Clin Exp Immunol 1999; **116**:1–3

REVIEW

Eotaxin and asthma: some answers, more questions

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(Accepted for publication 2 September 1998)

Cytokines and chemokines are characterized by their pleiotropicity and redundancy of function. Furthermore, any one cytokine will generally induce the release of one or more others. Consequently, in any inflammatory process it is usually straightforward to establish excessive production of a wide range of these inflammatory mediators. Discovery of a new cytokine or chemokine is typically followed by a flurry of scientific activity as researchers seek to establish its presence and possible role in their own particular disease. Such searches are seldom disappointing. What is more difficult in these situations is to identify the 'key players', so that a cogent and ultimately successful therapeutic approach can be formulated. The chemokine eotaxin has been suggested to be such a key player in the pathogenesis of asthma. It is the purpose of this brief review to examine the evidence for this proposition.

Eotaxin was first identified as an activity appearing in bronchoalveolar lavage fluid of guinea pigs in the course of antigen-induced pulmonary eosinophil infiltration which caused specific accumulation of indium-labelled eosinophils when injected into guinea pig skin [1,2]. The cDNA encoding the purified eotaxin protein was subsequently cloned [3]. Soon afterwards the human homologous eotaxin cDNA and genomic sequences were identified [4–6]. The human gene is located on chromosome 17 and comprises of three exons and two introns. The 5′-flanking region of the gene contains a number of consensus regulatory elements including binding sites for AP-1, NF-κB, NFIL-6, interferon-gamma (IFN-γ) response element and the glucocorticold receptor, suggesting possible regulation of expression of the gene by cytokines as well as glucocorticoids. Human eotaxin appears to be constitutively expressed, at least at the level of mRNA, at detectable concentrations in a variety of human tissues, and at relatively high concentrations in the small intestine and colon. The significance of this observation is not clear. Another recently described chemokine has rather confusingly been named eotaxin-2, not because of any close structural homology with eotaxin (in fact the molecules share only 39% of identical amino acids) but because it appears so far to have a spectrum of activities identical to those of eotaxin, although it is somewhat less potent [7,8].

Eotaxin is a member of the CC family of chemokines, so called because of their conserved two N-terminal cysteine residues (although natural eotaxin preparations seem to contain N-terminally truncated forms missing two or three amino acids [9]). The genes encoding these chemokines are clustered on chromosome 17. The hallmark of the CC chemokines in general is their ability to chemoattract and activate inflammatory leucocytes, particularly lymphocytes, monocytes, eosinophils and basophils, as well as some stromal cells such as endothelial and smooth muscle cells [10]. An important, and probably related function is their ability to induce diapedesis of cells across vascular endothelium, a function of obvious relevance to the propagation of tissue inflammation. A final notable effect of some of the CC chemokines, including eotaxin, is their ability to cause IgE-independent degranulation of basophils [11]. These activities of CC chemokines are mediated through a family of receptors belonging to the 7 transmembrane-spanning (serpentine) superfamily. At present, nine CC chemokine receptors (CCR1–9) have been described, and it seems likely that further receptors (as well as further CC chemokines) will be discovered, especially using techniques such as expression sequence tag screening [8]. In general, any particular CC chemokine can bind to several CC receptors. Eotaxin (and eotaxin-2) are exceptional in this regard, since they bind only to the CCR3 receptor. Thus far, only a limited range of cells has been found to express CCR3: eosinophils, basophils, 'Th2-type' T cells and microglial cells in the central nervous system [11–15]. Thus, only eotaxin and eotaxin-2 can chemoattract and activate these target cells specifically. While other CC chemokines (MCP-2, MCP-3, MCP-4 and RANTES) may exert a similar range of effects on these target cells by binding to CCR3 [12], these chemokines are less specific in the sense that they also bind to CC chemokine receptors other than CCR3 on a wider range of target cells.

Asthma is associated with chronic, T cell-mediated inflammation of the bronchial mucosa characterized by a striking and relatively specific influx of eosinophils [16]. Eosinophil products (granule basic proteins and membrane-derived lipid mediators) are believed to cause much of the bronchial mucosal damage which is thought ultimately to give rise to clinical symptoms. Release of mast cell and basophil products, IgE-mediated or otherwise, is additionally thought to cause acute exacerbation of asthma symptoms, e.g. after acute exposure to allergens or after exercise. One fundamental aspect of asthma research has been to determine the mechanism of this specific eosinophil influx. Local release of cytokines from 'Th2-type' activated T cells (particularly IL-5, which acts exclusively on eosinophils and their committed precursors, but also IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) which act on eosinophils but less specifically) is thought to be pivotal to this process, since these cytokines (and IL-5 specifically) can promote maturation of eosinophils in the bone marrow, increased adherence of eosinophils to vascular endothelium, and priming, activation and prolonged survival of these cells in the tissues [16]. One function not

