Cyclooxygenase-2-Derived Prostaglandin E_2 and Lipoxin A_4 Accelerate Resolution of Allergic Edema in *Angiostrongylus costaricensis*-Infected Rats: Relationship with Concurrent Eosinophilia[1]

Christianne Bandeira-Melo,* Magda F. Serra,* Bruno L. Diaz,* Renato S. B. Cordeiro,* Patricia M. R. Silva,* Henrique L. Lenzi,† Y. S. Bakhle,‡ Charles N. Serhan,§ and Marco A. Martins[2]*

In noninfected rats, challenge with allergen following local IgE sensitization induced a pleurisy marked by intense protein exudation that plateaued from 30 min to 4 h after challenge, reducing thereafter. Infection of rats with *Angiostrongylus costaricensis* induced a 5-fold increase in blood eosinophil numbers by 25 days postinfection, whereas the numbers of eosinophils in the pleural cavity ranged from normal to a weak increase. In infected rats, identically sensitized, challenge with Ag induced a much shorter duration of pleural edema with complete resolution by 4 h, but no change in the early edema response. In parallel, infection increased the number of eosinophils recovered from the pleural cavity at 4 h, but not at 30 min, following allergen challenge. Pretreatment with IL-5 (100 IU/kg, i.v.) also increased eosinophil numbers in blood and, after allergen challenge, shortened the duration of the pleural edema and increased pleural eosinophil numbers. There were increases in the levels of both PGE_2 and lipoxin A_4 (LXA_4) in pleural exudate. Selective cyclooxygenase (COX)-2 inhibitors, NS-398, meloxicam, and SC-236, did not alter pleural eosinophilia, but reversed the curtailment of the edema in either infected or IL-5-pretreated rats. Pretreatment of noninfected animals with the PGE analogue, misoprostol, or two stable LXA_4 analogues did not alter the magnitude of pleural exudation response, but clearly shortened its duration. These results indicate that the early resolution of allergic pleural edema observed during *A. costaricensis* infection coincided with a selective local eosinophilia and seemed to be mediated by COX-2-derived PGE_2 and LXA_4. ***The Journal of Immunology,* 2000, 164: 1029–1036.**

Heightened production of eosinophils leading to circulating and tissue eosinophilia is a hallmark of both helminth parasitic infections and allergic disorders. Other evidence indicates that these eosinophilic pathologies also share a dependence on the activity of cytokines preferentially released from Th2 lymphocytes, notably IL-4, IL-5 (1, 2), and IL-13 (3). Although there is still active discussion of the biological role of eosinophils, the prevalent view is that these cells do indeed exert two distinct functions, mediating protective immunity against parasites and causing tissue damage in allergic disorders (4, 5).

Based on the highly allergenic nature of helminthic parasites and on their particular ability to promote proallergic activities, including mastocytosis, IgE synthesis, and eosinophilia, several groups have postulated a causal link between helminthic infection and the development of allergic diseases (for review, see Ref. 6). However, both epidemiological and laboratory studies indicate that populations parasitized with helminths are actually less responsive to allergen challenge. For instance, there is an inverse relationship between helminth infection and incidence of allergies in human populations (7). Furthermore, decreases in both serum IgE and circulating eosinophil levels by treatment with antihelminthic drugs appeared clearly associated with enhancement of allergic reactivity (7, 8). According to these authors, the lower incidence of allergic reactions in humans infected with parasites would be accounted for by an increased polyclonal IgE production, causing receptor saturation and therefore suppression of specific IgE sensitization (for review, see Ref. 9).

Likewise, in experimental models of human allergic reactivity, helminth-infected rats are less reactive to cutaneous anaphylactic reactions than uninfected rats (10–12). Several other studies have demonstrated that tissue eosinophilia, caused by *Mesocestoide corti* or *Toxocara canis*, is closely related to down-regulation of inflammatory responses in different in vivo and ex vivo models (13–15). In line with these findings, previous studies by our group have demonstrated that rats undergoing localized eosinophilia induced by exogenous chemoattractants, or even expressing spontaneous eosinophilia, reacted to allergen-induced challenge with an attenuated pleural edema. This phenomenon, which was clearly reversed by either pharmacological or immunological blockade of the eosinophilia, also seemed to be dependent on PGs (16–18).

*Laboratory of Inflammation and †Laboratory of Pathology, Institute Oswaldo Cruz, Fiocruz, Brazil; ‡Leukocyte Biology Section, Imperial College School of Medicine, London, United Kingdom; and §Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Received for publication August 23, 1999. Accepted for publication November 1, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from Conselho Nacional de Pesquisas (CNPq) and British Council. Studies in the C.N.S. laboratory were supported by National Institutes of Health Grant GM38765. Synthesis of lipoxin stable analogs was supported by a sponsored research grant from Schering/Berlex (C.N.S. and Nicos A. Petasis).

[2] Address correspondence and reprint requests to Dr. Marco A. Martins, Laboratory of Inflammation, Department of Physiology and Pharmacodynamics. Oswaldo Cruz Institute, Fiocruz. Av. Brasil, 4365, Manguinhos, Rio de Janeiro, RJ, Brazil, 21045-900. E-mail address: mmartins@ioc.fiocruz.br

Copyright © 2000 by The American Association of Immunologists 0022-1767/00/$02.00

To define in more detail the interactions between parasitic infections and allergic inflammation, we have in this study investigated the effects of infection with *A. costaricensis* on allergen-evoked pleurisy in rats. Since, under our particular conditions, helminth infection reduced the duration of allergic edema, we further investigated the mechanisms involved in this phenomenon, assessing the contributions of the isoforms of cyclooxygenase (COX),[3] PGE_2, and lipoxin A_4 (LXA_4) to the regulation of this response to immunological challenge.

Materials and Methods

Animals

Wistar rats of either sex and weighing 150–200 g, purchased from the Oswaldo Cruz Foundation Breeding Unit (Rio de Janeiro, Brazil), were used.

Allergic pleurisy in passively sensitized rats

Rats were passively sensitized by means of an intrapleural (i.pl.) injection of murine IgE mAb to DNP (anti-DNP; 1 μg/cavity). Twenty-four hours later, the allergen, dinitrophenylated BSA (DNP-BSA; 1 μg/cavity), was injected i.pl. into sensitized and sham-sensitized animals (sensitization in which sterile isotonic saline replaced murine IgE anti-DNP). All i.pl. injections were performed during light ether anesthesia in a final volume of 100 μl using a 27.5-gauge needle adjusted to be 3 mm in length, and all solutions were prepared immediately before use. At different times after pleural stimulation, the animals were killed with terminal ether anesthesia and the thoracic cavity was rinsed with 3 ml of saline-containing heparin (10 IU/ml).

