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The eosinophil: A cytokine-producing cell?

Eosinophils play important roles in the allergic inflammation associated with bronchial asthma.¹ Although the mechanism of eosinophil involvement in allergic diseases is not fully understood, cationic granule proteins and lipid mediators released from eosinophils could damage tissues and cause dysfunction, thereby contributing to the pathogenesis of local inflammation. Because eosinophils are terminally differentiated end-stage leukocytes, in the past their capacity to transcribe and translate new proteins had been considered to be limited. However, recent studies suggest that eosinophils can synthesize and release cytokines. According to a report by Wong et al.,² who demonstrated transforming growth factor- α messenger RNA and protein in eosinophils infiltrating cancer tissues, a number of studies have identified cytokines produced by eosinophils. A review of current literature suggests that eosinophils can transcribe and/or translate various cytokines and growth factors including IL-1 α , IL-3, IL-5, IL-6, IL-8, transforming growth factor- α , transforming growth factor- β , macrophage inflammatory protein-1 α , granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor- α .³ These studies used peripheral blood eosinophils from both normal individuals and patients with eosinophilia of various causes, as well as tissue eosin-

Abbreviations used

BAL: Bronchoalveolar lavage
GM-CSF: Granulocyte-macrophage colony-stimulating factor

ophils resident at the sites of chronic inflammation (e.g., nasal polyps and intestines involved in inflammatory bowel diseases). However, little has been reported about the eosinophil's cytokine production in allergic inflammation and the potential roles of these cytokines in acute and chronic inflammatory responses. In addition, the stimuli and regulatory mechanisms for the eosinophil's cytokine production remain enigmatic.

In this issue of THE JOURNAL, Sullivan and Broide (pages 966-76) add to our knowledge by studying a model of allergic respiratory inflammation. This model involves the induction of allergic inflammation in the lungs of patients with asthma by segmental endobronchial allergen challenge, followed by bronchoalveolar lavage (BAL). Sullivan and Broide attempted to determine whether BAL eosinophils express GM-CSF mRNA and synthesize this protein by using in situ hybridization and immunocytochemical techniques. Peripheral blood specimens obtained from the same patients simultaneously with BAL or after total lung inhalation challenge with allergen were used as controls. In two prior studies, Broide et al.^{4,5} detected GM-CSF by ELISA in BAL fluids of the patients after segmental endobronchial allergen challenge and localized mRNA for GM-CSF in lymphocytes and eosinophils by in situ hybridization. In their report Sullivan and Broide have extended these studies and demonstrated that a

From the Department of Immunology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

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Reprint requests: Hirohita Kita, MD, Mayo Clinic, 200 First St., SW, Rochester, MN 55905

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segmental endobronchial challenge with allergen, but not diluent, induced a significant increase in the number of BAL cells (eosinophils and lymphocytes) that reacted with the GM-CSF RNA riboprobe and stained with an anti-GM-CSF monoclonal antibody. In contrast, peripheral blood eosinophils, either after inhalation allergen challenge or segmental endobronchial allergen challenge, showed no reactivity with these probes. Thus together with their prior observations, the current report adds substantial evidence to the notion that eosinophils infiltrating the lungs after the endobronchial allergen challenge produce GM-CSF.

These observations address two important issues concerning the pathophysiology of bronchial asthma and the regulatory mechanism of cytokine production by eosinophils. A key question regarding bronchial asthma is why, in some individuals, the acute asthma attack, rather than resolving, develops into chronic lung inflammation and impaired lung function. This question is particularly germane in cases of intrinsic bronchial asthma, and the study by Sullivan and Broide may provide an answer. We know that eosinophils likely play important roles in bronchial asthma by elaborating lipid mediators and toxic cationic proteins.¹ Furthermore, various functions of eosinophils are regulated by cytokines. For example, GM-CSF activates and enhances eosinophil leukotriene production and degranulation.⁶ GM-CSF also inhibits apoptosis and enhances longevity of eosinophils.⁷ In addition, eosinophils exposed to GM-CSF exhibit an enhanced chemotactic response to peptides and cytokines, the "priming effect" of GM-CSF.⁸ Therefore by considering the report by Sullivan and Broide, we can speculate that once eosinophils have infiltrated the sites of allergic inflammation, they produce GM-CSF. In turn, this GM-CSF enhances the recruitment of other eosinophils into the inflamed tissue and amplifies the effector functions of the eosinophils at sites of inflammation and those of the newly recruited eosinophils. The self-perpetuating nature of bronchial asthma may be explained by this autocrine secretion of GM-CSF by eosinophils.

