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Updates on cells and cytokines

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Human eosinophils

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Eosinophils are bone marrow-derived granulocytes that are normally not numerically prominent in either the peripheral blood or the tissues. In conjunction with specific conditions, including allergic diseases, helminthic infections, and some other often idiopathic diseases, eosinophilia develops. Because eosinophils are not capable of further cell division after exiting the marrow, they are terminally differentiated leukocytes; and much of the focus on understanding their functions has addressed the roles these distinct leukocytes may play as end-stage effector cells in those allergic, parasitic, and other conditions marked by augmented eosinophilia. Considerable progress has been made in understanding how eosinophil production and tissue distribution are regulated and how eosinophils may contribute to the immunopathogenesis of allergic diseases. Investigations in recent years have also identified new aspects of eosinophil function including their capacity to release a range of largely performed cytokines. Recent investigations also indicate that eosinophils have roles in tissue responses not necessarily marked by exuberant eosinophil tissue infiltration. Future studies will likely provide new understanding of the cellular function of eosino-

Abbreviations used

GM-CSF: Granulocyte-macrophage colony-stimulating factor
TGF: Transforming growth factor

phils and the diverse roles eosinophils play within the tissues in conjunction with fundamental mechanisms of immune responses to external antigens, organ transplantation, and neoplasia and mechanisms of tissue injury and repair.

EOSINOPHIL PRODUCTION AND DISTRIBUTION

The development and differentiation of eosinophils within the bone marrow are promoted by three cytokines. Granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, and IL-5 each promote eosinophilopoiesis, with IL-5 having the most cell-specific effects on the differentiation and production of eosinophils. IL-5 also acts acutely to release a pool of already developed eosinophils from the marrow into the circulation.

The steps that lead to eosinophil recruitment into certain sites of inflammation are multiple and require a combinatorial interplay of enhanced expression and/or function of integrins on intravascular eosinophils and their counterligands on the vascular endothelium, as well as the actions of eosinophil chemoattractant factors. Recent advances have helped delineate the adhesive pathways used by eosinophils in their interactions with the vascular endothelium. Several overlapping pathways are involved in the initial adhesion and subsequent transendothelial migration of eosinophils (as likewise

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TABLE I. Human eosinophil receptors for selected immunologic ligands

Receptors	Ligand	Cytokine receptors	Ligand
Complement			
C3a receptor	C3a	IL-1 receptor (CD121a)	IL-1
C5a receptor (CD88)	C5a	IL-2 receptor α chain (CD25)	IL-2
CR1 (CD35)	C3b	IL-3 receptor α chain (CD123)	IL-3
CR3 (Mac-1, CD11b/CD18)	C3bi, ICAM-1	IL-4 receptor (CD124)	IL-4
p150, 95 (CD11c/CD18)	C3bi	IL-5 receptor α chain	IL-5
Immunoglobulin			
Fc α R (CD89)	IgA	GM-CSF receptor α chain (CD116)	GM-CSF
Fc γ RI (CD64)*	IgG	Common β chain	IL-3/IL-5/GM-CSF
Fc γ RII (CD32)	IgG	CD4	IL-16
Fc γ RIII (CD16)*	IgG	Interferon- α receptor	Interferon- α
Fc ϵ RI (high-affinity R)	IgE	Interferon- γ receptor	Interferon- γ
Fc ϵ (CD23 variant)	IgE	TNF receptor	TNF- α
ϵ BP (Mac-2)	IgE	<u>Selected other</u>	
Chemokine			
IL-8 receptor (CDw 128)	IL-8	CD9	
CCR-1	MIP-1 α , RANTES, MCP-2	CD40*	
CCR-3	Eotaxin, RANTES, MCP-3, 4, 5	CD69*	
		MHC Class I	
		MHC Class II (HLA-DR, DQ)*	
		FAS (CD95)	
		CAMPATH-1 (CD52)	

ICAM, Intracellular adhesion molecule-1; TNF, tumor necrosis factor; MIP, macrophage inflammatory protein; MCP, monocyte chemotactic protein; MHC, major histocompatibility complex; FAS, fatty acid synthetase.

