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Modulation by lipid mediators of immune complex-induced lung inflammation in mice

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Abstract

The present study characterized a murine model of immune complex-induced pneumonitis and investigated the role of platelet-activating factor (PAF) and eicosanoids as mediators of lung neutrophil infiltration and hemorrhagic lesions. Rabbit antibodies to bovine serum albumin were injected into the airways and bovine serum albumin was injected intravenously into C3H/HePas and BALB/c mice. After 24 h, a significant increase in neutrophil infiltration and hemoglobin concentration in the bronchoalveolar lavage fluid and lung parenchyma was observed in both strains despite the C3H/HePas strain being 10 times more sensitive to PAF. Neutrophil influx and vascular lesions were not affected by pre-treatment of the mice with the PAF receptor antagonist, WEB 2170 (5-(2-chlorphenyl)carbonyl)-3,4-dihydro-10-methyl-3-((4-morpholinyl)-2H,7H-cyclopenta(4,5)thieno(3,2-*f*)(1,2,4)-triazolo-(4,3-*a*)(1,4)-diazepine). In contrast, neutrophil influx and vascular lesions were increased by the cyclo-oxygenase inhibitor, indomethacin, and reduced by the inhibitor of leukotriene synthesis, MK 886 (3-[1-(4-chlorobenzyl-3-*t*-butyl-thio-*t*-isopropyl-indol-2y-1]-2-2-dimethylpropanoic acid) and by the leukotriene B_4 receptor antagonist, RO 0254094 (2-[(5-carboxypentyl)-6-[6-[3,4-dihidro-4-oxo-8-propyl-2H-1-benzopyran-7-yl)hexyl] benzenepropanoic acid). Increased levels of leukotriene B_4, leukotriene C_4/D_4, thromboxane B_2 were found in bronchoalveolar lavage fluid 4 h after induction of the reaction. There is also a tendency to increased prostaglandins E_2 levels. Neutrophil infiltration and vascular lesions in immune complex-induced pneumonitis in mice are mediated by leukotriene B_4.

Keywords: Leukotriene B_4; Eicosanoid; Pneumonitis; Arthus reaction; Neutrophil; Hemorrhagic lesion

1. Introduction

Inhalation of antigen by previously sensitized individuals induces the formation of immune complexes which can be deposited in the alveoli, causing local inflammation with cell infiltration and release of inflammatory mediators (Pepys, 1969). Vascular lesions, hemorrhage and thickening of the bronchiolar walls are often detected. In consequence, dyspnea, dry cough, chest tightness and fever are observed a few hours after respiratory exposure to the sensitizing antigen. Frequent exposure to the sensitizing antigen can lead to chronic pneumonitis, a condition which can seriously affect pulmonary tissue and respiratory function. The incidence of immune complex-induced pneumonitis is particularly high in individuals exposed to organic dusts or to thermophilic fungi present in moldy materials or in air conditioning systems (Finnegan et al., 1985; Parker et al., 1992).

The Arthus reaction has been used as an experimental model to study the inflammation induced by immune complexes. This reaction was described in the beginning of this century as an acute hemorrhagic inflammation that developed in the skin of immunized rabbits following local injection of the antigen (Arthus, 1903). It has been later demonstrated that the hemorrhagic lesions are probably mediated by products released by activated neutrophils which migrate to the site of immune complex deposition (Stetson, 1951; Cochrane and Aikin, 1996).

An Arthus reaction induced in the lungs of experimental animals provides a useful model to study the mechanisms involved in lung diseases in which the pathological events are triggered by activated neutrophils. Johnson and Ward (1974) induced a passive Arthus reaction in rat lungs by simultaneous injection of antibodies into the airways and antigen intravenously. They observed a marked infiltration

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of neutrophils into the airways, increased vascular permeability and hemorrhage in the lung parenchyma. Although the participation of neutrophils in lung injury induced by immune complexes is well established (reviewed by Ward, 1994), the factors involved in the recruitment of these cells and the mechanisms involved in their activation are not completely understood. Cytokines such as tumor necrosis factor, interleukin-1 and -8, macrophage inflammatory protein-2 and cytokine-induced neutrophil chemoattractant have been shown to contribute to the recruitment and activation of neutrophils in immune complex-induced lung inflammation (Warren et al., 1989; Warren, 1992; Shanley et al., 1997).

