Evidence for Local Eosinophil Differentiation Within Allergic Nasal Mucosa: Inhibition with Soluble IL-5 Receptor

Lisa Cameron, Pota Christodoulopoulos, Francois Lavigne, Yutaka Nakamura, David Eidelman, Alan McEuen, Andrew Walls, Jan Tavernier, Eleanor Minshall, Redwan Moqbel and Qutayba Hamid

*J Immunol* 2000; 164:1538-1545; doi: 10.4049/jimmunol.164.3.1538
http://www.jimmunol.org/content/164/3/1538

**References**
This article cites 56 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/164/3/1538.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Evidence for Local Eosinophil Differentiation Within Allergic Nasal Mucosa: Inhibition with Soluble IL-5 Receptor

Lisa Cameron,* Pota Christodouloupolos,‡ Francois Lavigne,‡ Yutaka Nakamura,* David Eidelman,* Alan McEuen,‡ Andrew Walls,‡ Jan Tavernier,§ Eleanor Minshall,* Redwan Moqbel,¶ and Qutayba Hamid‡*

Eosinophil differentiation occurs within the bone marrow in response to eosinopoietic cytokines, particularly IL-5. Recently, however, eosinophil precursors (CD34/IL-5Rα cells) and IL-5 mRNA cells have been identified within the lungs of asthmatics, indicating that a population of eosinophils may differentiate in situ. In this report, we examined the presence of eosinophil precursors within allergic nasal mucosa and examined whether they undergo local differentiation following ex vivo stimulation. We cultured human nasal mucosa obtained from individuals with seasonal allergic rhinitis with either specific allergen, recombinant human IL-5 (rhIL-5), or allergen soluble IL-5Rα (sIL-5Rα), shown to antagonize IL-5 function. Simultaneous immunocytochemistry and in situ hybridization demonstrated that there were fewer cells coexpressing CD34 immunoreactivity and IL-5Rα mRNA following culture with allergen or rhIL-5, compared with medium alone. Immunostaining revealed that the number of major basic protein (MBP) immunoreactive cells (eosinophils) was higher within tissue stimulated with allergen or rhIL-5, compared with unstimulated tissue. In situ hybridization detected an increase in IL-5 mRNA cells in sections from tissue cultured with allergen, compared with medium alone. These effects were not observed in tissue cultured with a combination of allergen and sIL-5Rα. Colocalization analysis indicated this expression to be mainly, but not exclusively, T cell (44%) and eosinophil (10%) derived. Our findings suggest that a subset of eosinophils may differentiate locally within allergic nasal mucosa, in what appears to be a highly IL-5-dependent fashion, and imply that this process might be regulated in vivo by endogenous production of sIL-5Rα. The Journal of Immunology, 2000, 164: 1538–1545.

A cardinal feature of allergic disorders such as asthma and rhinitis is the elevated number of inflammatory cells, particularly eosinophils (1), within the respiratory mucosa. Activated eosinophils release a number of cytotoxic granule proteins, the most prominent being major basic protein (MBP) (2). MBP has been reported to damage respiratory epithelium (3) and to induce degranulation of other inflammatory cells (4, 5). Furthermore, eosinophils are a source of proinflammatory cytokines (IL-4, IL-6) (6–8), profibrotic (TGF-β) (9, 10), and hematopoietic cytokines (IL-5, IL-3, and GM-CSF) (11, 12).

IL-5 is critical for eosinophil development. In vitro, addition of this cytokine to progenitor cell cultures gives rise to eosinophil colonies (13, 14). Intravenous injection of rIL-5 into guinea pigs resulted in rapid blood eosinophilia (15). Furthermore, pulmonary eosinophilia was not observed within Ag-challenged IL-5-knockout mice and was diminished in wild-type mice pretreated with anti-IL-5 Abs (16, 17). IL-5 acts through a heterodimeric receptor (IL-5R) composed of a ligand-specific α-chain and the signal-transducing β-chain (18). Transcripts coding for the α-chain are differentially spliced, giving rise to either soluble or membrane-bound isoforms, which antagonize or mediate IL-5 function, respectively (19). IL-5 is thought to exert its effects at a relatively late period during eosinopoiesis, influencing terminal differentiation of CD34+/CD33+ progenitors (20). However, a recent report by Sehmi et al. has shown the expression of the α-subunit of the IL-5 receptor (IL-5Rα) on CD34+ cells and suggested that colocalization of these two markers may be indicative of eosinophil lineage-committed progenitors (CD34/IL-5Rα+) (21).

