

Eokines: Synthesis, Storage and Release from Human Eosinophils

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Eosinophils are prominent inflammatory cells in asthma and other allergic disorders, as well as in helminthic parasite infections. Recently, eosinophils have been reported to synthesize and store a range of regulatory proteins within their secretory granules (eokines). Eokines comprise a group of cytokines, chemokines, and growth factors which are elaborated by eosinophils. These proteins, and the messages which encode them, appear to be identical to those produced by lymphocytes and other tissues. Interestingly, immunoreactivity to many of these eokines has been found to co-localize to the eosinophil's secretory granules. In this review, we have discussed the repertoire of 18 eokines so far identified in eosinophils, and focused on four of these, namely, interleukin-2 (IL-2), IL-4, granulocyte/macrophage colony-stimulating factor (GM-CSF), and RANTES. These four eokines co-localize to the crystalloid granules in eosinophils, as shown in studies using subcellular fractionation and immunogold labeling in electron microscopy. During stimulation by physiological triggers, for example, with serum-coated particles, eosinophils release these mediators into the surrounding supernatant. In addition, eokines are likely to be synthesized within eosinophils rather than taken up by endocytosis, as show in detection of mRNA for each of these proteins using in situ hybridization, RT-PCR, and in the case of RANTES, in situ RT-PCR. Eokines synthesis and release from eosinophils challenges the commonly held notion that these cells act downstream of key elements in immune system, and indicate that they may instead belong to the afferent arm of immunity.

Key words: cytokines - chemokines - interleukin-2 - interleukin-4 - granulocyte/macrophage-colony-stimulating factor - RANTES

Since its early discovery, it has been difficult to describe clearly defined immunological roles for the eosinophil. To compound this problem, eosinophils have been frequently overlooked in their potential pathophysiological functions in studies of immunology and human disease. The eosinophil was originally believed to mediate an immunomodulatory function in acute phases of anaphylaxis by the action of released histaminase, which was thought to reduce histamine-induced inflammation brought on by mast cell degranulation. Later it was realized that eosinophils were critically important in helminthic parasite infections, although it was still uncertain as to whether they were engaged in self-protection or parasite destruction. In the last few years, workers in the field of eosinophil research have witnessed a dramatic transformation in the understanding of the pathophysiological function of eosinophils. It has become apparent that eosinophils may be capable of

direct proinflammatory reactions in atopic disease, through the release of toxic lipid and granule mediators following stimulation. Eosinophil-derived mediators can have potent effects on surrounding tissues in both *in vitro* and *in vivo* studies, including stimulation of further degranulation from mast cells and neighbouring eosinophils, deposition of cytotoxic granule major basic protein (MBP) in lung tissue, and epithelial desquamation. These events feature strongly in atopic asthma, and have led to speculation that the eosinophil may be a primary cell type involved in the initiation of atopic disease and its exacerbations.

Eosinophils have a newly discovered capacity to express and store a range of regulatory proteins in their secretory granules (eokines). These mediators are cytokines, chemokines, and growth factors. The potential ability of the eosinophil to produce these molecules has led to a fundamental reappraisal of the role of this cell in regulation of inflammatory reactions in atopic and parasitic diseases, and may hopefully shed more light on the function of eosinophils in immunity. The purpose of this review is to examine the repertoire of eokines so far identified in eosinophils, and to focus on the potential contribution of some of these

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eosinophil-derived molecules towards the pathology of eosinophil-related atopic inflammation and disease.

CYTOKINES, CHEMOKINES, AND GROWTH FACTORS IDENTIFIED IN HUMAN EOSINOPHILS

Cytokines, chemokines, and growth factors are rapidly synthesized in high concentrations upon stimulation of a wide range of tissues, including T cells, B cells, and other mononuclear cells. Granulocytic cells, including eosinophils, are also capable of synthesizing many of these inflammatory and chemotactic mediators. Following the discovery by del Pozo and co-workers in 1990 that murine peritoneal eosinophils express IL-1 α mRNA and release its translated product following stimulation by LPS, it has since been realized that not only are eosinophils capable of producing these regulatory mediators, but they also store many of them, often in association with their characteristic crystalloid secretory granules. Some of these stored cytokines can be released upon *in vitro* activation of eosinophils, suggesting that secretion of cytokines may occur by regulated exocytosis (as opposed to constitutive exocytosis). The Table shows cytokines, chemokines, and growth factors so far described in human eosinophils. The detection methods used for these mediators include immunocytochemistry, *in situ* hybridization, and *in situ* PCR on tissue sections, and ELISA assays on cells and supernatants.

