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Pathogenesis of Murine Experimental Allergic Rhinitis: A Study of Local and Systemic Consequences of IL-5 Deficiency

Hiroko Saito,* Koichiro Matsumoto,* Avram E. Denburg,* Lynn Crawford,* Russ Ellis,* Mark D. Inman,* Roma Sehmi,* Kiyoshi Takatsu,† Klaus I. Matthaei,‡ and Judah A. Denburg*‡*

Recent studies have demonstrated an important role for IL-5-dependent bone marrow eosinophil progenitors in allergic inflammation. However, studies using anti-IL-5 mAbs in human asthmatics have failed to suppress lower airway hyperresponsiveness despite suppression of eosinophilia; therefore, it is critical to examine the role of IL-5 and bone marrow responses in the pathogenesis of allergic airway disease. To do this, we studied the effects of IL-5 deficiency (IL-5−/−) on bone marrow function as well as clinical and local events, using an established experimental murine model of allergic rhinitis. Age-matched IL-5+/+ and IL-5−/− BALB/c mice were sensitized to OVA followed by 2 wk of daily OVA intranasal challenge. IL-5−/− OVA-sensitized mice had significantly higher nasal mucosal CD4+ cells and basophilic cell counts as well as nasal symptoms and histamine hyperresponsiveness than the nonsensitized group; however, there was no eosinophilia in either nasal mucosa or bone marrow; significantly lower numbers of eosinophil/basophil CFU and maturing CFU eosinophils in the presence of recombinant mouse IL-5 in vitro; and significantly lower expression of IL-5Rα on bone marrow CD34+CD45+ progenitor cells in IL-5−/− mice. These findings suggest that IL-5 is required for normal bone marrow eosinophilopoiesis, in response to specific Ag sensitization, during the development of experimental allergic rhinitis. However, the results also suggest that suppression of the IL-5-eosinophil pathway in this model of allergic rhinitis may not completely suppress clinical symptoms or nasal histamine hyperresponsiveness, because of the existence of other cytokine-progenitor pathways that may induce and maintain the presence of other inflammatory cell populations. The Journal of Immunology, 2002, 168: 3017–3023.

Although the role of IL-5 in the differentiation, proliferation, and migration of eosinophils in allergic inflammation has been well documented (1–9), it remains unclear how critical IL-5 is to the development of clinical disease. Indeed, recent studies using anti-IL-5 mAbs in vivo in human asthmatic subjects have failed to confirm that IL-5 is both necessary and sufficient to cause lower airway hyperresponsiveness, even though it appears responsible for the development of blood and tissue eosinophilia (10–12). In animal models involving IL-5 deficiency and/or overexpression, there are only a few studies in which both pathological and clinical variables have been evaluated (13–19).

Recent reports (3, 4, 20–37) have demonstrated an important role for the bone marrow as a source of eosinophils and other allergic inflammatory cells such as basophils, mast cells, or lymphocytes in upper or lower airway allergic inflammation. Bone marrow CD34+CD45+ progenitor cells are increased and phenotypically altered to preferentially differentiate—in response to al-

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2002-1767/02/S02.00

Material and Methods

Animals and OVA sensitization

Age-matched (8- to 10-wk-old, N6 BALB/c, female and male, crossed to the BALB/c from the C57BL/6 strain for 14 generations) IL-5+/+ and IL-5−/− mice were placed into one of two groups: 1) OVA/OVA group, which was given OVA sensitization followed by 2 wk of OVA intranasal daily challenge (IL-5+/+ and IL-5−/− mice, n = 10 each), and 2) sham/sham group, which was given normal saline instead of OVA in the same schedule (IL-5+/+ and IL-5−/− mice, n = 10 each). Under pathogen-free conditions, mice in the OVA/OVA group were sensitized using OVA Ag as follows. A total of 40 μg/kg OVA (Sigma-Aldrich, St. Louis, MO) diluted by sterile normal saline with aluminum hydroxide gel (alum adjuvant, 40 mg/kg) were administered to unanesthetized animals four times by i.p. injection on days 1, 5, 14, and 21. This was followed by daily challenge with OVA diluted by sterile normal saline intranasally (20 μl of 25 mg/ml OVA per mouse) from day 22 to 35 (Fig. 1).