Infection with parasite

The nematode *Angiostrongylus costaricensis* has been maintained in the Department of Pathology of Institute Oswaldo Cruz (Fiocruz, Rio de Janeiro, Brazil) through two hosts, mice and *Sarasinula sp* slugs. The infective larvae (third-stage larvae, L3) of this parasite, harvested from mollusc mucus, were counted under a dissecting microscope and diluted in saline. Unsensitized rats were infected by oral administration of 300 infective larvae (L3) of *A. costaricensis*. Twenty-five days later, infected rats were passively sensitized with an IgE anti-DNP-BSA and subsequently challenged as described above.

Pretreatment with IL-5

IL-5 i.v. (100 IU/kg) was diluted in sterile saline (final volume of 200 μl) and injected i.v. in naive rats, anesthetized with ether. One hour later, again anesthetized, rats were passively sensitized and subsequently challenged, as described above.

Pretreatment with COX inhibitors or eicosanoids

The COX inhibitors, indomethacin (2 mg/kg), aspirin (200 mg/kg), NS-398 (5 mg/kg), SC-236 (0.5 mg/kg), and meloxicam (1 mg/kg), were given i.p. 1 h before allergic challenge. Misoprostol (200 μg/kg) was orally administered 1 h before allergic challenge. In control groups, the inhibitors were replaced with their vehicles. The LXA_4 analogues, 15-methyl-LXA_4 and 15-epi-16-*p*-fluorophenoxy-LXA_4, were given locally 5 min before allergic challenge. All of the solutions were prepared immediately before use and, except indomethacin, NS-398, SC-236, and the LXA_4 analogues, which were dissolved in saline. Indomethacin was dissolved in 0.1 N NaOH, buffered with Tris, and neutralized with 0.1 N HCl. NS-398 and SC-236 were dissolved in DMSO and further diluted in saline. The LXA_4 analogues were dissolved in ethanol and further diluted in saline.

Measurement of the kinetics of vascular permeability increase, fluid accumulation, and total protein exuded

Microvascular permeability was assessed in several periods of time, including the first 10 min, from 10–30 min, from 30–60 min, from 60–120 min, and from 120–240 min postallergen challenge. The analysis was done by giving the Evans blue dye i.v. either 5 min before challenge, at 10, 30, 60, or 120 min postchallenge, and thus represents the state of the microvasculature in each of these periods. This type of sample contrasts with that for fluid accumulation, which measured all of the fluid present at the appropriate time, i.e., net accumulation with fluid exudation balanced against reabsorption, for the whole of the sampling period, ranging from 10–240 min postchallenge. Pleural washings were collected and its volume measured with a graduated syringe. Analysis of the plasma protein leakage was performed at different times after injection of allergen. The fluid collected from the pleural cavity was centrifuged (1300 $\times$ *g*) for 10 min, and the protein content of the supernatant was measured by the biuret technique (19). Evans blue dye exuded was quantified by means of spectrophotometric analysis at 650 nm.

Cell analysis

Total leukocytes from peripheral blood (from the tail vein) and pleural cavity were counted in Neubauer chambers by means of an optical microscope after dilution with Türk's solution (2% acetic acid). Differential analysis of cells from blood and pleural fluid was made in blood smears and cytospin preparations, respectively, stained with May-Grünwald-Giemsa dye under an oil immersion objective.

PGE_2, LTC_4, and LXA_4 measurements

Pleural exudates were collected with 1 ml of saline containing heparin (10 IU/ml) and indomethacin (50 μg/ml) to prevent further production of PGE_2. The pleural fluid samples were centrifuged at 1300 $\times$ *g* for 10 min at 0°C, and the supernatants were mixed with methanol (200 μl). The eicosanoids in the pleural supernatant were extracted with SEP-PAK C18 cartridges (Waters Associates, Milford, CT), as previously reported. PGE_2, LTC_4, and LXA_4 levels were measured with ELISA kits according to the manufacturer's instructions (Neogen, Lexington, KY). LXA_4 was further identified by liquid chromatography/mass spectrometry/mass spectrometry analysis using the LXA_4 molecular ion *m/z* 351 and a retention time of synthetic LXA_4 standard, as in Clish et al. (20).

Measurement of corticosterone in serum

Blood samples were taken from the abdominal aorta immediately after death. Serum corticosterone levels were determined using an RIA test kit, according to the instructions of manufacturer (ICN Pharmaceuticals, Costa Mesa, CA).

Materials

Murine anti-dinitrophenylated (DNP) mAb was kindly provided by Dr. A. Provoust-Danon (Unité d'Immuno-Allergie, Institut Pasteur, Paris, France). Indomethacin, Evans blue dye, DNP-BSA, and IL-5 were from Sigma (St. Louis, MO); aspirin was from Synthelabo France Laboratories (Paris, France); meloxicam (Movatec ampoules) was from Boehringer Ingelheim (Buenos Aires, Argentina); and NS-398 was from BIOMOL Research Laboratories (Philadelphia, PA). Misoprostol (Cytotec tablets) was a gift from BIOLAB (São Paulo, Brazil), and SC239 was kindly donated by Searle (Skokie, IL). The LXA_4 analogues used in these experiments were prepared by Dr. Nicos Petasis' laboratory (Department of Chemistry, University of Southern California) as part of a sponsored research program with the C.N.S. laboratory, Brigham and Women's Hospital/Harvard Medical School.

Statistical analysis

Data are reported as means ($\pm$SEM) and statistically analyzed by means of ANOVA, followed by the Newman-Keuls-Student's test. Differences were considered to be statistically significant when $p < 0.05$.

Results

Kinetics of allergic response

The kinetics of the development of the allergic edema are illustrated in Fig. 1, as changes in microvascular permeability measured by Evans blue dye in the pleural fluid (Fig. 1*a*) and as total volume of fluid accumulated in the pleural cavity (Fig. 1*b*). These two variables were measured at the same times after Ag challenge, but represent different types of sampling, as indicated in *Materials and Methods*. As shown in Fig. 1*a*, the increase in microvascular permeability induced by allergen challenge was restricted to the first 10 min postchallenge, with all subsequent assays revealing normal microvascular permeability. In contrast, the volume of pleural fluid essentially plateaued from 10 to 240 min (Fig. 1*b*), falling to background value 24 h postchallenge (data not shown).

[3] Abbreviations used in this paper: COX, cyclooxygenase; i.pl., intrapleural; LTC_4, 5(*S*)-hydroxy-6(*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; LXA_4, lipoxin A_4.