We have previously reported that an immune complex reaction induced in the rat lungs leads to the release of prostaglandin E_2, thromboxane and leukotriene B_4 into bronchoalveolar lavage fluid, to an influx of neutrophils and to the development of hemorrhagic lesions. Pre-treatment of the rats with platelet-activating factor (PAF) receptor antagonists or inhibitors of leukotrienes synthesis prevented the development of the lesions, whereas an antagonist of cysteinyl-leukotrienes and inhibitors of prostaglandins and thromboxane had no effect. These results suggested that in rats the lung hemorrhagic lesions are mediated by PAF and leukotrienes, possibly leukotriene B_4. Moreover, we have found that the release of leukotriene B_4 in this model is dependent on the prior release of PAF (Tavares de Lima et al., 1989, 1992). The present knowledge on the mechanisms involved in immune complex-induced pneumonitis was obtained mainly from studies with rats. However, there is evidence that the mechanisms as well as the mediators involved in this type of hypersensitivity vary among animal species (Williams and Jose, 1981; Zhang et al., 1991; Boughton-Smith et al., 1993).

The aim of the present study was to characterize a murine model of immune complex-induced pneumonitis and to investigate whether the pathological events are mediated by PAF and leukotrienes, as previously shown in rats. We found that a passive reverse Arthus reaction induced in the lungs of mice also leads to eicosanoids release, neutrophil infiltration, and development of hemorrhagic lesions. However, the lesions, which in rats are focal and restricted to the parenchyma, were diffused and extended to the airways. Furthermore, we show here that neutrophil infiltration and lung lesions are mediated by leukotriene B_4, whereas PAF does not seem to contribute significantly to the pathological events induced by immune complexes in the mouse lung.

2. Materials and methods

2.1. Animals and induction of the Arthus reaction

The BALB/c and C3H/HePas mice (male, 7 weeks old, weighing between 25–28 g) were obtained from our own animal facilities. Mice were anesthetized with i.p. injection of chloridrate of 2-(2,6-xilidine)-5,6-dihydro-4-h-1,3-tiasine (40 mg/kg) and chloral hydrate (250 mg/kg). A volume of 30 μl of rabbit immunoglobulin G antibodies to bovine serum albumin containing 250 μg of specific antibody protein was instilled intratracheally followed by i.v. injection of 2 mg of bovine serum albumin, in a volume of 100 μl. As control, groups of mice received the antibody intratracheally, as above and saline i.v. A group of non-manipulated animals was also included.

2.2. Evaluation of vascular permeability

Increased vascular permeability was evaluated by the Evans blue dye extravasation method (Wilhelm et al., 1958). The dye (20 mg/kg) was injected i.v. followed by i.p. administration of PAF (0.001–3 μg/mouse), in a volume of 100 μl. A stock solution of 1 mg/ml of PAF was prepared in ethanol and kept at −20°C in 10 μl aliquots. Further dilutions were made in 0.9% saline solution containing 0.12% of bovine serum albumin. Dilutions were freshly made on the day of experiment. After 20 min, the animals were killed with ether and the peritoneal cavity was washed with 2 ml of 0.9% saline solution containing 5 units of heparin/ml. The peritoneal lavage fluid was centrifuged for 10 min at $170 \times g$ and the Evans blue concentration in the supernatant was determined spectrophotometrically at 660 nm and calculated from a standard curve prepared with known concentrations of Evans blue dye.

2.3. Bronchoalveolar lavage

The animals were killed with an overdose of chloral hydrate. The trachea was cannulated and the airways were washed with 3 ml of phosphate-buffered saline (PBS) at 4°C, divided in four washes of about 0.8 ml each.