Tissue eosinophilia within the lungs and nose of individuals with allergic asthma and rhinitis has primarily been attributed to the influx of mature cells. Recent reports now suggest, however, that parallel mechanisms may also be at work. CD34+ progenitor cells have been detected within peripheral blood and lungs and are increased in number in atopic pigs compared with normals (22, 23). Furthermore, elevated numbers of cells producing the eosinopoietic cytokines IL-5, IL-3, and GM-CSF have been observed within the nasal mucosa of individuals with allergic rhinitis following allergen challenge (24). Collectively, these studies suggested the possibility of local eosinophil differentiation within respiratory mucosa. Robinson et al. have recently demonstrated the presence of eosinophil precursors (CD34-/IL-5Rα−) within the lungs and that their number was increased, as well as MBP immunoreactivity, in atopic asthmatics compared with normal controls (23). However, whether the change in eosinophil number was due to local differentiation of progenitor cells or infiltration from systemic circulation remains unknown.

*Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada; ‡Notre-Dame Hospital, Montreal, Quebec, Canada; §School of Medicine, University of Southampton, Southampton, United Kingdom; ¶Flanders Interuniversity Institute for Biotechnology, University of Ghent, Ghent, Belgium; and ¶Pulmonary Research Group, University of Alberta, Edmonton, Alberta, Canada

Received for publication August 9, 1999. Accepted for publication November 17, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by the Medical Research Council of Canada, the Inspira-Vector Networks Center of Excellence, and the J. T. Costello Memorial Research Foundation. L.C. is a recipient of a Canadian Society of Allergy and Clinical Immunology/ Merck Frosst fellowship. Q.H. is a Research Scholar of the Fonds de la Recherche en Santé du Québec. R.M. is an Alberta Heritage Senior Medical Scholar.

2 Address correspondence and reprint requests to Dr. Qutayba Hamid, Meakins-Christie Laboratories, McGill University, 3626 Saint Urban Street, Montreal, Québec, Canada H2X 2P2. E-mail address: hamid@meakins.lan.mcgill.ca

3 Abbreviations used in this paper: MBP, major basic protein; rhIL-5, recombinant human IL-5 protein; sIL-5R, soluble IL-5R; PNU, protein nitrogen unit.
To study the prospect of local eosinophil differentiation, we employed an explant system to exclude the possibility of cell infiltration and enable the examination of events occurring solely within the tissue. Human nasal mucosal tissue, obtained from patients with allergic rhinitis, exhibited fewer CD34/IL-5Ra+ cells, but more MBP-immunoreactive and IL-5 mRNA+ cells, following ex vivo stimulation with specific allergen or recombinant human (rh)IL-5. Soluble IL-5Ra (sIL-5Ra) was seen to inhibit the allergen-induced changes in the number of CD34/IL-5Ra+ cells and MBP+ cells, but not IL-5 mRNA+ cells. Our results show the presence of eosinophil precursors within allergic nasal mucosa and provide evidence for their local differentiation following stimulation, ex vivo.

Materials and Methods

**Tissue Culture**

Nasal mucosal tissue was obtained from the inferior turbinate of ragweed-sensitive individuals with seasonal allergic rhinitis, outside the ragweed season, that were not receiving any glucocorticosteroids. Tissue was resected from patients undergoing sinus surgery, who had given informed consent before the procedure, and was rinsed in medium before culture. The time frame between tissue removal and the commencement of culture was consistently less than 1 h, and tissue was in culture medium at all times during this transition period. Serial sections of tissue were placed on 0.4-μm well inserts (Millipore, Bedford, MA) in 2 ml of defined medium (25, 26) with 1) 250 μl of ragweed allergen (10–2000 protein nitrogen units (PNU); Hollister-Stier, Spokane, Washington), 2) rhIL-5 (12.5 ng/ml; Sigma, St. Louis, MO) for 5 min, which identifies eosinophil granules. With this technique, cells double positive for CD34 and chromotrope stained reddish-brown.

**Abs and probes**

Anti-human mAbs derived from hybridoma supernatants were used to detect MBP (BMK-13), an eosinophil marker (27); CD34 (QBEND/10, Se罗tec Kidlington, Oxford, England), to identify hemopoietic progenitors (28); CD3 (Dako), a pan T cell marker; and B1B1, a newly described Ab specific for basophil granule protein (29). Each Ab was diluted in a standardized diluting buffer (Dako): BMK-13 at 1/30, CD34 at 1/50, CD3 at 1/100, and B1B1 at 1/10. IL-5 and IL-5Ra cDNAs were inserted into pGEM vectors and linearized with the restriction enzymes BamHI or PstI, for antisense templates, and HindIII for sense templates, respectively. With these fragments, in vitro transcription was initiated with the RNA polymerase Sp6 and T7 in the presence of [3S]labeled UTP to generate radiolabeled antisense probes recognizing RNA message encoding IL-5 or IL-5Ra and sense probes (30).

