Online Repository Material for: *A humanized mouse model of anaphylactic peanut allergy*

METHODS

Mice

WT BALB/c and IgE$^{-/-}$ (*Igh-7*$^{-/-}$) mice were bred under specific pathogen-free conditions in a biosafety level 1 facility. Age-matched controls were used for experiments at 8-12 weeks of age.

Passive anaphylaxis

IgE-mediated anaphylaxis was assessed following passive sensitization of IgE$^{-/-}$ mice with mouse or human IgE. Mice were injected intravenously via the retro-orbital sinus with 10µg IgE from mouse (IgE anti-DNP clone SPE-7, Sigma-Aldrich) or human (chimeric IgE anti-NP clone JW8/1, AbD Serotec), and challenged 72hrs later by intraperitoneal injection with 500µg of the respective antigen (TNP-albumin from Sigma-Aldrich or NP-OVA from Biosearch Technologies).

Mast Cell Culture and Degranulation.

Mast cells were cultured from humanized mouse splenocytes or bone marrow under stimulation with recombinant human IL-3 and SCF (100ng/ml each, Shenandoah Biotechnology) with weekly passage. Purity was assessed by flow cytometry, staining against human FcεRI and c-Kit. Sera from peanut-allergic donors were collected under IRB protocols approved by Boston Children's Hospital, with written informed consent from all donors[1]. Mast cell degranulation assays using LAMP-1 as a read-out were performed according to procedures we have established for mouse and human basophil activation[1]. Mast cells were sensitized overnight with peanut-allergic serum containing 12 kU/L anti-PN IgE in 100µl final volume. Cells were stimulated for 10min at 37℃ with 4 µg/ml aqueous peanut extract in the presence of anti-LAMP-1 antibody (0.5µg/ml). Cells were promptly washed with cold buffer containing azide and analyzed by flow cytometry, gating on c-Kit^{+} viable cells.

Immunization and recall

huNSG mice were immunized to tetanus by i.p. injection with 1µg tetanus toxoid (Calbiochem) adsorbed to 1mg alum (aluminum hydroxide gel, Sigma-Aldrich) in saline on days 0 and 14, followed by weekly i.p. injections with 1µg tetanus toxoid. After 8 weeks, mice were sacrificed and splenocytes were fractionated using magnetic selection into mouse $CD11c^+$, human $CD11c^+$ and human $CD4^+$ cell types. For this purpose, mouse and human Fc gamma receptors were blocked using anti-mouse CD16/32 (clone 93) and human TruStain FcX, followed by staining with PE-conjugated antibodies against mouse CD11c (N418), human CD11c (Bu15) or human CD4 (OKT4) (all Biolegend). Anti-PE microbeads were then used prior to isolation over LS columns (Miltenyi Biotec). Dendritic cells (10^4) were pulsed overnight with 1µg/ml tetanus toxoid or 100µg/ml aqueous peanut extract (made as previously described[2]). Isolated T cells were labeled with CellTrace Violet (Invitrogen/ThermoFisher) according to the manufacturer's instructions, and $2x10^5$ cells from immune or non-immune mice were plated with pulsed dendritic cells. Soluble anti-CD3 (2µg/ml, OKT3, Biolegend) was added as a positive control. After 7 days, cells were stained for CD3, CD4 and viability, and proliferation was assessed in the $CD4^+CD3^+$ T cells.

Flow Cytometry

The following additional antibodies were used in flow cytometry experiments for the data in the online repository: APC anti-CD19 (HIB19, Biolegend), PE anti-IL-10 (JES3-19F1, Biolegend), Alexa Fluor 647 anti-LAMP-1 (H4A3, Biolegend), FITC anti-mouse $I\text{-}A^k/I\text{-}A^{g7}$ (10-3.6, Biolegend), PE-Cy7 anti-HLA-DR (L243, Biolegend), PE anti-human CD11c (Bu15, Biolegend), PE-Dazzle594 anti-mouse CD11c (N418, Biolegend), FITC anti-mouse c-Kit (2B8, BD Pharmingen), PerCP-Cy5.5 anti-mouse CD49b (DX5, Biolegend), PE-Cy7 anti-mouse FcεRI (MAR-1, Biolegend), and PE-Cy7 anti-human CD4 (OKT4, Biolegend).

