensate for the reduction in the number of B cells in Tg mice. Following the marked improvement of the development of human myeloid cells, we also confirmed development of a large number of human macrophages in various tissues, including the liver, lung, and spleen, in hu-HSC NOG IL-3/GM–Tg mice (Supplemental Fig. 1G).

Taken together, these data demonstrate that human myelopoiesis, especially the development of granulocytes, was dramatically improved in NOG IL-3/GM–Tg mice.

Development of human granulocytes in hu-HSC NOG IL-3/GM–Tg mice

One of the main characteristics of HSC-transplanted NOG IL-3/3–GM–Tg mice was the efficient development of human granulocytes. Therefore, we examined whether these cells contain typical subpopulations (i.e., basophils, eosinophils, and neutrophils). To analyze the morphology of human granulocytes from hu-HSC NOG IL-3/GM–Tg and non-Tg mice, human leukocytes were purified from the PB by eliminating mCD45+ cells. In the hu-HSC NOG IL-3/GM–Tg mice compared with non-Tg mice (Fig. 2E, Supplemental Fig. 1F). The increase in the total number of hCD45+ cells may compensate for the reduction in the number of B cells in Tg mice. Following the marked improvement of the development of human myeloid cells, we also confirmed development of a large number of human macrophages in various tissues, including the liver, lung, and spleen, in hu-HSC NOG IL-3/GM–Tg mice (Supplemental Fig. 1G).

Taken together, these data demonstrate that human myelopoiesis, especially the development of granulocytes, was dramatically improved in NOG IL-3/GM–Tg mice.

Development of mature basophils and mast cells in hu-HSC NOG IL-3/GM–Tg mice

Considering the pathogenic roles of basophils and mast cells in allergic responses, we were interested in determining whether these populations can be induced to enter a mature state in hu-HSC NOG IL-3/GM–Tg mice.

We examined whether human cells expressing FceRI or CD203c, functional and phenotypic markers, respectively, were present. As shown in Fig. 4A, there was a clear subpopulation of hFceRIε-hCD203cε- cells, which represented basophils and mast cells, in PB, BM, spleen, and BALF samples from hu-HSC NOG IL-3/GM–Tg mice but not non-Tg mice. The frequencies and numbers of hFceRIε-hCD203cε+ cells were significantly higher in hu-HSC NOG IL-3/GM–Tg mice than in non-Tg mice (Fig. 4B). These data suggest that IL-3 and/or GM-CSF support the development of FceR-expressing mature basophils and mast cells. We further distinguished mast cells and basophils by assessing hFcεRI expression. As shown in Fig. 4C, all hFcεRIε-hCD203cε+ cells were hFcεRIε+ (basophils) in human PBMCs. In contrast, ~20% of hFcεRIε-hCD203cε+ cells in the PB collected from hu-HSC NOG IL-3/GM–Tg mice were hFcεRIε+ (mast cells; Fig. 4C). When we compared the frequencies of basophils and mast cells in various tissues, ~70 and 50% of hFcεRIε-hCD203cε+ cells in the spleen and BM, respectively, were mast cells (Fig. 4D), suggesting that these cell types were differentially distributed in various tissues. When we stimulated these hFcεRIε-hCD203cε+ mast cells or basophils in BM of IL-3/GM–Tg mice, they showed elevated expression of CD63. The levels of CD63 were as high as those of basophils in human PBMCs, representing a marker of basophil and mast cell activation (37), which suggests that these cells were functional (Fig. 4E).