**Immunocytochemistry**

MBP immunoreactivity was detected by the avidin-biotin complex (ABC) method, as previously described (31). Anti-MBP was visualized using diaminobenzidine, with which positive cells appeared brown. B1B1+ cells were detected using the alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique (32) with the fast red chromogen. Positive cells were therefore red. The secondary and tertiary layer were repeated for maximum detection of B1B1 immunoreactivity. Negative control experiments were performed by replacing the primary Abs with an isotype-matched control.

**In situ hybridization**

This was conducted as previously described (30). Briefly, sections were permeabilized with proteinase K (1 μg/ml) and Triton X-100. Prehybridization was performed in 50% formamide in 2× SSC solution. For hybridization, 35S-labeled cDNA probes were applied and left overnight in a humid chamber at 42°C. Posthybridization washes of decreasing concentrations of SSC solution (4× to 0.1× SSC) were conducted at 42°C. Excess probe was destroyed with RNase A (20 μg/ml). Slides were dipped in Amersham (Oakville, Ontario, Canada) LM-2 emulsion and exposed for 11–15 days. Autoradiographs were developed in Kodak D-19 and counterstained with hematoxylin. Positive signal was identified as a collection of silver grains overlying the cells. Negative control experiments using sense probes and RNase treatment before antisense probe application were performed to confirm probe specificity.

**Simultaneous immunocytochemistry and in situ hybridization**

Eosinophil precursors, progenitor cells expressing receptors for IL-5, were identified as previously described (21). Briefly, alkaline phosphatase anti-alkaline phosphatase immunocytochemistry with Ab directed against the progenitor cell marker CD34 and the fast red chromogen was performed. Subsequently, sections underwent in situ hybridization with 35S-labeled cRNA probes for IL-5Ra mRNA. Eosinophil precursors were identified as those cells exhibiting both a red staining for CD34 and an accumulation of silver grains overlying the cells. Colocalization of IL-5 to T cells and eosinophils was also performed using this protocol with Abs to detect CD3 and MBP and immunoreactivity and cRNA probes for IL-5 mRNA.

**Colocalization of CD34 and carbol chromotrope 2R**

CD34 immunoreactivity was detected using the streptavidin peroxidase method (7) and the chromogen diaminobenzidine. Subsequently, slides were stained with 1% carbol chromotrope 2R (Sigma, St. Louis, MO) for 5 min, which identifies eosinophil granules. With this technique, cells double positive for CD34 and chromotrope stained reddish-brown.

**Quantification and statistics**

Using an Olympus light microscope (Carson Group, Markam, Ontario, Canada) at ×200 magnification, slides were analyzed for positive signal in a blinded fashion by two independent examiners. CD34 immunoreactivity associated with hemopoietic progenitor cells was counted, i.e., vessel wall cells and fibroblasts were excluded. For in situ hybridization, positive signal was determined as a discrete cluster of silver grains overlying the cell, which could be seen to encompass the nucleus under dark field illumination. By placing the graticule under the basement membrane, the number of positive cells was counted and reported as the mean of at least 6–8 fields (0.2 mm2). Data are represented within the text and figures as the mean ± SD. Significance was assessed using a Dunnett’s test for the comparison of multiple groups to one control. A paired Student t test was applied to the Δ values (i.e., ragweed minus medium alone) to determine the statistical difference between the various conditions (Δ ragweed vs Δ rhIL-5). Correlation analysis was applied using Pearson’s correlation coefficient, and a Bonferroni post hoc test was used. Values of p < 0.05 were considered significant (StatView version 7.1; SyStat, Evanston, IL).

**Results**

**Response of nasal mucosal explant tissue to ragweed allergen**

Although previous work has demonstrated no difference in symptom scores when doses of 10–1000 PNU of ragweed allergen were used in nasal provocation studies (33), the conditions for ex vivo challenge of nasal mucosal tissue with ragweed allergen had not been determined. We therefore performed a dose response with 10, 100, 1000, and 2000 PNU of ragweed allergen (n = 3). The number of MBP and IL-5 mRNA+ cells in sections of nasal mucosal tissue cultured for 24 h with ragweed allergen increased progressively from 10 PNU to 1000 PNU; however, no further elevation was noted at 2000 PNU. Experiments for this study were therefore conducted with 1000 PNU of ragweed allergen.

