IL-3 Induces Basophil Expansion In Vivo by Directing Granulocyte-Monocyte Progenitors to Differentiate into Basophil Lineage-Restricted Progenitors in the Bone Marrow and by Increasing the Number of Basophil/Mast Cell Progenitors in the Spleen

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IL-3 Induces Basophil Expansion In Vivo by Directing Granulocyte-Monocyte Progenitors to Differentiate into Basophil Lineage-Restricted Progenitors in the Bone Marrow and by Increasing the Number of Basophil/Mast Cell Progenitors in the Spleen

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Recent work has established important roles for basophils in regulating immune responses. To exert their biological functions, basophils need to be expanded to critical numbers. However, the mechanisms underlying basophil expansion remain unclear. In this study, we established that IL-3 played an important role in the rapid and specific expansion of basophils. We found that the IL-3 complex (IL-3 plus anti-IL-3 Ab) greatly facilitated the differentiation of GMPs into basophil lineage-restricted progenitors (BaPs) but not into eosinophil lineage-restricted progenitors or mast cells in the bone marrow. We also found that the IL-3 complex treatment resulted in ~4-fold increase in the number of basophil/mast cell progenitors (BMCPs) in the spleen. IL-3-driven basophil expansion depended on STAT5 signaling. We showed that GMPs but not common myeloid progenitors expressed low levels of IL-3 receptor. IL-3 receptor expression was dramatically up-regulated in BaPs but not eosinophil lineage-restricted progenitors. Approximately 38% of BMCPs expressed the IL-3-Rα-chain. The up-regulated IL-3 receptor expression was not affected by IL-3 or STAT5. Our findings demonstrate that IL-3 induced specific expansion of basophils by directing GMPs to differentiate into BaPs in the bone marrow and by increasing the number of BMCPs in the spleen.


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cells were isolated and stained with PE-labeled anti-FcRα. Data are representative of five independent experiments. B, A time course analysis of basophil numbers in the bone marrow. Two to five mice were used for each time point. Error bars show the SD from two to five mice. C, C57BL/6J mice were or were not injected with the IL-3 complex. Three days after injection, mice were killed and cells were isolated and stained with PE-labeled anti-FcRα and FITC-labeled anti-c-kit Abs. Basophils were defined as FcRα-“c-kit”. Eosinophils were stained with PE-labeled anti-FcRα and FITC-labeled anti-c-kit Abs. Mast cells were defined as FcRα-“c-kit” cells. Data are representative of five independent experiments.

Materials and Methods

Mice

C57BL/6J and B6.SJL-129P examined in this study were purchased from The Jackson Laboratory. A pair of CD45.1 mice was purchased from The Jackson Laboratory. A pair of CD45.1, C57BL/6J, and B6.SJL-129P mice were used for flow cytometric analysis of basophils, mast cells, and eosinophils. Cells were incubated with biotin-labeled lineage Abs for basophil or mast detection or with PE-labeled anti-Siglec-F Ab for eosinophil detection. Mast cells were defined as FcRα-“c-kit” cells. Eosinophils were stained with PE-labeled anti-FcRα and FITC-labeled anti-c-kit Abs. Basophils were defined as FcRα-“c-kit”, while mast cells were defined as FcRα-“c-kit” cells. Eosinophils were stained with Siglec-F and Siglec-F and CCR3 cells. For detecting donor-derived basophils, Pacific Blue-labeled anti-c-kit was used (clone 104, Biolegend).

Cytokine injection

IL-3 (10 μg) was mixed with either anti-IL-3 Ab (5 μg; MP2–8F8, BD Pharmingen) at room temperature for 1 min based on published methods. The cytokine and Ab mixture (0.2–0.3 ml of PBS) was injected into mice via the lateral tail vein. Three days after injection, mice were killed and cells obtained for flow cytometric analysis.

