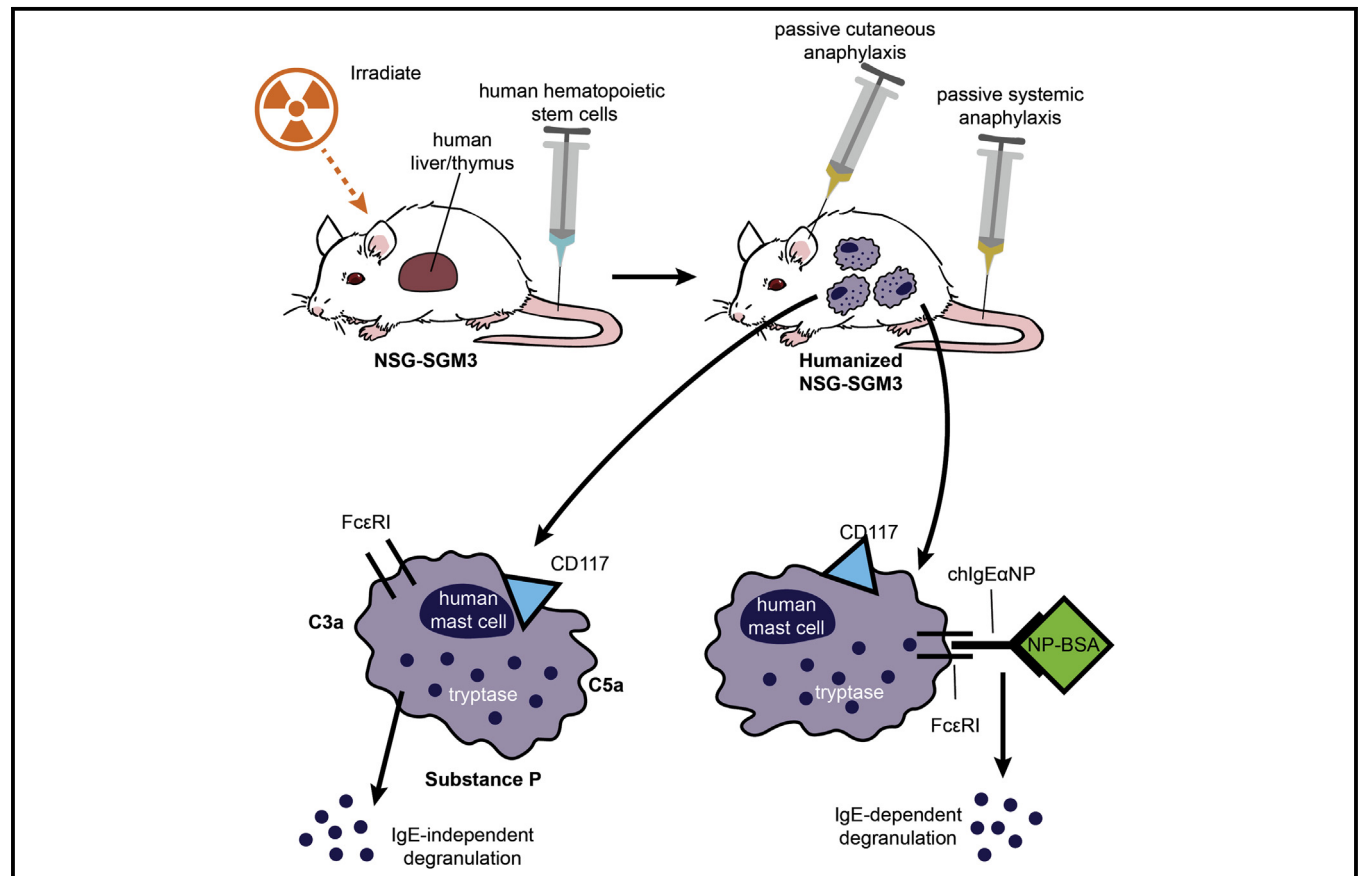


Humanized mouse model of mast cell-mediated passive cutaneous anaphylaxis and passive systemic anaphylaxis

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GRAPHICAL ABSTRACT



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Background: Mast cells are a critical component of allergic responses in humans, and animal models that allow the *in vivo* investigation of their contribution to allergy and evaluation of new human-specific therapeutics are urgently needed.

Objective: To develop a new humanized mouse model that supports human mast cell engraftment and human IgE-dependent allergic responses.

Methods: This model is based on the NOD-*scid* IL2 γ ^{null} SCF/GM-CSF/IL3 (NSG-SGM3) strain of mice engrafted with human thymus, liver, and hematopoietic stem cells (termed Bone marrow, Liver, Thymus [BLT]).

Results: Large numbers of human mast cells develop in NSG-SGM3 BLT mice and populate the immune system, peritoneal cavity, and peripheral tissues. The human mast cells in NSG-SGM3 BLT mice are phenotypically similar to primary human mast cells and express CD117, tryptase, and FcεRI. These mast cells undergo degranulation in an IgE-dependent and -independent manner, and can be readily cultured *in vitro* for additional studies. Intradermal priming of engrafted NSG-SGM3 mice with a chimeric IgE containing human constant regions resulted in the development of a robust passive cutaneous anaphylaxis response. Moreover, we describe the first report of a human mast cell antigen-dependent passive systemic anaphylaxis response in primed mice.

Conclusions: NSG-SGM3 BLT mice provide a readily available source of human mast cells for investigation of mast cell biology and a preclinical model of passive cutaneous anaphylaxis and passive systemic anaphylaxis that can be used to investigate the pathogenesis of human allergic responses and to test new therapeutics before their advancement to the clinic. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: Mast cell, humanized mice, NSG, NSG-SGM3, passive cutaneous anaphylaxis, PCA, passive systemic anaphylaxis, PSA

Type 1 hypersensitivity responses associated with eosinophils, basophils, and mast cells are thought to underlie the pathological allergic symptoms in more than 3 billion people worldwide.¹ Many of these symptoms are mediated by mast cell activation, leading to release of inflammatory mediators that drive much of the allergic reaction with pathological consequences.² Because of the severe allergic reactions that can be generated by mast cell activation, therapeutics targeting the prevention of mast cell activation or neutralization of the released downstream proinflammatory mediators are being developed.³

Animal models have been used for the study of allergic reactions and to test the potential efficacy of new drugs.² However, not all aspects of murine models of allergy reflect human allergic diseases because of the numerous differences in murine and human immune systems.⁴ To address this, investigators have been developing immunodeficient mice that can be engrafted with components of the human immune system, that is, “humanized” mice. The development of these mice has been reviewed.⁵ However, because of the lack of some species-specific factors, immune cells such as human myeloid cell populations develop poorly. This has been addressed by transgenic expression of human cytokines. For example, NOD.*cg-Prkdc*^{scid} IL2 γ ^{tm1Sug} (NOG) mice transgenically expressing human IL3 and GM-CSF were shown to develop human mast cells that can mediate a human IgE-driven passive cutaneous anaphylaxis (PCA) reaction upon antigen challenge.⁶

