*Brief Definitive Report*

# Regulation of Parasite-induced Eosinophilia: Selectively Increased Interleukin 5 Production in Helminth-infected Patients

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## Summary

Production of the eosinophilogenic cytokines interleukin 3 (IL-3), granulocyte/macrophage colony-stimulating factor (GM-CSF), and IL-5 by mitogen-stimulated peripheral blood mononuclear cells was compared between 11 noneosinophilic individuals and seven patients with helminth-induced eosinophilia. Both the kinetics and quantities of IL-3 and GM-CSF were similar in the two groups. In contrast, IL-5 production at both the protein and the mRNA level was markedly greater in the eosinophilic patients, an observation suggesting that IL-5 may be particularly important in mediating the selective eosinophilia seen in filarial and other helminth infections.

Eosinophilia is a characteristic feature of the host immune response to helminth infection. The T cell dependence of this eosinophilic response has been clearly demonstrated in a number of animal models (1, 2). More recently, several cytokines (IL-3, granulocyte/macrophage CSF [GM-CSF], and IL-5) have been shown to stimulate eosinophilopoiesis in vitro (3–5). While the effects of IL-3 and GM-CSF on in vitro bone marrow cell growth and differentiation extend to a broad range of cell types, IL-5 appears to exert its effects primarily on the development and maturation of eosinophils (6).

In the present study, we have examined the production of these eosinophilopoietic cytokines by PBMC from eosinophilic patients with filarial helminth infections and from normal individuals. While mitogen stimulation of PBMC from both the normal individuals and the eosinophilic patients induced similar amounts of IL-3 and GM-CSF, PBMC from the eosinophilic patients produced strikingly greater amounts of IL-5. This observation suggests that IL-5 may be a distinctive component of the mechanism(s) involved in eliciting the eosinophilia characteristically associated with filarial and similar helminth infections.

## Materials and Methods

*Study Population.* 11 healthy individuals without eosinophilia and seven patients with *Loa loa* infection were studied. The diagnosis of loiasis was documented either parasitologically (presence of microfilariae in the blood or adult worms in the subcutaneous tissue) or by strict clinical criteria (the concurrent findings of localized angioedema [Calabar swelling], antifilarial serum antibodies, and characteristic post-treatment responses) (7). These patients with loiasis (age range, 26–34 yr; four male, three female) had total white blood cell counts ranging from 4,700 to 14,700 (median, 12,000) and eosinophil counts ranging from 987 to 6,600 eosinophils/$\mu$l (median, 4,440 eosinophils/$\mu$l). The normal individuals had levels of eosinophils that did not exceed 500/$\mu$l.

*Cells and Culture Conditions.* PBMC were isolated from whole blood by density gradient centrifugation on Ficoll/Hypaque (8) and cryopreserved until used. Cryopreserved PBMC were thawed, washed twice with RPMI 1640 medium (Biofluids, Rockville, MD) containing 25 mM Hepes and 80 $\mu$g/ml gentamicin (C-RPMI), then resuspended at a final concentration of $5 \times 10^6$ cells/ml in C-RPMI supplemented with 10% FCS. For RNA analysis, $5 \times 10^7$ cells were cultured in 25-cm$^2$ tissue culture flasks (Corning, Corning, NY). For supernatant protein determination, $10^7$ cells were cultured in each well of 24-well, flat-bottomed tissue culture plates (Costar, Cambridge, MA). Cultures were incubated in a 5% $CO_2$-humidified atmosphere at 37°C for varying times with or without the addition of PMA (50 ng/ml; Calbiochem-Behring Corp., San Diego, CA) and ionomycin (1 $\mu$g/ml; Calbiochem-Behring Corp.).

*Northern (RNA) Analysis.* Total cellular RNA was extracted as described (9), and 15-$\mu$g samples were fractionated on a 1.2% agarose/0.66 M formaldehyde gel and transferred to a nylon membrane (Nytran; Schleicher & Schuell, Inc., Keene, NH) according to manufacturer's recommendations. Equivalent loading and quality of RNA were assured by ethidium bromide staining. Hybridization was carried out in a solution with a final concentration of 50% formamide, 5× Denhardt's solution, 1% SDS, 100 $\mu$g/ml denatured herring sperm DNA, 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, pH 7.7, 1 mM EDTA), and $10^6$ cpm/ml of $^{32}$P-labeled IL-5 riboprobe (described below) for 18–24 h. After hybridization, filters were washed twice in 1× SSPE/0.5% SDS at 65°C for 20 min each, then once in 0.1× SSPE/0.5% SDS for 60 min

at 65°C. Autoradiography was performed by exposing XAR film (Kodak, Rochester, NY) to washed filters at −70°C. Scanning densitometry was used to quantitate band intensities.