Clinical symptoms and specimens

Nasal symptoms were evaluated for each mouse in each group at the time points of days 28 and 35 by counting the number of sneezes and nasal
itching motions (nasal rubbing) for 10 min after OVA intranasal provocation. Nasal histamine responsiveness (NHR) was also measured by determining the concentration of histamine which caused sneezing and itching and was expressed as the limiting concentration of histamine (log_{10} pico grams per milliliter) as previously described (14). The mice in each group were euthanized by deep anesthesia using a solution that contained ketamine hydrochloride (Ketalar; Bimeda-MTC, Cambridge, Ontario, Canada) and Xylazine (Rompun; Bayer, Toronto, Ontario, Canada) diluted in normal saline, at 24 h postintranasal provocation. Nasal mucosa and bone marrow tissues were taken and immediately processed.

All procedures were performed in accordance with the ethical guidelines in the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care and approved by the Animal Ethics Committee of McMaster University (Hamilton, Ontario, Canada).

Tissue preparation

Nasal mucosal tissues were treated by the following methods (previously described in Ref. 43). Brieﬂy, specimens were cut into 1-mm-thick pieces and ﬁxed overnight in cold acetic containing protease inhibitors at −20°C before processing in glycolmethacrylate resin. The embedded tissues were cut into 4-μm thin sections using a microtome for ultra-thin sections (Ultra Cut; Leica Microsystems, Wetzlar, Germany) and recruited to the immunostaining procedure. Bone marrow cells were obtained from sternal or femoral bone marrow, suspended in McCoy’s 5+ culture medium, which was made from modiﬁed McCoy’s 5A medium and 15% FCS (Life Technologies, Grand Island, NY) with 1% penicillin-streptomycin and 0.35% 2-ME, as previously described (28). Total bone marrow cells, as well as mononuclear cells that were separated by density gradient centrifugation using LymphPrep (Nycosomed, Oslo, Norway) for 25 min at 2000 rpm in room temperature, were diluted to a concentration of 5 × 10^6/ml with PBS, and cytospin slides were prepared (Cytospin 3; Thermo Shandon, Sewickly, PA) on Silane-coated glass slides. Another set of mononuclear cells, which were isolated as described from femoral bone marrow, were incubated in plastic ﬂasks for 2 h at 37°C and 5% CO₂ to remove adherent cells and then prepared for methylcellulose culture or immunostaining.

Evaluation and quantitation of staining

In the lamina propria of the nasal mucosa, total numbers of cells expressing positive immunoreactivity for cellular surface markers or any of the intracellular cytokines were enumerated. The area of the nasal tissue was measured, excluding glands, using an eye piece with a grid: 10 high-power ﬁelds were randomly evaluated after the stained slides were coded by a person unconnected with the study and blinded from the investigator until all evaluations were complete. The cell count results were expressed as the number of cells per square millimeter of lamina propria. For bone marrow pathological evaluation, differential cell counts were performed by cytospin preparations of bone marrow cells after Dif-Quick stain, eosinophilic cells and basophilic cells on each slide were enumerated by light microscopy, 1000 bone marrow cells were counted, and the result was expressed as a percentage in total sternal bone marrow cells. CD34⁺ cells on bone marrow were also counted by light microscopy: 1000 mononuclear bone marrow cells were counted and the result was expressed as the percentage of positive cells in total mononuclear cells.

