

EDITORIAL

New concepts in effector functions of eosinophil cytokines

During the past decade, the field of allergic inflammation, particularly with regard to asthma, has witnessed a dramatic shift in perspectives concerning the mechanisms underlying this increasingly common disorder. The eosinophil, once considered an evolutionary redundancy in the immune response armamentarium against parasitic worm infections, has staged a comeback in the immunology of allergic inflammation as a critical component of this response. These inflammatory cells were identified as major effector cells in tissue allergic reactions that are recruited and activated in response to interleukin-4 (IL-4) and IL-5 associated with TH₂-type T-cells. Originally thought of as a 'bystander' cell, the eosinophil is now being recognized for its ability not only to generate up to 25 newly synthesized cytokines, chemokines and growth factors [1], but also to store these products in its unique crystalloid granules and small secretory vesicles in advance of their rapid release (in minutes) following stimulation. The recognition that eosinophils can produce cytokines, some of which have been demonstrated to exhibit bioactivity *in vitro*, has led to a significant re-evaluation of their potential role in allergic inflammation (Fig. 1). This is especially evident with regard to prolongation of allergic responses, activation of local inflammatory cells, and induction of further inflammatory cell infiltration from the blood into mucosal tissues such as the airways. This review seeks to understand the potential contribution of newly described eosinophil-derived cytokines to the allergic response, as well as the details of *de novo* production, storage and release of these immune regulatory molecules in developing and mature eosinophils.

New kids on the block

The capacity of the eosinophil to generate cytokines, chemokines and growth factors has been previously reviewed [1]. In particular, at least four new cytokines/growth factors have been described and characterized in human eosinophils in the last year. These are nerve growth factor (NGF), LIF, interferon- γ (IFN γ) and IL-9, which have a significant potential to modulate inflammatory processes in atopic disorders. In the case of NGF, this growth factor has recently been shown to be elevated in serum in a spectrum of allergic disorders, including asthma, rhinitis and urticaria-angioedema, with its largest increase observed in asthma [2]. Originally characterized for its

ability to stimulate neuronal cells, NGF has now been demonstrated to exert a pronounced effect on stimulation of T-cells, B-cells and granulocyte differentiation and proliferation [3–5]. In addition to its release from mast cells and B-cells [6], eosinophils have also been shown to express NGF mRNA and protein, and respond to NGF stimulation by secreting eosinophil peroxidase (EPO) [7]. What would be particularly interesting to explore is the possibility that eosinophil-derived NGF may have a role in influencing parasympathetic nerve function in the lungs, since eosinophils are observed to collect around nerve fibres in airway mucosa during allergic responses in animal studies [8].

Another possible neuroimmune substance elaborated by eosinophils is the cytokine LIF, a member of the IL-6 family. LIF gene expression is upregulated in airway mucosa following inflammatory challenge and augments contractility in explants from airways [9]. In the recent study by Zheng *et al.*, it was shown that eosinophils from both non-atopic and atopic individuals express LIF and LIF receptor mRNA and protein [10]. Similarly to the effects of NGF, LIF was able to induce moderate EPO secretion from eosinophils. Collectively, these findings demonstrate that eosinophils have the capacity to stimulate neural as well as immune tissues within the airway mucosa, potentially contributing to enhanced airway responsiveness to injury or challenge.

A hypothesis has recently been advanced suggesting that eosinophils, like T-cells, may express helper 1 and helper 2-like properties, since they were demonstrated to express IFN γ , IL-2 and IL-10 [11], which were shown to be stored intracellularly. Following activation of CD28 molecules on their surfaces by antibody-dependent cross-linking, eosinophils were found to release IL-2 and IFN γ into supernatant fluids. This was inhibited following concurrent incubation with secretory IgA, which, on its own, was able to induce secretion of IL-10. However, although it is highly significant that IL-2 and IFN γ expression has been characterized in resting and stimulated eosinophils, other laboratories have failed to detect measurable amounts of secreted IL-2 from stimulated eosinophils [12, M. Laviollette, personal communication], while no IFN γ mRNA expression was detected in eosinophils from normal donors [13]. In addition, only negligible quantities of IL-2 and IFN γ immunoreactivity were detected in flow cytometry analysis of eosinophils from spleen and granulomas