ELISAs

Total serum immunoglobulin levels were assessed by direct ELISA. Immunosorp ELISA plates (Nunc) were coated overnight with sera diluted in PBS, washed, blocked with 2% BSA and specific isotypes were detected with the following biotinylated isotype-specific antibodies: IgG1 (G17-1, BD Pharmingen), IgG2 (G18-21, BD Pharmingen), IgG3 (HP6047, Invitrogen/Thermo Fisher), IgG4 (G17-4, BD Pharmingen), IgA1/2 (G20-359, BD Pharmingen), IgD (goat polyclonal, Southern Biotechnology), IgM (goat polyclonal, Southern Biotechnology), and IgE (G7-26, BD Pharmingen). Purified IgM (Sigma-Aldrich), IgD (Athens Research), IgG1 (Sigma-Aldrich), IgG3 (Sigma-Aldrich), IgE (Abcam) and IgG4 (Affymetrix eBioscience) were purchased for use as standards. Chimeric IgG2 (clone JW183) and IgA (JW393A) for use as standards were obtained from AbD Serotec. PN-specific IgG isotypes were assessed by capture onto plates coated with peanut extract and detected using the isotype-specific antibodies described above. PN-specific IgG levels were quantified relative to a standard curve developed from peanut-allergic patient serum for which the isotype-specific levels of anti-peanut immunoglobulins had previously been roughly determined[1]. Total murine IgE was measured using goat polyclonal anti-mouse IgE (Southern Biotechnology) to capture and biotinylated anti-mouse IgE (R35-92, BD Pharmingen) to detect with IgE anti-TNP as a standard (clone C38-2, BD Pharmingen). Mouse mast cell protease-1 (MMCP-1) was quantified by ELISA according to the manufacturer's instructions (Affymetrix eBioscience).

Histology.

Tissue samples were fixed overnight in 10% formalin (Sigma-Aldrich) prior to processing and paraffin embedding at the Beth Israel Deaconess Medical Center Histology Core. Chloroacetate esterase staining with hematoxylin counter stain was performed as previously described[3]. Photomicrographs were taken through a Nikon E800 Eclipse microscope. Mast cells were

counted by an observer unaware of the samples' identities, and normalized to tissue area as measured using Image J software.

FIGURE LEGENDS

Figure E1. Analysis of mast cells in NSG SCF mice. CAE-stained sections of paraffin-embedded tissue are shown with insets highlighting example mast cells. Jejunal micrographs were taken at 20x magnification (scale bar indicates 100μm).

Table E2. Comparison of mast cell burden in huNSG mice to literature-reported values on adult humans.

	Human literature values		Experimental mouse values	
	Healthy	Atopic/Disease	NSG SCF	huNSG
Lung	44±8[4] 44.7[5] 155±21[6]	84±7[4] 91-102[5] 270±51[6] 250±60[7]	4±1	67±55
Intestine	187±23[8] 146±29[9] 38±13[10] 268[#11] 47[§11]	413±139[8] 243±41[9]	0 (n.d.)	256±140*
Stomach	60±25[12]	135±103[12]	13±8	111±24*
Skin	38.4±4[13] 48±3[14] 50±2[14]	71.8±13[13A] 596.5±278[13B] 720.6±176[13C] 142±69[15]	57±10	98±6*

Values indicate mean±SEM counts of mast cells per mm^2 by histological analysis. n.d., not detected.
*Statistically significant increase compared to unengrafted NSG SCF mouse.

[4]Farmer's lung.
[5]Extrinsic allergic alveolitis (hypersensitivity pneumonitis), 2 patients only.
[6]Nonfatal asthma.
[7]Severe asthma.
[8]Gastritis.
[11#]Carnoy's fixative. [§]Formalin fixation.
[12]Atopic/oral allergy syndrome.
[13]A) Unexplained anaphylaxis, B) urticaria pigmentosa, C) systemic mastocytosis.
[15]Atopic dermatitis.

