Inflammatory Mediation of Airway Hyperresponsiveness by Peripheral Blood Granulocytes*  

The Case for the Eosinophil  

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For many years, inflammation has been thought to play an important role in asthmatic hyperresponsiveness, but the mechanism(s) by which bronchoconstriction caused by the eosinophil, a minority constituent of the circulating blood, translocates to the conducting airways of lung and confers airway hyperresponsiveness. As implications are drawn from existing hypotheses, there is a virtual certainty that the conceptualization derived herein will be modified in the near future. Nevertheless, the data presented in this review summarize an increasing body of evidence implicating circulating granulocytes in airway hyperresponsiveness.

Effects of Granulocytic Infiltration

Evidence Implicating Polymorphonuclear Neutrophilic Leukocytes

While many investigators now agree that the infiltration of circulating granulocytes into the conducting airways of the lung is essential to the pathogenesis of asthma, there is still some controversy regarding the responsible cell(s). A role for the polymorphonuclear neutrophilic leukocyte (neutrophil) in causing airway hyperresponsiveness has been suggested by the histologic correlation of neutrophil infiltration into the lung during induced inflammatory states, such as those caused by ozone exposure or neurokinin-induced bronchoconstriction caused by the e-fiber stimulant, capsaicin. Other investigations have shown that the airways of neutrophil-depleted rabbits are less responsive to immune stimulation than in the nonneutrophilic state and that replacement of granulocytes restored increased airway responsiveness. Unfortunately, these studies have not specifically implicated either the eosinophil or the neutrophil, since no attempt was made to restore these two different granulocytes individually. While it also is true that neutrophil infiltration accompanies airway hyperresponsiveness such as that seen from ozone exposure, some studies have demonstrated that the hyperresponsive state pre-

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cedes cellular infiltration. In many respects, acute inflammation caused by ozone seems to model more closely an acute inflammatory state rather than the chronic inflammatory state of human asthma.

Recent investigations in guinea pig models of airway responsiveness have examined the role of the neutrophil in either causing or augmenting airway smooth muscle contraction in vitro. In these studies, metabolic burst activity was documented in neutrophils as evidence of ex vivo activation. While eosinophilic granulocytes (see below) were capable of inducing airway smooth muscle contraction and increased muscarinic responsiveness of airways after activation, activated human neutrophils had no effect of airway reactivity. While these investigations do not exclude a possible role for the neutrophil in the chronic induction of airway hyperresponsiveness through other more complex interactions, no granular protein or secretory product of the neutrophil has been shown to date to be involved directly in the induction of airway hyperresponsiveness. Neutrophils are capable of secreting substantial quantities of leukotriene D_4, which is a relatively weak chemotactant for human eosinophils. However, this 5-lipoxygenase derivative is synthesized by numerous cells in airways, and there is no indication that neutrophils play a direct role in the chemotaxis of other neutrophils or eosinophilic granulocytes.

Evidence Implicating Eosinophilic Granulocytes (Eosinophils)

The case for the eosinophil as the effector granulocyte causing airway hyperresponsiveness is, at present, substantially stronger than for the neutrophil. Eosinophils are found selectively in high concentrations in sputum, bronchoalveolar lavage fluid, and tissue biopsy specimens in human asthmatic states. Often, the numbers of these minority constituents of the circulating blood are selectively increased in the absence of significant changes in neutrophil population during asthmatic states in humans. There is evidence that eosinophils and/or their ghost proteins—the proteins stored in eosinophil granules (see below)—are present in all biopsy specimens of airways in humans who have active bronchial asthma, and there is further evidence that eosinophils and at least one of their core proteins are capable of causing both direct airway smooth muscle contraction and augmentation of airway smooth muscle responsiveness. In contrast to neutrophils, the core granular protein of the eosinophil (Fig 1), major basic protein (MBP), causes direct contraction of guinea tracheal smooth muscle in vitro. The MBP of eosinophils also augments airway responsiveness in primate species. In guinea pigs, airway smooth muscle contraction is dose related and dependent on an intact epithelium (Fig 1). Heat denaturation or removal of the epithelial layer attenuates completely both the direct contractile response of MBP and its augmentatory response of muscarinic contraction of airways (Fig 1). Major basic protein has been reported to stimulate the synthesis and release of bronchoactive prostanoids from cultured epithelial cells, although the mechanism by which it causes airway contraction is still unresolved. While there has been speculation that epithelium possesses a specific MBP receptor, this has not been demonstrated. Other studies have suggested that the extreme cationicity of MBP could account for its effects in causing airway hyperresponsiveness. However, the eosinophil cat-ionic protein is similar to MBP in this regard and has no effect on airway contractility.