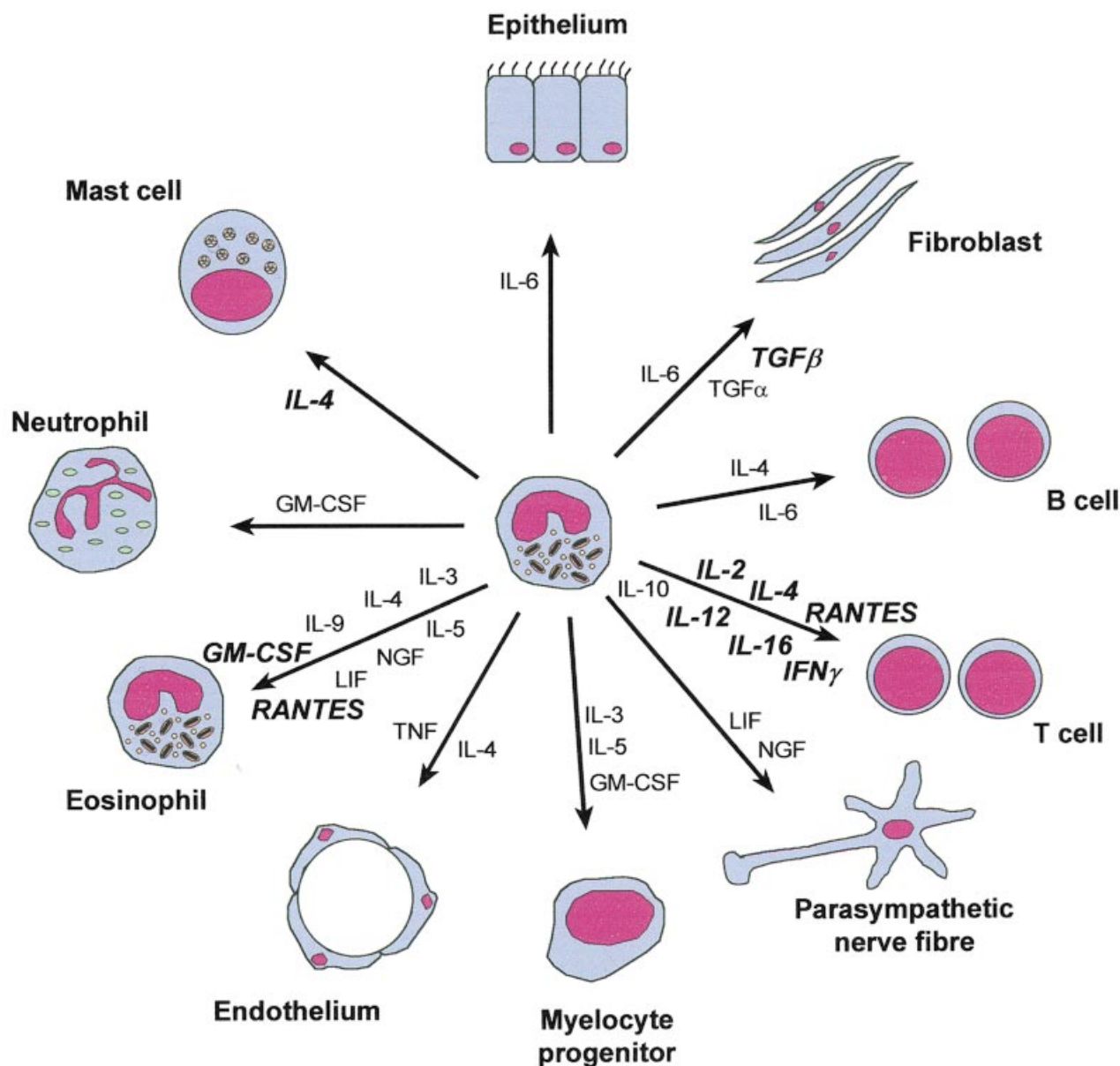


Fig. 1. Potential targets of eosinophil-derived cytokines. Cytokines shown in ***bold italics*** have been demonstrated to exert a biological effect on selected target tissues *in vitro*. Other cytokines are shown in plain text, which have not yet been demonstrated to exhibit bioactivity and/or bioavailability.

obtained from *Schistosoma mansoni*-infected mice [14]. It is clear that further experimental work will be necessary to reconcile these contrasting but interesting findings.

Recently, the TH₂ cytokine IL-9 was implicated as an important component of allergic responses, as suggested by its ability to enhance T-cell proliferation, IgE production from B-cells and haematopoietic progenitor proliferation and differentiation. In addition, T-cell-derived IL-9 has been demonstrated to be upregulated in airway mucosal cells in asthma along with other TH₂-type cytokines,

namely, IL-4, IL-5 and IL-13 [15]. Eosinophils also possess the capacity to express mRNA for IL-9 and IL-9 receptor [15]. These findings indicate that eosinophil-derived IL-9 may play an autocrine role in eosinophil function, as well as in enhancement of TH₂-type responses in the tissue.

Synthesis and storage of cytokines during maturation

An important question arising from these findings in mature peripheral blood and tissue eosinophils is: do

developing eosinophils have the ability to express these molecules? And, if they are capable of producing these, when does the expression of such immune mediators appear during their maturation?

In a recent study, we demonstrated that IL-3 and IL-5-induced maturing eosinophils generated *in vitro* by the use of the semi-solid culture medium, methylcellulose, do indeed possess the capacity to express cytokines during their maturation. These findings are in agreement with those of others showing that eosinophils produced from peripheral blood-derived progenitors obtained from atopic individuals are able to express IL-5 and GM-CSF while developing in culture [16]. IL-6 and RANTES protein do not appear to be expressed in freshly purified CD34⁺ cells, but were expressed in developing eosinophils after 16 days of culture [17]. Immunoreactivity to these cytokines co-localized