Bone marrow methylcellulose cultures

Nonadherent mononuclear cells (NAMNC) were cultured in 35 × 10^-mm tissue culture dishes (Falcon Plastics; BD Biosciences Labware, Franklin Lakes, NJ) in culture medium, which was made up of 0.9% methylcellulose (Dow Chemical, Midland, Michigan), 20% FCS and Iscove’s Dulbecco’s medium (with 1% penicillin-streptomycin, 0.35% 2-ME, and 0.1% BSA) and the following reconstituent mouse (rm) cytokines (R&D Systems, Minneapolis, MN): rmIL-5 (0.5, 1, 5, 10 ng/ml) with 1 × 10^9 NAMNC, rmIL-3 (5 ng/ml) with 5 × 10^4 NAMNC, or rmGM-CSF (5 ng/ml) with 2.5 × 10^4 NAMNC. With each batch of growth factor, dose response experiments were performed. After 6 days, colonies of ≥40 cells were counted using inverse microscopy and eosinophil/basophil (Eo/Baso)-CFU were classiﬁed using morphological and histological criteria (tight, compact, round refractile cell aggregates). To identify the differentiated cells from colonies as Eo/Baso-CFU, sample cells in each 10-day culture were evaluated, 3 ml of PBS was added to the sample in each culture dish, and then the sample was centrifuged at 1200 rpm for 10 min at 4°C. After the sample was resuspended in 1 ml of PBS, cytospin slides were created on Silane-coated glass slides and stained with Dif-Quick.

Immunofluorescence staining and flow cytometry analysis

Samples of 1 × 10⁶ NAMNC derived from femoral bone marrow tissues at the time point of 24-h postintranasal provocation on day 35 were suspended in 100 μl of washing buffer, which contained PBS with 0.02% sodium azide, 0.02% EDTA, and 1% BSA, then incubated with anti-mouse CD32/CD16 Ab (T21, rat IgG2a, κ, BD PharMingen) or iso-type-matched negative control for 10 min, then FITC anti-mouse CD45 Ab (rat IgG2a, κ, BD PharMingen) or isotype-matched negative control, and CyChrome anti-mouse CD45 Ab (rat IgG2b, κ; BD PharMingen) or isotype-matched negative control for 10 min, then PE (BD PharMingen) to label biontinized IL-5Rα-positive cells for 30 min at 4°C in the dark after incubation with anti-mouse CD32/CD16 Ab for 15 min at 4°C in the dark to reduce binding by streptavidin-PE, which could contribute to background. The cells were washed with 3 ml washing buffer, then incubated with streptavidin-conjugated PE (BD PharMingen) to label biontinized IL-5Rα-positive cells for 30 min at 4°C in the dark after incubation with anti-mouse CD32/CD16 Ab for 15 min at 4°C in the dark to reduce binding by streptavidin-PE, which could contribute to background. The cells were washed with 3 ml washing buffer twice, then fixed in 500 μl of PBS plus 1% paraformaldehyde and kept at 4°C in the dark until analysis 24 h later. For analysis, FACScan (BD Biosciences, San Jose, CA) was used as a flow cytometer with flow cytometry analysis.

**Figure 1.** Protocol for OVA sensitization and subsequent OVA intranasal challenge. Sham/sham mice were treated with diluent both during sensitization and challenge instead of OVA. In contrast, OVA/OVA mice were given daily OVA challenge intranasally from day 22 to 35 after OVA i.p. sensitization from day 1 to 21.
analysis software (CellQuest from BD Biosciences and FlowJo from Tree Star, San Carlos, CA). To measure CD34\(^{+}CD45^{+}\) progenitor cells a gating strategy was used as previously described (41, 42, 45), and IL-5Ra-positi-
ve CD34\(^{+}CD45^{+}\) progenitor cells were enumerated as a percentage of total CD34\(^{+}CD45^{+}\) progenitor cells in the marrow.

Statistics

For all cell counts of stained slides, slides were read randomly and in blinded fashion. The Mann-Whitney \(U\) test and ANOVA followed by Student’s Neuman-Keuls test were used for comparison of data between groups.

Results

Clinical symptoms

OVA-sensitized IL-5\(^{+/+}\) mice developed significant nasal symptoms of sneezing and nasal itching (rubs) during 2 wk of daily intranasal OVA challenge, as was previously reported (14). IL-5\(^{-/-}\) mice in the OVA/OVA group had significant nasal symptoms only at day 35, the number of sneezes and rubs being significantly higher compared with the sham/sham group. IL-5\(^{+/+}\) mice developed sneezing significantly more quickly than IL-5\(^{-/-}\): means \(\pm\) SE days after the first day of sensitization were 25.3 \(\pm\) 0.3 for IL-5\(^{+/+}\) mice and 31.2 \(\pm\) 0.7 for IL-5\(^{-/-}\) mice (\(p < 0.0001\)) (Fig. 2). In the comparison between OVA-sensitized IL-5\(^{+/+}\) and IL-5\(^{-/-}\) mice, there were significant differences in each symptom at day 28 (sneezing, \(p < 0.0001\); itching, \(p < 0.0001\)) and in the OVA/OVA group (sneezing, \(p < 0.02\); itching, \(p < 0.05\)).