Although recent studies suggest that eosinophils are capable of elaborating a number of cytokines,³ little has been reported regarding the regulatory mechanism or mechanisms for their cytokine production. Most previous studies have focused on already "activated" eosinophils, such as eosinophils from tissues and peripheral blood from patients with eosinophilia. What causes normal or

blood eosinophils to become activated and to produce cytokines is largely unknown; interferon- γ ⁹ and lipopolysaccharide¹⁰ are the only known soluble stimuli for GM-CSF production by eosinophils. In their report Sullivan and Broide have shown that GM-CSF is produced by BAL eosinophils after allergen challenge and that GM-CSF production is compartmentalized to the lungs and does not occur in the blood. However, this difference between lung and blood eosinophils could be explained by the different methods used in cell preparation; BAL cells were examined without further manipulation, and blood eosinophils were examined after several isolation steps through a Percoll gradient (Pharmacia, Uppsala, Sweden) centrifugation and a magnetic cell separation system for at least 3 hours. Nonetheless, if the authors' observations are correct, they suggest that eosinophils are activated *in vivo* for GM-CSF production while they are migrating from the circulation into the lungs. These observations are in agreement with a previous report by Sedgwick et al.,¹¹ who demonstrated that BAL eosinophils achieved a different stage of cellular activation compared with blood eosinophils after endobronchial allergen challenge. The signals that induce eosinophil activation in the lungs are not known; however, the local milieu created by various inflammatory cells and mediators is likely playing a key role; potential stimuli could be pulmonary vascular endothelial cells, bronchial epithelial cells, extracellular matrix proteins, cytokines, and mediators from inflammatory cells. In this regard, an *in vitro* study by Anwar et al.,¹² showing that eosinophils adherent to fibronectin (an extracellular matrix protein) synthesized and released GM-CSF, is intriguing. Thus eosinophils binding to vascular endothelium and migrating through the extracellular matrix proteins toward the inflammatory sites could express GM-CSF through their interaction with matrix proteins. Needless to say, considering its biologic action on eosinophils, IL-5, one of the predominant eosinophil-active cytokines in allergic reactions,¹³ would likely also be participating in the reaction. The regulation of cytokine production by eosinophils at the sites of allergic inflammation is clearly an important issue and remains to be elucidated further. Segmental endobronchial challenge with allergen, followed by BAL, will be a useful tool with which to address this issue.

There are several questions remaining before eosinophils are definitely recognized as cytokine-producing cells, however. To date, more than half

the studies of eosinophil cytokine production, including the report by Sullivan and Broide, have used in situ hybridization or immunohistochemistry to localize specific probes on eosinophils. Furthermore, a recent report demonstrated the localization of immunoreactive GM-CSF on the cores of eosinophil crystalloid granules by immunogold staining and electron microscopy.¹⁴ However, the specificities of these techniques need to be validated carefully because eosinophils contain extremely basic proteins including major basic protein, eosinophil peroxidase, and eosinophil cationic protein; and these proteins might nonspecifically bind to the probes. In fact, fluorescence-conjugated immunoglobulins do bind nonspecifically to tissue eosinophils.¹⁵ Similarly, nonspecific binding has been observed with nucleotide probes.¹⁶ In this regard, it is important to note that more than 20 enzymes and substances have been associated with eosinophils by immunochemical techniques; and some of them including mRNA, catalase, serine:pyruvate aminotransferase, and acyl coenzyme A oxidase have been localized onto the matrix and crystalloid core of eosinophil granules.¹⁷ Because the crystalloid core is likely a single substance, it is difficult to reconcile the localization of so many different substances to it, suggesting a potential risk of nonspecific binding of various probes to eosinophils or eosinophil granules. Furthermore, we have recently observed that purified eosinophil peroxidase and major basic protein produced false-positive results in some of the commercially available ELISA kits, especially when *o*-phenylenediamine was used as an enzyme substrate (H. Kita, Unpublished observations). Therefore to carefully evaluate the production of cytokines by eosinophils, one must bear in mind the biochemical characteristics of eosinophils. The immunochemical techniques need to be validated by a competition assay with authentic cytokines. Furthermore, the expression of mRNA could also be confirmed by using conceptually different techniques, such as Northern blot analysis and reverse-transcription polymerase chain reaction.

