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IL-25 elicits a multi-potent progenitor cell population that promotes Th2 cytokine responses

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Abstract

CD4^{pos} T helper (Th) 2 cells secrete interleukin (IL)-4, IL-5 and IL-13 and are required for immunity to gastrointestinal helminth infections[1]. However, Th2 cells also promote chronic inflammation associated with asthma and allergic disorders[2]. The non-hematopoietic cell-derived cytokines thymic stromal lymphopoietin (TSLP), IL-33 and IL-25 (IL-17E) have been implicated in inducing Th2 cell-dependent inflammation at mucosal sites[3-6], but how these cytokines influence innate immune responses remains poorly defined. Here we show that IL-25, a member of the IL-17 cytokine family, promotes the accumulation of a lineage negative (Lin^{neg}) multi-potent progenitor (MPP) cell population in the gut-associated lymphoid tissue (GALT) that promotes Th2 cytokine responses. The IL-25-elicited cell population, termed MPP^{type2} cells, was defined by expression of Sca-1 and intermediate expression of c-kit (c-kit^{int}) and exhibited multi-potent capacity, giving rise to cells of monocyte/macrophage and granulocyte lineages both *in vitro* and *in vivo*. Progeny of MPP^{type2} cells were competent antigen presenting cells and adoptive transfer of MPP^{type2} cells could promote Th2 cytokine responses and confer protective immunity

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Competing Interest statement J.E.T. and A.L.B. are stockholding employees of Amgen. M.A.K. and R.A.K. are employees of SPB, a subsidiary of Merck&Co.

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to helminth infection in normally susceptible *Il17e*$^{-/-}$ mice. The ability of IL-25 to induce the emergence of an MPP^{type2} cell population identifies a link between the IL-17 cytokine family and extramedullary hematopoiesis and suggests a previously unrecognized innate immune pathway that promotes Th2 cytokine responses at mucosal sites.

Keywords

IL-25 (IL-17E); Th2 cytokine responses; innate immunity; multi-potent progenitor; extramedullary hematopoiesis

Epithelial cell-derived TSLP, IL-33 and IL-25 promote the development of mucosal Th2 cytokine responses through the induction of IL-4 in distinct innate immune cell lineages[7]-[12]. TSLP elicits MHC class II^{pos} basophils that promote Th2 cytokine responses, while IL-33 treatment can activate basophils[13], eosinophils[14] and natural helper cells (NHCs)[15]. In contrast, IL-25 is thought to promote IL-4 production in either NKT cells or an undefined non-B/non-T (NBNT) c-kit^{pos} cell population[7], [14], [16]. Based on c-kit expression, IL-25-elicited NBNT cells were suggested to be a mast cell or mast cell precursor population[14], [16]. Hematopoietic stem cells (HSCs) are known to express c-kit, circulate through peripheral tissues and differentiate in response to microbial signals[17], [18]. However, whether IL-25 influences the population expansion or differentiation of peripheral HSC and whether these cells can influence CD4^{pos} Th2 cell responses has not been examined.

Following administration of IL-25 to IL-4/eGFP reporter mice[19], a 1.8-fold increase in the total cell numbers was observed in the mesenteric lymph nodes (mLN), with equivalent increases in the total numbers of CD4^{pos} and CD8^{pos} T cells, B cells and macrophages (Fig. 1a). In contrast, IL-25-treatment resulted in a 56-fold increase in a NBNT c-kit^{pos} cell population (Fig. 1b), indicating that the NBNT c-kit^{pos} cells exhibited the greatest relative population expansion following IL-25 administration. The IL-25-mediated population expansion of the NBNT c-kit^{pos} cells was associated with increased expression of *Il4*, *Il5* and *Il13* mRNA in the large intestine (Supplementary Fig. 1a), elevated levels of serum IgE (Supplementary Fig. 1b) and increased mucin production in the intestine (Supplementary Fig. 1c) [5].

Analysis of the IL-25-elicited cells revealed that in comparison to c-kit^{pos} mast cells, this cell population exhibited intermediate expression of c-kit (c-kit^{int}) (Supplementary Fig. 2a). Delivery of IL-25 elicited increased frequencies of c-kit^{int} cells in *W-sash* (W^{sh}) mice (Supplementary Fig. 2b), which lack classical mast cell populations[20] and induced equivalent expression of *Il13* mRNA and mucin responses in wild type (WT) and W^{sh} mice (Supplementary Fig. 2c and d), indicating that IL-25 promotes Th2 cytokine responses independently of mast cells.

Compared to control-treated animals (Supplementary Fig. 3a-c), administration of IL-25 increased the frequency of c-kit^{int} cells in all compartments of the GALT examined, including the mLN (Fig. 1c), the Peyer's patches (Fig. 1d) and cecal patch (Fig. 1e). However, IL-25 did not elicit this population in the spleen or bone marrow (data not shown), suggesting that IL-25-responsive cells may be located in the GALT. Further, analysis of

IL-25-elicited c-kit^{int} cells in the GALT revealed two distinct cell populations distinguished by expression of IL-4/eGFP (Fig. 1c-e, right panels), indicating that the IL-25-elicited c-kit^{int} cells are a heterogeneous population.

