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Pharmacological modulation of Paf-induced rat pleurisy and its role in inflammation by zymosan

[1]Marco A. Martins, Patricia M.R. e Silva, Hugo C. Castro Faria Neto, Patricia T. Bozza, Paulo M.F.L. Dias, Marcia C.R. Lima, Renato S.B. Cordeiro & *B. Boris Vargaftig

Dept. Fisiologia e Farmacodinâmica, Fundação Oswaldo Cruz, Av. Brasil, 4365, Caixa Postal 926, CEP 20010, Rio de Janeiro, Brazil and *Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur, INSERM 285, 25, Rue du Dr Roux, 75015 Paris, France

1 The intrapleural injection of Paf-acether into rats caused, at 30 min, a marked exudation accompanied by a reduction in the pleural leucocyte count. At 6 h, the exudate volume had decreased and a significant increase in the total leucocyte count, particularly eosinophils was noted.

2 Two Paf-acether antagonists, WEB 2086 and 48740 RP abrogated the pleural leucopenia observed 30 min after Paf-acether administration, whereas the exudation was inhibited only by the former. Pleurisy was also reduced by about 60% with dexamethasone, by about 45% with BW 755C or LY 171883, a mixed cyclo-oxygenase/lipoxygenase inhibitor and a peptido-leukotriene antagonist respectively, and by about 30% with indomethacin, flurbiprofen or piroxicam.

3 Repeated daily intrapleural injections of Paf-acether led to a state of progressive desensitization to Paf-acether itself, whereas responsiveness to 5-hydroxytryptamine was maintained. In addition, the Paf-induced auto-desensitization was largely inhibited by WEB 2086.

4 Pleurisy induced by zymosan, but not by carrageenin, was significantly reduced in Paf-acether-desensitized animals. These results were consistent with those obtained with WEB 2086 which supressed zymosan-induced but not carrageenin-induced pleurisy.

5 This study suggests that Paf-acether-induced pleurisy in the rat may be mediated by lipoxygenase arachidonic acid metabolites and that pleurisy induced by zymosan, but not by carrageenin, is largely dependent upon Paf-acether.

Introduction

Since the initial studies with Paf-acether (1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine), in the early seventies, several lines of evidence have accumulated indicating that this phospholipid may play an important role in acute inflammation. Paf-acether: (1) increases vascular permeability in several animal species (Wedmore & Williams, 1981; Humphrey *et al.*, 1982; Bjork & Smedegard, 1983; Pirotzky *et al.*, 1984; Hwang *et al.*, 1985), (2) is produced by or activates different cell types such as mast cells, platelets, macrophages, monocytes, endothelial cells, neutrophils, lymphocytes and eosinophils which are important participants in inflammation (for reviews see Braquet *et al.*, 1987 and Pinckard *et al.*, 1988), (3) is a potent inducer of oedema, hyperalgesia (Vargaftig & Ferreira, 1981) and pleurisy (Silva *et al.*, 1986; Tarayre *et al.*, 1986) and (4) is chemotactic for mononuclear and polymorphonuclear leucocytes (Shaw *et al.*, 1981; Archer *et al.*, 1985; Henocq & Vargaftig, 1988).

There is evidence for and against the participation of Paf-acether in the inflammatory reaction induced by carrageenin. Hwang *et al.* (1986) demonstrated that Paf-acether antagonists such as kadsurenone and L-652,731 reduce the first phase of the carrageenin-induced rat paw oedema. In addition, they detected a significant release of Paf-acether-like materials in the carrageenin-injected paw. In contrast, Cordeiro *et al.* (1986), reported that neither the antagonist BN 52021 nor selective desensitization to Paf-acether modify rat paw oedema induced by carrageenin.

Repeated daily intraplantar injections of Paf-acether or 2-methyl-carbamate-Paf lead to topical

[1] Author for correspondence.

cross-desensitization between these structurally related lipids under conditions where the responsiveness to 5-hydroxytryptamine (5-HT) was not modified (Cordeiro *et al.*, 1986). Therefore, the selective desensitization to Paf-acether, together with the development of specific Paf-acether antagonists, are effective tools for further elucidation of the physiopathological significance of this lipid.