In this regard, discrepancies in the observations of Sullivan and Broide in this issue of *THE JOURNAL* and in previous reports by the same group^{4,5} raise concerns. In the 1991 report, they found that after allergen challenge, the majority of GM-CSF mRNA-positive cells, as determined by in situ hybridization, were lymphocytes, with some contributions by macrophages.⁴ Although eosinophils constituted 37% of total BAL cells, eosinophils were not recognized as GM-CSF mRNA-positive cells.

In contrast, in the 1992 report, they claimed that 45% of BAL eosinophils were positive for GM-CSF mRNA. In addition, in their current report (pages 966-76), eosinophils and mononuclear cells are described as a group of cells that produce GM-CSF.⁵ It is difficult to understand these inconsistent results for eosinophils being GM-CSF mRNA-positive in one report and not in another. Indeed, the patient groups and bronchoprovocation procedures including the kinds and dosages of allergen and times of BAL seem to be similar in all three studies. However, the techniques used to detect cytokine mRNA in eosinophils are complicated and vulnerable to small changes in experimental conditions. Therefore caution may be needed to interpret these observations, as well as those of others regarding eosinophil cytokine production.

To date, a number of studies have claimed that cytokines are produced by eosinophils³; however, few studies have examined the physiologic importance of these eosinophil-derived cytokines. In this regard, several questions need to be addressed. For example, are the cytokines produced by eosinophils biologically active? How do the quantities of cytokines released from eosinophils compare with the quantities produced by immune cells specialized in cytokine production, such as lymphocytes and monocytes? So far, among the various cytokines made by eosinophils, GM-CSF and IL-3 are the only ones that have been detected on the basis of bioactivity.^{12,18} The amounts of cytokines produced by eosinophils also appear to have intriguing results. Peripheral blood eosinophils produced half as much IL-3, but only a twentieth of the IL-8, compared with the amounts produced by peripheral blood mononuclear cells.^{18,19} On the other hand, in vivo, eosinophils may be preferentially recruited into the airways and may constitute up to 70% of the infiltrating cells in allergic inflammation.¹¹ In such circumstances the relatively small quantities of cytokines produced by each eosinophil could collectively result in large amounts of cytokines. In addition, cytokines released within the small space surrounding the cell itself or cytokines bound and presented on the surface of the cells, the so-called "paracrine" or "intercrine" cytokines, may possess a strong capacity to regulate functions of other cells, especially other eosinophils. Another question regarding the physiologic relevance is whether eosinophils produce cytokines as primary cells, which are directly responding to exogenous stimuli without the help of other cells, or as secondary responders, which can produce cytokines only after primary respond-

ers (e.g., lymphocytes) have produced inflammatory mediators. At least in a murine model, lymphocytes likely play a primary role in cytokine production because depletion of CD4⁺ cells or anti-IL-5 treatment completely abrogated airway eosinophilia.²⁰ Finally, at the site of allergic inflammation, a localized milieu may be created so that cytokine production from eosinophils is optimized.

By virtue of recent advances in immunology and molecular biology, the information regarding the identities of cytokines produced by eosinophils has been rapidly increasing. In the future, these cytokines need to be evaluated by in vitro studies with isolated eosinophils and pure agonists to understand both the potential for eosinophils to produce these cytokines and the regulatory mechanism for their production. At the same time, in vivo studies need to be done to evaluate the physiologic significance of these eosinophil-derived cytokines. The report by Sullivan and Broide in this issue of THE JOURNAL potentially provides insight into the cytokines produced by eosinophils in allergic diseases. Production of cytokines by the eosinophil, an end-stage cell, is an attractive hypothesis because it adds another dimension to the cytokine network in allergic inflammation. This hypothesis needs to be carefully evaluated through both in vivo and in vitro studies before the eosinophil is recognized as a real "cytokine-producing cell."

Hirohito Kita, MD
Mayo Clinic
200 First St., SW
Rochester, MN 55905

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