

New millennium: The conquest of allergy

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Mechanisms of eosinophil-associated inflammation

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Increasing evidence supports a critical role for the eosinophil in disease. Here, the mechanisms underlying eosinophil-associated inflammation are reviewed including eosinophil constituents, eosinophil maturation and release from the bone marrow, and eosinophil tissue recruitment and activation. Eosinophil effector functions in bronchial asthma are summarized with particular attention to pulmonary M2 muscarinic receptors and bronchial hyperreactivity. Recent findings supporting roles for IL-5, the eosinophil, and its ribonucleases in viral immunity are presented. Overall, this information supports an expanded view of eosinophil participation in health and disease. (*J Allergy Clin Immunol* 2000;105:651-63.)

Key words: Eosinophil, bronchial asthma, IL-5, virus, immunity, localization, ribonuclease

About 120 years ago, Paul Ehrlich discovered the striking affinity of a coarsely granular leukocyte for the acidic dye eosin and coined the term "eosinophil" to describe this cell. During the next 40 years, much was learned about the eosinophil: elevated numbers of cells were associated with asthma and helminth infections and eosinophils were strikingly increased in animal tissues after acute anaphylaxis. The association of the eosinophil with anaphylaxis suggested that it ameliorated anaphylactic hypersensitivity. This hypothesis remained the dominant explanation for eosinophil function from the early part of this century until the 1980s. Now it is recognized that the eosinophil is armed with weapons able to kill helminths and to damage tissues and cause disease. Here, new information on the eosinophil is reviewed with emphasis on the mechanisms altering eosinophil function in disease. It is worthy of mention that the worldwide economic costs of one eosinophil-associated disease, bronchial asthma, are estimated to exceed the combined cost of the AIDS/HIV infection and tuberculosis.¹

Abbreviations used

BAL:	Bronchoalveolar lavage
BHR:	Bronchial hyperreactivity
CCR3:	Chemokine receptor 3
ECP:	Eosinophil cationic protein
EDN:	Eosinophil-derived neurotoxin
EPO:	Eosinophil peroxidase
HUVEC:	Human umbilical vein endothelial cell
ICAM:	Intercellular adhesion molecule
MBP:	Major basic protein
MBPH:	Major basic protein homolog
MMP:	Metalloproteinase
mRNA:	Messenger RNA
PAF:	Platelet-activating factor
RSV:	Respiratory syncytial virus
sIgA:	Secretory IgA
TIMP:	Tissue inhibitor of metalloproteinase
VCAM:	Vascular cell adhesion molecule
VLA:	Very late activation antigen

EOSINOPHIL CONSTITUENTS

Tables I and II list eosinophil-derived products including granule proteins, newly synthesized mediators such as leukotriene C₄, oxygen metabolites, and cytokines.

MATURATION OF EOSINOPHILS IN THE BONE MARROW

Eosinophils develop and mature in the bone marrow from IL-5-responsive CD34⁺ precursor cells (Fig 1). CD34⁺, a cell surface sialomucin-like glycoprotein, specifically marks hemopoietic progenitors. Allergen challenge of sensitive patients (who have both early and late allergic responses) causes resident bone marrow CD34⁺ cells to express the IL-5 receptor α -chain, but not the IL-3 receptor α -chain.³ The signals causing the expression of the IL-5 receptor on CD34⁺ progenitor cells are unknown. Although the most likely candidate is IL-5 itself, these results suggest a novel therapeutic target to control eosinophilia in sensitive patients.

Mice lacking the IL-5 receptor α -chain have been generated by homologous recombination and are unable to mount an eosinophil response.⁴ Helminth infection by

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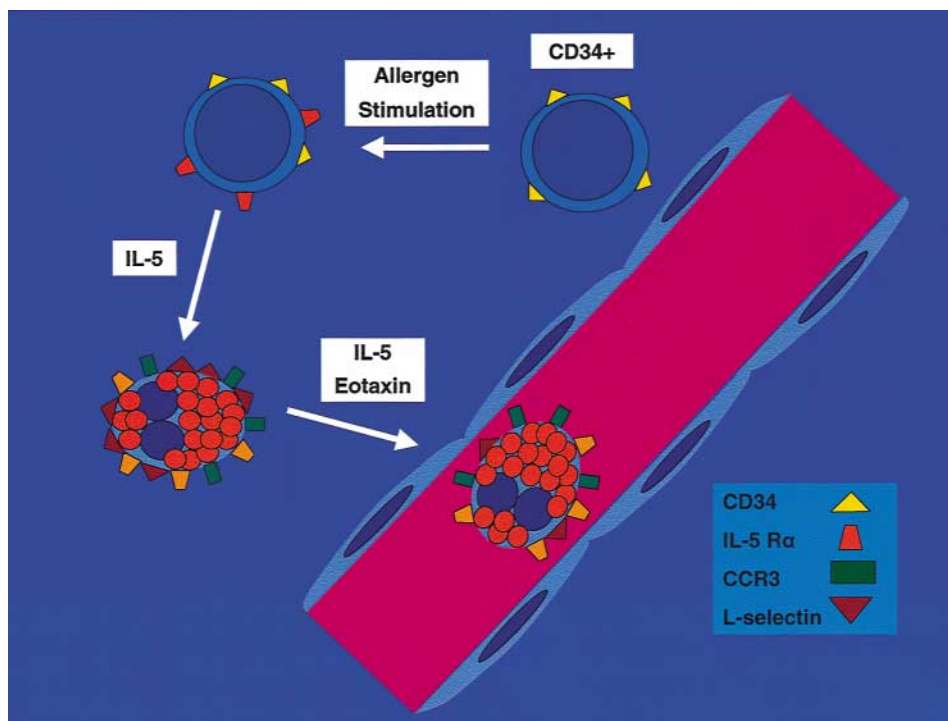


FIG 1. Eosinophil maturation in bone marrow. After allergen-induced late-phase reaction, CD34⁺ cells express IL-5 receptor α -chain. IL-5 drives the CD34⁺ and IL-5 receptor α -chain⁺ cells to eosinophil maturation. Under the stimulus of IL-5 or eotaxin, these mature cells shed L-selectin and migrate from the bone marrow to the blood.

TABLE I. Crystalloid granule proteins

	Site	M_r ($\times 10^{-3}$)	Isoelectric point	Cell content/ 10^6 cells	
				μg	$\mu\text{mol/L}$
Major basic protein (MBP)	Core	14	11.4	9	0.64
Eosinophil peroxidase (EPO)	Matrix	66	10.8	12	0.18
Eosinophil cationic protein (ECP)	Matrix	18-21	10.8	5	0.25
Eosinophil-derived neurotoxin (EDN)	Matrix	18-19	8.9	3	0.16
Major basic protein homolog (MBPH)	ND	14	8.7	ND	ND

M_r , Relative molecular mass; ND, not done.

