Mechanisms of Disease

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Eosinophilia

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MARKED accumulation of eosinophils occurs in several important disorders, such as allergic diseases, parasitic infections, and cancer. The level of eosinophils in the body is normally tightly regulated. In normal subjects, eosinophils account for only a small minority of peripheral-blood leukocytes, and their presence in tissues is primarily limited to the gastrointestinal mucosa. In certain disease states, however, eosinophils can selectively accumulate in the peripheral blood or any tissue in the body. Any perturbation that results in eosinophilia, defined here as an abnormal accumulation of eosinophils in blood or tissue, can have profound clinical effects. Eosinophilia may be harmful, because of the proinflammatory effects of eosinophils, or it may be helpful, because of the antiparasitic effects of these cells. This article focuses on recent advances in our understanding of the accumulation of eosinophils, as well as treatment approaches and the development of new therapeutic agents.

CLINICAL ASPECTS OF EOSINOPHILIA

Eosinophils normally account for only 1 to 3 percent of peripheral-blood leukocytes, and the upper limit of the normal range is 350 cells per cubic millimeter of blood. Eosinophilia occurs in a variety of disorders (Table 1) and is arbitrarily classified as mild (351 to 1500 cells per cubic millimeter), moderate (>1500 to 5000 cells per cubic millimeter), or severe (>5000 cells per cubic millimeter). The most common cause of eosinophilia worldwide is helminthic infections, and the most common cause in industrialized nations is atopic disease.

The differential diagnosis of eosinophilia requires a review of the patient’s history, which may reveal wheezing, rhinitis, or eczema (indicating atopic causes); travel to areas where helminthic infections (e.g., schistosomiasis) are endemic; the presence of a pet dog (indicating possible infection with Toxocara canis); symptoms of cancer; or drug ingestion (indicating a possible hypersensitivity reaction). Eosinophilia caused by drugs is usually benign but can sometimes be accompanied by tissue damage, as in hypersensitivity pneumonitis. In most cases, the eosinophilia resolves once the drug is withdrawn, but in some cases, such as the eosinophilia–myalgia syndrome due to the ingestion of contaminated tryptophan, the disease can persist despite withdrawal of the drug. Abnormal morphologic features of eosinophils, an increase in immature cells in the bone marrow or blood, or a karyotypic abnormality indicates the presence of eosinophilic leukemia. An accumulation of eosinophils that is limited to specific organs is characteristic of particular diseases, such as eosinophilic cellulitis (Well’s syndrome), eosinophilic pneumonias (e.g., Löffler’s syndrome), and eosinophilic fasciitis (Shulman’s syndrome). The association of eosinophilia with vasculitis, neuropathy, and a history of asthma indicates the presence of the Churg–Strauss syndrome. In the absence of an identifiable cause of moderate-to-severe eosinophilia and in the presence of end-organ involvement, the diagnosis of the idiopathic hypereosinophilic syndrome should be considered. This disorder occurs predominantly in men and is usually a progressive, fatal disease in the absence of effective medical management.

Diagnostic studies that should be performed in patients with moderate-to-severe eosinophilia and should be considered in patients with persistent mild eosinophilia include morphologic examination of a blood smear, urinalysis, and serial stool examinations for ova and parasites. Parasitic infections that cause eosinophilia are usually limited to helminthic parasites, with the exception of two enteric protozoans, Isospora belli and Dientamoeba fragilis. Strongyloides stercoralis infection is important to diagnose, because it can cause disseminated fatal disease in immunosuppressed patients; its detection often requires serologic testing. Other infections to rule out are those with filarial parasites, trichinosis, and T. canis infection. Bone marrow and chromosomal analysis (to detect hematologic cancer) and a tissue biopsy may be indicated.

Moderate-to-severe eosinophilia may persist in the absence of an identifiable cause or end-organ involvement. Patients with persistent, apparently be-
nign eosinophilia usually do not need therapy, and spontaneous resolution generally occurs within several years. However, such patients should have periodic clinical and echocardiographic examinations to detect eosinophil-mediated cardiac damage, which can occur insidiously at any time and which may not be correlated with the severity of eosinophilia. Although no therapy is indicated, it is often helpful to determine whether eosinophilia will resolve with a short trial of prednisone (1 mg per kilogram of body weight per day for three to five days). If so, this may be a suitable option in the event of future deterioration. Glucocorticoid-resistant cases may respond to drugs that are reserved for therapy rather than trials. The therapeutic approach to patients who have eosinophilia with an identifiable cause is discussed below.