Nasal histamine responsiveness

NHR correlated strongly with clinical symptoms (with sneezing, \(r = 0.691\) and \(p < 0.0001\); with itching, \(r = 0.811\) and \(p < 0.0001\)) (Fig. 3). NHR in OVA/OVA IL-5\(^{+/+}\) or IL-5\(^{-/-}\) mice was significantly higher than in sham/sham mice; however, in this case, too, IL-5\(^{-/-}\) mice developed NHR, manifested by nasal symptoms, later than IL-5\(^{+/+}\) mice.

Pathological changes in airway tissue

In IL-5\(^{+/+}\) mice, the numbers of allergic inflammatory cells in the nasal mucosa were increased in OVA/OVA mice (at 24-h postintrasal provocation on day 35) compared with sham/sham mice, including eosinophils (\(p < 0.01\)) and CD4\(^{+}\) cells (\(p < 0.05\)) but not basophilic cells (NS). In OVA/OVA IL-5\(^{-/-}\) mice, at 24-h postintranasal provocation on day 35, significantly higher numbers of CD4\(^{+}\) lymphocytes (\(p < 0.02\)) and increased basophilic cell counts were observed compared with sham/sham IL-5\(^{-/-}\) mice, without eosinophilia in the nasal mucosa, as shown in Table I.

Bone marrow analysis

In comparisons between the two groups, a significant increase in sternal bone marrow eosinophil counts was observed in OVA/
OVA IL-5\(^{+/+}\) mice compared with sham/sham IL-5\(^{+/+}\) mice; no

![FIGURE 2](http://www.jimmunol.org/) Nasal symptoms in IL-5\(^{+/+}\) and IL-5\(^{-/-}\) mice. Clinical al-
lergic nasal symptoms at days 0 (sham/sham group), 28, and 35 (OVA/
OVA group) in OVA sensitization protocol. a, Sneezes. b, Nasal rubs. Each
value represents mean \(\pm\) SE.

![FIGURE 3](http://www.jimmunol.org/) NHR in IL-5\(^{+/+}\) and IL-5\(^{-/-}\) mice. NHR is represented by
the limiting concentration of histamine that caused sneezing and itching at
days 0 (sham/sham group), 28, and 35 (OVA/OVA group) in OVA sensi-
tization protocol.

| Table I. Pathological changes in OVA-sensitized mice* |
|-----------------|-----------------|-----------------|
|                 | Sham/Sham Group | OVA/OVA Group   |
| Eosinophils     |                 |                 |
| IL-5\(^{+/+}\)  | 0.0 \(\pm\) 0.0 | 469.9 \(\pm\) 145.9\(^{bc}\) |
| IL-5\(^{-/-}\)  | 0.0 \(\pm\) 0.0 | 0.0 \(\pm\) 0.0   |
| CD4\(^{+}\) cells|                 |                 |
| IL-5\(^{+/+}\)  | 0.8 \(\pm\) 0.3\(^{b}\) | 34.6 \(\pm\) 12.0\(^{d}\) |
| IL-5\(^{-/-}\)  | 8.9 \(\pm\) 5.2  | 86.3 \(\pm\) 39.4\(^{d}\) |
| Basophilic cells|                 |                 |
| IL-5\(^{+/+}\)  | 10.6 \(\pm\) 5.0 | 23.1 \(\pm\) 6.4  |
| IL-5\(^{-/-}\)  | 10.9 \(\pm\) 5.8 | 26.0 \(\pm\) 5.9  |

* The number of eosinophils, CD4\(^{+}\) cells, and basophilic cells in nonsensitized and OVA-sensitized murine nasal tissue. Shown are mean \(\pm\) SE of cell numbers per square millimeter.