Modified from (and used with permission) Kita H, Adolphson CR, Gleich GJ. Biology of eosinophils. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, editors. Allergy: principles and practice. 5th ed. St Louis: Mosby; 1998. p 242-60.²

Angiostrongylus cantonensis in these mice showed increased numbers of mature worms in the brains of the knockout animals and a greater length of the parasite. Conversely, when IL-5 transgenic animals (which possess increased numbers of eosinophils) were challenged with *A cantonensis*, fewer worms were recovered from the brain and the parasites were shorter. These data underscore the importance of IL-5 as an eosinophil differentiation factor⁵ and they support the importance of eosinophils in resistance to helminths. Note that several prior experiments using antibodies to IL-5 in mice infected with *Trichinella spiralis* and *Schistosoma mansoni* had not shown altered parasite immunity to reinfection.^{6,7} Last, mice lacking the IL-5 receptor, as well as all the receptors for IL-3 and GM-CSF,⁸ still produce morphologically normal peripheral blood eosinophils albeit in reduced numbers, indicating that eosinophils can be gen-

erated in the absence of responsiveness to IL-3, GM-CSF, and IL-5. Nonetheless, these animals do not mount an eosinophil response to helminth challenge, and presumably their ability to defend themselves against such an infection is impaired.

IL-5 stimulates eosinophil precursors to synthesize granule proteins; Table III shows the 17 most abundant messenger RNA (mRNA) transcripts from IL-5-differentiated umbilical cord mononuclear cells.⁹ Five of these 17 transcripts code for granule proteins; the MBP, the most abundant mRNA molecule, constitutes more than 8% of the transcripts. This study also revealed a novel MBPH. The homolog differs from MBP in that it is almost 3 orders of magnitude less basic, isoelectric point 8.7, compared with MBP, isoelectric point 11.4, and it is only expressed in bone marrow-derived cells. MBPH has been isolated from human eosinophil granules, is cyto-

TABLE II. Secretory products of eosinophils

Cytokines and chemokines	Lipid mediators
IL-1	Leukotriene B ₄ (small amount)
IL-2	Leukotriene C ₄
IL-3	Leukotriene C ₅
IL-4	5-HETE
IL-5	5, 15-diHETE and 8, 15-diHETE
IL-6	5-oxo-15-hydroxy-6, 8, 11, 13-ETE
IL-8	PGE ₁ and PGE ₂
IL-10	6-keto-PGF ₁
IL-12	Thromboxane B ₂
IL-16	Platelet-activating factor (PAF)
GM-CSF	Enzymes
RANTES	Charcot-Leyden crystal protein
TNF- α	(?Lysophospholipase)
TGF- α	92-kd Gelatinase
TGF- β	Collagenase
MCP-1	Elastase (questionable)
MIP-1 α	
PDGF-B	
VEGF	
Reactive oxygen intermediates	
Superoxide anion	
Hydrogen peroxide	
Hydroxy radicals	

HETE, Hydroxyeicosatetraenoic acid; *diHETE*, dihydroxyeicosatetraenoic acid; *ETE*, eicosatetraenoic acid; *TGF*, transforming growth factor; *MIP*, macrophage inflammatory protein; *PDGF-B*, platelet-derived growth factor-B; *VEGF*, vascular endothelial growth factor.

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TABLE III. Seventeen most abundant transcripts of IL-5-differentiated umbilical cord mononuclear cells*

Description of GenBank sequence†	No. of matching clones (% abundance)‡
Eosinophil MBP mRNA	662 (8.12)
Eosinophil Charcot-Leyden crystal protein mRNA	243 (2.98)
UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase mRNA	107 (1.31)
EDN mRNA	103 (1.26)
Eosinophil MBPH mRNA	93 (1.14)
Secretory granule proteoglycan core gene	89 (1.09)
Elongation factor-1 α gene	87 (1.06)
Eosinophil peroxidase gene	78 (0.95)
Osteopontin mRNA	75 (0.92)
Ferritin light subunit mRNA	73 (0.89)
Cathepsin B mRNA	68 (0.83)
Transcription factor SP1 mRNA	64 (0.78)
CpG island DNA genomic <i>Mse</i> 1 fragment	59 (0.72)
IFN-inducible mRNA	54 (0.66)
HLA-DR antigen α -chain mRNA	44 (0.54)
Cathepsin D mRNA	41 (0.50)
Cytoplasmic β -actin gene	40 (0.49)

Modified from (and used with permission) Plager DA, Loegering DA, Weiler DA, Checkel JL, Wagner M, Clarke NJ, et al. A novel and highly divergent homolog of human eosinophil granule major basic protein. J Biol Chem 1999;274:14464-73.

*A complementary DNA library was created from IL-5-stimulated umbilical cord mononuclear cells harvested at days 10 to 12. At this time, the stimulated cells are vigorously expressing mRNA for eosinophil granule proteins and are EPO positive. Later, at about culture day 21, they become recognizable eosinophils.

†All mRNA or gene sequences listed are of human origin.

‡Absolute and percent abundance among 8146 total clones sequenced.

toxic for K562 tumor cells, and is able to activate neutrophils and basophils.⁹

MBP mRNA specifies the existence of an MBP proform (referred to as proMBP) containing an acidic propeptide rich in glutamic acid.¹⁰ ProMBP lacks the cytotoxic and cytostimulatory properties of MBP.¹¹ During eosinophil differentiation, proMBP is expressed before MBP. Later in eosinophil maturation, all eosinophils become MBP positive, and fewer cells are positive for proMBP; mature normal eosinophils do not contain the pro-portion of proMBP.¹² Double staining of immature eosinophils with use of antibodies specific for proMBP and MBP shows proMBP localization to large uncondensed granules, whereas MBP localizes to granules with electron-dense material in their centers. Thus during eosinophil differentiation, posttranslational processing of the pro-peptide of proMBP occurs in the granule, followed by MBP condensation, ultimately forming the characteristic crystalline core of the mature eosinophil.

