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Characterization and pharmacological modulation of antigen-induced peritonitis in actively sensitized mice

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1 The intraperitoneal (i.p.) injection of 1 or 10 μg ovalbumin to sensitized Balb/c mice led to an acute histamine release, firstly evidenced 1 min after the challenge and returning to basal levels 30 min thereafter. This phenomenon was unaccompanied by protein extravasation. A dose-dependent increase in the amounts of immunoreactive leukotriene (LT) C_4 and LTB_4 was observed in the peritoneal washing from sensitized mice 6 h after 1 or 10 μg ovalbumin administration. In separate experiments, the i.p. administration of 1 mg activated zymosan to non-immunized mice was followed by a marked protein extravasation, and by immunoreactive LTC_4 and LTB_4, but not histamine, release in mouse peritoneum 1 h after its injection.

2 Mediator release in the mice peritoneal cavity was concomitant with a transient neutrophil infiltration, which peaked at 6 h and returned to basal levels thereafter. An intense eosinophil accumulation starting at 24 h, peaking at 48 h and returning to basal values at 164 h, was also observed.

3 Ovalbumin (1 μg)-induced eosinophilia, observed at 24 h, was reduced by the pretreatment of the animals with dexamethasone (1 mg kg^{-1}, s.c.) or with the 5-lipoxygenase inhibitor, BWA4C (20 mg kg^{-1}, s.c.), whereas indomethacin (2 mg kg^{-1}, s.c.) and the platelet-activating factor (PAF)-antagonist SR 27417 (10 mg kg^{-1}, s.c.) were ineffective. These results indicate that metabolites of arachidonic acid of lipoxygenase pathway, but not cyclo-oxygenase derivatives or PAF, mediate antigen-induced eosinophil accumulation in the mouse peritoneum.

4 The histamine H_1 receptor antagonist drug, cetirizine (15–30 mg kg^{-1}, s.c.) markedly reduced ovalbumin-induced eosinophil accumulation under conditions where terfenadine was ineffective, suggesting that the effect of cetirizine was not related to the inhibition of the H_1 receptor effects of histamine.

5 The immunosuppressive agent, FK-506 (1–2 mg kg^{-1}, s.c.) and the protein synthesis inhibitor, cylcoheximide, when administered either *in situ* (0.06 ng/cavity) or systemically (5 mg kg^{-1}, s.c.), prevented antigen-induced eosinophil accumulation in the mouse peritoneum, contributing to the concept that substances (probably cytokines) originating from lymphocytes may be involved in the modulation of the eosinophilotactic response in this model.

6 The results of the present study indicate that the i.p. administration of ovalbumin to actively sensitized mice induced late eosinophil accumulation in the peritoneal cavity. This phenomenon, which may be in part mediated by the release of lipoxygenase metabolites and/or by newly generated factors, such as T-lymphocytes-derived eosinophilotactic cytokines, offers an interesting tool to investigate the mechanism of action of anti-allergic and anti-inflammatory drugs.

Keywords: Eosinophils; allergic response; zymosan; leukotrienes

Introduction

The participation of eosinophils in allergic reactions is suggested by the presence of specific receptors for anaphylactic immunoglobulins, adhesion molecules and pro-inflammatory mediators at their surface (Capron, 1992). Eosinophils are prominent inflammatory cells involved with allergic disorders, which are recruited in elevated numbers into the airways and the pleural cavity of several species, including guinea-pigs, mice and rats, following antigen challenge (Lellouch-Tubiana *et al.*, 1988; Gulbenkian *et al.*, 1990; Lima *et al.*, 1991; Okudara *et al.*, 1991). The ability of activated eosinophils to release eosinophil-derived cytotoxic proteins, such as major basic protein and eosinophil-derived neurotoxin (Gleich, 1990), has associated these cells with epithelial damage and tissue injury. In addition, the number of eosinophils in the airways correlates with the severity of the late phase asthma (Bousquet *et al.*, 1990), suggesting that their presence in inflamed tissues contributes to the perpetuation and the amplification of the disease. However, the mechanisms responsible for the attraction and the localisation of eosinophils at the site of allergic reactions remain, to be elucidated fully. Lipid mediators, such as platelet-activating factor (PAF), or leukotriene (LT) B_4 are potent chemotactic agents for eosinophils from different species (Ford-Hutchinson *et al.*, 1980; Lellouch-Tubiana *et al.*, 1988; Martins *et al.*, 1991; Coëffier *et al.*, 1991). Furthermore, it has been suggested recently that cytokines released by activated T-lymphocytes, such as interleukin-5 (IL-5), IL-3 or granulocyte-macrophage colony stimulating factor (GM-CSF), may play a role in eosinophil accumulation at the site of the allergic reactions (Owen *et al.*, 1987; Rothenberg *et al.*, 1988; Sanderson, 1992). This phenomenon may depend on the ability of those cytokines to induce eosinophil proliferation from their bone-marrow precursors and to enhance their survival. Thus, drugs inhibiting the pro-inflammatory activities of lipid mediators on one hand and those interfering with the activation of T-lymphocytes, on the other, are potentially useful in the treatment of allergic disorders.