SYNTHESIS AND STORAGE OF EOKINES

Eosinophils have so far been shown to express message and/or protein for at least 18 different cytokines, chemokines, and growth factors (Table). Since the quantity of these exceeds the scope of this review, we will focus on the possible mechanisms of synthesis, storage, and release of a selected group of eokines.

Eosinophils have been demonstrated to store interleukin-2 (IL-2), IL-4, GM-CSF, and possibly RANTES in their crystalloid granules (Figs 1, 2). The crystalloid granule, unique to these cells, is a membrane-bound organelle composed of a crystalline core (internum) which is almost entirely composed of the highly charged cationic major basic protein (MBP). Surrounding this core is the matrix, a liquid phase which contains concentrated amounts of three further cationic proteins among a plethora of other granule enzymes and proteins. These are eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPO). Many chemokines, cytokines, and growth factors co-localize to the matrix of the granules, although some have been reported to associate with the core.

Interleukin-2 - IL-2 is known to be an essential growth factor for T cells, and eosinophils respond by chemotaxis to this cytokine (Rand et al. 1991). An average of $6.8 \pm 0.4\%$ of freshly isolated, unstimulated peripheral blood eosinophils from

TABLE

Human eosinophil-derived cytokines, chemokine, and growth factors

Mediator detected in human eosinophils	Molecule detected	Quantity of protein stored (per 10^6 cells)	Release factors	Intracellular localization of stored protein	Reference
A. Cytokines					
Interleukin-1 α	mRNA protein	-	PMA	-	Weller et al. (1993)
Interleukin-2	mRNA protein	6 ± 2 pg	Serum-coated particles, PHA	Crystalloid granules (core)	Levi-Schaffer et al. (1996) Bossé et al. (1996)
Interleukin-3	mRNA protein	-	Ionomycin IFN γ	-	Kita et al. (1991) Fujisawa et al. (1994)
Interleukin-4	mRNA protein	108 ± 20 pg	Immune complexes Serum-coated particles	Crystalloid granules (core)	Nonaka et al. (1995) Moqbel et al. (1995) Möller et al. (1996a) Nakajima et al. (1996)
Interleukin-5	mRNA protein	-	Immune complexes	Crystalloid granules (core/matrix?)	Desreumaux et al. (1992) Broide et al. (1992) Desreumaux et al. (1993) Dubucquoi et al. (1994) Möller et al. (1996b)
Interleukin-6	mRNA protein	-	IFN γ	-	Hamid et al. (1992) Melani et al. (1993)

Mediator detected in human eosinophils	Molecule detected	Quantity of protein stored (per 10 ⁶ cells)	Release factors	Intracellular localization of stored protein	Reference
Interleukin-10	mRNA protein	-	[Constitutively released]	-	Nakajima et al. (1996)
Interleukin-16	mRNA protein	-	[Constitutively released]	-	Lim et al. (1996)
Interferon- γ (IFN γ)	mRNA	-	-	-	Lamkhioued et al. (1996)
Tumor necrosis factor- α (TNF- α)	mRNA protein	-	Immune complexes TNF α , LPS	-	Beil et al. (1993) Costa et al. (1993) Tan et al. (1993) Takanaski et al. (1994) Nakajima et al. (1996)
Granulocyte/Macrophage Colony-Stimulating Factor (GM-CSF)	mRNA protein	15.1 \pm 0.3 pg	Ionomycin LPS, IFN γ , Immobilized fibronectin, anti-CD40, anti-CD9	Crystalloid granules (core)	Moqbel et al. (1991) Kita et al. (1991) Ohno et al. (1991) Broide et al. (1992) Anwar et al. (1993) Takanaski et al. (1994) Levi-Schaffer et al. (1995) Ohkawara et al. (1996) Kim et al. (1997)

B. Chemokines

Interleukin-8	mRNA protein	-	C5a, fMLP GM-CSF - RANTES or PAF, Immune complexes TNF α , LPS	-	Braun et al. (1993) Takanaski et al. (1994) Miyamasu et al. (1995) Yousefi et al. (1995) Nakajima et al. (1996)
Macrophage Inflammatory Protein-1 α (MIP-1 α)	mRNA protein	-	-	-	Costa et al. (1993)
RANTES	mRNA protein	7.0 \pm 0.5 ng	Serum-coated particles, IFN γ	-	Ying et al. (1996)

C. Growth Factors

Transforming Growth Factor- α (TGF α)	mRNA protein	-	-	-	Wong et al. (1990) Elovic et al. (1994)
Transforming Growth Factor- β 1 (TGF- β 1)	mRNA protein	-	-	-	Wong et al. (1991) Ohno et al. (1992) Kadin et al. (1993) Ohno et al. (1996)
Platelet-Derived Growth Factor, B chain (PDGF-B)	mRNA	-	-	-	Ohno et al. (1995)
Heparin-Binding Epidermal Growth Factor-Like Binding Protein (HB-EGF-LBP)	mRNA	-	-	-	Powell et al. (1993)

- : not yet determined.

