The role of platelet-activating factor and peptidoleukotrienes in the vascular changes of rat passive anaphylaxis

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1 The role of platelet-activating factor (PAF) and peptidoleukotrienes as putative mediators of some of the vascular changes triggered by antigen was investigated in rats passively sensitized with monoclonal anti-DNP (2,4-dinitrophenyl) IgE.

2 Lethal anaphylaxis with respiratory distress, systemic hypotension, detachment of the intestinal mucosa, leukopenia and extravasation of protein-rich plasma was observed after antigen challenge of rats sensitized with partially purified monoclonal IgE at concentrations of 15 mg protein kg⁻¹.

3 Analysis of the peritoneal fluid obtained after i.v. challenge with DNP-BSA (bovine serum albumin) showed the presence of significant amounts of PAF (101 ± 8 pg/rat), whereas this mediator was undetectable in control animals. Leukotriene D₄ was the predominant peptidoleukotriene that could be recovered after antigen challenge, and showed an extremely high concentration (92 ± 15 ng/rat) as compared to PAF levels.

4 Extravasation of protein-rich plasma was observed shortly after challenge and reached a maximum at 30 min. Treatment of animals with i.v. PCA 4248 (1–2 mg kg⁻¹) and WEB 2086 (1 mg kg⁻¹), two chemically unrelated compounds which are antagonists of the PAF-receptor, produced a significant reduction of the extravasation of protein-rich plasma.

5 The same degree of protection could be afforded by MK-886, an inhibitor of leukotriene biosynthesis. Combined treatment with WEB 2086 and MK-886 provided greater inhibition of protein-rich plasma extravasation than either compound alone. PCA 4248 was also found to inhibit in a dose-dependent manner the systemic hypotension observed upon DNP-BSA challenge.

6 These data indicate that the lipid mediators PAF and peptidoleukotrienes are major effectors of the vascular disturbances observed in rat passive IgE-mediated anaphylaxis.

Keywords: Anaphylaxis; hypersensitivity; IgE; inflammatory mediators; leukotrienes; PAF; PAF antagonists; shock

Introduction

Various animal models have been employed as an approach to the study of human anaphylaxis. A major concern regarding the suitability of these models to characterize the network of inflammatory mediators involved, stems from the diverse classes of antibodies that can be elicited. For example, some studies on rat anaphylactoid syndromes produced by soluble immune complexes and IgG-aggregates have emphasized the role of several mediators released by mononuclear phagocytes as effectors of the extravasation of protein-rich plasma, systemic hypotension, release of lysosomal enzymes and interferences with haemostasis (Sánchez Crespo *et al.*, 1985; Doebber *et al.*, 1986; Fernández-Gallardo *et al.*, 1988). In addition, del Balzo *et al.* (1989) have emphasized the role of complement-derived anaphylatoxins in the amplification of cardiac anaphylaxis in the guinea-pig and another report has stressed the role of anaphylatoxins in the activation of platelets in the anaphylactoid syndrome elicited by soluble aggregates of IgG in the rat (Fernández-Gallardo *et al.*, 1989). This is in keeping with the results of Smedgård *et al.* (1989) who found the anaphylatoxin C5a to be the effector of platelet activation following endotoxin challenge in the rat, and with current views suggesting a major role in host defence for the generation of products by interaction of xenobiotics with biological fluids (Hellewell *et al.*, 1989).

On the other hand, classical views regarding the central role played by mast cells in the pathophysiology of anaphylaxis have been reinforced by a recent study carried out in genetically mast cell-deficient mice, which exhibited limited cardio-

pulmonary changes and no mortality after challenge with anti-IgE (Martin *et al.*, 1989). Since the description by Liu *et al.* (1980) of a hybridoma cell line that produces DNP-specific murine IgE, it has been possible to obtain large amounts of a single class of monoclonal antibody to be used in rats. This antibody permits a passive sensitization of circulating basophils and tissue mast cells that spares activation of the complement system, although a contribution of mononuclear phagocytes cannot be ruled out (Capron *et al.*, 1977; Rouzer *et al.*, 1982; Wei *et al.*, 1986). The purpose of this study was to determine the role of lipid mediators in the pathogenesis of vascular changes in IgE-mediated rat anaphylaxis.