Abbreviations used

BLT:	Bone marrow, Liver, Thymus
chIgE-anti-NP:	Chimeric human IgE-anti-hapten 4-hydroxy-3 nitrophenacetyl antibody
HSC:	Hematopoietic stem cell
NOG:	NOD. <i>cg-Prkdc</i> ^{scid} IL2 γ ^{tm1Sug}
NP:	Hapten 4-hydroxy-3 nitrophenacetyl antibody
NSG:	NOD. <i>cg-Prkdc</i> ^{scid} IL2 γ ^{tm1Wjl}
NSG-SGM3:	NOD- <i>scid</i> IL2 γ ^{null} SCF/GM-CSF/IL3
PCA:	Passive cutaneous anaphylaxis
PEC:	Peritoneal exudate cell
PSA:	Passive systemic anaphylaxis
SCF:	Stem cell factor

In this study, we have used NSG-SCF/GM-CSF/IL3 (NSG-SGM3) mice engrafted with human thymus, liver, and hematopoietic stem cells (HSC) (Bone marrow, Liver, Thymus [BLT]) to establish a robust humanized mouse model of human mast cell engraftment and function. NSG-SGM3 BLT mice mediate a robust human IgE-dependent PCA reaction and passive systemic anaphylaxis (PSA) reaction, modeling the responses seen in life-threatening anaphylaxis. NSG-SGM3 BLT mice will be important tools for investigation of human mast cell biology and for preclinical analysis of the efficacy of new therapeutics before their advancement to the clinic.

METHODS

Mice

NOD.*Cg-Prkdc*^{scid} IL2 γ ^{tm1Wjl} (NSG, JAX stock number 05557) and NOD.*Cg-Prkdc*^{scid} IL2 γ ^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloYszJ (NSG-SGM3, JAX stock number 013062) mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. The development of these mice has been described earlier.^{7,8} All animal use was in accordance with the guidelines of the Animal Care and Use Committees of the University of Massachusetts Medical School, Northwestern University, and the Jackson Laboratory.

Antibodies and flow cytometry

For analysis of human cells using flow cytometry, mAbs specific for mouse CD45 (clone 30-F11, BD Biosciences, Inc [BD], San Jose, Calif) and the following human antigens: CD45 (clone 2D1, BD), CD34 (clone 581, BD), CD3 (clone UCHT1, BD), CD4 (clone RPA-T4, BD), CD8 (clone RPA-T8, BD), CD33 (clone WM53, BD), CD117 (c-kit; clone A3C6E2, Miltenyi Biotec [MB], San Diego, Calif), FcεRI (clone AER-37 CRA1, MB), CCR3 (clone 5E8, BD), IL5RA (CDw125; clone A14, BD), CD64 (clone 10.1, BD), CD203c (clone FR 3-16A11, MB), CD88 (clone D53-1473, BD), CD69 (clone FN50, BD), CD49d (clone 9F10, BD), CD163 (clone GHI/61, BD), CD32 (clone FLI8.26, BD), CD20 (clone 2H7, BD), tryptase (clone AA1, Abcam, Cambridge, Mass), and F(ab')₂ fragment donkey antimouse IgG (H+L) (polyclonal, Jackson ImmunoResearch, West Grove, Pa) were purchased from BD, eBiosciences (San Diego, Calif), BioLegend (San Diego, Calif), or MB.

Chimeric human IgE-anti-hapten 4-hydroxy-3 nitrophenacetyl antibody (chIgE-anti-NP) is a human chimeric antibody with affinity directed against the hapten 4-hydroxy-3 nitrophenacetyl antibody (NP). The chIgE-anti-NP was concentrated (Amicon 30kMWCO centrifuge spin cell) from cell line JW8/5/13 (Sigma-Aldrich, Natick, Mass) supernatants⁹ grown in Hybridoma SFM (Life Technologies, Carlsbad, Calif). The chIgE-anti-NP antibody is composed of the human Fc ϵ chain and mouse anti-NP variable chain. The chIgE-anti-NP antibody was quantified in concentrated supernatant by ELISA and a known hu-IgE standard (Abcam). For the first ELISA, mouse-anti-hu-IgE-biotin (clone G7-26, BD) was used for capture. For the

second ELISA, FcεR1a (IgE receptor, R&D Systems, Minneapolis, Minn) was used for capture. The chIgE-anti-NP was also shown to bind NP-BSA (EC₅₀, 0.01 μg/mL) using BSA-NP (>20 NP) (BioResearch Technologies N-5050H-10) for capture. Goat anti-Hu-IgE (AbCam) followed by horseradish peroxidase-donkey anti-goat IgG (Rockland, Limerick, Pa) was used for detection in the ELISA with anti-IgE, FcεR1a, and NP-BSA ELISA.

Whole blood was collected in heparin, and single-cell suspensions were prepared from tissues of engrafted mice. Single-cell suspensions of 1×10^6 cells in 100 μL or whole blood were washed with fluorescence activated cell sorter buffer (PBS supplemented with 2% BSA) and then incubated with rat anti-mouse CD16/CD32 (clone 2.4G2) to block Fc binding. Specific antibodies were then added to the samples and incubated for 30 minutes at 4°C. Stained cell suspensions were washed and fixed with 2% paraformaldehyde. Alternatively, blood or single-cell suspensions were treated with red blood cell Lysis buffer for 5 minutes at room temperature using 0.8% NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA solution in water. The cells were washed twice with PBS and used for flow cytometry. For intracellular staining, cells were treated with 4% paraformaldehyde in PBS for at least 30 minutes and permeabilized with 100% ethanol for 20 minutes. Intracellular staining was performed in fluorescence activated cell sorter buffer.

At least 50,000 events were acquired on LSRII or FACSCalibur instruments (BD). Data analysis was performed with FlowJo (Tree Star, Inc, Ashland, Ore) software. In the figures, each symbol represents an individual mouse or independent data point.

Immunohistology

Tissues were fixed in buffered formalin, embedded with paraffin, and 5-μm thick sections were stained with pinacyanol erythrosinate for the detection of mast cells.¹⁰ For determination of human mast cells, 5-μm thick sections of paraffin-embedded, formalin-fixed tissues were stained with mouse antihuman tryptase (NeoMarkers, Fremont, Calif) and developed for visualization using the ABC kit (Vector Labs, Burlingame, Calif) and DAB Plus (Zymed Labs, South San Francisco, Calif) as described earlier.¹¹

Establishment of BLT mice

NSG-BLT and NSG-SGM3 BLT mice were established using a protocol for generation of NSG-BLT mice we have previously described.¹² Indicated groups of recipient NSG and NSG-SGM3 mice were irradiated with 200 cGy and 100 cGy, respectively.

In vitro culture of human mast cells recovered in the peritoneal exudate cells of NSG-SGM3 BLT mice and their calcium flux in response to stimulation

Peritoneal exudate cells (PECs) were recovered from NSG-SGM3 BLT mice by flushing the peritoneal cavity using 5 mL of complete RPMI media containing 10% FBS, penicillin/streptomycin, and L-glutamine. Cells from individual mice were then cultured in mast cell growth media (AIM-V CTS media supplemented with 100 ng/mL recombinant human SCF; R&D Systems) at 1.0 to 3.0×10^6 /mL. Floating cells were removed and placed into a new tissue culture flask for 2 to 3 consecutive days to remove adherent cells. Cells from individual mice were combined after verification of sterility. Half of the media was then replaced weekly and recombinant human SCF was supplemented to give a final concentration of 100 ng/mL. Mast cell phenotype was determined by toluidine blue staining and flow cytometry for double-stained c-kit⁺/FcεRI⁺ cells. Mast cells were used for studies as indicated in the text and cultures were not extended for more than 32 days.