Previous studies reported elevated expression of IL-25 and increased frequencies of a c-kit^{pos} cell population following exposure to the helminth parasite *Nippostrongylus brasiliensis*[16, 21]. Compared to uninfected controls (Supplementary Fig. 3d), increased frequencies and absolute numbers of c-kit^{int} cells were observed in the mLN of WT mice following infection with *Nippostrongylus* (Fig. 1f and g). Mice lacking expression of either *Il17rb* or *Il17ra* failed to exhibit IL-25-elicited population expansion of the c-kit^{int} cells (Supplementary Fig. 4a) or the development of IL-13 and mucin responses (Supplementary Fig. 4b and c), indicating that both IL-17RB and IL-17RA are required for the IL-25-mediated induction of this cell population. Furthermore, the total number of c-kit^{int} cells induced following *Nippostrongylus* infection were reduced following administration of αIL-25 mAb (infected + control IgG, 58981 ± 4975; infected + αIL-25 mAb, 26109 ± 3039).

To test whether IL-25-elicited c-kit^{int} cells influenced the development of antigen-specific or protective Th2 cytokine responses *in vivo*, CFSE-labeled ovalbumin (OVA)-specific TCR transgenic T cells were transferred alone or in combination with IL-25-elicited c-kit^{int} cells into naïve congenic recipients. As early as 48 hours–post OVA immunization, donor CD4^{pos} T cells began to proliferate (Fig. 2a, left panel) and accumulated at the site of immunization (Fig. 2b). In mice that also received IL-25-elicited c-kit^{int} cells, OVA-specific CD4^{pos} T cell proliferation was augmented (Fig. 2a, right panel) and there was a significant increase in the accumulation of antigen-specific cells (Fig. 2b). Further, mLN cells isolated from recipients of both OVA-specific CD4^{pos} T cell and IL-25-elicited c-kit^{int} cells secreted elevated levels of IL-13 compared to controls (Fig. 2c). *Trichuris*-infected *Il17e*$^{-/-}$ mice produced low levels of Th2 cytokines and parasite-specific IgG1 (Fig. 2d and e), impaired mucin responses (Fig. 2f) and were susceptible to infection (Fig. 2g)[22]. However, adoptive transfer of IL-25-elicited c-kit^{int} cells from WT mice into infected *Il17e*$^{-/-}$ mice resulted in elevated production of IL-4, IL-5 and IL-13 and parasite-specific IgG1 (Fig. 2d and e), increased mucin responses (Fig. 2f) and host protective immunity (Fig. 2g). Taken together, these data indicate that IL-25-elicited c-kit^{int} cells could promote antigen-specific Th2 cytokine responses and protective immunity to helminth infection.

IL-25-elicited c-kit^{int}-GFP^{neg} or c-kit^{int}-GFP^{pos} cells lacked expression of CD4 (Supplementary Fig. 5a), indicating that they were distinct from the CD4^{pos} NKT cell populations[7], and did not express other surface markers associated with CD4^{pos} T cells (Supplementary Fig. 5a). Further, the IL-25-elicited c-kit^{int} populations did not express B cell-, basophil- or eosinophil-associated surface markers (Supplementary Fig. 5b and c). The majority of IL-25-elicited c-kit^{int}-GFP^{neg} cells were T1/ST2$^{neg/lo}$ and IL-7Rα^{neg}, while IL-25-elicited c-kit^{int}-GFP^{pos} cells were T1/ST2$^{neg/lo}$ and heterogeneous for expression of IL-7Rα (Supplementary Fig. 5d), suggesting that both populations are distinct from NHCs[15]. Consistent with this, both c-kit^{int} populations expressed little or no mRNA encoding *Gata3, Junb, Maf, Stat6 and Il1rl1* (Supplementary Fig. 5e). Delivery of IL-25 resulted in increased frequencies of c-kit^{int} cells in the peritoneum and mesentery

(Supplementary Fig. 6a and b). However, while IL-25 treatment increased the cellularity in the mesentery, no changes were observed in the frequency of NHCs or in their expression of CD44 or Thy1.2 (Supplementary Fig. 6c). Taken together, these data indicate that IL-25-elicited c-kit^{int} cells are a unique population and are not T- or B-lymphocytes, NKT cells, basophils, eosinophils, mast cells or NHCs.

Hematopoietic stems cells (HSCs) and multi-potent progenitors (MPPs) express c-kit and Sca-1 and are characterized as lineage^{neg} [23, 24]. While HSCs are primarily localized in the bone marrow, they can circulate in the periphery[25-28] and have been implicated in immunosurveillance[17, 18]. IL-25-elicited c-kit^{int}-GFP^{neg} and c-kit^{int}-GFP^{pos} populations were Lin$^{neg/lo}$ (Supplementary Fig. 7), and the majority of the IL-25-elicited c-kit^{int}-GFP^{neg} and c-kit^{int}-GFP^{pos} cells expressed Sca-1, were CD150^{neg}, and exhibited heterogeneous expression of CD34 (Fig. 3a-c). Therefore the IL-25-elicited cell populations exhibited a surface phenotype consistent with a MPP-like cell. Although administration of IL-25 induced MPP-like cells in the GALT, the frequencies of MPPs, short-term and long-term HSCs in the BM were unchanged following IL-25-treatment (Supplementary Fig. 8a and b).

