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Lipid mediators, tumor necrosis factor and nitric oxide and their interactions in immune-complex-induced lung injury

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Abstract

We investigated the contribution of eicosanoids, platelet-activating factor, tumor necrosis factor and nitric oxide to the neutrophil influx and development of pulmonary haemorrhagic lesions following immune-complex-induced pneumonitis in rats and possible interactions between these mediators. Increased levels of leukotriene B_4 and tumor necrosis factor, measured by enzyme immunoassay and L-929 cytotoxicity assay, were found in the bronchoalveolar lavage 1 and 4 h after induction of the reaction, respectively, and their release was dependent on the previous generation of platelet activating factor. Antagonism of leukotriene B_4 receptors by RO-0254094 (2-[(5-carboxypentyl])oxy]-6-[6-[3,4-dihydro-4-oxo-8-propyl-2*H*-1-benzopyran-7-yl)oxy]hexyl] benzenepropanoic acid), inhibition of nitric oxide synthesis by L-NAME (N_W-nitro-L-arginine methyl ester) and antagonism of PAF-receptors by WEB-2170 (5-(2-chlorphenyl)-3-4-dihydro-10-methyl-3-((4-morpholinyl)carbonyl)-2*H*,7*H*-cyclopenta (4,5)thieno(3,2-*f*)(1,2,4)-triazolo-4,3,*a*)91,4)diazepine), significantly inhibited the intensity of haemorrhage, evaluated by the increased levels of extravascular hemoglobin in homogenates of lung tissues. Little evidence support the role of tumor necrosis factor in these lesions. The infiltration of neutrophils, evaluated by measuring myeloperoxidase in homogenates of lungs, was reduced by compounds L-663,536 (3-[1-(4 chlorobenzyl)-3-*t*-butyl thio-5-isopropylindol-2-yl]-2-2-dimethylpropanoic acid), WEB-2170 and L-NAME. These results indicate that neutrophil infiltration and haemorrhagic lesions in immune-complex-induced lung inflammation are mediated by platelet activating factor, leukotriene B_4 and nitric oxide and point out to interesting interactions between these mediators.

Keywords: Immune complex; PAF (platelet activating factor); Leukotriene B_4; NO (nitric oxide); TNF (tumor necrosis factor); Lung inflammation

1. Introduction

The deposition of immune complexes in the rat lung induces an inflammatory reaction that is characterised by increased vascular permeability, neutrophil migration and haemorrhagic lesions (Johnson and Ward, 1974). Numerous mediators have been shown to participate in immune-complex-mediated lesions in the lung and other organs, including complement-derived chemotactic factors, tumor necrosis factor (TNF), nitric oxide (NO), several cytokines, eicosanoids and platelet activating factor (PAF) (Warren et al., 1989; Tavares de Lima et al., 1989, 1992; Mulligan et al., 1991; Warren, 1991a,b).

These mediators also increase the expression of adhesion molecules leading to neutrophil infiltration into affected tissue. There is convincing evidence from in vivo studies that this type of lung injury is dependent on neutrophils for its expression. Activation of FcRγ receptors in neutrophils by immune complexes is followed by release of lysosomal enzymes, cationic proteins and reactive oxygen intermediates which are responsible for the tissue injury (for review see Ravetch and Kinet, 1991).

TNF and NO have also been implicated in immune-complex-induced lung injury. This cytokine but not NO appears to be responsible for the recruitment of neutrophils to the affected lungs (Warren et al., 1989; Mulligan et al., 1991). Concerning NO, there is evidence that this mediator may exert a deleterious effect in immune complexes pneumonitis by acting directly on the microvasculature (Mulli-

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gan et al., 1992). We have previously shown that the haemorrhagic lesions in a rat model of immune-complex-induced lung injury are mediated by PAF and 5-lipoxygenase-derived products. In addition, interactions between PAF and eicosanoids were suggested by the observation that the levels of leukotriene B_4 in bronchoalveolar lavage fluid were significantly reduced by PAF receptors antagonists, whereas those of prostaglandin E_2 were significantly increased (Tavares de Lima et al., 1992)

In the present study, we examined further the role of PAF and leukotriene B_4 in immune-complex-induced lung injury. The role of these mediators, as well as of TNF and NO in neutrophils recruitment to the lungs was also investigated. Furthermore, we evaluated the release of leukotriene B_4 and TNF into the bronchoalveolar space and possible interactions between these mediators with PAF and NO.