true for other leukocyte types); but for eosinophils, it is notable that they express two $\alpha 4$ integrins, not shared by neutrophils, yet found on basophils and some lymphocytes. The $\alpha 4\beta 1$ integrin (very late antigen-4) binds to vascular cell adhesion molecule-1 and to domains within tissue fibronectin. The expression of $\alpha 4\beta 7$ on eosinophils is intriguing, given the role that this integrin plays in binding to the mucosal vascular addressin MAdCAM-1 (as well as vascular cell adhesion molecule and fibronectin) and the expression of $\alpha 4\beta 7$ on a subset of mucosal trophic CD4⁺ memory T lymphocytes. MAdCAM-1 is known to be expressed on high endothelial venules of Peyer's patches and mesenteric lymph nodes and sinus-lining cells in the spleen. The common expression by eosinophils and some lymphocytes of $\alpha 4\beta 7$ may contribute to their colocalization within lymphoid tissues. Moreover, the $\alpha 4$ integrins have been shown to be involved in cognate cell-cell interactions, including those specifically between human lymphocytes and eosinophils. Studies with blocking monoclonal antibodies to the $\alpha 4$ component of $\alpha 4\beta 1$ and $\alpha 4\beta 7$ have demonstrated that such blockade can prevent eosinophil influx into cutaneous or pulmonary sites of elicited allergic reactions. Moreover, blockade of $\alpha 4$ integrins can have beneficial effects on allergic reactions even without inhibiting eosinophil influx, perhaps by blocking activation responses of $\alpha 4$ -bearing eosinophils and/or lymphocytes within the airways. Known eosinophil chemoattractants are multiple and include lipids, complement components, cytokines, and chemokines. An example of a recently recognized eosinophil chemoattractant chemokine is eotaxin. Integrin-mediated adhesion and signaling through extracellular matrix components likely

contribute further to enhanced functional responses of eosinophils.

CELL SURFACE RECEPTORS AND PROTEINS

Eosinophils either constitutively or after induction express on their external plasma membranes proteins that function as receptors for numerous immunologic ligands (Table I). These ligands include specific complement components, immunoglobulins, chemokines, and cytokines. For some of these ligands, such as C5a, the chemokines, and certain cytokines, directed migration of eosinophils is elicited. Some of these ligands directly promote effector responses, including enhanced respiratory burst activity and degranulation. Other ligands, including the eosinophil growth factor cytokines (GM-CSF, IL-3, and IL-5), augment eosinophil functional responses elicited by other stimuli and prolong the survival (by antagonizing apoptosis) of mature eosinophils. For several cytokines active on eosinophils, including IL-2, IL-16, and IL-4, traditional eosinophil effector responses are not stimulated, and the complete actions of these cytokines on eosinophils remain to be determined. IL-4 can differentially regulate expression of eosinophil transforming growth factor (TGF)- α and TGF- $\beta 1$. Other proteins appear to be expressed only on eosinophils after varied, as yet poorly understood, forms of activation in vivo; and these proteins include CD69, Class II major histocompatibility complex proteins and CD40. With the eosinophils' preferential localization to mucosal surfaces of the respiratory, gastrointestinal, and genitourinary tracts, IgA receptor expression on eosinophils may engage secretory IgA present in these

