Eosinophilia, activated eosinophils and human schistosomiasis

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Introduction

Eosinophils are proinflammatory cells that are associated with both allergic diseases and parasitic infections. In parasitic infections the eosinophil appears to be protective, but in allergic conditions eosinophil products are damaging and contribute to the tissue pathology of the disease. A particular property of eosinophils is that they can, like macrophages, exist in different states of activation. This has important consequences for the eosinophil in both its protective and pathological roles. In this commentary we concentrate on the protective role of eosinophils in helminth infections.

Eosinophilia

Helminth infections are commonly associated with eosinophilia. An increase in the number of circulating eosinophils is commonly observed in individuals infected with either Schistosoma mansoni or with S. haematobium. Granulomata from liver biopsy samples containing eggs of S. mansoni are infiltrated with eosinophils (Kephart et al. 1988). Baboons infected for 8 months with S. mansoni respond to a challenge by infective cercariae of S. mansoni with an eosinophilic reaction in the epidermis at the site of penetration (Seitz et al. 1987). Experiments in mice have shown that eosinophils kill schistosomula (larvae) in the skin at the site of infection (von Lichtenberg et al. 1976). Therefore local and systemic eosinophilia occur in schistosomiasis.

Local eosinophil accumulation can be induced by antigen–antibody complexes (Parish, 1972) and may involve eosinophil chemotactic factors such as PAF, LTD4, histamine and ECF-A (Wardlaw et al. 1986), which are secreted by sensitised mast cells. Both systemic and granulomatous eosinophilia appear to be T lymphocyte-dependent (Basten and Beeson, 1970). Granuloma formation in mice is prevented by antibody to CD4 lymphocytes. Eosinophil production is controlled by three T lymphocyte products: II-3, the pluripotent colony-stimulating factor (Yang et al. 1986), GM-CSF, a colony-stimulating factor for granulocytes and macrophages (Metcalf et al. 1986), and II-5, an eosinophil specific colony-stimulating factor (Sanderson et al. 1985). GM-CSF is secreted additionally by other cell types, including macrophages and endothelial cells. In mice, II-5 is produced by the Th2 subset of CD4 T cells, while II-3 and GM-CSF are produced by both Th1 and Th2 subsets (Cherwinski et al. 1987). Treatment of mice with antibody to II-5 abrogates both peripheral blood and granulomatous eosinophilia in mice infected with S. mansoni (Sher et al. 1990). Lymphocytes from acutely infected mice produce large quantities of II-5 in response to schistosome egg antigen.

Eosinophils may have a protective and/or a pathological role in disease. In allergic respiratory diseases the eosinophil is a proinflammatory cell that releases mediators that sensitize and damage bronchial tissue and induce bronchoconstriction and mucus secretion (Brujinzzel, 1989). In helminth infections there is evidence that the eosinophil has a protective function. Eosinophils isolated from peripheral blood kill schistosomula of S. mansoni (Butterworth et al. 1975, Vadas et al. 1979) and S. haematobium (Hagan et al. 1985a) in the presence of immune serum in vitro. S. mansoni killing is mediated by IgG1 and IgG3 (Khalife et al. 1989) and by IgE (Capron et al. 1984), and is blocked by IgG4 and by IgM (Khalife et al. 1986). Eggs of S. mansoni and of S. haematobium are also damaged by eosinophils in vitro (James and Colley, 1976; Sher et al. 1980). Purified eosinophil granule proteins MBP and ECP kill schistosomula of S. mansoni in vitro (Ackerman et al. 1985). Extracellular MBP has been localised by immunofluorescence on S. mansoni eggs in liver granuloma (Kephart et al. 1988).

Attempts to correlate eosinophil numbers with resistance to schistosomiasis have given variable results. In a study in the Gambia resistance to infection with S. haematobium was shown to correlate with blood eosinophil counts (Hagan et al. 1985b). Rates of reinfection with S. mansoni in a group of Kenyan children cured by chemotherapy were significantly reduced if the children had both detectable antibody and eosinophil counts above 400 mm$^{-3}$ (Sturrock et al. 1983). However, in a later study it was found that protection from reinfection with S. mansoni did not correlate with peripheral blood eosinophil counts (Butterworth et al. 1985). This latter lack of correlation with the single parameter of eosinophilia may be a reflection of the complexities of the system. Not only is there a requirement for IgG1, IgG3 or IgE for

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eosinophil-mediated cytotoxicity and blocking by IgM or by IgG4, but also eosinophils can exist in different states of activation.