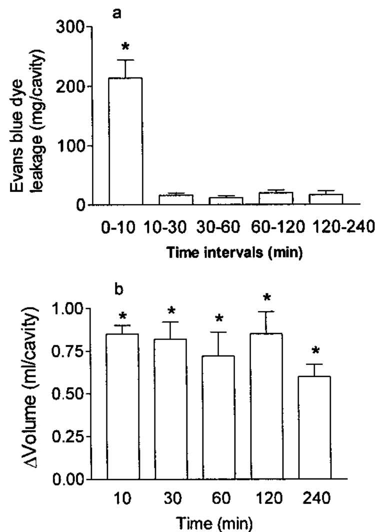

FIGURE 1. Kinetics of the development of the allergic edema illustrated as changes in microvascular permeability measured by Evans blue dye in the pleural fluid (*a*) and as total volume of fluid accumulated in the pleural cavity (*b*) during the first 4 h postallergen challenge. Results are expressed as the mean ± SEM from at least six animals. *, $p < 0.01$ as compared with nonsensitized challenged rats.

These kinetics suggest that the magnitude of the edema was determined by the early increase in microvascular permeability, and that the duration was determined by reabsorptive processes, with negligible continuing exudation over the 4-h period.

Interaction of infection by A. costaricensis *with Ag-induced pleurisy*

In noninfected rats, allergen challenge induced no increase in the eosinophil numbers in the pleural cavity at 30 min or at 4 h after challenge (Fig. 2) and no changes in peripheral blood leukocyte numbers. By contrast, 25 days after *A. costaricensis* infection, there was a marked and selective increase in eosinophil numbers in peripheral blood, from 0.2 ± 0.1 to $1.1 \pm 0.2 \times 10^3$ eosinophils/μl (mean ± SEM, $n = 8$, $p < 0.01$) in normal and infected rats, respectively.

Combination of these conditions, allergen challenge in infected rats, lead to marked changes in both eosinophils and protein exudation in the pleural cavity. The early responses, obtained 30 min postchallenge, for both eosinophil number and the pleural exudation of protein remained unaltered during *A. costaricensis* infection, as illustrated in Fig. 2, *c* and *e*. In contrast, analysis performed 4 h after allergen showed a striking increase in pleural eosinophil infiltration (Fig. 2*d*), together with a drastic decrease in the protein amount of pleural exudate in infected rats (Fig. 2*f*). The development of pleural eosinophilia was accompanied by a small reduction in the underlying blood eosinophilia (Fig. 2, *a* and *b*).

Pretreatment with IL-5

Intravenous stimulation with IL-5 (100 IU/kg) significantly increased circulating eosinophil numbers within 1 h, from 0.1 ± 0.1 to $0.5 \pm 0.1 \times 10^3$ eosinophils per μl (mean ± SEM, $n = 8$, $p < 0.01$), respectively, in normal and IL-5-treated rats, under conditions in which pleural protein content and leukocyte counts were not altered. As shown in Fig. 3, *c* and *e*, IL-5 pretreatment did not affect the allergic pleural exudation or eosinophil accumulation over 30 min, but reduced the allergic edema at 4 h after challenge and induced a selective pleural eosinophilia (Fig. 3, *d* and *f*).

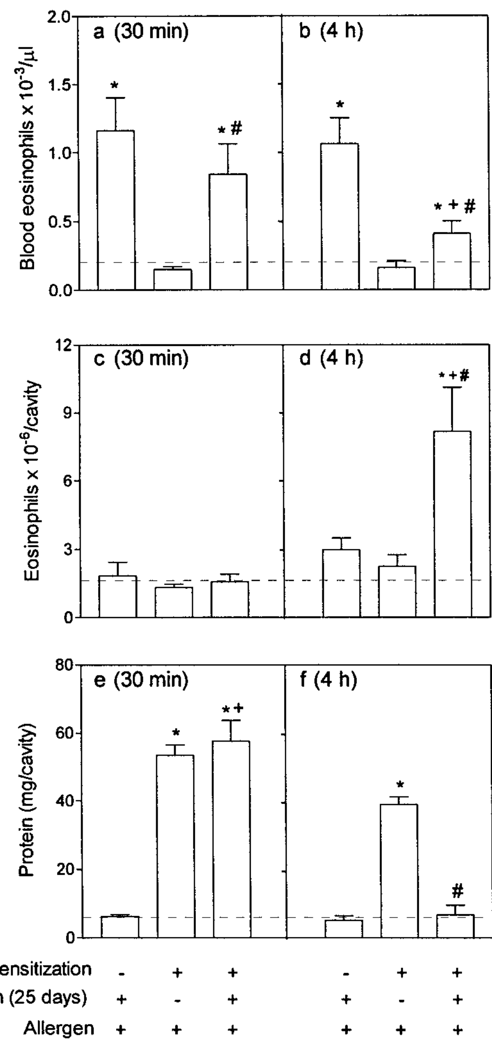

FIGURE 2. Curtailment of allergen-evoked edema following *A. costaricensis* infection in sensitized rats. Blood and pleural effluent samples were collected at 30 min and at 4 h postallergen challenge. Results are expressed as the mean ± SEM from at least seven animals. Dashed lines represent values from allergen-challenged sham-sensitized rats (negative control group). *, $p < 0.01$ as compared with the negative control group; +, $p < 0.01$ as compared with infected sham sensitized; and #, $p < 0.01$ as compared with infected sensitized rats.

Serum corticosterone and pleural fluid PGE_2, LXA_4, and LTC_4 levels

Total serum corticosterone was determined by RIA from samples collected 4 h after allergen challenge of *A. costaricensis*-infected rats. Corticosterone levels obtained in sham-sensitized animals (108 ± 47 ng/ml, $n = 6$, mean ± SEM) were not modified by either allergic challenge or helminth infection, showing values of 81 ± 28 and 106 ± 25 ng/ml, respectively. In addition, the combination of both treatments (allergic challenge and infection) also failed to evoke systemic alterations in corticosterone levels, with values of 101 ± 27 ng/ml.

