Pretreatment with rh-GMCSF, but not rh-IL3, enhances PAF-induced eosinophil accumulation in guinea-pig airways

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Intraperitoneal injections of recombinant human granulocyte-macrophage colony stimulating factor (rh-GMCSF, 50 µg kg⁻¹ daily) or interleukin-3 (rh-IL3, 50 µg kg⁻¹ daily) for two days, induced an increase in the percentage of bone marrow and pulmonary airway eosinophils in the guinea-pig. In addition, rh-IL3-treated animals exhibited an increase (21%) in blood neutrophils. Exposure of guinea-pigs to an aerosol of platelet activating factor (PAF) gives rise to a selective pulmonary eosinophil accumulation, maximal at 48 h. The eosinophilic response to PAF was significantly enhanced in rh-GMCSF-treated guinea-pigs but was suppressed in rh-IL3-treated animals.

Introduction Eosinophils are closely associated with allergies and asthma, and the inflammatory nature of these cells is well established, since toxic proteins secreted by eosinophils have the capacity to cause tissue damage (Frigas & Gleich, 1987; Tai *et al.*, 1987). In the guinea-pig, platelet activating factor (PAF) induces a selective accumulation of eosinophils within the airways (Lellouch-Tubiana *et al.*, 1988; Sanjar *et al.*, 1990). However, the eosinophilic response to PAF does not approach the intensity of an allergic reaction (Hutson *et al.*, 1988), which indicates an involvement of other mediators. Since the cellular response of sensitized animals (Samter, 1949; Sanjar *et al.*, 1989) or man (Kline *et al.*, 1932; Henocq & Vargaftig, 1988), to stimuli other than antigen, is qualitatively and quantitatively different from that of normal individuals, it is likely that sensitization in some way alters the response pattern. Recent studies may provide a molecular basis for such changed behaviour, since exposure of leucocytes to recombinant cytokines *in vitro* can induce a heightened responsiveness to activation stimuli (Owen *et al.*, 1987; Dahinden *et al.*, 1988). In the present study, the ability of recombinant human granulocyte-macrophage colony stimulating factor (rh-GMCSF) or interleukin-3 (rh-IL3) to enhance the eosinophilic response to an aerosol of PAF has been investigated in the guinea-pig.

Methods Dunkin-Hartley guinea-pigs (450–650 g) were given intraperitoneal injections of saline, rh-GMCSF (50 µg kg⁻¹, daily) or rh-IL3 (50 µg kg⁻¹, daily) for 2 days. Treated animals were divided into two groups; one group was exposed to PAF and the other not; in either case lungs were lavaged 48 h later. Guinea-pigs were exposed individually to an aerosol of PAF (10 ml of a 10 µg ml⁻¹ solution in sterile saline containing bovine serum albumin (BSA) 0.25%), generated in a Devilbiss nebulizer (flow rate of 71 min⁻¹) for 1 h (Sanjar *et al.*, 1990). Blood (5 ml) was collected by cardiac puncture in sodium citrate (2.8%) from ether anaesthetized guinea-pigs prior to death. Bronchoalveolar lavage (BAL) was performed with 4 × 10 ml aliquots of Hank's balanced salt solution (HBSS) containing disodium edetate (EDTA, 10 mM), HEPES (10 mM) and BSA (0.3%). Bone marrow was collected from femurs of guinea-pigs not exposed to PAF, by inserting an 18-gauge needle into the bone lumen and flushing with 10 ml of HBSS. Blood samples were layered over an equal volume of a mixture (10/16, v/v) of Hypaque (30% w/v)/methylcellulose (1% w/v) in order to sediment erythrocytes. The leucocyte-rich layer was washed and resuspended (1 ml) in HBSS. Cyto-

spin preparations of BAL, blood and bone marrow cells were stained by Leishman's stain for differential cell counting (light microscopy × 1000) and total BAL cells were counted in a micro cell counter (model cc-180, Sysmex, Switzerland). Results are presented either as cells (× 10⁴) ml⁻¹ BAL fluid or as percentage of cell populations. Student's *t* test (two tailed) for groups of unequal size was used for statistical analyses.

Results No change in eosinophil numbers was observed in BAL with rh-GMCSF (*n* = 14) or rh-IL3 (*n* = 9) treatment as compared with saline (*n* = 10) treatment (Figure 1). However, the percentage of eosinophils was higher in rh-GMCSF or rh-IL3 groups (saline, 5.8 ± 0.8%; rh-GMCSF, 13.4 ± 1.2% *P* < 0.0005; rh-IL3, 10.1 ± 1.6% NS). Similarly there was a small increase in the number of bone marrow eosinophils (saline, 6.2 ± 1.6% *n* = 3; rh-GMCSF, 10.5 ± 0.9% *n* = 6; rh-IL3, 10.1 ± 0.9% *n* = 3). No changes in blood eosinophils were observed in the different treatment groups; however, rh-IL3-treated guinea-pigs exhibited a significantly higher neutrophil population (saline, 44.3 ± 3.6%, rh-GMCSF, 45.9 ± 2.0%; rh-IL3, 53.0 ± 3.9% *n* = 9, *P* < 0.04). Exposure of saline- or rh-GMCSF-treated guinea-pigs to an aerosol of PAF resulted in an increase of both the total eosinophil numbers (Figure 1) and in the percentage of these cells (saline, 21.1 ± 2.5% *P* < 0.005; rh-GMCSF, 31.5 ± 4.9% *P* < 0.005).

Figure 1 Number of eosinophils recovered by bronchoalveolar lavage (BAL) from saline (open columns), rh-GMCSF (solid columns) or rh-IL3 (cross-hatched columns)-treated guinea-pigs either with (a) no PAF challenge or (b) 48 h after exposure to an aerosol of PAF (10 µg ml⁻¹). * indicates statistical significance (*t* test) of *P* < 0.001 when comparing PAF-exposed groups with guinea-pigs not exposed to PAF.

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Furthermore, the response of rh-GMCSF-treated animals was significantly greater ($P < 0.04$) than saline-treated controls (Figure 1). No increases in the percentage or numbers of eosinophils was observed in rh-IL3-treated animals following exposure to PAF (Figure 1).

Discussion This study demonstrates that administration of recombinant human cytokines (rh-GMCSF or rh-IL3) to guinea-pigs can lead to changes in leucocyte populations in blood, bone marrow or airways, when doses higher than those normally used to treat patients with leukopenia are given. However, the capacity of rh-GMCSF to increase the eosinophilic response of the guinea-pig to aerosolised PAF is of greater importance, since such an enhanced reactivity to a variety of stimuli is a feature of sensitized guinea-pigs (Samter,

1949; Sanjar *et al.*, 1989) or man (Kline *et al.*, 1932; Henocq & Vargaftig, 1988). These observations imply that exposure to cytokines, such as GMCSF, during sensitization may be responsible for the exaggerated eosinophil response. *In vitro* experiments reinforce such a proposal (Owen *et al.*, 1987). Under similar experimental conditions, and despite a similar increase in the percentage of airway eosinophils, rh-IL3-treated guinea-pigs did not respond to PAF. An increase in blood neutrophils may explain this phenomenon, since infected animals which have high blood or BAL neutrophils, do not give an eosinophilic response either to PAF or to antigen (unpublished observations). The study of allergic mediators in primed cells or animals may lead to greater understanding of cellular responses in disease processes.

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