**Activated eosinophils**

Peripheral blood eosinophils from different donors differ markedly in their cytotoxic activity and some correlation of activity with eosinophilia has been observed (David et al. 1980; Hagan et al. 1985b). Eosinophils with enhanced cytotoxic activity are described as 'activated'. They exhibit marked changes in biological activity over unactivated eosinophils, in a manner analogous to macrophage activation.

Eosinophils from children infected with *S. haematobium* frequently exhibit enhanced antibody-dependent cytotoxic activity (Hagan et al. 1985b) and therefore appear to be activated. Eosinophils from donors of high density exhibit IgG1, IgG2- or IgG3-dependent killing of schistosomula of *S. mansoni*. Eosinophils from donors of low activity require prior activation; the cytotoxic activity being mediated by IgG1 and IgG3, but inhibited by IgG2 (Khalife et al. 1989). A population of eosinophils of low density has been isolated from patients with idiopathic hypereosinophilia (HES) (Bass et al. 1980), and also from individuals with filarial infections (Khalife et al. 1986). They differ from normal-density eosinophils in mediating IgE instead of IgG-dependent cytotoxicity to schistosomula of *S. mansoni* (Capron et al. 1984). They have a reduced content of granule enzymes (Winqvist et al. 1982; Khalife et al. 1986) and reduced ability to produce toxic metabolites of oxygen (Prin et al. 1984). Morphologically HES low density eosinophils have a normal spherical shape but small granules (Peters et al. 1988), while activated normal-density eosinophils are distorted in shape, with extensive lamellipodia but with normal granules (Butterworth and Thorne, 1988).

**Eosinophil activation in vitro**

Enhancement of IgG-mediated cytotoxic activity of normal-density eosinophils can be achieved in vitro by incubation with soluble secretion products from mononuclear cells (Veith and Butterworth, 1983; Dessein et al. 1984) or with serum (Khalife et al. 1989) from patients with moderate eosinophilia. A number of component have now been identified in the secretion products of both lymphocytes and monocytes that possess the ability to enhance eosinophil cytotoxic activity. Three groups of activating factors have been described: colony stimulating factors, chemotaxins and activating factors. While all these factors enhance eosinophil cytotoxic activity, they differ somewhat in the spectrum of properties that they enhance (Table 1). The colony-stimulating factors IL-3, GM-CSF and IL-5 not only induce eosinophilopoiesis but also enhance cytotoxic activity of mature eosinophils (Dessein et al. 1982; Sanderson et al. 1985; Lopez et al. 1987). Properties of eosinophils that are enhanced by the colony stimulating factors are: survival in culture (Owen et al. 1987), adherence to plasma-coated glass, plastic and endothelial cells (Walsh et al. 1990a) and surface expression of the α-chain of the adherence molecule CR5 (CD11b) (Thorne et al. 1990). Degranulation is increased as shown by conversion of ECP from the stored to the secreted form (Tai and Spry, 1990) and increased IgA-induced release of EDN (Fujisawa et al. 1990). Production of LTC4 after triggering with the Ca ionophore A23187 is increased (Rothenburg et al. 1988; Silberstein et al. 1986) as is production of the toxic reduced oxygen metabolite superoxide (Vadas et al. 1983; Lopez et al. 1987; Lopez et al. 1988). Activation of eosinophils by colony-stimulating factors is associated with a change in shape and some loss of cell density (Lopez et al. 1986).