Fig. 4 summarizes the levels of the eicosanoids, PGE_2, LXA_4, and LTC_4, in pleural fluids at 4 h following allergen challenge. Infection with *A. costaricensis* led to a 5-fold increase of PGE_2 in pleural fluid over the values in noninfected animals (Fig. 4*a*). As illustrated in Fig. 4*b*, the LXA_4 content of pleural fluid was raised by infection alone and elevated further after allergen challenge. The generation of endogenous LXA_4 was confirmed by liquid

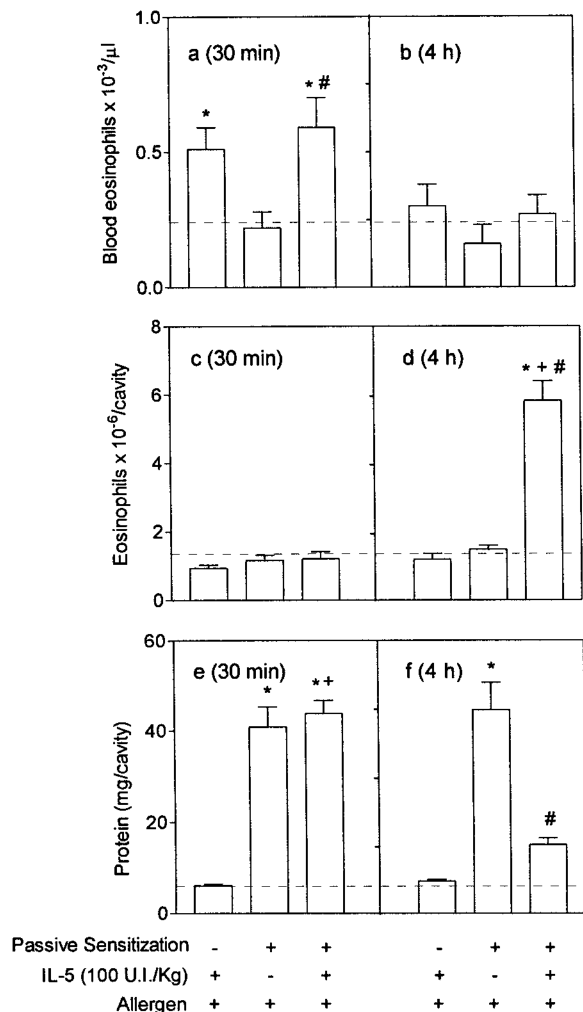

FIGURE 3. Curtailment of allergen-evoked edema following i.v. treatment with IL-5 (100 IU/kg) in sensitized rats. Blood and pleural effluent samples were collected at 30 min and at 4 h postallergen challenge. Results are expressed as the mean ± SEM from at least eight animals. Dashed lines represent values from allergen-challenged sham-sensitized rats (negative control group). *, $p < 0.01$ as compared with the negative control group; +, $p < 0.01$ as compared with infected sham sensitized; and #, $p < 0.01$ as compared with infected sensitized rats.

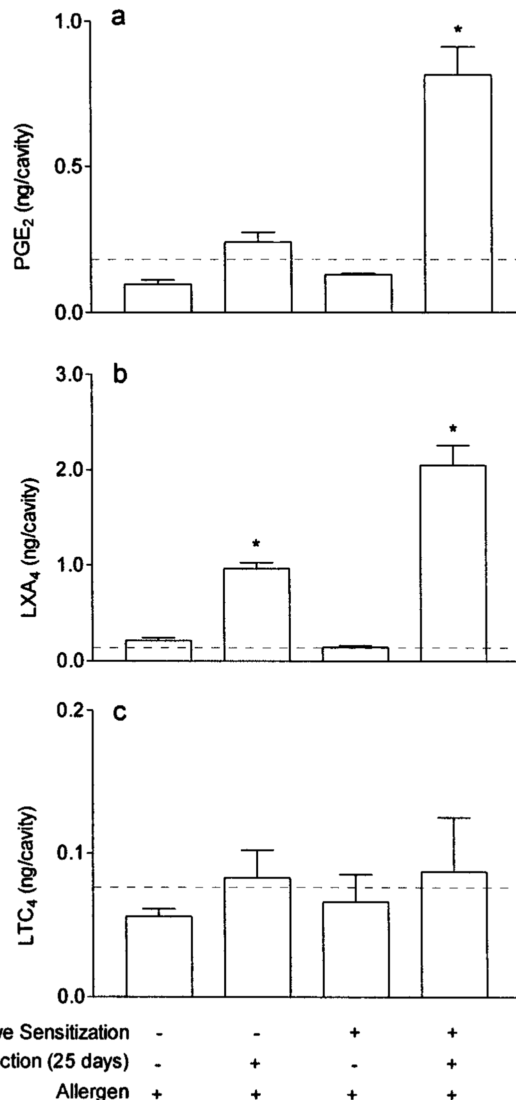

FIGURE 4. Effect of *A. costaricensis* infection on the generation of PGE_2 (*a*), LXA_4 (*b*), and LTC_4 (*c*) in the exudate of allergen-evoked pleurisy 4 h postchallenge. Results are expressed as the mean ± SEM from at least six animals. Dashed lines represent values from allergen-challenged sham-sensitized rats (negative control group). *, $p < 0.01$ as compared with the negative control group.

chromatography/mass spectrometry/mass spectrometry analysis (20). No significant changes in LTC_4 content of pleural fluid were noted after allergic challenge or after infection with the helminth (Fig. 4*c*).

Effect of COX inhibitors on allergic pleural eosinophils and exudation

Pretreatment with either of the nonselective COX inhibitors, indomethacin (2 mg/kg, i.p.) or aspirin (200 mg/kg, i.p.), 1 h before challenge failed to modify allergen-induced protein exudation in IgE passively sensitized rats (17). As summarized in Table I, these inhibitors also did not affect allergen-evoked pleural eosinophil accumulation observed at 4 h in rats with *A. costaricensis* infection, but they did reverse the reduction of the edema. Likewise, in rats with an IL-5-dependent eosinophilia, indomethacin (2 mg/kg, i.p.) also restored allergen-induced edema at 4 h without modifying the local eosinophilia (Table II).

We also used COX inhibitors with greater selectivity for the COX-2 isoform, meloxicam (1 mg/kg), NS-398 (5 mg/kg), or SC-236 (0.5 mg/kg) administered i.p. 1 h before allergen challenge. None of these treatments altered allergen-induced exudation response in passively sensitized rats without infection (data not shown). However, in infected animals, they restored allergic edema at 4 h to the level seen in uninfected rats (Fig. 5*B*), without affecting the concurrent pleural eosinophilia (Fig. 5*a*). In IL-5-pretreated rats, the highly selective COX-2 inhibitor, SC-236, also restored the allergic edema without modifying pleural eosinophilia (Table II).