The mechanisms of inflammatory reactions have been largely investigated in mice (Colorado *et al.*, 1991; Amorim *et al.*, 1992a,b; Perretti *et al.*, 1992), with particular emphasis on the role of eosinophil infiltration which accompanies helminthic infections (Sher *et al.*, 1990; Secor *et al.*, 1990). However, only few studies have focused on the effects of antigen challenge in sensitised mice in terms of cell migration and activation. In particular, Spicer *et al.* (1986) described

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the kinetics of cell infiltration in the peritoneal cavity of sensitized mice following antigen administration. Using a limited number of anti-inflammatory and anti-allergic drugs, they have also shown that metabolites of arachidonic acid or vasoactive amines are not involved in antigen-induced eosinophilia. However, the interactions between cell mobilisation and other inflammatory parameters, such as local mediator release, were not investigated. Consequently, the mechanisms responsible for the cell attraction and, particularly, those involved in the maintenance of eosinophils at the site of hypersensitivity reaction remain to be determined.

In an attempt to define better the role of different mediators and inflammatory cells in antigen-induced peritonitis in sensitized mice, we investigated the changes in cell distribution and mediator release in the peritoneal washing following ovalbumin administration to sensitized Balb/c mice. Changes in eosinophil distribution were modulated by different inhibitors of synthesis and antagonists of receptors for inflammatory mediators, and by drugs effective on the T-lymphocyte function.

Methods

Animals and sensitization procedure

Male Balb/C mice aged 8 weeks, weighing approximately 25–30 g raised at the Pasteur Institute (Paris, France), were actively sensitized by a subcutaneous (s.c.) injection of 0.4 ml 0.9% w/v NaCl (saline) containing 100 μg ovalbumin adsorbed in 1.6 mg aluminium hydroxide (Andersson & Brattsand, 1982). Seven days later, the animals received the same dose of ovalbumin in the presence of $Al(OH)_3$ and were used 7 days thereafter.

Antigen-induced peritonitis

Peritonitis was induced by the intraperitoneal (i.p.) injection of 0.4 ml of a solution containing 2.5 or 25 μg ml^{-1} ovalbumin diluted in sterile saline (1 or 10 μg of ovalbumin, as final doses injected per cavity). Control animals received the same volume of sterile saline. At various time intervals after antigen challenge (30 min–164 h), animals were killed by an overdose of ether and the peritoneal cavity was opened and washed with 3 ml of heparinised saline (10 U ml^{-1}). Approximately 90% of the initial volume was recovered. In rare cases, when haemorrhages were noted in the peritoneal cavity, the animals were discarded.

Zymosan-induced peritonitis

Non-immunized mice (25–30 g) were injected i.p. with 0.4 ml of a solution containing 2.5 mg ml^{-1} activated zymosan (AZ) (1 mg, as final dose injected) prepared according to Bruijnzeel *et al.* (1985). The peritoneal cavity was washed 1 or 4 h after the stimulation, as described above.

Leucocyte analysis

Total leucocytes present in the peritoneal lavages were counted in a Coulter counter ZM (Coultronics, Margency, France) and expressed as numbers of cells ml^{-1}. Differential cell counts were performed after cytocentrifugation (Hettich-Universal) and staining with Diff-Quik stain (Baxter Dade AG, Dudingen). At least 300 cells were counted and results were expressed as number of each cell population ml^{-1}.

Drug treatment

The dose of 1 μg of ovalbumin and the time of 24 h after challenge were selected to study the pharmacological modulation of ovalbumin-induced peritonitis.

The steroidal anti-inflammatory agent, dexamethasone (1 mg kg^{-1}), the non-steroidal anti-inflammatory drug, indomethacin (2 mg kg^{-1}) and the selective 5-lipoxygenase inhibitor, BWA4C (20 mg kg^{-1}) (Payne *et al.*, 1988) were injected s.c. 1 h before antigen challenge. The PAF antagonist, SR 27417 (10 mg kg^{-1}) (Herbert *et al.*, 1991) and the histamine H_1-antagonists, terfenadine (10 mg or 30 mg kg^{-1}, s.c.), or cetirizine (7.5–30 mg kg^{-1}) were administered s.c., 1 h before and 6 h after antigen challenge. The protein synthesis inhibitor cycloheximide was injected either i.p. at a dose of 0.06 ng/cavity 5 min before, or s.c. at 5 mg kg^{-1} 5 h before antigen challenge. The immunosuppressive compound FK-506 (0.5–2.0 mg kg^{-1}) (Yamamoto *et al.*, 1990) was injected s.c. 6 h and 5 min before ovalbumin provocation. All drugs were dissolved in sterile saline, except SR 27417, which was dissolved in 0.1 N HCl and saline (0.1:1, v/v), BWA4C, which was first dissolved in dimethylsulphoxide (DMSO) and further diluted in saline (0.1:1, v/v) and FK-506 which was dissolved in a mixture of ethanol, Tween 80 and saline (1:0.2:8.8; v/v/v). The appropriate vehicles were injected in control experiments.

Measurement of immunoreactive LTC_4-like material, LTB_4 and histamine in the peritoneal washing

Immunoreactive LTC_4-like material was measured in the supernatant from peritoneal washings (centrifuged at 1,200 *g* for 15 min at 4°C) by radioimmunoassay according to Aehringhaus *et al.* (1982) and to Young *et al.* (1991). Briefly, dextran-coated charcoal was used to separate unbound ligand by centrifugation at 1,200 *g* for 10 min at 4°C. The monoclonal anti-LTC_4 antibody employed (kindly provided by Dr U. Zor and Dr F. Kohen, Weizmann Institut, Rehovot, Israel) was 10% crossreactive with LTD_4 and less than 0.1% with LTA_4 and LTB_4. The sensitivity of the assay was approximately 15 pg of immunoreactive LTC_4 in 0.1 ml sample.

