CHAPTER 12

Eosinophil Cytokines in Allergy

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INTRODUCTION

Eosinophils are granulated white blood cells that form part of the innate immune system, and circulate at low levels in healthy individuals, preferentially recruiting to the gut mucosa after their egress from the bone marrow. Classically, eosinophils were considered to be protective against helminthic parasitic infections as part of their immune function, although recent studies have cast some doubt on this concept. A potential role for eosinophils may exist for other infectious pathogens such as viruses, particularly respiratory viruses. Other findings suggest that eosinophils may serve a role in immunosuppression of proinflammatory signals in homeostasis, particularly in the lungs and gut. While the specific immunological role of eosinophils is still incompletely understood, their contribution to human diseases such as allergy and asthma is more well defined. In many types of allergic inflammation, eosinophils are strikingly elevated, and they have been found to increase in blood, as well as accumulate in the gut mucosa, skin, and lung tissues. In allergic diseases such as atopic asthma, the numbers of eosinophils broadly correlate with severity of disease. Recruitment of eosinophils to tissues is orchestrated by a diversity of cells and mediators involving antigen-presenting cells, mast cells, T cells, B cells, epithelial cells, macrophages, neutrophils, and other cell types that release eosinophil-attracting factors.

Eosinophils are a source of over 35 immunomodulatory cytokines, chemokines, and growth factors (collectively referred to as cytokines for this chapter) that have a marked effect on the progression of immune and inflammatory responses (Fig. 12.1). This chapter is focused on the types of cytokines that are released from eosinophils and their role in the propagation of immune responses.
Eosinophils, or eosinophil-like cells such as heterophils, have been reported in invertebrates as well as vertebrates, suggesting an evolutionarily conserved role for eosinophils in immune modulation and host protection.\textsuperscript{5,13} Their role in immunity is still under intense investigation, with early evidence showing an association of eosinophils with helminthic parasite infections. However, evidence from studies with transgenic mice that lack eosinophils, such as the PHIL and \( \Delta \)dblGATA strains,\textsuperscript{14,15} have indicated that the role of eosinophils in protection of the host against helminths is more complex than previously recognized.\textsuperscript{16} More recent studies suggest a role for eosinophils in protection against respiratory virus infections.\textsuperscript{4} These recent findings contribute to an evolving area of research on the function of eosinophils in immunity.

In marked contrast, eosinophils are well known for their association with allergic inflammation. Organs, such as the lung and skin, that normally harbor very few eosinophils undergo a dramatic increase in tissue-recruited cells, including the lungs and upper airways, in a subset of patients with allergic asthma.\textsuperscript{9} Eosinophils recruit together with other inflammatory cells to sites of allergen exposure, such as the peribronchial regions of the lungs, which results in allergic exacerbations. Upon their activation, eosinophils are believed to contribute to

FIGURE 12.1  Cytokines, chemokines, and growth factors secreted by eosinophils that have a postulated role in allergic inflammation. Shown is a rendered image from CCL5/RANTES immunofluorescence in a human peripheral blood eosinophil.

ROLE OF EOSINOPHILS IN ALLERGY

Eosinophils, or eosinophil-like cells such as heterophils, have been reported in invertebrates as well as vertebrates, suggesting an evolutionarily conserved role for eosinophils in immune modulation and host protection.\textsuperscript{5,13} Their role in immunity is still under intense investigation, with early evidence showing an association of eosinophils with helminthic parasite infections. However, evidence from studies with transgenic mice that lack eosinophils, such as the PHIL and \( \Delta \)dblGATA strains,\textsuperscript{14,15} have indicated that the role of eosinophils in protection of the host against helminths is more complex than previously recognized.\textsuperscript{16} More recent studies suggest a role for eosinophils in protection against respiratory virus infections.\textsuperscript{4} These recent findings contribute to an evolving area of research on the function of eosinophils in immunity.

In marked contrast, eosinophils are well known for their association with allergic inflammation. Organs, such as the lung and skin, that normally harbor very few eosinophils undergo a dramatic increase in tissue-recruited cells, including the lungs and upper airways, in a subset of patients with allergic asthma.\textsuperscript{9} Eosinophils recruit together with other inflammatory cells to sites of allergen exposure, such as the peribronchial regions of the lungs, which results in allergic exacerbations. Upon their activation, eosinophils are believed to contribute to
bronchial symptoms including bronchoconstriction, mucus secretion, and coughing.

The inflammatory processes underlying allergic exacerbations are controlled by a complex network of cytokines that regulate bone marrow progenitor differentiation, migration to inflammatory foci, cell adhesion receptor expression, and immunoglobulin E (IgE) responses. The infiltration of eosinophils to inflammatory responses may further exacerbate inflammation by their ability to release a plethora of cytokines. Along with cytokines, eosinophils release a range of cationic proteins that are cytotoxic, including major basic protein (MBP), eosinophil peroxidase (EPX), eosinophil-derived neurotoxin (EDN), and eosinophil cationic protein (ECP). These factors have independent proinflammatory effects on tissues and are implicated in the exacerbation of allergic responses.

A large body of evidence from animal models has suggested that allergic inflammation arises from inappropriate polarization of the innate and adaptive immune systems toward a Th2 response, since a greater expression of Th2 cytokines (interleukin (IL)-4, IL-5, and IL-13) occurs in allergy. The primary roles for these cytokines are to promote IgE switching in B cells, which leads to binding of allergen-specific IgE to mast cells, along with enhancement of T cell differentiation to a Th2 phenotype, and suppression of Th1 responses. Th2 responses result in the maintenance of an abnormally hypersensitive status in reaction to usually innocuous allergens. These cytokines also serve to increase blood and tissue eosinophilia, enhance eosinophil survival in tissues, and initiate eosinophil degranulation. By prolonging eosinophil survival in tissues, Th2 cytokines are thought to prolong the ability of eosinophils to actively release their immunomodulatory mediators into tissues.

However, while the majority of asthma cases in patients fit the Th2 phenotype described in animal models, certainly not all of them may be categorized in this way. While the proportion of asthmatics with eosinophilia is not known, approximately 50% of patients with mild to severe asthma have some form of blood eosinophilia. Thus, eosinophils may be important contributors to allergic inflammation in around half of all cases of asthma.

Taken together, the network of cytokine signaling underlying allergic inflammation and asthma is highly complex. Th2 responses are strongly evoked in animal models of asthma and allergy; however, not all human asthma phenotypes are driven by Th2 cytokines. The contribution of eosinophil-derived cytokines to allergic diseases is not fully understood, although recent developments suggest that they may be implicated in the manifestation of allergic inflammation.
DEGRANULATION RESPONSES IN EOSINOPHILS

Eosinophils are densely packed with a unique type of secretory granules called crystalloid granules. These are so named because of the core of the granule which contains crystallized MBP, a highly charged cationic protein with cytotoxic effects upon its release.\(^{10}\) The crystalline core of the eosinophil secretory granule may be visualized by its electron-dense properties using transmission electron microscopy.\(^{10}\) Surrounding the core of the eosinophil granule is the matrix, which contains additional cationic proteins, including EPX, ECP, and EDN. Other components are also stored in the matrix including cytokines, which are released along with eosinophil granule proteins, although via distinct trafficking mechanisms (Fig. 12.2).