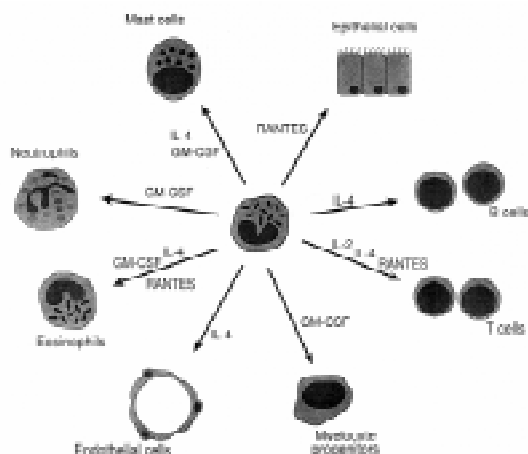


Fig. 1: potential targets for eosinophil-derived IL-2, IL-4, GM-CSF, and RANTES.

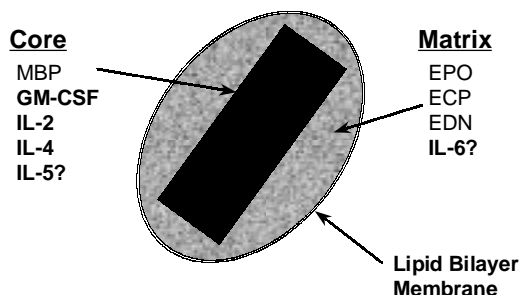


Fig. 2: localization of eosinophil-derived cytokines to the crystalloid granule.

atopic asthmatics exhibited granular IL-2 staining using immunocytochemistry (Levi-Schaffer et al. 1996). The percentage of IL-2⁺ blood eosinophils was elevated to 36 ± 8% in severe atopic asthmatics (Bossé et al. 1996). When cells were subjected to *in situ* PCR followed by *in situ* hybridization, IL-2 mRNA could be detected which was increased during incubation with GM-CSF and/or A23187. Freshly prepared, disrupted, unstimulated eosinophils store an average of 6 ± 2 pg/10⁶ cells of IL-2 as measured by ELISA, while eosinophils incubated with serum-coated Sephadex beads increased their content of IL-2 to 26 ± 7 pg of IL-2/10⁶ cells (Levi-Schaffer et al. 1996). Furthermore, using subcellular fractionation, IL-2 co-eluted with granule enzyme marker activities (MBP, ECP, EPO, arylsulfatase B, and β-hexosaminidase). However, as this procedure does not discriminate between core and matrix-associated granule proteins, immunogold labeling with a monoclonal antibody to IL-2 was carried out to determine the precise location of its storage.

Using electron microscopy on labeled sections, immunoreactivity to IL-2 co-localized to the core of secretory granules in freshly prepared unstimulated blood eosinophils. So far, IL-2 has only been identified in peripheral blood eosinophils, and no reports have appeared describing IL-2 immunoreactivity in eosinophils from tissue sections of biopsies obtained from atopic individuals.

A small amount of IL-2 release (2 ± 1 pg/10⁶ cells) can be stimulated by incubating serum-coated Sephadex beads with eosinophils freshly isolated from three out of six atopic asthmatic subjects (Levi-Schaffer et al. 1996). The potential physiological effects of eosinophil-derived IL-2 is likely to be of a juxtacrine or autocrine nature. Eosinophils themselves respond to IL-2 by chemotaxis and express the IL-2 receptor (CD25) (Rand et al. 1991). Another possible action of IL-2 released from eosinophils may be induction of T cell growth and activation at a local tissue level, which may act in conjunction with eosinophil-derived IL-4 to drive the response towards a Th2 phenotype. However, the bioactivity of eosinophil-derived IL-2 awaits further study.