Immunoreactive LTB_4 in the supernatants from peritoneal washings (centrifuged at 1,200 *g* for 15 min at 4°C) was determined by enzyme-linked-immunosorbent assay (Laboratoire des Stallergenes, Fresnes, France) according to Pradelles *et al.* (1985). The monoclonal antibody anti-LTB_4 was $<$ 0.1% crossreactive with LTC_4, LTD_4 and LTE_4. The sensitivity of the assay was 2 pg of LTB_4/0.1 sample.

For the histamine assay, 0.5 ml of the supernatant from peritoneal washings (centrifuged for 2 min at 12,000 *g* and at 4°C) were mixed with 0.5 ml of 0.8 N perchloric acid. After centrifugation for 10 min at 1,200 *g* and at 4°C, the supernatants were stored at 4°C. An automatic spectrofluorometric assay for histamine was performed, according to a previously published method (Lebel, 1983).

Protein assay

The fluids recovered from the peritoneal cavities were centrifuged for 2 min at 12,000 *g* at room temperature and the protein contents were measured in the supernatants by a standard dye-binding technique, as described by Bradford (1976).

Materials

Ovalbumin (5x crystallized) was from Immunobiological (Costa Mesa; U.S.A.); zymosan type A, cycloheximide, dexamethasone phosphate and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO. U.S.A.). Aluminium hydroxide (Andersson & Brattsand, 1982) was from Merck (Darmstadt, Germany). Heparin was from Choay (Paris, France) and the dye reagent for the protein assay (Bio-Rad Protein assay) was from Gmbh Laboratory (Ivry-Sur-Seine, France). Tween-80 was from Fluka Chemika (Buchs, Switzerland). Radiolabelled LTC_4 was from Amersham (Buckinghamshire); LTC_4 was from PRIMED-CNRS (Paris, France) and LTB_4 was from Cascade, Euromedex

(Strasbourg, France). BWA4C (N-(3-benzylcinnamyl)acetohydroxamic acid) was a gift from Dr S. Moncada (Wellcome Research Laboratories, Beckenham UK). SR 27417 [N-(2-dimethylaminoethyl)-N-(3-pyridinyl methyl) [4-(2,4,6-triisopropylphenyl)tetrahydrofuran] was kindly provided by Dr J.M. Herbert (Sanofi; France). Terfenadine was a gift from Dr M. Bloom (Merrell Dow; Paris, France). Cetirizine was kindly supplied by Dr J.P. Rihoux (UCB; Braine L'Alleud, Belgium). FK-506 (17-allyl-1,14-dihydroxy-12-(2-(4-hydroxy-3-methoxy-cyclohexyl)1-methylvinyl)-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo(22,3,10)4,9)octacos-18-ene-2,3,10,16-tetraone) was a gift from Dr K. Murato (Fujisawa Pharmaceutical; Osaka, Japan).

Statistical analysis

Data were analysed by standard statistical packages for one way analysis of variance (ANOVA) followed by Student's t test for unpaired values. P values of 0.05 or less were considered significant. Results are expressed as means ± standard error of the mean (s.e.mean).

Results

Kinetics of cellular distribution in peritoneal lavage from ovalbumin-challenged mice

The i.p. injection of 1 or 10 μg ovalbumin to sensitized mice did not modify the number of total cells in the peritoneal washing at 6 h, as compared to sensitized saline-challenged animals. In contrast, a significant increase in the number of total cells was noted at 24 and 48 h, but only for the higher dose of antigen (10 μg) (Figure 1). Ovalbumin induced a marked and dose-dependent increase in the number of neutrophils, which peaked at 6 h and returned to basal values thereafter (Figure 1). Challenge with either 1 or 10 μg ovalbumin also induced a dose-dependent eosinophil infiltration, starting at 24 h, and reaching a maximum at 48 h. At 164 h, the eosinophil counts returned to basal levels (Figure 1). No significant changes in the numbers of mononuclear cells were observed at any time-point considered (Figure 1). When non-immunized mice were injected with 10 μg ovalbumin, no changes in cellular distribution were detected at 24 h (data not shown).

Effect of antigen challenge and of AZ on protein extravasation in mouse peritoneal cavity

The i.p. administration of ovalbumin to sensitized mice failed to trigger protein extravasation above values measured in saline-challenged animals at any time considered (Table 1). In contrast, AZ (1 mg/cavity) was highly effective in promoting protein extravasation in the peritoneal cavity of non-immunized mice 1 h after stimulation. Indeed, 45.1 ± 9.2 μg ml^{-1} and 417.6 ± 41 μg ml^{-1} proteins were detected in the peritoneal washing from saline- and AZ-injected mice, respectively ($n = 6$; $P < 0.001$). Four hours after AZ administration, the levels of proteins were markedly decreased, but values were still significantly different, as compared to those measured in saline-injected animals (28.5 ± 9.8 μg ml^{-1} and 209.7 ± 21.7 μg ml^{-1} proteins for saline and AZ-injected mice, respectively, $n = 6$; $P < 0.001$).

