

Thymic Stromal Lymphopoietin-Mediated Extramedullary Hematopoiesis Promotes Allergic Inflammation

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SUMMARY

Extramedullary hematopoiesis (EMH) refers to the differentiation of hematopoietic stem cells (HSCs) into effector cells that occurs in compartments outside of the bone marrow. Previous studies linked pattern-recognition receptor (PRR)-expressing HSCs, EMH, and immune responses to microbial stimuli. However, whether EMH operates in broader immune contexts remains unknown. Here, we demonstrate a previously unrecognized role for thymic stromal lymphopoietin (TSLP) in promoting the population expansion of progenitor cells in the periphery and identify that TSLP-elicited progenitors differentiated into effector cells including macrophages, dendritic cells, and granulocytes and that these cells contributed to type 2 cytokine responses. The frequency of circulating progenitor cells was also increased in allergic patients with a gain-of-function polymorphism in *TSLP*, suggesting the TSLP-EMH pathway might operate in human disease. These data identify that TSLP-induced EMH contributes to the development of allergic inflammation and indicate that EMH is a conserved mechanism of innate immunity.

INTRODUCTION

Hematopoietic stem cells (HSCs) circulate through the bloodstream under steady-state conditions and accumulate in

peripheral tissues in response to stress signals such as injury or infection (Cheshier et al., 1999; Massberg et al., 2007; Wright et al., 2001). Peripheral HSCs are capable of differentiating into immune effector cells via a process termed extramedullary hematopoiesis (EMH) (Massberg et al., 2007; Nagai et al., 2006; Ratajczak et al., 2010; Saenz et al., 2010). Although the pathways and factors that regulate the ability of HSCs to patrol peripheral tissues and undergo EMH remain poorly defined, EMH has been reported to contribute to immunity and inflammation (Allakhverdi and Delespesse, 2012; Massberg et al., 2007; Nagai et al., 2006; Ratajczak et al., 2010; Saenz et al., 2010). For example, HSCs express germ-line encoded pattern-recognition receptors (PRRs) capable of identifying pathogen-associated molecular patterns (PAMPs) from viruses and bacteria (Massberg et al., 2007; Nagai et al., 2006). In addition, some HSCs express the receptors to type I and type 2 interferons (IFNs), respond to IFN- γ stimulation, and contribute to the development of chronic intestinal inflammation in an IFN- γ -dependent manner (Baldridge et al., 2010; Griseri et al., 2012; Takizawa et al., 2012). Collectively, these reports suggest a direct link between EMH and the mechanisms that promote proinflammatory cytokine responses associated with viral or bacterial infections. However, whether EMH is a conserved innate immune mechanism that promotes multiple modules of the immune system remains unknown.

Although the mechanisms through which mammalian hosts recognize viral, bacterial, and protozoan pathogens through PRRs and initiate T helper 1 (Th1) and Th17 cell responses are well defined, the evolutionarily conserved mechanisms through which Th2 cell-mediated immunity and inflammation are initiated remain less clear (Pulendran and Artis, 2012). Emerging studies suggest that epithelial cells provide essential signals that are

critically important for the development of type 2 cytokine-associated innate immune responses and the development of Th2 cell-dependent immunity and inflammation (Saenz et al., 2008; Wills-Karp and Finkelman, 2011; Ziegler and Artis, 2010). For example, the predominately epithelial cell-derived cytokines interleukin-25 (IL-25), IL-33, and TSLP promote type 2 cytokine-mediated immunity and inflammation by supporting dendritic cell, macrophage, mast cell, basophil, and innate lymphoid cell (ILC) responses (Saenz et al., 2008; Siracusa et al., 2011; Wills-Karp and Finkelman, 2011; Ziegler and Artis, 2010). Further, we have previously demonstrated that IL-25 promotes type 2 cytokine-mediated inflammation, in part by eliciting the population expansion of progenitor cells termed multipotent progenitor type 2 (MPP^{type2}) cells in the periphery of mice (Saenz et al., 2010, 2013). However, subsequent studies have reported that human CD34⁺ progenitor-like cells show little responsiveness to IL-25 signaling (Allakhverdi and Delespesse, 2012), suggesting that in some circumstances other pathways might promote type 2 cytokine-associated EMH.

In this study, we demonstrate that TSLP promoted EMH, which contributed to type 2 cytokine-associated inflammation. Systemic TSLP expression resulted in increases in the frequency and number of lineage negative (Lin[−]) CD34⁺ c-Kit⁺ progenitor cells in the spleen with a surface phenotype similar to that of bone marrow (BM)-resident granulocyte-monocyte progenitor (GMP) cells. In vitro and in vivo studies demonstrated that TSLP-elicited progenitor cells exhibited multipotent potential and developed into multiple myeloid effector cell populations. Genome-wide transcriptional profiling of TSLP-elicited progenitors revealed a distinct phenotype compared to BM-resident GMPs, suggesting that TSLP-elicited progenitors were skewed toward granulocyte development. In addition, TSLP-elicited progenitors exhibited an increased capacity to develop into mast cells and basophils and produced more IL-4 after differentiation compared to BM-resident GMPs. Adoptive transfer of TSLP-elicited progenitor cells promoted IL-4 production and protective immunity to *Trichuris muris*. In addition, Lin[−] CD34⁺ c-Kit⁺ cells were identified in the spleen and blood of human donors and were increased in the blood of allergic patients with a gain-of-function polymorphism in *TSLP* that is associated with elevated TSLP expression. Finally, a decrease in the percentage of progenitor-like cells was associated with increases in mature granulocyte populations and allergic inflammation in the esophageal biopsies of eosinophilic esophagitis (EoE) patients. These data suggest that a portion of the granulocyte populations found in the esophageal tissue in the context of active EoE might develop via EMH. Collectively, these studies identify a previously unrecognized pathway of TSLP-dependent EMH that contributes to Th2 cell-mediated inflammation and suggest that TSLP-elicited EMH might be a therapeutic target in the treatment of allergic inflammation in patients.

RESULTS

TSLP Elicits a Lin[−] CD34⁺ c-Kit⁺ Cell Population in the Spleen

Recent studies demonstrated that PRR-expressing HSCs enter the periphery, where they are capable of recognizing PAMPs and undergoing EMH that contributes to antiviral and antibacte-

rial immunity (Massberg et al., 2007; Nagai et al., 2006; Ratajczak et al., 2010). However, whether EMH operates in a broader immune context remains unknown. The epithelial cell-derived cytokine TSLP has been shown to be critically important in promoting type 2 cytokine-associated innate immune responses by influencing terminally differentiated cells (Kim et al., 2013; Siracusa et al., 2011; Ziegler and Artis, 2010). Further, we have previously reported that TSLPR is expressed on BM-resident progenitor cells and that TSLP can promote the development of basophils from BM-resident progenitors (Siracusa et al., 2011), suggesting that TSLP is capable of influencing central hematopoiesis in the BM. In addition to progenitor cells in the BM, TSLPR is expressed on CD34⁺ progenitor cells in the periphery (Allakhverdi et al., 2009; Osborn et al., 2004), although whether TSLP causes CD34⁺ progenitor cells to undergo EMH is unknown. To directly test whether TSLP can influence progenitor cell populations in the periphery, we employed hydrodynamic tail-vein injections of control cDNA (control-cDNA) or TSLP-encoding cDNA (TSLP-cDNA) as a method of TSLP overexpression (Siracusa et al., 2011) and examined CD34⁺ progenitor-like cell responses in the spleen and blood. Following injection of TSLP-cDNA, significant increases in the percentage and total number (Figures 1A–1C) of Lin[−] CD34⁺ c-Kit⁺ cells, a phenotype consistent with that of hematopoietic progenitors (Arinobu et al., 2005; Griseri et al., 2012), were observed in the spleen, and increased cell numbers were observed in the blood (see Figure S1A available online) compared to control-cDNA-injected mice.

To determine whether a similar population expansion of Lin[−] CD34⁺ c-Kit⁺ progenitor-like cells in the periphery occurred in the context of a natural stimulus associated with elevated expression of endogenous TSLP, we employed infection with *Trichinella spiralis*, a helminth parasite in which optimal Th2 cell responses are dependent on TSLP-TSLPR signaling (Giacomin et al., 2012), and monitored progenitor-like cell responses in the periphery. Critically, significant increases in the percentage and total number (Figures 1D–1F) of Lin[−] CD34⁺ c-Kit⁺ cells were observed in the spleens of infected mice compared to naive controls. In addition to supporting the development of protective immunity to some helminth parasites, TSLP also promotes allergic inflammation, including in the context of atopic dermatitis (AD) (Kim et al., 2013; Ziegler and Artis, 2010). Therefore, WT mice were treated topically with the vitamin D analog calcipotriol (MC903), a protocol that induced TSLP-dependent AD-like disease (Kim et al., 2013), and Lin[−] CD34⁺ c-Kit⁺ cells were evaluated. Topical treatment with MC903 resulted in a significant increase in the percentage and total number of Lin[−] CD34⁺ c-Kit⁺ cells in the spleen compared to ethanol (EtOH)-treated controls (Figures S1B and S1C). Collectively, these data demonstrate that TSLP production is associated with the population expansion of Lin[−] CD34⁺ c-Kit⁺ progenitor-like cells in the periphery in the context of type 2 cytokine-associated inflammation.

To test whether increases in Lin[−] CD34⁺ c-Kit⁺ cells in the spleen were dependent on TSLP-TSLPR-signaling, we treated *Trichinella*-infected mice with isotype control or an anti-TSLP neutralizing antibody and examined progenitor-like cell responses. Compared to controls, anti-TSLP-treated mice failed to demonstrate increases in the frequency or total number (Figures S1D–S1F) of splenic Lin[−] CD34⁺ c-Kit⁺ cells. Administration of anti-TSLP neutralizing antibody also resulted in