2.4. Cell counting

The bronchoalveolar lavage fluid was centrifuged at $170 \times g$ for 10 min at 4°C and the cell pellet was re-suspended in 1 ml of PBS. The number of cells was determined by counting in a Neubauer chamber. Differential cell counts were performed in fixed and stained cell suspensions (0.5% crystal violet dissolved in 30% acetic acid) or in cytocentrifuge preparations stained with a commercially available stain with characteristics similar to those of the traditional Wright–Giemsa stain (HEMA 3).

2.5. Assay of myeloperoxidase activity

To quantitate the neutrophil infiltration in the lung tissue we assayed the myeloperoxidase activity in lung homogenates (Warren et al., 1989). Whole lungs were homogenized with a Polytron homogenizer (20 s) in 1 ml of 0.1 M phosphate buffer pH 6.2 containing 0.5% hexade-

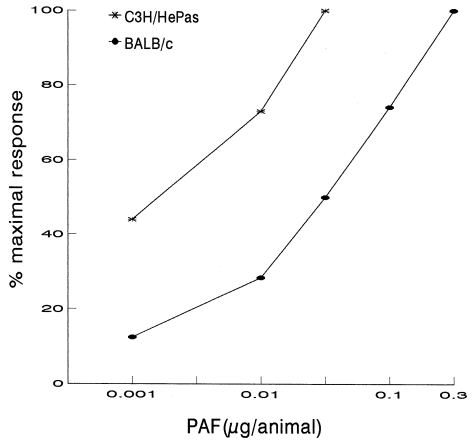

Fig. 1. Increased vascular permeability induced by PAF in C3H/HePas and BALB/c mice. PAF was injected i.p. and the permeability marker, Evans blue dye, was injected intravenously. Body weight in each mouse strain varied from 25 to 28 g. After 20 min the concentration of extravasated Evans blue was determined in peritoneal lavage fluid. Data are expressed as percent of the maximal permeability response of each strain.

cyltrimethyl ammonium bromide and 5 mM EDTA. The samples were then sonicated (5×10 s, 40 Hz) and centrifuged ($3000 \times g$, 30 min) at 4°C. Myeloperoxidase activity was assayed in 10 μl aliquots of the supernatants by measuring changes in absorbance at 460 nm resulting from decomposition of H_2O_2 in the presence of θ-dianisidine in a final volume of 1.5 ml as previously described (Henson et al., 1978). Data are presented as absorbance values and are not corrected for lung weight since variations were less than 10%. Whole lung weight varied from 140 to 150 mg both in BALB/c and C3H/HePas mice. Also, mice submitted to the Arthus reaction did not develop edema since the dry/wet ratio of lungs collected after 24 h was not significantly different from that of the control group (21.0 ± 0.54 and 19.7 ± 0.68, respectively, mean ± S.E.M. of 16 animals/group).

2.6. Evaluation of hemorrhagic lesions

The intensity of the lesions was evaluated by measuring the concentration of extravascular hemoglobin in lung fragments after extensive perfusion of the vascular bed with phosphate-buffered saline containing 5 UI/ml of heparin as previously described (Tavares de Lima et al., 1992). Fragments of the lung were incubated for 24 h in light-protected tubes with Drabkin's solution (8 ml of Drabkin/g tissue) at room temperature. The concentration of the extracted hemoglobin was determined colorimetrically at 550 nm wavelength and the results are expressed as milligrams of hemoglobin per gram of tissue. The concentration of hemoglobin in the bronchoalveolar lavage fluid was determined by incubation of the bronchoalveolar lavage fluid with Drabkin (1:1) for 1 h and is expressed as milligrams of hemoglobin per milliliter of bronchoalveolar lavage.