There are four major pathways by which eosinophil crystalloid granules are released to the cell exterior by a process known as degranulation. The first is classical exocytosis, in which the membrane of individual granules fuses with the plasma membrane to form contiguous structures.\(^{18,19}\) The second is compound exocytosis, whereby the crystalloid granules first fuse with each other (via homotypic fusion) and then fuse with the cell membrane through a single fusion pore.\(^{20–22}\) The third pathway of degranulation is known as piecemeal degranulation, where small, rapidly mobilizable secretory vesicles bud from the surface of crystalloid granules and shuttle materials to the cell membrane as part of the eosinophil’s tubulovesicular system.\(^{23–26}\) Finally,

![Diagram of eosinophil granule](image)

**FIGURE 12.2** Sites of storage and transport for eosinophil-derived cytokines. The eosinophil crystalloid granule consists of two subcompartments: the core, made up of crystallized MBP, and the matrix, which contains EPX, among other granule components. Cytokines are shown in their specific locations in secretory organelles. Small secretory vesicles also transport cytokines, including CCL5/RANTES, IL-4, and TGF\(_\alpha\).
degranulation may occur through necrosis or “cytolysis,” in which intact, membrane-bound crystalloid granules are released following the loss of cell membrane integrity.26–28

Degranulation from eosinophils is usually associated with marked tissue damage, as eosinophil granule proteins are cytotoxic and can induce epithelial damage, leading to increased inflammation.8,10 Excessive release of MBP, EPX, and ECP correlates with increased disease severity in allergy and asthma.10 Along with the release of eosinophil granule proteins, prestored immunomodulatory cytokines are also released, many of which are differentially secreted in distinct pathways from those of eosinophil granule proteins.29

Agonists that potently evoke degranulation from eosinophils include platelet-activating factor (PAF),30,31 opsonized bacteria or other surfaces,32 complement factors (especially C5a),33 and immunoglobulin complexes.34 In addition, a range of cytokines also induce or promote eosinophil degranulation, including granulocyte/macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), IL-3, IL-5, and CCL11/eotaxin.24,35–38 Most of these agonists are elevated in allergic inflammation and are predicted to contribute to eosinophil degranulation, leading to widespread tissue injury.

**CYTOKINES PRODUCED BY HUMAN EOSINOPHILS**

Human eosinophils produce over 35 cytokines, many of which are also expressed in their murine counterparts (Table 12.1). In most cases, both the mRNA transcript and protein expression have been identified for each cytokine in peripheral blood eosinophils obtained from nonatopic as well as atopic subjects. Interestingly, 10 of these cytokines have been found as preformed mediators packaged within eosinophil crystalloid granules, suggesting that eosinophil-derived cytokines may act immediately and directly on their microenvironment within minutes of cell activation.

Eosinophils from tissue sources also synthesize and release numerous cytokines, suggesting that eosinophils retain the ability to generate cytokines after their recruitment into peripheral tissues. Examples of tissue eosinophils that express cytokines have been observed in the respiratory system (nasal polyps, bronchial biopsies, bronchoalveolar lavage (BAL), sputum samples), the gastrointestinal tract (celiac mucosal biopsies), and in the skin.

The following sections elaborate on the expression of individual cytokines in eosinophils derived from blood and tissues. The most frequently used techniques for determining intracellular sites of cytokine
### TABLE 12.1  
Eosinophil-Derived Cytokines, Chemokines, and Growth Factors in Human and Mouse Studies

<table>
<thead>
<tr>
<th>Mediator detected in human eosinophils</th>
<th>Molecule detected</th>
<th>Mean quantity of protein stored (per $10^6$ cells)</th>
<th>Release factors</th>
<th>Intracellular localization of stored protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Cytokines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A proliferation-inducing ligand (APRIL)</td>
<td>mRNA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulocyte/Macrophage colony-stimulating factor (GM-CSF)</td>
<td>mRNA</td>
<td>15 pg</td>
<td>Ionomycin</td>
<td>Crystalloid granules (core)</td>
<td>40–46</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td></td>
<td>LPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferon-γ (IFN-γ)</td>
<td>mRNA</td>
<td>997 pg</td>
<td>Cytokines</td>
<td>Crystalloid granules, small secretory vesicles</td>
<td>11,47,48</td>
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<td>Protein</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Interleukin-1α</td>
<td>mRNA</td>
<td>–</td>
<td>PMA</td>
<td>–</td>
<td>49,50</td>
</tr>
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<td></td>
<td>Protein</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>mRNA</td>
<td>–</td>
<td>[Constitutively released]</td>
<td>–</td>
<td>51,52</td>
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<td>Protein</td>
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<td></td>
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</tr>
<tr>
<td>Interleukin-2</td>
<td>mRNA</td>
<td>6 pg</td>
<td>Serum-coated particles</td>
<td>Crystalloid granules (core)</td>
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<td>PHA</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>CD28 cross-linking</td>
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<tr>
<td>Interleukin-3</td>
<td>mRNA</td>
<td>–</td>
<td>Ionomycin</td>
<td>–</td>
<td>41,57</td>
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<td></td>
<td>Protein</td>
<td></td>
<td>IFN-γ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>mRNA</td>
<td>108 pg</td>
<td>Immune complexes</td>
<td>Crystalloid granules (core)</td>
<td>58–67</td>
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(Continued)
### TABLE 12.1 (Continued)

<table>
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<tr>
<th>Mediator detected in human eosinophils</th>
<th>Molecule detected</th>
<th>Mean quantity of protein stored (per 10⁶ cells)</th>
<th>Release factors</th>
<th>Intracellular localization of stored protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-5</td>
<td>Protein mRNA</td>
<td>—</td>
<td>Serum-coated particles Cytokines</td>
<td>Immune complexes Crystalloid granules (core/matrix?)</td>
<td>43,62, 68–73</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Protein mRNA</td>
<td>356 pg</td>
<td>Cytokines</td>
<td>Crystalloid granules (matrix)</td>
<td>48,74–76</td>
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<tr>
<td>Interleukin-9</td>
<td>Protein mRNA</td>
<td>—</td>
<td>—</td>
<td>Crystalloid granules</td>
<td>77–79</td>
</tr>
<tr>
<td>Interleukin-10</td>
<td>Protein mRNA</td>
<td>455 pg</td>
<td>Cytokines</td>
<td>Crystalloid granules</td>
<td>48,61,80</td>
</tr>
<tr>
<td>Interleukin-11</td>
<td>Protein mRNA</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>81</td>
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<tr>
<td>Interleukin-12</td>
<td>Protein mRNA</td>
<td>186 pg</td>
<td>Cytokines</td>
<td>Crystalloid granules</td>
<td>48,82</td>
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<tr>
<td>Interleukin-13</td>
<td>Protein mRNA</td>
<td>50 pg</td>
<td>Cytokines</td>
<td>Crystalloid granules</td>
<td>83,84</td>
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<td>Interleukin-16</td>
<td>Protein mRNA</td>
<td>—</td>
<td>[Constitutively released]</td>
<td>—</td>
<td>85,86</td>
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<tr>
<td>Interleukin-17</td>
<td>Protein</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>87</td>
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(Continued)
TABLE 12.1 (Continued)

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<tr>
<th>Mediator detected in human eosinophils</th>
<th>Molecule detected</th>
<th>Mean quantity of protein stored (per 10^6 cells)</th>
<th>Release factors</th>
<th>Intracellular localization of stored protein</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Interleukin-25</td>
<td>mRNA</td>
<td>–</td>
<td>Cytokines</td>
<td>–</td>
<td>88–90</td>
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<td>Protein</td>
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<td></td>
</tr>
<tr>
<td>Tumor necrosis factor-α (TNF)</td>
<td>mRNA</td>
<td>909 pg</td>
<td>Immune complexes</td>
<td>Crystalloid granules</td>
<td>44,48,61,91–94</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td></td>
<td>TNF</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LPS</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Cytokines</td>
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</table>

**B. Chemokines**

<table>
<thead>
<tr>
<th>CCL3/Macrophage inflammatory protein-1α (MIP-1α)</th>
<th>mRNA</th>
<th>–</th>
<th>–</th>
<th>–</th>
<th>92,95</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL5/Regulated on activation, normal T cell expressed and secreted (RANTES)</td>
<td>mRNA</td>
<td>7000 pg</td>
<td>Serum-coated particles</td>
<td>Crystalloid granules, small secretory vesicles</td>
<td>24,85,96</td>
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<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL11/Eotaxin</td>
<td>mRNA</td>
<td>16–22 pg</td>
<td>IFN-γ</td>
<td>Crystalloid granules</td>
<td>97–99</td>
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<td>Protein</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CCL13/Monocyte chemoattractant protein-4 (MCP-4)</td>
<td>mRNA</td>
<td>13 pg</td>
<td>Immune complexes</td>
<td>Crystalloid granules</td>
<td>99</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL17/Thymus activation regulated chemokine (TARC)</td>
<td>mRNA</td>
<td>–</td>
<td>TNF + IFN-γ or IL-4</td>
<td>–</td>
<td>100</td>
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(Continued)
### TABLE 12.1 (Continued)