Prior investigations have suggested that MBP may augment airway responsiveness by causing inhibition of an epithelial-derived inhibitory (relaxing) factor (EpDRF) that is tonically secreted. However, the magnitude of this augmentatory response is small (one log shift in the dose-response curve to muscarinic stimulation), and to date, the putative epithelial-derived inhibitory factor has not been identified. The existence of EpDRF is postulated through analogy to the endothelial-derived inhibitory factor. Removal of vascular endothelium profoundly altered the response of vascular smooth muscle to acetylcholine. In contrast, epithelial removal has minimal effect in altering the response to contractile or relaxing stimuli in some studies and no effect at all in others. Recently, it has been shown that epithelial removal in guinea pig tracheas causes no effect on baseline airway responsiveness in vivo or in vitro in tissues taken from the same animals (guinea pigs) unless airway responsiveness is normalized as percent maximal response, ie, absolute force of contraction is not altered by epithelial removal. These data suggest that the method for normalization could account entirely for the observations leading to the postulation of the yet unidentified EpDRF.

Role of 5-Lipoxygenase Metabolites in Induction of Airway Hyperresponsiveness Caused by Eosinophils

Recent investigations have suggested that intact eosinophils also cause direct activation of airway smooth muscle hyperresponsiveness after activation with a variety of stimuli, including platelet-activating factor, formylated tripeptide (fMLP), and phorbol ester. This mechanism appears to be related directly to release of 5-lipoxygenase metabolites, predominantly leukotriene C_4, which may be converted locally into leukotriene D_4. In guinea pig tracheas in vivo, this effect of eosinophil activation likely occurs independently of granular secretion of eosinophil
products (Fig 2). Pretreatment with activated eosinophils also augments the response of guinea pig trachealis to exogenous agonists, eg, methacholine. Blockade of the 5-lipoxygenase synthesis by the antagonist, A63162, also causes complete attenuation of airway contractile response and augmentation of muscarinic contraction caused by human cord blood-derived eosinophils activated by FMLP. This attenuation is accompanied by complete blockade of leukotriene C4 synthesis but has no effect on granular secretion of preformed eosinophil peroxidase. Thus, the first phase of airway hyperresponsiveness caused by eosinophils appears to relate to activation of metabolic function rather than by granular secretion.

It may be that in later phases, as eosinophils disintegrate after airway infiltration, there is release of granular protein, which first causes further stimulation of airway responsiveness, and which then is followed by cytotoxic effects of these proteins on epithelium (Fig 3). This leads to eventual sloughing of the epithelium. Although demonstrated in vitro and in cell culture, this later scenario of eosinophil function has not been demonstrated experimentally in vivo.

The mechanism regulating eosinophil secretion of 5-lipoxygenase metabolites also has not been elucidated. Human eosinophil secretion appears to depend on the activity of endogenous phospholipase A2.
Effect of cells activated with fMLP+cytochalasin B (CYB) as in Figure 5 on activate tension (AT) in guinea pig trachea in vivo. Contraction begins immediately after topical application of activated cells and is inhibited by pretreatment with the 5-lipoxygenase inhibitor, A63162 (adapted from Strek ME, White SR, Hsuie TR, Kulp GVP, Williams FS, Leff AR. Effect of mode of activation of human eosinophils on tracheal smooth muscle contraction in guinea pigs. Am J Physiol 1993; 264:L475-81. Similar data have been obtained for human eosinophils in explanted preparations of human airways. (PLA2) for secretion of both leukotriene synthesis and release and for secretion of the granular proteins of eosinophils (Fig 4). The mechanism by which PLA2 causes eosinophil activation and degranulation has not yet been elucidated. The PLA2 is itself a potent bronchoconstrictor in guinea pig airways; airway smooth contraction is caused in this case, however, by induction of synthesis of bronchoactive prostaglandins, and the response is blocked by exogenous preadministration of the cyclooxygenase inhibitor, indomethacin.

