

## Cytokines in the Modulation of Eosinophilia

Lúcia H Faccioli/<sup>+</sup>, BB Vargaftig\*, Alexandra I Medeiros, Adriana Malheiros

Departamento de Parasitologia, Microbiologia e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes 3900, 14049-900 Ribeirão Preto, SP, Brasil \*Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur/INSERM, Paris, France

*In this review we discuss our recently results showing interleukin 5 (IL-5) involvement in eosinophil migration and in the maintenance of eosinophilia in blood, bone marrow, lung and peritoneal cavity, in a visceral larva migrans syndrome model using guinea-pigs infected with Toxocara canis. We also describe the sequential release of TNF- $\alpha$  and IL-8 during the course of infection, and the interaction between these cytokines and IL-5 during infection. Finally we propose a new biological role for IL-5, at least in our model, as a modulator of IL-8 release and secretion.*

Key words: eosinophils - helminth Parasites - inflammation - cytokines

*Toxocara canis* is an intestinal parasite of dogs, and is the most common aetiologic agent of visceral larva migrans syndrome (VLMS) (Beaver et al. 1952). Tissue-migrating larvae of this parasite induce intense eosinophilia which reaches more than 90% of total leukocyte counts (Beaver et al. 1952). Although several investigators have suggested a direct correlation between eosinophilia and interleukin-5 (IL-5) in human helminthic infection (Limaye et al. 1990, Steel & Nutman 1993) and in experimental animal models (Yamaguchi et al. 1990, Parson et al. 1993), the mechanisms involved in blood and tissue eosinophilia in this model of helminthic infection remain unclear. The release of other eosinophil-related cytokines such as IL-8 and TNF- $\alpha$  and their interaction with IL-5 are currently being studied.

### WIDESPREAD EOSINOPHILIA AND EOSINOPHIL MIGRATION IN VLMS IS IL-5 DEPENDENT

Guinea-pigs infected orally with *T. canis* eggs showed widespread eosinophilia with a time-dependent increase of eosinophils in all compartments studied (Table I). In blood and bronchoalveolar lavage fluid (BALF) the number of eosinophils was significantly increased at 6 days post-infection reaching more than 90% of the total cell counts in BALF but decreased by day 24. In contrast to blood and BALF, the number of eosinophils in the peritoneal cavity increased significantly only at day 12 post-infection and increased progressively until day 24. The percentage of eosinophils in some

animals reached 55% at the peak of infection (Faccioli et al. 1996).

The development of eosinophilia in guinea-pigs infected with *T. canis* is accompanied by the release of two peaks of serum IL-5. The highest occurs soon after the stimulus, i.e., 1 day after infection, and the second occurs 18 days later (Fig. 1). Since IL-5 release correlated with the percentage of larvae recovered from the liver of guinea-pigs (Faccioli et al. 1996), led us to suggest that the eosinophilia against helminth larvae is initiated by the release of IL-5 when the parasites migrate from the intestine to the liver by stimulation of a specific cell population. The cytokine pattern that develops at this early stage probably is a T-cell independent pathway which may also influence the subsequent T-cell differentiation into Th2 type, which may be responsible for the second peak.

In our study, i.p. administration of TRFK-5, a monoclonal antibody (mAb) against IL-5, at the time of egg administration or one or three days later, drastically reduced the number of eosinophils in blood, BALF, peritoneal cavity and bone marrow by 18 days after infection (Table II). TRFK-5 administered 17 days after infection, and the animals killed 24 hr later, significantly inhibited the number of circulating eosinophils but was accompanied by an increase of mature eosinophils in bone marrow. The inhibition of circulating eosinophil numbers by different treatments with mAb, even when the antibody was given at the peak of blood eosinophilia, suggests that IL-5, apart from being required for terminal differentiation of eosinophils in bone marrow (Rennick et al. 1990), may also drive eosinophils from the bone marrow to blood and then to tissues (probably by up-regulating VLA-4 expression in eosinophils). Thus, even though the increase of serum IL-5 level shows only

<sup>+</sup>Corresponding author. Fax: + 55-16-635.6631. E-mail: faccioli@fmrp.usp.br

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TABLE I  
Eosinophils in guinea-pig with visceral larva migrans syndrome