In view of the potential importance of Paf-acether in inflammation, we have now studied the mechanism of pleurisy induced by this lipid in rats and, also, the potential involvement of Paf-acether in pleurisy induced by carrageenin or zymosan. Our results show that the pleurisy induced by zymosan is markedly reduced after the Paf-acether-induced desensitization, a finding which is not duplicated with carrageenin. In addition, pretreatment of animals with the Paf-acether antagonist WEB 2086 inhibited pleurisy induced by zymosan, but failed to modify that induced by carrageenin, suggesting an important role of Paf-acether in the inflammatory reaction due to the former, but not to the latter.

Methods

Animals and treatments

Male Wistar rats weighing 150–200 g were used. Indomethacin (2 mg kg^{-1}), pirozicam (1.8 mg kg^{-1}), flurbiprofen (1.0, 2.5, and 5.0 mg kg^{-1}) and BW 755C (25 mg kg^{-1}) were given intraperitoneally 1 h before the agonist. All drugs were diluted in sterile NaCl 0.9% solution (saline) except piroxicam which was dissolved in Tween 80 and further diluted with saline. Dexamethasone (Decadron, 0.5 mg kg^{-1}) was diluted in saline and given i.p. 12 and 1 h before the agonist. LY 171883 (Fleish *et al.*, 1985), a leukotriene D_4 antagonist, was dissolved in 1 M sodium hydroxide and subsequently neutralized with 2 M HCl to be administered (3 mg kg^{-1}, orally) 2 h before Paf-acether.

Paf-acether antagonists 48740 RP (Sédivy *et al.*, 1985) and WEB 2086 (Casals-Stenzel *et al.*, 1986; 1987) were given intrathoracically (i.t.) into the pleural cavity 5 min before Paf-acether at 2, 6 and 10 μg and 2, 6, 10 and 15 μg, respectively, in a volume of 0.1 ml. In some experiments, WEB 2086 was administered intraperitoneally (15 mg kg^{-1}) 1 h before Paf-acether, carrageenan or zymozan. The Paf antagonists were diluted in sterile saline, immediately before use, with the aid of 0.1 N HCl in the case of WEB 2086.

Induction of pleurisy

Pleurisy was induced by the i.t. injection of Paf-acether (0.5 to 16 μg/cavity), 5-hydroxytryptamine (100 μg/cavity), zymosan (1000 μg/cavity) or carrageenin (800 μg/cavity) in a final volume of 0.2 ml. Each experiment included an equivalent number of control animals receiving the same volume of sterile saline. The animals were killed 30 min or 6 h after Paf-acether, 1 h after 5-hydroxytryptamine and 4 h after carrageenin and zymosan. The pleural cavity was opened and rinsed with 3 ml of saline containing heparin (20 iu ml^{-1}). The fluid was collected and the volume measured with a graduated syringe.

Desensitization procedure

Auto-desensitization to Paf-acether was produced by successive daily i.t. administrations of Paf-acether (1 μg/cavity) for 5 successive days. In control groups saline was used as a substitute for Paf-acether. It is important to note that the exudative response induced by Paf-acether disappeared 24 h after i.t. injection of the lipid (data not shown). To verify the effect of Paf antagonists on Paf-induced desensitization, 6 μg doses of WEB 2086 were injected i.t. 5 min before each dose of Paf-acether administered daily for 3 days. On the 4th day, only Paf-acether (1 μg/cavity) was injected and the pleurisy determined. To test the potential existence of cross-desensitization, 5-hydroxytryptamine (100 μg/cavity), carrageenin (800 μg/cavity) or zymosan (1000 μg/cavity) were injected i.t. in Paf-desensitized animals.