**FIGURE 3.** Development of human granulocytes in hu-HSC NOG IL-3/GM–Tg mice. (A) Human leukocytes were negatively enriched by >95% in the PB from hu-HSC NOG IL-3/GM–Tg or non-Tg mice at 11 wk after HSC transplantation. The SSC1midCD66b+ fraction contained human granulocytes. (B) and (C) Smears of purified human leukocytes from hu-HSC NOG IL-3/GM–Tg and non-Tg mice were subjected to May–Giemsa staining. Red arrows identify eosinophils, which contained large red granules (left panel); basophils, which contained large red granules (middle panel) and immature neutrophils, which appear as small red granules (right panel). The data shown are representative of two independent experiments.
Tissue-resident human mast cells were identified by immunohistochemistry using anti-hMCC Abs (Fig. 5A). MCC+ cells were abundant in the spleen in hu-HSC NOG IL-3/GM–Tg mice, whereas only a few MCC+ cells were detected in non-Tg mice. Significant numbers of MCC+ cells were also identified in the lungs and stomach of hu-HSC NOG IL-3/GM–Tg mice but not non-Tg mice. Generally, human mast cells are classified on the basis of their protease contents (38). Mast cells containing tryptase and chymase (MCTC) localize primarily in the skin and any other tissues and resemble the connective tissue–type mast cells in the rodent. In contrast, mast cells containing tryptase (MCTs) but not chymase are dominant in mucosal tissues, including the lung and gastric mucosa, similar to mucosal-type mast cells in rodents. To clarify the subtypes of human mast cells, we stained sections from the spleen, lung, stomach, and skin from humanized Tg or non-Tg mice with anti-chymase and anti-tryptase mAbs. We detected abundant MCTCs with tryptase and chymase, whereas few MCTs solely expressed tryptase (Fig. 5B).

Taken together, these results suggested that a large number of IgER-expressing human basophils and mast cells developed in IL-3/GM–Tg mice. These human mast cells resemble MCTCs and are distributed in various tissues, including mucosal tissues. PCA responses in hu-HSC NOG IL-3/GM–Tg mice

Mast cells mediate allergic responses by releasing chemical substances, such as histamine or leukotrienes. A series of intracellular
processes leading to degranulation of mast cells was triggered by cross-linking of IgE-bound FcεRI by specific Ags. The presence of a large number of human mast cells and basophils in hu-HSC NOG IL-3/GM–Tg mice prompted us to examine whether those cells could mediate Ag-specific IgE–dependent PCA reactions in vivo. Animals were sensitized with NP-specific IgE and challenged with NP-conjugated BSA 1 d later. As demonstrated in Fig. 6A, hu-HSC NOG IL-3/GM–Tg mice showed strong local inflammatory responses manifested by the extravasation of Evans blue dye, but much weaker in non-Tg mice. This was an Ag-specific reaction because the extravasation of the dye was detected only in the dorsal skin, and not on the ventral side, where control hIgE was administered. Furthermore, our examination showed that the PCA response was mediated by human cells, because nonhumanized NOG mice showed no extravasation of Evans blue dye, which eliminated the possibility that murine mast cells were activated by hIgE and hAg complexes. MCC staining confirmed the localization of a large number of human mast cells in both the dorsal and ventral skin of hu-HSC NOG IL-3/GM–Tg mice, with fewer human mast cells in non-Tg mice (Fig. 6B). Moreover, immunohistochemistry of the serial sections revealed that most of the FcεRI+ cells in the dorsal skin of hu-HSC NOG IL-3/GM–Tg and non-Tg mice were positive for MCC (Fig. 6C), supporting that the PCA reaction was mediated by FcεRI-expressing human mast cells. Recently, Schafer et al. (39) demonstrated that the anaphylatoxins C3a and C5a enhance the IgE-mediated PCA reaction, including mast cell degranulation and inflammation, in mice. Indeed, we detected the expression of both hC3aR and hC5aR on mast cells in hu-HSC NOG IL-3/GM–Tg mice (Supplemental Fig. 2A). Then, to examine the role of the anaphylatoxins in our system, either C3aR or C5aR antagonist was administered to hu-HSC NOG IL-3/GM–Tg mice to inhibit binding of these anaphylatoxins on human mast cells during our NP-induced PCA responses. The PCA responses were strongly inhibited by the C3aR antagonist but not by the C5aR antagonist (Supplemental Fig. 2B, 2C). The modest effect of the C5aR antagonist may be attributed to the lack of murine C5 in NOD strains (40). These results suggest that the C3a anaphylatoxin plays an important role in the induction of PCA in hu-HSC NOG IL-3/GM–Tg mice.