**PHYSIOLOGIC FEATURES OF EOSINOPHILS**

Eosinophilia occurs as a result of four processes (Fig. 1): differentiation of progenitor cells and proliferation of eosinophils in bone marrow; interactions between eosinophils and endothelial cells that involve rolling, adhesion, and migration of eosinophils; chemotraction directing eosinophils to a specific location; and activation and destruction of eosinophils.

**Proliferation**

Eosinophils are produced in bone marrow from pluripotent stem cells. The latter cells differentiate first into hybrid precursors with properties of basophils and eosinophils and then into a separate eosinophil lineage. Three cytokines — interleukin-3, interleukin-5, and granulocyte–macrophage colony-stimulating factor (GM-CSF) — are particularly important in regulating the development of eosinophils. These cytokines are encoded by closely linked genes on chromosome 5q31 and bind to receptors that have a common beta chain and different alpha chains.

Of the three cytokines, interleukin-5 (also known as eosinophil-differentiation factor) is the most specific for the eosinophil lineage and is responsible for selective differentiation of eosinophils. Interleukin-5 also stimulates the release of eosinophils from bone marrow into the peripheral circulation. The critical role of interleukin-5 in the production of eosinophils is best demonstrated by genetic manipulation in mice. Overproduction of interleukin-5 in transgenic mice results in profound eosinophilia, and deletion of the interleukin-5 gene causes a marked reduction of eosinophils in the blood and lungs after an allergen challenge. The overproduction of one or more of the three cytokines occurs in humans with eosinophilia. Diseases involving eosinophilia without increases in other blood-cell lineages are usually accompanied by an overproduction of interleukin-5. The mechanisms of cytokine overproduction may involve a response of T-helper lymphocytes of the Th2 type in patients with allergic conditions (see below) or parasitic diseases, the malignant expansion of T-cell clones that produce interleukin-5 in some patients with lymphoma, or the activation of gene transcription due to a chromosomal translocation in some patients with leukemia.

**Adhesion and Migration**

The migration of eosinophils from the circulation into tissues involves a stepwise interaction between eosinophils and endothelial cells. The steps are mediated by adhesion molecules on endothelial cells and counter-ligands on eosinophils and are followed by the passage of eosinophils between endothelial cells (Fig. 1). Although the different types of leukocytes migrate into tissues in similar ways, their migration is mediated by different molecules. Eosinophils initially adhere to the endothelium by means of three selectins (adhesion molecules on endothelial cells) and their corresponding ligands. The rolling of circulating eosinophils on the endothelium is mediated primarily by P-selectin, whereas neutrophil rolling is mediated primarily by E-selectin. After cellular activation (e.g., by exposure to chemottractants such as platelet-activating factor or cotaxin), eosinophils adhere firmly to the endothelium through adhesion molecules of the integrin family. These include the CD18 family (β2 integrins) and very-late-antigen–4 (VLA-4) molecules (β1 integrins). The β2 integrins interact with intercellular adhesion molecule 1 (ICAM-1) on endothelial cells, whereas the β1 integrins interact with vascular-cell ad-
Adhesion molecule 1 (VCAM-1). Although the CD18–ICAM-1 pathway is used by all leukocytes, the VLA-4–VCAM-1 pathway is used by eosinophils and mononuclear cells but not by neutrophils. ICAM-1 is induced by a variety of proinflammatory mediators such as interleukin-1 and tumor necrosis factor α, whereas VCAM-1 is induced primarily by interleukin-4. Resting eosinophils normally express β1 and β2 integrins, but the level of expression of these adhesion molecules and their affinity for their appropriate endothelial receptors is increased by chemoattractants.22

Chemoatraction
The migration of eosinophils into tissues is initiated by local chemoattractant molecules. These molecules are likely to be responsible for both physiologic homing, in which eosinophils are directed into the lamina propria of the gastrointestinal tract, and the recruitment of eosinophils into inflamed tissues.
Numerous chemotactic substances act on eosinophils, including derivatives of arachidonic acid such as leukotriene B4, other lipid mediators such as platelet-activating factor, bacterial products, interleukins (e.g., interleukin-16), and various chemokines. Although all these substances mediate the recruitment of eosinophils, most are not selective for eosinophils. However, two newly described chemokines, eotaxin-1 and eotaxin-2, are relatively specific for eosinophils.25,26