Total and differential leukocyte counts

Total leucocytes from the pleural cavity were counted (in acetic acid 2%) in Neubauer chambers by means of an optical microscope. Differential counts were made with May-Grünwald-Giemsa dye in smears prepared in a cytocentrifuge (Incibras) and examined under an oil immersion objective. The fluid collected from the cavity was centrifuged for 5 min at 1500 r.p.m. (1000 *g*), and the total proteins were quantified in the supernatant by the Biuret technique in a spectrophotometer (Incibras MF 190).

Drugs

Paf-acether (1-O-hexadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) was from Bachem (Switzerland), 48740 RP (3-(3-pyridyl)-1H, 3H-pyrrolo-(1,2-c)-thiazole-7-carboxamide) from Rhône-Poulenc Santé (France), WEB 2086 (3-4-(2-chlorphenyl)-9-methyl-6H-thieno 3,2-f 1,2,4 triazolo-4,3-a 1,4-diazepin-2-yl-1-(4-morpholinyl)-1-propanone) was a gift from Dr H. Heuer (Boehringer Ingelheim, Federal Republic of Germany), LY 171883 (1- < 2-hydroxy-3-propyl-4- < 4 - (1H - tetrazol - 5 - yl) butoxy > phenyl > ethanone) was from Eli Lilly and Company, Indianapolis, U.S.A. The other drugs were obtained from the

following sources: 5-hydroxytryptamine (5-HT), zymosan, carrageenin (lambda) and indomethacin (Sigma); piroxicam (Pfizer); flurbiprofen was a gift from Dr Marcio Falci (Boehringer Ingelheim, S. Paulo, Brazil); BW 755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2pyrazoline (Wellcome Laboratories, Beckenham) and dexamethasone (Decadron (R), Merck, Sharp, and Dohme).

Statistical analysis

The data were analysed statistically by means of Student's two tailed t test for unpaired samples. P values of 0.05 or less were considered significant.

Results

Exudation and cell migration induced by intrapleural injections of Paf-acether

Table 1 shows that intrapleural injection of Paf-acether (1 μg/cavity) caused a significant exudation after 30 min, which was accompanied by protein extravasation and by a marked reduction in the total pleural leucocyte count. At 6h, the exudate volume and its protein content decreased from 867 ± 34 μl to 395 ± 20 μl and from 36.6 ± 2.4 μg to 12.2 ± 0.8 μg, respectively, under conditions where a significant increase in the total leucocyte count was noted (Table 1). Differential cell counts performed 30 min and 6 h, after intrapleural saline showed the predominance of mononuclear leucocytes in the cavity. When the differential counts were performed on samples collected 30 min after Paf-acether, a decrease in the number of neutrophils, eosinophils and mononuclear cells was noted. In contrast, at 6 h, the increase in the number of eosinophils was markedly above that of neutrophils and mononuclear cells (Table 1). As indicated in Figure 1, intrapleural injections of increasing amounts of Paf-acether (0.25–16 μg/cavity) yielded, at 30 min, bell-shaped

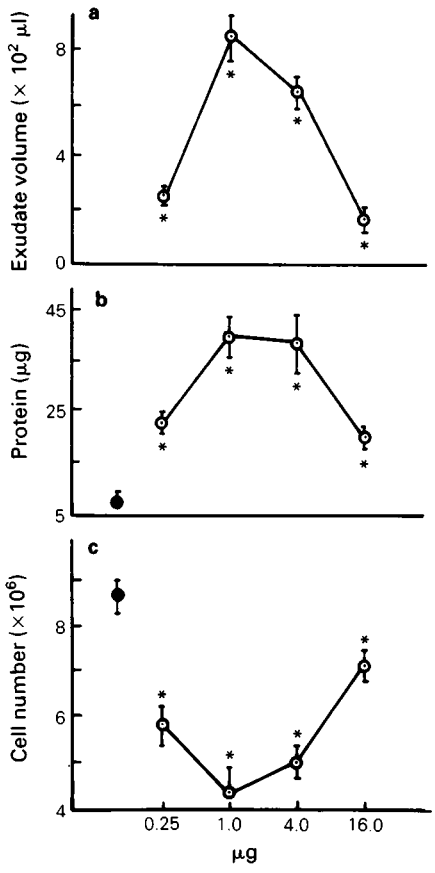

Figure 1 The biphasic dose-effect curves of exudate volume (a), extravasated protein (b) and reduction of pleural leucocyte counts (c) induced by i.t. injection of Paf-acether (0.25–16 μg/cavity). The analyses were made 30 min after injection of the lipid; (●) values obtained from animals injected with saline. Each point represents the mean from at least 6 animals with vertical bars showing s.e.mean and statistically significant ($P < 0.05$) differences are indicated by an asterisk.