We next examined whether the PCA reaction could be caused by patient-derived materials. We used the serum containing Japanese cedar pollen–specific hIgE obtained from pollinosis patients. After sensitization with the serum and subsequent injection with cedar pollen extract, strong extravasation of Evans blue dye into skin was detected in hu-HSC NOG IL-3/GM–Tg mice (Fig. 6D, 6E). Non-Tg mice showed a weak PCA reaction, probably due to the presence of only a few FcεRI+ cells (Fig. 6B), which may react with hIgE (Fig. 6D, 6E).

Collectively, these results suggested that human mast cells differentiated in hu-HSC NOG IL-3/GM–Tg mice have sufficient capacity to mediate allergic inflammatory responses in vivo and that the effector phase of allergic responses manifested by histamine release can be fully recapitulated in our hu-HSC NOG IL-3/GM–Tg mice.

![FIGURE 5. Histology of human mast cells in hu-HSC NOG IL-3/GM–Tg mice.](http://www.jimmunol.org/)

(A) Detection of human mast cells by immunohistochemistry. Sections of various tissues from hu-HSC NOG IL-3/GM–Tg or non-Tg mice were stained with an anti-human MCC Ab. Each brown dot represents an individual human MCC-expressing mast cell. Representative images from five mice are shown. (B) Multicolor immunofluorescence analysis of human mast cells. Frozen sections of each tissue from Tg or non-Tg mice were stained with anti-human mast cell tryptase (green) and MCC (red) Abs. For double-labeled cells, the separate images were pseudo-colored, one as green and the other as red, and then merged to create the yellow color. Images shown are representative of one of three mice.
Discussion

In the current study, we demonstrated that a novel IL-3/GM–Tg NOG mouse substrain has a strong ability to promote the development of a variety of human myeloid cells from HSCs. Strikingly, developed human mast cells mediated allergic responses, as shown in PCA tests. To our knowledge, this is the first model to demonstrate that human cells generated in mice can mediate human allergic reactions in a reliable and repeatable manner.

Two immunodeficient mouse strains carrying the human IL-3 and GM-CSF genes have been generated: IL-3/GM-CSF/SCF-Tg (SGM3-Tg) mice (30) and IL-3/GM-CSF-KI mice (41). It is noteworthy that there were considerable differences in phenotypes between our study and these previous studies. For example, in contrast to the high engraftment of hHSCs in our IL-3/GM–Tg mice, engraftment was reduced in SGM3-Tg mice compared with control mice, and there was no significant improvement in HSC engraftment in IL-3/GM–CSF–KI mice. In addition, there were no major differences in the frequency or number of macrophages, granulocytes, or T cells between IL-3/GM–CSF–KI mice and control mice. Those phenotypes were completely different from our IL-3/GM–Tg mice, which have the highest potential of these three strains from the point of view of HSC maintenance and myelopoiesis. These inconsistencies may be attributed, in part, to differences in the levels and distributions of cytokines. The amount of IL-3 was 4–6-fold higher in SGM3-Tg mice than in our IL-3/GM–Tg mice. Because high levels of IL-3 interfere with HSC proliferation, despite its pivotal role in the expansion of HSCs in in vitro culture systems (42), this increase in IL-3 in SGM3-Tg mice may explain the reduction in HSCs in SGM3-Tg mice. In IL-3/GM–CSF–KI mice, the expression patterns of hIL-3 and hGM-CSF were similar to those of endogenous mIL-3 and mGM-CSF. Accordingly, because of the high expression of GM-CSF, major effects were most evident in the lungs, where many human alveolar macrophages resided after HSC transplantation. Hence, the tissue-specific expression of IL-3 or GM-CSF in KI mice was sufficient for tissue-specific development and migration of human cells but not for systemically reconstituting entire human myeloid cells.

Intriguingly, the improved development of myeloid cells in our IL-3/GM–Tg mice was rather similar to that in the TPO-KI mice established by Rongvaux et al. (31), which also showed high engraftment of HSCs, enhanced myeloid cell development, and granulocyte differentiation. Although detailed analyses of granulocyte subpopulations have not been reported in TPO-KI mice,
Giemsa staining indicated the differentiation of neutrophil-like cells with highly lobulated nuclei, which were rarely detected in our IL-3/GM–Tg strain. Differences in cytokines (i.e., TPO versus IL-3/GM–CSF) influenced the lineage decisions of human myeloid cells, explaining the different results observed in these two strains.