2.7. Drug treatments

Groups of mice were i.v. treated, 30 min before induction of the Arthus reaction, with 10 mg/kg of WEB 2170 (5-(2-chlorphenyl)-3, 4-dihydro-10-methyl-3-((4-morpholinyl)carbonyl)-2H, 7H-cyclopenta(4,5)thieno(3,2-*f*)(1,2,4)-triazolo-(4,3-*a*)(1,4)-diazepine) or 2 mg/kg of indomethacin. Other groups were treated with 10 mg/kg of compound MK 886 (3-[1-(4-chlorobenzyl-3-*t*-butyl-thio-*t*-isopropyl-indol-2-yl]-2-2-dimethylpropanoic acid) or 5 mg/kg of RO 0254094 (2-[(5-carboxypentyl)-6-[6-[3,4-di-

Table 1
Neutrophil infiltration and hemorrhagic lesions in the lungs of BALB/c and C3H/HePas mice

		Neutrophil infiltration		Hemorrhagic lesions	
		Bronchoalveolar lavage PMN $\times 10^4$/ml	Lung tissue O.D.$^{a}_{460\ nm}$	Bronchoalveolar lavage (mg Hb/ml)	Lung tissue (mg Hb/g)
C3H/HePas	Basal[b]	0.33 ± 0.17	0.12 ± 0.05	0.014 ± 0.013	4.97 ± 0.93
	Control[c]	0.43 ± 0.33	0.17 ± 0.03	0.067 ± 0.090	6.49 ± 1.54
	AR[d]	20.6 ± 2.70[e]	0.99 ± 0.15[e]	1.263 ± 0.144[e]	25.03 ± 2.47[e]
BALB/c	Basal	0.27 ± 0.13	0.11 ± 0.03	0.017 ± 0.012	5.01 ± 1.99
	Control	0.47 ± 0.33	0.19 ± 0.04	0.049 ± 0.065	7.77 ± 1.58
	AR	23.3 ± 1.63[e]	0.87 ± 0.17[e]	1.379 ± 0.079[e]	27.81 ± 1.94[e]

Neutrophil infiltration and hemorrhagic lesions were evaluated 24 h after induction of the reaction. Neutrophil infiltration in the bronchoalveolar lavage fluid was measured by counting the number of polymorphonuclear (PMN) leukocytes and infiltration in lung tissue by determining the myeloperoxidase activity in homogenates of lung tissue. Hemorrhagic lesions were evaluated by measuring the concentration of hemoglobin (Hb) in the bronchoalveolar lavage fluid or in homogenates of lung tissue. Data represent the mean ± S.E.M. for 6 to 12 animals per group.

[a] The absorbance values from the myeloperoxidase assay were obtained for 10 μl of supernatants of whole lungs homogenized in 1 ml of phosphate buffer as described in Section 2.

[b] Basal levels were measured in naive animals.

[c] The control group received antibodies into the airways and saline i.v.

[d] Arthus reaction (AR) was induced by instillation of 250 μg rabbit immunoglobulin G antibodies to bovine serum albumin into the airways followed by i.v. injection of 2 mg of bovine serum albumin.

[e] $P < 0.05$ compared to control group of each mouse strain.

hidro-4-oxo-8-propyl-2H-1-benzopyran-7-yl) hexyl] benzenepropanoic acid), given per os, 3 h before induction of the reaction.

2.8. Extraction and quantification of eicosanoids

The cell-free bronchoalveolar lavage fluid was acidified with HCl 1 N to pH 3.4–3.6 and slowly passed through a Sep Pak C 18 column (pre-washed with 20 ml of ethanol and 20 ml of water). The column was washed with 10 ml of water and 1 ml of 35% of ethanol. The eicosanoids were then eluted from the column with 2 ml of absolute ethanol. The samples were filtered through 0.45 μm Millipore filters and dried under a stream of nitrogen. The quantification of the eicosanoids in the samples was performed by ELISA using commercially available kits.