PECs of NSG-SGM3 BLT mice were cultured to enrich for human mast cells and tested for calcium flux as described.¹³ Briefly, mast cells were treated with 100 ng/mL chIgE-anti-NP overnight. Cells were then loaded with 1.5 μM Fluo-4 NW at 1×10^5 cells/mL for 30 minutes at 37°C in loading buffer (Ca²⁺/Mg²⁺-free HBSS plus 2 mmol/L probenecid and 0.1% BSA). The cells were allowed to equilibrate to room temperature for 30 minutes and analyzed using flow cytometry for 100 seconds to establish baseline

before 50 ng/mL NP-BSA was added and the cells analyzed for calcium flux. Data were analyzed with FlowJo (TreeStar, Inc) to visualize a change in fluorescein isothiocyanate over time. Maximal response was determined following addition of 2 μM ionomycin at the end of the experimental period.

Mast cell degranulation

Mast cells obtained from the PECs of NSG-SGM3 BLT mice were cultured in the presence of chIgE-anti-NP overnight at indicated concentrations. The following day 50 ng/mL NP-BSA (BioResearch Technologies, Novato, Calif) was added for 30 minutes at 37°C to cross-link IgE and initiate mast cell degranulation. Culture supernatants were assayed for β-hexosaminidase release for 1.5 hours at 37°C in β-hexosaminidase substrate solution containing 4-nitrophenyl *N*-acetyl-β-D-glucosaminide in 0.09 mol citrate buffer at pH 4.5. Release of 4-nitrophenol was monitored at 405 nanometers.

PCA assay

The PCA assay for NSG-SGM3 BLT mice was performed as previously described.^{14,15} Briefly, NSG-BLT and NSG-SGM3 BLT mice were injected intradermally in the left ear with 100 ng of chIgE-anti-NP antibody in 10 μL of PBS and an equal volume of PBS was injected intradermally into the right ear. Twenty-four hours later, all mice were challenged intravenously with 500 μg of NP-conjugated BSA in PBS (Invitrogen, Carlsbad, Calif). Ear thickness was determined before and 1 hour after sensitization using an Engineers thickness gauge (Mitutoyo 0.0001 inch thickness gauge). At 24 hours after sensitization, ear thickness was again measured and blood and tissues were recovered for analysis.

PSA assay

The PSA assay as described for immunocompetent mice¹⁶ was adapted for humanized mice using chimeric human IgE mAb. NSG-BLT and NSG-SGM3 BLT mice were primed with intravenous injection of 1.6 μg of chimeric human IgE-anti-NP mAb in 200 μL and anaphylaxis was initiated 24 hours later by intravenous injection of 500 μg of NP-conjugated BSA in 100 μL of PBS. Anaphylaxis was observed as a significant decrease in core body temperature and observable symptom scores as described¹⁷ and adapted from Ganesan et al¹⁷ and Li et al.¹⁸ Two investigators unaware of the experimental conditions of each mouse assessed symptom scores.

Statistical analysis

Data are provided as means ± SEMs. Statistical significance was determined by using the 2-tailed Student *t* test, ANOVA (Dunnett test), the nonparametric Mann-Whitney test, or other nonlinear regression as appropriate. All analyses were done with GraphPad Prism software (La Jolla, Calif).

RESULTS

Human myeloid and mast cell engraftment in NSG-BLT and NSG-SGM3 BLT mice

NSG-BLT and NSG-SGM3 BLT mice were established and the human cell composition of the blood and peritoneal cavity (PEC) was determined 12 weeks after engraftment. As expected,^{12,19} high levels of human CD45⁺ cells were observed in the blood of both NSG-BLT and NSG-SGM3 BLT mice (Fig 1, A). Most of the CD45⁺ cells in the blood of the NSG-SGM3 BLT mice, but not in the NSG-BLT mice, were CD33⁺ (Fig 1, B), containing a significant proportion of CD117⁺CD203c⁺ mast cells (Fig 1, C). In contrast to the low percentages and numbers of human CD45⁺ cells detected in the PECs of NSG-BLT mice, high percentages and numbers of human CD45⁺ cells were observed in the PECs of NSG-SGM3 BLT mice (Fig 1, D and E). Most of these human CD45⁺ cells in the PECs of NSG-SGM3 BLT mice were CD33⁺ (Fig 1, F and G). Within