with that of eosinophil granule major basic protein (MBP), and was localized to large structures resembling immature granules on days 16 and 19. These large, apparently immature granules later appeared to undergo condensation into mature granules on day 23 and, particularly, day 28 of culture. At later stages of development, MBP no longer co-localized with IL-6 or RANTES and began to appear within the cores of granular structures [17]. In parallel with this study, we also observed that freshly prepared CD34⁺ cells express IL-4 and RANTES mRNA, which continued to be detected during IL-3 and IL-5-induced eosinophil development in culture [Velazquez *et al.* in press]. These developing eosinophils did not appear to express increasing amounts of transcripts during their maturation *in vitro* and instead, produced progressively less RANTES mRNA in comparison with the housekeeping gene transcript encoding β 2-microglobulin [Velazquez *et al.* in press]. In contrast, IL-4 and RANTES protein expression was abundant in these cells by day 21 of culture and was shown to be expressed in granular structures positive for MBP immunoreactivity. These findings suggest that changes in transcript levels in developing eosinophils do not necessarily correlate with the appearance of translated products.

Similarly, the presence of RANTES gene transcript is detectable but very low in mature peripheral blood eosinophils [18]. The stability of the transcript encoding RANTES in peripheral blood eosinophils suggests a turnover of message approximately every 4 h. This turnover may be accelerated during IFN γ stimulation, which appears to be due to increased rate of translation of a pool of RANTES gene transcripts in these cells, determined by the use of the translational inhibitor, cycloheximide [18]. The proposed increased rate of translation is in agreement with our earlier observation that IFN γ rapidly induced RANTES mobilization and release from eosinophils [19]. Therefore, the detection of relatively low amounts of gene transcripts in eosinophils does not correlate with low levels

of protein expression in these cells, and may instead be indicative of accelerated gene transcription and translation leading to an abundance of products.