Eosinophil mobilization from the bone marrow, investigated by in situ perfusion of the guinea pig hind limb,¹³ is strongly stimulated by IL-5 (Fig 1). IL-5 stimulates chemokinesis of bone marrow eosinophils in a concentration-dependent manner and causes their selective release. Eosinophils within bone marrow sinusoids

apparently migrate through the sinusoidal endothelium, gaining access to the sinus lumen. During transmigration eosinophils change their expression of adhesion molecules, shedding L-selectin and increasing β_2 -integrin. Blocking antibody to β_2 -integrin diminishes eosinophil release by about 40%, whereas an antibody to α_4 -integrin increases the rate of IL-5-stimulated eosinophil mobilization by about 40%, possibly by inhibiting eosinophil adhesion to bone marrow sinus endothelium. Eotaxin also stimulates eosinophil release from the guinea pig bone marrow in a selective concentration-dependent manner, and eotaxin and IL-5 synergistically mobilize eosinophils from the bone marrow.¹⁴ Thus eosinophil production in the bone marrow is under the control of IL-5, whereas migration through the bone marrow sinus endothelium is under the control of IL-5 and eotaxin.

Finally, eotaxin mobilizes IL-5-dependent eosinophil colony-forming progenitors from the bone marrow.¹⁴ These progenitors are rapidly cleared from the blood and may return to the bone marrow or be recruited to extravascular sites. Analyses of nasal polyp tissues from patients undergoing surgery for nasal obstruction revealed CD34⁺ mononuclear cells that morphologically resemble immature hemopoietic cells and that form myeloid colonies.¹⁵ Thus colony-forming cells released from the bone mar-

row by eotaxin may home to peripheral tissues and contribute to eosinophil-associated inflammation.

EOSINOPHIL TISSUE RECRUITMENT

Eosinophils selectively infiltrate diseased tissues, and recent studies have provided considerable insight into the molecular mechanisms of recruitment. Fig 2 summarizes the steps in this process. During tissue localization circulating eosinophils lightly tether to and roll on endothelial cells by binding to selectins and then adhere more firmly, changing their shape and spreading on the endothelial cell surface. Next, eosinophils migrate between endothelial cells, traverse the basement membrane barrier and, finally, enter the extravascular tissue compartment. Intuitively, these processes must result in stepwise cell activation sufficient to permit completion of vessel transmigration but not to cause degranulation and release of oxidants and other mediators; otherwise frank vasculitis would occur.

Recent experiments have tested the importance of selectin-mediated rolling by use of selectin-deficient mice and by *in vivo* tracking of eosinophils. Recall that P-selectin is expressed on endothelium and platelets, L-selectin is expressed on leukocytes including eosinophils, and E-selectin is expressed on endothelium. Mice deficient in both P- and E-selectin and in P- and L-selectin show significant elevations of blood eosinophils, about twice the baseline level, implying that fewer eosinophils are margined on the endothelium. After ragweed sensitization and challenge, P-, PE-, PL-, and ELP-selectin deficient mice showed a 2-fold reduction in the numbers of eosinophils recruited into the peritoneal cavities. These results point to the critical importance of P-selectin in this reduction because it is the common factor in the selectin combinations.¹⁶ They also are consistent with other reports that eosinophils do not interact importantly with E-selectin.^{17,18} Remarkably, even the animals devoid of selectin expression showed eosinophil migration into the peritoneal cavity (that was only reduced about 50%), indicating redundant mechanisms (apparently selectin independent) for eosinophil accumulation at sites of inflammation (see below).

Eosinophils selectively tether, roll, and accumulate on IL-4-activated endothelial cells expressing VCAM-1.^{19,20} Recent studies have tested the importance of selectins and β_1 - and β_2 -integrins for eosinophil interaction with IL-4-stimulated endothelial cells. *In vitro* studies of IL-4-stimulated human umbilical vein endothelial cells (HUVEC)²¹ showed that eosinophils quickly become adherent after minimal rolling, change shape, and spread on the endothelial cell monolayer. Endothelial cell P-selectin expression was increased by IL-4- and P-selectin-mediated eosinophil tethering. Endothelial cell VCAM-1 and its ligand on the eosinophil, VLA-4, also mediated eosinophil tethering and accumulation on IL-4-treated HUVECs. Blocking of P-selectin, VCAM-1, and VLA-4 totally abolished eosinophil tethering. Firm binding of eosinophils was only blocked by antibodies to α_4 - and β_2 -integrins. Thus *in vitro* eosinophils are able to

tether to IL-4-stimulated endothelial cell VCAM-1 through interactions with α_4 -integrins. TNF- α stimulation of HUVECs yielded somewhat different results.²² Here, rolling was largely α_4 -integrin dependent, whereas adherence was α_4 -integrin and E-selectin dependent.

Analyses of eosinophil accumulation in the peritoneal cavities of mice after ragweed sensitization and challenge also showed the importance of P-selectin.²³ P-selectin-deficient mice showed about a 75% reduction of eosinophil accumulation (roughly in keeping with the results above).¹⁶ Deficiency of the ICAM-1 also resulted in about a 70% reduction in eosinophil accumulation. Surprisingly, double mutant mice, deficient in both P-selectin and ICAM-1, only showed about a 60% reduction in eosinophil recruitment, about the same as the individual deficiencies. Treatment of double-mutant mice with anti-VCAM-1 caused nearly complete inhibition of eosinophil migration. Therefore mice evidently require P-selectin, ICAM-1, and VCAM-1 for eosinophil recruitment to the peritoneal cavity after ragweed sensitization and challenge.

Injection of IL-4 into a local site caused marked increases in leukocyte adhesion, and 50% of the cells recruited into the tissues were eosinophils.²⁴ IL-4 increased VCAM-1 expression but did not alter P-selectin or E-selectin expression. Although anti-P-selectin strikingly inhibited eosinophil rolling on vessels at the IL-4-treated sites, nonetheless, leukocyte recruitment was unaltered in mice treated chronically with P-selectin antibody or deficient in either P-selectin or P- and E-selectins. The residual leukocyte rolling was independent of L-selectin but was abolished by blockade of the α_4 -integrin. Overall, these results support the importance of the ligand pair, VCAM-1 and VLA-4, for the induction of IL-4-stimulated eosinophil tissue recruitment. As will be noted below, IL-4 also induces eotaxin,^{25,26} and thus eotaxin could participate in the selective recruitment of eosinophils.

Finally, the effects of C3a and C5a anaphylatoxins on eosinophil migration *in vivo* have been tested.²⁷ C3a is specifically chemotactic for eosinophils but not neutrophils.²⁸ By use of IL-1 β -stimulated rabbit mesentery, C3a caused a rapid and stable adhesion of rolling eosinophils, but not neutrophils, to postcapillary venules; however, it failed to cause transmigration of the adherent eosinophils. C5a caused both firm adhesion and transmigration of eosinophils and neutrophils through venular endothelium. *Ex vivo* treatment of eosinophils with anti- α_4 - and anti- β_2 -integrin antibodies blocked both C3a- and C5a-dependent adhesion to venular endothelium. Overall, these results support prior *in vitro* findings that C3a is an eosinophil-specific chemotactic mediator²⁸ by showing that it selectively influences eosinophil adhesion but not transmigration *in vivo*. In contrast, C5a is a complete activator of integrin-dependent adhesion, as well as transmigration, of both eosinophils and neutrophils.