Table 1 Exudative and cellular alterations induced by Paf-acether in rats

Time (h)	*Exudate* (μl)	*Total protein* (μg) Sal	Paf	*Total leucocytes* (× 10⁶) Sal	Paf	*Mononuclear cells* (× 10⁶) Sal	Paf	*Neutrophils* (× 10⁶) Sal	Paf	*Eosinophils* (× 10⁶) Sal	Paf
0.5	866.6* ±33.3	6.3 ±0.6	36.6* ±2.4	10.3 ±0.5	4.9* ±0.4	8.6 ±0.6	3.9* ±0.3	0.4 ±0.1	0.2 ±0.5	1.2 ±0.2	0.6* ±0.1
6.0	395.0* ±20.4	5.5 ±0.8	12.2* ±0.8	8.3 ±0.9	17.2* ±2.1	6.5 ±0.2	10.8* ±0.5	0.4 ±0.1	0.8 ±0.2	1.0 ±0.1	4.7* ±0.3

Exudate volume, protein extravasation and total and differential leucocyte counts were measured, in the pleural fluid, at 30 min and at 6 h after i.t. injection of Paf-acether (1 μg/cavity). Values in the table represent the mean ± s.e.mean from at least 6 animals and statistically significant differences are indicated by an asterisk.

dose-response curves for pleural exudation (Figure 1a), for protein (Figure 1b) and for the reduction in the number of leucocytes (Figure 1c),

Interference of potential inhibitors with Paf-acether-induced pleurisy

Figure 2 shows the effects of the *in situ* administration of WEB 2086 or 48740 RP in pleurisy triggered by Paf-acether (1 μg/cavity). WEB 2086 (2–15 μg/cavity) inhibited dose-dependently the exudate volume, the amount of extravasated protein and the reduction in the leucocyte count caused by Paf-acether, whereas 48740 RP (2–15 μg/cavity) inhibited only the latter. Table 2 summarizes the effect of several drugs on exudation induced by 1 μg of Paf-acether. Dexamethasone was the most effective among the tested compounds. The cyclo-oxygenase antagonists piroxicam, flurbiprofen and indomethacin, the dual cyclo- and lipoxygenase inhibitor BW 755C, and the leukotriene D_4 antagonist LY 171883 were also effective against Paf-induced pleurisy. It is noteworthy that in no instance was inhibition greater than 60%.

Auto-desensitization to Paf-acether-induced rat pleurisy

As shown in Figure 3a, repeated daily intrapleural injections of Paf-acether (1 μg) led to a progressive state of auto-desensitization, down to about 20% of initial response after 4 daily re-stimulations. The refractoriness to Paf-acether was selective since the responses to 5-hydroxytryptamine were maintained (Figure 3b). In addition, as indicated in Figure 4, the Paf-acether receptor antagonist WEB 2086 (10 μg/cavity) inhibited significantly the auto-

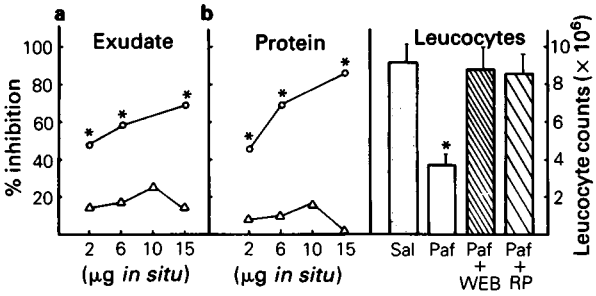

Figure 2 The effect of *in situ* administration of WEB 2086 (2–15 μg/cavity) (○) or 48740 RP (2–15 μg/cavity) (△) on exudate volume (a) and extravasated protein (b) induced by Paf-acether (1 μg/cavity). Section (c) shows the inhibitory effect of WEB 2086 (WEB, 6 μg/cavity) or 48740 RP (RP, 1 μg/cavity) on the reduction in numbers of pleural leucocyte induced by Paf-acether (1 μg/cavity). Each column represents the mean (with s.e.mean shown by vertical bars) from at least 6 animals and statistically significant ($P < 0.05$) differences are indicated by an asterisk.