Compartment	Days postinfection					
	3	6	12	18	24	Control
Blood	1.35±0.46	6.00±1.03 <sup>b</sup>	11.20±2.33 <sup>b</sup>	12.06±2.31 <sup>b</sup>	8.11±2.85 <sup>b</sup>	1.21±0.21
BALF	0.26±0.09	1.37±0.35 <sup>a</sup>	9.62±2.92 <sup>b</sup>	10.23±2.62 <sup>a</sup>	9.07±3.47 <sup>a</sup>	0.69±0.21
Peritoneal cavity	3.37±0.68	3.68±0.82 <sup>a</sup>	5.77±1.12 <sup>b</sup>	11.33±2.36 <sup>b</sup>	12.44±2.72 <sup>a</sup>	2.10±0.60
Bone marrow	6±2	9±2	14±2 <sup>a</sup>	17±2 <sup>a</sup>	16±4	7.5±1

In Blood, BALF and Peritoneal cavity the values represent mean ± SEM x 10<sup>5</sup> ml<sup>-1</sup> and in bone marrow mean ± SEM of the percentage of mature eosinophils. *Toxocara canis*-infected guinea-pigs n = 8-9 and noninfected animals n = 5-6. a: p < 0.05; b: p < 0.01.

TABLE II  
Eosinophils in *Toxocara canis*-infected guinea-pig treated or untreated with TRFK-5

Compartment	Time of sacrifice days	Days of treatment with TRFK-5 after egg administration					Irrelevant Ab at the time of infection (n=5)
		Non treated	0 (n=6)	1 (n=4)	3 (n=4)	17 (n=5)	
Blood	18	12.18±5.28	0.14±0.14 <sup>a</sup>	0.26±0.26 <sup>a</sup>	0.17±0.17 <sup>a</sup>	2.59±0.80 <sup>a</sup>	13.32±4.54
BALF	18	16.50±4.42	0.36±0.10 <sup>a</sup>	0.79±0.24 <sup>a</sup>	1.22±0.50 <sup>a</sup>	15.24±4.36	29.31±15.90
Peritoneal cavity	18	12.31±2.35	1.47±0.76 <sup>a</sup>	0.48±0.35 <sup>a</sup>	0.46±0.27 <sup>a</sup>	10.42±2.21	16.09±4.00
Bone marrow	18	9±1	3±1 <sup>a</sup>	1.25±0.25 <sup>a</sup>	2.6±1.3 <sup>a</sup>	15±2	9±1

In Blood, BALF and Peritoneal cavity the values represent mean ± SEM x 10<sup>5</sup> ml<sup>-1</sup> and in bone marrow mean ± SEM of the percentage of mature eosinophils. a: p < 0.05.

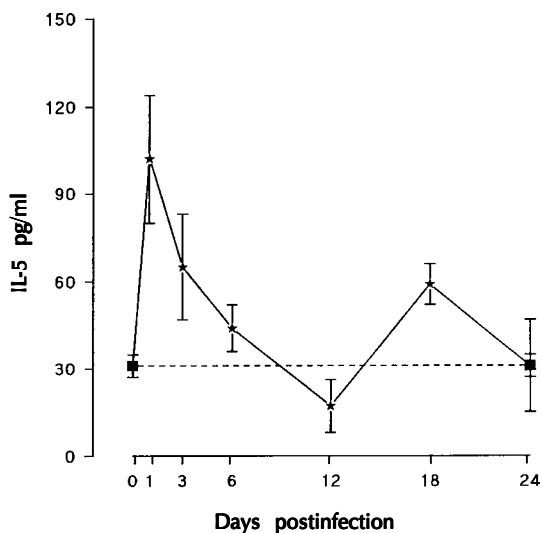


Fig. 1: IL-5 concentration in serum of *Toxocara canis*-infected guinea-pigs (n = 3-5). Basal IL-5 concentrations in noninfected animals were 31 ± 4 pg/ml (dotted line). IL-5 levels in the serum of guinea-pigs were measured by an enzyme-linked immunosorbent assay (Faccioli et al. 1996).

two peaks followed by levels close to the control during the rest of infection, the maintenance of the basal level of serum IL-5 appears to be essential for eosinophilia and to drive eosinophils during the infection.