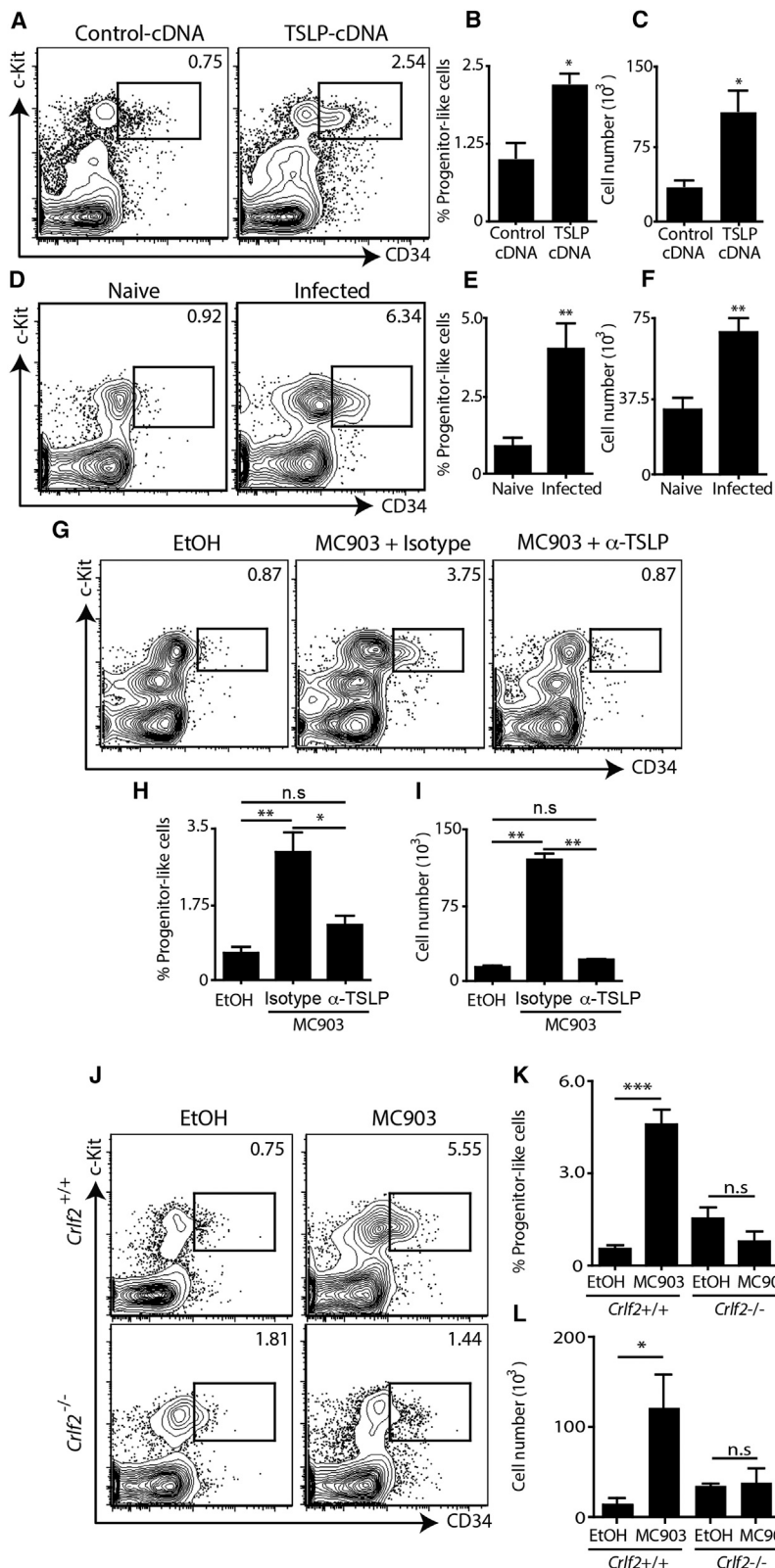


Figure 1. TSLP Promotes the Population Expansion of Progenitor-like Cells

WT mice were injected with control plasmid (control-cDNA) or TSLP-encoding cDNA plasmid (TSLP-cDNA), and the percentage (A and B), and total number (C) of Lin[−] CD34⁺ c-Kit⁺ progenitor-like cells in the spleen were quantified on day 5 after injection. WT mice were infected with *Trichinella spiralis*, and the percentage (D and E), and total number (F) of Lin[−] CD34⁺ c-Kit⁺ progenitor-like cells in the spleen were quantified on day 8 after injection. WT mice were treated with ethanol (EtOH) or MC903 for 7 days. Groups of MC903-treated mice were treated with isotype control or anti-TSLP neutralizing antibody, and the percentage (G and H), and total number (I) of progenitor-like cells in the spleen were quantified. *Crf2*^{+/-} mice or *Crf2*^{-/-} mice were treated with EtOH or MC903 for 7 days, and the percentage (J and K), and total number (L) of progenitor-like cells in the spleen were quantified. Results represent the mean $\pm$ SEM and numbers in the plots indicate the percentage of cells. (B, C, E, F, K, and L) Statistical analysis was performed with a Student's *t* test. (H and I) Statistical analysis was performed with a one-way ANOVA and Tukey's post hoc. (**p* < 0.05), (***p* < 0.01), (***)*p* < 0.001). All results are representative of at least three separate experiments of at least three mice per group per experiment. See also Figure S1.

number (Figures 1J–L) of Lin[−] CD34⁺ c-Kit⁺ cells compared to *Crf2*^{+/-} controls. Collectively, these gain- and loss-of-function studies demonstrate that exogenous and endogenous TSLP production is required for the population expansion of progenitor-like cells in the spleen.

TSLP-Elicited Lin[−] CD34⁺ c-Kit⁺ Cells Exhibit a GMP-like Phenotype

TSLP has been shown to regulate the population expansion and activation of multiple innate cell populations including ILCs (Kim et al., 2013; Siracusa et al., 2011; Spits et al., 2013). Although TSLP-elicited Lin[−] CD34⁺ c-Kit⁺ cells expressed CD127, ICOS, T1(ST2), and negative to low amounts of Sca-1 compared to simultaneously run controls, they lacked high expression of the ILC-associated surface markers CD90 and CD25 (Figure S2A) (Spits et al., 2013). Further, principle component analysis (PCA) of genome-wide transcriptional profiles revealed that TSLP-elicited Lin[−] CD34⁺ c-Kit⁺ cells were distinct from ILC populations (Figures S2B and S2C).

The surface markers c-Kit and CD34 are highly expressed on Lin[−] BM-resident progenitor cells, including GMPs (Arinobu et al., 2005; Griseri et al., 2012). Therefore, to better characterize the nature of TSLP-elicited Lin[−] CD34⁺ c-Kit⁺ cells in the periphery, their expression of

reduced progenitor cell responses following MC903 treatment (Figures 1G–I). Further, TSLPR-deficient (*Crf2*^{-/-}) mice failed to exhibit MC903-induced increases in the percentage or total

surface markers was examined and directly compared to that of Lin[−] BM-resident GMPs that express CD34 and c-Kit (Arinobu et al., 2005; Griseri et al., 2012). Consistent with published

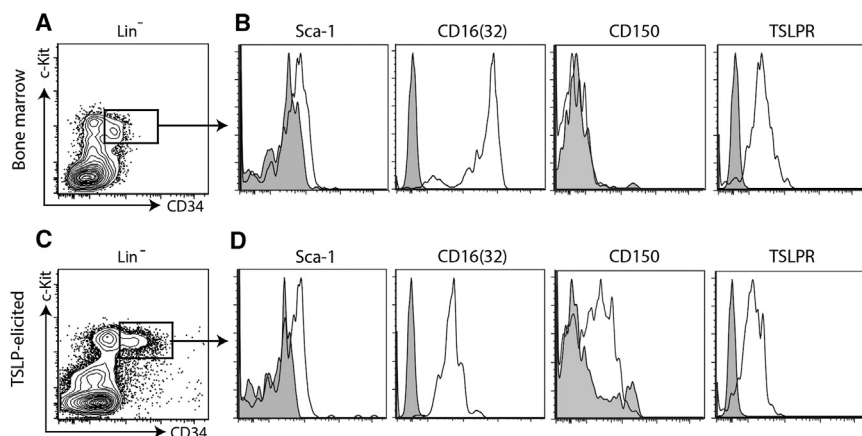


Figure 2. TSLP-Elicited Progenitor Cells Are GMP-like

BM-resident GMPs (A) were identified as $\text{Lin}^- \text{CD34}^+ \text{c-Kit}^+$, and TSLP-elicited splenic progenitor-like cells (C) were identified on day 5 after TSLP-cDNA injection as $\text{Lin}^- \text{CD34}^+ \text{c-Kit}^+$. Surface expression levels of Sca-1, CD16(32), CD150, and TSLPR were determined (black line) compared to fluorescence minus one (FMO) controls (filled gray) on BM-resident GMPs (B) or TSLP-elicited splenic progenitor cells (D). Results are representative of at least three separate experiments. See also Figure S2.

reports, BM-resident $\text{Lin}^- \text{CD34}^+ \text{c-Kit}^+$ GMPs were predominately Sca-1^{lo} , $\text{CD16(32)}^{\text{hi}}$, and CD150^- and also expressed the TSLPR (Figures 2A and 2B) (Arinobu et al., 2005; Griseri et al., 2012). Similar to BM-resident GMPs, $\text{Lin}^- \text{CD34}^+ \text{c-Kit}^+$ TSLP-elicited progenitor-like cells were Sca-1^{lo} , CD16(32)^+ , $\text{CD150}^{\text{int}}$, and TSLPR^+ (Figure 2C and 2D). These data demonstrate that TSLP-elicited progenitor cells in the spleen resemble BM-resident GMPs and are GMP-like.

TSLP-Elicited GMP-like Cells Exhibit Multipotent Potential In Vitro

To directly test whether TSLP-elicited $\text{Lin}^- \text{CD34}^+ \text{c-Kit}^+$ GMP-like cells exhibit multipotent potential, they were sort purified (Figures 3A and 3B) on day 5 after TSLP-cDNA injection and cultured in the presence of the hematopoietic cytokine IL-3. On day 5 after culture, CD11b^+ and CD11b^- cell fractions were readily identified (Figure 3C). Among the CD11b^+ fraction of cells, CD115^+ macrophages (Figure 3D) and Ly6G^+ neutrophils (Figure 3E) were identified, whereas within the CD11b^- fraction, $\text{Fc}\epsilon\text{RI}^+$ CD49b^+ basophils (Figure 3F) and $\text{Fc}\epsilon\text{RI}^+$ c-Kit^+ mast cells (Figure 3G) were identified. Cytologic analysis of progeny at day 5 after culture also revealed cell morphologies consistent with those of mature macrophages, neutrophils, basophils, and mast cells (Figure 3H). Consistent with the presence of mature granulocyte populations, robust levels of IL-4 were detected in IL-3-treated cultures (Figure 3I). In addition, an average of approximately 1×10^5 live cells were recovered from 1×10^4 input cells from IL-3-treated cultures (Figure 3J). Similar results were observed from cultures treated with stem cell factor (SCF) and IL-3 together (data not shown). Critically, TSLP-elicited GMP-like cells did not exhibit the ability to develop into lymphoid cells when cultured on Delta ligand-expressing OP9 cells in the presence of Flt3 ligand and IL-7 (data not shown) (Holmes and Zuniga-Pflucker, 2009). Collectively, these data indicate that TSLP-elicited progenitors proliferate and possess the potential to develop into cells of the myeloid lineage.

To further evaluate the developmental potential of TSLP-elicited GMP-like cells, we sort-purified and dispersed them in a methocult matrix. TSLP-elicited GMP-like cells were capable of developing into colony-forming unit granulocyte macrophage (CFU-GM), CFU-G, and CFU-M colonies, suggesting that they

possess similar developmental potential as BM-GMPs (Figure 3K) (Ito et al., 2003). Further, macrophages, mast cells, and basophils were identified by flow cytometric analysis in isolated single colonies (Figure 3L). Collectively, these data demonstrate that TSLP-elicited GMP-like cells possess multipotent potential in vitro.

TSLP-Elicited GMP-like Cells Exhibit Multipotent Potential In Vivo

To directly test the in vivo multipotent potential of TSLP-elicited GMP-like cells, we injected CD45.1^+ mice with TSLP-cDNA, and GMP-like cells were sort purified on day 5 after injection. Isolated TSLP-elicited progenitors were adoptively transferred into congenic mice that received a TSLP-cDNA injection 1 day prior to the cell transfer (Figure S3A). On day 5 after transfer, CD11b^+ and CD11b^- donor cells were identified (Figures S3B and S3C). Among the CD11b^+ fraction of cells, CD115^+ macrophage populations were identified (Figure S3D), whereas among the CD11b^- fraction, $\text{Fc}\epsilon\text{RI}^+$ CD49b^+ basophils and $\text{Fc}\epsilon\text{RI}^+$ c-Kit^+ mast cells (Figures S3E and S3F) were identified. In addition, in vivo transfers revealed that TSLP-elicited GMP-like cells also differentiated into small but detectable populations of CD11c^+ dendritic cells, CD11b^+ Ly6G^+ neutrophils and CD11b^+ Siglec-F^+ eosinophils (Figure S3G). Collectively, these studies demonstrated the multipotent potential of TSLP-elicited GMP-like cells to develop into multiple effector cell populations in vivo.