desensitization caused by repeated administration of Paf-acether.

Interference of Paf-induced desensitization and of WEB 2086 with carrageenin- or zymosan-induced rat pleurisy

Figure 5 shows that zymosan-induced pleurisy was markedly reduced in animals desensitized to Paf-acether under conditions where the response to carrageenin was preserved. The protective role against zymosan, assessed by cross desensitization with Paf-acether (about 50% inhibition, $P < 0.001$), was seen for both exudate volume (left panel) and for extravasated proteins (right panel).

Table 2 Effect of anti-inflammatory drugs on the pleural exudate induced by Paf-acether

Drug	*No. of rats*	*Dose* (mg kg^{-1})	*% inhibition*	*Degree of significance (P)*
Dexamethasone	5	0.5	62.2	<0.001
Indomethacin	5	2.0	26.9	<0.03
Flurbiprofen	4	1.0	30.9	<0.02
	5	2.5	28.4	<0.02
	6	5.0	37.0	<0.006
Piroxicam	6	1.8	38.1	<0.002
BW 755C	6	25.0	44.0	<0.001
LY 171883	6	3.0	47.9	<0.004

The anti-inflammatory drugs were administered 1 h before and the exudate volume was measured 30 min after the i.t. injection of Paf-acether (1 μg/cavity).

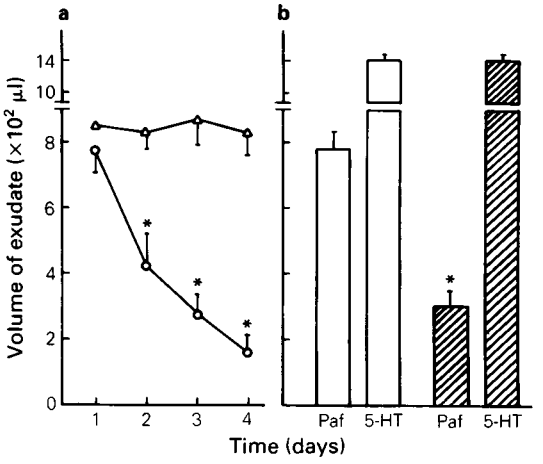

Figure 3 (a) The kinetics of the auto-desensitization induced by 1 to 4 repeated stimulations with 1 µg of Paf-acether (○), under conditions where the responsiveness was maintained after 1 up to 4 repeated injections of saline (△). (b) The effect of an i.t. injection of Paf (1 µg) or 5-hydroxytryptamine (5-HT, 100 µg) to animals which received one daily injection of Paf-acether (hatched columns) or saline (open columns) for 4 days. Each column represents the mean (with s.e.mean shown by vertical bars) from at least 6 animals. Statistically significant ($P < 0.05$) differences are indicated by an asterisk.

A close relationship between the kinetics of the progressive auto-desensitization induced by Paf-acether and the refractoriness to zymosan was also noted (Figure 6). The involvement of Paf-acether in zymosan-induced inflammation was reinforced by pretreating the animals with the Paf-acether antagonist WEB 2086 (15 mg kg^{-1}), which supressed the pleurisy induced by Paf-acether or zymosan, but not by carrageenin (Figure 7). In addition, a significant reduction in the zymosan-induced leucocyte migration was noted in animals either desensitized to Paf-acether or pretreated with WEB 2086 (Table 3).