Figure E3. Analysis of possible contributions of murine mast cells and IgE to anaphylaxis in NSG SCF mice. **A.** Representative panels showing flow cytometric identification of residual murine mast cells in humanized NSG SCF mice. **B.** Quantification of human and murine mast cells by flow cytometry. **C.** Serum levels of murine IgE and MMCP-1 in NSG SCF and immunocompetent mice. Values from huNSG mice are from sera post-challenge with PN. **D.** Assessment of the anaphylactogenic capacity of human and mouse IgE in the murine system. IgE-deficient *Igh7*$^{-/-}$ BALB/c mice (*n*=5) were passively sensitized with human□αNP IgE or mouse IgE□α□NP IgE and challenged. Anaphylaxis was assessed by measurement of core body temperature and mast cell degranulation followed by determining serum MMCP-1 levels. *P*<0.0001 by 2-way ANOVA. **E.** Anaphylactogenic capacity of human and murine IgE in NSG SCF mice (*n*=4) without human immune engraftment. **F.** Anaphylaxis elicited by human□αNP IgE and antigen challenge in a CD34^{+} stem cell (HSC)-engrafted huNSG mouse. **G.** Serum MMCP-1 levels elicited by antigen challenge of human or murine IgE-sensitized mice. Data are representative of two independent experiments. ***P<0.001 by unpaired t test (B) or Bonferroni post-test on ANOVA (E). n.d., not detected.

Figure E4. Serum antibody levels in humanized mice. **A.** ELISA determination of total serum levels of human IgE, IgA, IgD and IgM. **B.** Serum levels of IgG isotypes. Note that omalizumab (αIgE) is an IgG1 monoclonal. **C.** Serum levels of PN-specific IgG isotypes. The data shown summarize three independent experiments.

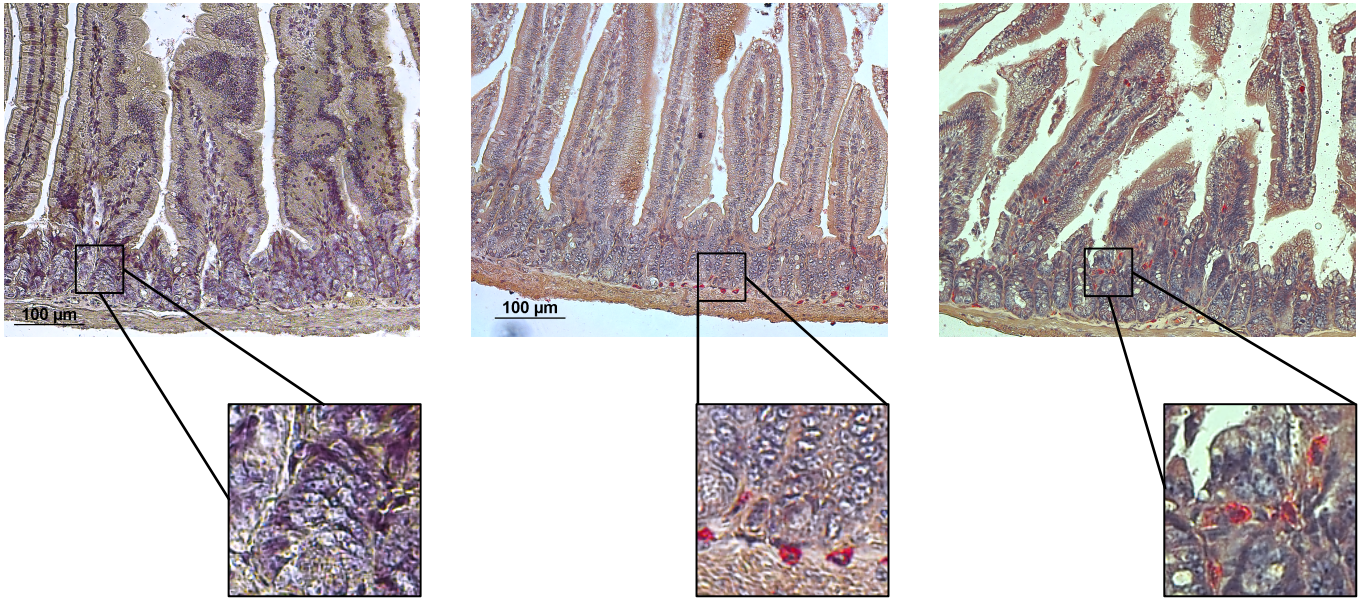
Figure E5. Analysis of murine and human dendritic cells in humanized NSG SCF mice. Flow cytometric identification of murine CD11c^{+}MHCII^{+} dendritic cells, and quantification of MHCII expression in **A.** the spleen and **B.** the jejunum. Representative flow cytometry plots showing human CD11c^{+}HLA-DR^{+} dendritic cells in the spleen and quantification of HLA-DR expression in **C.** the spleen and **D.** the jejunum. Data are representative of two experiments.