2. Materials and methods

2.1. Animals

Male Wistar rats (160–180 g) from our own animal facilities was used throughout the experiments.

2.2. Animal model of immune-complex-induced lung injury

Rats were anaesthetised with chloral hydrate (400 mg/kg, i.p.) and a cannula was inserted into the upper bronchus via a tracheotomy. Rabbit antiserum to ovalbumin (100 μl, containing 150 μg of specific antibody protein) was instilled intrabronchially in one of the lobes followed by the i.v. injection of 10 mg of ovalbumin in 0.5 ml. The control rats received intrabronchial antiserum plus saline i.v. or intrabronchial normal rabbit serum plus ovalbumin i.v.

2.3. Quantification of lung haemorrhage

The rats were killed at set time, the lungs were extensively perfused with Krebs–Heinseleit solution via pulmonary artery (10 ml/min) and samples of lung tissue, corresponding to the area of the reaction were removed. Samples of equivalent size were taken from the contralateral lobe, which was not affected by the reaction. The tissue samples were processed for haemoglobin determination by a colorimetric method previously described (Tavares de Lima et al., 1992). The concentration of haemoglobin was determined spectrophotometrically comparing the optical density at 546 nm of the lung samples with a standard curve of known haemoglobin concentrations. The concentration of haemoglobin found in each sample was corrected for the weight and expressed as mg of haemoglobin/mg tissue. The concentration of haemoglobin present in the fragment taken from the contralateral lobe of each animal was subtracted from the value of haemoglobin found in the affected lung and expressed as haemorrhagic index.

2.4. Bronchoalveolar lavage

The animals were killed by cervical dislocation and exsanguinated. A cannula was then inserted into the trachea and bronchoalveolar lavage was performed. Bronchoalveolar lavage for TNF measurements was performed using 5 ml of cooled serum-free RPMI 1640. For the analysis of eicosanoids, the lavage was performed with phosphate-buffered saline (PBS). Bronchoalveolar lavage fluids were centrifuged at $500 \times g$ for 10 min to remove cells and stored at -20°C until analysis.

2.5. Determination of TNF activity in bronchoalveolar lavage

TNF activity was measured by a cytotoxicity assay using L-929 tumor cells (Flick and Gifford, 1985). Briefly, 100 μl of diluted bronchoalveolar lavage samples was pipetted into 96-well microtiter plates containing target L-929 cells (5×10^4 cells/100 μl) in presence of actinomycin D (final concentration 5 μg/ml). The cells were incubated with the bronchoalveolar lavage samples for 20 h at 37°C. The supernatants were then discarded and the remaining viable adherent cells washed with PBS and stained by crystal violet for 15 min. The absorbance of samples was read at 620 nm (ELISA Titertek Multiskan). The TNF titre (units/ml) was defined as the reciprocal of the dilution that induces 50% of lysis L-929 cells.

2.6. Determination of leukotriene B_4 in bronchoalveolar lavage

Cell-free bronchoalveolar lavage fluid was assayed for leukotriene B_4 using enzyme immunoassay as described by Pradelles and Maclouf (1985). Briefly, 100 μl of each sample were incubated for 18 h with the acetylcholinesterase-conjugated eicosanoid and with the specific antiserum in 96-well microtiter plates coated with antibodies to immunoglobulin G. After approximately 2 h of the addition of substrate (acetylcholine), the optical density of the samples was determined at 412 nm in a microplate reader and the concentration of eicosanoids calculated from standard curve.

2.7. Tissue myeloperoxidase activity

Myeloperoxidase activity was used as an indicator for the presence of neutrophils in the lungs affected by the immune-complex reaction. The assay was based on that described by Goldblum et al. (1985) and Warren et al. (1990a). Samples of lung tissue were homogenised with a Polytron homogeniser (5×15 s, at a setting of 4 using 3 ml of PBS containing 0.5% of hexadecyltrimethylammo-

nium bromide and 5 mM EDTA, pH: 6.0). The homogenised samples were sonicated (6 × 10 s at 40 Hz) and then centrifuged at 3000 × *g* for 30 min. The myeloperoxidase activity in the supernatants was assayed by measuring the change in A_{460} resulting from the decomposition of H_2O_2 in the presence of *O*-dianisidine (Henson et al., 1978).