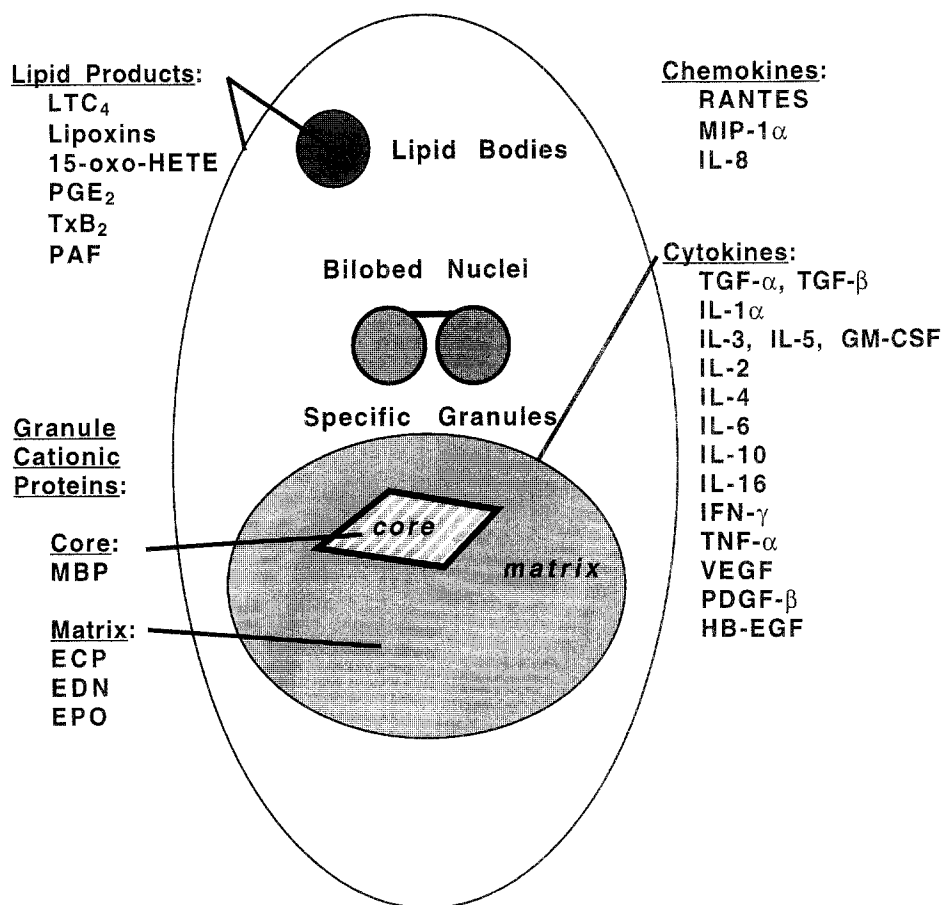


FIG. 1. Human eosinophil lipid and protein products.

mucosal sites. Analogously, the presence of IgE receptors on eosinophils is notable because concomitant increases in both IgE levels and eosinophil numbers are frequently found in helminthic and allergic diseases. Although immunoglobulin binding to IgA, IgE, and IgG receptors can induce eosinophil degranulation, the full range of activities of these receptors, perhaps also participating in antibody-mediated uptake of antigens for antigen presentation, remains to be elucidated.

EOSINOPHIL PRODUCTS

Lipids

On stimulation, eosinophils elaborate several bioactive lipids (Fig. 1), including platelet activating factor and products of the 5- and 15-lipoxygenase pathways and the cyclooxygenase pathway. Lipid bodies, intracellular lipid-rich domains, are induced to develop in many activated eosinophils in vivo and are sites for enhanced synthesis of both lipoxygenase- and cyclooxygenase-derived eicosanoids. The activities of eosinophil-derived lipids, which are generally proinflammatory, are multiple and include the potent smooth muscle contractile, vasoactive, and mucous

secretory activities of leukotriene C₄ and its peptidolytic derivatives, leukotrienes D₄ and E₄.

Granule proteins

Specific granules contain lysosomal hydrolases, as well as most proteins preferentially distinct for eosinophils (Fig. 1). The granule crystalloid core is composed of major basic protein; and the non-core granule matrix contains eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase. The biochemical and functional properties of each of these cationic proteins have been well studied by Gleich et al. and others.

Cytokines

Only in recent years has it been recognized that eosinophils are sources of cytokines and appear to store many, if not all, of these cytokines in cytoplasmic specific granules. The cytokines made by eosinophils include the myeloid-active and eosinophil-active growth factors (IL-3, IL-5, and GM-CSF), other growth factors (TGF-α, TGF-β1, vascular endothelial growth factor, platelet-derived growth factor-β, and heparin-binding epidermal growth factor), immunoregulatory cytokines

(IL-2, IL-4, IL-10, and interferon- γ), and proinflammatory cytokines (IL-1, IL-6, IL-8, and tumor necrosis factor- α). Moreover, the storage of these within specific granules provides a means for eosinophils, unlike lymphocytes that must synthesize cytokines *de novo*, to release a preformed pool of cytokines. Because these cytokines are stored with cationic proteins and moreover may be differentially packaged within the granule core or matrix, it is likely that mechanisms to selectively withdraw granule contents for ultimate secretion exist and that not all granule contents are simultaneously mobilized by conventional exocytosis. Degranulation pathways based on mobilization of selected granule contents within transport vesicles are suggested by experimental observations but still require full investigation to identify what is likely to be the physiologic means by which eosinophil granule cationic, cytokine, and other proteins are released extracellularly.