To assess the capacity of the c-kit^{int} MPP-like cell population to exhibit multi-potent potential, IL-25-elicited c-kit^{int}-GFP^{neg} or c-kit^{int}-GFP^{pos} cells were sorted and cultured *in vitro* in the presence of SCF and IL-3 (Fig. 3c-f). Un-fractionated bone marrow cells from naïve mice differentiated into a CD11b^{pos} macrophage-like population (Supplementary Fig. 9a, orange gate) and a CD11b^{neg} granulocyte population that could be identified as mast cells or basophils based on expression of c-kit and FcεRI in addition to cell morphology (Supplementary Fig. 9a and b). Sorted IL-25-elicited c-kit^{int}-GFP^{pos} cells gave rise to a CD11b^{neg} c-kit^{pos} FcεRI^{pos} mast cell population (Fig. 3c, red gate), but failed to give rise to CD11b^{pos} progeny. Consistent with this, the progeny of c-kit^{int}-GFP^{pos} cells were morphologically similar to mast cells (Fig. 3d). IL-25-elicited c-kit^{int}-GFP^{neg} input cells also gave rise to a CD11b^{neg} mast cell population (Fig. 3e, red gate). In addition, the c-kit^{int}-GFP^{neg} input cells gave rise to c-kit^{neg} FcεRI^{pos} basophils (Fig. 3e, blue gate) and CD11b^{pos} macrophages (Fig. 3e, orange gate). The multi-potent potential of the c-kit^{int}-GFP^{neg} cell population was confirmed by cell morphology (Fig. 3f). c-kit^{int}-GFP^{neg} cells cultured in the presence of SCF and IL-3 or using OP9 stromal cells[29] also gave rise to a CD11b^{pos} M-CSFR^{pos} Ly6C^{pos} MHC class II^{pos} cell population (Supplementary Fig. 10a and b), consistent with myeloid potential. IL-25-elicited c-kit^{int}-GFP^{neg} cells were isolated from WT CD45.2 donor mice and adoptively transferred into naïve CD45.1 congenic recipients. Six days following transfer, c-kit^{int}-GFP^{neg} donor cells differentiated into CD11b^{pos} cells as well as CD11b^{neg} c-kit^{pos} FcεRI^{pos} cells (Supplementary Fig. 11). Collectively, these data indicate that the c-kit^{int}-GFP^{neg} cells may represent a previously unrecognized IL-25-responsive MPP-like cell population in the GALT that selectively exhibits multi-potent potential *in vitro* and *in vivo*.

IL-25-elicited c-kit^{int} cell populations were sorted (Fig. 4a, left panel) and cultured *in vitro* in the presence of SCF and IL-3 for 8 days. The majority of the progeny derived from c-kit^{int}-GFP^{pos} cells were IL-4/eGFP^{pos} MHC class II^{neg}, while the c-kit^{int}-GFP^{neg}-derived progeny contained IL-4/eGFP^{pos} and MHC class II^{pos} populations (Fig. 4a, right panels). To test whether these cells could influence T cell proliferation and/or differentiation, progeny

from sorted c-kit^{int}-GFP^{neg}- or c-kit^{int}-GFP^{pos} cells were pulsed with OVA and co-cultured with CFSE-labeled OVA-specific TCR transgenic CD4^{pos} T cells. In the absence of antigen, T cells exhibited minimal proliferation and Th2 cytokine production (1% CFSE^{dim}, Fig. 4b (shaded histograms) and c). Antigen-pulsed c-kit^{int}-GFP^{pos}-derived progeny cells failed to induce T cell proliferation (Supplementary Fig. 12, black histogram) or production of Th2 cytokines. In contrast, antigen-pulsed progeny derived from c-kit^{int}-GFP^{neg} cells induced MHC class II-dependent T cell proliferation and production of IL-4 and IL-13 (Fig. 4b and c). Inclusion of anti-IL-4Rα mAb did not affect T cell proliferation (Fig. 4b), but resulted in decreased production of IL-4 and IL-13 (Fig. 4c), indicating that both MHC class II and IL-4R signaling are required for the c-kit^{int}-GFP^{neg}-derived progeny to influence Th2 cell differentiation. No IFN-γ was detected in any culture conditions (data not shown). These results indicate that IL-25-elicited c-kit^{int} cells contain a population of progenitors with multi-potent capacity, termed MPP^{type2} cells, whose progeny act to promote CD4^{pos} Th2 cell differentiation.

Collectively, these findings indicate that in addition to a c-kit^{int}-GFP^{pos} cell population that differentiates into mast cells, IL-25 elicits a MPP-like cell population that can differentiate into monocyte/macrophage and granulocyte lineages (Supplementary Fig. 13). Coupled with reports that peripheral HSCs express TLRs and can respond to microbial stimulation[17], [18], these findings indicate a previously unrecognized pathway in which peripheral MPP^{type2} cells can promote type 2 inflammation at mucosal sites and suggest an evolutionarily conserved pathway between the IL-17 cytokine family, extramedullary hematopoiesis and adaptive immunity.