TSLP-Elicited GMP-like Cells Are IL-25-Independent and Distinct from $\text{MPP}^{\text{type2}}$ Cells

Previous studies demonstrated that IL-25 promotes a $\text{Lin}^- \text{Sca-1}^+ \text{c-Kit}^+$ progenitor cell population termed $\text{MPP}^{\text{type2}}$ cells in the gut associated lymphoid tissues (GALT) that express low levels of CD34 (Saenz et al., 2010). Therefore, to determine whether TSLP-elicited GMP-like cells were dependent on IL-25-IL-25R signaling, WT or IL-25R-deficient mice ($\text{Il17rb}^{-/-}$) were treated with ethanol or MC903, and TSLP-elicited GMP-like cells were evaluated. Similar to WT mice (Figures S4A–S4C), IL-25R-deficient mice treated with MC903 exhibited a significant population expansion of TSLP-elicited GMP-like cells (Figures S4A–S4C). Next, we directly compared the genome-wide transcriptional profiles of TSLP-elicited GMP-like cells versus $\text{MPP}^{\text{type2}}$ cells.

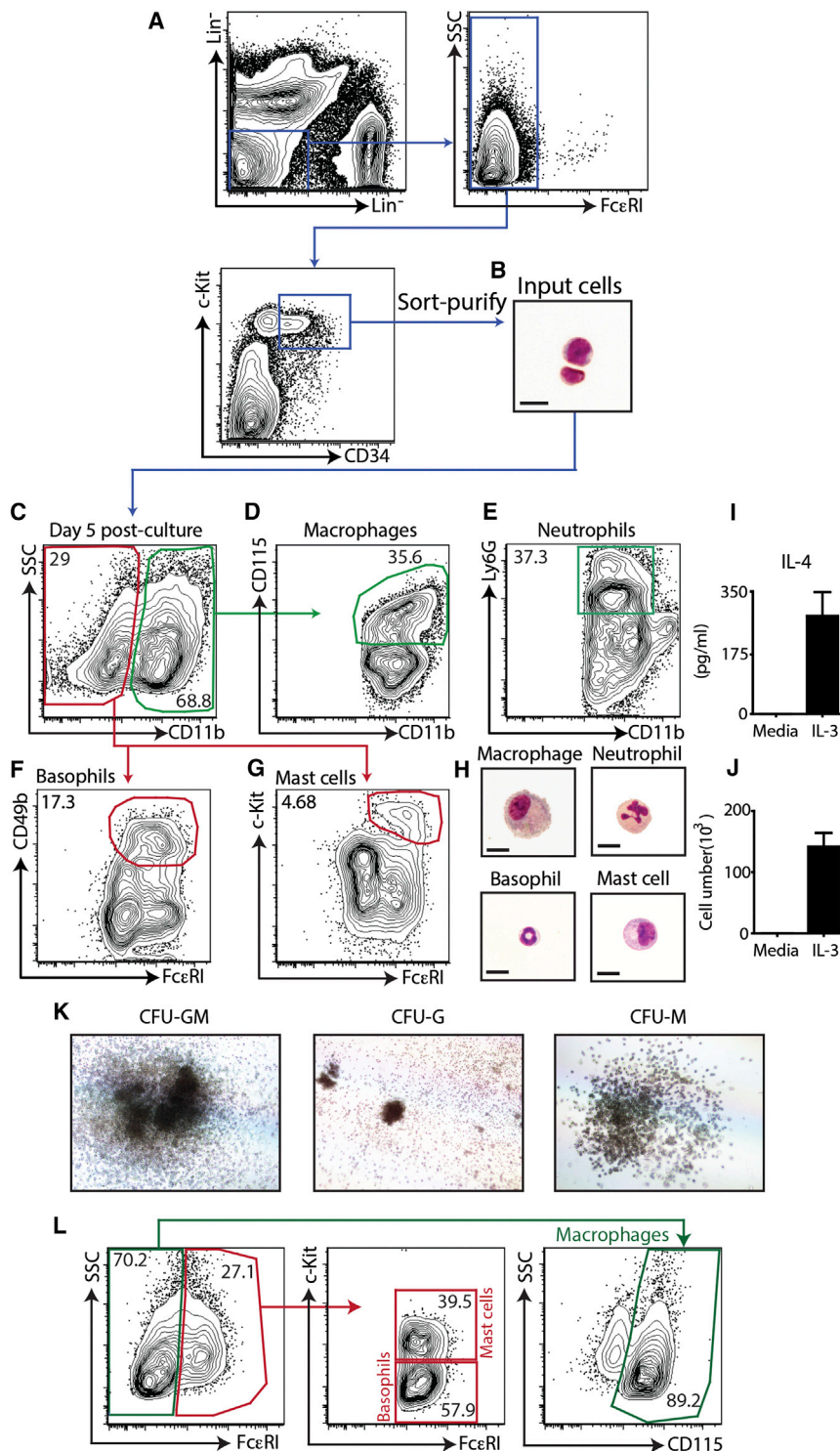


Figure 3. TSLP-Elicited GMP-like Cells Possess Multipotent Potential

Lin⁻ CD34⁺ c-Kit⁺ TSLP-elicited progenitor-like cells (A) in the spleen were sort purified on day 5 after TSLP-cDNA injection and cultured in media alone or 10 ng/mL of IL-3. On day 5 after culture, CD11b⁺ and CD11b⁻ cell fractions (C), CD11b⁺ CD115⁺ macrophages (D), CD11b⁺ Ly6G⁺ neutrophils (E), CD11b⁻ FcεRI⁺ CD49b⁺ basophils (F), and CD11b⁻ FcεRI⁺ c-Kit⁺ mast cells (G) were identified by flow cytometry. Cytology of sort purified TSLP-elicited progenitor cells (B) and post-culture progeny were performed (H). IL-4 levels (I) were determined by ELISA, and the number of live cells (J) were evaluated. Representative CFU-GM, CFU-G, and CFU-M colonies (K) identified post-methocult assay. Results represent the mean ± SEM and numbers in the plots indicate the percentage of cells. Representative flow cytometric analysis of a CFU-GM colony (L). All results are representative of at least three separate experiments. See also Figure S3.

TSLP-Elicited GMP-like Cells Are a Distinct Progenitor Cell Population

Recent studies have shown that progenitor cell populations accumulate in the periphery, undergo EMH, and contribute to the development of inflammation (Griseri et al., 2012; Saenz et al., 2010). However, the functional potential of peripheral progenitor cell populations and how they differ from BM-resident progenitor cells remain to be fully defined. To further explore the differences between TSLP-elicited GMP-like cells and BM-resident GMPs, we compared the genome-wide transcriptional profiles of these distinct cell populations. Critically, TSLP-elicited GMP-like cells and BM-resident GMPs differentially expressed 575 genes at a 2-fold or greater level (Figure 4A). These data suggest that TSLP-elicited GMP-like cells might possess unique functional potential.

To further compare TSLP-elicited GMP-like cells and BM-resident GMPs, we performed analysis of the 200 most differentially expressed genes (Figure 4B) by using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis et al., 2003). DAVID analysis revealed a transcriptional signature of BM-resident GMPs that was enriched for pathways associated with host defense

Consistent with differences in cytokine dependency, TSLP-elicited GMP-like cells exhibited substantially different genome-wide transcriptional profiles compared to IL-25-elicited MPP^{type2} cells (Figure S4D). Collectively, these data suggested that TSLP-elicited GMP-like cells represented a distinct cell population from MPP^{type2} cells.

against bacteria (Figure 4C; Figure S4E). In contrast, TSLP-elicited GMP-like cells were enriched for pathways consistent with biological adhesion, cell activation, growth factor binding, and cytokine binding (Figure 4C; Figure 4E).

To further compare the profiles of these two cell populations, we performed gene-set enrichment analysis (GSEA) (Siracusa

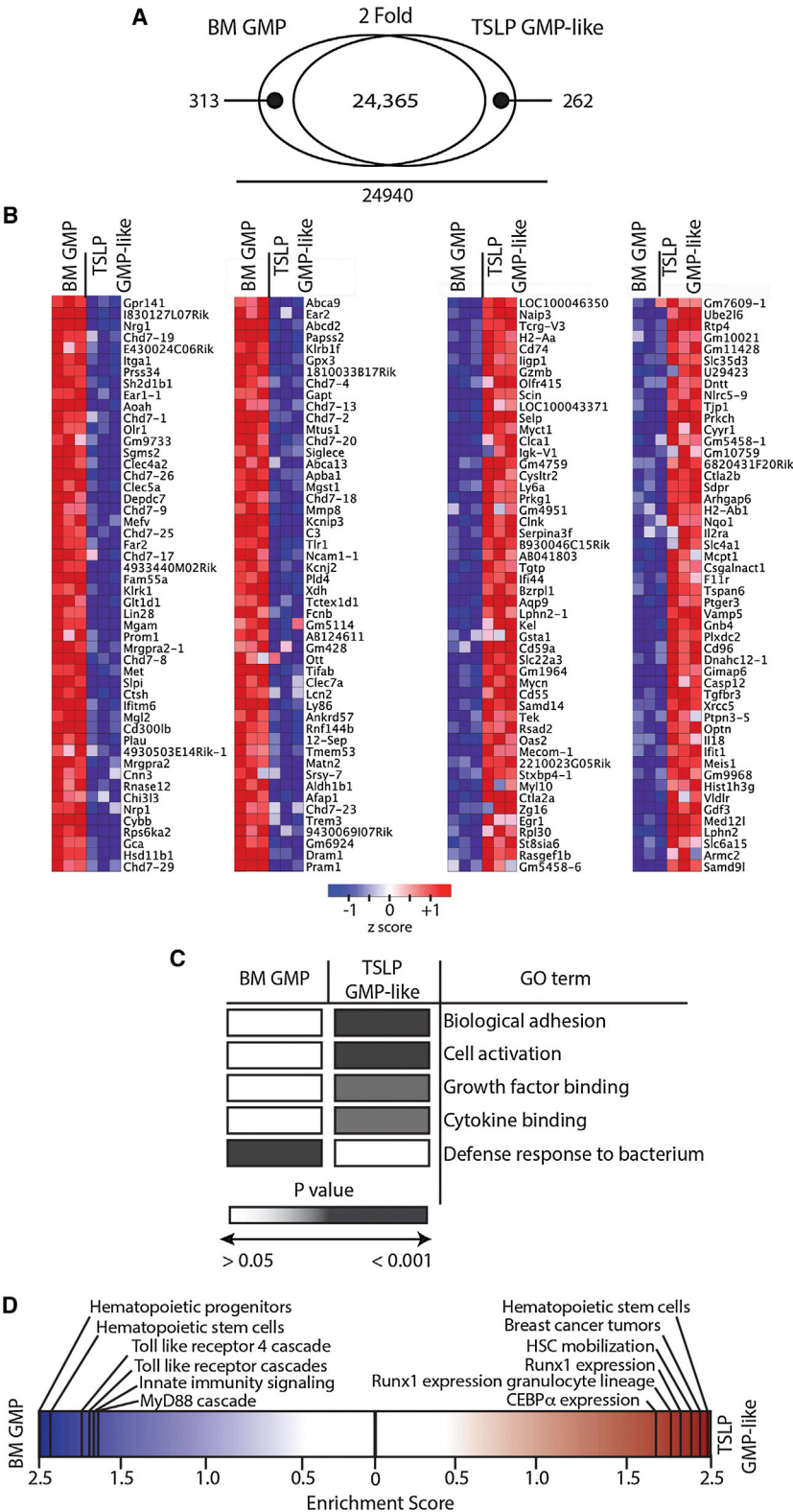


Figure 4. TSLP-Elicited GMP-like Cells Exhibit Distinct Transcriptional Profiles

BM-resident GMPs (BM GMP) or TSLP-elicited GMP-like cells (TSLP GMP-like) were sort purified on day 5 after TSLP-cDNA injection, and genome-wide transcriptional analysis was undertaken. Venn diagram illustrating shared and uniquely expressed genes at levels 2-fold or greater between BM-resident GMPs or TSLP-elicited GMP-like cells (A). Heatmap presentation of gene-expression profiles illustrating 100 of the top 200 genes with the greatest differences in expression (red, high; blue, low) in BM-resident GMPs versus TSLP-elicited progenitor-like cells (B). The top 200 differentially expressed genes were run through DAVID, and significantly enriched transcriptional pathways were identified (GO term) (C). GSEA analysis illustrating enrichment of coordinated gene sets. Gene sets enriched toward BM GMPs (blue gradient) and gene sets enriched in TSLP GMP-like cell gene signatures (red gradient) are indicated by enrichment scores (D). Data are representative of one experiment with three biological replicates per cell type. See also Figure S5.