**Progenitor cells within nasal mucosal explant tissue**

CD34 is expressed by hemopoietic progenitor cells as well as endothelial cells and fibroblasts (34, 35). As such, when counting the CD34+ cells, the vessels and fibroblasts were excluded as much as possible. A number of these CD34+ progenitor cells were seen scattered throughout the submucosal layer (Fig. 1a) in unstimulated tissue at 6 (Table I; n = 7, 19.9 ± 6.4) and 24 h (Table I; n = 11, 18.7 ± 4.2), and there were fewer following ex vivo exposure to ragweed allergen (13 ± 3.4, 11.7 ± 2.6; p < 0.01) as well as rhIL-5 (n = 5, 12.2 ± 1.9; n = 6, 14.4 ± 3.0; p < 0.05). sIL-5Ra molecules were used in this explant system to inhibit the action of IL-5 (18). We observed that culture with both ragweed and sIL-5Ra was associated with the presence of a similar number of CD34+ progenitor cells as unstimulated tissue (n = 6, 19.3 ± 3.7; p > 0.05), significantly higher than in sections of explant tissue cultured with ragweed alone (Table I; p < 0.01).
Eosinophil precursors within nasal mucosal explant tissue

Colocalization of CD34 and IL-5Rα mRNA has been suggested as a marker of eosinophil precursors (21). Fig. 2 illustrates the presence of CD34/IL-5Rα mRNA cells within nasal mucosa cultured for either 6 or 24 h in medium alone (11.1 ± 4.0, 12.2 ± 3.2; Fig. 1b). At both time points, the number of these cells was significantly less in tissue cultured with ragweed allergen (5.1 ± 1.5, 5.0 ± 1.8; p < 0.01) or rhIL-5 (5.2 ± 1.6, 7.6 ± 2.7; p < 0.01). When ragweed and sIL-5Rα were added together, more CD34/IL-5Rα mRNA cells were observed (11.8 ± 2.4) than nasal tissue cultured with ragweed alone (p, 0.01); the latter exhibited similar numbers as those in medium alone (12.2 ± 3.2). In fact, there were 60% fewer CD34/IL-5Rα mRNA cells within ragweed-stimulated than unstimulated tissue. In contrast, culture with ragweed allergen and sIL-5Rα resulted in only a 3% change (Fig. 2).

When the number of CD34/IL-5Rα mRNA cells was examined as a percentage of total CD34+ progenitor cells, we observed that 58% ± 0.1 and 65% ± 0.7 (6 and 24 h) of CD34+ progenitor cells were expressing IL-5Rα mRNA within unstimulated tissue. Following culture with ragweed allergen alone, these percentages were substantially lowered, 39% ± 0.1 and 41% ± 0.1 (p < 0.01). Incubation with rhIL-5 was also associated with a smaller percentage of progenitor cells expressing IL-5Rα mRNA at both 6 (42% ± 0.1) and 24 h (51% ± 0.1; p < 0.01), as compared with unstimulated tissue (Table I).

<table>
<thead>
<tr>
<th></th>
<th>6 h</th>
<th></th>
<th>24 h</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MA (n = 7)</td>
<td>RW (n = 7)</td>
<td>IL-5 (n = 5)</td>
<td>MA (n = 11)</td>
<td>RW (n = 11)</td>
<td>IL-5 (n = 9)</td>
</tr>
<tr>
<td>Total CD34+ PC</td>
<td>19.9 ± 6.4</td>
<td>13.0 ± 3.4*</td>
<td>12.2 ± 1.9*</td>
<td>18.7 ± 4.2</td>
<td>11.7 ± 2.6*</td>
<td>14.4 ± 3.0***</td>
</tr>
<tr>
<td>CD34+IL-5Rα+/CD34+ PC</td>
<td>0.58 ± 0.14 (58%)</td>
<td>0.39 ± 0.07* (39%)</td>
<td>0.43 ± 0.13 (43%)</td>
<td>0.65 ± 0.07 (65%)</td>
<td>0.42 ± 0.10* (42%)</td>
<td>0.51 ± 0.13* (51%)</td>
</tr>
<tr>
<td>Total IL-5Rα+ cells</td>
<td>12.9 ± 4.2</td>
<td>8.5 ± 2.1</td>
<td>8.6 ± 1.8</td>
<td>13.1 ± 3.3</td>
<td>9.6 ± 2.7*</td>
<td>10.5 ± 3.2***</td>
</tr>
</tbody>
</table>

* Data represented as the mean ± SD of the number of cells/high power field. MA, medium alone; RW, ragweed; PC, progenitor cells. *, p < 0.05 compared with medium alone; ***, p < 0.05 compared with ragweed.
MBP immunoreactivity