Figure E6. Presentation of antigens to human CD4^{+} T cells in humanized NSG SCF mice. Human CD4^{+} T cells from immunized or naïve humanized mice were assessed for recall proliferative responses following presentation of antigen by isolated CD11c^{+} dendritic cells of murine or human hematopoietic origin. Representative flow plots show the frequency of dividing (Violet CellTrace^{low}) cells among CD4^{+}CD3^{+} T cells after 7 days of culture. Similar data were obtained in a second experiment.

Figure E7. Culture techniques for *in vitro* experiments with human mast cell. **A.** Flow cytometry showing c-Kit and FcεRI expression in cultured human splenic mast cells. **B.** CAE-stained cultured human splenic mast cells **C.** *In vitro* degranulation (LAMP-1) following sensitization with peanut-allergic patient serum and peanut stimulation using culture splenic and bone marrow-derived mast cells. Data are representative of three independent experiments.

REFERENCES

1. Burton OT, Logsdon SL, Zhou JS, Medina-Tamayo J, Abdel-Gadir A, Noval Rivas M, et al. Oral immunotherapy induces IgG antibodies that act through FcgammaRIIb to suppress IgE-mediated hypersensitivity. J Allergy Clin Immunol 2014; 134:1310-7 e6.
2. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E signal inhibition during allergen ingestion leads to reversal of established food allergy and induction of regulatory T cells. Immunity 2014; 41:141-51.
3. Burton OT, Darling AR, Zhou JS, Noval-Rivas M, Jones TG, Gurish MF, et al. Direct effects of IL-4 on mast cells drive their intestinal expansion and increase susceptibility to anaphylaxis in a murine model of food allergy. Mucosal Immunol 2013; 6:740-50.
4. Pesci A, Bertorelli G, Olivieri D. Mast cells in bronchoalveolar lavage fluid and in transbronchial biopsy specimens of patients with farmer's lung disease. Chest 1991; 100:1197-202.