Methods Summary

Mice were treated i.p. with PBS or 0.4 μg recombinant IL-25 daily for 4 days or infected with *Nippostrongylus brasiliensis*. Mesenteric lymph nodes from IL-4/eGFP reporter mice were separated from the mesentery, homogenized and stained with anti-mouse fluorochrome-conjugated monoclonal antibodies against combined lineage markers and c-kit, FcεRIα, T1/ST2, and IL-7Rα. IL-25-elicited c-kit^{int} cells were sorted using a FACSAria and transferred into OVA/IFA-immunized WT mice or *Trichuris*-infected *Il17e*$^{-/-}$ mice and Th2 cytokine responses measured. IL-25-elicited c-kit^{int}-GFP^{pos} and c-kit^{int}-GFP^{neg} cell populations were either sorted and transferred into CD45.1 congenic recipients to assess *in vivo* differentiation or incubated *in vitro* in the presence of SCF (50 ng mL^{-1}) and IL-3 (10 ng mL^{-1}). Progeny were analyzed by cytospin or flow cytometry for surface expression of lineage-specific markers. Some progeny were co-cultured with CFSE-labeled OVA-specific TCR transgenic CD4^{pos} T cells in the presence of OVA peptide with or without the inclusion of blocking antibodies against MHC class II or IL-4Rα. CFSE dilution in CD4^{pos} T cells was assessed by flow cytometry and cell-free supernatants were analyzed for secretion of IL-4 and IL-13 by sandwich ELISA.

Methods

Mice

Balb/c, C57BL/6 and W^{sh} mice were obtained from Jackson Laboratory and IL-4/eGFP reporter mice were obtained from M. Mohrs (Trudeau Institute). *Il17e*$^{-/-}$ mice were provided by R.A. Kastelein (Schering-Plough Biopharma). *Il17ra*$^{-/-}$ and *Il17rb*$^{-/-}$ mice were provided by J.E. Tocker and A.L. Budelsky (Amgen). Animals were bred and housed in specific pathogen-free conditions at the University of Pennsylvania. All experiments were performed under Institutional Animal Care and Use Committee (IACUC) approved protocols and in accordance with the guidelines of the IACUC of the University of Pennsylvania. All mice used were 4-12 weeks of age. Mice were treated intraperitoneally with PBS or recombinant IL-25 (IL-17E) (0.4 μg; R&D Systems) daily for 4 days.

Flow cytometry, cell sorting and $CD4^{pos}$ T cell co-culture

Mesenteric lymph nodes from IL-4/eGFP reporter mice were separated from the mesentery, homogenized by passing through a 70 μm nylon mesh filter and stained with anti-mouse fluorochrome-conjugated monoclonal antibodies against CD3ε, CD4, CD8, TCRβ, TCRγδ, B220, CD19, CD11b, CD11c, MHC class II, Gr-1, NK1.1, Ter119, FcεRIα, c-kit, Sca-1, CD150, CD34, CD62L, CD69, CD127 (IL-7Rα), CD45.2, CD49b and CCR3 (eBioscience and BD Bioscience). T1/ST2 staining was performed using T1/ST2 biotinylated mAb (MD Biosciences) and PE- or eFluor450-conjugated streptavidin (eBioscience). Peritoneal exudate cells ('peritoneum') were collected using peritoneal lavage with injection of 10 mL PBS and aspirated using the same syringe. Mesentery was processed as previously described[15]. Cells were run on a BD FACSCanto II using DiVa software (BD Bioscience) and analyzed with FlowJo software (Version 8.7.1; Tree Star, Inc.). IL-25-elicited c-kit^{int} (GFP^{pos} or GFP^{neg}) cell populations were sorted using a FACSAria (BD Bioscience) and for *in vitro* differentiation studies were incubated in the presence of SCF (50 ng mL^{-1}; R&D systems) and IL-3 (10 ng mL^{-1}; R&D systems). Following in vitro culture, progeny were assessed for expression of CD11b, MHC class II, CD115 (M-CSFR), Ly6C, FcεRIα, and c-kit (eBioscience) by flow cytometry as described above. Naïve OVA-specific $CD4^{pos}$ T cells were isolated from DO11.10 mice as previously described[14]. T cells were stained with fluorochrome-conjugated monoclonal antibodies against CD4, CD62L, and CD44 (eBioscience), re-suspended in 2% FBS in HBSS with 2 mM EDTA (Gibco) with DAPI (1 μg mL^{-1}; Molecular Probes) and naïve T cells sorted based on live cells ($DAPI^{neg}$) $CD4^{pos}$ $CD62L^{hi}$ $CD44^{lo}$ using a FACSAria (BD Bioscience). T cells were labeled with CFSE (Molecular Probes) and co-cultured in the presence of OVA peptide (1 μg mL^{-1}) and blocking antibodies to MHC class II (5 μg mL^{-1}; M5/114; eBioscience) or IL-4Rα (5 μg mL^{-1}; mIL4R-M1; BD Bioscience) in complete medium (DMEM Iscove's with 10% (vol/vol) heat-inactivated FBS, 2 mM glutamine, 100 U mL^{-1} of penicillin, 100 μg mL^{-1} of streptomycin, 25 mM HEPES and 50 μM β-mercaptoethanol). Cell-free supernatants were assessed for cytokine production by standard sandwich ELISA (eBioscience) following 4h stimulation with 50 ng mL^{-1} PMA, 750 ng mL^{-1} ionomycin (Sigma Aldrich).