EOSINOPHIL CHEMOATTRACTANTS

Since the discovery of eotaxin as an eosinophil chemoattractant in a guinea pig model of asthma,²⁹ murine

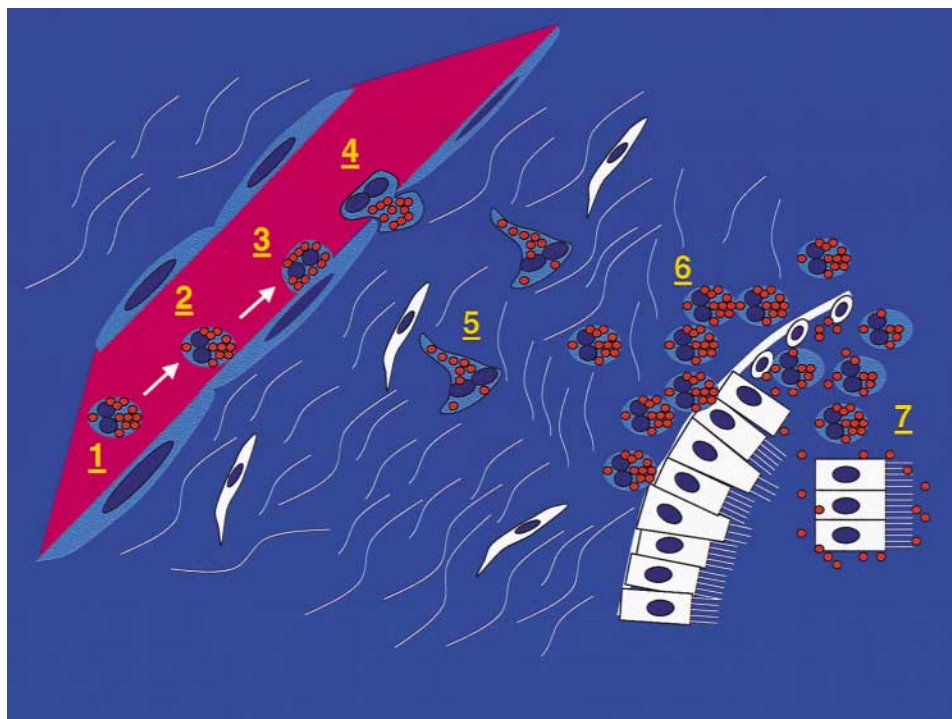


FIG 2. Schematic summary of eosinophil recruitment to and activation in bronchial tissues. At 1, the eosinophil is moving in the peripheral blood. At 2, the eosinophil has tethered to the vessel wall by adhesion molecules (for example, endothelial P-selectin and eosinophil very late activation antigen [VLA]-4 with endothelial vascular cell adhesion molecule [VCAM]-1) and rolls along the endothelium. At 3, the eosinophil has more firmly adhered to the endothelium (by the eosinophil β_1 - and β_2 -integrins and the endothelial intercellular cell adhesion [ICAM] molecules) and has flattened on the surface. At 4, the eosinophil is undergoing transmigration between endothelial cells into the connective tissues. At 5, the eosinophils (with use of VLA-4 and VLA-6) interact with connective tissue matrix proteins (eg, fibronectin and laminin), become "primed" and partially activated under the influence of various factors (eg, cytokines such as IL-5, chemokines such as RANTES and eotaxin, and lipid mediators such as PAF), and migrate toward their eventual destination. At 6, eosinophils become fully activated (by interactions with cytokines, lipid mediators, Igs, complement fragments, and β_2 integrins) and start to degranulate. Finally, at 7, the eosinophils have degranulated, releasing toxic mediators (eg, granule proteins, lipid mediators, oxygen metabolites, proteases, and cytokines) and disrupting cells in the bronchial epithelium.

and human eotaxin have been described.^{25,30} Considerable new information highlights the importance of eotaxin in disease. For example, eotaxin is constitutively expressed at high levels in the normal human large and small bowel (where eosinophils normally reside)³¹ and is up-regulated in ulcerative colitis and lesions of Crohn's disease.³⁰ Expression of eotaxin mRNA in endothelial cells, the gatekeepers for leukocyte migration, is stimulated by TNF- α and IL-1 α ; respiratory epithelial cells also express eotaxin mRNA in response to stimulation with IL-1 α and TNF- α .³² The possibility that mast cell-derived TNF- α and IL-1 α stimulate eotaxin production in epithelial and endothelial cells is intriguing and would link IgE-mediated mast cell stimulation to eosinophil recruitment. Interestingly, eotaxin expression is induced in eosinophils themselves after culture with IL-3.³⁰

To investigate the importance of eotaxin in bronchial asthma, eotaxin levels were analyzed in bronchoalveolar lavage (BAL) fluids from patients with atopic asthma, and eotaxin mRNA levels were measured in the airway cells.³³ BAL fluid contained high concentrations of

eotaxin, and the respiratory epithelium and submucosa showed increased expression of eotaxin mRNA and protein. Eotaxin was present predominantly in macrophages, with smaller contributions from T cells and eosinophils. Eotaxin was also expressed in respiratory smooth muscle after stimulation with TNF- α and IL-1 β .³⁴ Furthermore, strong signals for eotaxin mRNA and eotaxin immunoreactivity were observed in smooth muscle from asthmatic airways, suggesting that airway smooth muscle contributes to eosinophil inflammation in asthma through eotaxin production. Fibroblasts from lung³⁵ and skin³⁶ also can be stimulated by IL-4 to secrete eotaxin. Finally, although both IL-4 and IL-13 induce expression of eotaxin mRNA and protein in the murine lung, IL-13 is considerably more potent than IL-4.³⁷

A second eotaxin, eotaxin-2, has now been identified and promotes chemotaxis and calcium mobilization of human eosinophils, but not neutrophils or monocytes.³⁸ Cross-desensitization studies concluded that eotaxin-1 and eotaxin-2 and MCP-4 share the same receptor, chemokine receptor 3 (CCR3). Thus a second eotaxin exists and

exerts its activity through the CCR3 receptor. Finally, a third eotaxin, eotaxin-3, has been identified.³⁹ Eotaxin-3 appears to bind to CCR3 and its mRNA is up-regulated by IL-4 in HUVEC; in contrast, TNF- α and IL-1 β did not alter the expression of eotaxin-3 in these endothelial cells. Thus, currently 3 species of eotaxin are recognized. The results in patients noted above suggest the importance of eotaxin in human disease, but the precise contributions of the various eotaxins await elucidation.