2.9. Drugs used

Bovine serum albumin, rabbit immunoglobulin G antibody to bovine serum albumin, indomethacin, hexadecyltrimethyl ammonium bromide, EDTA, and θ-dianisidine were purchased from Sigma (St. Louis, USA). PAF (C16) was purchased from Novabachem (Switzerland); HEMA 3 was from Biochemical Sciences (NJ, USA); Drabkin's solution was from Wiener Lab. (Rosario, Argentina) and ELISA kits for determination of prostaglandin E_2, thromboxane B_2, leukotriene B_4 and leukotriene C_4/D_4 were from Cayman Chem. (Ann Arbor, MI, USA). The following compounds were received as gifts: WEB 2170 from Boehringer-Ingelheim (Germany); MK 886 from Merck-Frosst (Canada) and RO 0254094 from Hoffmann La Roche (USA).

2.10. Statistical analysis

Data are expressed as the means ± S.E.M. Statistical evaluation of the data was carried out by analysis of variance and sequential analysis of differences among means was done by Tukey's contrast analysis. $P < 0.05$ was considered significant.

3. Results

The following experiments were performed in two strains of mice, C3H/HePas and BALB/c, based on the previous observation that these strains show a different sensitivity to PAF (Vásquez-Bravo et al., 1993). We confirmed this result by analyzing the vascular response to i.p. injection of increasing doses of PAF and found that the maximal response to PAF in C3H/HePas mice was obtained with the dose of 0.03 μg of the agonist whereas a dose 10 times higher was required to induce an equivalent response in BALB/c mice (Fig. 1).

Infiltration of inflammatory cells was evaluated by counting cells in bronchoalveolar lavage fluid and by measuring myeloperoxidase activity in homogenates of lung tissue. Table 1 shows that, 24 h after induction of the Arthus reaction, both strains exhibited significantly higher numbers of polymorphonuclear leukocytes in the bronchoalveolar lavage fluid compared with the control groups. Neutrophils represented 97 to 99% of the polymorphonuclear cells present in bronchoalveolar lavage fluid. The myeloperoxidase activity in lung homogenates was also significantly elevated in both strains of mice at this time (Table 1). The presence of neutrophils in the lung parenchyma was confirmed by histopathological analysis (data not presented).

The intensity of the hemorrhagic lesions was evaluated by measuring the amount of extravascular hemoglobin present in the bronchoalveolar lavage fluid or homogenates of lung tissue. After 24 h of induction of the Arthus