Discussion

Intrathoracic injections of Paf-acether induced, at 30 min, a marked exudative response accompanied by a reduction in pleural leucocyte count. After 6 h, the volume of exudate was reduced and a significant increase in total number of leucocytes present was noted, confirming studies by Tarayre *et al.* (1986). Exudation is indeed induced by Paf-acether (Wedmore & Williams, 1981; Humphrey *et al.*, 1982; Handley *et al.*, 1984; Pirotzky *et al.*, 1984; Tarayre *et al.*, 1986), and probably results from an initial short-

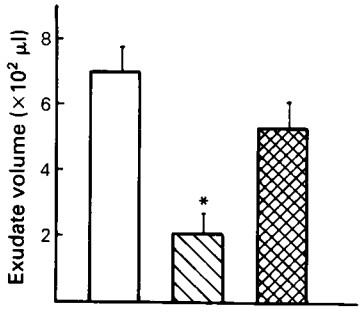

Figure 4 The effect of the i.t. injection of Paf-acether (1 µg/cavity) in animals pretreated with 1 daily injection of saline (open column), Paf-acether (1 µg/cavity) (hatched column) or Paf-acether (1 µg/cavity) plus WEB 2086 (6 µg/cavity) (cross-hatched column), for 3 days. Each column is the mean (with s.e.mean shown by vertical bars) from at least 6 animals. Statistically significant ($P < 0.05$) difference between treated and untreated Paf-stimulated animals is indicated by an asterisk.

lasting increase of the permeability of the endothelium (Bussolino *et al.*, 1987) of pleural vessels. Such an effect, a hallmark of acute inflammation, was probably accompanied by the acquisition of adhesive properties to infiltrated leucocytes, which should account for the reduction in their number, incoming cells becoming unavailable for counting, because of margination (Born & Planker, 1979). However, the possibility that leucocytes disappear as a conse-

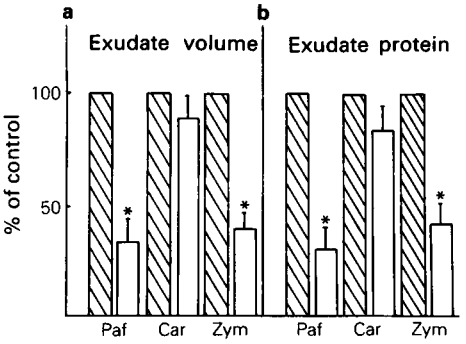

Figure 5 The effect of the i.t. injection of Paf-acether (1 µg/cavity), zymosan (zym, 1000 µg/cavity) or carrageenin (Car, 800 µg/cavity) on exudate volume (a) and extravasated protein (b) of animals pretreated with 1 daily injection of Paf-acether (1 µg/cavity) (open columns) or saline (hatched columns), for 4 days. The exudate volume was determined 30 min after the last injection of Paf-acether or 4 h after zymosan and carrageenin. Each column is the mean (with s.e.mean shown by vertical bars) from at least 6 animals. Statistically significant ($P < 0.05$) difference between Paf-treated and untreated animals is indicated by an asterisk.

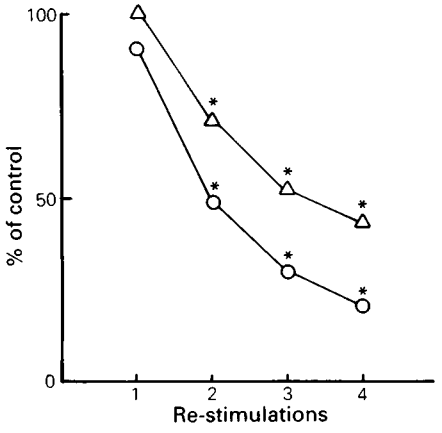

Figure 6 Modifications of the pleural exudation induced by 1 μg of Paf-acether (○) or 1000 μg of zymosan (△) observed in animals pretreated with 1 to 4 re-stimulations of Paf-acether (1 μg/cavity) or of saline in the control groups. The exudate volume was determined 30 min after the last injection of Paf-acether or 4 h after zymosan. Horizontal axis represents the number of pre-injections of Paf-acether. Each point is the mean of at least 6 animals. Statistical significances ($P < 0.05$) are indicated by an asterisk.

quence of cell aggregation and/or lysis cannot be ruled out.