Once the eosinophil starts its migration through the blood vessel, it must traverse the basement membrane zone, containing various structural molecules, such as type IV collagen, laminin, entactin, and heparan sulfate proteoglycans. To traverse this region, which appears to act as a barrier to cells migrating from blood vessels, eosinophils use metalloproteinases (MMP), especially MMP-9. For example, eosinophils in the lungs of patients with asthma express mRNA for MMP-9.⁴⁰ Eosinophil transmigration through an artificial basement membrane (Matrigel, Becton-Dickinson, Bedford, Mass) is induced only by PAF and eotaxin in the presence of IL-5⁴¹; this migration through the Matrigel barriers is inhibited by an antibody to MMP-9.⁴² Administration of a tissue inhibitor of MMP (TIMP) blocks the antigen-induced infiltration of eosinophils and lymphocytes in a murine model of asthma.⁴³ Thus TIMPs may be important regulators of eosinophil migration and may be therapeutically useful in eosinophil-associated diseases, including bronchial asthma.

EOSINOPHIL ACTIVATION

Once the eosinophil has migrated into an inflamed tissue, it becomes activated and releases mediators (see Tables I and II). The activators and regulators of eosinophil function have been reviewed in detail elsewhere.² Numerous stimuli, including Ig receptors (especially for secretory IgA [sIgA] and IgG), as well as cytokines (such as IL-5, GM-CSF, and IL-3), potentially activate the eosinophil and likely participate in eosinophil-associated diseases. Although the high-affinity receptor for IgE, Fc ϵ RI, has been proposed as a stimulus for eosinophil activation,⁴⁴ recent tests of this hypothesis with murine⁴⁵ and human^{46,47} eosinophils have not confirmed an important role of this receptor. These results discourage belief that IgE is critically involved in eosinophil activation. In contrast, cross-linking of the IgG receptor, Fc γ RII (CD32), enhances eosinophil survival, evidently by autocrine production of GM-CSF, and stimulates apoptosis of adherent eosinophils, through a β_2 -integrin-dependent mechanism.⁴⁸ Secretory IgA is a very potent stimulus for eosinophil degranulation²; its potency is mediated by the secretory component, which binds to the eosinophil and synergizes with cytokines or IgG for superoxide production.⁴⁹

Eosinophil activation by cytokines and Igs is critically dependent on β_2 -integrins, especially Mac-1 (α M β_2).⁵⁰ β_2 -Integrins participate in eosinophil superoxide anion production and in degranulation stimulated by PAF, GM-

CSF, cross-linking of Fc γ RII (CD32),⁴⁸ sIgA,⁴⁹ and IgG.⁵¹ The physiologic ligand for Mac-1 is ICAM-1, and ICAM-1-coated surfaces promote eosinophil activation in a β_2 -integrin-dependent manner.⁵²

Finally, eosinophil activation through various secretagogues, such as IL-5 and IgG, results in detectable PAF in supernatants,⁵³ suggesting that PAF functions as an endogenous eosinophil stimulant. A test of this hypothesis revealed that structurally distinct PAF antagonists inhibit both IgG- and IL-5-induced eosinophil superoxide production and degranulation.⁵³ Moreover, inhibition of cytosolic phospholipase A₂, a key enzyme for PAF synthesis, attenuated both PAF production and eosinophil effector functions. PAF also promotes the formation of lipid bodies in eosinophils, and lipid bodies provide enzymes that generate eicosanoids, such as PGE₂ and leukotriene C₄.⁵⁴ Although prior tests of PAF antagonists in patients with asthma have not shown efficacy, more recent studies with newly developed and more potent PAF receptor antagonists have revealed beneficial effects.⁵⁵ Thus PAF antagonists may find a place in treatment of eosinophil-associated diseases.

EOSINOPHIL EFFECTOR FUNCTIONS

Considerable evidence supports the eosinophil's ability to inflict tissue damage in bronchial asthma,⁵⁶ and Fig 3 summarizes these mechanisms. For example, the eosinophil granule proteins are toxic to respiratory epithelium, and concentrations of these proteins in the toxic range are present in respiratory secretions from patients with asthma.⁵⁷ The toxicity to the respiratory epithelium is manifested by epithelial cell desquamation, frank damage to epithelial cells, alterations in ciliary beating, and epithelial secretion.^{56,57} Eosinophil granule proteins also potentially increase vascular permeability at concentrations in the nanomolar range⁵⁸; these concentrations are exceeded by those present in respiratory secretions⁵⁷ and estimated in tissues (MBP concentrations from about 1×10^{-7} to 1×10^{-5} mol/L).⁵⁹ Eosinophil granule proteins also stimulate various cells, including human basophils, rat mast cells, neutrophils, respiratory goblet cells and basophils, as well as platelets.⁵⁶

BRONCHIAL HYPERREACTIVITY AND MBP

Considerable evidence supports the ability of eosinophil granule MBP to induce bronchial hyperreactivity (BHR), one of the defining criteria of bronchial asthma. First, BHR is significantly correlated with peripheral blood eosinophilia in patients with asthma, both atopic and nonatopic.⁵⁶ Second, investigations of patients with bronchial asthma showed significant correlations between the percent of BAL eosinophils and BHR, as measured by methacholine PC₂₀ and between the quantities of eosinophil granule MBP and methacholine PC₂₀.⁶⁰ Third, induction of eosinophilia in cynomolgus monkeys by repeated inhalation of *Ascaris suum* extracts produced increased BAL eosinophilia and

