Interleukin-5 and the Posttreatment Eosinophilia in Patients with Onchocerciasis

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Abstract

To understand the role of the eosinophilopoietic cytokine IL-5 in humans, the posttreatment eosinophilic response in a group of microfilaria (mf)-positive patients with onchocerciasis (n = 10) was examined before and after treatment with diethylcarbamazine (6 mg/kg for 7 d). Sequential blood samples were assessed at 24 and 1 h before treatment (baseline values), then at frequent intervals over the next 14 d. Symptom scores, skin microfilariae (mf), and peripheral blood eosinophil counts were recorded as a function of time after treatment, and serum levels of IL-5 were quantitated by a highly sensitive (sensitivity ≥ 20 pg/ml) monoclonal-based ELISA. Pretreatment eosinophil counts ranged from 240 to 1,186 eosinophils/μl (geometric mean, 675), and the mf counts from 10 to 218 per mg skin (geometric mean, 79). After an initial decline in the peripheral eosinophil count to 28±8% of pretreatment levels at 8 h after beginning treatment, the eosinophil counts steadily increased over the next 2 wk, reaching a maximum at 14 d (257±38% of pretreatment levels). Serum levels of IL-5 rose sharply from pretreatment levels to a peak of 70.5±11 pg/ml by 24 h after treatment. Serum IL-5 remained elevated over the next 2–3 d and declined toward baseline by ~ 6 d after treatment, at which time the eosinophil levels were steadily increasing. IL-3 and granulocyte macrophage colony-stimulating factor, two other cytokines implicated in eosinophilopoeisis, were not detectable in the serum at any time before or after treatment. The rise in serum IL-5 before the posttreatment eosinophilia seen in this group of patients with onchocerciasis demonstrates a temporal relationship between IL-5 and the subsequent development of eosinophilia and implicates IL-5 as an important mediator of eosinophilia in humans. (*J. Clin. Invest.* 1991. 88:1418–1421.)
Key words: interleukin-5 • eosinophilia • onchocerciasis • Mazzotti reaction • diethylcarbamazine

Introduction

Blood and tissue eosinophilia is a characteristic response to infection with a variety of helminth parasites. Recently, IL-5 has been implicated in mediating this response in vitro both in bone marrow cultures (1) and in cross-sectional studies of eosinophilic individuals (2). Although other cytokines, particularly IL-3 and granulocyte macrophage colony-stimulating factor (GM-CSF),[1] have been shown to stimulate eosinophilopoeisis directly in vitro and to induce eosinophilia in vivo (3), only IL-5 appears to be eosinophil (and probably basophil) specific in its colony-stimulating activity (4, 5). The role of IL-5, however, has not been examined prospectively in a physiologic in vivo eosinophilic response in humans.

Treatment of many helminth infections—including lymphatic filariasis (6), schistosomiasis (6), and onchocerciasis (7, 8)—has consistently been shown to cause a characteristic rise in peripheral blood eosinophil levels, the magnitude of which is felt to relate to the parasite burden (6, 8). Therefore, because this eosinophilia is such a predictable sequela of the treatment of onchocerciasis, the relationship of IL-5 to the induction of this response could be directly assessed in *Onchocerca*-infected individuals.

Methods

Study population. 10 male patients from Tamale, Ghana, aged 18–51 yr with proven onchocerciasis, were selected for study (Table I). Identification and quantification of *O. volvulus* microfilariae, recovered from skin snips of scapulae, iliac crests, thighs, and calves using a Walzer-type corneoscleral punch, established the diagnosis and intensity of infection as previously described (7). The intensities of infection ranged from 10 to 218 mf/mg of skin. Two individuals without evidence of onchocerciasis (endemic normal individuals) were also studied. These individuals were from the same region of Ghana and were clinically and parasitologically free of onchocercal infection. Stool microscopic examination for other parasites was performed at the initiation of the study (Table I).

Treatment protocol. Patients and controls received 200 mg of diethylcarbamazine (DEC) orally once a day for 7 d, as previously described (6). The study was performed at a time (1982) in which DEC was the only available treatment for onchocerciasis. No patient had received DEC previously. Informed consent was obtained from all subjects. This study was carried out under protocols approved by the National Institute of Allergy and Infectious Diseases and by the Ghanaian Ministry of Health. Blood was collected twice before treatment and at 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, and 336 h after initiation of DEC treatment. WBC (Coulter Electronics, Inc., Hialeah, FL) and complete differential counts were performed within 4 h of blood collection; 500 cells were counted for each differential. Sera were stored in liquid nitrogen or at −70°C until use. Aliquots of serum used in this study had never been thawed.