OVA immunization, helminth infections and adoptive transfers

3-5 ×10^6 CFSE-labeled OVA-specific CD45.2 $CD4^{pos}$ T cells were transferred i.v. into CD45.1 congenic recipient mice and 24 hours later immunized i.p. with 100 μg OVA emulsified in IFA, with one cohort receiving 5×10^4 IL-25-elicited c-kit^{int} cells i.p. Proliferation of OVA-specific $CD4^{pos}$ T cells in the spleen was assessed two days post-immunization and cytokine production measured by ELISA. *Trichuris muris* infections were performed as previously described[22]. *Trichuris*-infected *Il17e*$^{-/-}$ mice were left untreated or given 5×10^4 IL-25-elicited c-kit^{int} cells at day 10 post-infection. Worm counts were performed at day 20 post-infection. Mesenteric lymph nodes cells were collected at necropsy, plated in medium alone or polyclonally stimulated with 1 μg ml^{-1} each of αCD3 and αCD28 (eBioscience). Following 48 h, cell-free supernatants were assessed for cytokine production by sandwich ELISA (eBioscience). *Trichuris*-specific IgG1 antibody titers were analyzed by ELISA as described previously[22]. Total serum IgE was measured using the OptEIA IgE ELISA kit according to the manufacturer's instructions (BD Biosciences). For *Nippostrongylus brasiliensis* infections, WT mice were infected s.c. with 500 infective third-stage larvae (L3) and treated with 0.5 mg of anti-IL-25 or control IgG (from J.E.T. and A.L.B.) on days 0, 2, 4, 6 and 8. Mesenteric LN cells from infected mice were assessed at day 10 post-infection for the induction of c-kit^{int} cells. For *in vivo* differentiation assays, 3×10^4 CD45.2 IL-25-elicited c-kit^{int}-GFP^{neg} cells were FACS-purified and transferred i.p. into CD45.1 congenic recipient mice. Recipient mice were treated 4 times with 1 μg each of SCF and IL-3 and differentiation of donor cells was assessed on day 6 post-transfer.

HSC differentiation assays

FACS-purified cell populations were plated onto semi-confluent OP9 stromal cells (ATCC # CRL-2749), as previously described[30]. Monolayers were irradiated (3000 rad) prior to co-culture with FACS-purified populations. Cells were incubated in the presence of SCF (50 ng mL^{-1}; R&D systems) and IL-3 (10 ng mL^{-1}; R&D systems).

Histology and cytospin preparation

Colon sections were fixed in 4% (vol/vol) paraformaldehyde and embedded in paraffin wax. 4 μm sections were stained with Periodic acid-Schiff/Alcian blue. Purified cell populations were subjected to cytospin (Cytospin 3, Thermo Fisher Scientific) and stained by Diff-quick for analysis of cellular morphology.

Real-time PCR

RNA from colonic tissues of mice was isolated by TRizol extraction (Invitrogen) or collected from sorted cell populations using RNeasy Mini kit (Qiagen). Whole tissues were homogenized with a tissue homogenizer (TissueLyzer; Qiagen) and cDNA was prepared with SuperScript Reverse Transcriptase (Invitrogen). Quantitative real-time PCR analysis used commercial QuantiTect primer sets for *Il4*, *Il5*, *Il13*, *Gata3*, *Maf*, *Junb*, *Stat6* and *Il1rl1* (Qiagen) and SYBR Green chemistry (Applied Biosystems). All reactions were run on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). Target genes were normalized for endogenous β-actin levels and relative quantification of samples were compared to controls.

Statistical analysis

Results are shown as means ± s.e.m for individual animals. Statistical significance was determined by Student's *t*-test. Results were considered significant at $P < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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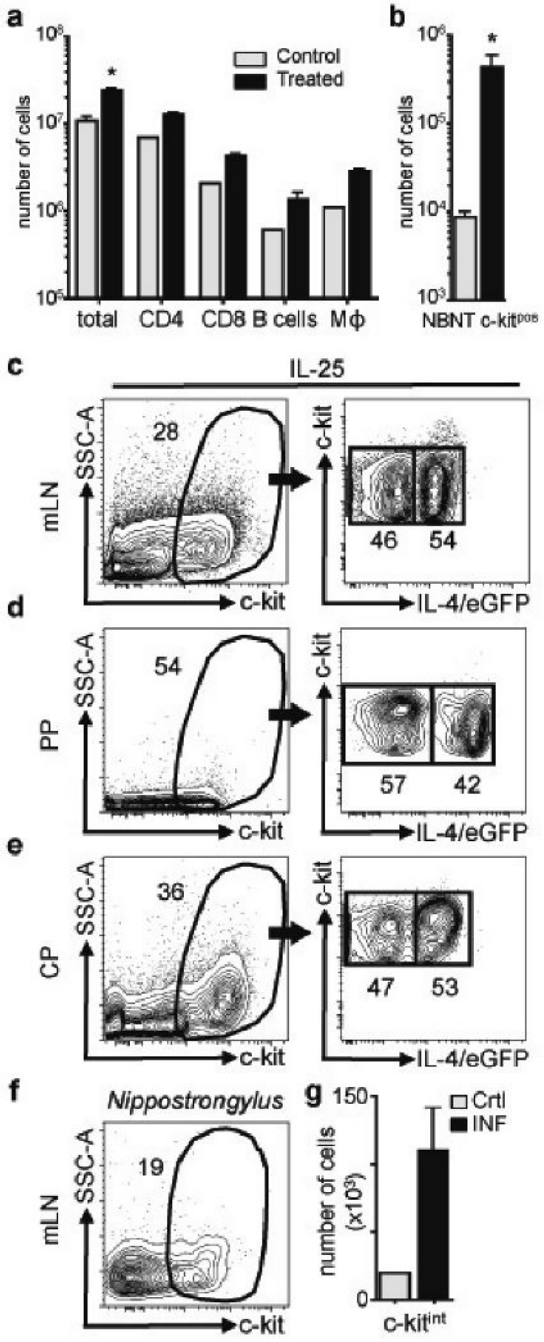