Effect of antigen challenge and of AZ on histamine, immunoreactive LTC_4-like material and LTB_4 release in mouse peritoneal washing

Histamine release was detected in the peritoneal washing of sensitized mice 1 min after antigen challenge. The levels of histamine progressively decreased and returned to basal levels at 30 min (Table 1). In contrast, the i.p. injection of 1 mg AZ was not followed by histamine secretion at any time point considered (data not shown). A dose-dependent release of immunoreactive LTC_4-like material in the peritoneal washing was observed 6 h after antigen challenge, but values reached statistical significance, as compared to saline-injected mice, only for 1 μg ovalbumin (Table 1). The i.p. administration of 1 mg AZ to non-sensitized mice triggered the release of high amounts of immunoreactive LTC_4-like material, which peaked 1 h after its injection (saline-injected mice = 0.05 ± 0.04 ng ml^{-1}; AZ-injected mice = 10.1 ± 0.8 ng ml^{-1}; $n = 6$; $P < 0.001$) and returned to basal levels at 4 h. Higher concentrations of immunoreactive LTB_4 were measured in the peritoneal washing from sensitized mice after the i.p. injection of 1 or 10 μg ovalbumin, as compared to saline-challenged

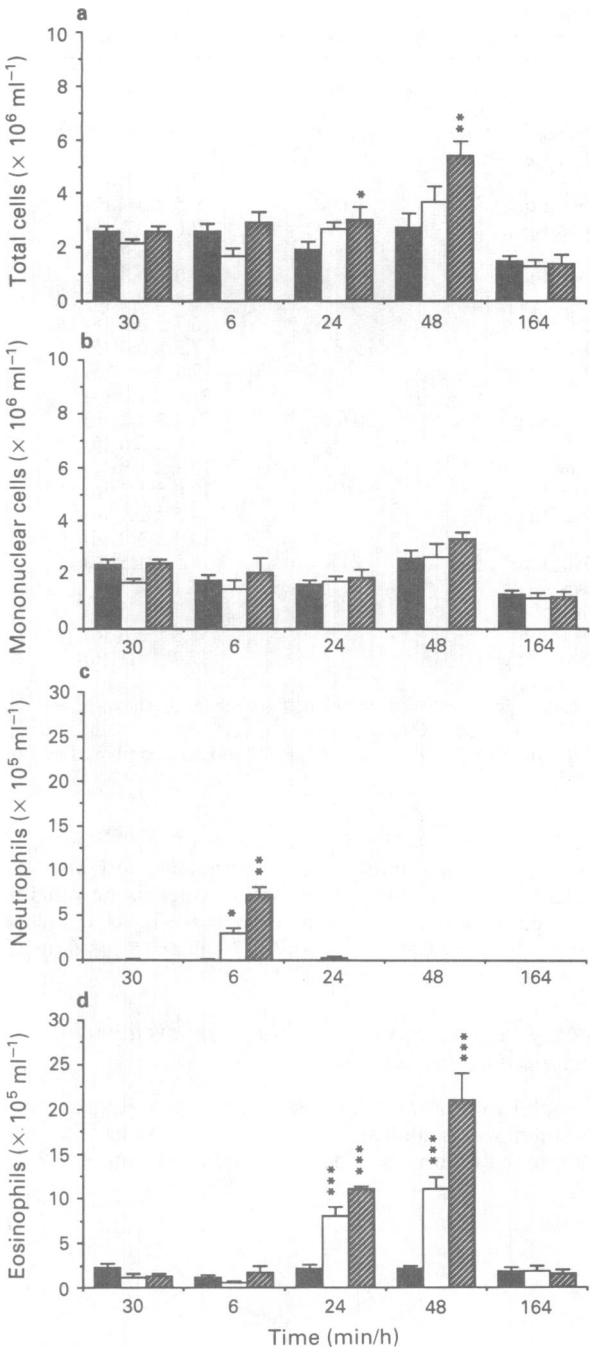

Figure 1 Kinetics of total cell (a), mononuclear cell (b), neutrophil (c) and eosinophil (d) distribution in the peritoneal cavity of sensitized mice. Cells were counted and differentiated at various time-intervals (30 min–164 h) after the injection of either saline (■), or 1 (□), or 10 μg (▨) ovalbumin. Results are expressed as mean ± s.e.mean of 5–11 experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, as compared to saline-injected animals.

Table 1 Kinetics of antigen-induced protein extravasation, histamine, immunoreactive leukotriene C_4 (LTC_4)-like material and LTB_4 release in the peritoneal washing from sensitized Balb/C mice