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1. *Abbreviations used in this paper:* DEC, diethylcarbamazine; GM-CSF, granulocyte macrophage colony-stimulating factor; NIP, nitroiodophenyl.

Table I. Study Population: Pretreatment

Patient group	Sex/age	Skin microfilariae	Eos/liter	IL-5*	Other parasites
		mf/mg skin		*pg/ml*	
Onchocerciasis					
1	M/21	10	525	<20	None
2	M/51	72	250	ND	*Balantidium coli*
3	M/39	88	495	159	None
4	M/21	185	852	<20	None
5	M/21	102	814	<20	None
6	M/18	218	908	25	*S. mansoni*
7	M/20	203	1186	44	Hookworm
8	M/20	56	832	<20	None
9	M/18	104	702	25	None
10	M/30	18	240	<20	Hookworm
Endemic normal					
11	M/21	0	123	<20	None
12	M/24	0	124	<20	None

* Limits of detectability, <20 pg/ml.

Assay for quantitation of serum IL-5, IL-3, and GM-CSF. Cytokines were quantified in patient sera by immunoenzymetric assays described previously (2). "U"-bottom polyvinylchloride microtiter plates were coated with a 100 μl/well volume of the appropriate capture antibody (see below) diluted in PBS for 2 h at 37°C. The plates were washed three times with 0.15 M NaCl plus 0.05% Tween 20 using a Microwash II (Skatron Inc., Sterling, VA) automatic plate washer. The samples were incubated for 2 h at room temperature. After washing, the appropriate nitroiodophenyl (NIP)-derivatized detecting MAb (see below) was added in 0.01 M PBS plus 1 mg/ml BSA plus 0.05% Tween 20 (PBS-BSA-Tween) and incubated for 1 h at room temperature. After washing, bound NIP-MAb was detected by incubating a monoclonal anti-NIP (J4) immunoperoxidase conjugate (800 ng/ml) in PBS-BSA-Tween for 1 h at room temperature. Finally, after washing, the plates were developed using of the peroxidase chromogenic substrate 2-2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., St. Louis, MO) (1 mg/ml) in the presence of H_2O_2. Plates were read using a microtiter plate reader (Molecular Devices, Menlo Park, CA). Unknown values were interpolated from standard curves prepared using purified recombinant standards graciously provided by Schering Research (Bloomfield, NJ). The following antibody pairs (coating antibody and NIP [hapten]-derivatized detecting antibody, respectively) and recombinant standards were used in the cytokine immunoenzymetric assays carried out in this study: for IL-5, JES1-39D10 (10 μg/ml) and JES1-5A10-NIP (1 μg/ml) with L cell–derived recombinant standard; for IL-3, BVD8-3G11 (1 μg/ml) and BVD3-1F9-NIP (1 μg/ml) with yeast-derived standard; and for GM-CSF, BVD2-23B6 (10 μg/ml) and BCD2-21C11-NIP (1 μg/ml) with *E. coli*–derived recombinant standard.

Data analysis. Unless otherwise stated, data are expressed as geometric means. Correlations were assessed by Spearman rank.

Results

Pretreatment assessment. Pretreatment blood eosinophil counts and serum IL-5 levels were determined as the means of the two pretreatment observations for each of the 10 patients with onchocerciasis and the two endemic normal individuals (Table I). Although there was a broad range of eosinophilia (240–1,186/μl) among the patients with onchocerciasis, these pretreatment levels were significantly elevated ($P < 0.05$) when compared to the endemic uninfected individuals or normal North American blood donors (data not shown). Interestingly, the pretreatment eosinophil levels were positively correlated with the levels of skin microfilariae ($P < 0.007$).

Serum levels of IL-5 pretreatment were below the limits of detectability (< 20 pg/ml) in five of the 10 patients; in the other four, there were measurable levels of serum IL-5 (range, 25–159 pg/ml). There was, however, no relationship between the pretreatment levels of serum IL-5 and the pretreatment levels of blood eosinophils or skin microfilariae.

Effects of microfilaricidal treatment on blood eosinophils and serum IL-5 levels. Data for two representative individuals, one with microfiladermia and one uninfected endemic control, are illustrated in Fig. 1. When DEC was given to the patient with onchocerciasis, blood eosinophil levels dropped within 1–3 h of the first dose and reached their nadir at ~ 8–12 h (Fig. 1 *A*). After this transient eosinopenia, the eosinophil levels rose to pretreatment values by 72–96 h and peaked at 6 d. In contrast, blood eosinophil levels in the endemic normal individual did not vary significantly after receiving the same dose of DEC (Fig. 1 *A*). Serum IL-5 levels were assessed in parallel (Fig. 1 *B*). In the patient with onchocerciasis, serum IL-5 remained at baseline for 8 h and then rose to a peak at 24 h. Soon thereafter, the serum IL-5 levels diminished, returning to pretreatment levels by 72 h. As expected, the uninfected individual showed no change in serum IL-5 levels over the 2-wk period of observations (Fig. 1 *B*).