Methods

In vivo experimental design

Male Sprague-Dawley rats of about 200 g body weight were used throughout the study. These animals were passively sensitized according to the method of Orange *et al.* (1977) by the i.p. administration of about 0.2 ml of anti-DNP (2,4-dinitrophenyl) IgE in a phosphate-buffered isotonic saline solution, pH 7.4. The solution injected contained, unless otherwise stated, about 3 mg of total protein as assessed by the method of Bradford (1976). Animals were used for experiments within a period of 24 h following sensitization, in view of previous reports showing a decline of maximal response thereafter (Leng *et al.*, 1988). Rats were anaesthetized by i.p. injection of Nembutal at a dose of 50 mg kg⁻¹, and tracheostomized with a polyethylene catheter (PE 250, Vigon, France). A femoral artery was cannulated with a polyethylene catheter (PE 50) for

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recording mean arterial pressure or for taking blood samples. A jugular vein was also cannulated (PE 10) and used for administration of drugs. Platelets and leukocytes were counted in blood samples of 44.5 μ l anticoagulated with EDTA-K₂ using a Coulter counter model Plus I.

Assay of protein-rich plasma extravasation

Extravasation of protein-rich plasma was studied as described (Sánchez Crespo *et al.*, 1982). Briefly, rats received an i.v. infusion of ¹²⁵I-labelled bovine serum albumin (BSA) (about 200,000 c.p.m.) 10 min before DNP-BSA challenge. At different times thereafter, blood samples were taken to measure radioactivity, and extravasation was expressed as percentage reduction of the initial radioactivity per ml of blood. The spontaneous drop in the ¹²⁵I-labelled BSA count was regularly less than 9% during a 60 min period in control animals, and this value was subtracted from the drop in the count in the anaphylaxis experiments. The haematocrit value was obtained together with blood cell counts in the Coulter counter analysis.

Assay and characterization of PAF in peritoneal fluid

At 30 min after DNP-BSA challenge or at the time of lethal anaphylaxis, peritoneal fluid was obtained by lavage with 2 ml of isotonic saline solution. PAF was extracted by the procedure of Bligh & Dyer (1959), and processed as described (López Díez *et al.*, 1989). Briefly, after evaporation to dryness, the lipid extract was resuspended in the medium used as a mobile phase for straight-phase high performance liquid chromatography (h.p.l.c.). This was carried out in a Spheri-5 silica column using as a mobile phase 96% propan-2-ol/hexane (1/1, supplemented with 0.005% acetic acid) and 4% water which was linearly increased to 8% during a 15 min period (Blank & Snyder, 1983). The column was eluted for 60 min at 2 ml min⁻¹, and the fractions collected, evaporated to dryness, and resuspended in 100 μ l of a N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES)-saline buffered medium supplemented with 0.25% BSA to carry out a bioassay on rabbit platelets according to the procedure described by Henson (1976). Briefly, rabbit platelet-rich plasma was obtained by low speed centrifugation and incubated with [³H]-5-hydroxytryptamine ([³H]-5HT) at a concentration of 1 μ Ci ml⁻¹. After two washes to remove plasma and non-incorporated [³H]-5-HT, platelets were resuspended at a concentration of 10⁸ ml⁻¹, and incubated with different concentrations of the h.p.l.c. fractions or a standard solution of PAF. The amount of PAF contained in the samples was corrected for recovery and expressed in pg as assessed from comparison with the activity of the standard. The limit of sensitivity of this assay was 5 pg.