FACS analysis and sorting

For flow cytometric analysis of basophils, mast cells, and eosinophils, cells were incubated with anti-FcγRIII/II (2.4G2) at 4°C for 10 min and stained with FITC-labeled anti-c-kit (2B8), biotin-labeled anti-FcRα (MAR-1), and PE-labeled Siglec-F (E50–2440) Abs, followed by streptavidin-allophycocyanin. Basophils were further characterized with PE-labeled anti-Thy1.2 (30-H12) or PE-labeled anti-CD49b (DX5) Abs. Basophils were defined as FcRα-“c-kit”, while mast cells were defined as FcRα-“c-kit” cells. Eosinophils were stained with Siglec-F and Siglec-F and CCR3 cells. For detecting donor-derived basophils, Pacific Blue-labeled anti-c-kit was used (clone 104, Biolegend).

CMPs, GMPs, BaPs, BMCPs, and EoPs were FACS sorted or analyzed according to the published protocols with some of the Abs labeled with different fluorochromes. CMPs and GMPs were identified as Lin-IL-7RαCD45.1+, CD34highCD19lowIL-5RαhighCD122low, while mast cells were defined as FcRα-“c-kit”. Eosinophils were stained with Siglec-F and Siglec-F and CCR3 cells. For detecting donor-derived basophils, Pacific Blue-labeled anti-c-kit was used (clone 104, Biolegend).

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cells. For staining of BMCPs, spleen cells were incubated with biotin-labeled lineage markers, followed by streptavidin-PE-Cy5 (BD Biosciences), PE-labeled anti-FcRII/III (BD Biosciences), allophycocyanin-labeled anti-c-kit (BD Biosciences), and FITC-labeled anti-β7 integrin (Biolegend, FIB504) mAbs were also added to the staining mix. The number of BMCPs was calculated by multiplying the total number of spleen cells by the percent of Lin− c-kit+ BMPCs.

Figure 2. Further characterization of FcεRIα− c-kit− cells. A, C57BL/6J mice were injected with the IL-3 complex. Three days after injection, bone marrow cells were isolated and stained with biotin-labeled anti-FcεRIα Ab, followed by streptavidin-conjugated APC, FITC-labeled anti-c-kit Ab, and PE-labeled anti-CD49b or PE-labeled anti-Thy1.2 Ab. B, FcεRIα− c-kit− cells were isolated by FACS sorting and stained with Giemsa. Cells were analyzed by light microscopy (×100) or by transmission electron microscopy (a Philips CM-10 electron microscope). Cells were acquired by CyAn (DakoCytomation) and analyzed using FlowJo software (Tree Star). The number of CMPs and GMPs was calculated by multiplying the total number of bone marrow cells by the percent of Lin− c-kit− CD34+ FcεRII/IIIhigh cells, respectively. BaPs were identified as Lin−CD34+ c-kit+ FcεRIα− cells. The numbers next to the sorting gates indicate the percentage of GMPs, CMPs, or BaPs in total bone marrow cells (left panel) or within the gated populations (right panel). B. Sorted progenitors (1000 cells) were cultured in complete modified Iscove’s medium containing 1% methylcellulose in the presence of IL-3 for 6 days. The numbers of colonies were counted. The percentage of basophils was determined by FACS analysis.

Figure 3. STAT5 is critical for basophil development and IL-3-driven basophil expansion. WT or STAT5−/− FL cell reconstituted mice were or were not injected with the IL-3 complex. Three days after injection, the percentage of donor-derived basophils was analyzed by FACS. The numbers indicate the percentage of basophils within donor-derived bone marrow cells. The total number of donor-derived basophils was calculated by multiplying the total number of donor-derived bone marrow cells in the reconstituted mice by the percentage of donor-derived basophils. Three to five chimera mice were used for each group. Data are representative of one of three independent experiments with similar results.