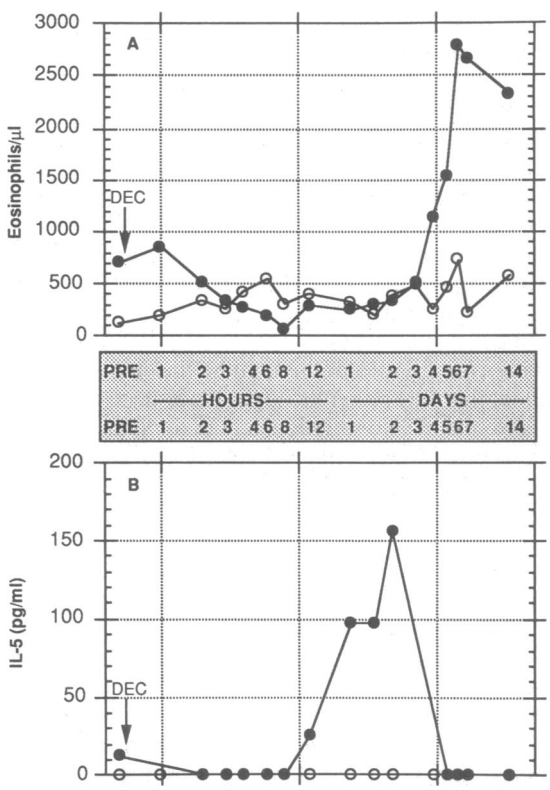

Figure 1. Posttreatment changes of serum IL-5 levels and blood eosinophils in onchocerciasis. Eosinophil levels (*A*) and serum IL-5 levels (*B*) in a representative patient with onchocerciasis (*solid circles*) and a normal individual (*open circles*) before and sequentially after administration of DEC. Time is plotted logarithmically on the *x* axis.

Similar findings were seen in all 10 of the treated patients with onchocerciasis (Fig. 2). After initial declines in the peripheral eosinophil counts to 28.5±8% of pretreatment levels at 8 h after treatment, the eosinophil counts steadily increased over the next 2 wk, reaching a maximum at 14 d (257±38% of pretreatment levels). Serum levels of IL-5 rose sharply from pretreatment levels by 8–12 h, reaching a peak (over baseline) of 70.5 pg/ml by 24 h after treatment. Serum IL-5 remained elevated for the next 2–3 d and slowly returned toward baseline by 6 d posttreatment, a time at which blood eosinophil levels were steadily increasing. Interestingly, on an individual level, there was a positive correlation ($P < 0.01$) between the time to the peak IL-5 values (range, 12–48 h; mean, 35 h) and the time to the peak eosinophilia (range, 3–14 d; mean, 185 h [7.7 d]) as well as a relationship ($P < 0.04$) between the time to induction of IL-5 (as determined by levels above baseline [range, 3–24 h; mean, 9.2 h) and time to peak eosinophilia (range, 3–14 d; mean, 185 h [7.7 d]) or eosinophilia above pretreatment levels (range, 48–168 h [2–7 d]; mean, 87.5 h [3.6 d]) ($P < 0.01$). There was no relationship, however, between the levels of IL-5 and the levels of eosinophilia seen posttreatment, nor was there a correlation between the patients' skin microfilariae and IL-5 levels.

In none of the serum samples tested was there any measurable GM-CSF or IL-3 (data not shown).