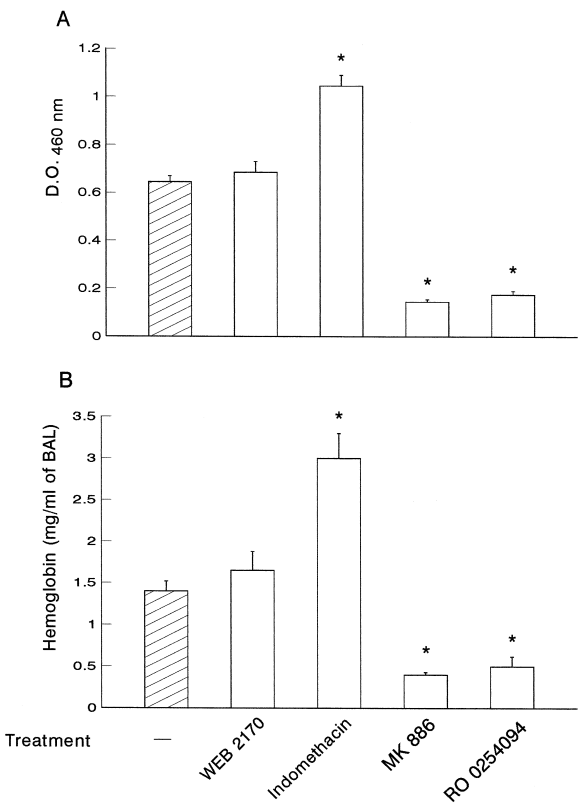

Fig. 2. Effect of treatments on neutrophil infiltration (A) and hemorrhagic lesions (B) in the lungs induced by the Arthus reaction. Groups of BALB/c mice were treated i.v. with one of the following drugs or their vehicles, 30 min before induction of the Arthus reaction: WEB 2170 (10 mg/kg) or indomethacin (2 mg/kg). Compounds MK 886 (10 mg/kg) or RO 0254094 (5 mg/kg) were given p.o. 3 h before induction of the reaction. Groups treated with the vehicles of the drugs were not significantly different from a non-treated group. Neutrophil infiltration was evaluated by measuring myeloperoxidase activity in homogenates of lung tissue and hemorrhagic lesions were evaluated by measuring the hemoglobin content in bronchoalveolar lavage fluid (BAL) 24 h after induction of the Arthus reaction. Data represent the means ± S.E.M. for 6–12 animals/group. * $P < 0.05$ compared to non-treated group. ND = not done.

reaction in the lungs, there was a significant increase in the concentration of extravascular hemoglobin in the bronchoalveolar lavage fluid and lung tissue of both strains compared with the control group. No significant difference was found between strains regarding neutrophil infiltration and hemorrhage (Table 1).

Treatment of BALB/c mice with a PAF receptor antagonist, WEB 2170 (5 mg/kg), prior to induction of the Arthus reaction did not affect the lung hemorrhage (non-treated: 1.40 ± 0.08; WEB treated: 1.52 ± 0.10 mg of hemoglobin/ml). Even a higher dose of WEB 2170 (10 mg/kg) did not change the number of neutrophils that infiltrated the lung tissue (Fig. 2A) or the hemoglobin content of the bronchoalveolar lavage fluid (Fig. 2B). This dose of WEB 2170 was effective in inhibiting the increased vascular permeability induced by i.p. injection of 0.3 μg of PAF (control: 2.01 ± 0.28; PAF: 11.80 ± 0.37; PAF + WEB: 3.16 ± 0.40 μg Evans blue/ml).

Fig. 2A shows that the number of neutrophils that infiltrated the lung parenchyma 24 h after induction of the Arthus reaction, was significantly inhibited by the 5-lipoxygenase inhibitor, MK 886, and by the leukotriene B_4 receptor antagonist, RO 0254094, suggesting that leukotriene B_4 is responsible for this event. Pre-treatment with indomethacin increased significantly the neutrophil infiltration, suggesting that prostanoids may have an inhibitory effect upon neutrophil migration in this model.

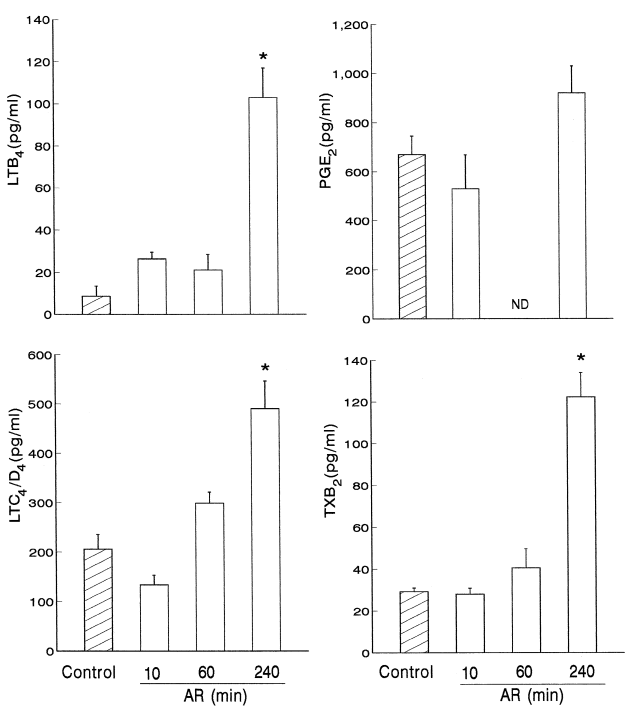

Fig. 3. Concentration of eicosanoids in the bronchoalveolar lavage fluid of BALB/c mice collected at different times after induction of the Arthus reaction. Since the control group, in which antibody was instilled in the airways and saline was injected i.v., and the non-manipulated group had similar levels of eicosanoids, the data were combined and are thus shown as a single control. Data represent the means ± S.E.M. for four to seven animals/group. * $P < 0.05$ compared to control group. ND = not done.