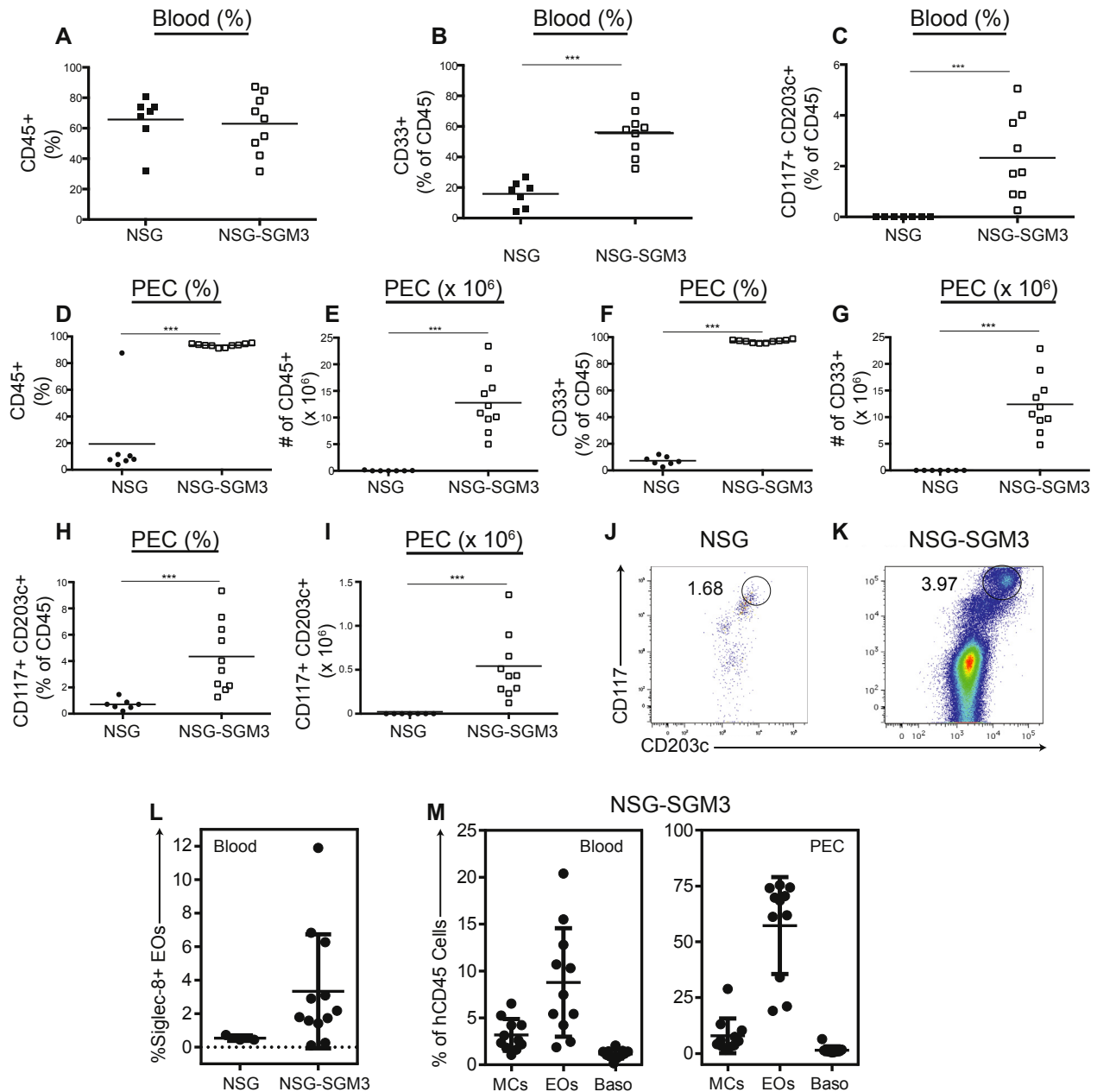


FIG 1. Human cells in the blood and peritoneal lavage of NSG-BLT and NSG-SGM3 BLT mice. The percentage and number of human cells in the blood and PECs 8 to 10 weeks after transplantation (**A-I**). **J** and **K**, Representative histograms. **L**, Siglec-8+ EOs cells. **M**, Percent CD45+ cells that are Siglec-8+ mast cells (MCs), eosinophils (EOs), or basophils (Baso). Horizontal bars represent mean percent. *** $P < .001$ (Mann Whitney). Blood: $N = 7$ for NSG-BLT and $N = 9$ for NSG-SGM3 BLT. PECs: $N = 7$ for NSG-BLT and $N = 10$ for NSG-SGM3 BLT.

this human CD45+ population, a significant number of cells were CD117+CD203c+ (Fig 1, **H** and **I**). Representative histograms of human CD45+ PECs stained with CD117 and CD203c in NSG-BLT and NSG-SGM3 BLT mice are shown (Fig 1, **J** and **K**).

Siglec-8 is a late maturation marker detectable on mast cells, on eosinophils, and at low levels on basophils in humans.²⁰ The human CD45+ cells in NSG-SGM3 BLT mice were analyzed for their expression of Siglec 8 to define their level of maturation. Many of the blood cells and PECs in NSG-SGM3 BLT mice that expressed Siglec 8 were eosinophils, whereas only few PECs in NSG-BLT mice were Siglec 8+ (Fig 1, **L**). To further define the Siglec 8+ cells, we costained with an antibody recognizing

FcεRI. Three populations of cells were observed in the blood and PECs of NSG-SGM3 BLT mice (Fig 1, **M**): FcεRI+ Siglec-8low (putative basophils), FcεRI+ Siglec-8+ (putative mast cells), and FcεRI− Siglec-8+ (putative eosinophils).

Tissue distribution of human mast cells in NSG-SGM3 BLT mice

To further investigate the distribution of human mast cells, tissues from NSG-BLT and NSG-SGM3 BLT mice were recovered and stained for human tryptase, a marker of human

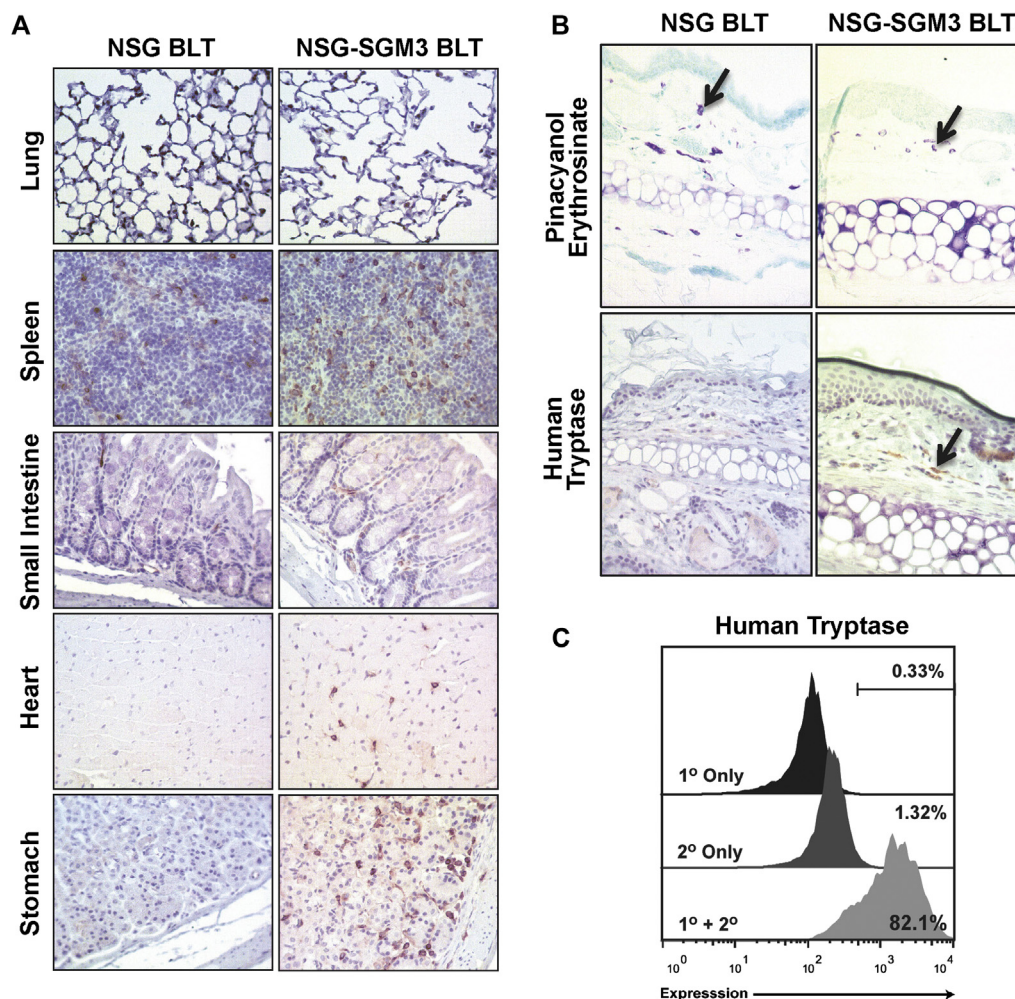


FIG 2. Representative images of tissues of NSG-SGM3 BLT mice stained for human tryptase. **A**, Human tryptase-positive cells (brown) in NSG-BLT and NSG-SGM3 BLT mice. **B**, Mast cells by pinacyanol erythrosinate and tryptase staining. **C**, PECs cultured for 32 days were >90% mast cells by CD117 and FcεRI expression and were stained with human tryptase antibody. Data are representative of PECs from pooled cohorts evaluated in 2 independent experiments.

mast cells.²¹ Human tryptase-positive cells were observed in the lung and spleen of both NSG-BLT and NSG-SGM3 BLT mice (Fig 2, A). Although small numbers of tryptase-positive cells were seen in the small intestine of NSG-BLT mice, these were present in higher numbers in NSG-SGM3 BLT mice tissues.