Relevance to the Human Airways

It remains extremely difficult to design experimental interventions that provide direct evidence for specifically inflammatory cells in causing airway hyperresponsiveness. Rather than implying a function, the coincidental presence of a particular cell type suggests the need for further investigation. For this reason, most of the studies cited above have been related to animal models. Limited data are becoming available from human airway studies in ex vivo implant preparations. These data, though preliminary, seem to validate most assumptions made from animal studies implicating a contractile role for the human eosinophil on human airway smooth muscle. Human eosinophils, but not human neutrophils, cause constriction of explanted human airways after cellular

Effect of phospholipase A2 on endogenous activation of granular secretion of eosinophil peroxidase (EPO) from isolated human eosinophils. Activation with fMLP + cytochalasin B as above causes increased secretion of EPO, which is blocked by the phospholipase A2-antagonist, mepacrine. Excess substrate (arachidonic acid, AA) restores EPO secretion to normal, further indicating that a pathway enzyme (phospholipase A2) has been inhibited. The mechanism by which phospholipase A2 is activated in the endogenous state is unknown (adapted from White SR et al, J Clin Invest 1995; 91:2118-25.)

Effect of eosinophil infiltration into human airways. At later stages, there is a cytotoxic effect that likely accounts for the epithelial denudation (see arrow) found in human asthma. Left, Histologic section from a biopsy specimen of an airway of a human asthmatic (hematoxylin-eosin, original magnification X160). Right, The same section if stained by fluorescent monoclonal antibody for the eosinophil major basic protein (MBP). Virtually the entire cellular infiltrate is eosinophils (immuno-fluorescent antibody stain, original magnification X160) (from Gleich GJ et al, 26 reproduced by permission of the publisher).
activation.\textsuperscript{13} Muscarinic responsiveness of human airways also is augmented by human eosinophils. These studies and more extensive investigations in animal studies suggest that models may now be in place to establish some fundamental mechanisms in the inflammatory augmentation of airway responsiveness in humans.

**HONING IN ON THE CONDUCTING AIRWAY: MOLECULAR ADHESION MOLECULES**

The mere presence of blood eosinophils does not imply a human asthmatic state. In many circumstances, \textit{eg}, drug reactions, eosinophilia myalgia syndrome,\textsuperscript{26} eosinophilia is not accompanied by airway hyperresponsiveness. In other circumstances, where eosinophils infiltrate the lung but not the conducting airways (\textit{eg}, eosinophilic pneumonia, histiocytosis X), airway hyperresponsiveness also does not occur. Finally, many humans with asthma do not have eosinophilia, and so the nature of the eosinophil response in human asthma is unique and highly specific. The remarkable specificity of the mechanism by which eosinophils, a minority constituent of the human blood, hone in selectively on conducting airways and confer hyperresponsiveness to previously normoreactive tissues is only beginning to be understood.

To a large extent, eosinophilic infiltration of human airways results directly from adhesion of surface molecule on both the eosinophil and the vascular endothelium of the airway. Because many of these adhesion molecules are shared by the neutrophil, the specificity of eosinophil chemotaxis through molecular adhesion still is not elucidated. Eosinophils possess some adhesion molecules on their surface that are not shared by neutrophils (\textit{eg}, VLA-4), and there is a specific endothelial adhesion molecule (VCAM), which binds specifically to VLA-4. Yet, most inflammatory stimuli that upregulate surface adhesion molecules on eosinophils have no effect on VLA-4.\textsuperscript{27} Surface expression of VLA-4 is increased after exposure to fibronectin, suggesting that this molecule may be more important in chemotaxis toward the lumen of airway \textit{after} initial adhesion to the endothelium and diapedesis.\textsuperscript{28}