Assay and characterization of peptidoleukotrienes

Collection of peritoneal fluid was carried out as described for PAF. The extraction procedure was a modification of that of Clancy & Hugli (1983). Briefly, each sample was adjusted to a total volume of 3 ml with saline solution, combined with 90 μ l 5 M formic acid and 1.5 ml propan-2-ol, vortexed for 5 min at room temperature, and then mixed with 3.5 ml anhydrous ether. After further vortexing, the organic layer was taken, combined with 45 μ l 10 M NH₄OH and the solvent removed under N₂. The extraction efficiency for LTC₄ was above 70%, as determined with a radiolabelled standard. Reverse-phase h.p.l.c. separation of the eicosanoids was carried out with a dual pump Kontron system model 420 (Kontron Instruments, Zurich, Switzerland), fitted with a Spheri-5 RP18 column (220 $\times$ 4.6 mm, 5 μ m) and a 15 $\times$ 3.2 mm guard column, using as a system of solvents methanol/water/acetic acid (60/40/0.1, pH 5.4 [adjusted with 10% ammonium hydroxide] + 1 mM EDTA). The column was isocratically eluted for 30 min, and then the strength of the mobile phase increased by a methanol

gradient from 0% to 25% between 30 and 45 min, and from 25 to 35% between 50 and 70 min. Peaks of peptidoleukotrienes were detected by on line u.v. absorbance at 280 nm, and identified by comparison with authentic standards, analysis of u.v. absorbance spectra, and immunoreactivity with the antibody used for the radioimmunoassay (RIA). The quantities of leukotrienes were estimated by integration of the peak areas using a Spectra-Physics apparatus (San Jose, CA, U.S.A.).

Materials and drugs

Monoclonal anti-DNP IgE was obtained from an IgE-secreting hybridoma grown in ascites in BALB/c mice and was partially purified by ammonium sulphate precipitation followed by dialysis (Liu *et al.*, 1980). The final preparation of protein used to carry out passive sensitization contained about 7 mg ml⁻¹ specific IgE, as indirectly inferred from a classical precipitation reaction using antigen and several dilutions of the antibody solution (Hudson & Hay, 1976). This estimated content of specific antibody in the sample was consistent with the data obtained from FPLC gel filtration chromatography of a 200 μ l sample in a Superose-12 column (Pharmacia LKB, Uppsala, Sweden) and measurement of the protein present in the peak showing a molecular weight analogous to mouse IgE. Analysis by sodium dodecyl sulphate/polyacrylamide gel electrophoresis under reducing conditions, of the immunoprecipitate formed upon incubation of DNP-BSA with IgE anti-DNP showed the presence of two bands compatible with both the heavy and light chains of mouse IgE as described by Liu *et al.* (1980).

DNP-BSA containing 20 mol of DNP per mol of BSA was prepared according to the method of Eisen (1964), by using BSA from Sigma Chemical Company (St. Louis, MO, U.S.A.) and 2,4-dinitrobenzenesulphonic acid from Aldrich-Europe, Beerse, Belgium. PCA 4248 (2-[phenylthio]ethyl 5-methoxycarbonyl-2,4,6-trimethyl-1,4-dihydropyridine-3-carboxylate) was provided by ALTER Laboratories, Madrid, Spain (Ortega *et al.*, 1990). The solubilization of this product was carried out under protection of light exposure by mixing 10 mg of the compound with 20 μ l dimethylsulphoxide (DMSO) under a stream of N₂, followed by the addition of 1 ml polyethyleneglycol-400 and 1 ml of distilled water. This solution could be diluted in saline solution for i.v. administration, as required, WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6H-thieno-(3,2-f)(1,2,4)-triazolo-(4,3-a)(1,4)-diazepine-2-yl)-1-(4-morpholinyl)-1-propane), a kind gift from Dr H.O. Heuer, Boehringer Ingelheim, Ingelheim, F.R.G., was solubilized in a mixture of 0.1 M HCl and saline (1:20 v/v). MK-886 (3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid [Gillard *et al.*, 1989]), leukotriene B₄ (LTB₄), LTC₄, LTD₄ and LTE₄, used as standards for reverse-phase h.p.l.c. were a kind gift from Dr C.B. Pickett, Merck Frosst, Pointe Claire-Dorval, Quebec, Canada. [³H]-hexadecyl-PAF, and reagents for RIA of LTC₄, LTD₄ and LTE₄, were purchased from Amersham International, U.K. MK-886 was solubilized in 0.1% methyl cellulose and given by mouth, at a dose of 10 mg kg⁻¹, 2 h before anaphylactic challenge, according to the manufacturer's instructions. PAF was obtained from Bachem Feinchemikalien AG, Bubendorf, Switzerland.