Effect of the PGE analogue, misoprostol, or LXA_4 analogues on allergic pleural exudation

In the first set of experiments, the pleural exudation in response to allergen challenge was analyzed at three points after allergen challenge, 15 min, 1 h, and 4 h. As shown in Table III, the edema was maintained from 15 min to 4 h, and for the first two time points,

Table I. *Reversal by indomethacin or aspirin of the accelerated resolution of allergic edema during* A. costaricensis *infection in sensitized rats*[a]

Condition	Treatment	Protein (mg/cavity)	Eosinophil × 10^6/cavity
Sham-sensitized		5.6 ± 0.6	1.0 ± 0.6
+ Infection		5.4 ± 0.4	2.1 ± 0.7
Sensitized		47.2 ± 8.0*	1.4 ± 0.1
+ Infection		14.6 ± 1.5$^+$	5.1 ± 1.1$^+$
	Indomethacin	46.4 ± 7.8#	5.4 ± 0.6
	Aspirin	42.3 ± 3.1#	4.9 ± 0.5

[a] COX inhibitors were administered 1 h before i.pl. injection of allergen (DNP-BSA). All groups were challenged with allergen and 4 h later protein and eosinophils in pleural fluid were measured. Each value represents the mean ± SEM from at least eight animals. Although allergen challenge increased edema, which was markedly reduced in infected rats, this reduction was essentially abolished by pretreatment with the nonselective COX inhibitors, indomethacin and aspirin. Note that the pleural eosinophilia remained unaffected by the COX inhibitors.

*, $p < 0.001$ as compared with sham-sensitized group; +, $p < 0.001$ as compared with sensitized group; #, $p < 0.001$ as compared with *A. costaricensis*-infected sensitized group.

this response was not affected by oral pretreatment with the synthetic PG analogue, misoprostol (200 μg/kg). However, misoprostol did inhibit the edema at 4 h postchallenge, to about 55% of the previous value.

In the next set of experiments with two analogues of LXA_4, exudation was assessed at 15 min and 4 h only. A similar pattern of activity was shown by 15-methyl-LXA_4 (Fig. 6, *c* and *d*) and 15-epi-16-*p*-fluorophenoxy-LXA_4 (Fig. 6, *a* and *b*). These LXA_4 stable analogues that resist rapid metabolic inactivation of LXA_4 did not affect exudation measured at 15 min (Fig. 6, *a* and *c*) after allergen challenge, but reduced that at 4 h, in a dose-dependent manner (Fig. 6, *b* and *d*).

Discussion

The goal of this study was an analysis of the interaction between parasitic infection and allergic inflammation. To elucidate the mechanisms responsible for this interaction, we have used two in vivo models. In one, we combined infection with the helminth, *A. costaricensis,* and allergen-induced pleurisy in rats passively sensitized with an IgE anti-DNP-BSA. Because infection with *A. costaricensis* induced a marked blood eosinophilia, we used, for comparison, another model in which blood eosinophilia was induced by pretreatment of noninfected animals with IL-5 before sensitization.

In both models, we observed a curtailment of the duration of the allergic edema, concomitantly with pleural eosinophil accumulation. In both models, there was clear evidence for the involvement of COX-2 in the accelerated resolution of allergen-induced edema. We were also able to mimic the ability of infection or IL-5 pretreatment to shorten the duration of the allergic edema response by systemic administration of misoprostol, a synthetic PGE analogue, or local, i.pl. treatment with LXA_4 analogues.

Despite abundant evidence of the decreased incidence of allergic disorders in patients or experimental animals with existing helminth parasitic infection, the mechanisms underlying this mutual

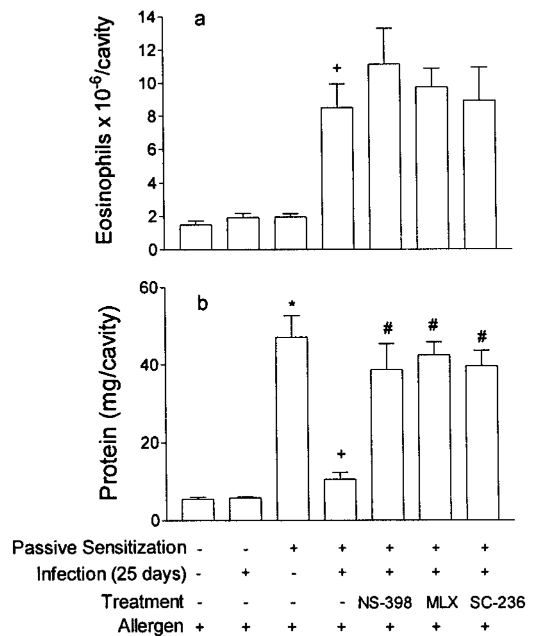

FIGURE 5. Pretreatment with two COX-2-preferring inhibitors, NS-398 and meloxicam, or a COX-2-selective inhibitor, SC-236, does not alter pleural eosinophilia (*a*), but reversed the curtailment of the edema (*b*) evoked by allergen in *A. costaricensis*-infected rats. NS-398, meloxicam, and SC-236 were administered 1 h before challenge. Results are expressed as the mean ± SEM from at least six animals. *, $p < 0.01$ as compared with naive challenged control group; +, $p < 0.01$ as compared with sensitized rats; +, $p < 0.01$ as compared with sensitized noninfected rats; and #, $p < 0.01$ as compared with infected and sensitized rats.

Table II. *Reversal by indomethacin or SC-236 pretreatment of the accelerated resolution of allergic edema during pleural eosinophilia caused by IL-5 in passively sensitized rats*[a]

Condition	Treatment	Protein (mg/cavity)	Eosinophils × 10^6/cavity
Sham-sensitized		5.6 ± 0.2	1.0 ± 0.1
+ IL-5		7.0 ± 0.3	0.9 ± 0.1
Sensitized		41.7 ± 3.1*	1.2 ± 0.1
+ IL-5		18.8 ± 2.2$^+$	3.6 ± 0.2$^+$
	Indomethacin	39.8 ± 2.1#	4.0 ± 0.5
	SC-236	36.8 ± 2.4#	3.8 ± 0.4

[a] Both COX inhibitors (i.p.) and IL-5 (i.v.) were administered 1 h before i.pl. injection of allergen (DNP-BSA). All groups were challenged with allergen, and 4 h later protein and eosinophils in pleural fluid were measured. Each value represents the mean ± SEM from at least eight animals. Here IL-5 pretreatment halved the protein exudation and increased 3 fold the eosinophil accumulation in pleural fluid after allergen challenge. Both the nonselective COX inhibitor, indomethacin, and the highly selective COX-2 inhibitor, SC-236, restored the protein exudation but did not affect the eosinophilia.

*, $p < 0.001$ as compared with sham-sensitized group; +, $p < 0.001$ as compared with sensitized group; #, $p < 0.001$ as compared with IL-5 pretreated and sensitized group.