In addition, high numbers of human tryptase-positive cells were detected in the cardiac and stomach tissues of NSG-SGM3 BLT mice but were absent in similar tissues of NSG-BLT mice (Fig 2, A). Tissues from nonhuman cell-engrafted NSG and NSG-SGM3 mice were uniformly negative for human tryptase-positive cells (data not shown). Focusing on the skin, because previous humanized mouse studies have described PCA reactions, we initially used pinacyanol erythrosinate staining to show the presence of mast cells within the skin tissues of ears for both NSG-BLT and NSG-SGM3 BLT strains (Fig 2, B, upper panels). Quantifying the number of human mast cells by tryptase staining in the truncal skin of 3 NSG-SGM3 BLT mice, we observed an average of 15.2 ± 1.4 per each of ten 400× fields examined in each mouse. However, staining for human tryptase demonstrated that human mast cells were absent in the skin of NSG-BLT mice (Fig 2, B, lower panels). In addition, cultured

PECs from NSG-SGM3 BLT mice contained high numbers of human tryptase-positive cells (Fig 2, C).

Mast cells in NSG-SGM3 BLT mice express CD117 and FcεRI

Human mast cells are known to express high levels of CD117 and FcεRI.²² As shown in Fig 3, a distinct population of human PECs expressed CD117. This population of CD117+ cells also expressed high levels of CD203c and FcεRI and lower levels of IL5RA, CD69, CD88, and CD32. They were negative for expression of CD64, CD16, CD49d, CD163, CD3, CD20, and IgE (Fig 3). These phenotypic characteristics are similar to those of mast cells in the blood of humans.²²

Culture of PECs from NSG-SGM3 BLT mice in the presence of human stem cell factor enriches for human mast cells

We next determined whether we could generate sufficient numbers of primary human mast cells from the PECs of NSG-SGM3 BLT

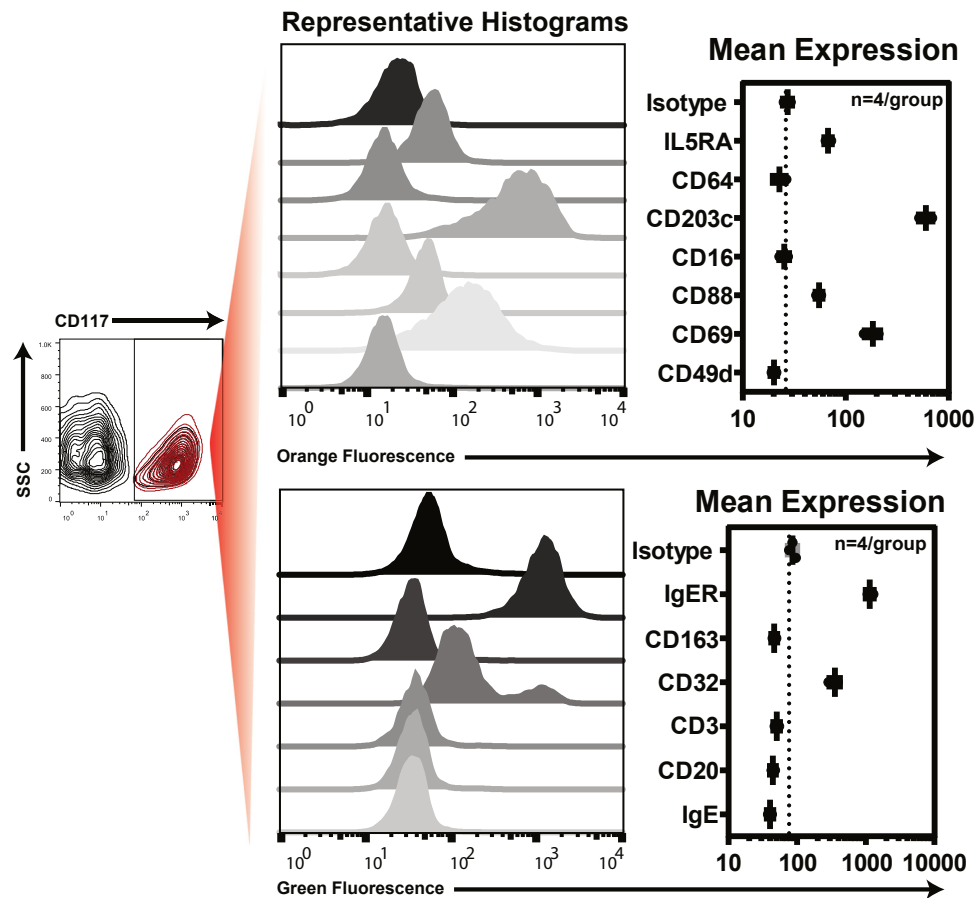


FIG 3. Phenotype of human CD117+ cells in the peritoneal exudate of NSG-SGM3 BLT mice. Peritoneal cells pooled from 4 NSG-SGM3-BLT mice were recovered and labeled with CD117 (left histogram) and human-specific antibodies of the indicated surface receptors. Representative overlay plots are shown for cells gated on CD117+ population (middle column). Scatter graphs of mean fluorescence intensity, a measure of mean expression level of each cell surface receptor, are plotted (right column). SSC, Side scatter.

mice for *in vitro* studies (Fig 1). We first observed that human stem cell factor (SCF) in culture was essential (Fig 4, A). A 16-hour short-term culture in the presence of human SCF led to an increase in the purity of human mast cells but not in their number (Fig 4, B). At 12 days, we were able to obtain 7 to 11×10^6 human mast cells of 90% or more purity in culture from PECs of 4 to 12 NSG-SGM3 BLT mice (Fig 4, B). These human mast cells were phenotypically identical to primary human mast cells present in the bone marrow and skin of individuals.

NSG-SGM3 BLT peritoneal lavage mast cells degranulate in an IgE-dependent and -independent manner

NSG-SGM3 BLT day 18 cultured PEC mast cells were sensitized overnight with 100 ng/mL of chIgE anti-NP, which was verified by monitoring IgE (Fig 5, A, upper panel) and FcεRI (Fig 5, A, lower panel) on the surface of mast cells. These overnight sensitized mast cells degranulated and released β-hexosaminidase into the supernatants in a dose-dependent manner with EC₅₀ of 8.3 ng/mL of chIgE-anti-NP using a fixed concentration of 50 ng/mL chIgE-anti-NP (Fig 5, B). Sensitized

mast cells loaded with fluo-4 NW initiated an intracellular calcium flux after challenge with NP-BSA (Fig 5, C and D). Maximal degranulation and calcium flux in the PECs was quantified by addition of ionomycin (Fig 5, B, C, and D). Function of the human mast cells was further confirmed by their response to non-IgE-mediated molecules, including C3a, C5a, and Substance P (Fig 5, E and F). These data document that the human mast cells recovered from the peritoneal cavity of NSG-SGM3 BLT mice are phenotypically similar to primary human mast cells, bind human chIgE, and can functionally respond in an IgE-dependent manner by calcium flux, degranulation, and release of β-hexosaminidase.