2.8. Drug treatments

Groups of rats were treated with the following drugs at doses chosen from relevant published reports of their activity, 30 min before inducing the immune-complex reaction: WEB-2086, 1 mg/kg, i.v.; WEB-2170, 5 mg/kg, i.v.; BN-52021, 5 mg/kg, i.v.; L-NAME, 30 mg/kg, i.v.; chlorpromazine, 1.25 mg/kg, i.p. The compounds L-663,536 (10 mg/kg) and RO-0254094, (1 mg/kg) were administered orally 3 h before induction of the immune-complex reaction.

2.9. Drugs used

N_W-nitro-L-arginine methyl ester (L-NAME), ovalbumin (grade II), oyster glycogen, hexadecyltrimethylammonium bromide, *O*-dianisidine, hydrogen peroxide, azide, were all purchased from Sigma (St. Louis, MO, USA); Chlorpromazine (2 chloro-10-(3-dimethylaminopropyl phenothiazine) was obtained from Rhodia-Farma (Brazil); Chloral hydrate was supplied by Reagen Química (Brazil). The following drugs were received as gifts: BN-52021 (3-(1, 1-demethylethyl)hexahydro-1,4,7*b*-trihydroxy-8-alpha-methyl-9*H*-1,7-alpha-epoximethanol)-1*H*,6*H*-cyclopenta(*c*)furo(2,3-*b*)furo (3′,12′:3,4) cyclopenta(1,2-*d*)furan-5,9,12(4*H*)-trione) from the Institute Henri Beaufour (France), WEB-2170 (5-(2-chlorphenyl)-3-4-dihydro-10-methyl-3-((4-morpholinyl) carbonyl)-2*H*,7*H*-cyclopenta(4,5)thieno(3,2-*f*)(1,2,4)-triazolo-4,3,*a*) 91,4) diazepine) and WEB-2086 (3-{4-(2) chlorophenyl)9-methyl 6*H*-thieno-[3,2-*f*]-[1,2,4,-triazol[4,3 α,]-[1,4]-diazepin 2:1-(4-morpholinyl)}-1-propanone) from Boehringer Ingelheim (Germany), RO-0254094 (2-[(5-carboxypentyl])-oxy]-6-[6-[3,4-dihydro-4-oxo-8-propyl-2*H*-1-benzopyran-7-yl)oxy]hexyl] benzenepropanoic acid) from Hoffman-La Roche (USA), L-663,536 (3-[1-(4 chlorobenzyl)-3-*t*-butyl thio-5-isopropylindol-2-yl]-2-2-dimethylpropanoic acid) from Merck Frost (Canada).

2.10. Statistical analysis

The TNF data were expressed as median values and were compared by an extension of the median test followed by multiple comparison test. All other data were analysed by Student's *t*-test and expressed as the mean ± S.E.M. Statistical significance was defined as $P < 0.05$ (Zar, 1984).

3. Results

3.1. Release of leukotriene B_4 and TNF in the bronchoalveolar lavage and their modulation

In a previous study we found that peak release of leukotriene B_4 occurs 1 h after inducing the immune-complex reaction in the lung (Tavares de Lima et al., 1989). Here we show that release of leukotriene B_4, measured at this time, was significantly reduced by pretreatment of the rats with the PAF receptor antagonist WEB-2170. Pretreatment with inhibitors of NO and TNF synthesis, L-NAME and chlorpromazine, respectively, did not change the amount of leukotriene B_4 in the bronchoalveolar lavage (Fig. 1). When TNF activity was measured in bronchoalveolar fluid as a function of time, significant TNF activity was only found at 4 h after induction of immune-complex lung injury (64 units/ml). The levels of TNF activity in bronchoalveolar lavage collected 1 h and 24 h after induction of the reaction were not significantly different from the control (5 and 2 units/ml, respectively). Pretreatment with L-NAME or with the leukotriene synthesis inhibitor, L-663,536 did not affect the levels of TNF. However, two PAF receptors antagonists, BN-52021 and WEB-2170 significantly reduced the TNF activity. In addition, chlorpromazine, a drug reported to inhibit TNF biosynthesis in other systems (Gadina et al., 1991) also decreased the levels of TNF in our model (Fig. 2).