Similar results were found for the lung hemorrhagic lesions (Fig. 2B). The hemorrhagic lesions induced by the Arthus reaction in mice lungs were significantly inhibited by pre-treatment with MK 886 or RO 0254094, which indicates that they are possibly mediated by leukotriene B_4. Prostanoids seem to have a protective role also against the development of the lung lesions since pre-treatment with indomethacin significantly potentiated the lesions.

Analysis of the kinetics of eicosanoid release into the bronchoalveolar lavage fluid showed a closely similar pattern to that for the increases in leukotrienes B_4, C_4/D_4, and thromboxane B_2 4 h after induction of the Arthus reaction in the lung but not at earlier times. There was a trend for an increase of prostaglandin E_2; however, due to higher baseline values this increase did not reach statistical significance (Fig. 3).

4. Discussion

The results presented here show that an Arthus reaction induced in mice lung elicited inflammatory changes such as neutrophil infiltration, hemorrhagic lesions and release of inflammatory mediators. Neutrophil infiltration and increased vascular permeability in the lung, 4 h after induction of a similar reaction in mice, has been previously reported by Bozic et al. (1996). Here we show neutrophil infiltration after 24 h and describe for the first time the occurrence of hemorrhagic lesions in this animal species.

We evaluated the neutrophil infiltration by counting the cells in the bronchoalveolar lavage fluid and by measuring myeloperoxidase activity in lung tissue homogenates. We found an intense infiltration of neutrophils in both compartments 24 h after induction of the Arthus reaction, corroborating previous findings in rats (Johnson and Ward, 1974; Brieland et al., 1987; Tavares de Lima et al., 1992).

The intensity of the vascular damage caused by the Arthus reaction seems to correlate with neutrophil infiltration and activation in the lung (Mulligan and Ward, 1992; Mulligan et al., 1995). Vascular damage in this study was evaluated by the presence of hemorrhagic lesions measured 24 h after induction of the Arthus reaction. The extent and intensity of these lesions were estimated from the amount of extravascular hemoglobin present in the homogenates of lung tissue or from the hemoglobin present in the bronchoalveolar lavage fluid. Other factors, such as congestion, thrombus formation and hemodynamic alterations, can also cause red cell retention and thus contribute to the increased hemoglobin concentration at the reaction site. However, independently of the mechanisms responsible for the hemoglobin increase, it is clear that this phenomenon was restricted to the animals in which the Arthus reaction was induced. Hemorrhagic lesions caused by a reverse passive Arthus reaction induced in the lung have already been reported in the rat, but in this animal the

hemorrhage is focal and restricted to the parenchyma (Tavares de Lima et al., 1992). We showed here that in mice the hemorrhage was diffuse and extended to the airways, as shown by the increased hemoglobin levels found in the bronchoalveolar lavage fluid.

In previous work we have demonstrated that in the rat these lesions are mediated by PAF (Tavares de Lima et al., 1992). However in the mouse, PAF does not seem to have a relevant role. In the present work treatment of mice with a PAF receptor antagonist WEB 2170 (Heuer et al., 1986) prior to induction of the Arthus reaction did not modify the number of neutrophils infiltrating the lung tissue or the intensity of the hemorrhagic lesions. In addition, the intensity of the vascular lesions was similar in BALB/c and C3H/HePas mice despite their clearly different sensitivity to PAF. We are confident that the dose of WEB 2170 used in these experiments(10 mg/kg) was effective to antagonize PAF receptors because even a smaller dose (5 mg/kg) already abolished the PAF-induced vasopermeability in the same strain. The participation of PAF as mediator of the inflammation triggered by immune complexes has been well documented: it contributes to increased vasopermeability in rabbit skin (Hellewell and Williams, 1986), rat pancreas and peritoneal cavity (Jancar et al., 1988; Steil et al., 1995) besides mediating lung hemorrhagic lesions in the rat (Tavares de Lima et al., 1992). The observation that PAF was not relevant for the lung injury in the mouse suggests that, depending on the species, different pathways can be triggered by the immune complex to induce tissue damage.