NSG-SGM3 BLT mice develop an antigen-specific IgE-dependent PCA reaction

In the PCA assay, no ear swelling was observed in NSG-BLT mice at any time point examined (Fig 6). In contrast, ear swelling was clearly evident in the ears of NSG-SGM3 BLT mice sensitized with NP-BSA but not in the PBS-injected ear at 1 hour after challenge. As expected, ear swelling was also observed in the sensitized but not PBS-injected ears of NSG-SGM3 BLT mice at 24 hours (Fig 6). No ear swelling was observed using the human-specific agents when 2 nonengrafted NSG-SGM3 mice were tested in the PCA assay, confirming the requirement for

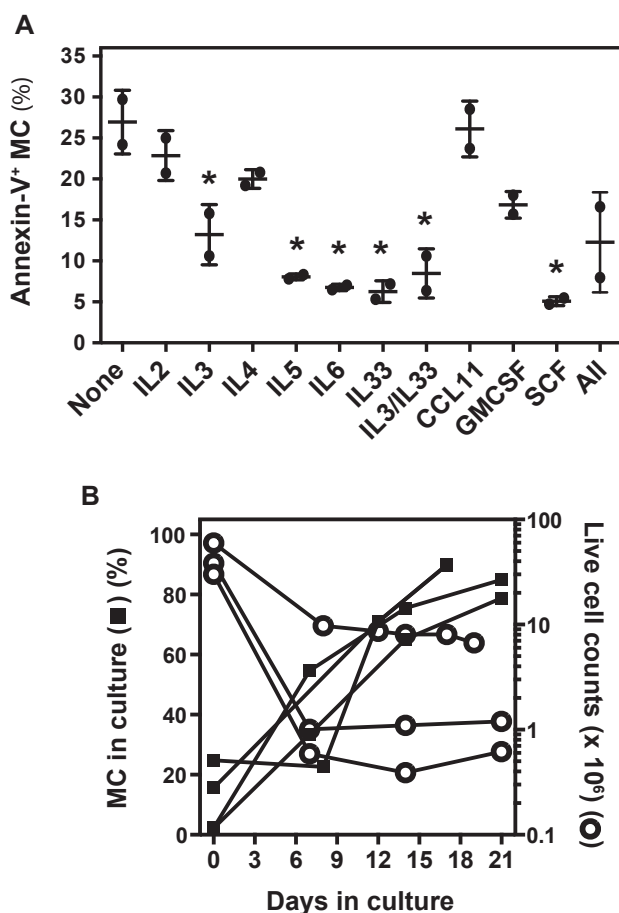


FIG 4. Culture of human mast cells from the peritoneal cavity of NSG-SGM3 BLT mice. **A**, PECs from NSG-SGM3 BLT mice cultured for 16 hours in the presence of human cytokines (100 ng/mL). The percentage of mast cells (gated on FcεRI, >70% of total cells) with surface Annexin-V binding was quantified. Cytokines that supported mast cell survival (* $P < .05$). **B**, Representative experiments cultured in the presence of SCF for up to 21 days (3 independent experiments, $N = 11, 5, 5$).

human mast cells to develop a PCA reaction in this model (data not shown).

NSG-SGM3 BLT mice develop an antigen-specific IgE-dependent PSA reaction

In the PSA assay, systemic anaphylaxis in sensitized NSG-SGM3 BLT mice was observed within 10 minutes of challenge (Fig 7). All IgE-primed mice demonstrated a decline in body temperature and increased symptom scores, with fatal PSA responses being observed in 2 mice.

DISCUSSION

In humanized mice generated by injection of human CD34+ HSC, engraftment is predominately in the peripheral lymphoid tissues with few human cells populating the mucosal or peripheral tissues where mast cells typically reside. When thymic fragments are implanted before injection of autologous CD34+ HSC (BLT model), a robust human mucosal as well as a peripheral tissue immune system is generated. Moreover, the absolute number of human mast cells generated is higher in

NSG-SGM3 BLT mice than is observed in NSG-SGM3 CD34+ cell-engrafted mice (unpublished observations, data from Michael Brehm, 2016). Therefore, our experiments were performed using NSG-SGM3 BLT mice. Furthermore, humanization of NSG-SGM3 did not change the numbers of murine mast cells (unpublished observations, data from Michael Brehm, 2016). In both the BLT and CD34+ cell engraftment models, the generation of myeloid cells, including mast cells, is poor without the addition of species-specific cytokines required for human myeloid cell development. The use of NSG-SGM3 BLT mice allows robust engraftment of human mast cells in hematopoietic tissues as well as multiple organs and peripheral tissues.

Due perhaps to the rise in anaphylactic diseases such as food allergy, there has been significant interest in developing a mouse model of human mast cell-mediated anaphylaxis. These efforts have led to a recent report based on human CD34+ HSC engraftment into NOG mice transgenically expressing human IL-3 and GM-CSF.⁶ The authors used this model to demonstrate a passive PCA reaction defined by extravasation of Evans Blue dye; ear swelling was not quantified.⁶ We reasoned that addition of SCF (also known as KIT-ligand), important in mast cell development, would enhance mast cell development in mice also expressing IL3 and GM-CSF. Indeed, NSG-SGM3 BLT mice develop high numbers of human mast cells in multiple tissues, including sites of mucosal barriers and cardiac tissue. We do not know how the mast cell numbers we observed in the NSG-SGM3 BLT mice correspond to that observed in humans because mast cell numbers in human tissues can dramatically differ between individuals.²³ These mast cells have many phenotypic markers that are similar to those in humans, and functionally respond in an IgE-dependent and -independent manner. Sensitization and intradermal antigen challenge of NSG-SGM3 BLT mice leads to a strong PCA swelling reaction and for the first time we documented the ability of humanized mice to generate a robust PSA reaction. Moreover, these mice had high numbers of human mast cells in the peritoneal cavity of NSG-SGM3 BLT mice, which are a source of human mast cells that can easily be recovered and used in *ex vivo* experiments.

Human mast cell development in NSG-human SCF transgenic mice engrafted with HSC has been reported, but no functional analyses were performed.²⁴ Additional articles reporting mast cell development in humanized mice include small numbers present in HSC-engrafted NSG and NOG mice, which were too low in number for *in vivo* functional assays.^{25,26} Furthermore, previous studies using humanized NSG-SGM3 mice have not studied human mast cell development or function.^{8,19,27} Finally, Weigmann et al²⁸ have described an adoptive transfer model in which human PBMCs obtained from individuals with various allergic responses were injected into NSG mice followed by challenge with the allergen of interest orally or rectally. The mice showing circulating human IgE and displaying T-cell responses to the allergens also demonstrated histological inflammation of the colon after challenge, suggesting that this model may be used to study allergen-associated IgE-dependent colitis.²⁸

Importantly, the inducement of robust anaphylactic reactivity in NSG-SGM3 BLT mice supports the concept that the downstream mediators of anaphylactic responses are conserved because the human mast cell-derived mediators must work via the murine receptors to produce these anaphylactic responses. A likely mediator for such effects would be the bioactive amine