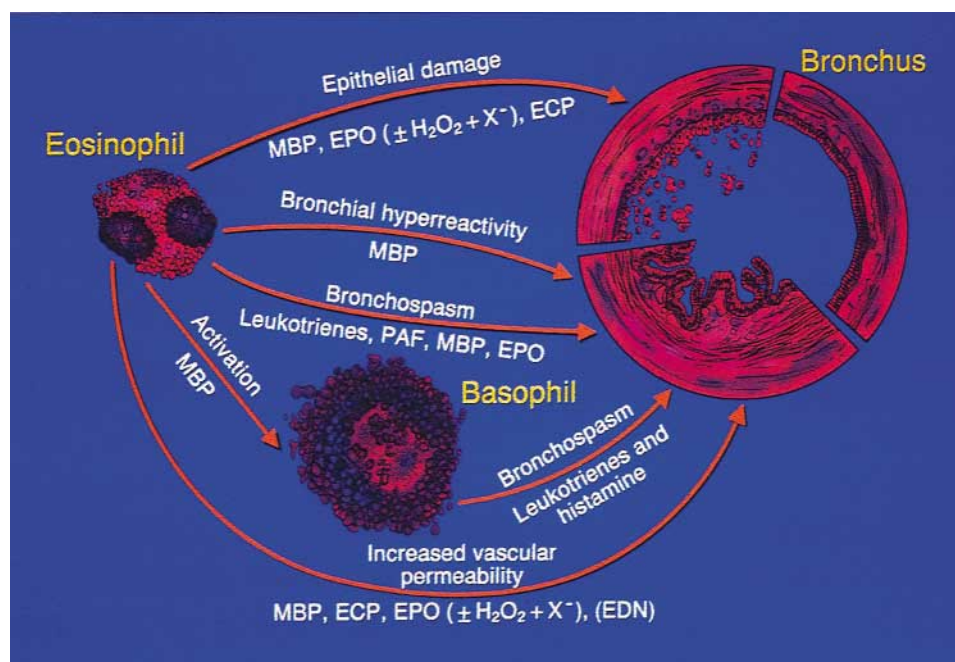


FIG 3. Proposed role of eosinophils in bronchial asthma. Bronchus, *top right*, shows a normal portion; *top left*, epithelial damage resulting from toxic eosinophil granule proteins; *bottom left*, edematous airway with smooth muscle hyperplasia. X, Halide anions, such as Cl^- , Br^- , or I^- . (Modified from Gleich GJ, Adolphson CR. The eosinophil and bronchial asthma: evidence for a critical role of eosinophils in pathophysiology. In: Sanderson CJ, editor. Interleukin-5: from molecule to drug target for asthma. Vol 125. New York: Marcel Dekker; 1998. p 1-37.⁵⁶)

increased quantities of MBP.⁶¹ Both eosinophil numbers and MBP concentrations in the BAL fluids correlated significantly with methacholine PC_{100} values. Interestingly, at week 2 of *A suum* administration, striking BAL eosinophilia was present, but BHR had not increased and MBP levels in BAL fluids were not significantly different from control levels. By week 4, BHR had increased strikingly and BAL eosinophilia and MBP levels were maximal. Here, MBP levels appeared to be a better correlate of BHR than numbers of eosinophils. Fourth, instillation of eosinophil granule proteins directly into the respiratory tract of cynomolgus monkeys showed that MBP and EPO cause an increase in respiratory resistance, whereas only MBP caused BHR.⁶² Fifth, the mechanism by which MBP causes BHR has been investigated with poly-L-lysine used as a substitute for MBP; activation of C fibers emerged as an important mechanism.⁶³ A subsequent report concluded that bradykinin generation was also critical for BHR induced by MBP or poly-L-lysine.⁶⁴ Sixth, MBP application to rings of guinea pig tracheal epithelium significantly augmented the rings' reactivity to acetylcholine and histamine, presumably through an interaction with the epithelium.⁶⁵ Seventh, in vivo experiments in guinea pigs showed that direct application of MBP to respiratory epithelium caused active force generation by smooth muscle.⁶⁶ Finally, mice deficient in MBP by homologous recombination fail to develop BHR and their eosinophils lack crystalline cores.⁶⁷

Overall, these results suggest that MBP alters BHR through an interaction with epithelium. As discussed below, evidence also exists that MBP is able to cause BHR through interaction with M2 muscarinic receptors. However, instillation of MBP into the airways does not alter M2 muscarinic receptor function, indicating that these two mechanisms differ (Fryer and Gleich, unpublished observations).

BHR AND M2 MUSCARINIC RECEPTOR FUNCTION

The possibility that excessive activity of the cholinergic nerves contributes to the pathophysiologic mechanisms of bronchial asthma is supported by numerous observations, including an early treatment of asthma by inhaling smoke from the stramonium plant containing atropine.⁶⁸ Acetylcholine released onto M3 muscarinic receptors causes bronchoconstriction.⁶⁹ Normally, the release of acetylcholine from parasympathetic nerves is inhibited by prejunctional M2 muscarinic receptors on the nerves. The M2 receptor can be stimulated with acetylcholine or muscarinic agonists, such as pilocarpine, resulting in decreased vagally induced bronchospasm through inhibition of acetylcholine release.⁶⁸ Loss of M2 receptor function or blockade of these receptors with antagonists increases release of acetylcholine and vagally induced bronchospasm. Antigen-challenged guinea pigs show bronchial hyperresponsiveness, which

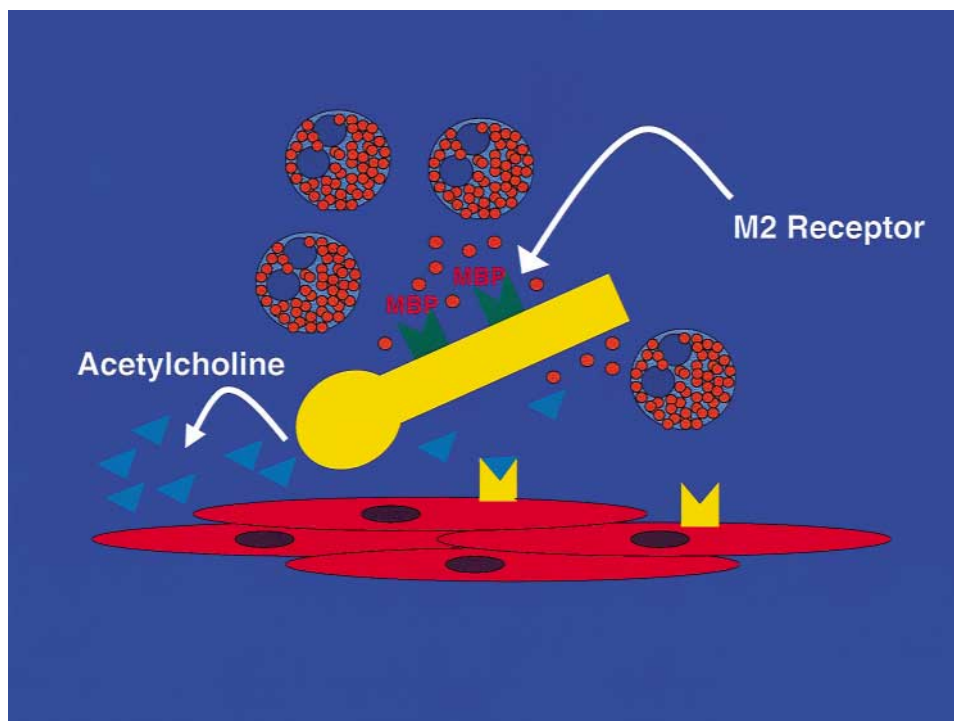


FIG 4. Interaction among eosinophils, MBP, and M2 muscarinic receptors in the lung. M2 receptors are present on terminals of the vagus nerve. M3 receptors are present on respiratory smooth muscle. Stimulation of the M2 receptor decreases acetylcholine release from terminals of the vagus nerve. Stimulation of M3 receptors causes muscle contraction. Eosinophils selectively infiltrate areas around nerves and degranulate. MBP released from the eosinophil allosterically blocks the M2 receptor, thereby increasing acetylcholine release from the terminals of the vagus nerve.