5. Heard BE, Nunn AJ, Kay AB. Mast cells in human lungs. J Pathol 1989; 157:59-63.
6. Carroll NG, Mutavdzic S, James AL. Distribution and degranulation of airway mast cells in normal and asthmatic subjects. Eur Respir J 2002; 19:879-85.
7. Balzar S, Chu HW, Strand M, Wenzel S. Relationship of small airway chymase-positive mast cells and lung function in severe asthma. Am J Respir Crit Care Med 2005; 171:431-9.
8. Beil WJ, Schulz M, McEuen AR, Buckley MG, Walls AF. Number, fixation properties, dye-binding and protease expression of duodenal mast cells: comparisons between healthy subjects and patients with gastritis or Crohn's disease. Histochem J 1997; 29:759-73.
9. Strobel S, Busuttil A, Ferguson A. Human intestinal mucosal mast cells: expanded population in untreated coeliac disease. Gut 1983; 24:222-7.
10. Bacci S, Faussone-Pellegrini S, Mayer B, Romagnoli P. Distribution of mast cells in human ileocecal region. Dig Dis Sci 1995; 40:357-65.
11. Strobel S, Miller HR, Ferguson A. Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. J Clin Pathol 1981; 34:851-8.
12. Bagnato GF, Di Cesare E, Caruso RA, Gulli S, Cugliari A, Morabito Lo Prete A, et al. Gastric mucosal mast cells in atopic subjects. Allergy 1995; 50:322-7.
13. Garriga MM, Friedman MM, Metcalfe DD. A survey of the number and distribution of mast cells in the skin of patients with mast cell disorders. J Allergy Clin Immunol 1988; 82:425-32.
14. Eady RA, Cowen T, Marshall TF, Plummer V, Greaves MW. Mast cell population density, blood vessel density and histamine content in normal human skin. Br J Dermatol 1979; 100:623-33.
15. Ackermann L, Harvima IT. Mast cells of psoriatic and atopic dermatitis skin are positive for TNF-alpha and their degranulation is associated with expression of ICAM-1 in the epidermis. Arch Dermatol Res 1998; 290:353-9.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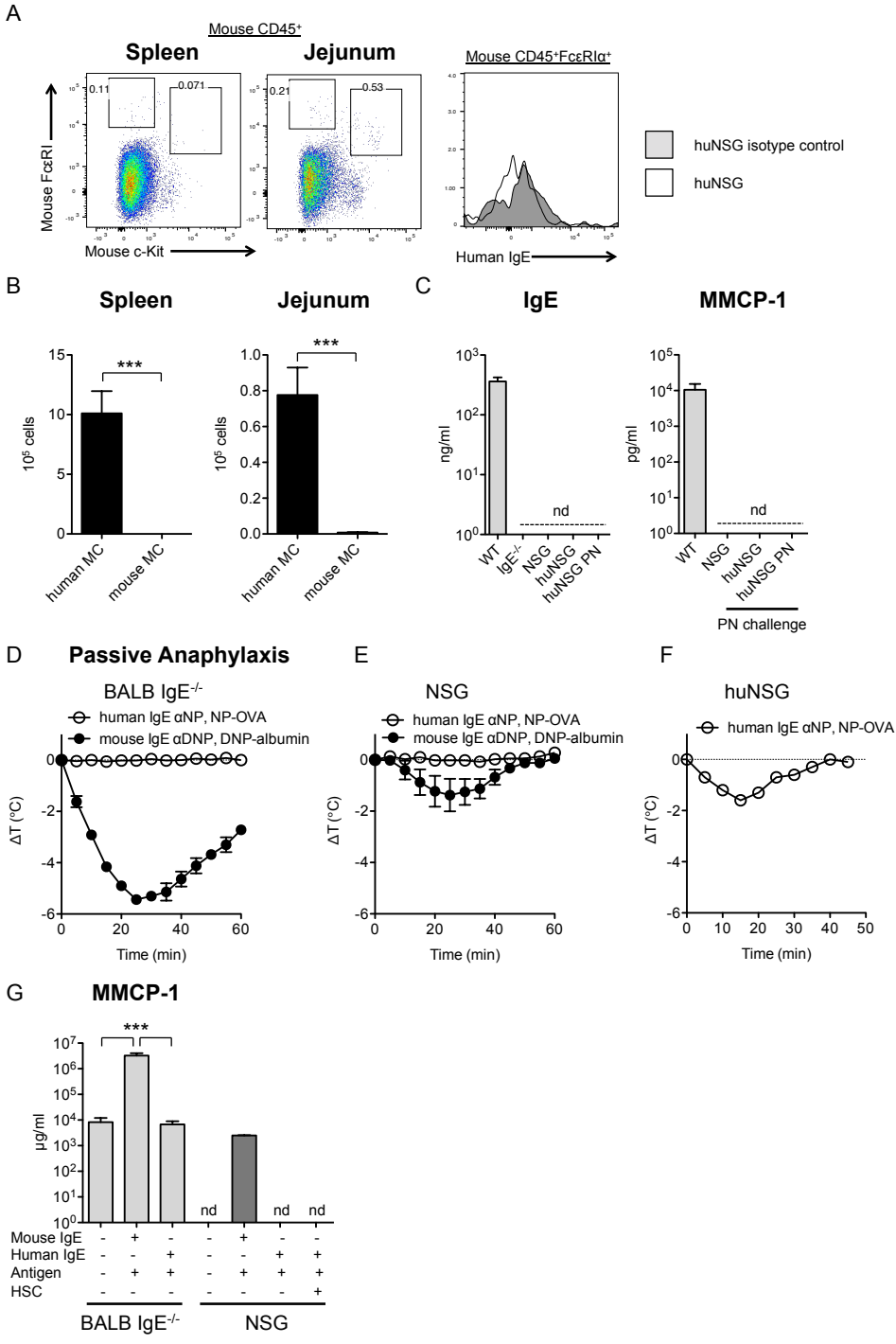