Figure 4. Isolation of CMPs, GMPs, and BaPs. A. FACS sorting gates are shown. CMPs and GMPs were identified as Lin−IL-7Rα− Sca-1− c-kit− CD34+ FcεRII/IIIhigh and FcεRII/IIIhigh cells, respectively. BaPs were identified as Lin−CD34+ c-kit− FcεRIα− cells. The numbers next to the sorting gates indicate the percentage of GMPs, CMPs, or BaPs in total bone marrow cells (left panel) or within the gated populations (right panel). B. Sorted progenitors (1000 cells) were cultured in complete modified Iscove’s medium containing 1% methylcellulose in the presence of IL-3 for 6 days. The numbers of colonies were counted. The percentage of basophils was determined by FACS analysis.
weeks later, cells were isolated from the bone marrow of the reconstituted mice and were subjected to flow cytometric analysis.

**Statistical analysis**

All of the error bars in this report represent the SD. The difference between two samples was analyzed with Student’s t-test.

**Results**

**IL-3 specifically and dramatically increases the number of basophils in vivo**

Treatments with the IL-3 complex (IL-3 plus anti-IL-3 Ab) for 8 days induced the expansion of basophils, mast cells, and eosinophils (6). Because basophils might have expanded more rapidly than mast cells, we tested a short-term expansion protocol. Three days after a single injection of the IL-3 complex, the total number of bone marrow, spleen, and peritoneal cells was comparable between two groups with and without the IL-3 complex injection. Interestingly, we observed an increase in the number of FceRIα<sup>+</sup> c-Kit<sup>+</sup> basophils by about 8-fold in the bone marrow (Fig. 1A, C, and D). The increase caused by a single injection lasted for 6 days (Fig. 1B). By contrast, injection with IL-3 or with the sham control (anti-IL-3 Ab alone) did not increase the number of FcεRIα<sup>+</sup> c-Kit<sup>+</sup> basophils (Fig. 1A).

Binding IL-3 with anti-IL-3 Ab has been shown to increase the half-life of IL-3 significantly (28). No significant changes in the number of FcεRIα<sup>+</sup> c-Kit<sup>+</sup> mast cells were detected in the peritoneal cavity (Fig. 1, C and D) (p > 0.05). Injection of the IL-3 complex only caused a 2-fold increase in the number of Siglec-F<sup>+</sup> eosinophils in the bone marrow, peripheral blood, and the spleen (Fig. 1, C and D). Further phenotypical and morphological analyses of the isolated cells revealed that the increase in basophils was due to an increase in the number of mature basophils, as indicated by the increase in the expression of FcεRIα and c-Kit.

**FIGURE 5.** IL-3 greatly expands the number of BaPs and BMCPs. A, C57BL/6J mice were or were not injected with the IL-3 complex i.v. Three days after injection, CMPs, GMPs, and BaPs in the bone marrow were analyzed by FACS. BaPs were identified as Lin<sup>+</sup>IL-7Rα<sup>+</sup>Sca-1<sup>+</sup> c-Kit<sup>+</sup>CD34<sup>-</sup>FcyRII/III<sup>+</sup> and FcyRII/III<sup>+</sup> cells. BaPs were identified as Lin<sup>+</sup>IL-7Rα<sup>+</sup>Sca-1<sup>+</sup> c-Kit<sup>+</sup>CD34<sup>-</sup>FcyRII/III<sup>+</sup> cells. BMCPs were identified as Lin<sup>+</sup>IL-7Rα<sup>+</sup>Sca-1<sup>+</sup> c-Kit<sup>+</sup>CD34<sup>-</sup>FcyRII/III<sup>+</sup> Cells. The percentages indicate the percent of cells with defined phenotypes within the Lin<sup>+</sup>IL-7Rα<sup>+</sup>Sca-1<sup>+</sup> c-Kit<sup>+</sup> cell population as indicated. B, The table summarizes the total number of CMP, GMP, and BaPs in the bone marrow or BMCPs in the spleen per mouse with or without IL-3 injection. The data are average of seven mice in the no-injection group and nine mice in the IL-3 injected group. SDs are shown. The calculation methods are described in the Materials and Methods section. C, Bone marrow cells from C57BL/6J mice that were or were not injected with the IL-3 complex were analyzed for EoPs. EoPs were defined as Lin<sup>+</sup>Sca-1<sup>+</sup>CD34<sup>-</sup>c-Kit<sup>+</sup>IL-5Rα<sup>+</sup> cells. The percentages indicate the percent of cells with defined phenotypes within the gated cell population. D, WT or STAT5<sup>−/−</sup> FL cell reconstituted mice were or were not injected with the IL-3 complex. Three days after injection, BaPs in the bone marrow were analyzed by FACS. The numbers within the FACS plots indicate the percentage of BaPs within the gated cell population. The absolute number of donor-derived BaPs was calculated by multiplying the total number of donor-derived bone marrow cells in the reconstituted mice by the percentage of donor-derived BaPs.
FceR1α⁺c-kit⁻ cells revealed that expanded FceR1α⁺c-kit⁻ cells resembled those basophils reported previously (Fig. 2 and Ref. 29). These results indicate that short-term IL-3 treatment specifically induces expansion of basophils in vivo.