Chemokines are a family of low-molecular-weight (8 kD to 10 kD) chemotactic cytokines that regulate leukocyte movement. Most chemokines interact with eosinophils by binding to a chemokine receptor (CCR-3) that is relatively restricted to eosinophils. In mice with no gene for eotaxin-1, eosinophils do not migrate to the intestinal tract; in addition, after allergen challenge, they are reduced in the lungs.27 The chemoattractive effect of eotaxin is augmented by interleukin-5,12,28 It is remarkable that two distinct steps in the accumulation of tissue eosinophils (proliferation and chemotraction) are regulated by molecules that are relatively specific for eosinophils and that act synergistically in promoting their accumulation.

Survival and Destruction in Tissue

Eosinophils, unlike neutrophils, can survive in tissues for extended periods (perhaps weeks), depending on the cytokines in the microenvironment.29 Only eosinophils and basophils have receptors for interleukin-3, interleukin-5, and GM-CSF present on both the precursor cells in bone marrow and the circulating cells. The lifespan of tissue eosinophils is not known, but interleukin-3, interleukin-5, and GM-CSF inhibit eosinophil apoptosis for at least 12 to 14 days in vitro and in explants of allergic sinus tissue.30 In contrast, eosinophils survive for less than 48 hours in the absence of these cytokines.31 Tissue eosinophils can also regulate their own survival through an autocrine pathway (Fig. 1).31,32

**PATHOGENESIS OF EOSINOPHILIA IN CLINICAL DISORDERS**

Moderate-to-severe eosinophilia occurs as a pathophysiologic response to infection with helminthic parasites. Eosinophilia induced by parasitic infection is dependent on interleukin-5 produced by Th2 lymphocytes (discussed below). Eosinophils participate in the immune response against helminthic parasites by discharging their cytotoxic granular contents onto the parasites, which kills them.33 However, depletion of eosinophils in mice with antibodies to interleukin-5 does not always increase their susceptibility to helminthic infections.34 Since tissue eosinophilia is a hallmark of atopic diseases and eosinophils are a major effector cell in these disorders, allergic diseases serve as a prototype for understanding the pathogenesis and consequences of eosinophilia.

**Genetic Aspects of Atopy**

Twenty to 30 percent of people inherit a predisposition to atopy and the associated production of IgE antibodies against common environmental antigens. Several genes are likely to be responsible, and mapping studies have identified candidate genes that include the gene for the high-affinity IgE receptor and a locus near the genes for interleukin-4 and interleukin-5 on chromosome 5q31,35,36 The occurrence of eosinophilia has recently been genetically mapped to a locus near the class I genes of the major histocompatibility complex on chromosome 6.39

**Late-Phase Response**

After exposure to allergen, many patients with allergies have a progressive clinical response that begins in three to four hours, reaches a peak at about eight hours, and subsides in several days. This process, known as the late-phase response, is accompanied by an influx of inflammatory cells containing many eosinophils (Fig. 2). The inflammatory component of the response is believed to be primarily responsible for the chronic inflammation in patients with repeated exposure to an allergen (e.g., house-dust mites). Eosinophils under the control of T cells are the essential effector cells in the late-phase response.