Table III. *Effect of misoprostol on allergen-induced pleural edema observed 15 min, 1 h, or 4 h after challenge in passively sensitized rats*[a]

Condition	Protein (mg/cavity)		
	15 min	1 h	4 h
Sham-sensitized	4.1 ± 0.4	7.0 ± 0.8	5.6 ± 0.8
Sensitized	41.6 ± 2.2*	45.9 ± 5.2*	45.0 ± 5.2*
+ Misoprostol	45.5 ± 2.4	41.9 ± 2.8	25.5 ± 2.7$^+$

[a] Misoprostol was given orally 1 h before i.pl. injection of allergen (DNP-BSA). All groups were challenged with allergen and protein exudation measured at the time shown. Each value represents the mean ± SEM from at least eight animals. Protein exudation in response to the challenge increased by 15 min and remained constant thereafter for at least 4 h. Misoprostol, a PGE analogue, reduced exudation only at 4 h and not in the early stages of the response.

*, $p < 0.001$ as compared with sham-sensitized group; +, $p < 0.001$ as compared with sensitized group.

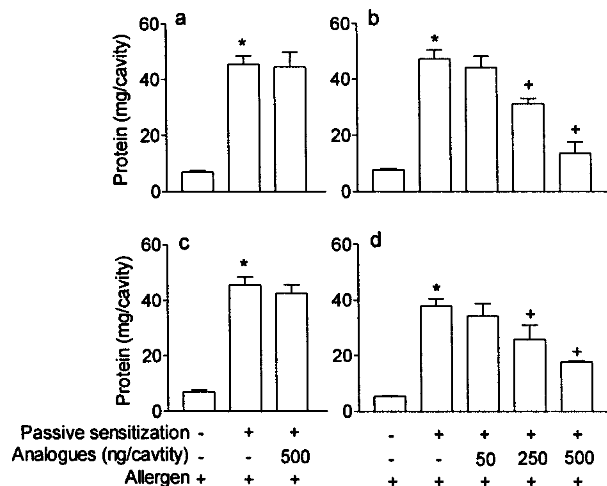

FIGURE 6. Effect of LXA_4 analogues, 15 metil-LXA_4 (50–500 ng/cavity) (*a* and *b*) and 15-epi-16-*p*-fluorophenoxy-LXA_4 (50–500 ng/cavity) (*c* and *d*), on allergen-induced protein extravasation noted at 15 min (*a* and *c*) and 4 h postchallenge (*b* and *d*). Results are expressed as the mean ± SEM from at least six animals. ∗, $p < 0.001$ as compared with nonsensitized rats; +, $p < 0.01$ as compared with sensitized rats; +, $p < 0.01$ as compared with sensitized untreated rats.

exclusion have been poorly investigated. Nevertheless, several attempts have been made to define the mechanisms responsible for the inverse relationship between parasitic infection and allergies. The presently prevailing hypothesis proposes the elevated synthesis of nonspecific polyclonal IgE in helminthic infection as being responsible for the suppression of allergic inflammation, because such an increased synthesis could lead to a saturation of Fcϵ receptors (9). Another explanation considers the suppressive mechanism to be inhibition of allergen-specific IgE synthesis by the polyclonal response against the parasites (7).

Other studies have suggested that allergic activation of mast cells is inhibited by a variety of factors, including the parasitosis-related soluble Ag/IgE complex (21), or rat C3a fragments (22) or human IgG4 (23). However, all of these possible mechanisms operate by inhibiting the capacity of mast cells to react to stimulation with allergen, a process essential for the initiation of the allergic response. As such, these explanations are unlikely to apply to our experimental findings, as the failure in allergic response we observed in this study was always a failure of duration and not of initiation, of the allergic response. Indeed, initiation of allergic responses is most likely to be controlled by factors such as the production or binding of anaphylactic Abs, or receptor activation. In our experiments, both noninfected and helminth-infected rats were sensitized with the same amount of IgE anti-DNP mAb and mounted comparable allergic pleural exudatory responses, showing no differences in the initial development of the edema.

A further mechanism involved in the lower occurrence of allergic reaction in parasitized individuals, which might also account for our observation of shorter duration of edema in *A. costaricensis*-infected rats, is overproduction of anti-inflammatory corticosteroid hormones (24, 25) induced by the helminth infection. However, in our model, we did not find any alteration in serum corticosterone level in *A. costaricensis*-infected rats even after allergic challenge, indicating that yet another mechanism should underlie the curtailment of allergic edema noted in infected animals in our model.

It is particularly relevant to note in this work that helminth parasitic infection is perhaps the most powerful stimulus for blood and tissue eosinophilia. Consistent with this, we found a marked increase in peripheral blood eosinophil numbers following *A. costaricensis* infection in rats. This eosinophilia was long lasting and it did not affect the onset of the pleural edema following Ag challenge. We have also previously shown that pleural eosinophilia, induced by local administration of eosinophil attractants such as platelet-activating factor, ECF-A, bacterial LPS, and pleural wash from LPS-treated rats (16–18), also promotes an attenuation of allergen-induced pleural edema within 4 h. Selective inhibition of eosinophil influx by either immunological or pharmacological means diminished the curtailment of allergic edema, reinforcing the relationship between eosinophilia and down-regulation of allergic edema (16, 18).

A constant concomitant of the shorter lasting allergic edema in helminth-infected rats was local, i.e., pleural, eosinophilia. It was also remarkable that the allergic edema was modified only at the later stages of the overall response when eosinophil accumulation in the pleural cavity had occurred and not in the early stages, at about 30 min after Ag challenge, when eosinophil numbers in the pleural cavity were still low. Because eosinophilic responses in helminthic infections are known to be dependent on IL-5 (2), we also assessed another model in which the direct administration of IL-5 was used to induce eosinophilia in uninfected animals. In agreement with other studies (26–28), injection of IL-5 induced circulating eosinophilia that, like that observed in *A. costaricensis*-infected rats, gave rise to a pleural eosinophil infiltration within 4 h and an accelerated resolution of allergic edema without affecting its onset. These observations and our earlier work suggested that some component of the process of eosinophilia exerted an anti-inflammatory effect.

One possible mediator of the phenomenon we have described in this work, i.e., the shortening of allergic edema related to a local eosinophilia, is PGE_2. Although this PG can act as a proinflammatory agent, inducing vasodilatation and synergizing with other proinflammatory mediators to promote protein exudation and hyperalgesia, it has also been shown to down-regulate inflammatory responses, including allergic reactions, impairing activation of pivotal leukocytes (29). PGE_2 is one of the eicosanoids produced by eosinophils (30, 31), and Buijs et al. (15) had noted that parasitic infection increased the biosynthesis of PGE_2 concurrent with eosinophilia. Furthermore, allergic stimulation raised PGE_2 production in pleural cavities that were experiencing a concurrent selective eosinophilia induced by eosinophil chemoattractants (17, 18).