**STAT5 signaling is essential in basophil development and IL-3-driven basophil expansion**

It has been shown that IL-3 activates STAT5 (30) and that STAT5 signaling is required for the mast cell development, survival, and functions (31, 32). Although basophils and mast cells share many similarities, basophils do differ significantly from mast cells. Thus, it merits an investigation of the role of STAT5 in basophil development and IL-3-driven basophil expansion. In this study, we chose to evaluate the role of STAT5 in basophils in an experimental system where the STAT5 protein was completely absent. The complete absence of STAT5 results in perinatal death (20). We generated STAT5⁻⁻ chimeric mice by injecting embryonic day 14.5 fetal liver (E14.5 FL) cells into sublethally irradiated (600 rad) recipient mice. Mice reconstituted with STAT5⁻⁻ FL cells contained 10% of donor-derived cells, while mice reconstituted with WT littermate control FL cells contained 80% of donor-derived cells. Total number of basophils was greatly reduced in STAT5⁻⁻ mice (Fig. 3, a 93% reduction), demonstrating that basophil development was greatly impaired in the absence of STAT5 signaling. Although the percentage of STAT5⁻⁻ FL cell-derived basophils increased in response to IL-3, the total number of STAT5⁻⁻ FL cell-derived basophils failed to increase significantly (Fig. 3). These results demonstrate that STAT5 is required for basophil development and IL-3-driven expansion of basophils.

**FIGURE 6.** BaPs but not EoPs up-regulate IL-3 receptor expression independent of IL-3 or STAT5 signaling. A, Bone marrow or spleen cells were prepared from C57BL/6J mice that were or were not injected with the IL-3 complex 3 days before the assay. IL-3 receptor expression on CMPs, GMPs, BMCPs, BaPs, basophils, EoPs, and eosinophils was analyzed by FACS. Mean fluorescence intensity (MFI). The numbers indicate the percentage of the defined cells within the gated cell population. Data are representative of two independent experiments. Bone marrow cells were prepared from C57BL/6J, IL-3⁻⁻ (B), or WT or STAT5⁻⁻ chimeric mice (C). IL-3 receptor expression on CMPs, GMPs, BaPs, and/or basophils was analyzed by FACS. The numbers indicate the percentage of the defined cells within the gated cell population. Data are representative of two independent experiments.
IL-3 greatly facilitates developmental transition from GMPs to BaPs in the bone marrow

To analyze the mechanisms by which IL-3 induces the dramatic expansion of basophils in vivo, we first established the methods for analyzing CMPs, GMPs, and BaPs in the bone marrow according to published protocols (Fig. 4A and Ref. 24–26). Using the defined sorting gates, we were able to achieve a 52-fold enrichment for CMPs, a 36-fold enrichment for GMPs, and a 5-fold enrichment for BaPs (Fig. 4B). The BaP-derived colonies were highly enriched with basophils, consisting of 87% basophils (Fig. 4B). Mature basophils failed to generate any colonies (data not shown). Thus, the relative low colony-forming efficiency of the enriched basophil progenitors may reflect a lower proliferation capacity for such committed basophil progenitors.