Interleukin-4 - IL-4 is regarded as a critical cytokine in initiation and maintenance of the allergic phenotype, and its effects include switching of T cells to the Th2 subset as well as B cells to the IgE-producing phenotype (Del Prete et al. 1988, Mosmann & Coffman 1989). It also exhibits chemotactic activity towards eosinophils (Dubois et al. 1994). By a combination of RT-PCR and Southern blot analysis of reverse-transcribed eosinophil mRNA, IL-4 mRNA has been detected in highly purified blood eosinophils from atopic asthmatics (Moqbel et al. 1995). An average of 22 ± 5% of these cells were found to be positive for IL-4 immunoreactivity using immunocytochemical staining, which was granular in appearance. Measurement of IL-4 by ELISA assay on lysed, unstimulated eosinophils yielded an average of 108 ± 20 pg/10⁶ cells of IL-4 inside the cells. Although the number of IL-4⁺ eosinophils in cytopins of bronchoalveolar lavage (BAL) cells from atopic asthmatics was not significantly different from those of nonatopic normal controls, the majority of eosinophils infiltrating allergen-induced cutaneous late-phase (6-hr) reactions were immunoreactive for IL-4 (84 ± 4%). The work of Nonaka and colleagues (1995) has shown that while IL-4⁺ eosinophils could not be detected in the bronchial mucosa of nonasthmatics, some IL-4⁺ eosinophils could be detected in both diluent-challenged and allergen-challenged tissues of mild atopic asthmatics. Furthermore, immunostained sections of nasal polyp tissues showed that as many as 44% of eosinophils were positive for IL-4. Highly puri-

fied peripheral blood eosinophils from normal subjects constitutively express IL-4 mRNA and its product in the absence of external stimuli, although no released IL-4 protein could be detected in supernatants even in the presence of strong physiological stimuli (TNF α and/or immobilized secretory IgA; Nakajima et al. 1996). Interestingly, this study showed that exogenous IL-5 enhanced levels of stored IL-4 in purified eosinophils from normal subjects. In immunogold studies, IL-4 has been localized to the crystalloid core compartment of the secretory granules in eosinophils from peripheral blood (Moqbel et al. 1995) and bronchial biopsies of atopic asthmatics (Möller et al. 1996a). These reports have extended the observation of IL-4⁺ eosinophils in peripheral blood to tissue eosinophils from atopic subjects, and suggest that IL-4 is synthesized *in vivo* by eosinophils as a part of their pathophysiological function.

By incubation of blood eosinophils for 45 min with serum-coated Sephadex beads *in vitro*, IL-4 release could be detected in supernatants at 34 ± 6 pg/10⁶ cells (Moqbel et al. 1995). Nonaka and co-workers (1995) also demonstrated IL-4 release from eosinophils, detecting 47 ± 21 pg/10⁶ cells in supernatants of peripheral blood eosinophils incubated with immobilized secretory IgA for 18 hr. These amounts are physiologically relevant, and suggest that eosinophils could potentially provide an initial, rapidly released reservoir of IL-4 for switching naïve precursor Th cells to the Th2 phenotype in local allergic and inflammatory reactions. In support of this notion, mice infected intraperitoneally with *Schistosoma mansoni* eggs displayed an early rise in IL-4 which was derived from peritoneal eosinophils, and which was also responsible for the subsequent priming of naïve T cells in the peritoneum (Sabin et al. 1996). Other potential actions of eosinophil-derived IL-4 include enhancement of local IgE production, upregulation of VCAM-1 receptors on endothelial cells, and local chemotaxis of nearby eosinophils. These interesting scenarios await further investigation to determine the physiological role of eosinophil-derived IL-4.

Granulocyte/Macrophage Colony-Stimulating Factor - Eosinophils have been demonstrated to transcribe message for GM-CSF and store its products within their intracellular secretory granules. GM-CSF is a maturation and growth factor for myeloid progenitors (including eosinophils) and is also involved in priming, activation, and prolongation of the survival of mature eosinophils. In peripheral blood eosinophils from atopic asthmatics, GM-CSF mRNA was found to be expressed and upregulated in response to IFN γ or A23187 using *in situ* hybridization (Moqbel et al. 1991). Co-localization of GM-CSF mRNA to eosinophils