Membrane trafficking and release of cytokines

Eosinophils have been recognized for their ability to store preformed cytokines, chemokines and growth factors intracellularly as a prelude to their release. Over half of all these substances appear to be stored within the large crystalloid granules, mainly in the granule matrix, although a few (GM-CSF, IL-2, IL-4 and potentially, IL-5) have been localized to the core of the granule by immunogold labelling [19]. Interestingly, eosinophils also possess a separate population of small secretory vesicles which have so far been identified as being positive for RANTES [19] and possibly TGF α [20]. These secretory vesicles migrate at a lower buoyant density than that of crystalloid granules following equilibrium sedimentation on Nycodenz gradients, indicating that they belong to a separate intracellular compartment. In addition, these small secretory vesicles are highly sensitive to stimulation by IFN γ and are rapidly mobilized in less than 30 min to secrete RANTES to the outside of the cell. The crystalloid granules, which also contain RANTES immunoreactivity in their matrix, released this chemokine more slowly in response to IFN γ , while the bulk of MBP immunoreactivity remained localized in their cores. Thus, the MBP-containing crystalloid granules do not appear to mobilize towards the plasma membrane during IFN γ stimulation [19]. These observations suggest that the small, rapidly mobilizable population of secretory vesicles may be 'shuttling' RANTES from the crystalloid granules to the exterior. The mechanisms underlying this mobilization are currently under investigation in our laboratory.

Bioactivity and bioavailability of eosinophil-derived cytokines

Many eosinophil-derived cytokines have been shown to exert bioactivity *in vitro* following their release or by the use of eosinophil sonicates (Fig. 1). Earlier studies have shown that eosinophil-derived IL-4, GM-CSF, IL-12, IL-16 and RANTES are all capable of inducing bioactive effects on neighbouring mast cells, eosinophils and/or T-cells [1]. The release of IL-4 from eosinophils is thought to be important in driving the initiation of a TH₂-type response during *Schistosoma mansoni* infection of mice [21]. In human studies, eosinophils have also been demonstrated to express mRNA for IL-4 and IL-5 at early stages of inflammation (6 h following allergen-induced late nasal responses), as shown in the elegant study by Nouri-Aria *et al.* in this issue [22]. These findings suggest that

eosinophils could have a more important role in establishing and maintaining a TH₂-type response than previously appreciated.

More recent findings have implicated a number of newly described roles for other eosinophil-derived cytokines, particularly IL-2, IFN γ and TGF β . The bioactivity of IL-2 and IFN γ was demonstrated by the ability of supernatant fluids from CD28-stimulated eosinophils to stimulate proliferative activity in an IL-2-dependent cell line, and IFN γ -induced MHC Class II expression on Colo 205 cells, respectively [11]. These findings suggest that IL-2 and IFN γ are both bioactive and bioavailable upon stimulation of eosinophils. The implication of this study is that eosinophils may potentially harbour cytokines which can influence the status of T-cell activation in inflammation, as well as exerting antiviral effects. The authors also suggested that eosinophils may contain cross-regulatory signals allowing them to release TH₁- vs TH₂-type cytokines. However, further studies are required to confirm these observations, particularly given their rather controversial nature.