<table>
<thead>
<tr>
<th>Mediator detected in human eosinophils</th>
<th>Molecule detected</th>
<th>Mean quantity of protein stored (per $10^6$ cells)</th>
<th>Release factors</th>
<th>Intracellular localization of stored protein</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>CCL22/Macrophage-derived chemokine (MDC)</td>
<td>Protein mRNA</td>
<td>–</td>
<td>TNF + IFNγ or IL-4</td>
<td>–</td>
<td>100</td>
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<tr>
<td>CCL23/Myeloid progenitor inhibitory factor 1 (MPIF-1)</td>
<td>Protein mRNA</td>
<td>–</td>
<td>Cytokines</td>
<td>–</td>
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<tr>
<td>CXCL1/Groα</td>
<td>Protein mRNA</td>
<td>95 pg</td>
<td>Cytokines</td>
<td>Crystalloid granules</td>
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<tr>
<td>CXCL5/Epithelial-derived neutrophil-activating peptide 78 (ENA-78)</td>
<td>Protein mRNA</td>
<td>1500 pg</td>
<td>Cytokines</td>
<td>–</td>
<td>103</td>
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<td>CXCL8/Interleukin-8</td>
<td>Protein mRNA</td>
<td>–</td>
<td>C5a</td>
<td>–</td>
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(Continued)
TABLE 12.1 (Continued)

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<tr>
<th>Mediator detected in human eosinophils</th>
<th>Molecule detected</th>
<th>Mean quantity of protein stored (per 10^6 cells)</th>
<th>Release factors</th>
<th>Intracellular localization of stored protein</th>
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<tr>
<td>CXCL9/Interferon γ induced protein 10 (IP-10)</td>
<td>mRNA</td>
<td>–</td>
<td>TNF + IFNγ or IL-4</td>
<td>–</td>
<td>100,109</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
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<tr>
<td>CXCL10/Interferon γ induced protein 10 (IP-10)</td>
<td>mRNA</td>
<td>–</td>
<td>TNF + IFNγ or IL-4</td>
<td>–</td>
<td>100,109</td>
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<td>CXCL11/Interferon γ induced T cell α chemoattractant (I-TAC)</td>
<td>mRNA</td>
<td>–</td>
<td>IFNγ</td>
<td>–</td>
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<td></td>
<td>Protein</td>
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<tr>
<td>C. Growth factors</td>
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<tr>
<td>Heparin-binding epidermal growth factor-like binding protein (HB-EGF-LBP)</td>
<td>mRNA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>110</td>
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<td></td>
<td>Protein</td>
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<td>Nerve growth factor (NGF)</td>
<td>mRNA</td>
<td>10 pg</td>
<td>–</td>
<td>–</td>
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<td>Protein</td>
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<tr>
<td>Platelet-derived growth factor, B chain (PDGF-B)</td>
<td>mRNA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>112</td>
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<td>Stem cell factor (SCF)</td>
<td>mRNA</td>
<td>9 pg</td>
<td>Chymase</td>
<td>Crystalloid granules</td>
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storage in eosinophils include immunocytochemistry, immunofluorescence, subcellular fractionation, immunogold labeling, and immunofluorescence using confocal microscopy analysis. In this chapter, a specific focus will be made on eosinophil-derived cytokines that have a potential role in allergy or that have been identified in tissues from allergic patients.

**GM-CSF (Granulocyte/Macrophage Colony-Stimulating Factor)**

The principal role of GM-CSF in the immune system is to stimulate the production of granulocytes (neutrophils, eosinophils, and basophils) in the bone marrow. In allergic disease, GM-CSF facilitates allergic airway inflammation and promotes eosinophilic inflammation, Th2 responses, mucus production, and airway hyperresponsiveness together with other cytokines. Further, GM-CSF promotes cutaneous anaphylaxis reactions in mice expressing humanized immune systems. In eosinophils, GM-CSF promotes maturation, differentiation, and survival by delaying apoptosis. GM-CSF also enhances survival of adherent eosinophils in an autocrine manner and degranulation as well as superoxide production from human eosinophils.

TABLE 12.1 (Continued)

<table>
<thead>
<tr>
<th>Mediator detected in human eosinophils</th>
<th>Molecule detected</th>
<th>Mean quantity of protein stored (per 10⁶ cells)</th>
<th>Release factors</th>
<th>Intracellular localization of stored protein</th>
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<tr>
<td>Transforming growth factor-α (TGFα)</td>
<td>mRNA</td>
<td>–</td>
<td>Cytokines</td>
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<td>114–118</td>
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<td>Transforming growth factor-β (TGF-β)</td>
<td>mRNA</td>
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<td>Vascular endothelial growth factor (VEGF)</td>
<td>mRNA</td>
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Eosinophils isolated from human peripheral blood produce significant levels of GM-CSF. Early studies identified GM-CSF as a prominent cytokine that was released by eosinophils following stimulation by calcium ionophore (ionomycin) or bacterial lipopolysaccharide. It was also demonstrated that GM-CSF prolonged eosinophil survival in an autocrine manner, and that the effect of GM-CSF on delay of apoptosis was inhibited by the immunosuppressive drug cyclosporin A.

In confirmation of findings in blood eosinophils, tissue eosinophils express GM-CSF. For example, GM-CSF-positive eosinophils have been detected in nasal biopsies from patients with allergic rhinitis and bronchial biopsies from asthmatic subjects. Following endobronchial challenge in human asthmatics, eosinophils are recruited to the peribronchial regions, and these were shown to express GM-CSF. Moreover, sputum eosinophils from asthmatic subjects express GM-CSF as evaluated by immunocytochemistry. Thus, these findings suggest that GM-CSF is a critical eosinophil-derived cytokine that serves an important role in maintaining viability as well as eosinophil effector functions in allergy.

GM-CSF is one of the 10 cytokines that have so far been identified as preformed mediators that are stored within eosinophil crystalloid granules (Fig. 12.2). The intragranular localization of GM-CSF appears to be associated with the matrix subcompartment that surrounds the crystalline core. This early observation was among the first reports describing the ability of eosinophils to store preformed cytokines, which could be later released in a rapid manner in response to inflammatory signals.

**Interferon-γ**

IFN-γ is the sole member of the type II class of interferons, and serves as a potent proinflammatory Th1 cytokine. Its release is mainly from innate immune NK and NKT cells in response to viral, bacterial and some protozoal infections. IFN-γ is classically known for its role in downregulation of Th2-driven allergic inflammation, leading to reduced eosinophilia, in mouse models of allergic inflammation. In allergic asthma, IFN-γ is predominantly associated with virus-induced asthma exacerbations.

Stimulation of eosinophils by IFN-γ has been proposed to promote allergic inflammation through a number of mechanisms in response to virus infections, suggesting a role for IFN-γ in autocrine signaling in eosinophils. The effects of IFN-γ on eosinophils are to enhance cytotoxicity, increase cytokine expression such as GM-CSF, induce secretion of IL-3, IL-6, and CCL5/RANTES, and promote IL-5- or GM-CSF-induced superoxide release. IFN-γ also enhances...
eosinophil survival, although not as potently as GM-CSF or IL-5, while it suppresses degranulation responses from eosinophils induced by secretory IgA, suggesting a complex effect by this cytokine on eosinophil secretory functions.

Perhaps counterintuitively to the classical model of the Th2 skewed responses in allergy, IFN-γ is also expressed in eosinophils, cells which are usually considered to be associated with Th2 responses. IFN-γ is among the most abundantly secreted cytokines generated by human eosinophils (Table 12.1). Secretion of IFN-γ from eosinophils was shown to occur following stimulation with Th1, Th2, and inflammatory signals such as TNF-α. Eosinophils also store significant quantities of IFN-γ, suggesting that this may be released immediately within minutes of stimulation.

In Balb/c mice, eosinophil-derived IFN-γ has bioactive effects on surrounding tissues was shown to contribute to airway hyperresponsiveness in a manner independent of T cells, indicating an important immunomodulatory role for eosinophil-derived cytokines in lung inflammation.

The intracellular sites of storage for IFN-γ in eosinophils have not yet been determined, and this remains an area of interest for future studies.

Interleukin-1α

IL-1α, also known as hematopoietin 1, is a member of the IL-1 family of 11 cytokines and is produced mainly by macrophages, neutrophils, epithelial cells, and endothelial cells. This cytokine was originally named IL-1 until it was recognized that this consisted of two distinct cytokines, IL-1α and IL-1β. IL-1α is a potent proinflammatory cytokine associated with inflammation, fever, and sepsis. In allergic inflammation, IL-1α is thought to promote epithelial release of GM-CSF and IL-33, leading to allergic sensitization in mice exposed to house dust mite (HDM). Eosinophils respond to IL-1α by undergoing degranulation and adhesion to endothelial cells.