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clearly attributable to these cytokines is the induction of diapedesis of cells across vascular endothelium. This role is potentially ideally filled by the CC chemokines. In particular, eotaxin and eotaxin-2 may attract eosinophils, basophils and 'Th2-type' T cells into tissues, and specifically so via interaction with the CCR3 receptor. It is little wonder, then, that these molecules have become one of the current icons of the asthma researcher.

There exists considerable evidence in support of this cooperative scenario between eotaxin and IL-5 in the mechanism of specific eosinophil influx in the tissues from studies on animals. Thus, in guinea pigs accumulation of eosinophils in the skin following subcutaneous injection of eotaxin was time-dependently augmented by concomitant i.v. infusion of IL-5, which released eosinophils from the bone marrow into the circulation [17]. IL-5 alone, when given subcutaneously, did not cause local eosinophil accumulation. Eotaxin, when injected directly into the arterial supply of the guinea pig femoral bone marrow, caused a rapid and selective release of eosinophils into the draining vein [18]. Eosinophil progenitor cells, which have recently also been shown to express CCR3 [19], were also released, an effect not observed with IL-5. Eotaxin administered subcutaneously to IL-5 gene-deleted mice failed to evoke local eosinophil accumulation unless exogenous IL-5 was administered concurrently [20]. The mechanisms by which eotaxin evokes this 'two-way traffic' of eosinophils (into tissues but out of the bone marrow) are as yet poorly defined, but these may at least partly involve interactions of β_1- and β_2-integrin adhesion molecules (CD11b/CD18, VLA-4) on eosinophils with counter-receptors (intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)) on local endotlielial cells [21–24]. Interestingly, IL-4 may also attract eosinophils through secondary eotaxin release [9,24].

What of human asthma? Two recent studies [25,26] have demonstrated elevated numbers of cells expressing eotaxin mRNA and protein in the bronchial mucosa of atopic asthmatics compared with controls. Eotaxin mRNA expression correlated positively with local eosinophil numbers and inversely with disease severity. One of these studies [25] in addition showed CCR3 expression on local infiltrating eosinophils. These studies are consistent with the hypothesis that eotaxin, through its specific action on CCR3, plays a role in the specific recruitment of eosinophils to the asthmatic bronchial mucosa, a phenomenon which in turn regulates asthma severity. The potential cellular sources of eotaxin in inflammatory reactions are beginning to be delineated. One study referred to above [25] clearly identified both epithelial and endothelial cells as the principal cells expressing eotaxin mRNA in the asthmatic bronchial mucosa. Similarly, human epithelial cell lines have been shown to release eotaxin *in vitro* after cytokine (tumour necrosis factor-alpha (TNF-α), IL-1β, IFN-γ) stimuli [27]. Human [26] as well as animal [28] studies implicate T cells as a probable potential source, while eosinophils themselves have been shown to secrete the eotaxin in response to IL-3 stimulation [29]. Skin fibroblasts secrete eotaxin in response to TNF-α and IL-4 stimulation [9].

To return to the question posed at the beginning of this article, it seems reasonable from these data to suggest that eotaxin may play a key role in asthma pathogenesis. Reservations remain, however. First, knowledge of the entire repertoire of CC chemokines and their receptors is probably incomplete. It is always possible that further CC chemokines with a spectrum of activities similar to eotaxin will be discovered (in this regard, eotaxin-2 is already with us). Second, little or nothing is known about the molecular mechanisms which result in eotaxin release in inflammatory processes in general and asthma in particular. It is possible that a knowledge of these mechanisms will be more revealing than simply a knowledge of the existence of eotaxin itself. Finally, owing to the redundancy of action of chemokines, it is always possible that targeting of one particular chemokine in a disease process will not be particularly effective since other chemokines may assume similar roles. This may explain why, in a model of antigen-induced pulmonary eosinophil accumulation in mice, targeted deletion of the eotaxin gene reduced, but did not abolish, pulmonary cosinophil infiltration [30]. Having said this, targeting of the CCR3 receptor would seem to be a much more promising therapeutic proposition in asthma, since this would block the effects not only of eotaxin and eotaxin-2, but also of those other CC chemokines known to bind to this receptor. Further advances in this direction are eagerly awaited.

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