Another recent study has shown that sonicates prepared from eosinophils exhibit a significant stimulatory effect on lung- and skin-derived fibroblasts in culture [23]. Eosinophil sonicates were shown to induce mitogenesis and lattice retraction in lung and dermal fibroblasts, although they only stimulated collagen production from dermal fibroblasts. The mitogenic and lattice retraction effects were partially reversed by the addition of antibody to TGF β . However, this study did not include an effect by anti-TGF β on collagen synthesis by fibroblasts, which would have been of benefit to the conclusion that eosinophil-derived TGF β induces collagen synthesis from dermal fibroblasts. In addition, although a bioactive role for TGF β was indicated based on these findings, it was not established whether eosinophil-derived TGF β might be bioavailable during inflammatory responses. In any case, these observations suggest that eosinophil-derived TGF β may play a repair role in inflammation by promoting fibroblast growth and activation. Eosinophils may also contribute to airway remodelling by exerting their effects on the extracellular matrix, and cause structural abnormalities such as stromal fibrosis and basement membrane thickening in asthmatic inflammation. These findings also corroborate earlier studies showing that eosinophils express mRNA encoding TGF β ₁ in the airways which are upregulated in asthma [24].

Future directions

These recent observations add further evidence that eosinophils not only synthesize and store important inflammatory cytokines, but that they also produce them at early stages of development; additionally, almost a third of these have so far been shown to be bioactive, at least *in*

vitro. Eosinophil-derived cytokines have been demonstrated to be released in a selective, piecemeal manner which correlates with *in situ* evidence showing that the majority of tissue eosinophils undergo piecemeal degranulation during upper airway allergic inflammation [25]. Cytokines are released from eosinophils in response to physiological stimuli, indicating that they may play a multitude of inflammatory roles to presumably mediate the immune response in an autocrine and/or juxtacrine manner during allergy. Although eosinophils generally produce and release a smaller amount of these cytokines than related immune and tissue cells, their overwhelming infiltration and presence during allergic exacerbation suggests that eosinophil-derived cytokines may exert a cumulative effect on the surrounding tissue due to their abundance. Evidence for the direct contribution of eosinophil-derived cytokines and chemokines to allergic responses remain circumstantial, as is the evidence for all other inflammatory cells in allergic inflammation. More work is needed, focused on dissecting the precise immunobiological and functional dimensions of eosinophil-stored and -released cytokines, to underscore the relevance of cytokines elaborated by eosinophils during allergy and asthma.

References

- 1 Lacy P, Moqbel R. Eosinophil cytokines. In: Marone G. ed. *Human Eosinophils: Biological and Clinical Aspects*. Basel, Switzerland: Karger AG, 2000: 134–55.
- 2 Bonini S, Lambiase A, Bonini S et al. Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc Natl Acad Sci USA* 1996; 93:10955–60.
- 3 Thorpe LW, Perez-Polo JR. The influence of nerve growth factor on the *in vitro* proliferative response of rat spleen lymphocytes. *J Neurosci Res* 1987; 18:134–9.
- 4 Otten U, Ehrhard P, Peck R. Nerve growth factor induces growth and differentiation of human B lymphocytes. *Proc Natl Acad Sci USA* 1989; 86:10059–63.
- 5 Matsuda H, Coughlin MD, Bienenstock J, Denburg JA. Nerve growth factor promotes human hemopoietic colony growth and differentiation. *Proc Natl Acad Sci U S A* 1988; 85:6508–12.
- 6 Levi-Montalcini R, Skaper SD, Dal Toso R, Petrelli L, Leon A. Nerve growth factor: from neurotrophin to neurokin. *Trends Neurosci* 1996; 19:514–20.
- 7 Solomon A, Aloe L, Pe'er J et al. Nerve growth factor is preformed in and activates human peripheral blood eosinophils. *J Allergy Clin Immunol* 1998; 102:454–60.
- 8 Costello RW, Schofield BH, Kephart GM, Gleich GJ, Jacoby DB, Fryer AD. Localization of eosinophils to airway nerves and effect on neuronal M2 muscarinic receptor function. *Am J Physiol* 1997; 273:L93–103.
- 9 Knight D, McKay K, Wiggs B, Schellenberg RR, Bai T. Localization of leukaemia inhibitory factor to airway epithelium