There were similar numbers of MBP immunoreactive cells within the submucosa of unstimulated tissue following either 6 (n = 7, 3.0 ± 2.3) or 24 h of culture (n = 10, 2.1 ± 1.1; Fig. 3 and Fig. 4a). Furthermore, these numbers were not significantly different from within tissue blocked immediately following resection (n = 3, 2.2 ± 0.46; p > 0.05). This baseline level of eosinophils may be attributed to the fact that most of these patients were also allergic to house dust mite; however, significant increases were observed within nasal tissue that was cultured for 6 (n = 7, 10.1 ± 5.2) or 24 h with ragweed allergen (n = 10, 9.4 ± 3.5; p < 0.01), the majority of which were found just beneath the basement membrane (Fig. 1c and 4b). An inverse correlation was observed between the increase in the number of MBP-immunoreactive cells and the reduction in CD34/IL-5Rα+ cells in ragweed-stimulated vs unstimulated tissue (r² = 0.64, p < 0.01). At both 6 and 24 h, the numbers of MBP+ cells was also greater in explant tissue cultured with rhIL-5 (n = 6, 8.2 ± 4.8; n = 9, 6.2 ± 1.7) than in medium alone (p < 0.01, Fig. 4c), which was less than what we observed following culture with ragweed allergen (p < 0.01, Fig. 4b). Combination of ragweed and sIL-5Rα molecules was associated with fewer MBP+ cells (n = 6, 1.9 ± 1.6; Figs. 2 and 4d) than ragweed alone (p < 0.01, Fig. 4b), numbers similar to unstimulated tissue (Fig. 4a).

Colocalization of carbol chromotrope 2R staining and CD34 immunoreactivity

Since the Abs employed to detect CD34 and MBP were both mouse monoclonals, they could not be used in colocalization studies to identify immature eosinophils. Instead, this was achieved by colocalizing CD34 immunoreactivity to chromotrope 2R, which identifies the eosinophil granules. The number of chromotrope+ cells was increased, similar to what was observed for MBP immunoreactivity, within tissue cultured with ragweed allergen (n = 5, 8.6 ± 1.7) compared with medium alone (2.0 ± 1.6, p < 0.01). There was a population of double positive (CD34 and chromotrope) cells, but their numbers were not different in stimulated (1.4 ± 0.9) compared with unstimulated tissue (2.6 ± 0.9, p > 0.05; Fig. 5). However, when the CD34+/chromotrope+ cells were examined as a percentage of total cell population, we observed a 14.6% increase per total CD34+ progenitor cells and a 27.2% reduction per total chromotrope+ cells. Since the absolute number of double positive cells remained relatively constant, these results suggest the progression from CD34+/chromotrope+, to CD34+/chromotrope+ and finally to the CD34+/chromotrope+ cells.

BB1 immunoreactivity

Basophils also arise from a CD34+ progenitor, express the IL-5R, and produce MBP, although at much lower levels than the eosinophil (36–38). For this reason, we employed a basophil-specific Ab, BB1 (29), to identify these cells within nasal mucosal tissue. Our observations demonstrate that there are basophils within unstimulated tissue (n = 5; 5.8 ± 0.8), but that the number of these cells was not significantly different following ragweed stimulation (6.8 ± 2.2, p > 0.05).

IL-5 gene expression

The number of IL-5 mRNA+ cells within nasal mucosal tissue was greater following both 6 and 24 h culture with ragweed allergen (n = 7, 10.1 ± 5.1; n = 9, 10.6 ± 3.2) compared with medium alone (3.8 ± 2.3, 3.5 ± 1.6; p < 0.001). There was no significant difference in the number of these cells within tissue cultured with rhIL-5 for either 6 h (n = 4, 4.6 ± 5.2) or 24 h (n = 8, 3.2 ± 2.6). Furthermore, there were similar numbers of IL-5 mRNA+ cells in tissue cultured with ragweed and sIL-5Rα molecules (n = 5, 8.6 ± 2.4), compared with ragweed only (10.0 ± 3.3, p > 0.05). Colocalization studies to phenotype the cells producing IL-5 mRNA demonstrated that, within sections from nasal mucosal tissue cultured with ragweed allergen, 44.9% ± 2.0 were associated with CD3+ cells (T cells) and 10.0% ± 8.3 with MBP+ cells (eosinophils). Furthermore, we also determined the proportion of these cell populations undergoing IL-5 gene transcription; 13% ± 0.8 of CD3+ cells were IL-5 mRNA+, whereas 5% ± 4.0 of the MBP+ cells were IL-5 mRNA+ (n = 4, Table II).

Discussion

Before this report, the concept of eosinophil-committed progenitors differentiating within the inflammatory lesion had been proposed (23, 39, 40). To date, however, no studies designed to directly test this possibility have been performed. Here, we developed and employed an explant system to examine the local changes in precursor and maturing eosinophil numbers following ex vivo stimulation. Our data provide strong evidence to indicate that a population of eosinophils do indeed differentiate locally within allergic nasal mucosa, that this process is highly IL-5 dependent, and imply that endogenous production of the sIL-5Rα may regulate this event.