was achieved by a combination of *in situ* hybridization and staining with chromotrope 2R or EG2 immunoreactivity. Activated eosinophils were also observed to display specific immunoreactivity with an anti-GM-CSF monoclonal antibody. The transcript for GM-CSF has also been detected in normal peripheral blood eosinophils (Nakajima et al. 1996). In tissue sections, GM-CSF mRNA has been detected in eosinophils in association with nasal polyposis (Ohno et al. 1991), in which approximately 30% of infiltrating eosinophils expressed this transcript. Eosinophils staining positive for GM-CSF mRNA have also been detected in BAL cells obtained from asthmatic subjects after endobronchial allergen challenge (Broide et al. 1992). In unstimulated eosinophils from atopic asthmatic subjects, the mean concentration of stored GM-CSF was 15.1 ± 0.3 pg/10⁶ cells (Levi-Schaffer et al. 1995). Following subcellular fractionation of unstimulated eosinophils across a linear gradient, GM-CSF was found to co-elute almost exclusively with marker granule proteins and enzymes. Immunogold staining with anti-GM-CSF showed preferential localization of gold particles with the cores of eosinophil secretory granules, suggesting that eosinophil GM-CSF is stored as a preformed mediator rather than being immediately released after *de novo* synthesis.

GM-CSF can be released from peripheral blood eosinophils during *in vitro* incubation of cells with ionomycin or LPS (Kita et al. 1991, Takanaski et al. 1994). Ionomycin-induced release of GM-CSF resulted in the release of 45 ± 20 pg/10⁶ cells in supernatants of cultured eosinophils (Kita et al. 1991), while LPS induced the release of 23 ± 8 pg/mL of GM-CSF from 5×10^5 freshly isolated peripheral blood eosinophils (Takanaski et al. 1994). LPS failed to stimulate significant GM-CSF release from a maximal number of potentially contaminating mononuclear cells or neutrophils, demonstrating that the LPS-induced release of GM-CSF is exclusively from eosinophils in these preparations. The significance of GM-CSF synthesis and release from eosinophils is that this cytokine may act in an autocrine and/or paracrine manner to prime eosinophils and enhance their survival *in vivo*. Priming of eosinophils would lead to enhanced exocytosis of eosinophil granule proteins, which mediate a number of inflammatory actions and tissue injury. In this manner, eosinophil-derived GM-CSF may prolong the allergic late phase response in which eosinophils are continually activated in a self-exacerbating cycle of inflammation.

RANTES - Eosinophils have also been shown to be capable of synthesizing and storing the potent CC chemokine RANTES. RANTES is chemotactic for eosinophils, basophils, monocytes/mac-

rophages, CD4⁺/CD45RO⁺ memory T cells, and mast cells, with maximal potency for eosinophils and memory T cells (Schall et al. 1990, Kameyoshi et al. 1992, Baggiolini & Dahinden 1994, Taub et al. 1995). Highly purified peripheral blood eosinophils from atopic asthmatics have been shown to express mRNA for RANTES by RT-PCR (Ying et al. 1996), which has been confirmed by others (Lim et al. 1996). The presence of RANTES transcript was detected by *in situ* hybridization, and 4-17% of blood eosinophils were found to be positive for RANTES mRNA, which increased to 22-30% following *in vitro* stimulation with IFN γ (1000 U/ml for 16 hr) (Ying et al. 1996). Immunoreactivity to RANTES was detected in 4-13% of unstimulated cells, consistent with translation of mRNA for RANTES. Stimulation of eosinophils with IFN γ also enhanced the percentage of cells expressing RANTES protein to 11-20%. Incubation of eosinophils with ionomycin did not increase the percentage of cells expressing RANTES mRNA; however, it did increase the percentage of cells positive for RANTES immunoreactivity (to 6-21%). In eosinophils from normal, healthy subjects, RANTES mRNA was upregulated following 3 hr of stimulation with immobilized immunoglobulins or TNF α (Nakajima et al. 1996). The capacity of human eosinophils to synthesize and translate mRNA encoding RANTES in association with allergic inflammation has also been demonstrated in allergen-induced late-phase cutaneous reactions in atopic subjects (Ying et al. 1996). Unstimulated blood eosinophils from atopic individuals have approximately 7.0 ± 0.5 ng/10⁶ cells of RANTES stored inside them as determined by ELISA. It has not yet been established where RANTES is stored within eosinophils, but preliminary data suggest that it may be localized to the small granules.

The release of RANTES may be evoked by serum-coated Sephadex beads from purified blood eosinophils. An average of 1.7 ± 0.2 ng/10⁶ cells of RANTES was released into supernatants of *in vitro*-stimulated eosinophils as detected by ELISA (Ying et al. 1996). The apparently high concentrations of RANTES released in supernatants and from lysed cells is probably artefactual, and likely to be due to a contaminating serum component. Recent experiments in our laboratory using more stringently washed serum-coated surfaces has shown that approximately 150-500 pg/10⁶ cells of RANTES is usually released under these conditions.