Most of what is known about molecular adhesion of eosinophils is derived from studies of human neutrophils. This rather brief description serves more as an introduction to the issues at hand, rather than a mechanistic explanation of what still is a poorly described phenomenon. In the first phase of adhesion, the upregulated eosinophils begin to roll along the endothelial surface of the blood vessel and likely form an early, weak bond between its own adhesion molecule, termed L-selectin, and the endothelium intracellular adhesion molecule-1 (ICAM-1). This bond with L-selectin is broken almost simultaneously while stronger bonds are formed with the eosinophil adhesion molecules, Mac-1 and, probably also, LFA-1, another surface adhesion molecule on the eosinophil that binds with ICAM-1 on the endothelium. The downregulation of L-selectin is likely to weaken this bond, and this promotes further diapedesis through the endothelium as bonding occurs with LFA-1 and Mac-1. Further chemotaxis in the direction of the lumen is promoted by bonding of the VLA-4 molecule, which is contained on the surface of eosinophils, but not neutrophils. Recent studies indicate that VLA-4 may bind directly and specifically to the parenchymal matrix of the airway and that this binding may also upregulate eosinophil secretion of eicosanoids. Experimental blockade of the ICAM-1 receptor in the monkey prevents both eosinophils chemotaxis into the lumen and increased airways.
responsiveness to methacholine in allergen-sensitized animals.

There is recent evidence to suggest that eosinophils may be directed initially to the conducting airways by selective regulation of surface adhesion molecules that are shared by both eosinophils and neutrophils. Prior investigations have shown that L-selectin function is downregulated and Mac-1 adhesion molecule expression is upregulated selectively by the cytokine IL-5, which is secreted by T lymphocytes. This effect is not seen for human neutrophils, which lack an IL-5 receptor (Fig 5). Thus, the initial steps of selective diapedesis of eosinophils could relate to the selective activation of surface adhesion molecules that are common to neutrophils through selective activation of a specific cytokine receptor on the surface of the eosinophils. Alexander and coworkers have shown recently that T-cell inhibition by administration of cyclosporine A caused a slight decrease in responsiveness in human asthmatics, presumably by inhibition of T-cell-eosinophil communication of this type.

Do Adhesion Molecules Also Activate Eosinophils?

Eosinophils in the lumen of humans after allergen challenge have a lower density profile than those in the peripheral blood of the same individuals. Thus, the process of cellular migration itself appears to be related to eosinophil activation. Hypothetically, this could be the result of upregulation of eosinophil activity by the process of adhesion and diapedesis itself. Ex vivo experimental models described above indicate that stimulation of cellular receptors on eosinophils stimulates secretion of 5-lipoxygenase products and eosinophil granular protein. Interestingly, the cytokine IL-5, which primes that eosinophil for selective endothelial adhesion, does not itself cause upregulation of the secretory or bronchoconstrictor function of the eosinophil. It thus is interesting to speculate that the process of molecular adhesion itself contributes substantially to the activation of eosinophils as they enter the conducting airways.

Conclusions

While many cells and mediators eventually interact to mediate the airway hyperresponsiveness of human asthma, the eosinophil appears most likely to be the responsible granulocyte and to play a central role in airway hyperresponsiveness. This cell or its ghost proteins are invariably present in active human asthma. However, histologic correlation does not imply function. In experimental models, including some limited studies in explanted human airways, eosinophils have been shown to confer hyperresponsiveness on normally reactive airways. This is clearly the initial result of 5-lipoxygenase activation and is independent of early-stage granular protein secretion. Experimentally, the MBP of eosinophils also upregulates airway responsiveness in an epithelium-dependent manner, suggesting a possible later activity of this infiltrating granulocyte. In contrast to the eosinophil, there is no evidence that isolated neutrophils can induce airway hyperresponsiveness. This may, of course, represent a failure to demonstrate an as yet undiscovered mechanism of activation for this cell.

Of particular interest is the mechanism by which eosinophils selectively enter the conducting airways of asthmatics through chemotaxis that appears currently to be directly related to selective activation of surface adhesion molecules on eosinophils. This may be related to specific cytokine activation of these surface adhesion molecules on eosinophils in the initial adhesion and diapedesis phase, since these same molecules are shared by neutrophils. Once adherent to the epithelium, eosinophils undergo increased metabolic burst and secretory activity that correspond to the activation associated experimentally with airway hyperresponsiveness. This also may be the result of molecular adhesion.

While it is likely that focus on a single cell type will not elucidate fully the complex syndromes of human asthma, further investigations into eosinophil function should provide insight into the complex cell-cell interaction by which normally reactive tissues are converted into hyperreactive airways of the human asthmatic state.

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