- and its amplification of contractile responses to tachykinins. *Br J Pharmacol* 1997; 120:883–91.
- 10 Zheng X, Knight DA, Zhou D et al. Leukemia inhibitory factor is synthesized and released by human eosinophils and modulates activation state and chemotaxis. *J Allergy Clin Immunol* 1999; 104:136–44.
 - 11 Woerly G, Roger N, Loiseau S, Dombrowicz D, Capron A, Capron M. Expression of CD28 and CD86 by human eosinophils and role in the secretion of type 1 cytokines (interleukin 2 and interferon γ): inhibition by immunoglobulin A complexes. *J Exp Med* 1999; 190:487–95.
 - 12 Levi-Schaffer F, Barkans J, Newman TM et al. Identification of interleukin-2 in human peripheral blood eosinophils. *Immunology* 1996; 87:155–61.
 - 13 Nakajima H, Gleich GJ, Kita H. Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. *J Immunol* 1996; 156:4859–66.
 - 14 Rumbley CA, Sugaya H, Zekavat SA, El Refaei M, Perrin PJ, Phillips SM. Activated eosinophils are the major source of Th2-associated cytokines in the schistosome granuloma. *J Immunol* 1999; 162:1003–9.
 - 15 Shimbara A, Christodopoulos P, Soussi-Gounni A et al. IL-9 and its receptor in allergic and nonallergic lung disease: increased expression in asthma. *J Allergy Clin Immunol* 1999; 104:108–15.
 - 16 Gauvreau GM, O'Byrne PM, Moqbel R et al. Enhanced expression of GM-CSF in differentiating eosinophils of atopic and atopic asthmatic subjects. *Am J Respir Cell Mol Biol* 1998; 19:55–62.
 - 17 Mahmudi-Azer S, Velazquez JR, Lacy P, Denburg JA, Moqbel R. Immunofluorescence analysis of cytokine and granule protein expression during eosinophil maturation from cord blood-derived CD34⁺ progenitors. *J Allergy Clin Immunol* 2000; 105:1178–84.
 - 18 Velazquez JR, Lacy P, Moqbel R. Replenishment of RANTES mRNA expression in activated eosinophils from atopic asthmatics. *Immunology* 2000; 99:591–9.
 - 19 Lacy P, Mahmudi-Azer S, Bablitz B et al. Rapid mobilization of intracellularly stored RANTES in response to interferon- γ in human eosinophils. *Blood* 1999; 94:23–32.
 - 20 Egesten A, Calafat J, Knol EF, Janssen H, Walz TM. Subcellular localization of transforming growth factor- α in human eosinophil granulocytes. *Blood* 1996; 87:3910–8.
 - 21 Sabin EA, Kopf MA, Pearce EJ. *Schistosoma mansoni* egg-induced early IL-4 production is dependent upon IL-5 and eosinophils. *J Exp Med* 1996; 184:1871–8.
 - 22 Nouri-Aria KT, O'Brien F, Noble W, Jacobson MR, Rajakulasingam K, Durham SR. Cytokine expression during allergen-induced late nasal responses: IL-4 and IL-5 mRNA is expressed early (at 6 hours) predominantly by eosinophils. *Clin Exp Allergy* 2000; 30 (BSLtocomplete).
 - 23 Levi-Schaffer F, Garbuzenko E, Rubin A et al. Human eosinophils regulate human lung- and skin-derived fibroblast properties *in vitro*: a role for transforming growth factor β (TGF- β). *Proc Natl Acad Sci USA* 1999; 96:9660–5.
 - 24 Minshall EM, Leung DY, Martin RJ et al. Eosinophil-associated TGF- β 1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997; 17:326–33.
 - 25 Erjefalt JS, Greiff L, Andersson M et al. Allergen-induced eosinophil cytolysis is a primary mechanism for granule protein release in human upper airways. *Am J Respir Crit Care Med* 1999; 160:304–12.

R. MOQBEL
P. LACY

*Pulmonary Research Group
Department of Medicine
574 HMRC University of Alberta
Edmonton
Alberta
Canada
T6G 2S2*