\(^{a}\) Value of \(p < 0.01\) compared to IL-5\(^{-/-}\).

\(^{b}\) Value of \(p < 0.01\) compared to sham/sham group.

\(^{c}\) Value of \(p < 0.01\) compared to sham/sham group.

\(^{d}\) Value of \(p < 0.05\) compared to sham/sham group.
differences were seen between the IL-5−/− mice in the two groups (Fig. 4). Also, a significantly higher percentage of eosinophils in the bone marrow was detected in IL-5+/+ mice than in IL-5−/− mice in the OVA/OVA group ($p < 0.01$); however, there was no significant difference between IL-5+/+ mice and IL-5−/− mice in the sham/sham group. Basophilic cells and CD34+ cells also increased in the bone marrow in the OVA/OVA mice of both IL-5+/+ and IL-5−/− strains when compared with sham/sham mice. There was no significant difference in the number of basophilic cells or CD34+ cells between mice of either strain in the OVA/OVA group.

**Eo/Baso-CFU analysis**

In 6-day methylcellulose assays, the number of Eo/Baso-CFU that grew from NAMNC derived from murine bone marrow increased significantly in the presence of rmIL-5, rmIL-3, or rmGM-CSF in OVA/OVA, compared with sham/sham, IL-5+/+ mice (Fig. 5). In IL-5+/+ mice, the number of Eo/Baso-CFU was higher in the OVA/OVA group than in the sham/sham group in the presence of rmIL-3 or rmGM-CSF; however, in the presence of rmIL-5 in vitro, the number of Eo/Baso-CFU did not increase significantly in the OVA/OVA group. Furthermore, in the comparison between IL-5+/+ and IL-5−/− mice, there were significantly higher numbers of Eo/Baso-CFU grown in the presence of rmIL-5 in OVA/OVA IL-5+/+ mice ($p < 0.02$), whereas in the presence of rmIL-3 or rmGM-CSF there were no significant differences between IL-5+/+ and IL-5−/− mice.

**IL-5Ra expression on CD34+ CD45+ progenitor cells in murine bone marrow**

As shown in Table II, there were significant differences in IL-5Ra expression on CD34+ CD45+ progenitor cells between IL-5+/+ and IL-5−/− mice. In the OVA/OVA group, there were no significant differences in the number of basophilic cells or CD34+ cells between mice of either strain in the OVA/OVA group.

**FIGURE 4.** Bone marrow changes in murine allergic rhinitis. The percentages of eosinophils (A), basophilic cells (B), and CD34+ cells (C) in the bone marrow. Hatched bar, result from the OVA/OVA group; open bar, result from the sham/sham group.

**FIGURE 5.** Eo/Baso-CFU in 6-day methylcellulose cultures of murine bone marrow. Eo/Baso-CFU in the presence of 5 ng/ml each rmIL-5 (A), rmIL-3 (B), and rmGM-CSF (C). Hatched bar, result from the OVA/OVA group; open bar, result from the sham/sham group.
Table II. IL-5Rα expression on murine bone marrow CD34+ CD45+ progenitor cells

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<th>Sham/Sham Group</th>
<th>OVA/OVA Group</th>
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<tbody>
<tr>
<td>IL-5+/+</td>
<td>4.14 ± 0.64</td>
<td>11.97 ± 2.77a</td>
</tr>
<tr>
<td>IL-5+/-</td>
<td>2.01 ± 0.55</td>
<td>2.48 ± 0.30</td>
</tr>
</tbody>
</table>

* Each value is shown as a percentage of IL-5Rα+ CD34+ CD45+ cells in bone marrow CD34+ CD45+ progenitor cells.

a Values of p < 0.05.

b Values of p < 0.001.

c Values of p < 0.02 compared to sham/sham group.

and IL-5−/− mice, in both sham/sham and OVA/OVA groups, with IL-5+/+ mice showing higher IL-5Rα expression. Looking at IL-5+/+ mice across groups, the OVA/OVA group had significantly higher IL-5Rα expression on CD34+ CD45+ cells.