**Regulation of Eosinophils by Th2 Lymphocytes and Mast Cells**

Mast cells participate in the initial events after exposure to allergen, but their importance in orchestrating eosinophilia is uncertain (Fig. 2).40 After IgE-triggered activation, mast cells may promote inflammation of the airways with eosinophils by producing proinflammatory mediators (e.g., interleukin-1 and tumor necrosis factor α) and eosinophil-directed cytokines (e.g., interleukin-4 and interleukin-5). These substances, in turn, induce chemokines that attract eosinophils. However, mast cells do not appear to be required in some animal models of allergic disease. In allergen-sensitized mice with a deficiency of mast cells and allergen-sensitized mice with a targeted deletion of the gene for IgE, recruitment of eosinophils into the lungs is not impaired after allergen challenge.41,42 In contrast, helper T lymphocytes are essential for the late-phase response, because they produce three cytokines that promote allergic responses: interleukin-4 and interleukin-13, both of which regulate IgE and VCAM-1 production, and interleukin-5. The helper cells that orchestrate this type of response are Th2 cells (Fig. 2). In contrast, Th1 cells produce interferon-γ and tumor necrosis factor β.14,15 Genetic factors and the conditions of antigen exposure determine the relative contribu-
tions of mast cells and T cells in the regulation of eosinophils. Antigen-presenting cells not only activate Th2 cells but also secrete proinflammatory mediators that induce resident cells (e.g., epithelial cells) to produce the chemokines that attract eosinophils.

Proinflammatory and Cytotoxic Effects

Once eosinophils arrive at an inflammatory focus, they may undergo apoptosis with rapid clearance by macrophages, but if they are stimulated by interleukin-3, interleukin-5, or GM-CSF, they survive for prolonged periods and have increased responsiveness to other activating agents. Eosinophils activated in this way express numerous receptors for cytokines, immunoglobulins, and complement.

Eosinophils produce unique toxic inflammatory mediators, which are stored in granules and synthesized after cellular activation. The granules contain a crystalloid core composed of major basic protein and a matrix composed of eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase (Fig. 3). These cationic proteins share certain proinflammatory properties but differ in other ways. For example, at concentrations similar to those in fluids from patients with eosinophilia, major basic protein, eosinophil peroxidase, and eosinophil cationic protein have cytotoxic effects on respiratory epithelium. In addition, eosinophil cationic protein and eosinophil-derived neurotoxin are ribonucleases.

Eosinophil cationic protein can cause voltage-insensitive, ion-nonselective toxic pores in the membranes of target cells, and these pores may facilitate the entry of other toxic molecules. Major basic protein directly increases smooth-muscle reactivity by causing the dysfunction of vagal muscarinic M2 receptors. It also triggers the degranulation of mast cells and basophils. In addition, eosinophils amplify the inflammatory cascade by producing their own chemoattractants (e.g., RANTES [regulated upon activation normal T-cell expressed and secreted], eotaxin, and platelet-activating factor), which accelerate the recruitment of eosinophils into the inflammatory focus.

Further damage is caused by hydrogen peroxide and halide acids, which are generated by eosinophil peroxidase, and by superoxide, which is generated by the respiratory-burst–oxidase pathway in eosinophils. Eosinophils also generate large amounts of the cysteiny leukotriene, leukotriene C₄, which is me-
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Figure 3. An Eosinophil and Its Contents.

The granules of eosinophils contain a crystalloid core composed of major basic protein and a matrix composed of eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin. Eosinophils also produce a variety of cytokines, some of which are stored in the granules, and lipid mediators that are generated after cellular activation. Eosinophils express one predominant chemokine receptor that interacts with multiple chemokines.

DRUGS THAT INTERFERE WITH EOSINOPHILIA OR EOSINOPHIL PRODUCTS

Patients with eosinophilia of any magnitude who have end-organ involvement should be treated with the goal of reducing eosinophil counts or blocking the effect of eosinophil products. Numerous drugs inhibit the production of eosinophils or the production or action of their products (Fig. 4). These agents include glucocorticoids, myelosuppressive drugs, and interferon alfa (Table 2). Glucocorticoids, the most effective agents for reducing eosinophilia, suppress the transcription of a number of genes for inflammatory mediators, including the genes for interleukin-3, interleukin-4, interleukin-5, GM-CSF, and various chemokines. In addition, glucocorticoids inhibit the cytokine-dependent survival of eosinophils. In most patients, treatment with systemic or topical (inhaled or intranasal) glucocorticoids causes a rapid reduction in eosinophils, but a few patients have a resistance to glucocorticoids, with persistent eosinophilia despite high doses. The mechanism of resistance to glucocorticoids is unclear, but a reduced level of glucocorticoid receptors and alterations in activator protein 1, a transcription factor, are at least partly responsible in some patients. Patients with glucocorticoid resistance sometimes require other therapy, such as myelosuppressive drugs (hydroxyurea or vincristine) or interferon alfa. Interferon alfa appears to be especially promising, because it inhibits the degranulation and effector function of eosinophils. Cyclophils (e.g., cyclosporine) have also been used, because they block the transcription of numerous eosinophil-active cytokines (e.g., interleukin-5 and GM-CSF) (Fig. 4).