RANTES is the only eokine which has been reported to have biological effects on other cells; eosinophil-derived RANTES has been shown to exhibit chemotactic activity towards other eosinophils *in vitro*, and this effect was inhibited by a

mean of 68% by a specific anti-RANTES antibody (Ying et al. 1996). The release of RANTES from tissue eosinophils during inflammatory responses may serve as a potent chemotactic signal for other tissue eosinophils, circulating eosinophils, and memory T cells. It is interesting to note in this context that infiltration of eosinophils and memory T cells into lung tissues is a prominent feature of human asthma, and may indicate a primary role for RANTES in asthmatic inflammation.

CONCLUSIONS

The detection of mRNA for each of the eokines described here suggests that eosinophils possess the capacity to synthesize these molecules themselves, rather than endocytosing them from the surrounding milieu. Furthermore, density gradient centrifugation of homogenized unstimulated eosinophils has shown that the buoyant density at which cytokine immunoreactivity sedimented was greater than that of endosomes, suggesting storage of cytokines within the very dense crystalloid granules.

Given that eosinophils are apparently capable of producing so many cytokines, chemokines, and growth factors, it should be remembered that the protein detection methods used so far to analyse eokine expression and storage are all based on antibody recognition. Eosinophils are probably the most difficult cells in the body to analyse with specific antibody binding methods, as they contain highly concentrated pockets of positively charged granule proteins that attach nonspecifically to antibodies. Thus, many of the protein detection results so far reported for eosinophils have to be taken on board with a degree of caution, as some of these may represent false positive results due to nonspecific antibody binding. It is therefore mandatory that a carefully chosen blocking regime is employed to prevent false positive results. Similar problems have been encountered with the use of riboprobes, which can adhere to the same granule proteins to give an erroneous positive signal. The use of RT-PCR has helped to overcome uncertainty regarding message expression in eosinophils, but this is also a finicky method due to its inherent randomness in sequence detection and the difficulty of obtaining 100% pure eosinophil preparations. If cytokine expression is to be fully evaluated in eosinophils, it will be essential to investigate the bioavailability and bioactivity of eokines. The study of eokine bioactivity is currently in progress in several laboratories including our own, and new insights into the functioning of eosinophil-derived molecules will hopefully eventuate in the next few years.

From the findings so far, eosinophils exhibit a remarkable degree of biological versatility and heterogeneity. Stored cytokines in eosinophils may

serve as a rapidly mobilizable reservoir of inflammatory mediators in allergic reactions association with eosinophils, in contrast to T cells and other cells which do not store these compounds. The expression of these molecules by eosinophils suggest that these cells may play an afferent role in the immune system, since eosinophils can express MHC Class II and present antigen to T cells (Del Pozo et al. 1992, Weller et al. 1993) and produce IL-4 upon activation (Moqbel et al. 1995, Nonaka et al. 1995). It is likely that the actions of many eosinophil-derived cytokines are mediated by putative paracrine, autocrine, or juxtacrine signalling mechanisms, since the quantities of cytokines released are low in comparison to levels achieved by *in vitro* stimulation of T cells. However, local release of cytokines would suggest that a number of interesting scenarios occur in tissue inflammation. The release of highly concentrated packets of cytokines in close proximity to target cells may result in a cascade of inflammatory responses as part of an amplification process. In this manner, the eosinophil may be involved in amplifying or modulating the local allergic response in asthma, thus contributing to tissue damage, while on the other hand it may promote putative protective immune reactions, as in helminthic infections. At the other end of the spectrum, it is possible that in spite of the circumstantial evidence in favour of a possible effector role for this cell, eosinophil cytokine production may be a redundant process in immune and inflammatory events. However, as mentioned earlier, murine studies of eosinophil-derived IL-4 suggest a role for this mediator in mounting a Th2 response in reaction to helminthic infection (Sabin et al. 1996). This observation awaits further investigations using human tissues.

These findings have brought about exciting times for eosinophil research. The eosinophil is rapidly becoming the focus of attention in atopic disease. There is now a large body of evidence available showing strong clinical correlation between increasing numbers of eosinophils infiltrating lung tissue and severity of disease in asthma. Sadly, the rate of asthma affliction is rising steeply in many countries around the world. As we discover more about the immunobiology of eosinophils, we may hopefully increase the number of ways of treating atopy, and develop new treatment regimes for these clinical conditions.

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