Five of the numerous substances known to be chemotactic for eosinophils have also been shown to exhibit eosinophil-activating activity. These are the lipid mediator PAF (Moqbel et al. 1990a), the tetrapeptides ECF-A, histamine (Anwar et al. 1980), LTB4 (Moqbel et al. 1983) and a basic glycoprotein HILDA from alloreactive T cells stimulated with specific antigen and IL-2 (Moreau et al. 1987; Goddard et al. 1988). PAF is the most potent of the chemotactic factors. It enhances both IgG-mediated (Walsh et al. 1990b), and IgE-mediated (Moqbel et al. 1990a) killing of schistosomula of *S. mansoni*. It increases adherence of eosinophils to cultured endothelial cells (Kimani et al. 1988; Lamas et al. 1988) and to plasma-coated glass (Walsh et al. 1990a), producing flattened elongated cells of reduced density (Yukawa et al. 1989). Expression of both CD11b and the IgE receptor CD23 are enhanced on the cell surface (Thorne et al. 1990; Tanaka et al. 1989). PAF induces rapid release of granule enzymes and increases production of superoxide (Kroegel et al. 1988). It increases the production of LTC4 both spontaneously and after triggering with Ca ionophore, opsonised zymosan or IgE-coated schistosomula (Tamura et al. 1988; Moqbel et al. 1990b).

Three factors have been described that act only on mature cells and are neither eosinophil colony-stimulating factors nor chemotaxins. These factors are TNFa (Silberstein and David, 1986), EAF (Thorne et al. 1986) and ECEF (Lenzi et al. 1985). They can be separated by gel filtration and by anion-exchange chromatography. TNF has a molecular weight of 17×10^3 and pi 5.3; EAF has molecular weight 40×10^3 and pi 4.4; ECEF has monomer molecular weight 10×10^3 and pi 3.8 (Silberstein and David, 1987). TNF enhances adherence to endothelial cells and to gelatinised plastics (Lamas et al. 1988) and increases expression of the surface receptor CD11b (Thorne et al. 1990). TNF has little effect on either degranulation or production of superoxide (Whitcomb et al. 1989) or A23187-induced production of LTC4 (Silberstein and David, 1986). EAF increases IgG- but not IgE-dependent killing of schistosomula and adherence to IgG-coated schistosomula (Veith et al. 1984). It increases expression of the surface receptor molecule CD11b (Thorne et al. 1990). Both EAF and ECEF enhance eosinophil degranulation (Thorne et al. 1985; Caullfield et al. 1985), spontaneous production of superoxide (Pincus et al. 1984; Thorne et al. 1985) and triggered production of LTC4 (Dessein et al. 1990; Elsas et al. 1987; Fitzharris et al. 1986).

While most investigations of the phenomenon of eosinophil activation have concentrated on identification of individual mediators it is likely that in vivo several factors will be present together at a particular site. It is particularly interesting, therefore, that several of the factors described above act synergistically. TNF, EAF and GM-CSF act synergistically to increase the expression of CD11b (Thorne, Richardson and Mazza, unpublished results). In mice GM-CSF and IL-5 act synergistically to stimulate the chemokinesis of eosinophils. The chemo-
kinetic activity that is secreted by spleen cells from *S. mansoni* infected animals stimulated with soluble egg antigen (Colley, 1972) can only be neutralised by a combination of antibodies to GM-CSF and II-5 (Secor et al. 1990).

### Inhibitors of eosinophil activation

A further complication in the assessment of the role of activating factors in vivo is the presence of endogenous inhibitors. Silberstein and co-workers (1990) have identified a protein (molecular weight 80-100 x 10^3) in sera of donors of eosinophils that are unresponsive to TNF. This protein, ECI, inhibits both TNF- and GM-CSF-enhanced cytotoxicity. It inhibits TNF-enhanced adherence to plastic, but has no effect on PMA-induced production of H_2O_2. It is of interest that ECI was found in all serum samples, but was in a latent form in some individuals and was detectable only after purification.

IL-4 acts as an inhibitor of eosinophil function. It inhibits IgG-dependent killing of schistosomula, IgG-mediated degranulation and expression of IgG receptors (Baskar et al. 1990). It is of interest that IgE-dependent functions were unaffected, since IL-4 induces B-cells to secrete IgE in a process that is enhanced by II-5 (Pene et al. 1988).