Statistical analyses

Data are expressed as the mean $\pm$ s.e.mean. For comparison of two groups of samples normally distributed, the Student's two-tailed *t* test was used to analyse differences for significance. For comparison of two groups of samples not normally distributed, the Mann-Whitney U test was used. Differences in the survival rate were evaluated by the χ^2 -test with the Yates' correction. Statistical procedures were performed using a database and statistical package (Sigma, Horus Hardware Co., Madrid, Spain). A *P* value <0.05 was considered significant.

Results

Effect of DNP-BSA challenge on systemic arterial pressure

Sensitized animals showed respiratory distress and a drop of mean arterial pressure after i.v. challenge with 0.7 mg kg^{-1} of DNP-BSA (Figure 1). This was not observed in either non-sensitized rats receiving DNP-BSA nor in those having received IgE heated for 1 h at 56°C , i.e. a treatment that destroys the biological effect of IgE by affecting the portion of molecule which interacts with cell receptors (Dorrington & Bennich, 1973). The hypotension occurred immediately after challenge, but the record frequently showed a modest elevation after the initial drop, and then a protracted decline which lagged until the death of the animal (Figure 1). The hypotension was dependent on the dose of antibody used for sensitization, and lethal anaphylaxis was observed in those animals that had received more than $15 \text{ mg protein kg}^{-1}$ of anti-DNP IgE. Since this dose usually spared the death of the animal for up to 40 min after challenge, it was selected for experiments intended to show pharmacological modulation of the anaphylactic reaction. Under these conditions, arterial pressure dropped from 108 ± 5.0 to $49.4 \pm 4.1 \text{ mmHg}$ 2 min

after antigen challenge (Table 1). This was followed by a further drop to $35.7 \pm 8.3 \text{ mmHg}$ during the next 30 min. Two out of 16 animals showed recovery to the basal level at 2 and 35 min after challenge, whereas the remainder showed a prolonged shock state until death occurred. Animals treated with PCA 4248 (1 mg kg^{-1} , i.v., 10 min before DNP-BSA challenge) showed a decline of arterial pressure from 110 ± 4.0 to $69.7 \pm 9.5 \text{ mmHg}$, but in contrast to the drop observed in the rats not treated with the drug, this was not followed by secondary reduction during the ensuing minutes. Prechallenge arterial pressure levels were reached in most animals within 40 min, whereas only 2 out of 15 rats showed lethal anaphylaxis during the period of observation (Table 1).

Histological examination including transmission electron microscopy was carried out in specimens of small intestine from control and DNP-BSA challenged sensitized animals. Whereas few changes were observed in the peritoneal serosa, the mucosa showed prominent changes in the epithelial layer with wide areas detached. The lamina propria showed oedema and cellular infiltration with a prominent presence of eosinophils, which were identified by examination of their granules. The lumen of the vessels was occupied by red cells, which is most probably due to the occurrence of haemoconcentration (Figure 2).

Figure 1 Effect of intravenous challenge with DNP-BSA on mean arterial pressure in both non-sensitized and passively immunized rats, and effect of a previous treatment with PCA 4248. Rats received 0.7 mg kg^{-1} DNP-BSA at the times indicated by the arrows. PCA 4248 was injected i.v. 10 min before DNP-BSA. These are typical recordings selected from 3 non-sensitized rats, and 15 or 16 animals in each group of sensitized animals.