IL-1α was the first cytokine discovered in eosinophils, where it was characterized for its expression in peritoneal eosinophils from mice infected with the larvae of the parasite *Mesocestoides corti*. Human eosinophils were first shown to synthesize IL-1α in association with cytokine-induced human leukocyte antigen DR (HLA-DR) expression. IL-1α mRNA transcripts and protein expression were elevated by stimulation with phorbol ester myristate (PMA), suggesting that human eosinophils can process antigens (such as allergens), and express the costimulatory cytokine IL-1α, for HLA-DR presentation to T cells. Later studies confirmed that eosinophils may function as antigen-presenting cells.
cells in mouse models of allergic inflammation. The release of IL-1α from eosinophils may serve an important role as antigen-presenting cells in promoting allergen presentation to T cells in subjects with eosinophilia.

No studies have indicated intracellular sites of IL-1α storage or trafficking in eosinophils. As IL-1α is an unusual cytokine that does not contain a signal peptide fragment, it is unlikely to be transported out of the cell via vesicular trafficking. The proposed transport mechanisms for IL-1α are described elsewhere.

**Interleukin-1β**

IL-1β, also called human leukocytic pyrogen, lymphocyte-activating factor and other names, and has similar properties to IL-1α in immune function by promoting inflammatory responses, fever, and sepsis. Like IL-1α, IL-1β is a member of the IL-1 family of 11 cytokines, which regulates and initiates proinflammatory reactions. IL-1β is implicated in allergic disease, including atopic dermatitis and bronchial asthma, and has been shown to support T cell survival, upregulate IL-2 receptor expression on lymphocytes, enhance DC recruitment, increase antibody production from B cells, and promote B cell proliferation as well as elevate Th2 cytokines. For these reasons, IL-1β is considered to be an important therapeutic target for treatment of allergic inflammation. In eosinophils, IL-1β acts in a similar manner to IL-1α by inducing degranulation and cell adhesion, although different eosinophil subpopulations responded selectively to each isoform of IL-1.

Interestingly, it was only recently that eosinophils were shown to constitutively release IL-1β as an immunomodulatory cytokine. Eosinophil-derived IL-1β was shown to be involved in promoting the synthesis and secretion of IL-17 from activated CD4+ T cells. Eosinophils also elaborate IL-1β in the gastrointestinal lamina propria where they promote secretory IgA production. Since Th17 cells have been implicated in the pathogenesis of allergic airway inflammation, the ability of eosinophils to generate IL-1β indicates that this may be an important mechanism whereby eosinophils promote differentiation of T cells to Th17 phenotype.

Similarly to IL-1α, IL-1β lacks a signal peptide sequence, and there are no reports indicating the sites of storage or transport of IL-1β in eosinophils.

**Interleukin-2**

An essential growth factor for T cells, IL-2 is a critical cytokine for key functions of the immune system and has pivotal roles in tolerance...
and immunity. Its predominant target is T cells, particularly in the thymus where it prevents the development of autoimmunity. In peripheral lymphoid tissues, IL-2 promotes differentiation of T cells into effector T cells and memory T cells. In allergic inflammation, IL-2 contributes to the establishment of the allergic phenotype following early phase allergen exposure leading to IL-4 release,\(^\text{152}\) and was recently demonstrated to be important in the generation of Th2 memory cells that promote allergic airway responses in asthma.\(^\text{153}\) IL-2 also has a direct effect on eosinophils which express the IL-2 receptor (CD25), by promoting chemotaxis.\(^\text{154}\)

Eosinophils constitutively express IL-2 at the mRNA and protein levels, suggesting that eosinophil-derived IL-2 may contribute to T cell proliferation in peripheral tissues.\(^\text{53–56}\) The release of IL-2 may be induced by serum-coated particles and phytohemagglutinin (PHA) from human peripheral blood eosinophils. Eosinophil-derived IL-2 was shown to be bioactive, as supernatants from eosinophils treated with anti-CD28 induced T cell proliferation and MHC II expression on a colon carcinoma cell line.\(^\text{55}\) Few studies have reported the expression of IL-2 in tissue eosinophils, however, and this remains an area of interest for future investigations to determine the role of eosinophil-derived IL-2 in allergic disease.

Intracellular sites of storage for IL-2 have been characterized in eosinophils. The majority of IL-2 was found stored as a preformed mediator within eosinophil crystalloid granules,\(^\text{53}\) with some cytoplasmic staining evident in a minority of freshly isolated peripheral blood eosinophils. Within the granules, IL-2 appeared to be predominantly localized to the crystalline core.\(^\text{53}\) These findings suggesting that eosinophils have the capacity to synthesize, store, and release IL-2 from the crystalloid granules, allowing the granules to serve as a reservoir for the rapid release of IL-2 in inflammatory reactions associated with allergy.

**Interleukin-3**

An essential growth factor for myelocytic cells and granulocytes, IL-3 is required as a pluripotent growth factor along with GM-CSF to promote the proliferation of precursors toward a myeloid phenotype. It is produced by a number of immune cells, primarily T cells, NK cells, and mast cells. In allergic diseases, IL-3 is elevated following allergen exposure in the skin\(^\text{155}\) and in bronchial asthma.\(^\text{155–158}\) This cytokine is frequently observed to increase in correlation with GM-CSF and IL-5 during allergic responses.\(^\text{155,157,158}\) The function of IL-3 in these conditions is suggested to be through augmentation of IgE synthesis from B cells\(^\text{159}\) and increased histamine release from basophils.\(^\text{160}\) In eosinophils, IL-3 functions together with IL-5 and GM-CSF to promote differentiation
from human bone marrow progenitor cells into mature eosinophils. IL-3 alone is insufficient to serve as a chemoattractant for mature peripheral blood eosinophils, although low concentrations are capable of enhancing chemotactic effects of PAF and other agonists. Eosinophils from atopic patients show an increased sensitivity to IL-3 compared to normal individuals. IL-3 also promotes eosinophil survival and activation to generate hypodense eosinophils, and is capable of promoting degranulation and superoxide production from human eosinophils.

IL-3 was among the first cytokines to be characterized in eosinophils, and its release can be evoked by GM-CSF. Eosinophils isolated from human peripheral blood synthesize IL-3 at the mRNA and protein levels, and secrete IL-3 in response to calcium ionophore and IFN-γ stimulation. Thus, eosinophils marginated into tissue sites that actively secrete IL-3 are likely to prolong their own survival by autocrine signaling. While IL-3 has been shown to increase in allergic inflammation based on biopsies from individuals with atopic rhinitis, the expression of IL-3 in tissue eosinophils was not directly demonstrated but instead showed a correlation between numbers of eosinophils and IL-3 mRNA+ cells.

To date, the intracellular sites of storage or trafficking of IL-3 have not yet been elucidated, and this remains an area of interest.

Interleukin-4

Of all the cytokines studied in eosinophils to date, perhaps the best characterized has been IL-4, a cytokine essential for the development of the Th2 response and the production of IgE by B cells, placing IL-4 in a central effector role in allergic inflammation. In eosinophils, IL-4 has been shown to promote chemotaxis in cells obtained from atopic patients. Interestingly, early studies suggested that stimulation of eosinophils with IL-4 may also promote the development of Th1 responses. IL-4-treated eosinophils released the Th1 cytokine IL-12, which in turn induced the expression of IFN-γ from Th1 cells during culture of T cells with eosinophil-conditioned media. Conversely, IL-4 also promotes the expansion of Th2 cytokine-producing eosinophils in vivo in a mouse model of asthma, both in the airways and in bone marrow progenitor cells. The finding that eosinophils may release Th1 or Th2 cytokines, depending on the stimulation and microenvironment, suggests that eosinophils possess a more nuanced control of cytokine responses in allergic inflammation than may be previously recognized.