FIGURE 3. The number of MBP-immunoreactive cells within nasal mucosal explant tissue. More MBP+ cells were observed following culture with allergen or rIL-5 than medium alone at both 6 and 24 h (*, p < 0.01). Significantly more MBP+ cells were observed following culture with ragweed compared with rhIL-5 (*, p < 0.01). Combination of ragweed + sIL-5Rα inhibited the increase in MBP+ cells seen following culture with ragweed alone (*, p < 0.01).
We show that both CD34$^+$ progenitor and CD34/IL-5R$^+$ cells are present within allergic nasal mucosa, the latter accounting for over 58% of the total progenitor cell population. Whether these eosinophil precursors arise from the bone marrow, previously associated with a population of CD34/IL-5R$^+$ (21), and accumulate in the tissue or undergo lineage commitment locally could not be determined here. However, CD34$^+$ progenitor cells have been demonstrated within the peripheral blood and lungs of both atopic and nonatopic control subjects (22, 23), and higher numbers of CD34/IL-5R$^+$ cells have been detected in the lungs, but not the blood, of atopic asthmatics compared with atopic as well as nonatopic controls (23). Furthermore, it has recently been suggested that IL-5 may induce the expression of its own membrane-bound receptor (41). Together with the present finding that IL-5 mRNA$^+$ cells are increased following ex vivo stimulation, these studies suggest that the presence of CD34$^+$ progenitor cells are a characteristic feature of both normal and allergic mucosal tissue and that the local environment may indeed provide the necessary stimuli for eosinophil lineage commitment within the tissue itself. A further implication of this work is that the migration of progenitor cells, like mature cells, to the inflammatory lesion may be a controlled mechanism. Progenitor cell chemokines such as stromal cell-derived factor (SDF)-1 (42) and their expression within the tissue are potentially interesting areas of investigation.

The purpose of studying the nasal mucosa in an explant system was to eliminate the possibility that changes in cell numbers may be due to their infiltration, an issue inherent to most in vivo studies. Fewer eosinophil precursors and more MBP$^+$ cells ($r^2 = 0.64$) within stimulated, compared with unstimulated, tissue supports the notion that CD34/IL-5R$^+$ cells may undergo local differentiation, giving rise to the observed increase in MBP$^+$ cells. This is in line with previous work by Eidelman et al. demonstrating increased MBP immunoreactivity in explanted slices of rat lung following culture with specific allergen (43). The fact that there were similar numbers of MBP$^+$ cells within unstimulated tissue after 24 h of culture as that blocked immediately following resection indicates that the difference in eosinophil number was not merely due to cell death within unstimulated tissue. Furthermore, the increased proportion of immature eosinophils (CD34$^+$/chromotrope$^+$ cells) per total number of progenitor cells, with the accompanying reduction per total number of eosinophils, in stimulated compared with unstimulated tissue, suggests a progression along the differentiation pathway. We cannot rule out the possibility that a proportion of the CD34/IL-5R$^+$ or MBP$^+$ cells may be partly associated with tissue basophils. Until recently, no reagents were available to us for the immunodetection of these cells, and, as such, their presence within allergic tissue has not been well documented. Using a newly developed basophil-specific Ab (29), we demonstrated no change in cell number in stimulated compared with unstimulated tissue, indicating that the observed increase in MBP immunoreactivity was eosinophil derived. Although we have not examined chemokine expression, the consistent observation that MBP$^+$ cells migrate toward and infiltrate the epithelial layer within stimulated tissue indicates the likelihood that the epithelium is producing eotaxin and/or MCP-4, which occurs in vivo following intranasal challenge with specific allergen (44, 45). Although these data

![FIGURE 4. Representative MBP immunostaining 24 h following culture in the four different conditions. Note the presence of MBP$^+$ cells within unstimulated tissue (a), but that there were substantially more with tissue cultured with ragweed Ag (b) or rhIL-5 (c). Furthermore, d demonstrates that culturing tissue with ragweed + sIL-5Ra inhibited the increase in MBP$^+$ cells.](http://www.jimmunol.org/)
The more striking reduction in CD34/IL-5R mRNA was observed within ragweed-stimulated compared with unstimulated tissue (\( p < 0.05 \)). Although there seemed to be fewer CD34\(^{+}\)/chromotrope\(^{+} \) cells per total chromotrope\(^{+} \) cells within tissue cultured with ragweed as compared with medium alone, this did not reach significance.