Extravasation of protein-rich plasma

Antigen challenge of sensitized rats produced a time-dependent drop of the plasma ^{125}I -labelled BSA count. Figure 3 shows the temporal course of protein-rich plasma extravasation and the protection provided by PCA 4248 ($1\text{--}2 \text{ mg kg}^{-1}$, i.v., 10 min before antigen challenge). Extravasation could be demonstrated as early as 2 min after challenge, and showed a decrease to $52 \pm 12\%$ of the initial blood-associated ^{125}I -labelled BSA count by 30 min. Rats treated with PCA 4248 at a dose of 1 mg kg^{-1} , showed a reduction of the ^{125}I -labelled BSA blood count to $76 \pm 10\%$ of the prechallenge level at 30 min, and to $80 \pm 5\%$ when PCA 4248 was administered at a dose of 2 mg kg^{-1} . This represents $58 \pm 7\%$ and $70 \pm 4\%$ inhibition, respectively, when expressed as a percentage of the response observed in vehicle-treated animals. The existence of extravasation of protein-rich plasma could also be demonstrated by the haematocrit value, which increased from $44 \pm 1\%$ to 50 ± 4 , 30 min after challenge ($n = 8$), compared to 44 ± 3 and 43 ± 7 in a group of animals pretreated with PCA 4248 at the dose of 1 mg kg^{-1} . WEB 2086, a compound structurally unrelated to PCA 4248 but sharing the PAF-receptor antagonistic property (Casals-Stenzel *et al.*, 1987), was tested at the dose of 1 mg kg^{-1} , i.v., 10 min before DNP-BSA. As shown in Table 2, it was also found to blunt protein-rich plasma extravasation. MK-886 (10 mg kg^{-1} , orally, 2 h before DNP-BSA) produced a diminution of protein-rich plasma extravasation similar to that observed with both PAF-receptor antagonists. Combined administration of MK-886 and WEB 2086 showed a reduction of extravasation higher than that provided by treatment with either drug alone (Table 2).

The leukocyte count decreased following DNP-BSA challenge to reach $59 \pm 15\%$ of the prechallenge level 5 min thereafter (Figure 4). This was followed by a recovery to

Table 1 Effect of PCA 4248 on mean arterial pressure and survival rate during passive anaphylaxis

PCA 4248	Mean arterial pressure			% survival	n
	Prechallenge	At 2 min	At 30 min		
Vehicle	108 ± 5.0	49.42 ± 4.1	35.71 ± 8.3	12.5	16
1 mg kg^{-1}	110 ± 4.0	69.66 ± 9.5	$103 \pm 10.0^*$	86.6*	15
2 mg kg^{-1}	112 ± 5.5	56.32 ± 4.6	$112 \pm 5.5^*$	81.2*	16

Rats were treated as described in the legend to Figure 1 and in Methods. Mean arterial pressure was continuously recorded for up to 40 min, when most animals pretreated with vehicle died. Mean arterial pressure values registered 2 and 30 min after DNP-BSA challenge were taken to construct this table. Survival rate was recorded during a period of observation of 40 min. Data represent mean $\pm$ s.e.mean.

* Indicates $P < 0.001$ as compared to vehicle-treated animals (χ^2 with the Yates' correction).

Figure 2 Micrographs of specimens of small intestine from rats sensitized with anti-DNP IgE and challenged with 0.7 mg of DNP-BSA. The sample was obtained 30 min after antigen challenge, fixed by immersion, and processed for both light microscopy and transmission electron microscopy: (a) shows detachment of the epithelium from the lamina propria (arrowheads) of an intestinal villus. Electron micrograph (b) shows an area of intestinal mucosa denuded of the epithelial layer, infiltration of eosinophils (arrows), and occupation of the vascular lumen by red blood cells. Electron micrograph (c) shows the detached epithelium of a villus with infiltrating eosinophils (arrows) in the central area.