Eosinophil differentiation in 10-day methylcellulose bone marrow culture

As shown in Table III, sham/sham IL-5−/− mice had a significantly higher ratio of immature eosinophilic cells in 10-day cultures compared with sham/sham IL-5+/+ mice; along these lines, OVA/OVA IL-5−/− mice had significantly lower ratios of mature eosinophils compared with OVA/OVA IL-5+/+ mice.

Discussion

Eosinophilia has been studied as an important phenomenon in allergic disorders. There have been many studies of IL-5 as an important factor in eosinophilic inflammation, because this cytokine controls the differentiation, proliferation, and migration of eosinophils. However, other cytokines, such as GM-CSF, IL-4, and IL-13, or chemokines, such as eotaxin, also influence the inflammatory process (2, 9, 31, 46). Whether eosinophils take part in the pathogenesis of allergic disease or behave as bystanders has been the subject of much discussion recently (10–12, 54). Views that cast doubt on the pathogenetic role of eosinophils have come from mainly clinical studies in which data have been gathered after a relatively short period of sensitization and Ag challenge, and in which protocols varied widely, making comparisons of the results difficult. In this study, for the first time, the roles of IL-5 and eosinophils in upper airway allergic inflammation were studied over a relatively protracted period.

In our model, significant nasal symptoms were surprisingly observed in IL-5-deficient animals, although these were delayed compared with wild-type mice. For a better understanding of the pathological basis for this clinical result, we measured NHR and inflammatory changes in the nasal mucosa. NHR was, like nasal symptoms, significantly higher in OVA/OVA compared with wild-type mice. For a better understanding of the subject of much discussion recently (10–13, or chemokines, such as eotaxin, also influence the inflammatory process (2, 9, 31, 46). Whether eosinophils take part in the pathogenesis of allergic disease or behave as bystanders has been the subject of much discussion recently (10–12, 54). Views that cast doubt on the pathogenetic role of eosinophils have come from mainly clinical studies in which data have been gathered after a relatively short period of sensitization and Ag challenge, and in which protocols varied widely, making comparisons of the results difficult. In this study, for the first time, the roles of IL-5 and eosinophils in upper airway allergic inflammation were studied over a relatively protracted period.

In our model, significant nasal symptoms were surprisingly observed in IL-5-deficient animals, although these were delayed compared with wild-type mice. For a better understanding of the pathological basis for this clinical result, we measured NHR and inflammatory changes in the nasal mucosa. NHR was, like nasal symptoms, significantly higher in OVA/OVA compared with sham/sham IL-5−/− mice, but again this was significantly delayed compared with wild-type mice. The pathological changes observed in OVA/OVA IL-5−/+ mice were the same as previously reported (14): the numbers of eosinophils, basophilic cells, and CD4+ lymphocytes were increased, while IL-5-deficient mice showed no eosinophilia in either sham/sham or OVA/OVA groups and the numbers of other cellular populations, basophilic cells, and CD4+ lymphocytes were elevated and showed no significant differences when comparing OVA/OVA IL-5−/+ to IL-5−/− mice. These results demonstrate that eosinophils are not the only cells responsible for the development of NHR in allergic rhinitis; rather, nasal mucosal mast cells and basophils might be just as important in the expression of both NHR and clinical symptoms. Also, the possibility of differences between human and mouse biology, including Erk/Baso function, should be considered. Although some papers have discussed comparisons of murine and human cell functions (55–58), this is an area in which further work is needed to explore the contribution of these and other types of cells, such as macrophages, lymphocytes, neutrophils, and various epithelial cells, to allergic responses.