Strongly suggest local eosinophil differentiation, they do not in any way exclude a role for eosinophil infiltration. Elevated numbers of peripheral blood (46, 47) as well as tissue (27, 48) eosinophils in subjects with allergic disorders are well documented. Our present work indicates, however, that, in addition to the infiltration of mature eosinophils, a population may originate from within the target organ itself.

Culture with rhIL-5 protein was associated with similar, albeit less pronounced, effects on the number of CD34\(^{+}\)/IL-5R\(^{+} \) and MBP\(^{+} \) cells as observed following culture with specific allergen. The more striking reduction in CD34/IL-5R mRNA\(^{+} \) cells in response to rhIL-5 at 6 (rather than 24 h) could be due to protein degradation. Alternatively, since the total number of IL-5R\(^{+} \) mRNA\(^{+} \) cells was higher within IL-5- than ragweed-stimulated tissue at 24 h (but not 6 h), it is possible that IL-5 may induce synthesis of IL-5R mRNA, as has been previously suggested (41). Although it appeared that there were more IL-5 mRNA\(^{+} \) cells following 6 h culture with rhIL-5 compared with control, it was not statistically different, indicating that this cytokine has no or little effect on the expression of its own mRNA. These results imply that allergen induces a more broad-spectrum response than rhIL-5 alone. Presumably, activation of mast cells and release of IL-4, likely to induce T cell production of IL-5 (49), is the predominant pathway involved. In fact, in a concurrent study, we have observed increased numbers of mast cells and T cells producing IL-4 mRNA within allergen-stimulated compared with unstimulated tissue (50).

Culturing tissue with sIL-5R\(^{+} \) almost completely attenuated the ragweed-induced change in eosinophil precursor and MBP-immunoreactive cell numbers, indicating the obligatory nature of IL-5 for eosinophil differentiation. Although this finding is contrary to work by Clutterbuck (13) and Shalit (14) et al. demonstrating the IL-5-independent ability of IL-3 and GM-CSF to induce the development of eosinophil colonies, it is in agreement with recent work by Popken-Harris et al. illustrating that rhIL-5 is sufficient for the formation of both pro- and mature MBP (51). Furthermore, Robinson et al. have now suggested that the effects of GM-CSF and IL-3 may be mediated through the production of IL-5 itself (41), which would be in agreement with the present findings. However, sIL-5R\(^{+} \) did not inhibit ragweed-induced IL-5 gene expression, further substantiating the observation that this cytokine does not seem to control its own synthesis. The drastic effect of sIL-5R\(^{+} \) on the ragweed-induced eosinophil differentiation leads one to consider whether endogenous production of soluble receptors may regulate IL-5 function, in vivo. Indeed, eosinophil-differentiated HL-60 cells and eosinophils derived from CD34\(^{+} \) cord blood cells have been shown to produce mainly IL-5R mRNA coding for the soluble isoform of the \( \alpha \)-chain (18) and Yasueta et al. (52) have shown that the expression of sIL-5R\(^{+} \) mRNA in bronchial biopsies of atopic and nonatopic asthmatics directly correlated with the patients’ FEV\( \_1 \). As such, factors that induce production of the soluble isoform of the IL-5R may prove highly useful for aborting eosinophil development as well as other IL-5-mediated events.

An increased number of IL-5 mRNA\(^{+} \) cells is a well recognized characteristic of allergic respiratory mucosa (48, 53) and has been considered to be the consequence of inflammatory cell infiltration. Here, we demonstrate the production of IL-5 mRNA following ex vivo stimulation, providing direct evidence that local inflammatory cells increase their cytokine production following allergen exposure. Although we show that a significant proportion of IL-5 mRNA is T cell derived, eosinophils were also seen to produce this cytokine. However, these two cell types accounted for only 54% of the total IL-5 mRNA\(^{+} \) cells, in contrast to previous in vivo findings demonstrating that 88% of IL-5 mRNA\(^{+} \) cells were T cells and eosinophils (54). This underlines the importance of other sources of IL-5, particularly mast cells (54), within allergic nasal mucosa. Regardless of the source, it appears that enough IL-5 is made locally to induce changes in the number of eosinophil precursors and MBP-immunoreactive cells, lending support to the idea that in vivo circulating cells may enter the tissue and rely on the local cytokine environment for phenotype acquisition.