The most striking characteristic of our NOG IL-3/GM–Tg strain was that a large number of fully functional human basophils and mast cells were able to develop from HSCs. The numbers of these cell populations in conventional humanized mice are too small in which to analyze the functional maturity and characteristics of these populations, especially in vivo situations, although two reports demonstrated the presence of these cells in conventional NOG mice (43, 44). The initial study of human mast cells in humanized mice was reported by Kambé et al. (43). They showed that a few MCTs were present in the lung and stomach of conventional humanized NOG mice and that both types of human mast cells were differentiated in these animals. This result is inconsistent with our findings, and there might be several reasons for the discrepancy. Kambé et al. (43) identified these mast cell phenotypes by an immunoenzyme technique using anti-chymase (clone: B7) and anti-tryptase (clone: G3) mAbs, whereas we used directly labeled mAbs for our double-staining results. These different techniques and mAb clones could explain this inconsistency. Also, MCTs require IL-4 to suppress chymase expression (45). The production of IL-4 from human T cells in humanized mice was markedly lower than that in human PB, and there were large variances in the amounts of IL-4 inhumanized mice (46). Varying levels of IL-4 may be responsible for the poor development of MCTs in our mouse colony. Therefore, administration of hIL-4 to humanized mice may support human mast cell development.

Recently, Takagi et al. (32) demonstrated that the development of human mast cells was significantly improved in their new hSCF-Tg NSG strain compared with normal NSG mice. However, the expression of hFcεRI on mast cells was not evaluated, and the ability of their strain to mediate human allergic responses in vivo was not addressed. Although IL-3/GM–Tg mice have no source of hSCF, the human mast cells developed in our IL-3/GM–Tg mice may be stimulated by mSCF, which is known to cross-react with human c-kit on mast cells (24). It will be interesting to compare the development of human mast cells between IL-3/GM–Tg and SCF-Tg mice, because this comparison may be useful in future investigations of human mast cells.

Previously, hIgE-mediated allergic responses were studied using hPBMC-engrafted mice (47–49). Weigmann et al. (49) demonstrated that colon inflammation was induced by rectal or oral challenge of allergens in NSG mice transplanted with allergic patient-derived hPBMCs. In these models, it is thought that patient-derived B cells produced an allergen-specific IgE Ab in mice, which, in turn, triggered allergic inflammation upon administration of the specific allergen. Although some aspects of allergic symptoms were mimicked in these models, suggesting that they may serve as an attractive model for human allergies, there are several caveats to the current study. For example, the release of histamine and any granules would become insufficient because of the small number of human basophils or mast cells in hPBMC grafts. In addition, xenogenic GVHD occurred easily in NOD-scid-IL-2γ−/− mice when they were transplanted with hPBMCs (15). Xenogenic GVHD symptoms may sometimes be confused with allergic inflammation, and careful examinations are necessary. From these points, we conclude that our IL-3/GM–Tg mouse system is superior to PBMC-induced models because of the stable presence of many human mast cells and basophils in mouse environments; moreover, these cells did not develop GVHD, enabling long-term experimentation.

In summary, we generated a novel mouse strain, IL-3/GM–CSF–Tg NOG, which is one of the most suitable strains for inducing human hematopoiesis, including the production of granulocytes and mast cells, from HSCs. In addition, it is of note that the effector phase of the human allergic response was induced by in vivo–developed human mast cells. This new mouse strain will be an indispensable platform for analyzing human allergies in vivo. Furthermore, by combining this strain with other Tg strains expressing HLA, IL-4, or IL-21, the complete recapitulation of human allergies will be possible in humanized mice. These models will enable the analysis of cellular and molecular mechanisms responsible for allergies and may become useful tools for developing antiallergy drugs in the near future.

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Disclosures

The authors have no financial conflicts of interest.

References

A HUMAN ALLERGY MODEL USING A HUMANIZED MOUSE