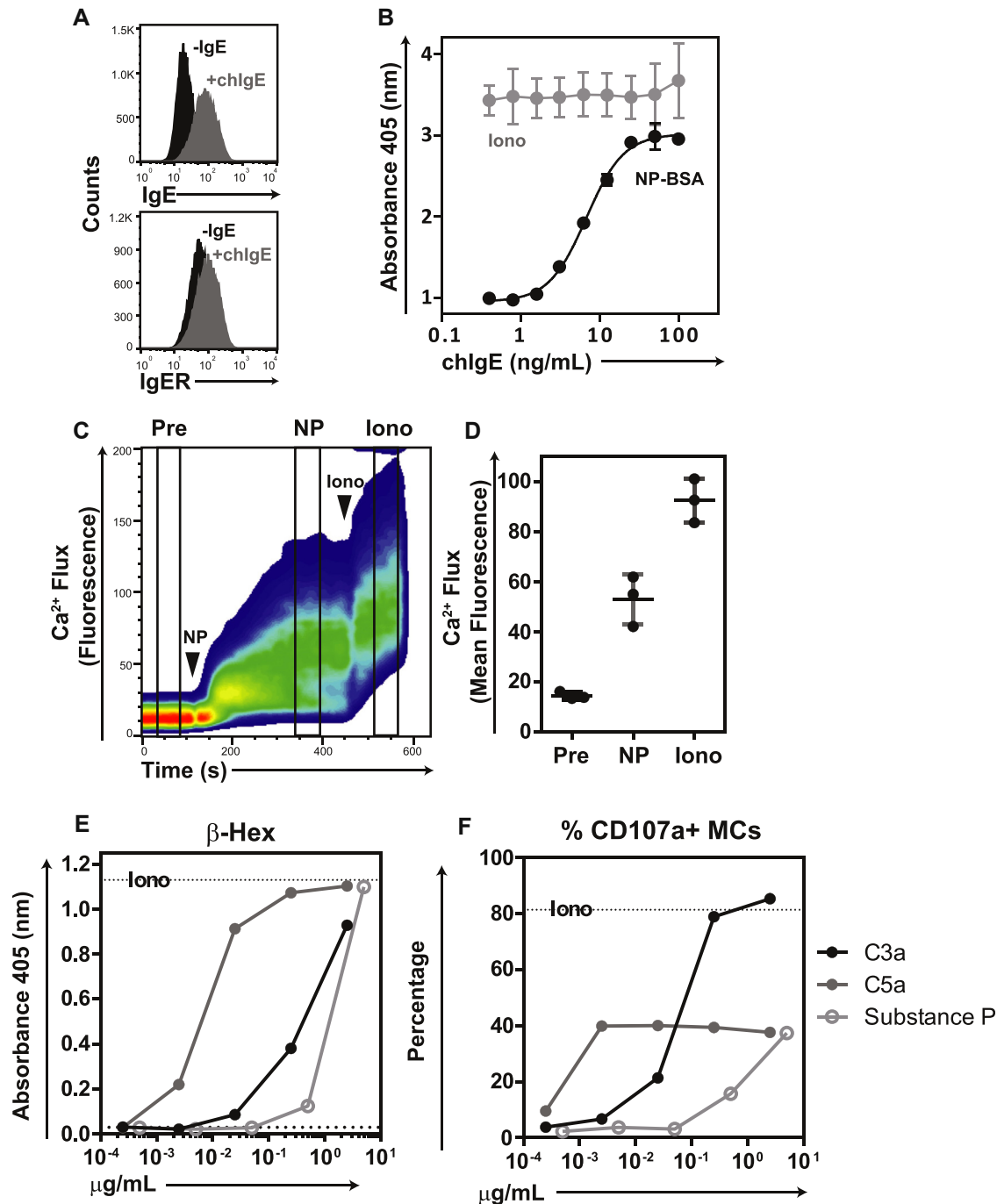


FIG 5. NSG-SGM3 BLT PECs bind human IgE, and degranulate in an IgE-dependent and -independent manner. Histograms of sensitized NSG-SGM3 BLT PEC cultures (>90% mast cells) (**A**) incubated with NP-BSA or lono (**B**). **C**, Calcium flux of panel A cells. **D**, MFI replicates of panel C. **E**, NSG-SGM3 BLT PEC mast cell day 34 cultures (20,000 mast cells/well) treated with ionomycin (2 μM) or varying concentrations of Substance P, C3a, C5a. **F**, Percentage of CD107a+ cells. lono, ionomycin, representative of 2 independent experiments; MFI, mean fluorescence intensity; NP, NP-BSA; pre, baseline values.

histamine because it is conserved across species and stored within mast cell granules, but these granules also contain several protease mediators.²⁹ Moreover, in the NOG-IL3/GM-CSF model, complement C3 was implicated in the extravasation of Evan's blue dye following antigenic challenge.⁶ In addition, various species show conserved lipid-derived mediators that are synthesized from arachidonic acid by mast cells, including

prostaglandins and leukotrienes. Cytokines, chemokines, and growth factors are also released by activated mast cells but these are unlikely to be contributing to anaphylactic responses within the immediate reactivity and are generally more species diverse. However, of note is that the late-phase response in the PCA model occurred and this has been shown to be regulated via cytokine products, including TNF.³⁰ Chemokines released by human

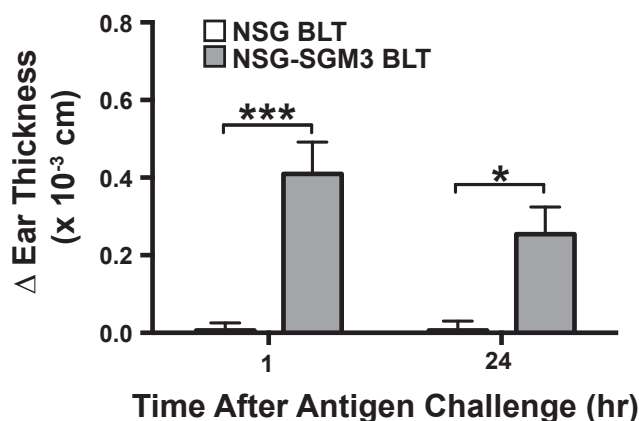


FIG 6. NSG-SGM3 BLT mice exhibit pronounced PCA upon antigen challenge. NSG-BLT and NSG-SGM3 BLT were sensitized intradermally with human chIgE-anti-NP and 24 hours later injected intravenously with NP-BSA as described in the [Methods](#) section. The ear thickness of the ears was determined at 1 and 24 hours after challenge. * $P < .05$ and *** $P < .001$.

mast cells include CCL5 and CXCL8 and growth factors include vascular endothelial growth factor, GM-CSF, and importantly SCF. The transgenic expression of SCF in the NSG-SGM3 mice permits high levels of human mast cells to develop independently of their activation. However, it remains to be determined which of these many downstream mediators of PCA and PSA reactions are species cross-reactive and are ultimately responsible for the robust anaphylactic reactions observed. Furthermore, the role of basophils in such models has remained elusive and requires further investigation.

We observed the engraftment of human mast cells in multiple tissues. Mast cells are often classified as to their location or protease subtype, and typically are grouped as mucosal or connective tissue-type subpopulations of mast cells.³¹ The presence of mast cells in the spleen, lung, skin, and small intestine has been commonly considered one of the front lines of host immune defense against environmental pathogens.²² The development of the NSG-SGM3 BLT model containing high levels of human myeloid cells including mast cells, particularly in the mucosal and connective tissues, now provides a potential model for defining the roles of mast cells in defense against key human pathogens because mast cells have been suggested to provide antibacterial, antihelminth, and antiviral responses to human-specific pathogens that cannot otherwise be studied in small animal models. Furthermore, the role of mast cells as a reservoir for HIV is a concept that has been suggested,^{32,33} but remains uncertain,³⁴ and could be tested directly in this model.