Human peripheral blood eosinophils from atopic donors produce IL-4 and release this cytokine in response to serum-coated surfaces and
cytokines. Eosinophils purified from the blood of healthy donors also produce and secrete IL-4 in response to CCL11/eotaxin, suggesting that IL-4 is constitutively expressed in blood eosinophils under normal conditions. This is consistent with mouse studies of eosinophil-derived cytokines. In IL-4 reporter 4get mice, eosinophils constitutively express IL-4 transcript at early stages of ontogeny. Moreover, instillation of IL-4 into mice leads to proliferation of IL-4-expressing eosinophils, suggesting that IL-4 promotes differentiation of bone marrow progenitor cells into eosinophils that express Th2 cytokines including IL-4. An earlier study showed that, among the non-T and non-B cell populations in lung and spleen tissues in mice infected with *Nippostrongylus brasiliensis*, eosinophils are among the most abundant IL-4-expressing cells. Using 4get mice, eosinophils were found to be the most prevalent IL-4-expressing cells that infiltrated the lungs of mice infected with *N. brasiliensis*. Eosinophils expressing IL-4 have also been observed in mice infected with fungal *Cryptococcus neoformans*, in which they formed the majority of cells expressing IL-4.

IL-4 production from eosinophils has been shown to occur in the spleen following adjuvant stimulation of B cell responses, based on observations from eosinophil-deficient ΔdblGATA mice injected with alum. Alum injection into ΔdblGATA mice led to attenuation of early B cell priming and IgM production, suggesting that eosinophils may play an important role in promoting the adaptive immune response to vaccines containing adjuvants.

In parallel with findings on blood-derived human eosinophils, tissue eosinophils from human subjects also express IL-4 as shown in airway, lung, and skin biopsy samples. For example, in nasal biopsies obtained from subjects with allergic rhinitis, tissue eosinophils expressed IL-4. As many as 44% of the tissue eosinophils present in nasal polyp tissues were found to be positive for IL-4. Moreover, following allergen-induced cutaneous late-phase reactions, the majority of tissue-infiltrating eosinophils (84%) were IL-4 after 6 h. This was further demonstrated in lung tissues, where eosinophils from bronchial biopsies taken from atopic asthmatics and normal nonatopic subjects were shown to express IL-4 mRNA. Finally, in skin biopsies of allergic individuals, around 20% of tissue eosinophils were positive for IL-4 mRNA at 24 h following challenge, and this increased to 50%—60% for protein expression of IL-4.

Eosinophil-derived IL-4 is also one of the few eosinophil-derived cytokines that have been shown to have a direct bioactive role in tissues in numerous reports. Several studies have suggested that eosinophil-derived IL-4 is important in priming naïve T cells and activation of mast cell IL-5 release during helminthic parasite infection, for example with *Schistosoma mansoni*. However, recent studies suggest that
eosinophil-derived IL-13 instead of IL-4 may play a more prominent role in establishing allergic inflammation in a mouse model.\textsuperscript{172} The pathophysiological significance of eosinophil-derived IL-4 in human disease is yet to be determined.

IL-4 is one of at least 10 cytokines that have been identified as a preformed, stored mediator that is located within the crystalloid granules of eosinophils\textsuperscript{59} (Fig. 12.2). While this report suggested IL-4 colocalized with the core of the crystalloid granules, later studies using immunogold electron microscopy suggest that IL-4 was located within crystalloid granules as well as small secretory vesicles that are granule-associated vesiculotubular carriers (so-called eosinophil sombrero vesicles).\textsuperscript{63,64,66,67} These small secretory vesicles are important in trafficking of IL-4, and their membrane trafficking mechanisms are described in more detail elsewhere.\textsuperscript{29}

In summary, IL-4 production from eosinophils may be important in a variety of allergy and immune responses that are only just beginning to be understood. These observations will continue to shape our understanding of the biological role of eosinophils in allergy.

**Interleukin-5**

Similarly, to IL-4, IL-5 has been extensively investigated for its role in allergic inflammation. While IL-5 does not have a direct role in skewing immune responses toward a Th2 phenotype, it is important in downstream Th2-associated events in response to allergens. IL-5 is an essential cytokine involved in the terminal differentiation and proliferation of precursor cells in the bone marrow into an eosinophilic phenotype. This cytokine acts on CD34\textsuperscript{+} cells together with IL-3 and GM-CSF to promote eosinophil development.\textsuperscript{173,174} IL-5 has an important effector role in mouse and human eosinophils, including prolongation of survival, induction of chemotaxis, priming, and degranulation.\textsuperscript{36,38,175–177} Anti-IL-5, as well as anti-IL-5 receptor, are promising new treatments for eosinophilic asthma and may be able to promote a steroid-sparing regime for atopic asthmatics.\textsuperscript{178–182} Interestingly, eosinophils also express IL-5, suggesting that IL-5 may have an autocrine or paracrine role in promoting eosinophil differentiation, survival, and activation.

Eosinophil expression of IL-5 was first characterized in tissue biopsies from human subjects. A significant percentage of eosinophils in gut mucosa tissue samples from patients with active celiac disease were found to be positive for IL-5, and following administration of a gluten-free diet, the numbers of IL-5\textsuperscript{+} eosinophils declined.\textsuperscript{68} However, IL-5\textsuperscript{+} eosinophils are not associated with all disorders of the gut, as intestinal
mucosal eosinophils in Crohn’s disease were not found to be positive for IL-5 transcript.\textsuperscript{70} 

In allergic airway disease, eosinophils in nasal biopsies from subjects with allergic rhinitis correlated with elevated IL-5\textsuperscript{+} mRNA in tissue cells.\textsuperscript{157} Endobronchial or segmental challenge of atopic asthmatics led to increased IL-5\textsuperscript{+} eosinophils infiltrating the airways.\textsuperscript{43} Eosinophils also express IL-5 mRNA and protein in bronchial biopsies of atopic asthmatics as well as normal nonatopic subjects.\textsuperscript{72} In complementary studies, \(\sim20\%\) of tissue eosinophils were positive for IL-5 mRNA in skin biopsies of allergic individuals 24 h following challenge, which increased in correlation with IL-5 protein expression.\textsuperscript{62,73} 

Human peripheral blood eosinophils also express IL-5, and its release may be evoked by immune complexes.\textsuperscript{70,71} Moreover, IL-5 is also found as a preformed, stored cytokine located within the eosinophil crystalloid granule.\textsuperscript{70,71} Immunogold electron microscopy analysis of IL-5 protein storage indicated that IL-5 was localized to the core of the crystalloid granule.\textsuperscript{71} Thus, eosinophils have the capability of releasing IL-5 into surrounding tissues that has been already synthesized and stored within its secretory granules, suggesting that eosinophils may be able to promote the survival and activation of other newly recruited cells in an autocrine manner.

**Interleukin-6**

IL-6 is a pleiotropic proinflammatory cytokine that has a diversity of roles in the immune system, with a predominant function in stimulation of immune responses after trauma or infection, particularly in acute phase reactions. This cytokine is also important in allergic asthma, where it is elevated in serum and bronchoalveolar lavage samples both at baseline and following allergen challenge.\textsuperscript{183–185} Recent studies indicate that IL-6 has a potential role in determining the adaptive immune response in allergy. Specifically, in vitro studies show that IL-6 promoted differentiation of effector CD4\textsuperscript{+} T cells to a Th2 phenotype and suppressed Th1 differentiation.\textsuperscript{186} Together with this, IL-6 is an essential cofactor together with IL-4 in isotype switching of B cells to produce IgE.\textsuperscript{187} IL-6 also has a role in priming of granulocytes, suggesting a direct role in activation of the innate immune system. Eosinophils express IL-6 and this is one of the 10 cytokines that has been identified as being stored as a preformed mediator in eosinophil crystalloid granules\textsuperscript{76} (Fig. 12.2). Stimulation of human peripheral blood eosinophils in vitro by IFN\(\gamma\) and other inflammatory cytokines induces intracellular mobilization of IL-6 prior to its release.\textsuperscript{48,74–76} Whether eosinophil-derived IL-6 is important in allergic inflammation is not known.
Interleukin-9

IL-9 was first characterized as a T cell and mast cell growth factor which is predominantly generated by T cells. The production of IL-9 is associated with the Th2 phenotype in allergy, and IL-9 has a prominent role in the establishment of the allergic phenotype in mouse models. IL-9 is also important in human allergy as segmental allergen challenge of atopic asthmatics led to increased IL-9 expression in lymphocytes present in bronchoalveolar lavage fluid. In the mouse model of eosinophilic tissue inflammation, IL-9 acts by promoting an influx of eosinophils and enhancing their differentiation, maturation, and survival. Eosinophils may also augment established Th2 responses by secreting IL-9 in response to proinflammatory cytokines TNFα and IL-1β. The expression of IL-9 mRNA and protein products was shown in both peripheral blood eosinophils obtained from atopic asthmatics and normal healthy individuals. Tissue eosinophils also demonstrated IL-9 mRNA expression in asthmatic airways. These studies indicate that eosinophils have the capacity to express and secrete IL-9 in allergic inflammation.