Figure 3 Time course of the effect of PCA 4248 on the extravasation of ^{125}I -labelled BSA during passive anaphylaxis. PCA 4248 or vehicle were administered i.v. to IgE-sensitized rats 10 min before i.v. challenge with DNP-BSA (0.7 mg kg^{-1}). At the times indicated, $100 \mu\text{l}$ blood samples were taken via a femoral catheter to measure ^{125}I -BSA. Results are expressed as percentage of the basal ^{125}I -BSA count rate. Vehicle-treated rats (Δ); rats treated with PCA 4248 at the dose of 1 mg kg^{-1} ($\circ$) and 2 mg kg^{-1} ($\bullet$). Data represent mean of 7 to 11 animals in each group; s.e.mean shown by vertical bars. * Indicates $P < 0.05$; ** $P < 0.01$.

$80 \pm 20\%$ by 30 min. The platelet count showed variations of less than 14% throughout the experiments, which suggests the absence of a true thrombocytopenia linked to platelet activation and organ sequestration.

Presence of PAF in peritoneal fluid in IgE-sensitized rats

The volume of fluid collected from the peritoneum of DNP-BSA challenged rats was $3.1 \pm 0.2 \text{ ml}$ ($n = 22$, range $2.7\text{--}4.2 \text{ ml}$) and the recovery of protein was $8.2 \pm 0.8 \text{ mg}$ (range $5.3\text{--}10.9 \text{ mg}$). Ten control rats contained $0.8 \pm 0.1 \text{ ml}$ of peritoneal fluid and $1.9 \pm 0.3 \text{ mg}$ of protein. The number of peritoneal cells was $9.3 \pm 2.6 \times 10^6 \text{ ml}^{-1}$ (range $6.4 \times 10^6\text{--}1.3 \times 10^7 \text{ cells ml}^{-1}$) and did not significantly differ from the number obtained from normal controls ($9.0 \pm 2.4 \text{ cells ml}^{-1}$). IgE-sensitized animals at the time of lethal anaphylaxis or killed 30 min after antigen challenge, were found to contain

Figure 4 Variations of leukocyte and platelet counts during IgE-mediated anaphylaxis. Blood samples were taken to assess platelet ($\circ$) and leukocyte counts (Δ). Data represent mean of 3 to 6 animals; s.e.mean shown by vertical bars.

Table 2 Inhibition by WEB 2086 and MK-886 of protein-rich plasma extravasation in rat passive anaphylaxis

Treatment	Dose	Extravasation (% inhibition)	P
Vehicle	—	0	
WEB 2086	1 mg kg ⁻¹	47.5 ± 5.2	<0.01*
MK-886	10 mg kg ⁻¹	53.7 ± 4.4	<0.01*
MK-886 + WEB 2086	10 mg kg ⁻¹ + 1 mg kg ⁻¹	70.7 ± 4.4	<0.05**

WEB 2086 was administered i.v., 10 min before DNP-BSA challenge. MK-886 was given orally 2 h before challenge. The inhibition of [¹²⁵I]-BSA extravasation was studied at 30 min after DNP-BSA challenge, and is expressed as percentage inhibition of the response observed in vehicle-treated animals. Data represent mean ± s.e.mean. * As compared to vehicle-treated animals. ** As compared to rats treated with either MK-886 or WEB 2086 alone.

PAF in the peritoneal fluid (Table 3), whereas only 1 out of 10 control animals showed 19 pg PAF (13 pg mg⁻¹ protein), and the remaining 9 animals showed values below the detection limit of 5 pg. The amounts of PAF assayed in a group of rats undergoing i.p. challenge with the same dose of DNP-BSA were slightly lower than those detected in animals undergoing i.v. challenge (Table 3). Characterization of the lipid product obtained from the peritoneum as authentic PAF was based on its time of retention of 21 min in straight-phase h.p.l.c., i.e. analogous to that of a [³H]-hexadecyl-PAF standard, as well as the loss of its platelet-activating properties upon treatment with phospholipase A₂ from *Naja naja* venom.