Figure 1. IL-25 elicits a c-kit^{int}-GFP^{neg} and c-kit^{int}-GFP^{pos} cell population in the GALT

a, b, Cell numbers from IL-25-treated IL-4/eGFP reporter mice. Numbers of total mLN cells or of T cells (CD4^{pos} or CD8^{pos}), B cells (CD19^{pos}) and macrophages (CD11b^{pos} MHC class II^{pos}) (**a**) and total numbers of non-B non-T (NBNT) c-kit^{pos} cells (**b**). **c-e**, Frequency of c-kit^{int} cells in mLNs (**c**), Peyer's patches (PP) (**d**) and cecal patch (CP) (**e**) was assessed by flow cytometry. **f, g**, Frequencies (**f**) and total numbers (**g**) of c-kit^{int} cells in control or *Nippostrongylus brasiliensis*-infected (INF) mice. Crtl = control. Plots shown are gated on live, CD4^{neg} CD8^{neg} CD11b^{neg} CD11c^{neg} and B220^{neg} cells or as indicated. *, $P < 0.05$.

Error bars indicate s.e.m. Data in **a-e** are representative of more than five independent experiments (control, n=16; IL-25-treated, n=34). Data in **f-g** are representative of at least two independent experiments (Crtl, n=2; INF, n=6).