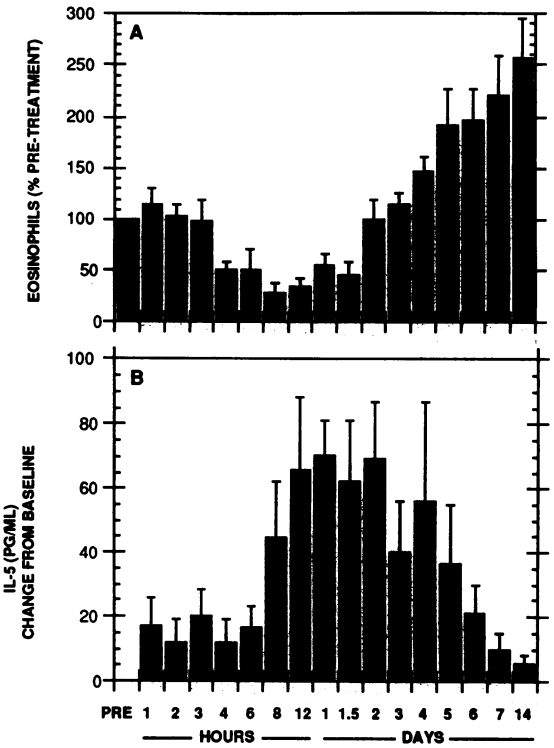

Figure 2. Posttreatment changes in serum IL-5 and blood eosinophil numbers in 10 patients with onchocerciasis. Mean eosinophil levels SEM expressed as a percent of the pretreatment eosinophil level (*A*) and mean serum IL-5 levels SEM expressed as an absolute concentration above pretreatment baseline values (*B*) are plotted as a function of time after the first dose of diethylcarbamazine.

Discussion

Parasitic helminth infections, along with atopic diseases, are the conditions most commonly associated with tissue and peripheral blood eosinophilia. In rodents infected with either *Nippostrongylus braziliensis* (9) or *Schistosoma mansoni* (10), this parasite-induced tissue and peripheral blood eosinophilia has been shown to be mediated by IL-5. In humans, the role of IL-5 has been, until recently, limited to in vitro studies. In these in vitro studies, human IL-5 has been clearly shown to be a potent stimulus for eosinophil growth and differentiation (1). In contrast to IL-3 and GM-CSF (the other cytokines implicated in mediating eosinophilia), IL-5 has also been shown to be induced at high levels (mRNA and protein) in lymphocytes of eosinophilic patients with filarial infections (2), an observation suggesting a critical role for this lymphokine in parasite-induced eosinophilia. The present study extends these earlier in vitro findings to the in vivo situation and, in so doing, demonstrates the role of IL-5 as a critical mediator of the posttreatment eosinophilia seen in human onchocerciasis.

Eosinophilia has been commonly reported after treatment of infections with *S. mansoni* (6) and most of the filariae including *W. bancrofti* (6), *B. malayi, O. volvulus* (7, 8), *Loa loa* (unpublished), and *M. ozzardi* (11). Previously, it had been shown that magnitude of the posttreatment eosinophilia in *W. bancrofti* infections was a direct function of the pretreatment parasite burden (i.e., numbers of blood microfilariae), an observation suggesting that the magnitude of the eosinophil response depends on the degree of antigenic stimulation induced by the parasites cleared from the blood (6). In the present study, however, it was the pretreatment levels of eosinophils that were positively associated with skin microfilarial densities.

The most important issue raised by the present study concerns the kinetics of IL-5 appearance and its temporal relationship to the rise in blood eosinophils. The fact that the eosinophilic response took several days to develop and 7–14 d to peak suggests that the increase in circulating eosinophils reflected active bone marrow eosinophilopoeisis rather than compartmental shifts. In addition, the relatively constant relationship between the time to the peak of serum IL-5 and the time to the peak eosinophilia is consistent with IL-5's action on eosinophilic progenitors in the bone marrow, a hypothesis already suggested after the administration of anti-IL-5 antibody to helminth-infected rodents (12).

The role of IL-5 in eosinophilic conditions not caused by parasites remains unclear, although observations on the idiopathic hypereosinophilic syndrome (13) and on the eosinophilia-myalgia syndrome (14) have implicated IL-5 as an important mediator. Additionally, T cells from patients with reactive eosinophilia have been shown to produce IL-5 upon IL-2 stimulation in vitro (15); and in eosinophilic patients undergoing IL-2/LAK cell tumor immunotherapy, serum IL-5 has been found to be elevated, with kinetics of appearance of peak eosinophilia analogous to that observed in the present study (16). That IL-5 (and not GM-CSF or IL-3) was mediating this response was demonstrated by the complete absence of either of these in any of the serum samples tested.

The present findings, while clearly implicating IL-5 as the major mediator of the post-treatment eosinophilia seen in onchocerciasis, do not specifically address the nature of the stimulus required for the induction of this eosinophilopoeitic cytokine. There are hypotheses suggesting that either particular structural motifs found in parasite antigens are "eosinophilo-

genic" or that the chronic antigenic stimulation found in helminth infections plays the major role in inducing eosinophilia. By whatever mechanism, the present findings clearly demonstrate that acute stimulation of the immune system by parasite antigens (as seen with treatment of filarial infections) overcomes the regulatory mechanisms that keep IL-5 production in check. With the induction of IL-5 comes the predictable rise in peripheral blood eosinophilia seen after treatment of onchocerciasis (and presumably, other helminth infections). This physiologic response to parasite antigen release should provide an excellent model for continued study of the in vivo regulation of eosinophilia, of the nature of its determinants, and of the effector mechanisms underlying it.

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