The reduction in pleural leucocyte count was followed, at 6 h, by a marked cell infiltration into the pleural cavity. Since Paf-acether is a potent chemotactic agent for leucocytes, as indicated by *in vivo* (Colditz & Movat, 1984) and *in vitro* assays (Shaw *et*

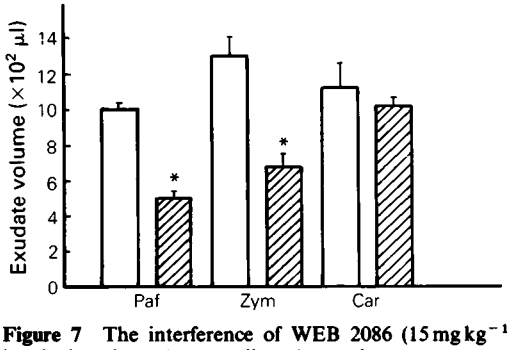

Figure 7 The interference of WEB 2086 (15 mg kg^{-1} hatched columns) or saline (control groups, open columns) with the pleurisy induced by Paf-acether (1 μg/cavity), zymosan (Zym, 1000 μg/cavity) or carrageenin (Car, 800 μg/cavity). The treatments were given, intraperitoneally, 1 h before the agonists. Each column represents the mean (with s.e.mean shown by vertical bars) from at least 6 animals and statistically significant ($P < 0.05$) differences are indicated by an asterisk.

Table 3 Zymosan-induced leucocyte accumulation after the desensitization to Paf-acether or pretreatment with WEB 2086

Treatment	*Total leucocytes* (× 10^5) *Saline*	*Zymosan*
None	50.8 ± 9.8	605.6 ± 19.8
Desensitization to Paf	68.0 ± 13.3	243.2 ± 42.0*
WEB 2086 (15 mg kg^{-1})		277.6 ± 22.3

WEB 2086 (15 mg kg^{-1}) was administered intraperitoneally 1 h before zymosan and the leucocyte counts was performed 4 h later. Values in the table represent the mean ± s.e.mean from at least 6 animals and statistically significant differences are indicated by an asterisk.

al., 1981) it is likely that they invade the pleural cavity by active chemotaxis. Paf-acether has been shown to be highly selective for eosinophils (Wardlaw *et al.*, 1986; Lellouch-Tubiana *et al.*, 1987; Hakansson *et al.*, 1987) and indeed a four fold increase in their number was noted, under conditions where neutrophils and mononuclear cells infiltration into the pleural cavity was increased only 2 fold.

To clarify further the nature of the Paf-acether receptor which is involved in the development of pleurisy in rats, we used the receptor antagonists WEB 2086 (Casals-Stenzel *et al.*, 1986; 1987) and 48740 RP (Sédivy *et al.*, 1985; Lefort *et al.*, 1988). Over the dose-range of 2 to 15 μg/cavity, WEB 2086 dose-dependently inhibited both the volume of exudate and the extravasated protein 30 min after Paf-acether, whereas 48740 RP was inactive. However, both antagonists, WEB 2086 and 48740 RP, suppressed the reduction by Paf-acether of the number of free pleural leucocytes, strongly suggesting that exudate volume, extravasated protein and the drop in the leucocyte count are receptor-mediated phenomena. The close-relationship among the bell-shaped dose-response curves for these 3 events suggests that they may be linked via a single mechanism. However, since the pretreatment with 48740 RP abrogated the reduction in the leucocyte count without interfering with the volume of exudation and its content of proteins, these mechanisms are in fact distinct. It is noteworthy that compound 48740 RP is also inactive against the Paf-acether-induced rat paw oedema, under conditions where it blocks leucocytosis, thrombocytopenia and haemoconcentration induced by the lipid (Martins *et al.*, 1987). These observations are consistent with the interpretation that there are, at least two classes of receptors for Paf-acether which can be clearly distinguished by compound 48740 RP.