Upon treatment with the IL-3 complex, we observed that the number of BaPs increased dramatically by ~8-fold (Fig. 5, A and B). CMP numbers did not change significantly by the IL-3 treatment, while the number of GMPs increased moderately (Fig. 5, A and B). The IL-3 treatment did not significantly enhance the development of GMPs into EoPs (Fig. 5C). Total number of BaPs was greatly reduced in STAT5−/− chimeric mice (Fig. 5D, a 93% reduction). Although the percentage of STAT5−/− FL cell-derived BaPs increased in response to IL-3, the total number of STAT5−/− FL cell-derived BaPs failed to increase significantly (Fig. 5D). Together, these results demonstrate that IL-3 expands basophils by directing GMPs to differentiate into BaPs in the bone marrow.

An additional pathway for basophil differentiation has also been reported. Akashi and colleagues (24) have described a subset of BMCPs in the spleen, which expresses high levels of β integrin. BMCPs have been proposed as the immediate progeny of GMP in the bone marrow and are thought to give rise to mature basophils without going through the intermediate BaP developmental stage. We found that the IL-3 complex treatment resulted in ~4-fold increase in the number of BMCPs in the spleen (Fig. 5, A and B). This result demonstrates that the IL-3 complex treatment also expands basophils in the spleen by directing BMCPs to differentiate into basophils.

GMPs begin to express IL-3 receptor expression independent of IL-3 and STAT5 signaling

To understand why a short-term IL-3 treatment resulted in the expansion of basophils in a remarkable fashion, we examined the developmental stage at which IL-3 receptor begins to be expressed. The IL-3Rα-chain expression on CMPs, GMPs, BMCPs, BaPs, basophils, EoPs, and eosinophils was measured using six-color FACS analysis. We found that CMPs did not express a detectable IL-3Rα-chain while ~7% of GMPs expressed the IL-3Rα-chain (Fig. 6A), consistent with previous report (33). Approximately 38% of BMCPs expressed the IL-3Rα-chain. Nearly all BaPs and basophils expressed the IL-3Rα-chain at high levels. The up-regulation of IL-3 receptor expression did not occur on EoPs or eosinophils (Fig. 6A).

Although IL-3 treatment increased the percentage of IL-3 receptor-expressing GMPs by 2-fold (presumably by inducing the division of the IL-3 receptor-expressing GMPs), IL-3 treatment did not increase the mean fluorescence intensity of IL-3 receptor expression on progenitors at any developmental stages examined (Fig. 6A; p > 0.05). The IL-3 receptor expression on myeloid progenitors was not affected in the absence of IL-3 (Fig. 6B) or in the absence of STAT5 signaling (Fig. 6C). Together, our data indicate that selective up-regulation of the IL-3 receptor on BaPs and BMCPs might explain the remarkable expansion of basophils as a result of the IL-3 treatment.

Discussion

Expansion of basophils is imperative to the development of type-2 immunity. In this study, we sought to examine the mechanisms by which IL-3 dramatically increases the number of basophils. We used a short-term in vivo expansion system to examine the effects of IL-3 on the differentiation of myeloid progenitors into basophil lineage. In our short-term expansion experimental system, we found that IL-3 induced basophil-specific expansion. This result differs from that observed by Finkleman and colleagues (6). They used an 8-day IL-3 treatment protocol and observed that IL-3 induced dramatic expansion of mast cells and modest expansion of basophils and eosinophils in the spleen (6). The discrepancy might be explained by the different time points at which we chose to analyze basophil numbers. Three-day treatment with the IL-3 complex resulted in a 4-fold increase in the number of basophils in the spleen but did not increase the number of mast cells or eosinophils. Consistent with these observations, we found that the 3-day IL-3 treatment regimen caused a 4-fold increase in the number of BMCPs. Compared with the degree of increase in the number of BaPs, the fold of increase in the number of BMCPs was lower. We believe that this lower increase is due to a lower percentage of BMCPs that express the IL-3 receptor. Mast cells are known to live longer and undergo a slower maturation process (19, 34). Eight days of treatment with the IL-3 complex might preferentially induce mast cell expansion. Our time course experiment, showing that IL-3-induced basophil expansion that lasted up to seven days, was consistent with this notion. Taken together, our data demonstrate that a short-term IL-3 treatment leads to basophil-specific expansion in the bone marrow and in the spleen.