Previous reports from our group and others (3, 4, 20–37, 59) have shown that a systemic up-regulation of the bone marrow may play a pivotal role in the development and maintenance of not only lower, but also upper, airway allergic inflammation, as exemplified by asthma and allergic rhinitis, respectively. In the present study, we performed detailed examination of bone marrow cellular and molecular events, as well as function, in response to allergen sensitization and challenge in our model of experimental rhinitis. While marrow eosinophils, basophilic cells, and CD34+ cells were increased after Ag sensitization and nasal Ag challenges in wild-type mice, there was no increase of eosinophils in the marrow of IL-5-deficient mice after challenge. Nonetheless, all other inflammatory cell types were increased to the same extent as in wild-type mice, accompanied by the same nasal mucosal pathologic changes. Thus, though IL-5−/− deficiency resulted in suppression of Ag-dependent eosinophilic progenitor differentiation (i.e., reduced bone marrow Erk/Baso-CFU), the actual number of progenitor cells was not lower at baseline than in wild-type mice. This defective functional response of eosinophilic progenitors was attended by reduced IL-5Rα expression on CD34+ CD45+ bone marrow cells in IL-5−/− mice both before and after Ag sensitization and challenge, with defective up-regulation following Ag sensitization.

The functional consequences of reduced IL-5Rα expression on CD34+ CD45+ bone marrow cells in IL-5−/− mice were seen in 6-day methylcellulose assays. While the number of marrow Erk/Baso-CFU increased significantly in vitro in the presence of rmIL-5, rmIL-3, or rmGM-CSF in OVA/OVA IL-5−/+ animals, it was only in the presence of rmIL-3 or rmGM-CSF, but not IL-5 in vitro, that Erk/Baso-CFU increased in the OVA/OVA IL-5−/− mice. Related to this, in sham/sham IL-5−/− mice there was a significantly higher ratio of immature eosinophilic cells in colonies enumerated at day 10 compared with significantly higher ratios of

Table III. Eosinophil differentiation from murine bone marrow derived NAMNC in 10-day methylcellulose semisolid culture assay in the presence of rmIL-5 (5 ng/ml)

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<th>Sham/Sham Group</th>
<th>OVA/OVA Group</th>
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<tbody>
<tr>
<td>IL-5+/+</td>
<td>22.6 ± 3.3</td>
<td>53.1 ± 3.9a</td>
</tr>
<tr>
<td>IL-5+/-</td>
<td>25.7 ± 1.4</td>
<td>11.5 ± 2.4</td>
</tr>
<tr>
<td>Mature eosinophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature eosinophils</td>
<td>26.0 ± 3.0b</td>
<td>15.6 ± 2.3</td>
</tr>
<tr>
<td>Other cell types</td>
<td>51.4 ± 3.9b</td>
<td>31.3 ± 4.2c</td>
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* The number of mature eosinophils, immature eosinophilic cells, and other cell types. Shown are mean ± SE of the percentage of total cells.

b Value of p < 0.002 compared to IL-5−/−.

c Value of p < 0.01 compared to IL-5−/+.
that these latter cytokines can replace IL-5 functionally, giving rise to small numbers of eosinophils and, more importantly, to normal numbers of basophilic cells, which can probably account for the persistence of clinical symptomatology and nasal hyperresponsiveness, albeit delayed. Lantz et al. (61) have shown IL-3 dependency of basophilic responses in mice; whether upper airway inflammation and bone marrow responses are defective in IL-3-deficient mice remains to be investigated. To clarify the role of basophilic cells in the systemic and local pathogenesis of allergic rhinitis, investigations of IL-3 deficiency and/or IL-3 expression in this model would be an important future direction.

In conclusion, IL-5 is required for the development of tissue and marrow eosinophilia, the formation of Eo/Baso-CFU, and the early development of symptoms, but not for other components of the inflammatory response in murine experimental allergic rhinitis. Our findings confirm that IL-5 is required for normal bone marrow eosinophiliopoiesis, in response to specific Ag sensitization during the development of experimental allergic rhinitis. However, the results also point out that the suppression of the IL-5-eosinophil pathway in the pathogenesis of allergic rhinitis (and also, by inference, asthma) may not fully suppress clinical symptoms or airway hyperresponsiveness due to the possible existence of other cytokine-progenitor pathways that may induce and maintain the presence of other inflammatory cell populations.

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References
34. Denburg, J. A. 1999. The nose, the lung and the bone marrow in allergic inflammation. Allergy 54(Suppl. 57):73.