Studies have shown that MBP immunoreactivity (BMK-13) is absent in CD34\(^{+} \) cells before IL-5 stimulation (55), that MBP message is produced as early as day 3 (56), and MBP protein, or at least pro-MBP, is produced after 6 days of culture with IL-5 (51). Here, we demonstrate an increase in the number of MBP-immunoreactive cells after only 6 h of culture with allergen or rhIL-5. At present, we cannot explain these rapid kinetics; however, they are consistent with the work of Eidelman et al. demonstrating an increased number of MBP\(^{+} \) cells after 6 h of culturing slices of rat lung with specific allergen (43). Furthermore, the fact

**FIGURE 5.** Percentage of immature eosinophils within nasal mucosal explant tissue. An increased proportion of CD34\(^{+}\)/chromotrope\(^{+} \) cells per total CD34\(^{+} \) progenitor cells was observed within ragweed-stimulated compared with unstimulated tissue (\( * p < 0.05 \)). Although there seemed to be fewer CD34\(^{+}\)/chromotrope\(^{+} \) cells per total chromotrope\(^{+} \) cells within tissue cultured with ragweed as compared with medium alone, this did not reach significance.

**Table II. Colocalization of IL-5 mRNA to T cells and eosinophils**

<table>
<thead>
<tr>
<th>PhenoType of IL-5 mRNA (^{+} ) Cells (^{a} )</th>
<th>Proportion of T cells and Eosinophils Expressing IL-5 mRNA (^{b} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Percentage Type Percentage</td>
</tr>
<tr>
<td>IL-5/CD3</td>
<td>44.0 ± 2.0 CD3/IL-5 13.0 ± 0.8</td>
</tr>
<tr>
<td>IL-5/MBP</td>
<td>10.0 ± 8.3 MBP/IL-5 5.0 ± 4.0</td>
</tr>
</tbody>
</table>

\(^{a}\) Percentage (\( n = 4 \); mean ± SD) of IL-5 mRNA\(^{+} \) cells coexpressing CD3 or MBP.

\(^{b}\) Percentage of CD3\(^{+} \) and MBP\(^{+} \) synthesizing IL-5 mRNA in nasal mucosal explants after 24 h culture with ragweed allergen.
that we identified immature eosinophils (CD34+/chromotrope+) within sections of unstimulated tissue implies that the nasal mucosa of these individuals was sensitized and as such may be primed for rapid MBP production. Recent in vitro studies of eosinophil progenitor differentiation have revealed that there is a pattern of granulogenesis and cationic protein condensation, including the proteolytic processing of pro-MBP to MBP, within the secondary granules (55, 51). Whether BMK-13 binds to pro-MBP as well as the mature protein is not known. Consequently, the observed increase in MBP immunoreactivity at 6 h could be attributed to cleavage of pro-MBP, rather than de novo protein synthesis. Although we did not see eosinophils that were clearly intravascular, the possibility that the airway microvasculature retains a population of marginated eosinophils that transmigrate toward the airway mucosa following allergen challenge cannot be ruled out. Considering the relatively few MBP+ cells detected within unstimulated tissue, however, it is unlikely that the increase observed here could be accounted for in this way.

Cell culture, while greatly advancing our understanding of cell and cytokine function, is a limited system lacking the important elements of cell-cell and cell-matrix interactions, as well as complex intercytokine networking. The strength of the explant technique is two-fold. First, it side-steps the difficulty of recreating an “in vivo-like” environment, and, second, it allows for the delineation of local vs systemic events. Using nasal mucosal explant tissue, we have demonstrated a concomitant decrease in eosinophil precursors and increase in MBP-immunoreactive cells following ex vivo stimulation and that sIL-5Rα inhibits these changes. This study provides strong evidence to support the concept that a subset of eosinophils may differentiate locally within allergic tissue and indicates that this event is highly IL-5 dependent. In addition, these findings suggest that endogenous production of sIL-5Rα functions as a regulator of this process.

Acknowledgments
We thank Ms. Elsa Schotman and Ms. Cathy Fragiskatos for their invaluable technical expertise and assistance.

References
9. Robinson, D. R., R. Damia, K. Zeiberg, E. Molet, N. Thapar, A. M. A. Irani, and R. M. Nieder. 1990. Identification of messenger RNA for IL-4 in human eosinophils with granule mRNA. Cell culture, while greatly advancing our understanding of cell and cytokine function, is a limited system lacking the important elements of cell-cell and cell-matrix interactions, as well as complex intercytokine networking. The strength of the explant technique is two-fold. First, it side-steps the difficulty of recreating an “in vivo-like” environment, and, second, it allows for the delineation of local vs systemic events. Using nasal mucosal explant tissue, we have demonstrated a concomitant decrease in eosinophil precursors and increase in MBP-immunoreactive cells following ex vivo stimulation and that sIL-5Rα inhibits these changes. This study provides strong evidence to support the concept that a subset of eosinophils may differentiate locally within allergic tissue and indicates that this event is highly IL-5 dependent. In addition, these findings suggest that endogenous production of sIL-5Rα functions as a regulator of this process.