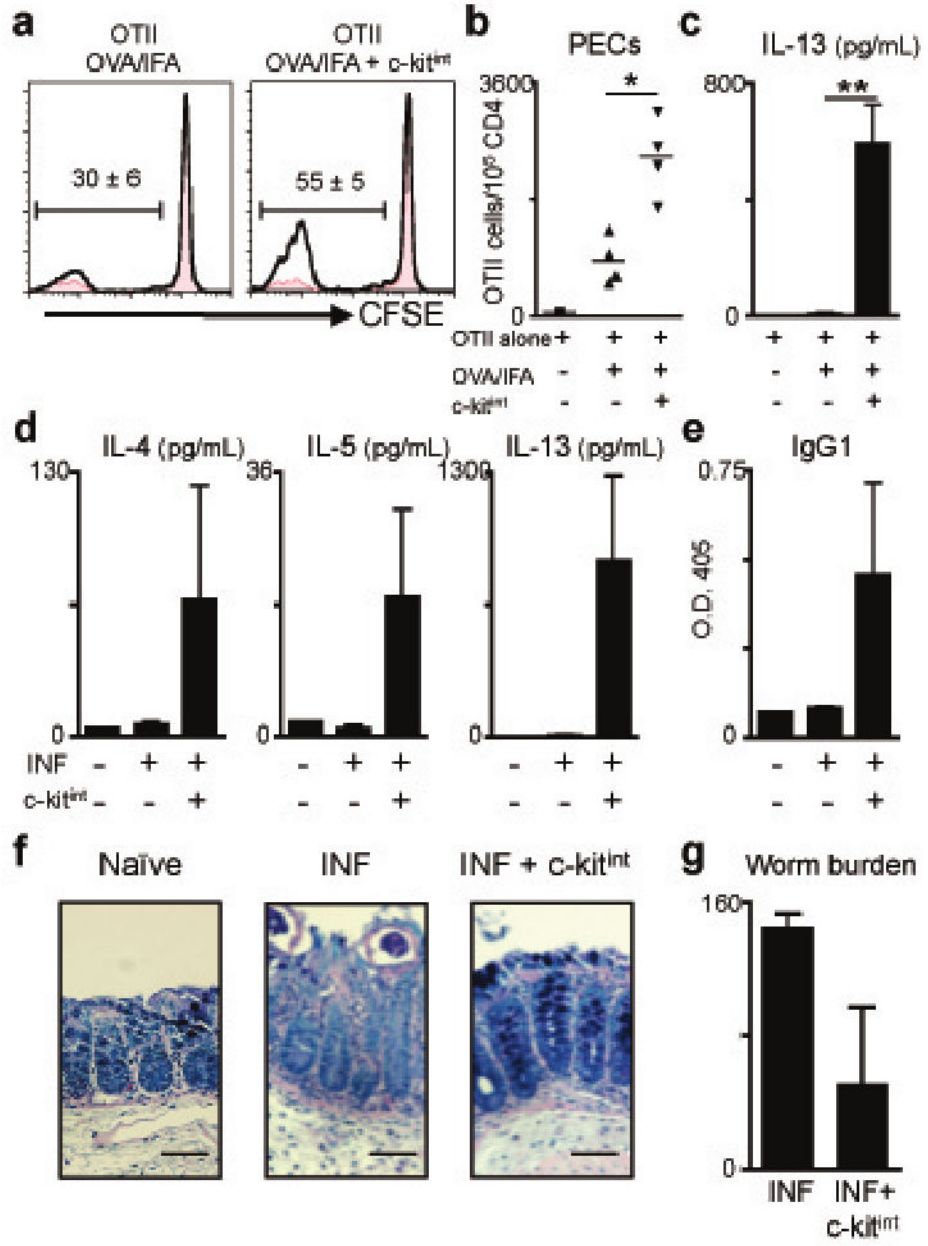

Figure 2. IL-25-elicited c-kit^{int} cells promote Th2 cytokine-dependent responses *in vivo*

a-c, CFSE-labeled CD45.2 OVA-specific CD4^{pos} T cells were adoptively transferred i.v. into CD45.1 congenic recipients and mice were immunized i.p. with OVA/IFA in the presence or absence of IL-25-elicited c-kit^{int} cells. Proliferation of donor CD45.2 cells in recipient mice receiving T cells alone (red shaded histogram) or T cells and OVA/IFA immunization ± c-kit^{int} cells (black histograms) was measured by flow cytometry (**a**). Frequency of donor CD4^{pos} cells/total CD4^{pos} T cells isolated from the peritoneum (**b**). IL-13 production from αCD3/αCD28-stimulated mLN cells was measured by ELISA (**c**). Data in **a-c** are representative of two independent experiments (n=7). *, $P < 0.05$, **, $P < 0.01$. **d-g**, Adoptive transfer of c-kit^{int} cells protects *Trichuris*-infected (INF) *Il17e*$^{-/-}$ mice. Cytokine production by αCD3/αCD28-stimulated mLN cells (**d**), *Trichuris*-specific serum

IgG1 antibody titers (**e**), intestinal mucin responses (**f**), and number of worms from *Trichuris*-infected mice (**g**) were assessed at day 20 post-infection. Scare bar, 50 μm.

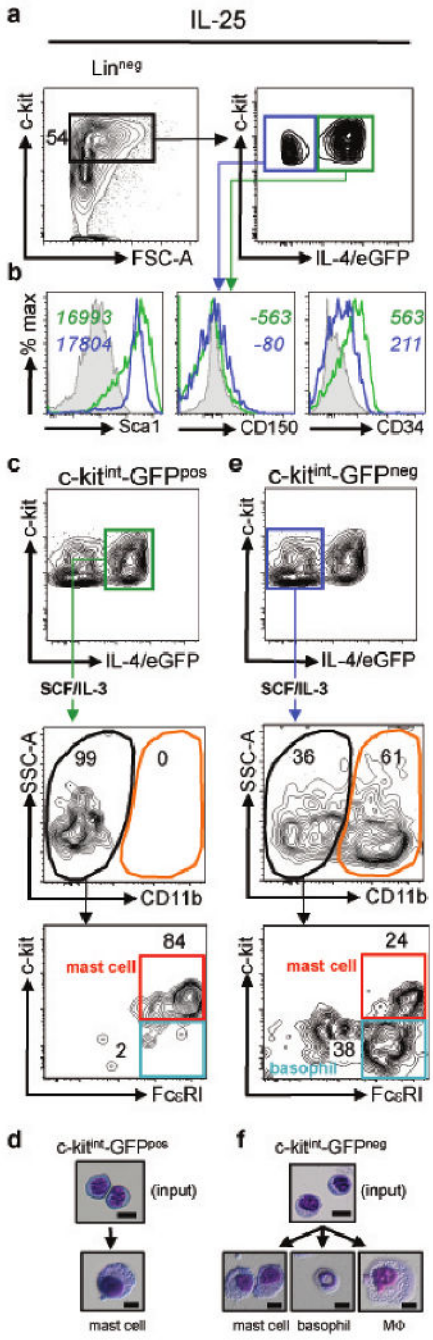

Figure 3. IL-25-elicited c-kit^{int} cells exhibit multi-potent capacity

a, b, Frequencies of c-kit^{int} cells in the mLNs of IL-25-treated IL-4/eGFP reporter mice (**a**) and expression of HSC markers by c-kit^{int}-GFP^{neg} (**blue histograms**) or c-kit^{int}-GFP^{neg} (**green histograms**) cells from IL-25-treated IL-4/eGFP reporter mice (**b**). Number *(italics)* indicates the mean fluorescent intensity. Plots shown are gated on live, lineage^{neg} cells (CD3ε, CD8α, CD8β, TCRβ, TCRγδ, B220, CD19, CD11b, CD11c, Gr-1, NK1.1 and Ter119) or as indicated. Data in **a and b** are representative of two independent experiments (control, n=4; IL-25-treated, n=7). **c**, **e**, Flow cytometric analysis of myeloid cell and

granulocyte differentiation of FACS-purified lineage$^{neg/lo}$ c-kit^{int}-GFP^{pos} (**c**) or c-kit^{int}-GFP^{neg} (**e**) cells from IL-25-treated IL-4/eGFP reporter mice following *in vitro* culture in SCF and IL-3. **d, f**, Cytospin preparation of progeny derived from IL-25-elicited c-kit^{int}-GFP^{pos} cells (**d**) or c-kit^{int}-GFP^{neg} cells (**f**). Scale bar, 20 μm. Data in **c-f** are representative of at least three independent experiments.