### Table 1. Enhancement of eosinophil functions by activating factors

<table>
<thead>
<tr>
<th>Cytotoxicity</th>
<th>CSFs</th>
<th>PAF</th>
<th>EAF</th>
<th>TNF</th>
<th>ECEF</th>
</tr>
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<tbody>
<tr>
<td>Adherence</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Plasma-coated glass</td>
<td>+</td>
<td>+</td>
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<tr>
<td>IgG-coated targets</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Surface receptors</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FcεRI (CD23)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>CR3 (CD11b)</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Degranulation (spontaneous)</td>
<td>EPO</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ECP</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>MBP</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Degranulation (triggered)</td>
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<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>EDN</td>
<td>(IgG/IgA)</td>
<td>(C3b)</td>
<td>(C3b)</td>
<td>(C3b)</td>
<td>(C3b)</td>
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<tr>
<td>ECP</td>
<td>(IgG/IgA)</td>
<td>(C3b)</td>
<td>(C3b)</td>
<td>(C3b)</td>
<td>(C3b)</td>
</tr>
<tr>
<td>Reduced oxygen metabolites</td>
<td>NBT reduction</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Chemiluminescence</td>
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<td>Superoxide</td>
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<td>Lipid mediators</td>
<td>LTC_4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proliferation of precursor cells</td>
<td>+</td>
<td>(IgE/C3b)</td>
<td>(IgG)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Survival</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Chemotaxis</td>
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<td>0</td>
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<tr>
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<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reduced cell density</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+, Stimulates; 0, no effect.

### Table 2. Production of eosinophil-activating factors by different cell types

<table>
<thead>
<tr>
<th>Cell</th>
<th>Activating factor</th>
<th>Induced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes</td>
<td>EAF</td>
<td>Antigen</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>IL-3</td>
<td>Antigen, mitogen</td>
</tr>
<tr>
<td>Mast cells</td>
<td>IL-3</td>
<td>Antigen*</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>GM-CSF</td>
<td>IL-1, TNF</td>
</tr>
</tbody>
</table>

*1gE-mediated.

### Role of accessory cells in eosinophil activation

Lymphocytes, monocytes, mast cells and endothelial cells all appear to play a role in eosinophil activation (Table 2). CD4 lymphocytes secrete the colony-stimulating factors II-
3, GM-CSF and IL-5 in response to antigenic stimulation. In addition the lymphocytes have an indirect role, since they produce IL-2, which enhances IL-5 production, and γ-IFN, which enhances TNF production (Philip and Epstein, 1986) and cytotoxic activity. Monocytes secrete activating factors LAF, ECEF and TNF spontaneously or in response to antigens or mitogens. Monocyte products that play an indirect role in eosinophil activation are II-1, which enhances GM-CSF production, and TNF, which enhances production of GM-CSF and production of TNF itself. Alveolar macrophages from asthmatics secrete GM-CSF and, in the presence of LPS, TNF (Howell et al. 1983). Mast cells respond to antigenic stimuli with production of the colony-stimulating factors (Broudy et al. 1986; Munker et al. 1986) and also production of the lipid mediator PAF. Endothelial cells secrete GM-CSF and PAF (Braquet et al. 1986). Since adherence of eosinophils to endothelial cells is enhanced by GM-CSF and by PAF (Walsh et al. 1990a) local production of eosinophil-activating factors could be highly significant.

**Eosinophil activation in schistosomiasis**

Eosinophil activation in vitro has been studied extensively. Colony-stimulating factors from lymphocytes, activating factors from monocytes and the potent chemotactic factor PAF from mast cells and endothelial cells selectively enhance properties of eosinophils that contribute to their function as cytotoxic cells. The evidence that activated eosinophils play a protective role in vivo in human schistosomiasis is now only beginning to emerge. Eosinophils from a group of children infected with *S. haematobium* were tested for their cytotoxic activity (Hagan et al. 1985b). Eosinophils from subjects with high peripheral blood eosinophil counts were more active in killing *S. haematobium* schistosomula than were eosinophils from subjects with low eosinophil counts. Children with high eosinophil counts were less likely to be reinfections after treatment than children with low eosinophil counts.

Blood mononuclear cells from children infected with *S. mansoni* secrete eosinophil activating activity when incubated in vitro without further stimulation (Butterworth et al. 1986). Kerri and co-workers (1987) described an eosinophil colony-stimulating factor resembling IL-5 in serum samples from patients with helminth infections. In recent work we have demonstrated that eosinophil activating activity is present in serum samples from children infected with *S. mansoni* (Khalife et al. 1989; Mazza et al. 1990). One component of the active sera is IL-5, but the levels are too low to account for the total eosinophil activating activity present. Further work is required to identify the other activating factors and to determine their importance in resistance to schistosomiasis.

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**References**


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