Based on in vitro data, IL-3 is believed to be a hematopoietic factor that has broad effects; it has been shown to promote macrophage, eosinophil, basophil, and mast cell differentiation (35). In vitro, we have consistently observed that bone marrow progenitors gave rise to 10–20% of basophils in the presence of IL-3. Yet, the in vivo effect of IL-3 stimulation is remarkably specific in expanding the number of basophils. Our analysis of IL-3 receptor expression patterns on progenitors may provide part of the explanation. Acquisition of IL-3 receptor expression by myeloid progenitors indicates the readiness of particular myeloid progenitors to respond to IL-3 robustly. We found that at the GMP developmental stage, 7% of GMPs began to express low levels of IL-3 receptor. At the BaP stage, all BaPs expressed high levels of the IL-3 receptor. Up-regulation of the IL-3 receptor did not occur on EoPs. Acquisition of IL-3 receptor expression appears to be controlled stochastically. Mice deficient in IL-3 have normal IL-3 receptor expression throughout basophil development. Thus, IL-3 does not appear to regulate its own receptor expression. Furthermore, expression of IL-3 receptor on basophils did not require STAT5 signaling.

The present study revealed that the development of STAT5−/− FL-derived basophils was greatly impaired in the chimera model when sublethally irradiated recipient mice were used. We also observed that the mice reconstituted with STAT5−/− FL cells contained only 10% of donor-derived cells, while those reconstituted with WT FL cells contained 80% of donor-derived cells. STAT5 is activated by multiple cytokines including IL-2, IL-3, IL-5, IL-7, SCF, Flt-3 ligand, GM-CSF, and erythropoietin (36) and was shown to play a critical role in hematopoietic stem cell functions (37–39); thus, we cannot rule out the possibility that STAT5 deficiency might have affected the earlier hematopoietic stem cell development. Indeed, STAT5 has been demonstrated to play a critical role in hematopoietic stem cell functions (40). One of the caveats of interpreting the STAT5 data obtained in this study is that we cannot identify the exact developmental stage at which STAT5 is required. STAT5 could be required at the GMP to BaP stage; alternatively, it could act at a much earlier stage. Ideally, if basophil-specific Cre mice were available, we would cross the basophil-specific Cre mice with STAT5 flox/flox mice. Because basophil-specific Cre mice were not available, a more definitive answer to which developmental stage STAT5 is required awaits the
generation of basophil-specific Cre mice. Although our results do not determine the exact developmental stage at which STAT5 is necessary, our study demonstrates that STAT5 is required for basophil development and for IL-3 to induce basophil expansion in vivo.

We propose that up-regulation of the IL-3 receptor on GMPs can serve as a mechanism for innate type cells to coordinate with adaptive immunity to generate strong immune responses. Under various disease conditions, CD4+ T cells have been shown to be the major cellular source of IL-3 (12–14). It is conceivable that IL-3 receptor-expressing GMPs can respond robustly to IL-3 produced by CD4+ T cells. As the result of the interaction between CD4+ T cells and myeloid progenitors, basophils can expand to critical numbers within a short period of time and these expanded basophils might then produce IL-4 to augment Th2 immune responses (4, 6, 12, 41) or to maintain B cell memory responses (8).

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Disclosures

The authors have no financial conflict of interest.

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