cells, respectively (Figure 4D; Figure 4F). Consistent with published reports, GSEA of BM-resident GMP profiles identified expression patterns enriched for Toll-like receptor (TLR) pathway signaling and innate immune surveillance (Figure 4D; Figure 4F) (Massberg et al., 2007; Nagai et al., 2006). In contrast, GSEA of TSLP-elicited GMP-like cells revealed gene-expression profiles enriched for genes associated with breast cancer tumors, hematopoietic stem cell mobilization, Runx1 expression, Runx1 expression associated with granulocyte development, and CEBP α expression (Figure 4D; Figure 4F). Critically, EMH and TSLP expression have been linked to the progression of breast cancer, and both Runx1 expression and CEBP α expression are associated with the commitment and differentiation of progenitor cell populations into granulocyte cell lineages (Arinobu et al., 2005; Millar et al., 2009; Mukai et al., 2012; Pedroza-Gonzalez et al., 2011; Wang and Darvishian, 2006). These data suggest that TSLP-elicited GMP-like cells might have a heightened capacity to develop into granulocytes.

TSLP-Elicited GMP-like Cells Exhibit Enhanced Capacity to Promote Type 2 Cytokine Responses

The data presented above suggest that TSLP-elicited GMP-like cells might be skewed toward differentiating into granulocyte populations compared to BM-resident GMP populations. To directly test this, we directly compared the multipotent potential of these

et al., 2011). Both BM-resident GMPs and TSLP-elicited progenitor cells were enriched for gene signatures consistent with hematopoietic stem cells and hematopoietic progenitor

cells, respectively (Figure 4D; Figure 4F). Consistent with published reports, GSEA of BM-resident GMP profiles identified expression patterns enriched for Toll-like receptor (TLR) pathway signaling and innate immune surveillance (Figure 4D; Figure 4F) (Massberg et al., 2007; Nagai et al., 2006). In contrast, GSEA of TSLP-elicited GMP-like cells revealed gene-expression profiles enriched for genes associated with breast cancer tumors, hematopoietic stem cell mobilization, Runx1 expression, Runx1 expression associated with granulocyte development, and CEBP α expression (Figure 4D; Figure 4F). Critically, EMH and TSLP expression have been linked to the progression of breast cancer, and both Runx1 expression and CEBP α expression are associated with the commitment and differentiation of progenitor cell populations into granulocyte cell lineages (Arinobu et al., 2005; Millar et al., 2009; Mukai et al., 2012; Pedroza-Gonzalez et al., 2011; Wang and Darvishian, 2006). These data suggest that TSLP-elicited GMP-like cells might have a heightened capacity to develop into granulocytes.

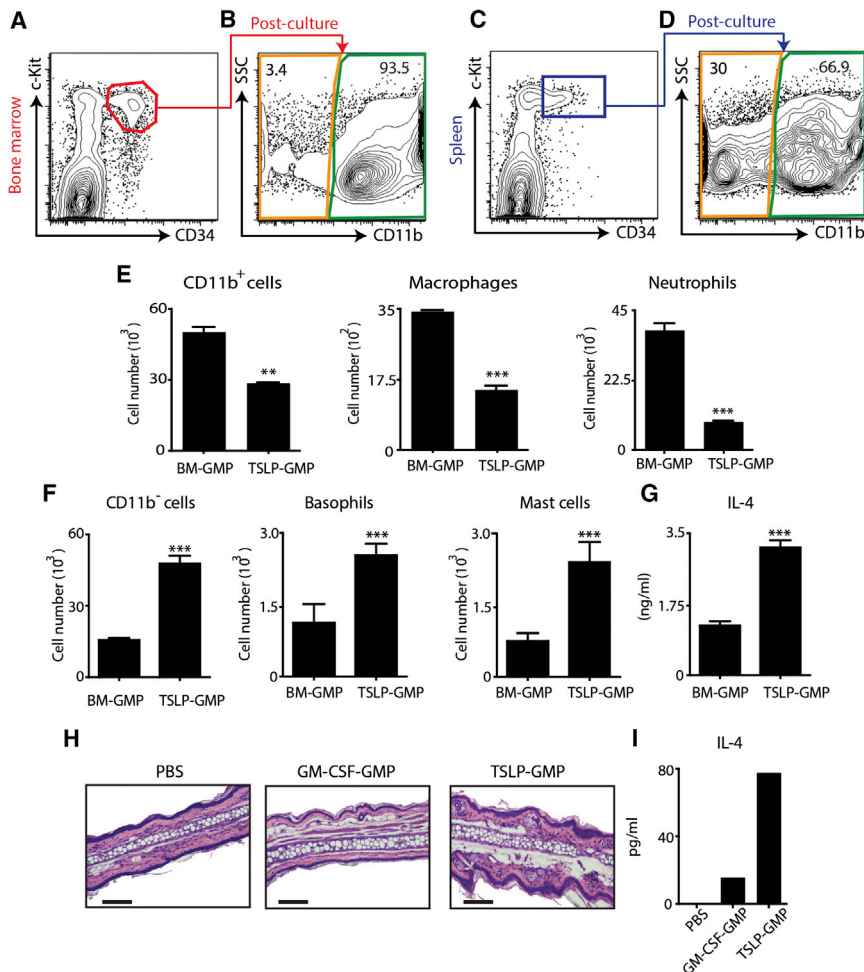


Figure 5. TSLP-Elicited GMP-like Cells Preferentially Differentiate into IL-4-Producing Granulocytes

BM-resident GMPs (A) or TSLP-elicited GMP-like cells (C) were sort-purified and cultured with 10 ng/mL of IL-3. CD11b⁺ and CD11b⁻ cell fractions were quantified in BM-resident GMP cultures (BM-GMP) (B) or TSLP-elicited GMP-like cell cultures (TSLP-GMP) (D). The total number of CD11b⁺ cells, CD11b⁻ cells, macrophages, neutrophils, basophils, or mast cells was identified in BM-GMP cultures or TSLP-GMP cultures (E and F). IL-4 was measured in after culture supernatants by ELISA (G). GM-CSF + LPS initiated GMP-like cells or TSLP-elicited GMP-like cells were sort purified from the spleens of mice and transferred in the skin of naive mice. Pathology (H) and spontaneous IL-4 production in the draining LN (I) were compared to PBS injected controls. (A–D) Representative flow cytometric plots. (E–G) Results represent the mean $\pm$ SEM and are compiled from three separate experiments. Numbers in the gates indicate the percentage of cells. (H and I) Results are representative of three separate experiments. Statistical analysis was performed with a two-way ANOVA. (**p < 0.01), (***)p < 0.001). Scale bars represent 100 μ m.

two populations. Specifically, BM-resident GMPs (Figure 5A) and TSLP-elicited GMP-like cells (Figure 5C) were sort-purified and cultured with the hematopoietic cytokine IL-3. On day 5 after culture, CD11b⁺ and CD11b⁻ cell fractions were readily identified in cultures seeded with BM-resident GMPs (Figure 5B) or TSLP-elicited GMP-like cells (Figure 5D). Although there was no difference in the number of live cells recovered from either set of cultures (data not shown), BM-resident GMPs developed into more CD11b⁺ macrophages and neutrophils than did TSLP-elicited GMP-like cells (Figure 5E). Conversely, TSLP-elicited GMPs developed into more CD11b⁻ basophils and mast cells (Figure 5F). Further, increases in the number of basophils and mast cells also correlated with increases in the levels of IL-4 in the supernatants of TSLP-elicited GMP-like cell cultures (Figures 5F and 5G). Collectively, these data indicated that TSLP-elicited progenitor cells possessed an enhanced capacity to differentiate into mast cell and basophil populations compared to BM-resident progenitors and thus might be more potent at promoting type 2 cytokine responses than other progenitor cell populations.

Previous studies have demonstrated that multiple cytokines and growth factors promote the accumulation of peripheral progenitor cells in the context of Th1 and Th17 cell-associated inflammation (Griseri et al., 2012; Papayannopoulou, 1999;

Schuettpelz and Link, 2013). Our findings demonstrate that TSLP elicits a distinct GMP-like cell population in the context of type 2 cytokine responses (Figure 1), suggesting that TSLP-elicited GMP-like cells might be specialized to promote Th2 cell responses. To directly test this, we sort purified splenic TSLP-elicited GMP-like or GM-CSF + LPS-elicited GMP-like cells, injected them into the skin of naive mice, and monitored their ability to promote pathology and type 2 cytokine production (Saenz et al., 2013). Consistent with an ability to promote type 2 cytokine responses, TSLP-elicited GMP-like cells were more potent inducers of skin inflammation characterized by a mixed dermal inflammatory infiltrate and edema (Figure 5H) and IL-4 production in the LN (Figure 5I). These data suggested that TSLP-elicited GMPs were primed to promote type 2 cytokine responses.