*Probes.* An IL-5-specific riboprobe of high specific activity was generated by in vitro transcription of a 565-bp EcoRV/NheI fragment of the full-length human IL-5 cDNA (kind gift of R. Coffman, DNAX Research Institute) cloned into the PGEM 3Z transcription vector (Promega Biotec, Madison, WI), using T7 polymerase and $^{32}$P-CTP as per manufacturer's recommendations (Gemini II riboprobe kit; Promega Biotec). An α-actin probe was generated by nick translation of a 2-kb fragment of the full-length human cDNA (kind gift of M. Sneller, NIH) using a commercial nick translation labeling kit (Bethesda Research Laboratories, Gaithersburg, MD).

*Cytokine Measurement.* Cytokines were measured in supernatants of mitogen-stimulated PBMC by sandwich ELISA. IL-5 was measured as previously described (10). GM-CSF ELISA was also performed as previously described (10), except that a nitroiodophenyl (NIP)-conjugated mAb (21C11-NIP) was used (0.5 μg/ml). For IL-3, plates were coated with a rat IgG1 monoclonal anti-IL-3 (6G8) (10 μg/ml), and, after incubation with supernatant samples, a rat polyclonal anti-IL-3 (1:200) was added. Next, a cocktail of mouse anti-rat IgG2a, IgG2b, and IgG2c mAbs (1 μg/ml) was added, followed by a goat anti-mouse immunoperoxidase conjugate (1:2,000). The plates were developed as described for the other cytokines. Each of the ELISAs was highly specific for its respective cytokine and showed no crossreactivity with any other cytokines.

*Statistical Analysis.* Unless otherwise stated, the data were compared using student's *t* test on logarithmically transformed data.

## Results

*Kinetics of IL-5 mRNA and Protein Production after Mitogen Stimulation.* The kinetics of IL-5 production by PMA/ionomycin-stimulated PBMC were studied in both eosinophilic patients ($n$ = 3) and normal individuals ($n$ = 3). Representative results show that the kinetics of IL-5 mRNA were similar in the mitogen-stimulated PBMC from both the normal and eosinophilic individuals (Fig. 1 *A*); IL-5 mRNA was clearly detectable by 3 h, peaked by ~6 h, and subsequently declined. IL-5 protein production induced by PMA/ionomycin was assessed in parallel (Fig. 1 *B*). IL-5 was detectable by 3 h in the PBMC supernatants of all three eosinophilic patients and by 6 h for the normal individuals. Thereafter, IL-5 continued to accumulate, with the rate of accumulation being greater in supernatants from the eosinophilic individuals' cells. By 24 h, the levels of IL-5 protein in the supernatant began to plateau in both groups.

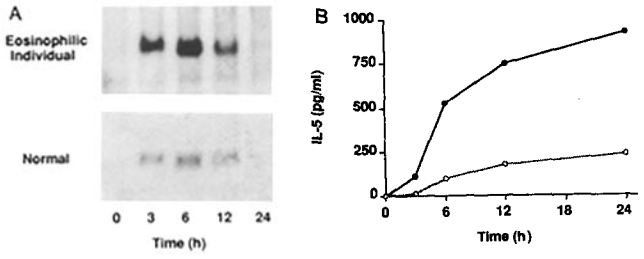


**Figure 1.** Kinetics of IL-5 production after mitogen stimulation. PBMC from a normal individual and an eosinophilic patient were cultured with medium alone (time = 0 h) or with PMA + ionomycin for 3, 6, 12, or 24 h. (*A*) Northern analysis for IL-5 mRNA. Detection of IL-5 mRNA by Northern analysis required a threefold longer exposure for the normal individual than for the eosinophilic patient (6 vs. 2 d). (*B*) Supernatant IL-5 concentration from the same cultures: eosinophilic patient (●); and normal individual (○).

*Comparison of IL-5 mRNA Accumulation after Mitogen Stimulation in Normal and Eosinophilic Individuals.* After demonstrating that peak accumulation of IL-5 mRNA in PMA/ionomycin-stimulated PBMC occurred at 6 h, we compared the relative amounts of IL-5 mRNA induced in three normal and seven eosinophilic individuals. Strikingly greater amounts of IL-5 mRNA were induced in each of the seven eosinophilic individuals (Fig. 2, *bottom*). Equivalent loading and RNA transfer were verified by reprobing the same filter with an α-actin cDNA probe (Fig. 2, *top*).

*Comparison of IL-3, GM-CSF, and IL-5 Protein Production after Mitogen Stimulation of PBMC from Normal and Eosinophilic Individuals.* As both IL-3 and GM-CSF (in addition to IL-5) have been implicated in the induction of eosinophilia (3, 4), the production of all these cytokines after PMA/ionomycin stimulation was compared directly in normal ($n$ = 11) and eosinophilic ($n$ = 6) individuals (Fig. 3). Having determined that the production of IL-3 and GM-CSF after PMA/ionomycin stimulation was maximal at 24 h and that the kinetics of production were similar in eosinophilic and normal individuals (data not shown), we assessed the levels of these three cytokines in 24-h PBMC culture supernatants. Neither for IL-3 (Fig. 3 *A*) nor for GM-CSF (Fig. 3 *B*) were there significant differences in PBMC responses between the normal and eosinophilic individuals. In marked contrast, however, strikingly greater levels of IL-5 were found in the PBMC supernatants from the eosinophilic patients (Fig. 3 *C*). IL-5 levels ranged from 18 to 481 pg/ml in the 11 normal, and from 527 to 1519 pg/ml in the six eosinophilic patients, with the geometric mean of the supernatant IL-5 concentration ~13-fold higher in the group of eosinophilic patients than in the normal individuals (880 vs. 68 pg/ml, $p < 0.001$).