The presence of mast cells in the cardiac tissue is interesting because these cells would not be in “the front line” of immune defense and are considered as somewhat of a separate lineage of mast cells from the typical connective and mucosal types.²⁹ Cardiac mast cells have been associated with the development of cardiac fibrosis, cardiovascular disease, and myocardial remodeling.³⁵ They also appear critical in the development of pathological cardiac responses to Hymenoptera stings from Apoidea (bees), Vespoidea (wasps, hornets, and yellow jackets), and Formicidae (ants), which result in an increased load of cardiac mast cells together with coronary stenosis favoring myocardial hypoxia.³⁶ The role of mast cells in mediating these

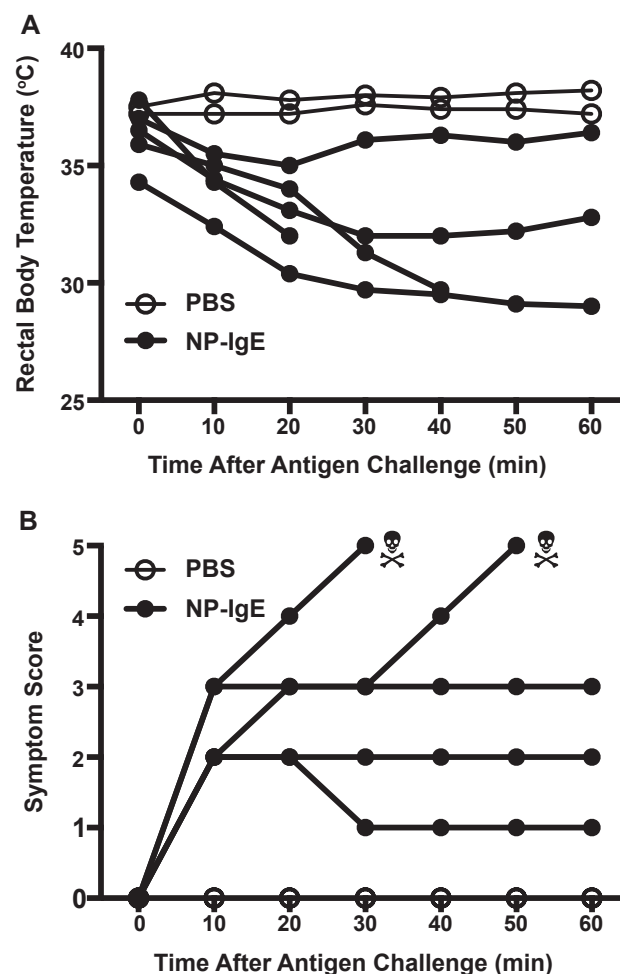


FIG 7. NSG-SGM3 BLT mice exhibit pronounced PSA upon antigen challenge. NSG-SGM3 BLT mice were sensitized by intravenous injection of chimeric human IgE anti-NP antibody and challenged 24 hours later by intravenous injection of NP-BSA in PBS. Systemic anaphylaxis was measured by monitoring rectal body temperature (**A**) and by observable symptom scores (**B**), as described in the [Methods](#) section.

responses can now be more easily investigated. There is the potential to model allergic diarrhea in NSG-SGM3 BLT mice, but, because of the experimental length of these models (eg, Osterfeld et al³⁷) and the established issues because of the development of graft versus host disease with NSG-SGM3 BLT mice as they age,¹² such experiments would be difficult and fraught with variability.

Humanized mouse models have several limitations in the B-cell compartment that make it unlikely that antigen-specific IgE would be generated within the mice. Therefore, active immunization to induce an IgE antibody response will be an extremely difficult approach for testing food or environmental allergens in NSG-SGM3 BLT mice. An alternative approach could be using serum from polysensitized patients. In this approach, using the methodology that we have used for the PSA but with challenges of various allergen extracts, the allergens triggering the most severe reactions might be determined without the need for patient challenges.

Previous approaches to obtain enough human mast cells for *ex vivo* study have involved complex manipulations of tissue digestion, culturing, directed differentiation of stem cells,

and/or purification. For example, reports of directed differentiation of human mast cells from embryonic stem cells have been described, but requires complex and long-term culturing with expertise in both embryonic stem cell culture and expensive recombinant factors for the directed differentiation into mast cells.³⁸ Embryonic stem cell–derived human mast cells have many of the phenotypic and functional responses of mast cells, but may or may not be completely mature. Additional approaches generate human mast cells from different hematopoietic cell populations such as CD34+ or CD133+ progenitor cells isolated from cord blood or peripheral blood cells. These cultures typically take 6 to 7 weeks of differentiation in cytokine-supplemented medium,^{39–41} but a recent report suggested that human mast cell populations could be generated from human CD34+ blood cells after only 3 weeks of culture but only 4% to 20% of the mast cells appeared to be mature mast cells that contained tryptase and chymase, and expressed FcεRI and CD117.

Isolation of primary human mast cells has been described.^{42–45} The benefits of using primary human mast cells compared with mast cell lines or *in vitro* differentiated mast cells is clear and not limited to realistic levels of protein expression (tryptase, FcεR1, Substance P receptor, Complement receptor, etc) and robust effector responses.^{45,46} The protocols for isolation of primary human mast cells, however, are time/resource consuming and dependent on access to and availability of donor human skin or lung. Even when sources are available, human skin isolated mast cell yields are poor and highly variable ($0.1\text{--}1 \times 10^6/10\text{g}$ tissue).^{42,43} Expansion protocols have been developed allowing for recovery of approximately 15×10^6 mast cells (~100-fold expansion) after 8 weeks of culture but quantity and reliability are still inadequate.⁴²

A major technical advance of the NSG-SGM3 BLT model is that it now provides ready availability of human mast cells for *ex vivo* experiments without the need for tissue digestion. Large numbers of human mast cells are resident in the peritoneal cavity and can easily be recovered for culture. Also, long-term expansion cultures are not necessary because on average 1×10^6 mast cells are recovered per PEC lavage of individual mice. Although we show using the current short-term culture conditions that mast cell numbers do not increase in culture over time, we describe a culture system that rapidly leads to highly enriched large numbers of human mast cells. Overall, we found that the reactivity of human mast cells recovered from NSG-SGM3-BLT mice is superior to the published activity of primary human mast cells and human mast cells cultured from progenitors.⁴⁷ Moreover, we demonstrate *in vivo* functional human mast cell activity in NSG-SGM3 BLT mice using both the PCA and PSA assays.

Conclusions

We have established a robust humanized mouse model of human mast cell engraftment and function that provides models of human mast cell–mediated PCA and PSA reactions. Furthermore, high numbers of human mast cells can be recovered from the peritoneal cavity and cultured *in vitro*, providing a readily accessible source of human mast cells for study. NSG-SGM3 BLT mice will be important tools for investigation of human mast cell biology and for preclinical analysis of the efficacy of new therapeutics for anaphylaxis reactions before their advancement to the clinic.

Key messages

- NSG-SGM3 BLT mice engrafted with human HSCs develop high numbers of functional human mast cells.
- Human HSC-engrafted NSG-SGM3 BLT mice generate robust PCA reactions and robust PSA reactions.

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