To further evaluate the ability of TSLP-elicited GMP-like cells to contribute to the development of type 2 cytokine responses in vivo, TSLP-elicited GMP-like cells were transferred into *Trichuris muris*-infected TSLPR-deficient mice (*Cr1f2*^{-/-}). Consistent with previous reports (Taylor et al., 2009), *Trichuris*-infected *Cr1f2*^{-/-} mice exhibited decreased production of type 2 cytokines (IL-4, IL-5, and IL-13), decreased serum immunoglobulin E (IgE) titers, a loss of goblet cell hyperplasia, and increased worm burdens (Figures 6A–6D) compared to *Cr1f2*^{+/+} mice. Critically, adoptive transfer of TSLP-elicited GMP-like cells into infected *Cr1f2*^{-/-} mice (*Cr1f2*^{-/-} + GMP-like) was sufficient to partially restore type 2 cytokine production (Figure 6A), serum IgE levels (Figure 6B), and goblet cell hyperplasia (Figure 6C) and significantly reduce worm burdens (Figure 6D). Together, these data suggest that TSLP-elicited

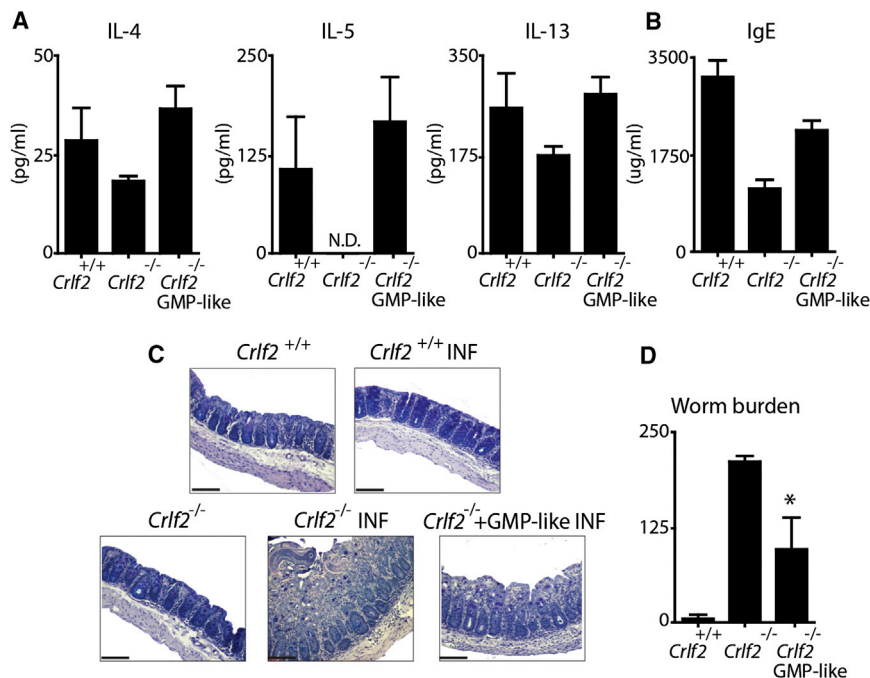


Figure 6. TSLP-Elicited GMP-like Cells Contribute to Type 2 Cytokine Responses In Vivo

Crfl2^{+/+} or *Crfl2*^{-/-} mice were infected with *Trichuris muris*, and on days 10–15 after infection, one group of *Crfl2*^{-/-} mice received TSLP-elicited GMP-like cells (*Crfl2*^{-/-} + GMP-like). On day 21 postinfection, mesenteric lymph node (mLN) cells were stimulated with *Trichuris* antigen, and IL-4, IL-5, and IL-13 production (A) and serum IgE titers (B) were determined. Intestinal mucin production by PAS/alcian blue staining was examined (C), and worm burdens were determined (D). Results represent the mean $\pm$ SEM. Results are representative of two separate experiments (*Crfl2*^{+/+}; n = 8) (*Crfl2*^{-/-}; n = 8) (*Crfl2*^{-/-} + GMP-like; n = 6). Statistical analysis was performed with a two-tailed Mann-Whitney t test. (*p < 0.05). Scale bars represent 100 μ m.

GMP-like cells are capable of contributing to type 2 cytokine responses in vivo.

Human GMP-like Cell Responses Are Associated with a Gain-of-Function Polymorphism in TSLP and Allergic Inflammation

To determine whether a similar population of Lin⁻ CD34⁺ c-Kit⁺ progenitor-like cells could be identified in the spleens of humans, we collected splenic tissue from ten human organ donors and examined it for the presence of progenitor-like cells. Critically, Lin⁻ CD34⁺ c-Kit⁺ cells were found in all ten human samples examined (Figure 7A). Further, Lin⁻ CD34⁺ c-Kit⁺ GMP-like cells were also identified in the peripheral blood of human subjects (Figure 7B). To determine whether human GMP-like cells possess multipotent potential, we sort purified Lin⁻ CD34⁺ c-Kit⁺ cells from the blood of patients and performed methocult assays. Consistent with murine GMP-like cells, human progenitor-like cells readily produced CFU-GM, indicating they were GMP-like (Gribaldo et al., 1999) (Figure 7C).

Next, we examined the frequencies of Lin⁻ CD34⁺ c-Kit⁺ cells in the peripheral blood (Figure 7B) of allergic patients suffering from EoE. EoE is an allergic inflammatory disease that causes symptoms such as dysphagia and food impaction (Noti et al., 2013). Although the causes of EoE remain unknown, a subset of EoE patients possess a gain-of-function single-nucleotide polymorphism (SNP) rs3806932 in *TSLP* that is associated with elevated TSLP expression and elevated granulocyte responses in the periphery (Noti et al., 2013; Rothenberg et al., 2010; Sherrill et al., 2010). Critically, allergic patients with the SNP rs3806932 (SNP+) presented with increased frequencies of Lin⁻ CD34⁺ c-Kit⁺ cells in their peripheral blood compared to patients lacking a gain-of-function polymorphism (control) (Figure 7D). These data indicate that the TSLP-EMH pathway is conserved and active in mice and

humans and might contribute to the development of allergic inflammation.

The symptoms of EoE are thought to result from chronic esophageal inflammation and pathophysiological changes in esophageal tissues associated with

the accumulation of granulocytes such as basophils and eosinophils (Noti et al., 2013). However, whether mature granulocytes are recruited to the esophageal tissue or whether these cells develop in the tissue via EMH remains unknown. Therefore, we evaluated the relationship between progenitor-like cells and granulocytes in esophageal biopsies from control patients, patients with active EoE, and patients with inactive EoE. Critically, patients with active EoE had significantly decreased frequencies of progenitor-like cells in their biopsies compared to healthy controls. Further, frequencies of progenitor-like cells were restored back to control levels in patients with inactive EoE, demonstrating that progenitor cells were negatively associated with active disease (Figure 7E). Additionally, reductions in progenitor-like cells were associated with a significant increase in frequencies of Fc ϵ RI⁺ granulocytes in esophageal biopsies (Figure 7F). Collectively, these data suggest that progenitor-like cells in esophageal tissues of patients with active EoE might differentiate via EMH into mature granulocytes and contribute to the development of allergic inflammation associated with EoE.

DISCUSSION

In this study, we demonstrated a previously unrecognized role for TSLP-elicited EMH in contributing to the development of type 2 cytokine-dependent immunity and inflammation. Exogenous and endogenous TSLP induced the population expansion of murine Lin⁻ CD34⁺ c-Kit⁺ progenitor cells in the context of type 2 cytokine-associated infection or inflammation, and disruption of the TSLP-TSLPR signaling pathway prevented the accumulation of progenitor cells in the periphery. TSLP-elicited Lin⁻ CD34⁺ c-Kit⁺ cells were found to phenotypically resemble BM-resident GMPs, indicating that they possess GMP-like qualities. However, genome-wide transcriptional profiling and functional assays demonstrated that TSLP-elicited

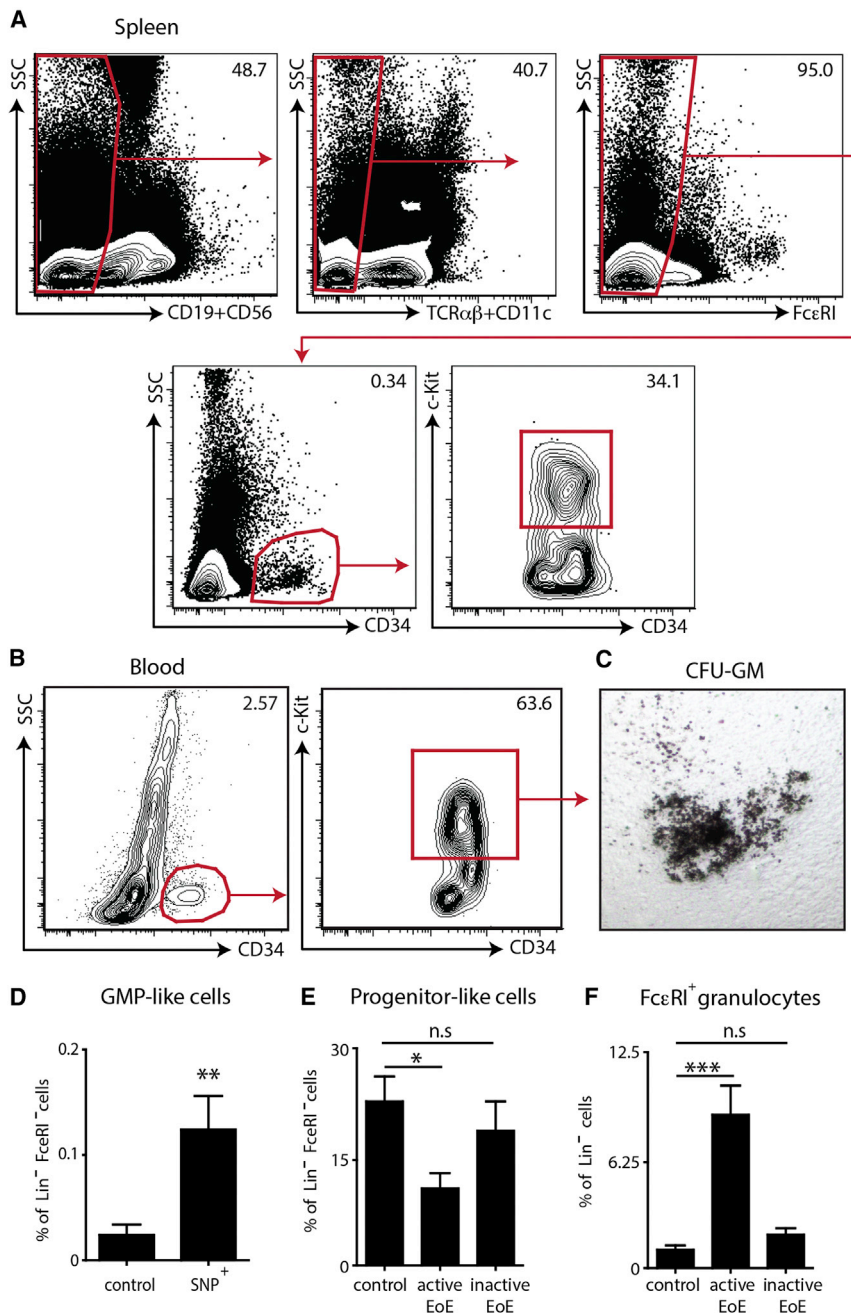


Figure 7. Progenitor Cell Responses Are Associated with a Gain-of-Function Polymorphism in *TSLP* and Allergic Inflammation

Lin⁻ (CD3⁻, TCRαβ⁻, CD11c⁻, CD19⁻, CD56⁻, FcεRI⁻) CD34⁺ c-Kit⁺ cells were identified in the spleens of 10 human organ donors (A). Lin⁻ FcεRI⁻ CD34⁺ c-Kit⁺ progenitor-like cells were identified (percentage of parent gate is displayed) (B) and sort purified from the blood of healthy patients, and CFU-GM colonies were identified after methocult assay (C). Lin⁻ CD34⁺ c-Kit⁺ progenitor-like cells were quantified in the peripheral blood of allergic patients with no copies of SNP rs3806932 (SNP⁻, n = 37) (D). Esophageal biopsies were taken from healthy control patients (n = 19), patients with active EoE (n = 16), or patients with inactive EoE (n = 15), and the percentage of Lin⁻ FcεRI⁻ CD34⁺ c-Kit^{variable} progenitor-like cells (E) and Lin⁻ FcεRI⁺ granulocytes (F) were determined by flow cytometric analysis. Results represent the mean ± SEM, and numbers in the plots indicate the percentage of cells. Statistical analysis was performed with a two-tailed Mann-Whitney t test. (*p < 0.05), (**p < 0.01), (***)p < 0.001). (C) Results are representative of two blood donors and eight technical replicates.

ated with elevated expression of *TSLP* have significantly increased progenitor cell populations in their peripheral blood. Further, we showed that reduced progenitor-like cell populations were associated with increased granulocyte populations in the context of EoE. These data suggest that granulocyte populations found in the esophageal tissue in the context of active EoE might be derived, at least in part, from local progenitor cells that undergo EMH. These translational studies suggest a link between elevated *TSLP* expression, an increased potential for EMH, and increased granulocyte responses in human patients suffering from allergic inflammation.