Detection of peptidoleukotrienes in peritoneal fluid

Reverse-phase h.p.l.c. analysis of peritoneal fluid obtained from passively immunized rats at times similar to those mentioned for PAF recovery, showed the presence of several major peaks displaying absorbance at 280 nm, an u.v. absorbance spectrum compatible with leukotrienes, and reactivity with an antibody used for the combined RIA of LTC₄, LTD₄, and LTE₄. The identity of these compounds was further confirmed by comparison of their times of retention with those showed by standards of LTC₄ (16.4 ± 0.2 min), LTD₄ (35.1 ± 0.1 min), LTB₄ (38.3 ± 0.1 min), and LTE₄ (39.4 ± 0.2 min). LTD₄ was the predominant leukotriene in antigen-challenged rats, whereas little LTE₄ was detected. LTC₄ was only found in animals sensitized with 37 mg protein kg⁻¹ of the anti-DNP IgE preparation, which probably indicates that LTC₄ accumulates because of enzymatic overload at the gamma-glutamyl transpeptidase step due to massive release of leukotrienes. In experiments with rats sensitized with 15 mg protein kg⁻¹ of anti-DNP IgE, the amount of LTD₄ that could be detected in the peritoneal fluid was 92 ± 15 ng/rat (n = 8), whereas non-sensitized rats contained less than 5.0 ± 0.3 ng/rat (n = 15). Rats pretreated with MK-886 prior to DNP-BSA challenge showed a 73% inhibition of LTD₄ production, and those treated with PCA 4248 showed a generation of LTD₄ similar to that of vehicle treated animals (Table 4). The amount of LTD₄ measured in a group

of 6 passively sensitized rats challenged with i.p. DNP-BSA was 68 ± 12 ng/animal (Table 4), i.e. slightly lower than the quantities detected in animals undergoing i.v. challenge. The pattern of leukotriene distribution was not modified when i.p. L-cysteine, which inhibits the conversion of LTD₄ to LTE₄ was omitted (Sok *et al.*, 1980). A problem observed during the development of these experiments was the identification of a major peak that eluted at a retention time of 10–12 min, and which was present in all of the samples including those from non-sensitized animals as well as those from animals pretreated with MK-886. This peak was tentatively characterized as a sulphoxide on the basis of its u.v. spectrum, since it showed a maximal absorbance at 285 nm. In addition, it had a retention time analogous to that of a leukotriene sulphoxide prepared by treatment of LTC₄ with an excess of H₂O₂ at room temperature (Foster *et al.*, 1988).

Discussion

This study was intended to define the role of lipid mediators in rat IgE-mediated passive anaphylaxis. Some stress was placed on PAF, a phospholipid mediator characterized as 1-O-hexadecyl/octadecyl-2-acetyl-sn-glycero-3-phosphocholine (Demopoulos *et al.*, 1979; Blank *et al.*, 1979; Benveniste *et al.*, 1979), the receptor for which in guinea-pig lung has recently been cloned and characterized as a member of the superfamily of G protein-coupled receptors (Honda *et al.*, 1991). The role of PAF in anaphylaxis has been established in the rabbit (Pinckard *et al.*, 1979), guinea-pig (Darius *et al.*, 1986), mouse (Terashita *et al.*, 1987) and sheep (Tomioka *et al.*, 1989). However, there is only one report on the role of PAF in the shock elicited in rats, and this refers to animals immunized to produce IgG (Damas, 1989). Our data confirm previous studies as to the occurrence of systemic hypotension, transient leukopenia and extravasation (Sanyal & West, 1958; Ross *et al.*, 1986; Leng *et al.*, 1989), and show that the lesions observed in the small intestine are similar, if not identical, to the ischaemic necrosis described in rats following administration of either PAF, combinations of PAF and endotoxin

Table 3 Quantitation of PAF in peritoneal fluid from sensitized rats challenged with DNP-BSA

Pretreatment	Challenge	PAF (pg/animal)	PAF (pg mg ⁻¹ protein)	n
Vehicle	i.v.	101 ± 8	13 ± 3	8
PCA 4248	i.v.	172 ± 19	20 ± 2	4
Vehicle	i.p.	88 ± 12	9 ± 1	10

Peritoneal fluid was obtained at the time of death or at 30 min after either i.v. or i.p. challenge with DNP-BSA. Protein content was assayed by the method of Bradford. The limit of sensitivity of PAF assay was 5 pg. Data represent mean ± s.e.mean.