Our present experiments have directly confirmed an anti-inflammatory role for PGE_2 and further suggested that LXA_4 in this model may also contribute to the overall impact. Thus, levels of PGE_2 were increased at the late stages, but only in animals both infected and challenged. Administration of the synthetic PG misoprostol, which acts on the same receptors as PGE_2, caused a shortening of duration of allergic edema in uninfected rats without affecting the onset of edema. Finally, inhibition of PGE_2 biosynthesis with a range of COX inhibitors reversed the curtailment of the edema in infected or IL-5-treated rats, again without affecting onset. All of these findings would support an important contribution from PGE_2 in mediating the accelerated resolution of edema. Biosynthesis of PGE_2 is catalyzed by COX, which is now known to exist as two isoforms, both expressed by eosinophils. COX-1 is a constitutively expressed enzyme, whereas COX-2 is strongly induced by proinflammatory agents such as cytokines and endotoxin (for review, see Ref. 32). The inhibitors used in this study included nonselective inhibitors of both isoforms (indomethacin, aspirin) and more selective COX-2 inhibitors (meloxicam, NS 398, and SC

236) (32, 33). None of them affected the onset of edema or the subsequent eosinophilia, leading to the conclusion that, in these conditions, neither isoform was crucial to the onset of the edema. These findings would contrast with the clear suppression of other forms of inflammatory edema (e.g., paw edema, air pouch exudation, carrageenin pleurisy) by COX inhibitors, and this discrepancy may reflect the allergic stimuli used in this study. However, at the later stages of the experiment (4 h), the efficacy of the selective COX-2 inhibitors SC 236 and NS 398 would imply that this isoform was crucially involved in the formation of the PGE_2 that induced the early resolution of the edema. This suggestion of a late, anti-inflammatory effect of COX-2, instead of the more widely recognized early, proinflammatory action, has recently been supported by results from another rat pleurisy model, induced by carrageenin (34). In these studies, although the selective COX-2 inhibitor NS 398 and indomethacin both inhibited inflammation at 2 h, later administration at 48 h caused an exacerbation of the pleurisy, comparable with our observations. Our experiments also showed no effect of COX inhibition on the development of pleural eosinophilia either in infected rats or after IL-5 pretreatment, a finding in agreement with others showing that eosinophil accumulation is not affected by COX inhibitors (35, 36).

A degree of selectivity of stimulation of eicosanoid production and hence of action at the later stages of the allergic edema (about 4 h) is inferred from the lack of increase in LTC_4, whereas LXA_4 was increased either by infection alone or, more strikingly, after allergen challenge in infected animals. Here too, synthetic analogues of the endogenous LXA_4 were able to reduce edema at 4 h, but not at 15 min, after challenge. Eosinophils are known to secrete LXA_4 (37), and this lipoxin exhibits anti-inflammatory actions in both in vitro and in animal assays, acting as an endogenous stop signal to inflammatory reactions (38).

In conclusion, our results suggest that local increases in COX-2-derived PGE_2, and in LXA_4, occurring some hours after the initiating allergen challenge, accelerate the resolution of pleural edema evoked by allergen in *A. costaricensis*-infected or IL-5-treated rats. Although the source of these eicosanoids is not definitely known, it is most likely that they are derived from the eosinophils concurrently accumulated in the pleural cavity. The exact effects of these eicosanoids by which the accelerated resolution of allergic edema is achieved (for instance, increased blood or lymphatic flow) remain to be determined. However, our findings do provide a new, localized mechanism by which the eosinophilia induced by parasitic infections can decrease inflammatory reactions to Ag challenge, and one that does not affect the initiation of the response, but its duration. Whether or not eosinophilia can modulate other signs of inflammation such as pain, and what is the real impact of a shorter duration of edema in the totality of the inflammatory response remain to be established. Our results would also support the general concept that COX-2 can mediate some tissue repair processes, as in wound healing (see, for instance, Refs. 39 and 40) and resolution of edema (34; present results). The inhibition of such processes may emerge as unwanted effects of selective COX-2 inhibitors in inflammatory disease.

Acknowledgments

We thank Dr. Clary Clish of the Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital, Massachusetts General Hospital, for his efforts in the identification of eicosanoids by liquid chromatography/mass spectrometry/mass spectrometry analyses. We are also indebted to Mr. Edson Alvarenga and Ms. Juliane Pereira da Silva for their technical assistance.