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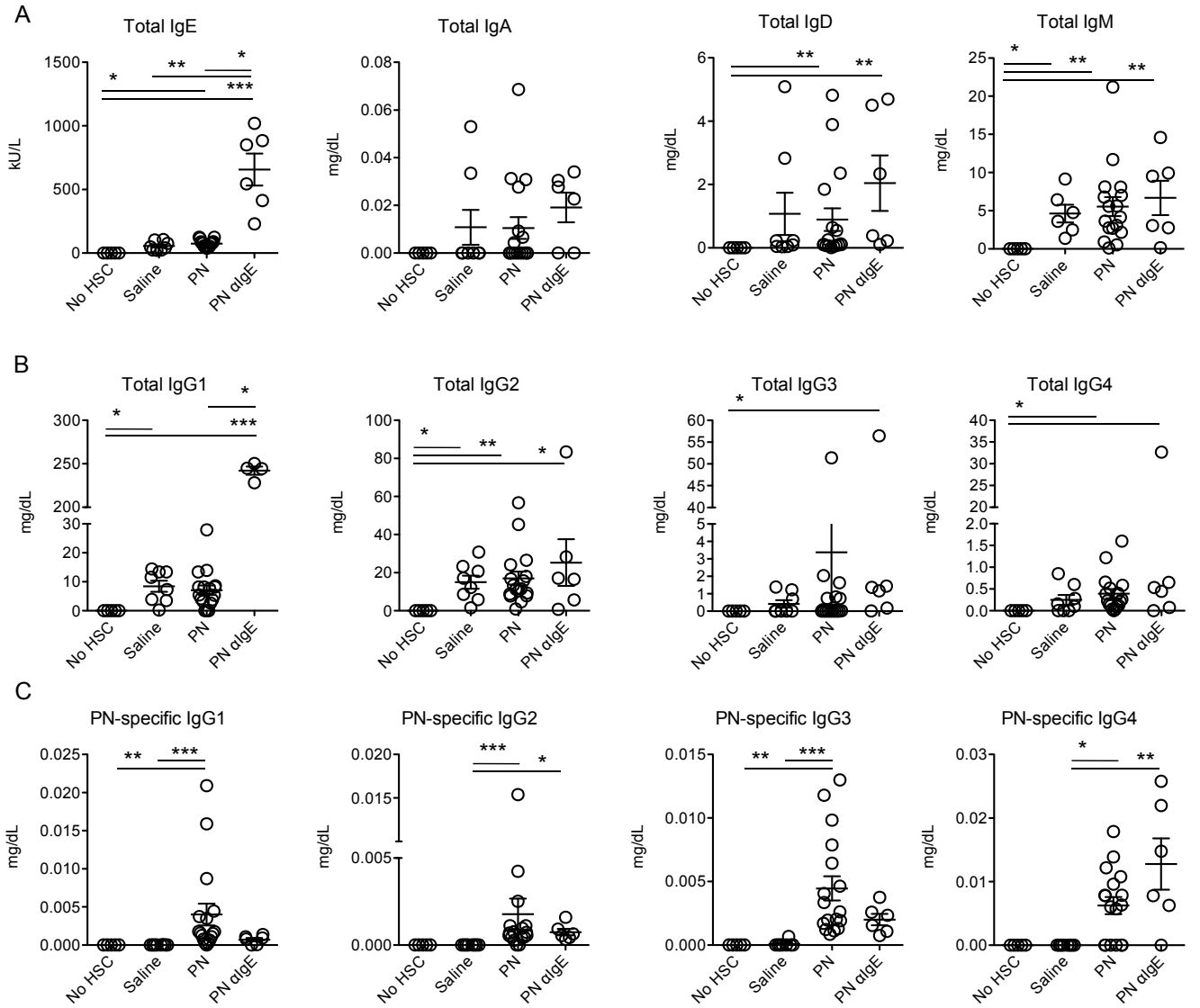
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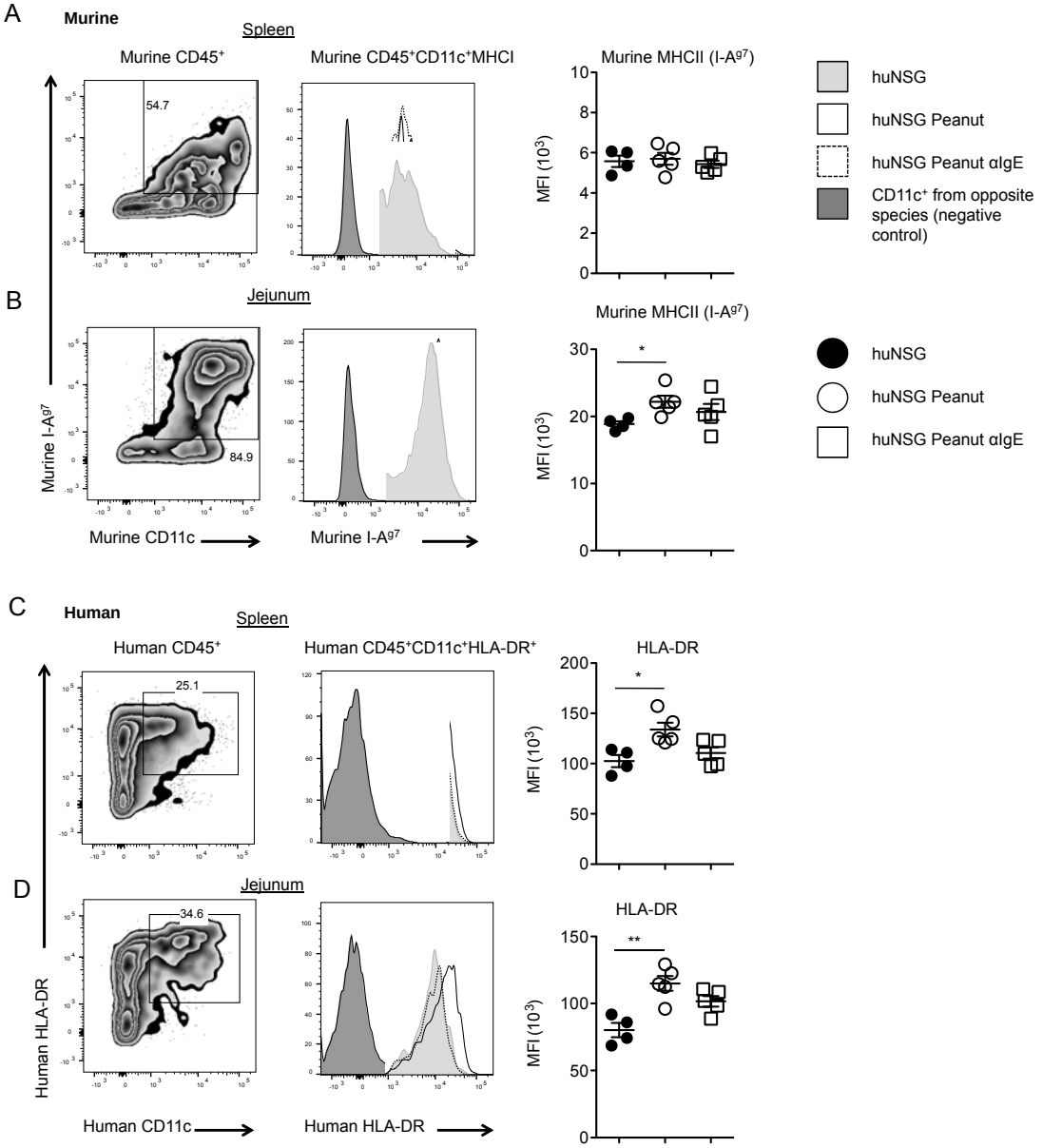
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huNSG Peanut αIgE
CD11c+ from opposite species (negative control)
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Jejunum
Murine MHCII (I-Ag7)
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Murine I-Ag7
Murine CD11c
Murine I-Ag7
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huNSG
huNSG Peanut
huNSG Peanut αIgE
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Human CD45+CD11c+HLA-DR+
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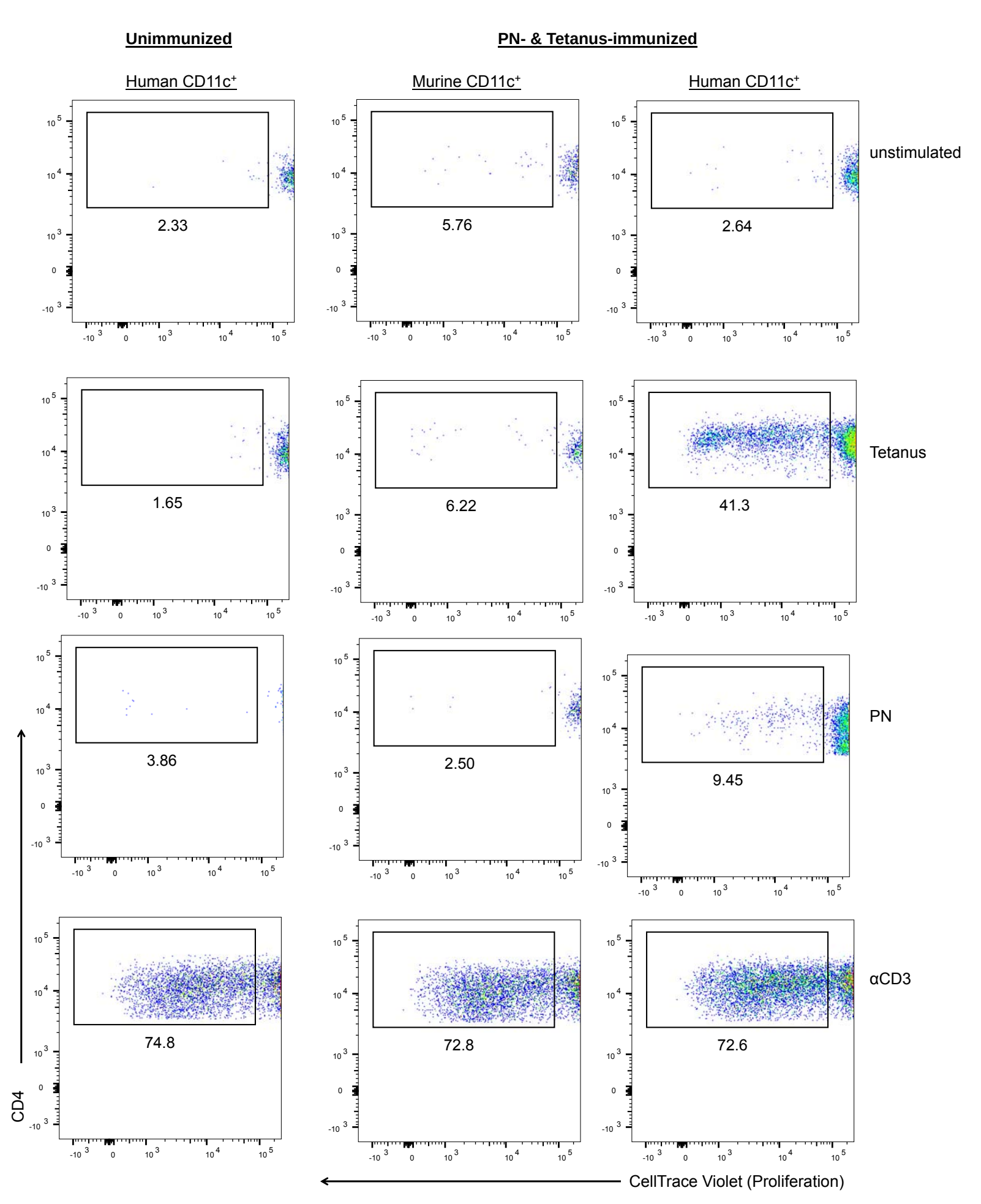
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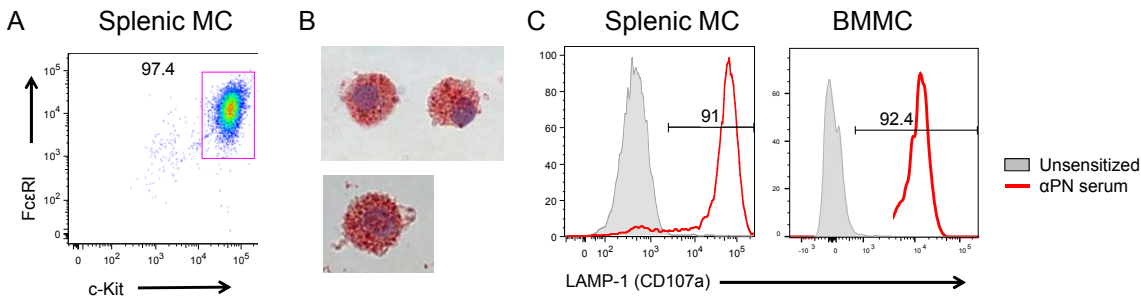
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