The intracellular sites of storage and trafficking of IL-9 has not yet been reported for eosinophils, and this is an area of ongoing investigation.

Interleukin-10

The immunosuppressive cytokine IL-10 plays a major role in immune regulation and the promotion of immune tolerance. Its former name is human cytokine synthesis inhibitory factor, and it is primarily produced by monocytes, Th2 cells, and other innate and adaptive immune cells. The main function of IL-10 is to downregulate the production of Th1 cytokines and to prevent antigen presentation in macrophages. IL-10 is also known for its immunosuppressive role in allergic inflammation by suppressing cytokine secretion. In parallel with this, IL-10 inhibits the effects of bacterial lipopolysaccharide on survival and cytokine production by human peripheral blood eosinophils.

Human peripheral blood eosinophils have been shown to express and release IL-10. Tissue eosinophils from nasal mucosa samples also demonstrated increased IL-10 expression after nasal allergen challenge. These findings indicate that eosinophil-derived IL-10 may enhance allergic inflammation, as IL-10 acts in concert with IL-4 to mediate the growth, differentiation, and isotype switching of activated B cells. However, because IL-10 is known for its immunosuppressive properties in allergic inflammation, this may suggest that eosinophil-derived IL-10 may have a more complex role in the modulating the allergic phenotype than these reports suggest. In helminth infections, eosinophil-derived IL-10 was shown to have a role in
proliferation of myeloid dendritic cells and CD4\(^+\) T cells, leading to protection of intracellular *Trichinella spiralis* larvae.\(^8^0\) This striking observation suggests that eosinophils may serve a protective role for helminth larvae, counterintuitive to the classically held notion that eosinophils kill helminths to protect the host. These findings show that a significant functional diversity exists for eosinophils and their cytokines that was not previously appreciated until the development of eosinophil-deficient mouse strains.

The intracellular sites of synthesis, storage, and trafficking of IL-10 have not yet been elucidated in eosinophils, and this remains to be investigated in future studies.

### Interleukin-11

A multifunctional cytokine derived from the bone marrow stromal cells, IL-11 has an important role in platelet function and bone development. In allergy, little is known regarding IL-11 function in Th2 responses, although its expression is upregulated in bronchial biopsy specimens from patients with asthma,\(^8^1\) suggesting that it may have a role in chronic remodeling in asthmatic airways. In this study, eosinophils were shown to express IL-11 mRNA along with airway epithelial cells. Mouse models of allergic airway inflammation have demonstrated a role for IL-11 in inhibiting asthma-associated inflammation while promoting airway fibrosis.\(^1^9^6\) There have been no other reports of eosinophil-derived IL-11, so the function of eosinophil-derived IL-11 in allergic diseases has not been determined and its intracellular location not yet been investigated.

### Interleukin-12

IL-12 is a Th1 cytokine that is predominantly generated by cells in the innate immune system. Its role is associated with the differentiation of naïve T cells into Th1 cells. In allergy, IL-12 is known to downregulate allergic inflammation following its release, along with IFN\(\gamma\).\(^1^3^5\) Recombinant human IL-12 has been shown to reduce peripheral blood and sputum eosinophils in patients with mild allergic asthma, although no effects on airway hyper-responsiveness or the late asthmatic reaction were observed.\(^1^9^7\) In human peripheral blood eosinophils, IL-12 has been demonstrated to induce apoptosis in an antagonistic manner with IL-5.\(^1^9^8\)

Eosinophils also produce IL-12 in response to proinflammatory cytokine treatment. Stimulation of human peripheral blood eosinophils with IL-4 led to the secretion of IL-12, which in turn induced expression of...
IFN-\(\gamma\) from Th1 cells.\(^{82}\) IL-12 is also one of a number of cytokines secreted by eosinophils stimulated by Th1, Th2, and proinflammatory cytokines.\(^{86}\) In patients with dermatitis, it has been shown that eosinophil-derived IL-12 induced a switch from Th2 to Th1 responses in late phase allergic skin reactions.\(^{82,199}\)

The location of IL-12 protein expression in eosinophils has not yet been determined.

**Interleukin-13**

IL-13 is a prominent Th2 cytokine that is released by many cell types, particularly from Th2 cells. The effects of IL-13 on immune cells are similar to those of IL-4 as these cytokines share a common receptor subunit (the \(\alpha\) subunit), although IL-13 plays a predominant role in allergic inflammation by promoting bronchial hyperreactivity and overproduction of mucus.\(^{200}\) IL-13 also promotes isotype switching of B cells to produce IgE.\(^{201}\) In the mouse model, IL-13 is an important mediator of allergic asthma,\(^ {200}\) and was shown to induce eosinophil recruitment to the airways in an IL-5 and CCL11/eotaxin-dependent manner.\(^ {202}\) More recently, anti-IL-13 has been promoted as a treatment for severe asthma in human patients, although its effects have not resulted in improvements in asthma control and other clinical parameters.\(^ {203}\) Further studies are in process to determine if targeting of more than one cytokine may result in improved asthma symptoms.

The receptor for IL-13 is expressed in an eosinophilic cell line,\(^ {204}\) although there are few reports describing its direct effects on eosinophils. Conversely, IL-13 is synthesized and secreted by eosinophils.\(^ {83,84}\) IL-13 synthesis and expression has been characterized in human peripheral blood eosinophils from patients with bronchial asthma, atopic dermatitis, and hypereosinophilic syndrome.\(^ {84}\) In addition, tissue eosinophils from nasal polyps were shown to express IL-13, and its release could be induced by activation with cytokines or CD28 ligation.\(^ {83,84}\) Eosinophil-derived IL-13 was also shown to be bioactive by inducing CD23 expression on B cells.\(^ {83,84}\)

A recent study has demonstrated that eosinophil-derived IL-13 may be required for allergic airway responses.\(^ {205}\) This was determined using IL-13\(^ {2-/-}\) eosinophils that were adoptively transferred into \(\Delta\text{dblGATA}\) mice. In the computational model for this study, it was found that IL-13 production by eosinophils was integral to the development of allergic asthma.\(^ {205}\)

The intracellular site of storage of IL-13 in eosinophils is associated with the crystalloid granules, suggesting that IL-13 is a preformed mediator that is released upon stimulation of degranulation.\(^ {83}\) This was
determined by subcellular fractionation and immunogold electron microscopy analysis of human peripheral blood eosinophils.

Interleukin-16

IL-16 was formerly known as lymphocyte chemoattractant factor, and serves as a proinflammatory factor that is chemotactic for immune cells, including T cells, monocytes, and eosinophils. It acts through CD4 as its signal-transducing receptor, and is elevated in allergic responses in nasal tissues and bronchoalveolar lavage fluid of histamine-challenged asthma patients. In human eosinophils, IL-16 promotes leukotriene C\(_4\) generation and IL-4 secretion, suggesting that this cytokine activates eosinophils to facilitate an allergic inflammatory response.

Human peripheral blood eosinophils synthesize and secrete IL-16 that was shown to be bioactive for T cells by inducing cell migration. Thus, human eosinophils may have the ability to alter CD4\(^+\) T cell and memory T cell activities. IL-16 was also demonstrated to be stored as a preformed mediator in eosinophils, with intense granular staining. Further studies on the intracellular storage and trafficking of IL-16 would shed more light on how this is secreted from eosinophils.

Interleukin-17

The predominant role of IL-17 in immune responses is mainly proinflammatory, and it is the major cytokine secreted by Th17 cells. In severe asthma, IL-17 is considered to be a key factor in the pathophysiology of airway disease, and it has been shown to enhance Th2-mediated eosinophilic airway inflammation in mouse models of asthma. A direct effect of IL-17 on eosinophils has not yet been reported.