References

1. Robinson, D. S., Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A. M. Bentley, C. Corrigan, S. R. Durham, and A. B. Kay. 1992. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med. 326:298.*
2. Tagboto, S. K. 1995. Interleukin-5, eosinophils and the control of helminth infections in man and laboratory animals. *J. Helminthol. 69:271.*
3. Li, L., Y. Xia, A. Nguyen, Y. H. Lai, L. Feng, T. R. Mosmann, and D. Lo. 1999. Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells. *J. Immunol. 162:2477.*
4. Ovington, K. S., and C. A. Behm. 1997. The enigmatic eosinophil: investigation of the biological role of eosinophils in parasitic helminth infection. *Mem. Inst. Oswaldo Cruz 92:93.*
5. Weller, P. F. 1994. Eosinophils: structure and functions. *Curr. Opin. Immunol. 6:85.*
6. Pritchard, D. I. 1992. Parasites and allergic disease: a review of the field and experimental evidence for a 'cause-effect' relationship. In *Allergy and Immunity to Helminths: Common Mechanisms or Divergent Pathways?* R. Moqbel, ed. Taylor and Francis, London, p. 38.
7. Lynch, N. R., I. Hagel, M. Perez, M. C. Diprisco, R. Lopez, and N. Alvarez. 1993. Effect of antihelminthic treatment on the allergic reactivity of children in a tropical slum. *J. Allergy Clin. Immunol. 92:404.*
8. Lynch, N. R. 1992. Influence of socio-economic level on helminthic infection and allergic reactivity in tropical countries. In *Allergy and Immunity to Helminths: Common Mechanisms or Divergent Pathways?* R. Moqbel, ed. Taylor and Francis, London, p. 51.
9. Bell, R. G. 1996. IgE, allergies and helminth parasites: a new perspective on an old conundrum. *Immunol. Cell Biol. 74:337.*
10. Turner, K. J., E. H. Fisher, and P. G. Holt. 1982. Host age determines the effects of helminthic parasite infestation upon expression of allergic reactivity in rats. *Aust. J. Exp. Biol. Med. Sci. 60:147.*
11. Turner, K. J., E. H. Fisher, and P. G. Holt. 1982. Suppression of allergic reactions in helminth-parasitized rats of low-IgE-responder phenotype. *Clin. Immunol. Immunopathol. 24:440.*
12. Turner, K. J., K. Shannahan, and P. G. Holt. 1985. Suppression of allergic reactivity by intestinal helminths: susceptibility is a function of IgE responder phenotype. *Int. Arch. Allergy Appl. Immunol. 78:329.*
13. Cook, R. M., N. R. J. Musgrove, and H. Smith. 1988. Relationship between neutrophil infiltration and tissue eosinophilia in the rat. *Int. Arch. Allergy Appl. Immunol. 87:105.*
14. Buijs, J., M. W. E. C. Egbers, W. H. Lokhorst, H. F. J. Savelkoul, and F. P. Nijkamp. 1995. *Toxocara*-induced eosinophilic inflammation: airway function and effect of anti-IL-5. *Am. J. Respir. Crit. Care Med. 151:873.*
15. Buijs, J., M. W. E. C. Egbers, and F. P. Nijkamp. 1995. *Toxocara canis*-induced airway eosinophilia and tracheal hyperreactivity in guinea pigs and mice. *Eur. J. Pharmacol. 293:207.*
16. Bandeira-Melo, C., P. M. R. Silva, R. S. B. Cordeiro, and M. A. Martins. 1995. Pleural fluid eosinophils suppress local IgE-mediated protein exudation in rats. *J. Leukocyte Biol. 58:395.*
17. Bandeira-Melo, C., Y. Singh, P. M. R. Silva, R. S. B. Cordeiro, and M. A. Martins. 1996. Involvement of prostaglandins in the down-regulation of allergic plasma leakage observed in rats undergoing pleural eosinophilia. *Br. J. Pharmacol. 118:2192.*
18. Bandeira-Melo, C., R. S. B. Cordeiro, P. M. R. Silva, and M. A. Martins. 1997. Modulatory role of eosinophils in allergic inflammation: new evidence for a rather outdated concept. *Mem. Inst. Oswaldo Cruz 92:37.*
19. Gornall, A. G., C. J. Bardawill., and M. M. David. 1949. Determination of serum protein by means of the biuret reaction. *J. Biol. Chem. 177:751.*
20. Clish, C. B., J. A. O'Brien, K. Gronert, G. L. Stahl, N. A. Petasis, and C. N. Serhan. 1999. Local and systemic delivery of a stable aspirin-triggered lipoxin prevents neutrophil recruitment in vivo. *Proc. Natl. Acad. Sci. USA 96: 8247.*
21. Stampfli, M. R., M. Rudolf, S. Miesher, J. M. Pachlopnik, and B. M. Stadler. 1995. Antigen-specific inhibition of IgE binding to the high-affinity receptor. *J. Immunol. 155:2918.*
22. Erdei, A., S. Andreev, and I. Pecht. 1995. Complement peptide C3a inhibits IgE-mediated triggering of rat mucosal mast cell. *Int. Immunol. 7: 1433.*
23. Koike, T., A. Tsutsumi, and Y. Nawata. 1989. Prevalence and role of IgG anti-IgE antibody in allergic disorders. *Monogr. Allergy 26:165.*
24. Barnard, C. J., J. M. Behnke, A. R. Gage, H. Brown, and P. R. Smithurst. 1998. The role of parasite-induced immunodepression, rank and social environment in the modulation of behavior and hormone concentration in male laboratory mice (*Mus musculus*). *Proc. R. Soc. Lond. B. Biol. Sci. 265:693.*
25. Bailenger, J., J. B. Chanraud, P. Marcel, and A. Annes. 1981. Parasitism and corticosteronemy in rats during repeated infestations with *Strongyloides ratti. Ann. Parasitol. Hum. Comp. 56:317.*
26. Collins, P. D., S. Marleau, D. Griffiths-Johnson, P. J. Jose, and T. J. Williams. 1995. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J. Exp. Med. 182:1169.*
27. Mould, A. W., K. I. Matthaei, I. G. Young, and P. S. Foster. 1997. Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J. Clin. Invest. 99:1064.*
28. Rothenberg, M. E., R. Ownbey, P. D. Mehlhop, P. M. Loiselle, M. van de Rijn, J. V. Bonventre, H. C. Oettgen, P. Leder, and A. D. Luster. 1996. Eotaxin triggers

eosinophil-selective chemotaxis and calcium flux via a distinct receptor and induces pulmonary eosinophilia in the presence of interleukin 5 in mice. *Mol. Med. 2:334.*

29. Weissmann, G. 1993. Prostaglandins as modulators rather than mediators of inflammation. *J. Lipid Mediators 6:275.*
30. Hubsher, T. 1975. Role of the eosinophil in the allergic reactions. I. EDI: an eosinophil-derived inhibitor of histamine release. *J. Immunol. 114:1379.*
31. Hubsher, T. 1975. Role of the eosinophil in the allergic reactions. II. Release of prostaglandins from human eosinophilic leukocytes. *J. Immunol. 114:1389.*
32. Vane, J. R., Y. S. Bakhle, and R. M. Botting. 1998. Cyclooxygenases 1 and 2. *Annu. Rev Pharmacol. Toxicol. 38:97.*
33. Gierse, J. K., J. J. McDonald, S. D. Hauser, S. H. Rangwala, C. M. Koboldt, and K. Seibert. 1996. A single amino acid difference between cyclooxygenase-1 (COX-1) and -2 (COX-2) reverses the selectivity of COX-2 specific inhibitors. *J. Biol. Chem. 271:15810.*
34. Gilroy, D. W., P. R. Colville-Nash, D. Willis, J. Chivers, M. J. Paul-Clark, and D. A. Willoughby. 1999. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat. Med. 5:698.*
35. Tarayre, J. P., M. Aliaga, M. Barbara, N. Tisseyre, S. Vieu, and J. Tisne-Versailles. 1992. Model of bronchial allergic inflammation in the brown Norway rat: pharmacological modulation. *Int. J. Immunopharmacol. 14:847.*
36. Zuany-Amorim, C., D. Leduc, B. B. Vargaftig, and M. Pretolani. 1993. Characterization and pharmacological modulation of antigen-induced peritonitis in actively sensitized mice. *Br. J. Pharmacol. 110:917.*
37. Serhan, C. N., U. Hirsch, J. Palmblad, and B. Samuelsson. 1987. Formation of lipoxin A by granulocytes from eosinophilic donors. *FEBS Lett. 217:242.*
38. Serhan, C. N. 1997. Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? *Prostaglandins 53:107.*
39. Shigeta, J., S. Takahashi, and S. Okabe. 1998. Role of cyclooxygenase-2 in the healing of gastric ulcers in rats. *J. Pharmacol. Exp. Ther. 286:1383.*
40. Schmassmann, A., B. M. Peskar, C. Stettler, P. Netzer, T. Stroff, B. Flogerzi, and F. Halter. 1998. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. *Br. J. Pharmacol. 123:795.*