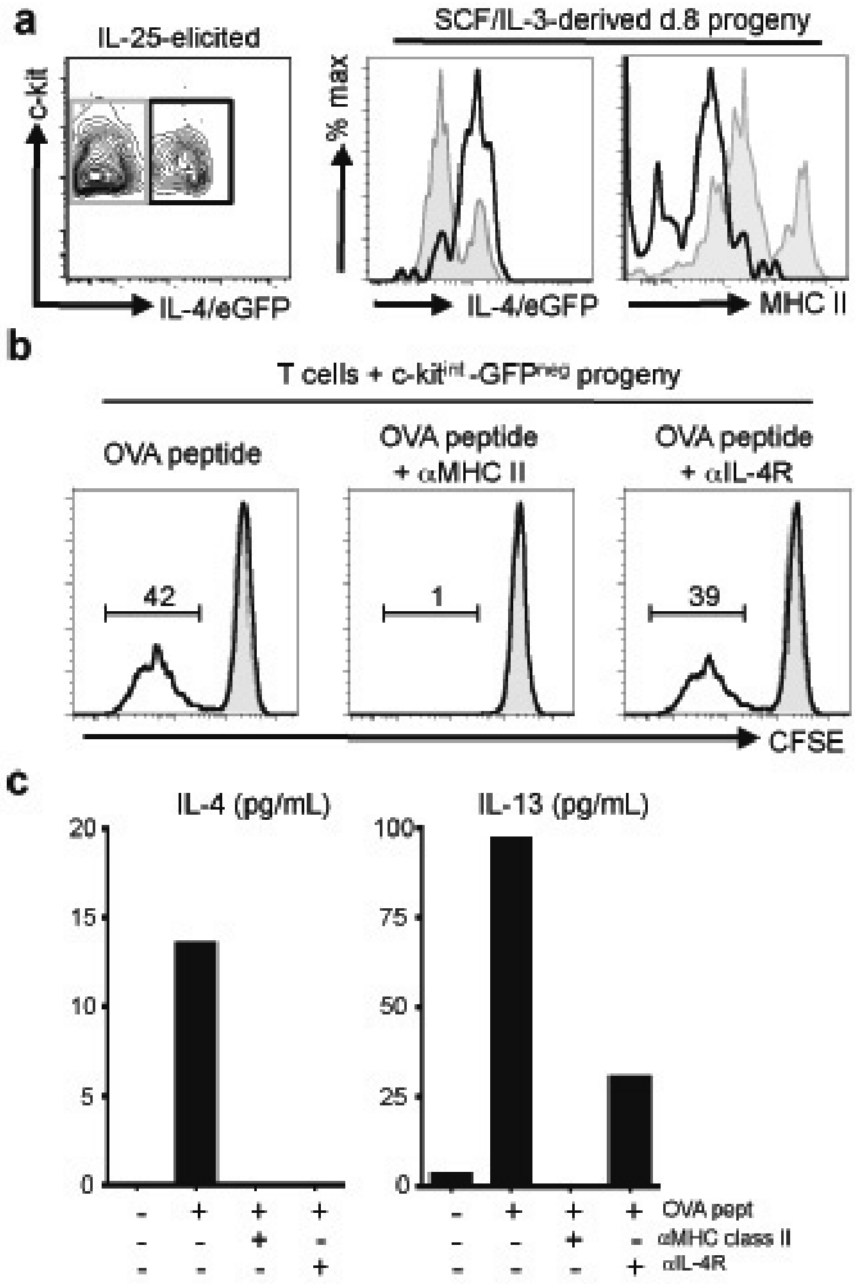

Figure 4. Progeny from IL-25-elicited c-kit^{int}-GFP^{neg} cells promote Th2 cell differentiation

a, FACS-purified IL-25-elicited c-kit^{int} cells were cultured for 8 days in SCF and IL-3 and the resulting progeny from c-kit^{int}-GFP^{pos} (**black histogram**) or c-kit^{int}-GFP^{neg} (**gray shaded histogram**) cells were assessed for expression of IL-4/eGFP and MHC class II. Results in (**a**) are representative of three independent experiments. **b**, CFSE-dilution by OVA-specific CD4^{pos} CD62L^{hi} CD44^{lo} T cells following 4 day co-culture with progeny from FACS-purified IL-25-elicited c-kit^{int}-GFP^{neg} cells in the presence of OVA peptide with or without addition of mAb against MHC class II or IL-4Rα. Gray histogram indicates CFSE-dilution by OVA-specific CD4^{pos} T cells cultured in medium alone. **c**, IL-4 and IL-13

protein levels in cell-free supernatants from (**b**) were assayed by ELISA. Results in **b-c** are representative of at least two independent experiments.