can be related to loss of neuronal M2 receptor function.⁶⁸ Furthermore, in guinea pigs sensitized and challenged with ovalbumin, eosinophil depletion by anti-IL-5⁷⁰ or inhibition of eosinophil migration into the lungs⁷¹ prevents the loss of M2 receptor function. Therefore loss of M2 receptor function is mediated by eosinophils. In addition, eosinophils localize around nerves⁷² and release MBP there. MBP is an allosteric antagonist for the M2 receptor.⁷³ The possibility that MBP released from eosinophils binds to the M2 receptor and causes increased vagal responsiveness was tested using heparin, which avidly binds MBP. Indeed, heparin restored M2 receptor function.⁷⁴ Moreover, an antibody to guinea pig eosinophil MBP prevented M2 receptor dysfunction and BHR,^{75,76} supporting MBP as the molecule blocking the M2 receptor and causing hyperreactivity in antigen-challenged guinea pigs. Fig 4 shows these results diagrammatically. In this model, MBP interacts with an acidic moiety on the M2 muscarinic receptor and causes its dysfunction. Guinea pigs sensitized and challenged with ovalbumin also demonstrate hyperreactivity to histamine.⁷⁷ The histamine-stimulated hyperreactivity is abolished by vagotomy and by atropine, but it is not altered by pilocarpine (implying that the M2 receptor is blocked). Further, blockade of the eosinophil influx into the airways or neutralization of eosinophil granule MBP prevented the development of histamine hyperreactivity in these animals.⁷⁷ Information regarding the effects of

inflammatory cells on neuronal M2 muscarinic receptor functions in the lung has recently been reviewed.⁷⁸

EOSINOPHILS AND VIRAL INFECTIONS

Viral respiratory tract infections are an important cause of asthma exacerbations.⁷⁹ Infection with respiratory syncytial virus (RSV) is especially important in infancy. In addition, rhinoviruses are a major cause of asthma exacerbations in school-aged children. Viral infections enhance mediator release during the immediate reaction to antigen and increase recruitment of eosinophils to the lung after antigen challenge.⁸⁰ The mechanisms by which rhinoviruses enhance airway inflammation are not established, but chemokines and cytokines likely play a role, including RANTES and GM-CSF.⁷⁹

To investigate the mechanisms of viral infection, a mouse model of RSV infection has been developed. RSV infection causes BHR in association with increased IFN- γ expression and with modest increases in eosinophils and neutrophils. Administration of anti-IL-5 prevents development of BHR and blocks the increased numbers of eosinophils after infection.⁸¹ Mice deficient in IL-5 do not develop BHR or BAL eosinophilia during RSV infection.⁸² Finally, depletion of CD8 cells blocks development of BHR after RSV infection and IL-5 levels are decreased, suggesting that CD8 T cells are critical for IL-5 production, eosinophil infiltration, and the development of BHR.⁸³

Recent experiments in guinea pigs have tested whether respiratory viral infections might have different outcomes in sensitized animals compared with nonsensitized animals.⁸⁴ As noted earlier, ovalbumin sensitization and challenge causes M2 muscarinic receptor dysfunction and BHR that is IL-5, eosinophil, and MBP dependent.⁷⁸ A previous observation suggested that antigen sensitization before virus infection alters the resulting inflammation. Ordinarily, CD8 T cells respond to viral protein stimulation by generating IL-2 and IFN- γ , leading to an influx of neutrophils and mononuclear cells in the airway.⁸⁵ However, when mice were sensitized to a nonviral protein such as ovalbumin before virus infection, virus-specific T cells released IL-4 and IL-5, resulting in eosinophil-associated pulmonary inflammation. Therefore antigen sensitization may alter the inflammatory response to a subsequent viral infection. Nonsensitized guinea pigs infected with virus developed vagal hyperreactivity that was not altered by antibody to IL-5.⁸⁴ This result agrees with prior findings showing that viral infection or IFN- γ treatment reduces M2 receptors.⁸⁶ In contrast, animals previously sensitized to ovalbumin developed eosinophil-associated bronchial inflammation, and the vagus-mediated BHR was prevented by pretreatment with anti-IL-5 before virus infection. Pretreatment with antibody to guinea pig MBP prevented virus-induced vagal reactivity in the sensitized guinea pigs, and heparin administration reversed virus-induced BHR.⁸⁴ Fig 5 shows that the viral content of the lungs from sensitized animals is significantly decreased compared with that of nonsensitized virus-infected guinea pigs. Pretreatment with anti-IL-5 did not affect the lung viral content of nonsensitized virus-infected guinea pigs, but it strikingly increased viral content in the sensitized virus-infected animals. Interestingly, sensitized virus-infected animals treated with anti-MBP did not show a significant change in viral content. These results indicate that the viral immunity in the sensitized and virus-infected animals is IL-5 dependent and suggest that eosinophils mediate viral neutralization in this model. The mechanism for eosinophil mediation of viral neutralization in the guinea pig model is obscure; the inability of anti-MBP antibody to alter viral titers suggests that MBP is not responsible.