#### SERUM TNF $\alpha$ AND IL-8 LEVELS IN VLMS

Since little is known about the presence of other cytokines related to eosinophil recruitment in the model of *T. canis* infection, we determined the release of two relevant cytokines related to eosinophils migration, TNF- $\alpha$  (Weg et al. 1995, Lukacs et al. 1995a) and IL-8 (Collins et al. 1993, Sehmi et al. 1993). TNF- $\alpha$  levels increased very early in the serum of infected guinea-pigs, reaching levels 87% above those of noninfected animals 4 hr after egg inoculation and remaining above control levels up to 48 days post-infection (Fig. 2a). *T. canis* larvae persist in different organs for long periods of time in infected animals (Kayes & Oaks 1976) and release excretory-secretory antigens during migration (Parsons et al. 1986). The prolonged and sustained TNF- $\alpha$  activity in blood may be ex-

plained either by the persistent presence of larvae in the tissues and/or by the release of those excretory-secretory antigens. TNF- $\alpha$  is involved in eosinophil recruitment (Weg et al. 1995, Lukacs et al. 1995a) and has also been reported to occur in diseases presenting elevated number of eosinophils, such as late (Gosset et al. 1991) and early (1 to 8 hr) asthmatic reactions during airway inflammation (Lukacs et al. 1995a). Finally, bronchoalveolar leukocytes from patients with bronchial asthma secrete high levels of TNF (Cembrzyanska-Novak et al. 1993).

The IL-8 serum levels showed a similar profile to that of blood eosinophilia, increasing significantly only between 6 and 12 days post-infection and peaking between days 18 and 24 (Fig. 2b). These increases occurred at the same time as the increase in circulating eosinophil numbers, and the decrease of IL-8 in serum was followed by a reduction in blood eosinophil counts. This suggests that IL-8 contributed to eosinophil recruitment. IL-8 has been described as an eosinophil chemoattractant *in vivo* and *in vitro* (Collins et al. 1993, Sehmi et al. 1993, Erger & Casale 1995). However, it appears that IL-8 is only able to induce eosinophil migration after being primed by IL-5 (Moser et al. 1992, Warringa et al. 1992, Sehmi et al. 1993). Based on these data and on the data presented here, we suggest that IL-8 requires pre-priming with IL-5 to induce eosinophil recruitment, *in vivo*. Thus, despite the release of high amounts of IL-8 in this model, the presence of IL-5 was essential for eosinophil migration, as demonstrated in antigen-challenged guinea-pigs (Coeffier et al. 1994) and *in vitro* (Moser et al. 1992, Warringa et al. 1992, Sehmi et al. 1993). However, the exact mechanisms involved in this process are not yet completely understood, and further studies using anti-IL-8 Ab will be essential to determine the contribution of

IL-8 to the eosinophil recruitment and lung inflammation (Faccioli et al. 1996) occurring in this model. The observation that serum IL-8 level started to increase only after serum TNF release suggests that TNF may be involved in the induction of IL-8 release in this model. Indeed, several reports have shown that TNF is a potent inducer of IL-8 (Kunkel et al. 1990, Kwon et al. 1994). IL-8 is present after TNF therapy in patients with chronic hepatitis (Sheron & William 1992) and is released *in vitro* from pulmonary smooth muscle and endothelial cells stimulated with TNF (Lukacs et al. 1995b).

#### IL-5 MODULATES IL-8 SYNTHESIS AND RELEASE DURING HELMINTHIC INFECTION AND IN SUPERNATANTS OF LPS-STIMULATED GUINEA-PIG ADHERENT PERITONEAL CELLS

During the course of infection we observed a sequential release of TNF- $\alpha$  and IL-8 in connection with IL-5 release (Faccioli et al., 1996). We also examined the relationship between IL-5 levels and IL-8 and TNF- $\alpha$  release *in vivo* by inducing IL-5 depletion with anti-IL-5 mAb. Intraperitoneal injection of anti-IL-5 mAb (2 mg/animals) into *T. canis*-infected guinea-pigs only at the time of egg administration of several doses (0.3 mg/animal on days 0, 1, 3 and 17 post-infection) inhibited blood eosinophil counts by 95% to 100%, as shown in Fig. 3a. When the animals were treated with a single dose of anti-IL-5 mAb, a 159% increase in IL-8 was observed in serum (Fig. 3c). Moreover, when infected animals were treated with several doses, eosinophilia was also suppressed and serum IL-8 levels were increased by 216% (Fig. 3c). By contrast, no alteration in serum TNF concentration was observed regardless of the treatment (Fig. 3b). Infected animals treated with irrelevant mAb showed no significant differences in serum