EOSINOPHIL "ACTIVATION"

It has long been recognized that eosinophils from eosinophilic donors exhibit metabolic, morphologic, and functional changes indicating that they have been "activated" *in vivo*. It has been tempting to consider the functional state of eosinophils as a binary alternative, either normal or "activated." As studies of the varied responses of eosinophils *in vivo* and *in vitro* have accumulated, it is now clear that there is not a singular activation state of eosinophils. One often-used measure of eosinophil activation in the tissues was based on staining with a specific monoclonal antibody to a form of eosinophil cationic protein. More recent studies, however, provided evidence that staining with this reagent is tissue fixation- and processing-dependent and not simply due to differences in the "activation" of variably glycosylated eosinophil cationic protein. Moreover, studies of eosinophils in the blood and airways after experimental antigen challenge indicate that eosinophils activated by one measure, "hypodensity," do not exhibit another measure of eosinophil activation, enhanced HLA-DR expression as found on airway eosinophils. Although the eosinophil-active growth factor cytokines contribute to some enhanced functional responses associated with eosinophil "activation," these cytokines alone do not elicit all measures of eosinophil activation, such as enhanced expression of Fc ϵ RI or CD40 found on eosinophils obtained from subjects with allergy. Other cytokines or tissue- or extracellular matrix-derived activating stimuli are likely to be involved as well in augmenting specific functional capabilities of eosinophils.

EOSINOPHIL FUNCTIONS

Allergic inflammation

It is abundantly clear that eosinophils are major participants in the immunopathogenesis of allergic inflammation. They are characteristically recruited to such sites, and their cationic proteins are released, as are their lipid mediators. Studies of the activities of the cationic proteins provide a wealth of evidence that they contrib-

ute to damage and dysfunction of other cell types in sites of allergic inflammation. Further, experimental studies in animals that block eosinophil recruitment or activation ameliorate the severity of allergic inflammation and its physiologic sequelae. The additional roles that eosinophils might play on the basis of their elaboration of cytokines or their roles as antigen-presenting cells in promoting allergic inflammation remain to be defined.

Parasite host defense

Infections with helminthic parasites elicit eosinophilia mediated by IL-5. On the basis of *in vitro* studies demonstrating that eosinophils function as helminthotoxic effector cells, the major beneficial function of eosinophils was hypothesized to be action in host defense against helminthic parasites. This putatively beneficial role is contrasted with the deleterious effects of eosinophils identified for allergic diseases. Recent studies with anti-IL-5 antibody-treated, helminth-infected mice, however, have questioned this role. In mice infected with helminths, neutralizing anti-IL-5 antibody has abrogated infection-induced blood, marrow, and tissue eosinophilia. In these anti-IL-5-treated (or anti-IL-4-treated) eosinophil-deficient mice, intensities of infections (both primary and secondary) have not been greater than in eosinophilic mice. These findings argue against a major role for eosinophils as helminthotoxic effector cells. Although it is possible that other host defense mechanisms against helminths are sufficiently redundant that eosinophil ablation is not deleterious, at least in mice these findings with depletion of eosinophils raise the possibility that eosinophils do not have a predominant helminthotoxic effector function in host defense. Thus the nominally beneficial function of eosinophils in parasite host defense remains to be validated.

Other functions

Although the focus of interest on eosinophil function has been on those allergic, parasitic, and other diseases marked by prominent tissue eosinophilia, it is likely that eosinophil infiltration in tissues in other responses is both underappreciated by conventional histologic stains and likely to have central roles. Such processes include wound healing, the development of fibrosis, organ transplant rejection, and possibly the evolution of neoplasia. Moreover, because eosinophils in health localize in mucosal epithelial tissues of the respiratory, gastrointestinal, and lower genitourinary tracts, the beneficial roles of eosinophils in the ongoing immune response of mucosal sites in the absence of disease remain to be elucidated. An area that requires investigation involves the largely unknown mechanisms for eosinophil recruitment and localization in normal mucosal tissue sites and the roles for these eosinophils that likely reside in tissues for weeks in duration. Whether eosinophils traffic bidirectionally to and from the lumen; whether eosinophils process particulate antigens, including inhaled aeroallergens, within lumenal sites; and whether eosinophils, by means of Fc-receptor-mediated mechanisms and IgE, IgA, and/or

IgG anti-allergen antibodies, can function to present antigens to lymphocytes require further study. These studies would elucidate whether eosinophils have heretofore unknown roles in the effector limb of allergic responses.

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