		Parameters			
Stimulus	*Time* (min-h)	*Proteins* ($\mu g\ ml^{-1}$)	*Histamine* ($ng\ ml^{-1}$)	*LTC_4* ($ng\ ml^{-1}$)	*LTB_4* ($pg\ ml^{-1}$)
Saline		7.8 ± 0.9 (5)	9.1 ± 1.9 (6)	0.2 ± 0.1 (6)	ND
Ova 1 μg	1 min	9.5 ± 0.7 (7)	26.6 ± 5.7 (6)*	0.4 ± 0.2 (6)	ND
Ova 10 μg		7.6 ± 0.6 (6)	30.3 ± 1.9 (6)***	0.2 ± 0.0 (6)	ND
Saline		8.5 ± 0.9 (6)	6.0 ± 1.5 (6)	0.2 ± 0.1 (6)	ND
Ova 1 μg	5 min	10.0 ± 0.8 (6)	13.1 ± 2.3 (6)*	0.2 ± 0.0 (6)	ND
Ova 10 μg		8.9 ± 0.7 (7)	17.9 ± 3.9 (6)*	0.2 ± 0.2 (6)	ND
Saline		6.7 ± 0.5 (5)	5.0 ± 1.4 (6)	0.2 ± 0.2 (4)	ND
Ova 1 μg	15 min	7.2 ± 0.4 (5)	4.5 ± 0.4 (6)	0.2 ± 0.0 (5)	ND
Ova 10 μg		7.7 ± 0.6 (5)	11.3 ± 1.2 (6)***	0.3 ± 0.1 (5)	ND
Saline		21.0 ± 1.0 (6)	3.4 ± 0.4 (6)	0.8 ± 0.5 (6)	54.0 ± 5.0 (5)
Ova 1 μg	30 min	26.1 ± 4.2 (6)	1.9 ± 0.4 (6)	1.4 ± 0.2 (6)	63.0 ± 16.0 (5)
Ova 10 μg		21.9 ± 2.6 (6)	3.2 ± 0.7 (6)	1.1 ± 0.7 (6)	49.0 ± 6.0 (5)
Saline		15.1 ± 1.9 (6)	7.5 ± 0.8 (6)	1.9 ± 0.6 (6)	5.0 ± 1.0 (5)
Ova 1 μg	6 h	18.0 ± 1.5 (6)	4.8 ± 0.5 (6)	4.4 ± 0.6 (12)**	156.0 ± 40.0 (11)*
Ova 10 μg		14.5 ± 2.1 (6)	4.6 ± 0.4 (6)	2.6 ± 0.1 (6)	162.0 ± 70.0 (6)*
Saline		19.4 ± 4.0 (6)	6.1 ± 0.3 (6)	1.8 ± 0.7 (6)	5.0 ± 0.8 (4)
Ova 1 μg	24 h	18.5 ± 1.5 (6)	5.3 ± 0.5 (6)	0.5 ± 0.2 (6)	5.0 ± 1.0 (12)
Ova 10 μg		23.1 ± 2.5 (4)	4.0 ± 0.3 (5)	1.9 ± 0.9 (5)	18.0 ± 7.0 (6)
Saline		31.7 ± 8.5 (5)	6.6 ± 0.8 (6)	2.2 ± 0.2 (5)	5.0 ± 0.8 (5)
Ova 1 μg	48 h	32.6 ± 3.6 (6)	7.3 ± 0.8 (6)	2.5 ± 0.7 (5)	4.0 ± 0.9 (5)
Ova 10 μg		37.0 ± 4.1 (6)	3.5 ± 0.3 (6)	2.2 ± 0.5 (4)	9.0 ± 3.0 (5)

Results are expressed as mean ± s.e.mean of the number of experiments indicated in parentheses.
ND = not done; Ova = ovalbumin.
*$P<0.05$; **$P<0.01$ and ***$P<0.001$, as compared to saline-injected mice.

animals (Table 1). The i.p. injection of AZ induced the release of increased amounts of immunoreactive LTB_4 above the basal values 1 h after its injection, whereas no difference was noted after 4 h, as compared to saline-injected preparations (saline-injected mice = 77 ± 14 $pg\ ml^{-1}$; AZ-injected mice = 349 ± 65 $pg\ ml^{-1}$, $n = 5-6$; $P<0.01$).

Effect of dexamethasone, BWA4C and cycloheximide on antigen-induced eosinophilia

Eosinophil infiltration triggered by the i.p. injection of 1 μg ovalbumin was significantly reduced by 1 $mg\ kg^{-1}$ s.c. dexamethasone (Figure 2). The s.c. administration of BWA4C (20 $mg\ kg^{-1}$) significantly inhibited eosinophil accumulation in the mice peritoneal cavity at 24 h (Figure 2). The dose of BWA4C selected suppressed ovalbumin (1 μg)-induced LTC_4-like material generation, as measured at 6 h in the peritoneal fluid. Indeed, 4.5 ± 0.6 and 1.3 ± 0.4 $ng\ ml^{-1}$ LTC_4 were measured in the peritoneal washings from antigen-challenged vehicle- or BWA4C-treated mice, respectively ($n = 6$, $P<0.01$).

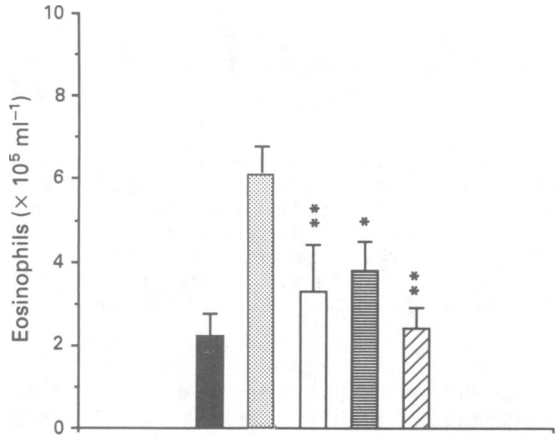

Figure 2 Eosinophil distribution in the peritoneal washing from sensitized mice challenged i.p. 24 h before with either saline (■), or with 1 μg ovalbumin and pretreated by a single s.c. injection of 0.1:1 v/v DMSO-saline (▩), or of dexamethasone (□, 1 $mg\ kg^{-1}$,) or of BWA4C (▤, 20 $mg\ kg^{-1}$) 1 h before the challenge; (▨) represents the number of eosinophils measured in the peritoneal washing from ovalbumin-challenged mice which had received an i.p. injection of 0.06 ng cycloheximide 5 min before antigen provocation. Data are presented as mean ± s.e.mean of 6 experiments. *$P<0.05$ and **$P<0.01$, as compared to antigen-challenged vehicle-injected animals.