TSLP was originally isolated from a mouse thymic stromal cell line and was first defined as a lymphocyte growth

GMP-like cells were more potent mast cell and basophil precursors and produced more IL-4 than BM-resident GMPs. Critically, adoptive transfer of *TSLP*-elicited GMP-like cells preferentially promoted type 2 cytokine production compared to GM-CSF + LPS-elicited GMP-like cells and was sufficient to partially restore Th2 cell responses and protective immunity to *Trichuris* in normally susceptible mice. These data demonstrated that in contrast to other peripheral progenitor cell populations, *TSLP*-elicited GMP-like cells appear to preferentially promote type 2 cytokine responses.

Employing translational approaches, we also demonstrated that EoE patients with a gain-of-function polymorphism associ-

factor (Ziegler and Artis, 2010). It is now appreciated that *TSLP* can promote type 2 cytokine-mediated inflammation by regulating multiple terminally differentiated cell types (Al-Shami et al., 2004; Allakhverdi et al., 2007; Ito et al., 2005; Kim et al., 2013; Lu et al., 2009; Reche et al., 2001; Siracusa et al., 2011; Ziegler and Artis, 2010). The data presented in this manuscript identify a previously unrecognized role for *TSLP* as a critical regulator of EMH and suggest that in addition to activating terminally differentiated cells and promoting the central hematopoiesis of basophils, *TSLP* regulates EMH and influences the development of multiple myeloid effector cells in peripheral tissues.

Recent studies have demonstrated that epithelial cell-derived cytokines critically support type 2 cytokine-associated innate immune responses (Ho et al., 2007; Noti et al., 2013; Saenz et al., 2010; Saenz et al., 2008; Siracusa et al., 2011; Wills-Karp and Finkelman, 2011; Ziegler and Artis, 2010). For example, IL-25, IL-33, and TSLP promote the accumulation of ILCs that contribute to the development of type 2 cytokine-mediated inflammation (Spits and Cupedo, 2012; Walker et al., 2013). In addition to promoting ILC responses, our new findings demonstrated for the first time that TSLP supports the development of multiple myeloid effector cells and elicits type 2 cytokine responses by directly regulating the presence of progenitor cells in the periphery and by promoting EMH.

TSLP is strongly associated with multiple human disease states and the development of type 2 cytokine-mediated inflammation in the skin, lung, and digestive tract. For example, genetic analyses of patient populations have demonstrated that gain-of-function mutations in the genes encoding TSLP or its receptor are associated with the development of asthma, allergic rhinitis, and EoE (Bunyavanich et al., 2011; Harada et al., 2010; Hunninghake et al., 2010; Sherrill et al., 2010). Further, exaggerated TSLP production has been observed in patients suffering from AD, asthma, and EoE (Sherrill et al., 2010; Soumelis et al., 2002; Ying et al., 2005). Critically, our findings demonstrated that patients with a gain-of-function polymorphism in *TSLP* and suffering from EoE presented with exaggerated frequencies of Lin[−] CD34⁺ c-Kit⁺ cells in their peripheral blood. Further, we demonstrated that reduced progenitor cell populations were associated with increased mature granulocyte responses and allergic inflammation in the esophagus, suggesting that TSLP-elicited EMH might contribute to EoE-associated inflammation in humans. These data provide a link between elevated *TSLP* expression and progenitor cell responses in the periphery of human patients suffering from type 2 cytokine-associated disease. However, more extensive genetics-based studies are necessary to evaluate the relationships between a gain-of-function polymorphism in *TSLP*, EMH, and EoE.

TLR-expressing HSCs are known to exit the BM and are thought to patrol the periphery in a manner that allows them to provide an immediate source of myeloid cells that can contribute to host microbial defense (Massberg et al., 2007; Nagai et al., 2006). Further, peripheral tissue compartments such as the spleen and blood are thought to provide a reservoir of undifferentiated myeloid cells that are primed for rapid deployment and function as critical regulators of inflammation (Swirski et al., 2009). More recently, it has been shown that GMPs accumulate in the spleen and colon following the induction of chronic intestinal inflammation and contribute to proinflammatory responses in the gut (Griseri et al., 2012). However, the role of EMH in promoting the induction of type 2 cytokine-mediated inflammation has remained less well defined. The data presented here demonstrate that TSLP-elicited EMH directly contributes to Th2 cell responses and suggest that EMH is capable of influencing multiple modules of the innate and adaptive immune system.

In conclusion, data presented here demonstrating that TSLP-elicited EMH promotes type 2 cytokine-mediated inflammation—coupled with previous reports demonstrating the contributions of EMH in other settings (Griseri et al., 2012; Massberg

et al., 2007; Nagai et al., 2006; Swirski et al., 2009)—indicate that EMH might be a conserved aspect of innate immunity in multiple infectious and inflammatory contexts. The alteration of stem cell populations by modifying transcription factors or epigenetic events is recognized for its therapeutic potential to replace damaged cells or to regenerate damaged tissues (Robinton and Daley, 2012; Sharkis et al., 2012). Our present study suggests that in addition to the therapeutic potential of targeting stem cells in regenerative medicine, altering the migration and differentiation potential of pluripotent HSCs might represent a therapeutic approach to regulate type 2 inflammation in the context of helminth infections, asthma, allergies, and cancer.

EXPERIMENTAL PROCEDURES

Mice

C57BL/6 wild-type (WT) mice were purchased from Charles River Laboratory. *Cr1f2*^{−/−} mice and *Il17rb*^{−/−} were provided by Amgen via Charles River Laboratory. Congenic mice were purchased from NCI. Mice were maintained in specific pathogen-free facilities at the University of Pennsylvania. All protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC).

Flow Cytometry

Cells were stained with monoclonal anti-mouse fluorescently conjugated antibodies: Sca-1 (D7), c-Kit (2B8), CD4 (GK1.5), CD8 (SK1), CD5 (53-73), NK1.1 (PK136), CD11b (MI/70), CD11c (N418), CD45.1 (A20), CD45.2 (104), CD16(32) (93), CD127 (A7R34), ICOS (7E.17G9), CD90 (53-2.1), and CD25 (PC61.5) from eBioscience; CD34 (RAM34) from BD Pharmingen; and T1(ST2) biotinylated mAb from MD Biosciences; or monoclonal anti-human fluorescently conjugated antibodies: CD19 (HIB19), FcεRI (AER-37), CD34 (4H11), and c-Kit (61D3) from eBioscience; TCRαβ (ID26) and CD56 (B159) from BD Pharmingen; and CD11c (551077) from Miltenyi Biotec. Anti-mouse TSLPR antibody was provided by Amgen. Samples were acquired on a BD LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo software (v9.2, Tree Star). Cell sorting was performed with a FACSAriaII (BD Bioscience).

Microarray Gene-Expression Profiling

TSLP-elicited GMP-like cells (Lin[−] CD34⁺ c-kit⁺) were sort-purified from the spleen of C57BL/6 mice on day 5 after TSLP-cDNA treatment. BM-resident GMPs (Lin[−] Sca1[−] c-kit⁺ CD34⁺ CD16[32][−]) were sort-purified from mice that received a control-cDNA injection. IL-25-elicited MPP^{type2} cells (Lin[−] T1[ST2][−] IL-7Rα[−] CD90[−] CD25[−] c-kit⁺) were sort purified from the MLN and PECs of C57BL/6 mice on day 7 after IL-25 treatment (Saenz et al., 2013). Lineage markers included CD3ε, CD4, CD8α, CD19, CD11b, CD11c, and NK1.1. Three biological replicates were collected, each consisting of 50,000 cells. mRNA was isolated, amplified, and hybridized to the Mouse Gene 1.0ST genechip (Affymetrix). The GEO accession number for the transcriptional profiles reported in this paper is GSE52485. With the ClassNeighbors function in GenePattern, differentially expressed genes (fold-change > 2, p < 0.05) were identified. Gene pathways were analyzed with DAVID. GSEA of the top 100 differentially expressed genes from each biological group was performed. PCA (Saenz et al., 2013) and Euclidean distance measurements were performed with R (R Core Team), and hierarchical clustering analysis was performed with Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm. Analysis plot compares transcriptional profiles for TSLP-elicited GMP-like cells (TSLP-GMP), natural helper cells (NHC) (GSE18752), lung-resident ILCs (lung ILC) (GSE46468), splenic lymphoid tissue-inducer cells (LTi) (GSE46468), and fetal lymphoid tissue-inducer cells (fLTi) (GSE18752).

In Vitro Differentiation Assay

Spleens of TSLP-cDNA-treated mice were isolated on day 5 after injection, and single-cell suspensions were made. GMP-like cells were identified as Lin[−] (CD3, CD4, CD5, CD8, CD19, CD11b, CD11c, NK1.1, and FcεRI), and

CD34⁺ c-Kit⁺ cells were sort purified. We plated 1×10^4 GMP-like cells in 200 μ l of complete RPMI in the presence or absence of 10 ng/mL of rIL-3 (R&D Systems). Fresh rIL-3 was added on day 3 after transfer. On day 5, cell populations were evaluated, and cytokine was measured by standard ELISA (Saenz et al., 2010).

Methocult Assays

TSLP-elicited GMP-like cells were sort-purified and processed according to the manufacturer's protocol (Methocult GF M3434, STEMCELL Technologies). GMP-like cells were sort-purified from the blood of healthy human donors and processed according to the manufacturer's protocol (Methocult H4035 Optimum, STEMCELL Technologies).

In Vivo Differentiation Assay

We sort purified 3×10^4 TSLP-elicited GMP-like cells from the spleen of CD45.1⁺ mice and adoptively transferred intraperitoneally (i.p.) into CD45.2⁺ congenic mice that received a TSLP-cDNA injection 1 day prior to the cell transfer. On day 5 after transfer, peritoneal cells were collected and flow cytometric analysis was performed.

MC903, Antibody Treatments, Hydrodynamic Tail-Vein Injections, and *Trichinella spiralis* Infections

C57BL/6, *Crfl2*^{-/-}, or *Il17rb*^{-/-} mice were treated as previously described (Kim et al., 2013). C57BL/6 mice were administered Rat IgG isotype or anti-TSLP neutralizing antibody (i.p.) as previously described (Kim et al., 2013). C57BL/6 administered cDNA plasmids as previously described (Siracusa et al., 2011). Mice were infected with 300 *Trichinella* muscle larvae by oral gavage and sacrificed on day 8 after infection (Giacomini et al., 2012).

Intradermal Injections

We suspended 2×10^4 sort purified BM-resident GMPs or TSLP-elicited GMP-like cells in 50 μ l of PBS and injected them intradermally into C57BL/6 mice. Controls were PBS injected. At day 5 after infection, draining LNs were collected and spontaneous cytokine production was evaluated after 48 hr by ELISA (eBioscience).

Trichuris muris Infection

WT and *Crfl2*^{-/-} mice were infected with *Trichuris muris* as previously described (Taylor et al., 2009), and on days 5–10 after infection, one group of *Crfl2*^{-/-} mice received $3 \times 10^4 - 2 \times 10^5$ sort purified TSLP-cDNA-elicited GMP-like cells from WT mice. On day 21 postinfection, *T. muris* antigen-specific type 2 cytokine responses, serum IgE, pathology, and worm burdens were evaluated.