Eosinophils have been shown to express IL-17 in peripheral blood, sputum, and bronchoalveolar lavage fluid. Levels of IL-17 were significantly higher in peripheral blood eosinophils from subjects with asthma than in control subjects. These findings indicate that eosinophil-derived IL-17 may be an important factor in eosinophilic asthma. However, there are no reports regarding the intracellular sites of synthesis or storage of IL-17 in eosinophils.

Interleukin-25

Also known as IL-17E, IL-25 is produced by epithelial cells along with numerous innate immune cells, and plays a prominent role in enhancing Th2 cytokine production. IL-25 may be important in promoting Th2 cytokine-mediated allergic inflammation along with
thymic stromal lymphopoietin (TSLP) by activating the function of adaptive Th2 memory cells through dendritic cells. The expression of IL-25 is elevated in tissue biopsies from patients with chronic asthma and atopic dermatitis.

While they do not express significant levels of IL-25 receptor and do not respond to IL-25 treatment, eosinophils purified from the peripheral blood of atopic and nonatopic subjects generate IL-25 constitutively and upon activation with eosinophil-specific cytokines. It was further demonstrated that eosinophil-derived IL-25 induced IL-5 production from Th2 memory cells, along with modest levels of IL-4 and IL-13. Eosinophil-derived IL-25 production was confirmed in independent studies of Churg–Strauss patients, where eosinophils were found to be the main IL-25-producing cells in blood.

No studies have been reported describing the intracellular sites of storage and trafficking of IL-25 in human eosinophils, and this remains an area of interest for future investigations.

CCL5/RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted)

Chemokine (C-C motif) ligand 5 (CCL5), also known as RANTES, is classified as a chemotactic cytokine, or chemokine. It is highly chemotactic for T cells, eosinophils, and basophils, and acts on the G protein-coupled CCR5 receptor for its effector actions. CCL5/RANTES acts as a major regulator of local immune responses, and targets immune cells to sites of inflammation. In allergic diseases, CCL5/RANTES is elevated along with other cytokines in tissue samples from atopic patients, where it is thought to have a role in recruitment of T cells and eosinophils.

The production of CCL5/RANTES during respiratory virus infections is suggested to exacerbate allergic airway disease. In eosinophils, CCL5/RANTES not only induces chemotaxis but also induces cell activation by elevation of Ca\(^{2+}\), along with upregulation of adhesion molecules, respiratory burst, and degranulation.

Peripheral blood eosinophils from humans express CCL5/RANTES mRNA and protein. The release of CCL5/RANTES from eosinophils may be evoked by cytokines such as IFN\(\gamma\) and opsonized particles. Expression of CCL5/RANTES in tissue eosinophils from human subjects was also confirmed in studies which showed that around 15% of the total CCL5/RANTES\(^+\) population of cells in nasal mucosal biopsies from seasonal rhinitis patients were eosinophils. In late-phase cutaneous reactions following allergen challenge in atopic subjects, eosinophils recruited to the skin also expressed increased CCL5/RANTES mRNA and protein. These findings suggest that both
peripheral and tissue eosinophils express CCL5/RANTES, and that this is released during allergic inflammation.

Eosinophil-derived CCL5/RANTES has been shown to be bioactive, as it has direct chemotactic effects on lymphocytes in culture. In these studies, the bioactivity of eosinophil-derived CCL5/RANTES was inhibited by treatment of eosinophil supernatants with antibody to CCL5/RANTES. Thus, human eosinophils have the capacity to regulate the function of T cells and to elicit the accumulation of eosinophils through an autocrine mechanism.

Eosinophils elaborate CCL5/RANTES from preformed stores within the matrix of their crystalloid granules. The release of CCL5/RANTES was inducible by IFN-γ, which led to intracellular mobilization of this chemokine prior to its release. Specifically, IFN-γ evoked the redistribution of CCL5/RANTES from the crystalloid granules to a rapidly mobilized pool of small secretory vesicles within 10 minutes of stimulation, leaving MBP+ crystalloid granules behind. After 16 h of IFN-γ stimulation, CCL5/RANTES was found replenished in crystalloid granules, suggesting that eosinophils have the ability to undergo sustained rounds of cytokine secretion. These findings indicate the eosinophils have the ability to selectively and differentially release cytokines and granule proteins in response to specific inflammatory stimulation.

The mechanism of selective cytokine release from eosinophils has been associated with a tubulovesicular system which consists of the small secretory granules containing CCL5/RANTES along with IL-4. This membrane transport system is hypothesized to form the mechanism associated with piecemeal degranulation, a type of degranulation that is commonly observed in tissues from allergic subjects. The tubulovesicular system allows the trafficking of granule contents to the cell surface through vesicles and tubules that directly bud from the surface of the crystalloid granule. This specialized mechanism of cytokine transport was first characterized with CCL5/RANTES in eosinophils.

Some of the specific membrane fusion machinery has also been investigated in association with CCL5/RANTES secretion. The small secretory vesicles containing CCL5/RANTES were found to colocalize with the SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor), VAMP-2 (vesicle-associated membrane protein-2), in human peripheral blood eosinophils. SNARE proteins are the hypothetical universal fusion proteins that regulate docking of granules and vesicles to target membranes including the plasma membrane. Fusion of CCL5/RANTES small secretory vesicles is proposed to require binding to cognate target membrane SNAREs, known as SNAP-23 and syntaxin-4.
CCL11/Eotaxin

CCL11/Eotaxin is an important eosinophil-specific chemokine that is associated with the recruitment of eosinophils into sites of inflammation. It is generated in the lungs of asthmatic patients and has a role in targeting eosinophils at inflammatory foci. Mouse models of allergic airway inflammation indicate a central role for CCL11/eotaxin in recruitment of eosinophils to the airways. Gene knockout of CCL11/eotaxin leads to markedly reduced tissue eosinophil numbers, which is associated with reduced allergic inflammation in the gastrointestinal system, subcutaneous regions, and the airways. CCL11/eotaxin acts on cells through its G protein-coupled receptor, CCR3, and eosinophils respond to CCR3 ligand binding by undergoing chemotaxis, Ca\(^{2+}\) mobilization, degranulation, and respiratory burst.

Human peripheral blood eosinophils express CCL11/eotaxin and release this chemokine in response to stimulation by complement factors and immune complexes. In human airway tissues, endobronchial or segmental challenge with allergen evokes elevated numbers of eosinophils positive for CCL11/eotaxin labeling. The intracellular sites of storage of CCL11/eotaxin have not yet been described in detail beyond an apparent association with the crystalloid granules in eosinophils based on conventional immunocytochemical staining. This remains an area of interest for future studies.

CCL17/TARC and CCL22/MDC

The roles of CCL17/TARC (thymus- and activation-regulated chemokine) and CCL22/MDC (monocyte-derived chemokine) in the immune system are associated with induction of chemotaxis in T cells, particularly Th2 cells, by binding to the chemokine receptor CCR4. These two chemokines are found secreted in parallel from dendritic cells and macrophages. In allergic inflammation, CCL17/TARC has been shown to be elicited upon segmental allergen exposure in asthmatics, which correlated with elevated recruitment of Th2 cells that preferentially express CCR4. Interestingly, eosinophils do not respond significantly to CCL17/TARC stimulation in correlation with an absence of CCR4 expression, at least in mouse cells. Eosinophils were also shown to elicit CCL17/TARC and CCL22/MDC in a mouse model of allergic inflammation, leading to effector T cell recruitment and the establishment of airway hyperresponsiveness.

While there is no detectable CCR4 in eosinophils, human eosinophils express and release CCL17/TARC and CCL22/MDC in response to stimulation by TNF and IFN\(\gamma\) or IL-4. These are among the few

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cytokines that are actively synthesized and not stored by eosinophils. These intriguing findings suggest that eosinophils may contribute to allergic inflammation by promoting the recruitment of Th2 cells through the release of Th2-specific chemokines. Moreover, the release of CCL17/TARC and CCL22/MDC may occur through a distinct intracellular trafficking pathway from those associated with preformed, stored cytokines. This remains an area of interest for future studies.

**CXCL8/Interleukin-8**

CXCL8/IL-8 (chemokine C-X-C motif ligand 8, along with its mouse homolog, keratinocyte-derived chemokine, CXCL1/KC) is widely expressed throughout the body, with predominant secretion from macrophages, epithelial cells, airway smooth muscle cells, and endothelial cells. Its role in immunity is to attract neutrophils and other cell types through CXCR1 and CXCR2 to sites of inflammation and infection. In allergic inflammation, CXCL8/IL-8 has been associated with virus-induced exacerbations of asthma and airway hyperresponsiveness. Eosinophils from atopic individuals respond to CXCL8/IL-8 by undergoing chemotaxis in vitro; however, CXCL8/IL-8 challenge of nasal mucosa in allergic patients did not result in significant tissue eosinophil recruitment.