Antigen-induced eosinophilia was also suppressed by the *in situ* administration of 0.06 ng cycloheximide 5 min before ovalbumin injection (Figure 2). A significant reduction in the number of eosinophils was also observed when cycloheximide was administered by the s.c. route at 5 $mg\ kg^{-1}$, 5 h before antigen challenge. Indeed, 9.6 ± 1.9 and 4.1 ± 0.4 × $10^5\ ml^{-1}$ eosinophils were found in the peritoneal washing from saline- and cycloheximide-treated mice, respectively ($n = 6$, $P<0.001$).

Effect of indomethacin and SR 27417 on antigen-induced eosinophilia

Pretreatment of sensitized mice with indomethacin (2 mg kg^{-1}, s.c.) 1 h before ovalbumin administration, was ineffective against eosinophilia (Figure 3). The s.c. treatment with the PAF-antagonist SR 27417 (10 $mg\ kg^{-1}$) 1 h before and 6 h after antigen challenge led to a slight, but not statistically significant, reduction of eosinophil accumulation in the peritoneal washing (Figure 3).

Effect of terfenadine and cetirizine on antigen-induced eosinophilia

The s.c. treatment with cetirizine (15–30 $mg\ kg^{-1}$, 1 h and 6 h after the challenge) dose-dependently reduced the eosinophilia induced by 1 μg ovalbumin (Figure 4). The administration of 10 or 30 $mg\ kg^{-1}$ terfenadine under the same conditions was ineffective. Indeed, 5.6 ± 0.1 × $10^5\ ml^{-1}$, 3.6 ± 0.1 × $10^5\ ml^{-1}$ and 6.2 ± 0.1 × $10^5\ ml^{-1}$ eosinophils were counted in the peritoneal lavage from ovalbumin untreated or terfenadine (10 or 30 $mg\ kg^{-1}$)-treated mice, respectively ($n = 5-6$, difference not statistically significant). In separate experiments, non-immunized mice were treated s.c.

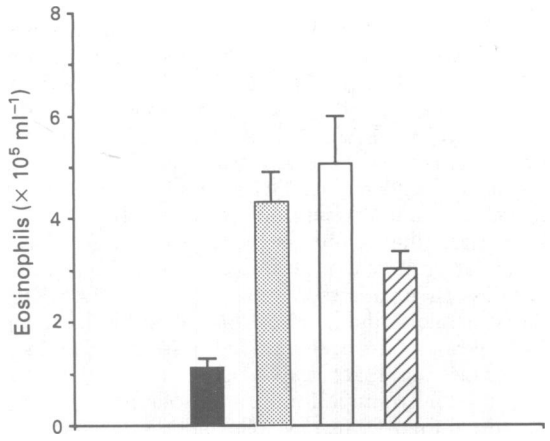

Figure 3 Effect of indomethacin and of the PAF antagonist, SR 24417, on antigen-induced eosinophil accumulation in the peritoneal cavity of sensitized mice. Animals were challenged with i.p. saline (■), or with 1 μg ovalbumin and pretreated either with a s.c. injection of 0.1 N HCl-saline (0.3:1; v/v) 1 h before and 6 h after the challenge (▩), or with 2 mg kg^{-1} indomethacin 1 h before the challenge (□), or with SR 27417, injected s.c. twice, 1 h before and 6 h after the challenge (▨). Each point represents the mean ± s.e.mean of 6 experiments.

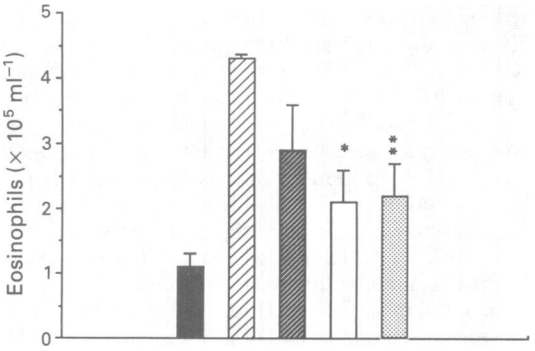

Figure 4 Eosinophil counts in the peritoneal washing from sensitized mice challenged i.p. 24 h before either saline (■), or with 1 μg ovalbumin (▨). Ovalbumin-challenged animals were treated by two s.c. injections of saline (▨), or of cetirizine (▨, □, ▩, 7.5, 15 or 30 mg kg^{-1}, respectively) 1 h before and 6 h after the challenge. Results are expressed as mean ± s.e.mean of 5–6 experiments. *$P<0.05$ and **$P<0.01$, as compared to ovalbumin-challenged vehicle-injected animals.

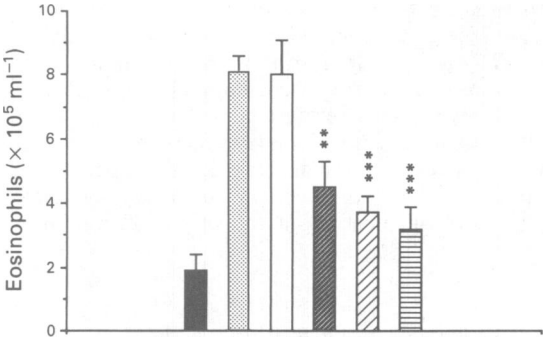

Figure 5 Effect of FK-506 on antigen-induced eosinophil accumulation in the peritoneal washing from sensitized mice killed 24 h after the challenge. Animals were injected i.p. either with saline (■), or with 1 μg ovalbumin (▩) and they were treated s.c. with the vehicle (ethanol, Tween 80 and saline, 1:0.2:8.8; v/v/v; □), or with 0.5 mg kg^{-1} (▨), 1 mg kg^{-1} (▨) or 2 mg kg^{-1} (▤) FK-506, injected twice, 6 h and 5 min before the challenge. Results are expressed as mean ± s.e.mean of 5–11 experiments. *$P<0.05$, **$P<0.01$ and ***$P<0.001$, as compared to antigen-challenged untreated mice.

with 30 mg kg^{-1} of cetirizine or terfenadine and challenged with 1 mg/cavity histamine 18 or 24 h later. Protein extravasation, as measured by Evans blue dye technique 30 min after the stimulation, was inhibited to a similar extent in cetirizine or terfenadine-treated animals (data not shown).