We then investigated whether prostaglandins or leukotrienes are involved in the neutrophil influx and hemorrhagic lesions in mice lungs. To this purpose we used specific inhibitors of their synthesis or antagonists of their receptors, at doses chosen from relevant published reports of their activity. We found that treatment of BALB/c mice with the lipoxygenase inhibitor, compound MK 886 (Gillard et al., 1989), significantly inhibited both pathological events. A selective leukotriene B_4 receptor antagonist, RO 0254094 (Yagaloff et al., 1995), inhibited both neutrophil infiltration and hemorrhagic lesions, suggesting that leukotriene B_4 mediates these events. In contrast, treatment of the animals with the cyclo-oxygenase inhibitor, indomethacin (Vane, 1971), increased the number of neutrophils that infiltrated the lung parenchyma and the concentration of hemoglobin in the bronchoalveolar lavage fluid. This potentiating effect of indomethacin is probably due to inhibition of prostaglandin E_2 release. These results suggest that cyclo-oxygenase products may have a protective role against the genesis of the lesions, possibly by inhibiting neutrophil influx or activation. Another possibility is that inhibition of cyclo-oxygenase leaves more substrate available for the lipoxygenase pathway with the subsequent increased production of leukotrienes, which, as we showed here, have pro-inflammatory effects. Leukotrienes have been implicated as mediators of hemorrhagic lesions caused by immune complexes in mouse skin (Zhang et al., 1991) and rat lung (Tavares de Lima et al., 1992), but this is the first demonstration that they mediate lung lesions in the mouse. In the rat lung we have shown that the hemorrhagic lesions induced by immune complex were mediated by PAF and leukotriene B_4 and that PAF induced the further release of leukotriene B_4 (Tavares de Lima et al., 1992, 1998). Thus, the difference between rat and mouse models is that in mice the release of leukotriene B_4 seems to be independent of PAF. In both species, leukotriene B_4 seems to be the effector molecule of the tissue injury induced by immune complexes. In the rat, PAF plays a central role, possibly because the release of leukotriene B_4 is dependent on the prior release of PAF.

The release of leukotriene B_4 was detected in the bronchoalveolar lavage fluid but only at 4 h after induction of the Arthus reaction. Similarly, leukotriene C_4/D_4 and thromboxane release was observed only at this time. For prostaglandin E_2, due to the high levels found in the control group, the increased levels measured 4 h after induction of the reaction did not reach statistical significance. It is possible that in a larger series of experiments the difference would be significant.

In summary, our results show that local deposition of immune complexes in the mouse lung causes the release of eicosanoids into the bronchoalveolar space, na influx of neutrophils and hemorrhagic lesions. The hemorrhagic lesions, which are dependent on the infiltration and activation of neutrophils, seem to be mediated by leukotriene B_4 both in the mouse and rat models.

There are several lung diseases where immune complex formation and neutrophil activation play a central role in the pathogenesis. In occupational lung diseases, although some of the symptoms might have an allergic component, the tissue lesions that are characteristic of this group of diseases are dependent on immune complex formation. Recent therapeutic approaches to the Arthus reaction focus on the use of recombinant soluble human complement receptor type I (CD35) to control the activation of the complement cascade (Rossi et al., 1992), the utilization of a recombinant soluble form of human FcγRII genetically engineered to interfere with neutrophil activation via FcγRII and the ensuing release of inflammatory mediators (Ierino et al., 1993), and the utilization of recombinant chimeric selectin molecules to block polymorphonuclear cell recruitment (Mulligan et al., 1993). Based on the results presented here, we propose another approach which consists of using drugs that inhibit leukotriene synthesis or antagonize leukotriene B_4 receptors in human lung diseases triggered by immune complexes.

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