Table 4 Quantitation of leukotriene D₄ (LTD₄) in peritoneal fluid from DNP-challenged rats

Pretreatment	Challenge	LTD ₄ (ng/animal)	LTD ₄ (ng mg ⁻¹ protein)	n
Vehicle	i.v.	92 ± 15	11 ± 3	8
PCA 4248	i.v.	101 ± 26	12 ± 2	6
MK-886	i.v.	25 ± 6	3 ± 1	9
Vehicle	i.p.	68 ± 12	7 ± 3	6

PCA 4248 was administered i.v., 10 min before DNP-BSA at a dose of 1 mg kg⁻¹. MK-886 was given orally at a dose of 10 mg kg⁻¹ 2 h before DNP-BSA. DNP-BSA was given either i.v. or i. p., as indicated.

(González-Crussi & Hsueh, 1983; Hsueh *et al.*, 1986), or tumour necrosis factor (Sun & Hsueh, 1988). The evidence in favour of PAF as a mediator of passive anaphylaxis provided in this study are its appearance in the peritoneal exudate of antigen-challenged rats, and the beneficial effect of two selective PAF-receptor antagonist on the evolution of the shock. Furthermore, the infusion of PAF into experimental animals causes a number of pathophysiological changes similar to those observed during systemic anaphylaxis (McManus *et al.*, 1980; Sánchez Crespo *et al.*, 1982), including bowel necrosis (Hsueh *et al.*, 1986).

Extravasation of protein-rich plasma is a prominent event in anaphylaxis, but its mediation has not been fully ascertained. In a report by Leng *et al.* (1988) the LTD₄-receptor antagonist L-649,923 and the 5-lipoxygenase inhibitor ONO-LP-049 showed no effect on skin extravasation, a moderate effect on peritoneal extravasation, and no effect on systemic hypotension. Our data indicate that either an inhibitor of leukotriene biosynthesis or a PAF-receptor antagonist may blunt protein-rich plasma extravasation, whereas the combined interference with both families of mediators enhances the effect of either drug alone. We have not detected a reduction of platelet count following DNP-BSA challenge. This is at variance with the anaphylactoid syndrome produced by soluble aggregates of IgG, and might be due to the lack of complement activation and anaphylatoxin formation in IgE-mediated anaphylaxis.

PAF was detected in the peritoneal exudate of sensitized rats at concentrations lower than those of LTD₄, but much higher than could be expected from a mere contamination of samples with blood, as previous studies (Caramelo *et al.*, 1984;

Chang *et al.*, 1987) have reported a mean content of 2.8 pg PAF per mg protein in rat blood, i.e. 4.6 to 7 fold lower than the concentration found here. This finding was rather unexpected in view of the prominent effect of the two PAF-receptor antagonists on the course of rat passive anaphylaxis. The observation is also at variance with studies on human whole blood, where the formation of PAF and leukotrienes in response to ionophore A23187 challenge reaches similar levels (Fradin *et al.*, 1989) as if both families of lipid mediators originated from a common 1-O-alkyl-2-araquidonyl-*sn*-glycero-3-phosphocholine precursor by the action of a phospholipase A₂ to yield lyso-PAF and nonesterified arachidonate.

The predominance of leukotrienes observed in the present study possibly stems from the nature of the cell types involved. Thus, the activation of submucosal mast cells, which do not generate PAF, but produce peptidoleukotrienes (Mencia-Huerta & Benveniste, 1979), might be a major explanation for this finding. Alternative explanations could be an ineffective recovery of PAF due to intracellular retention (Lynch & Henson, 1986), a formation of PAF by mononuclear phagocytes not located in the peritoneal cavity (Mencia-Huerta & Benveniste, 1979; Iñarra *et al.*, 1983), or a predominant production of PAF-analogues of the 1-acyl subclass for which the current PAF-bioassays show limited sensitivity (Sturk *et al.*, 1989; Triggiani *et al.*, 1990).

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