Effect of FK-506 on antigen-induced eosinophilia

Eosinophilia induced by the i.p. injection of 1 μg ovalbumin was inhibited by the s.c. administration of 0.5–2.0 mg kg^{-1} FK-506 6 h and 5 min before the challenge (Figure 5). Indeed, a significant reduction (50%) in the number of eosinophils was observed even with the lower dose of FK-506 (0.5 mg kg^{-1}). Pretreatment with 1 or 2 mg kg^{-1} FK-506 decreased by 54% and 60%, respectively the numbers of eosinophils measured in the peritoneal washing 24 h after antigen challenge.

Discussion

The i.p. injection of ovalbumin to sensitized Balb/C mice induced a transient neutrophil infiltration in the peritoneal cavity, which peaked at 6 h and was not accompanied by an increase in the total number of cells. At 24 h, when the number of neutrophils had returned to basal levels, a dramatic eosinophil augmentation, which peaked at 48 h and resolved at 164 h, was observed. The mechanism explaining the early recruitment of neutrophils to the site of inflammatory reaction secondary to antigen challenge is not fully elucidated. However, similar kinetics for cell infiltration induced by antigen challenge have been described in other species. Hutson *et al.* (1988) and Tarayre *et al.* (1992) reported that antigen provocation of sensitized guinea-pigs and rats respectively, is followed by an early rise in the number of neutrophils in the bronchoalveolar lavage fluid, followed by late eosinophil infiltration.

Eosinophil numbers are augmented in several diseases, such as pollen-sensitive rhinitis (Bentley *et al.*, 1992), asthma (Azzawi *et al.*, 1992), parasite infection (Secor *et al.*, 1990) and rheumatoid arthritis (Venge, 1990). One of the intriguing aspects of eosinophilia is its biological specificity, since an increase in the number of eosinophils may occur in the absence of a rise in other leucocyte populations (Strath & Sanderson, 1986; Maxwel *et al.*, 1987). In confirmation, no changes in the other cell types accompanied antigen-induced eosinophil accumulation in our experiments. In fact, eosinophil increment triggered by 10 μg ovalbumin reflected an augmentation in the number of total cells, as evaluated at 24 and 48 h. Our present findings are partly in conflict with those of Spicer *et al.* (1986), who showed that the injection of ragweed pollen extract into the peritoneal cavity of actively sensitized mice induced eosinophil accumulation, which was accompanied by mononuclear cell increase.

The mechanisms underlying hypersensitivity reactions involve the immune activation of antibody-bound cells, followed by the release of vasoactive amines, such as histamine and 5-hydroxytryptamine, and of newly generated mediators, such as PAF and leukotrienes (Drews, 1990). In the present study, we demonstrated a very early histamine secretion in the peritoneal cavity following antigen challenge, resulting most likely from mast cell degranulation (Prouvost-Danon *et al.*, 1972). In contrast, antigen challenge was followed by a late release of immunoreactive LTC_4 and LTB_4 in the peritoneal washing, which peaked at 6 h. Even though the ability of sensitized mast cells to generate peptido – leukotrienes following antigen stimulation is well-established (Mencia-Huerta *et al.*, 1983), the different kinetics for histamine and for immunoreactive LTC_4 and LTB_4 release presently reported suggests that a cell type other than the mast cell accounts for leukotriene production. Indeed, it has been reported that mononuclear cells and eosinophils are capable of generating

peptido-leukotrienes following antigen challenge (Rankin *et al.*, 1982; Shaw *et al.*, 1985).

Although the injection of ovalbumin triggered cell accumulation in the mouse peritoneal cavity, no protein extravasation was detected at any time point considered. This phenomenon is not due to a low immune response induced by immunization, since the intraplantar injection of ovalbumin to mice sensitized by the same procedure as used in this study elicited a marked paw oedema (unpublished results). Furthermore, the ability of AZ to increase the protein content in the peritoneal cavity suggests that the model described here, as others reported recently (Perretti *et al.*, 1992), allows the evaluation of protein extravasation and mediator release induced by pro-inflammatory stimuli. Taken together, these results suggest that antigen-induced cell migration into the peritoneal cavity of sensitized mice is unrelated to an increase in the vascular permeability. This concept has already been proposed by Griswold *et al.* (1991), who demonstrated that the i.p. injection of LTC_4 to the mouse induced protein leakage but not polymorphonuclear leucocyte infiltration, whereas the i.p. administration of LTB_4 led to polymorphonuclear infiltration without protein extravasation.