Since Paf-acether induces the release of arachidonate derivatives in several experimental models

(Voelkel *et al.*, 1982; Lefort *et al.*, 1984; Jancar *et al.*, 1987), the possibility must be considered that, at least part of the Paf-acether-induced exudative reaction is secondary to eicosanoid release. Previous studies by our group (Cordeiro *et al.*, 1986), using cyclo-oxygenase and lipoxygenase inhibitors, suggested the involvement of leukotrienes, but not of prostaglandins, in rat paw oedema induced by Paf-acether. These results are consistent with the data of Tarayre *et al.* (1986) who detected significant increase in the amount of leukotriene C_4, but not of prostaglandin-like materials, in the rat pleural exudate after Paf-acether. The hypothesis that the leukotrienes participate in the Paf-induced inflammatory reaction is now reinforced by the observation that the compound LY 171883 a peptido-leukotriene antagonist (Fleisch *et al.*, 1985) significantly suppressed Paf-induced rat pleurisy. The pleural exudatation was also significantly inhibited by the suppression of both cyclo- and lipoxygenase products with dexamethasone and BW 755C, whereas cyclo-oxygenase inhibitors, indomethacin, piroxicam and flurbiprofen only slightly inhibited the phenomenon. Together these data strongly suggest that Paf-induced rat pleurisy is largely dependent upon leukotrienes and to a lesser extent upon cyclo-oxygenase metabolites of arachidonate.

The desensitization to Paf-acether after prior exposure to this lipid is observed in several experimental models, such as platelet aggregation (Demopoulos *et al.*, 1979; Henson, 1981; Lalau-Keraly & Benveniste, 1982), smooth muscle contraction (Findlay *et al.*, 1981; Tokomura *et al.*, 1983; Detsouli *et al.*, 1985), macrophage secretion (Maridonneau-Parini *et al.*, 1985), neutrophil reactivity (Smith *et al.*, 1984; Bureau *et al.*, 1987) and lung mechanical changes (Halonen *et al.*, 1980; Lefort *et al.*, 1984). Topical and selective auto-desensitization to Paf-acether-induced rat paw oedema has been proposed previously as a tool to investigate its involvement in inflammation reactions (Cordeiro *et al.*, 1986). In this study we demostrated that daily repeated intrapleural injections of Paf-acether lead to a progressive state of auto-desensitization, under conditions where the responsiveness to 5-hydroxytryptamine was maintained. In addition, it was noted that the antagonist WEB 2086 suppressed Paf-induced auto-desensitization, clearly indicating that this phenomenon is selective, and depends upon the interaction of Paf-acether with specific receptors.

The auto-desensitization process and the receptor antagonist WEB 2086 were used to investigate the potential involvement of Paf-acether in the pleural inflammation triggered by carrageenin and zymosan in rats. Our findings showed that the response to carrageenin, unlike zymosan, was not modified after the treatment with WEB 2086 or after desensitization to Paf-acether. These results agree with the demonstration by Cordeiro *et al.* (1986) that neither desensitization to Paf-acether nor the Paf antagonist BN 52021 block carrageenin-induced oedema, which is thus independent of Paf-acether. In contrast, the pleural exudation and cell migration stimulated by zymosan were significantly inhibited by either WEB 2086 pretreatment or Paf-acether-induced desensitization, strongly suggesting that zymosan may trigger its inflammatory effects via generation of Paf-acether. It is of interest to point out that a marked generation of Paf-acether from rat peritoneal macrophages stimulated with zymosan has been demonstrated (Mencia-Huerta & Benveniste, 1981), thus indicating that indeed zymosan may trigger the *in vivo* release of Paf-acether.

In conclusion, our findings indicate that Paf-acether-induced rat pleurisy may be dependent on leukotrienes and to a lesser extent on cyclo-oxygenase metabolites. In addition, the results obtained with the Paf-acether desensitized animals, and also with WEB 2086, support the hypothesis that the inflammatory reaction triggered by zymosan but not that one induced by carrageenin, may be largely dependent of Paf-acether.

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