Drugs that interfere with eosinophil chemotactic signals include recently approved leukotriene antagonists and inhibitors. The 5-lipoxygenase inhibitors (e.g., zileuton) block the rate-limiting step in leukotriene synthesis and inhibit the generation of the eosinophil chemoattractant leukotriene B₄ and the sulfidopeptide leukotrienes, leukotrienes C₄, D₄, and E₄ (Fig. 4). These drugs therefore decrease airway infiltration by eosinophils during the late-phase response. Drugs (e.g., zafirlukast) that block the receptor for leukotriene D₄, which is also a receptor for leukotriene C₄ and leukotriene E₄, prevent the muscle contraction and increased vascular permeability mediated by eosinophil-derived leukotrienes. These drugs have been found to decrease exercise-induced bronchoconstriction and improve baseline lung obstruction in patients with asthma. Some of the third-generation antihistamines (e.g., cetirizine) inhibit the vacuolization and accumulation of eosinophils after an allergen challenge and directly inhibit eosinophils in vitro. Cromolyn and nedocromil inhibit the effector function of eosinophils, such as antibody-dependent cellular cytotoxicity. Phosphodiesterase inhibitors raise intracellular cyclic AMP concentrations in eosinophils, and this in turn inhibits intracellular signaling, leading to decreased activation of eosinophils.

The identification of molecules that specifically regulate the function or production of eosinophils offers new therapeutic strategies. Antibodies against interleukin-5 are especially promising, because they have been effective in animals with allergic airway disease. A humanized form of an antihuman interleukin-5 monoclonal antibody has been developed. Another approach involves blocking the interaction of interleukin-5 or eotaxin with its receptor. In preliminary in vitro experiments, a monoclonal antibody directed against human eotaxin blocked the adhesion of eosinophils to cultured endothelium, and this approach appears promising as a new therapeutic strategy.

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antibody against eosinophil chemokine receptor 3 (CCR-3) inhibited all ligands for this receptor (Fig. 4). The production of interleukin-5 may also be inhibited by modulating the immune response to allergens so that a Th2-lymphocyte response does not predominate. This has been accomplished in animals by administering interleukin-12 during allergic sensitization. Interleukin-12 inhibited the production of interleukin-4 and interleukin-5 and reduced pulmonary eosinophilia after exposure to allergen. Another molecular target interrupts the adhesion of eosinophils to the endothelium through the interaction of CD18 with ICAM-1 or VLA-4 with VCAM-1 (Fig. 4). In addition, phosphodiesterase inhibitors that are specific for leukocyte isoenzyme type IV are being developed. Lastly, lidocaine and sulfonylurca-receptor blockers have been shown to inhibit interleukin-5 activity on eosinophils, and lidocaine appeared to be promising in a preliminary clinical trial in patients with asthma.

**SUMMARY**

Eosinophilia occurs in a large number of diseases, and in some of them, eosinophils are the principal effector cells. The production of eosinophils involves the proliferation and differentiation of hematopoietic progenitor cells, and the accumulation of eosinophils involves interactions between eosinophils and endothelial cells, chemotaxis and cellular activation, and a balance between the survival and apoptosis of eosinophils.
cosinophils. An understanding of these processes gives the clinician an insight into the pathogenesis of disorders involving eosinophils and an appreciation of the increasing number of drugs available to treat these disorders. The identification of molecules specifically involved in eosinophilia (e.g., interleukin-5 and eotaxin) offers hope for the development of new drugs that specifically target eosinophil pathways.

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**TABLE 2. PHARMACOLOGIC APPROACHES TO BLOCKING EOSINOPHILIA OR THE ACTION OF EOSINOPHILS.**

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<td>Glucocorticoids</td>
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<td>Myelosuppressive drugs</td>
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<td>Leukotriene inhibitors and antagonists</td>
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<td>Agents that block the VLA-4–VCAM-1 pathway</td>
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*ICAM-1 denotes intercellular adhesion molecule 1, VLA-4 very late antigen 4, and VCAM-1 vascular-cell adhesion molecule 1.*