Peripheral blood eosinophils from normal and atopic individuals are very well characterized for their ability to express and release CXCL11/IL-8 in response to stimulation by a variety of factors and cytokines. Further, eosinophils accumulating in the airways following allergen challenge express CXCL8/IL-8. These findings indicate that eosinophils may recruit neutrophils and other cells expressing receptors for CXCL8/IL-8 to sites of allergic inflammation. The role of eosinophil-derived CXCL8/IL-8 in the context of allergic inflammation has not yet been determined, and its intracellular sites of transport to the cell membrane are yet to be investigated.

**TGFα (Transforming Growth Factor-α)**

A cytokine with growth factor functions, TGFα is a mitogenic factor with a role in cell proliferation, differentiation, and maturation. Its receptor is epidermal growth factor receptor (EGFR) and it has important roles in wound healing and tissue remodeling in response to inflammatory stimuli. The role of TGFα in allergic inflammation has not been described in detail, although it is hypothesized to be important in keratinocyte-mediated wound healing in response to allergen challenge. Further, remodeling in asthmatic airways is postulated to

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be associated with TGFα production in response to IL-13 in airway epithelial cells.\textsuperscript{238}

TGFα was the second cytokine to be described for its expression in eosinophils. It was shown to be expressed at mRNA and protein levels in tissue eosinophils infiltrating interstitial regions adjacent to human colonic or oral carcinomas.\textsuperscript{114} This was soon followed by the observation that TGFα\textsuperscript{+} tissue eosinophils could be detected in a rabbit model of healing cutaneous wounds,\textsuperscript{115} and in the nasal mucosa of individuals with allergic rhinitis.\textsuperscript{116} The release of TGFα from human eosinophils may be triggered by cytokine stimulation.\textsuperscript{118}

Detailed immunogold analysis of human peripheral blood eosinophils showed that TGFα is present as a preformed, stored mediator in eosinophil crystalloid granules as well as small secretory vesicles,\textsuperscript{117} suggesting that this cytokine is trafficked through the tubulovesicular system associated with IL-4 and CCL5/RANTES transport. These findings indicate that eosinophils utilize their tubulovesicular system for differential release of several key cytokines in a pathway that may be distinct from classical or compound exocytosis of crystalloid granules.

\textbf{TGFβ (Transforming Growth Factor-β)}

First identified in human platelets, TGFβ is a member of the TGFβ superfamily of cytokines that is secreted by a variety of cell types. TGFβ is produced as three highly homologous isoforms, and is bound as an immobilized, latent precursor form to extracellular matrix proteins in many tissues throughout the body.\textsuperscript{239} On its release into an active form, it controls many types of cellular function including proliferation, differentiation, apoptosis, and angiogenesis, as part of its role in wound healing, and most cells possess receptors for TGFβ.\textsuperscript{239,240} TGFβ is also an important target for drug treatment in numerous clinical trials specifically focused on cancer therapy, but may also be a promising target for allergic disease.\textsuperscript{240} In allergic disease, TGFβ plays a prominent role in the development of asthma, allergic rhinitis, and eczema, along with gastrointestinal complaints.\textsuperscript{241,242} Mutations in the TGFβ receptor (TGFβR) were recently discovered in patients with Loeys–Dietz syndrome, resulting in elevated rates of allergic disease and increased eosinophil numbers.\textsuperscript{241,242} High levels of TGFβ have been found in patients with asthma, suggesting an important role in airway remodeling.\textsuperscript{243} Eosinophils also respond to low concentrations of TGFβ by undergoing chemotaxis,\textsuperscript{244} although TGFβ inhibits IL-5 activation of eosinophils.\textsuperscript{245}

Perhaps unsurprisingly, human eosinophils from both blood and tissue sources also express TGFβ, and there are numerous reports

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demonstrating this. In normal individuals, the airway epithelium appears to be the major site of TGFβ expression, while in asthmatics, many reports have shown that eosinophils recruited to the airways are the main source of TGFβ. Eosinophils from bronchial biopsies of patients with severe asthma were shown to exhibit greater expression of TGFβ than those of normal control subjects, with up to 75% of tissue eosinophils being positive for TGFβ. Moreover, eosinophil-derived TGFβ has bioactive effects in vitro, as TGFβ from eosinophils has been shown to regulate fibroblast proliferation and differentiation, suggesting a role for eosinophils in wound healing. In support of this finding, eosinophils were found to express TGFβ along with IL-13 following intradermal allergen challenge, leading to increased repair and remodeling in human atopic skin.

However, in spite of substantial studies showing that eosinophils are a major source of TGFβ, and another report describing TGFα storage in eosinophil crystalloid granules and secretory vesicles, there are no reports of the intracellular sites of TGFβ synthesis and/or storage in eosinophils.

TNF (Tumor Necrosis Factor-α)

A major proinflammatory cytokine that has pleiotropic roles in local and systemic inflammation, TNF is one of several cytokines that is a target for pharmacological intervention in numerous chronic inflammatory disorders. TNF is also implicated in airway inflammation in asthma, and may be important in refractory asthma. TNF is a highly potent activator of monocytes, T cells, neutrophils, and endothelial cells, and also acts by enhancing eosinophil adhesion and cytotoxicity. TNF contributes to allergic inflammation by promoting antigen-specific IgE production and induction of Th2 cytokines. Eosinophils respond to TNF activation by undergoing respiratory burst, and releasing matrix metalloprotease-9, but do not undergo full degranulation, based on EDN release. However, TNF acts synergistically with IL-5 to induce degranulation. Eosinophils synthesize and secrete TNF, and represent a potential source of this proinflammatory cytokine in allergy and chronic inflammation. Purified peripheral blood eosinophils from atopic individuals spontaneously release TNF in culture, and normal eosinophils stimulated with immobilized immunoglobulins or cytokines express mRNA for this cytokine. TNF is one of the 10 cytokines that has been detected as a preformed, stored mediator in eosinophil granules based on immunogold electron
microscopy\textsuperscript{91} (Fig. 12.2). Ultrastructural immunogold analysis demonstrated that TNF was localized to the matrix compartment of eosinophil crystalloid granules in patients with hypereosinophilic syndrome\textsuperscript{91} and Crohn’s disease.\textsuperscript{257} These findings indicate the eosinophil-derived TNF may orchestrate inflammatory processes in an exacerbating or modulatory manner.

SUMMARY

It is clear from many studies that eosinophils have the capacity to release a large array of cytokines and that many of these have been demonstrated to have immunoregulatory roles in allergic responses in both human and mouse models of allergic inflammation. A large proportion of cytokines are found as preformed mediators stored in eosinophil crystalloid granules or similar secretory organelles, and many of these have been demonstrated to have bioactive roles in immunity. Many other cytokines derived from eosinophils that are not described in detail in this chapter may also contribute to allergic inflammation, and these are listed in Table 12.1. Note that this chapter does not include all of the functions of eosinophil-derived cytokines in homeostasis, fat deposition, antiviral effects, and diseases such as cancer. There have been a series of recent striking developments implicating eosinophils in many other immune-related functions, that demonstrate a pivotal role for eosinophils and their cytokines in plasma cell maintenance in the bone marrow,\textsuperscript{39} of immunosuppressive effects in the lungs\textsuperscript{6} and gastrointestinal tract,\textsuperscript{7} as well as implicating a role for these intriguing cells in the formation of beige fat.\textsuperscript{258,259} What is evident is that eosinophils are only now being recognized as important immune modulatory cells in maintaining the health of the organism and repressing damaging signals from pathogens and their impact on the immune system.

Taken together, these reports indicate that eosinophils serve an important role in immunity, with a major role for their cytokines, chemokines, and growth factors in augmentation of inflammatory responses in allergic diseases. We look forward to future studies in which the specific functions of each of these eosinophil-derived cytokines is determined for its contribution to allergic inflammation, and to investigate the mechanisms of cytokine trafficking and release for the purpose of development of novel therapeutic targets.

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