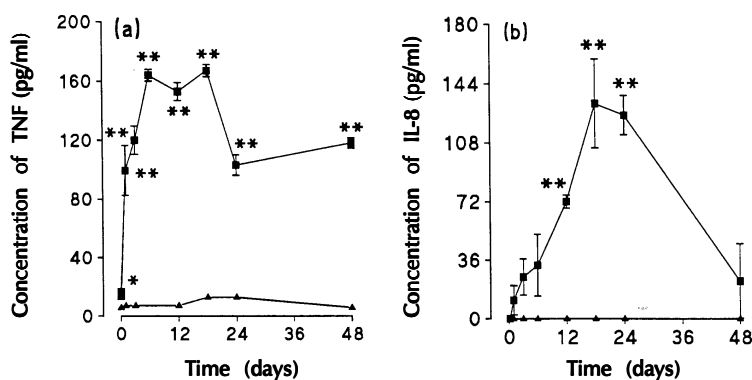


Fig. 2: serum TNF- $\alpha$  (a), and IL-8 (b) levels in *Toxocara canis*-infected (n) guinea-pigs sacrificed at different times post-infection ( $n = 4-5$  per day). Asterisks indicate a significant difference between infected and non-infected animals (s). \*  $p < 0.05$  and \*\*  $p < 0.01$ . Serum cytokines were measured using human ELISA kits.

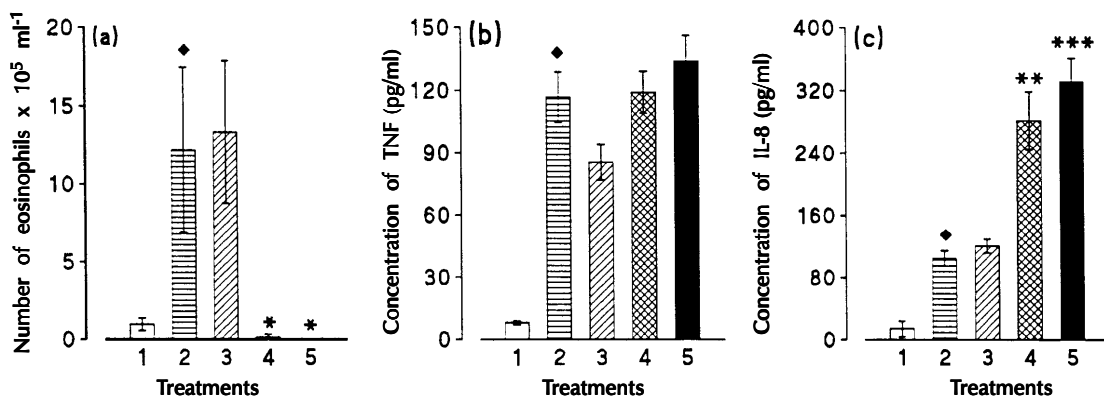


Fig. 3: blood eosinophil counts (a), serum TNF (b), and IL-8 (c) levels in untreated *Toxocara canis*-infected guinea-pigs (column 2), and in infected animals treated with irrelevant Ab (column 3), or treated with anti-IL-5 Ab once at the time of infection (column 4) or with several doses (0.3 mg/animal on days 0, 1, 3 and 17 post-infection) (column 5). Animals were sacrificed 18 days postinfection. Diamonds indicate a significant difference between untreated infected animals ( $n = 5-6$ ) and non-infected controls (column 1,  $n = 6$ ).  $\cup$   $p < 0.05$ . Asterisks indicate a significant difference between untreated infected animals ( $n = 5-6$ ) and infected animals treated with anti-IL-5 Ab ( $n = 7$ ). \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$  (Faccioli et al. unpublished data).

IL-8 and TNF levels (Faccioli et al. unpublished data).