Human Patients and Sample Preparation

Pediatric patients at the University of Pennsylvania Penn-CHOP Joint Center for Digestive, Liver and Pancreatic Medicine or the Center for Pediatric Eosinophilic Disorders at CHOP were analyzed and were provided to M.-L.W. and A.J.B. under protocols approved by the CHOP IRB. EoE patients were defined as previously described (Noti et al., 2013). Patients with inactive EoE were defined as previously described (Noti et al., 2013). Peripheral blood from genotyped pediatric patients was collected and processed as previously described (Noti et al., 2013).

Written consent was obtained from all participants or their parents or legal guardians, and for pediatric participants, verbal assent from the child was additionally obtained as described in IRB approved protocols.

Statistical Analysis

Results represent the mean $\pm$ SEM unless indicated otherwise. For murine studies, statistical significance was determined by unpaired Student's t test, one-way ANOVA, or two-way ANOVA as indicated. For patient studies, a nonparametric, two-tailed Mann-Whitney t test was used to compare means. Statistical analyses were performed with Prism GraphPad software v5.0 (*p < 0.05), (**p < 0.01), (**p < 0.001).

ACCESSION NUMBERS

The GEO accession number for the transcriptional profiles reported in this paper is GSE52485.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and can be found with this article online at <http://dx.doi.org/10.1016/j.immuni.2013.09.016>.

AUTHOR CONTRIBUTIONS

M.C.S., S.A.S., E.D.T.W., B.S.K., L.C.O., and D.A. designed and performed the research. A.J.B., K.R.R., A.C., M.-L.W., J.M.S., and D.L.F. provided human samples, and P.M.S. and H.H. provided genotype information. M.R.C. provided reagents and advice. M.C.S., S.A.S., E.D.T.W., B.S.K., C.G.Z., and D.A. analyzed the data. M.C.S. and D.A. wrote the paper.

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Supplemental Information

Thymic Stromal Lymphopoietin-Mediated Extramedullary Hematopoiesis Promotes

Allergic Inflammation

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Supplemental Inventory

Supplemental Figures and Tables

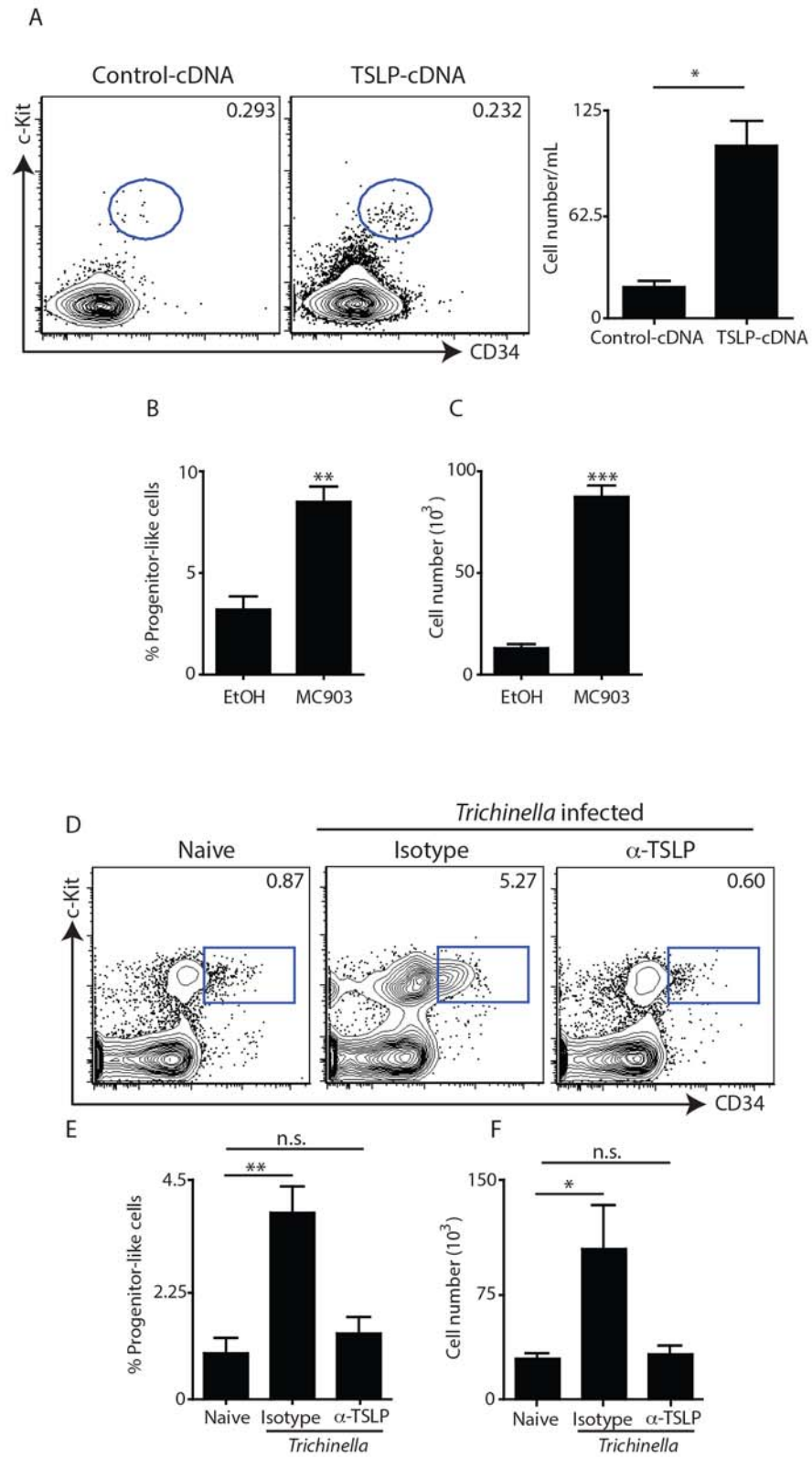
Figure S1, related to Figure 1

Figure S2, related to Figure 2

Figure S3, related to Figure 3

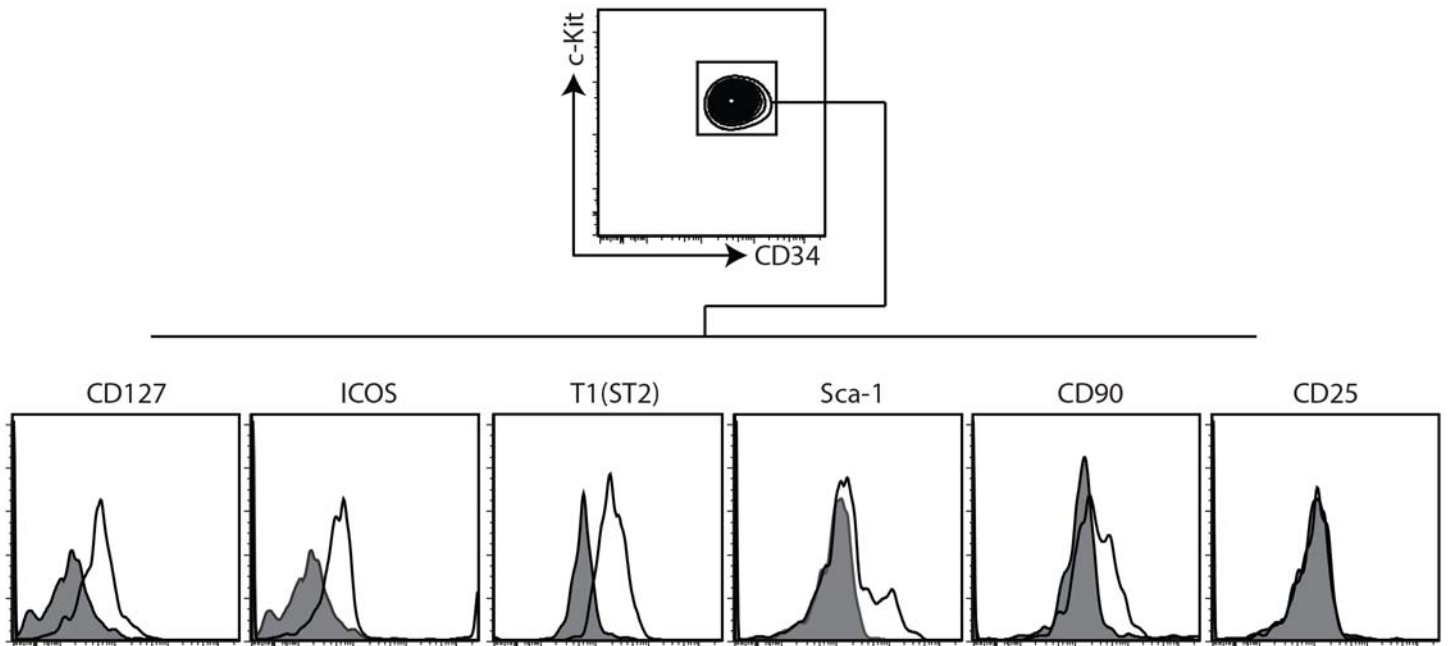
Figure S4, related to Figure 4

Supplementary Figure 1

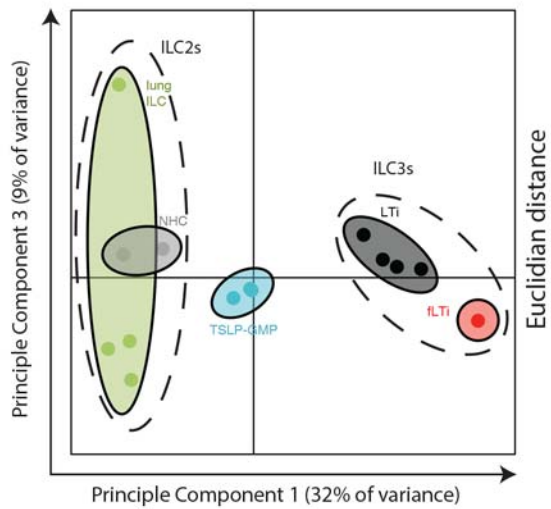


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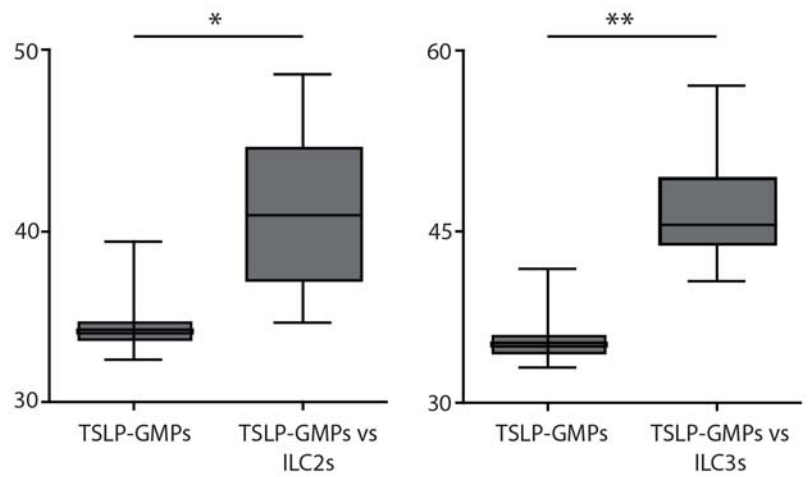
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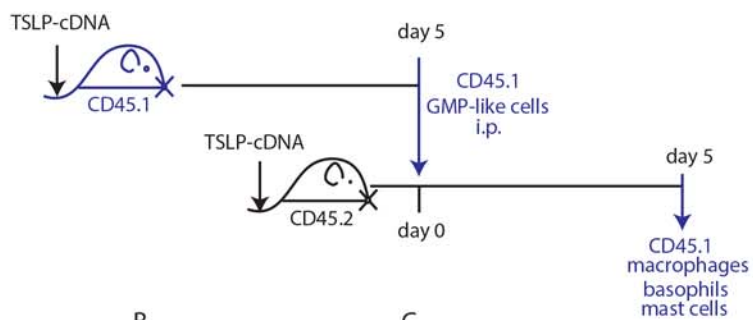