Other experiments have shown that two eosinophil granule proteins can neutralize viruses. Both the ECP and EDN possess ribonuclease activity, and EDN is roughly 100 times more potent as a ribonuclease than is ECP.⁸⁷ These molecules have a striking similarity to bovine ribonuclease A⁸⁸ and other members of the ribonuclease A gene superfamily.⁸⁹⁻⁹² Studies of the genes coding for EDN and ECP suggest that these molecules evolved by gene duplication and show a surprisingly high rate of divergence.⁹³ A careful study of primate genomes showed that the EDN and ECP genes have been evolving at very rapid rates, and nearly 30% amino acid sequence divergence was noted between human and New World monkey EDN genes.⁹⁴ Overall, the genes encoding EDN and ECP have accumulated nonsilent mutations at rates greater

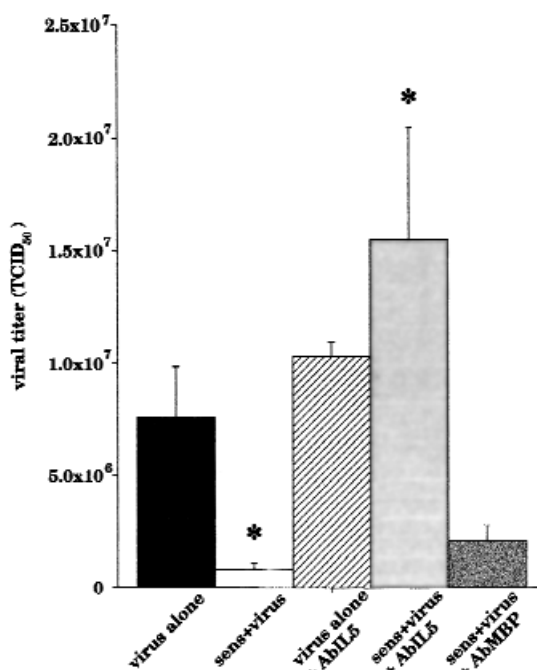


FIG 5. Viral titers from the lungs of virus-exposed guinea pigs. Sensitized virus-infected guinea pigs (white bar, $n = 19$) showed a significant decrease in viral titer compared with nonsensitized virus-infected guinea pigs (black bar, $n = 11$, $P = .04$). Pretreatment with antibody to IL-5 had no effect on nonsensitized virus-infected guinea pigs (hatched bar, $n = 3$) but caused a significant increase in recovered virus from sensitized virus-infected animals (light gray bar, $n = 11$, $P < .0001$). Sensitized virus-infected guinea pigs treated with antibody to MBP (dark gray bar, $n = 7$) continued to show decreased viral titers compared with untreated sensitized virus-infected animals. (Reproduced from Adamko DJ, Yost BL, Gleich GJ, Fryer AD, Jacoby DB. Ovalbumin sensitization changes the inflammatory response to subsequent parainfluenza infection: eosinophils mediate airway hyperresponsiveness, M₂ muscarinic receptor dysfunction, and antiviral effects. *J Exp Med* 1999;190:1465-77⁸⁴ by copyright permission of the Rockefeller University Press.)

than all other functional coding sequences in primates, suggesting responsiveness to unusual evolutionary pressures. In spite of these genetic changes, the basic features critical for ribonuclease activity have been preserved.

Therefore experiments were conducted testing the abilities of EDN and ECP to neutralize viruses, with RSV used as a target. The RSV genome is a molecule of single-stranded RNA, and, as noted previously, RSV is one of the most important respiratory pathogens worldwide. Purified eosinophils added to RSV viral suspensions caused a concentration-dependent drop in virus titer; placental ribonuclease inhibitor completely eliminated this antiviral effect, suggesting that ribonuclease activity is critical.⁹⁵ Furthermore, recombinant human EDN also neutralized extracellular virions in vitro, and an EDN mutant lacking ribonuclease activity did not neutralize RSV. Interestingly, ribonuclease A lacked the antiviral activity. Thus an eosinophil granule protein that is elevated in respiratory secretions (see below) after

viral infection is able to kill infectious RSV virions. The interaction between RSV and EDN was both saturable and specific, and a mutant EDN devoid of ribonuclease activity inhibited the antiviral activity of EDN.⁹⁶ Analyses of EDN chimeric molecules have pointed to the N-terminal segment as possessing sequences mediating its interaction with RSV.⁹⁶ Analysis of ECP also demonstrated antiviral activity for RSV, although this activity was about 50-fold less than that of EDN.⁹⁷

It is known that RSV-infected airway epithelial cells produce eosinophil chemoattractants⁹⁸⁻¹⁰⁰ and that RSV activates human eosinophils.¹⁰¹ Moreover, both EDN and ECP are increased in respiratory secretions from patients with RSV infection.¹⁰⁰⁻¹⁰² Overall, this information points to an important role for the eosinophil in viral immunity and provides a molecular basis for this role. Also, EDN-related protein structures could be used as novel antiviral agents. Finally, these findings may explain, in part, the striking evolutionary changes in the genes for EDN and ECP.

CLINICAL HORIZONS

Better methods to appraise eosinophil participation in disease are needed. MBP levels are a useful indicator of eosinophil degranulation, but normal serum or plasma contains appreciable and variable MBP concentrations (likely in the form of proMBP). Thus detection of a modest elevation of MBP is often problematic. Although measurement of ECP in biologic fluids is useful, ECP and EDN are present in neutrophils.¹⁰³ Therefore fluids from neutrophil-rich lesions could show elevated levels of these proteins. Further, the mAb EG2, believed to recognize only activated eosinophils, cannot reliably distinguish between stimulated and unstimulated eosinophils.¹⁰⁴

Better treatments for eosinophil-associated diseases are needed. Impressive evidence supports a role for the eosinophil in many diseases including asthma, atopic dermatitis, chronic urticaria, chronic eosinophilic pneumonia, and the hypereosinophilic syndrome. Glucocorticoids are still the mainstay in these diseases. The latest iterations of their structures have yielded remarkably potent agents, but they still possess potential side effects. Humanized monoclonal anti-IL-5 is presently in clinical trials of asthma, and the results of studies in severe asthma and other eosinophil-associated diseases will be interesting. Eotaxin and CCR3 are appealing drug targets, and blocking agents are under development. Because IL-4 is important in T_H2 development and eosinophil infiltration, soluble IL-4 receptor is also under investigation and may be useful for therapy of eosinophil-associated diseases.

Leukotriene antagonists are beneficial, especially in certain patients with mild to moderate asthma, but their effectiveness in severe asthma remains to be established.¹⁰⁵ Ursodeoxycholic acid inhibits eosinophil degranulation in patients with primary biliary cirrhosis,¹⁰⁶ and one wonders whether it would be useful in other eosinophil-associated diseases. Polyanions, such as polyglutamic acid and heparin, neutralize the toxicity of eosinophil granule proteins and block the ability of MBP

to cause BHR¹⁰⁷; their therapeutic usefulness warrants trial. Lidocaine mimics certain effects of glucocorticoids in vitro, and two clinical studies have shown a beneficial effect in asthma.^{108,109} Treatment with humanized mAb to IgE strikingly reduces the levels of blood IgE, diminishes the late phase of the IgE-mediated allergic reaction, and benefits patients with asthma.¹¹⁰ Finally, IFN- α dramatically reduces total leukocyte and eosinophil numbers in patients with the hypereosinophilic syndrome,¹¹¹ and it may be useful in other eosinophil-associated diseases.

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