To corroborate the results obtained *in vivo*, we carried out experiments *in vitro* to examine the effect of recombinant IL-5 (rIL-5) on IL-8 release in supernatants of LPS-stimulated adherent peritoneal cells and on IL-8 and IL-5 mRNA expression. IL-8 was determined in supernatants of LPS-stimulated guinea-pig adherent peritoneal cells pre-incubated or not with rIL-5 (Fig. 4). LPS-induced IL-8 release was inhibited (64% to 66%), by rIL-5 addition. Moreover, when rIL-5 was added to the cells before LPS, a marked inhibition of IL-8 release and IL-8 mRNA expression occurred, which was prevented by anti-IL-5 mAb treatment, indicating specificity (Faccioli et al. unpublished data)

These data suggest a regulatory role of IL-5 acting on IL-8 synthesis and perhaps on secretion, *in vivo*. Thus, the inhibitory effects of IL-5 on IL-8 expression and synthesis may represent an endogenous down-regulating mechanism for eosinophil inflammation. It is possible that IL-5 is required to prevent IL-8 over production and release. Moreover, as discussed above, we suggest that serum IL-8, in the presence of IL-5, acts as an eosinophil chemoattractant. However, since IL-5 is decreased during the course of infection an increase in serum IL-8 occurs which, above a certain level, may act as a modulator of eosinophil release from bone marrow and tissue eosinophil migration. Thus, it is possible that the increase in serum IL-8 levels in *T. canis*-infected guinea-pigs after anti-IL-5 mAb treatment may contribute to the inhibition of eosinophilia. IL-8, which is a potent chemotactic factor for neutrophils has been shown to in-

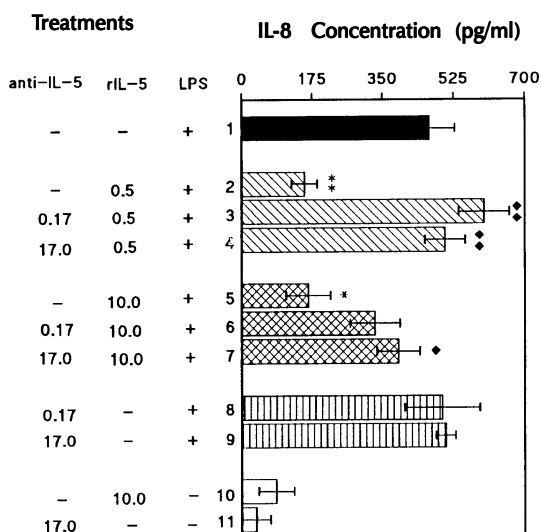


Fig. 4: inhibition of IL-8 generation into supernatants of LPS-stimulated (500 ng/ml) guinea-pig adherent peritoneal cells pre-treated with 0.5 ng/ml or 10 ng/ml of rIL-5. Data are presented as means  $\pm$  SEM ( $n = 4$ ). Asterisks indicate differences between the amount of IL-8 released by LPS-stimulated adherent peritoneal cells pre-incubated without or with rIL-5. \*  $p < 0.05$  and \*\*  $p < 0.01$ . Diamonds indicate differences between the amount of IL-8 released by LPS-stimulated adherent peritoneal cells pre-treated with rIL-5 in the absence or presence of anti-IL-5 Ab (0.17 ng/ml or 17 ng/ml).  $\cup$   $p < 0.05$  and  $\cup\cup$   $p < 0.01$  (Faccioli et al. unpublished data).

hibit migration to the skin when administered at high concentrations by the i.v. route (Hechtman et al. 1991).

The present results describe a new function of IL-5 as a modulator of IL-8 synthesis and secre-

tion and may help in understanding and ultimate control of diseases associated with eosinophilia and high IgE levels (Nutman et al. 1989). IL-8 has been shown to selectively inhibit IgE production (Kimata et al. 1992, 1995) and thus may be involved in the control of serum IgE levels *in vivo*. Production of high amounts of IL-5 in such diseases may down regulate IL-8 levels and consequently contribute to the maintenance of high IgE levels during infection.

In summary, our results demonstrate that in *T. canis*-induced eosinophilia there is a sequential release of TNF- $\alpha$  and IL-8 which occur in parallel to or after serum IL-5 release. Thus, in this model, IL-5 appears to be the main factor involved in both induction of eosinophilia and eosinophil migration. Also, IL-5 may modulate eosinophilic inflammation by down-regulating IL-8 synthesis and secretion.

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