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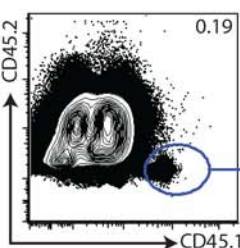


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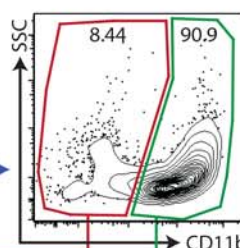
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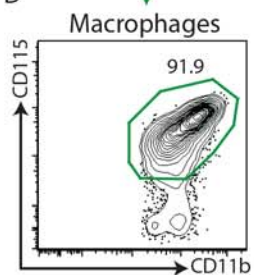
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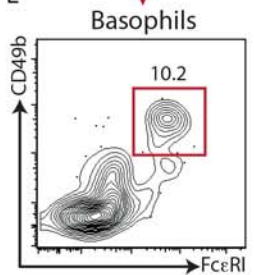
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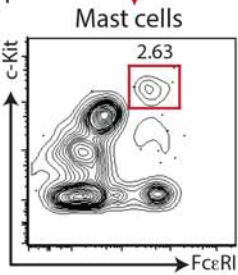
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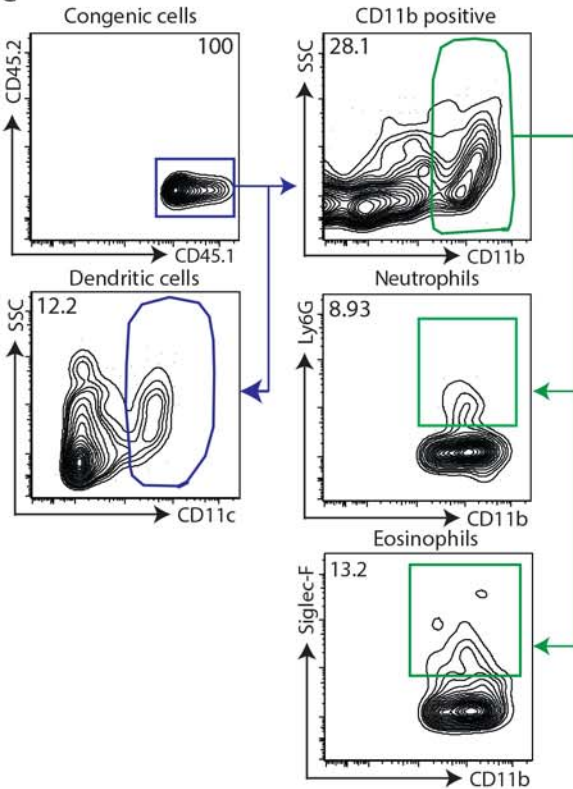
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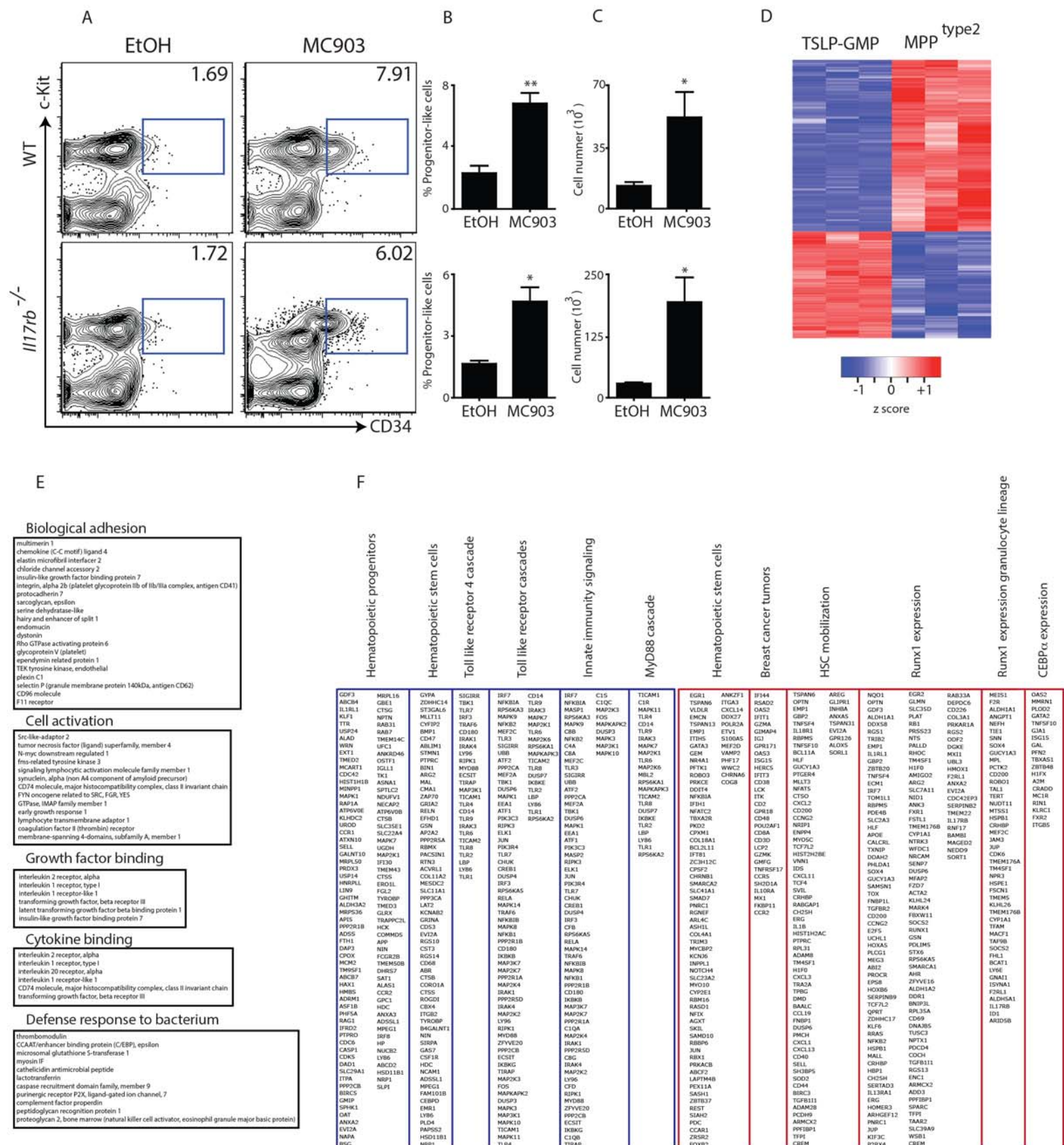
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Supplementary Figure 4



Supplementary Figure 1. Progenitor-like cells expand in response to allergic stimuli. Mice were injected with control plasmid (Control-cDNA) or TSLP-encoding cDNA plasmid (TSLP-cDNA), and Lin⁻ CD34⁺ c-Kit⁺ progenitor-like cells in the blood were quantified on day 5 post-injection (A). Mice were treated with ethanol (EtOH) or MC903 for seven days, and the percentage (B) and total number (C) of Lin⁻ CD34⁺ c-Kit⁺ progenitor-like cells were examined. Results are representative of at least 3 independent experiments of at least 3 mice per group per experiment. Results represent the mean +/- SEM and numbers in the plots indicate the percentage of cells. Statistical analysis was performed using a Student's t-test. (*, $p<0.05$) (, $p<0.01$) (***, $p<0.001$). WT mice were infected with *Trichinella spiralis* and treated with isotype control or anti-TSLP neutralizing antibody, and the percentage (D, E), and total number (F) of progenitor-like cells in the spleen were quantified. Results represent the mean +/- SEM and numbers in the plots indicate the percentage of cells. Statistical analysis was performed using a one-way ANOVA and Tukey's post hoc. (*, $p<0.05$), (**, $p<0.01$). Results are representative of 2 separate experiments of 5 mice per group per experiment. Figure S1, related to Figure 1.**

Supplementary Figure 2. TSLP-elicited GMP-like cells are distinct from innate lymphoid cells. Lin⁻ CD34⁺ c-Kit⁺ TSLP-elicited GMP-like cells were evaluated for expression of CD127, ICOS, T1/ST2, Sca-1, CD90 and CD25 (open histograms) and compared to isotype controls (shaded histograms)

(A). Principle component analysis was performed evaluating the transcriptional profiles of TSLP-elicited GMP-like cells, ILC2s and ILC3s
(B). Euclidian distances were determined between transcriptional data sets
(C). Results represent the mean +/- SEM. Statistical analysis was performed using a Student's t-test. (*, $p < 0.05$), (**, $p < 0.01$). Figure S2, related to Figure 2.

Supplementary Figure 3. TSLP-elicited GMP-like cells exhibit multipotent potential *in vivo*. Lin⁻ CD34⁺ c-Kit⁺ splenic TSLP-elicited progenitor-like cells were sort-purified from CD45.1 congenic mice on day 5 post-TSLP-cDNA injection and transferred (i.p.) into TSLP-cDNA injected CD45.2 disparate mice that received a TSLP-cDNA injection day -1 prior to cell transfer (A). On day 5 post-transfer, CD45.1⁺ CD45.2⁻ donor cells were identified in the peritoneal cavity (B), and CD11b⁺ and CD11b⁻ fractions (C) were evaluated. CD11b⁺ CD115⁺ macrophages (D), CD11b⁻ FcεRI⁺ CD49b⁺ basophils (E) and CD11b⁻ FcεRI⁺ c-Kit⁺ mast cells (F), were identified by flow cytometry. In addition, CD45.1⁺ CD45.2⁻ donor CD11c⁺ dendritic cell, CD11b⁺ Ly6G⁺ neutrophil and CD11b⁺ Siglec-F⁺ eosinophil populations were identified flow cytometry. Results are representative of 3 separate experiments. Figure S3, related to Figure 3.

Supplementary Figure 4. TSLP-elicited GMP-like cells are distinct from other progenitor cell populations. WT or *Il17rb*^{-/-} mice were treated with

ethanol (EtOH) or MC903 for seven days, and the percentage (A-B) and total number (C) of Lin⁻ CD34⁺ c-Kit⁺ progenitor-like cells were examined. The genome-wide transcriptional profiles of TSLP-elicited GMP-like cells and IL-25-elicited MPP^{type2} cells were compared (D). Results are representative of at least 3 separate experiments of at least 3 mice per group. Results represent the mean $\pm$ SEM and numbers in the plots indicate the percentage of cells. Statistical analysis was performed using a Student's t-test. (*, $p < 0.05$), (**, $p < 0.01$). The top 200 differentially expressed genes for BM-resident GMPs or TSLP-elicited GMP-like cells were run through the Database for Annotation, Visualization and Integrated Discovery (DAVID), and significantly enriched transcriptional pathways were identified (GO term). Gene lists comprising each GO term is illustrated (E). Gene expression profiles of BM-resident GMPs or TSLP-elicited GMP-like cells were run through Gene set enrichment analysis (GSEA), performed with software available at <http://www.broadinstitute.org/gsea/index.jsp>, illustrating enrichment of coordinated gene sets that correspond to previously defined biological processes. Genes comprising gene sets are illustrated (F).