## Discussion

Though eosinophil responses in most individuals are tightly regulated, the presence of helminth infection appears to overcome these regulatory mechanisms so that high levels of circulating eosinophils are consistently produced. While the stim-

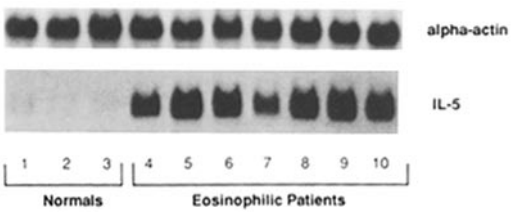


**Figure 2.** Maximal IL-5 mRNA accumulation in normal (lanes *1–3*) and eosinophilic individuals (lanes *4–10*). Northern analysis of IL-5 (*bottom*) and α-actin (*top*) mRNA accumulation in PBMC stimulated for 6 h with PMA + ionomycin.

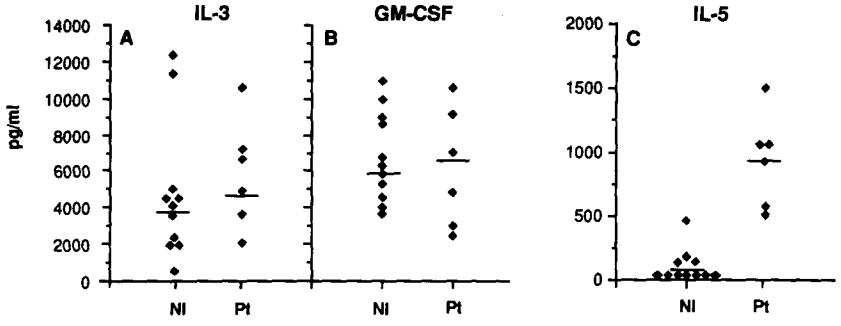


**Figure 3.** IL-3, GM-CSF, and IL-5 levels after mitogen stimulation. PBMC from six eosinophilic patients (Pt) and 11 normal individuals (NI) were stimulated with PMA + ionomycin for 24 h, and cell-free supernatants were assayed for IL-3 (*A*), GM-CSF (*B*), and IL-5 (*C*) protein by ELISA. Each data point represents a single patient, and the geometric mean for the group is indicated by a horizontal line.

ulation of eosinophilopoiesis in vitro has been described for IL-3, GM-CSF, and IL-5, only IL-5 appears to have a restricted (eosinophil) specificity. In the present investigation, stimulation of PBMC by PMA/ionomycin elicited relatively little IL-5 production in the normal individuals but very high levels of this cytokine in patients with helminth-induced eosinophilia. That the levels of both IL-3 and GM-CSF were similar in the normal and eosinophilic individuals argues in favor of a clonal expansion of IL-5-producing cells. Indeed, as human IL-5 has thus far been shown to be produced only by $CD4^+$ T cells, our findings suggest that the increased IL-5 production by the eosinophilic patients may be due to increased numbers of IL-5-secreting $CD4^+$ cells in their PBMC.

In murine helminth infections, eosinophilia (mediated by IL-5 [11, 12]) and elevation in IgE (mediated by IL-4 [11]) have been shown to be regulated by a single Th subset (Th2) capable of producing both IL-4 and IL-5, but not IL-2 or IFN-$\gamma$ (13). While an equivalent T cell subset has not been formally demonstrated in humans, there is evidence that antigen-specific IL-4/IL-5–secreting, IFN-$\gamma$/IL-2–nonsecreting T cell clones can be established from patients with filarial infections (unpublished data), and such cells may well play a role in mediating the eosinophilia associated with helminth infections. Further testing of this hypothesis will require additional cell fractionation studies, and ultimately, single cell analysis by in situ hybridization and/or Elispot.

It is not yet clear how ubiquitous is the involvement of IL-5 in eosinophilic conditions not caused by parasites, but observations of another eosinophilic state (the idiopathic hypereosinophilic syndrome) have also implicated either undefined soluble T cell–derived factors (14), or IL-5 directly (15). In addition, T cells from patients with reactive eosinophilia have been shown to produce IL-5 upon IL-2 stimulation in vitro (16), and IL-5 has been found to be elevated in the serum of patients undergoing IL-2/lymphokine-activated killer therapy who subsequently developed eosinophilia (J. S. Abrams, manuscript submitted for publication). These observations, taken together with the findings from the present study, suggest that IL-5 may be the common denominator in a variety of eosinophilic states, and that its enhanced production may be responsible for the abnormally elevated eosinophil levels seen not only in parasitic infections but in other disorders as well.

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