In order to investigate the mechanisms responsible for antigen-induced late eosinophil accumulation in the mouse peritoneum, different pharmacological tools were used in the present study. Our results showing that ovalbumin-induced eosinophilia observed at 24 h was suppressed by the steroidal anti-inflammatory agent, dexamethasone, are in agreement with those of Spicer *et al.* (1986). Since dexamethasone is a drug with a large spectrum of effects, it is difficult to determine which of its activities is involved with inhibition. In fact, inhibition by dexamethasone of eosinophil recruitment during late allergic reactions may result from decreased circulating numbers, from the inhibition of the local production of chemo-attractant factors and from an interference with their chemotaxis and diapedesis (Zweiman *et al.*, 1976; Dunsky *et al.*, 1977). In addition, glucocorticoids are very effective in inhibiting cytokine production by activated T-lymphocytes (Schleimer, 1990). In our experimental conditions, it can be speculated that, at least in part, dexamethasone may act via the inhibition of phospholipase A_2 (Flower *et al.*, 1988) and, consequently, of the arachidonic acid metabolism, since pretreatment with the selective 5-lipoxygenase inhibitor, BWA4C, reduced antigen-induced eosinophil infiltration in the peritoneal cavity. Our findings differ from those of Spicer *et al.* (1986), who claimed that the effect of dexamethasone did not involve the inhibition of the arachidonic acid metabolism. In this model, the participation of lipoxygenase derivatives is also suggested by our findings of increased amounts of LTB_4 in the peritoneal washing 6 h after antigen challenge. Since LTB_4 is chemotactic for guinea-pig (Coëffier *et al.*, 1991) and human (Bruijnzeel *et al.*, 1990) eosinophils, it can be hypothesized that eosinophil accumulation induced by antigen provocation is in part related to *in situ* LTB_4 production. Conversely, neither indomethacin nor the PAF antagonist, SR 27417, significantly modified the eosinophil accumulation in the peritoneal cavity following antigen challenge, indicating that neither cyclo-oxygenase derivatives nor PAF mediate antigen-induced eosinophil infiltration in the mouse peritoneum.

The histamine H_1-receptor antagonist, cetirizine, markedly reduced eosinophil accumulation under conditions where terfenadine was ineffective. Since both drugs were equally effective in inhibiting histamine-induced vascular leakage, the effects of cetirizine on eosinophil infiltration secondary to antigen challenge are probably not related to the antagonism of the H_1 receptor for histamine. Similar findings were described by Leprevost *et al.* (1988), who demonstrated that cetirizine, but not polaramine, another histamine H_1 receptor antagonist, inhibited the *in vitro* guinea-pig eosinophil migration triggered by PAF. More recently, Kyan-Aung *et al.* (1992) demonstrated that cetirizine impairs N-formyl-methionyl-leucyl-phenylalanine (fMLP)- or IL-1-induced adhesion of eosinophils, but not neutrophils, to endothelial cells. These results suggest that inhibition by cetirizine of antigen-induced eosinophil recruitment may result from its ability to act on the expression of adhesion molecules on endothelial cells.

The systemic administration of cycloheximide suppressed the late eosinophil accumulation induced by antigen challenge, suggesting that newly-generated material(s) may be responsible for eosinophil infiltration observed in the present study. Interestingly, when cycloheximide was injected i.p. at a very low dose, a similar degree of inhibition was observed. These results suggest that cycloheximide may act via the inhibition of the local expression of adhesion molecules and/or by suppressing the production of chemotactic factor(s) for eosinophils.

Recent studies indicate that some cytokines promote eosinophil chemotaxis in various species, including mice. These cytokines are IL-3 and GM-CSF, which are involved in monocyte-macrophage and eosinophil proliferation (Lopez *et al.*, 1986; 1987; 1988) and IL-5, which acts specifically on the eosinophil lineage (Lopez *et al.*, 1988). IL-5 is primarily produced by activated Th2 lymphocytes, even though mast cells (Plaut *et al.*, 1989) and eosinophils (Desreumaux *et al.*, 1992) are also a source for this cytokine. The immunosuppressive agent FK-506 was also effective in decreasing eosinophil accumulation triggered by antigen challenge, a marked inhibition being achieved with the dose of $2\,mg\,kg^{-1}$. The ability of FK-506 to inhibit selectively the *in vitro* T cell cytokine production has been widely described (Kino *et al.*, 1987; Sawada *et al.*, 1987; Harding *et al.*, 1989; Sierkierka *et al.*, 1989). More recently, Hatfield & Roehm (1992) demonstrated that FK-506 also blocked *in vitro* production of mast cell-derived cytokines, such as IL-2, IL-3, IL-4 and GM-CSF, at concentrations comparable to those effective on T cells. Our results suggest that the inhibitory effect of FK-506 may result from its ability to prevent the formation of eosinophilotactic cytokines responsible for eosinophil accumulation triggered by antigen challenge. The concept that IL-5 may be involved in *in vivo* eosinophil recruitment has been already supported by Kaneto *et al.* (1991), who showed that pretreatment of sensitized mice with a specific antibody against IL-5 decreased the antigen-induced eosinophil influx in the peritoneal cavity. In addition, the same authors demonstrated that peritoneal cells from immunized mice were able to produce IL-5, when cultured in the presence of the specific antigen. In conclusion, the present study demonstrates that the i.p. administration of ovalbumin to actively sensitized mice induces late eosinophil accumulation in the peritoneal cavity. This phenomenon, which may be in part mediated by the release of lipoxygenase metabolites and/or by newly generated factors, such as T-lymphocyte-derived eosinophilotactic cytokines, offers an interesting tool to investigate the mechanism of action of anti-allergic and anti-inflammatory drugs.

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