3.2. Pharmacological modulation of lung haemorrhage

We have shown previously that the PAF receptors antagonists, BN-52021 and WEB-2086 and the 5-lipoxygenase inhibitor, L-663,536, but not the peptidoleukotriene B_4 receptor antagonist, L-660,711, signifi-

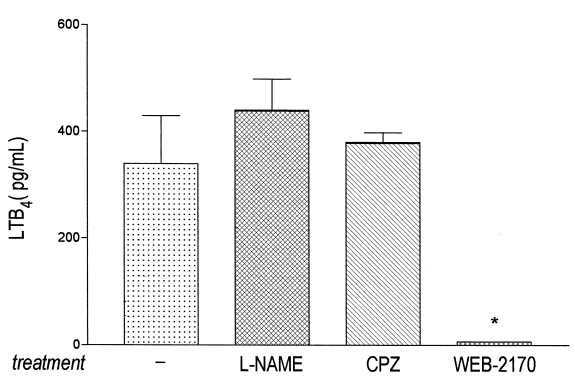

Fig. 1. Effect of treatments on the concentration of LTB_4 in the bronchoalveolar lavage fluid of rats with immune-complex pneumonitis. Enzyme immunoassay was used to quantify the concentration of LTB_4. The immune-complex reaction was induced by intrabronchial instillation of antibodies to ovalbumin (150 μg) followed by intravenous administration of ovalbumin (10 mg) and bronchoalveolar lavage performed 1 h later. The rats were treated 30 min before the induction the reaction with L-NAME (30 mg/kg i.v.), Chlorpromazine (CPZ, 1.25 mg/kg i.p.) or WEB-2170 (5 mg/kg i.v.). Data represent the mean ± S.E.M. of 4–6 experiments. * $P < 0.05$ comparing treated with nontreated group.

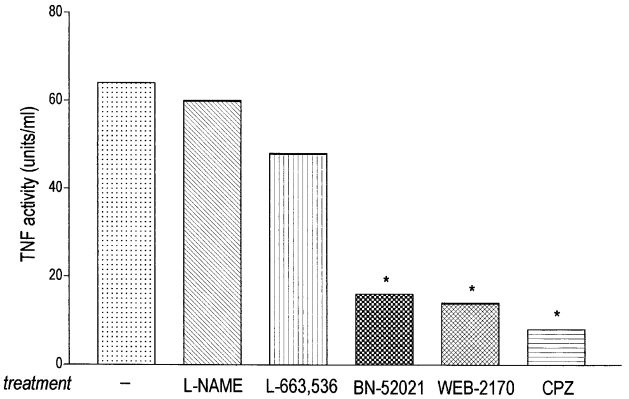

Fig. 2. Effect of treatments on the levels of TNF in the bronchoalveolar lavage fluid of rats with immune-complex pneumonitis reaction. The animals were treated 30 min before induction of the immune complex reaction with L-NAME (30 mg/kg i.v.), L-663,536 (10 mg/kg p.o.), BN-52021 (5 mg/kg i.v.), WEB-2170 (5 mg/kg i.v.) or chlorpromazine (CPZ, 1.25 mg/kg i.p.). The immune-complex reaction was induced by intrabronchial instillation of antibodies to ovalbumin (150 μg) followed by intravenous administration of ovalbumin (10 mg). A BAL was made 4 h later and the TNF activity was determined by L-929 cytotoxicity assay. Data represent the median of 5–9 rats. $* P < 0.05$ comparing treated with non treated group.

cantly reduced the haemorrhage in this model of acute lung injury suggesting the involvement of PAF and leukotriene B_4 in this event (Tavares de Lima et al., 1992). Here we investigated the effect of another PAF receptor antagonist, WEB-2170, given before or after induction of the reaction on the intensity of lung haemorrhage measured 24 h after induction of immune-complex inflammation. We also investigated the effect of a selective leukotriene B_4 receptor antagonist (RO-0254094) and of inhibitors of NO (L-NAME) and TNF synthesis (chlorpromazine). Fig. 3 shows that WEB-2170 significantly inhibited the haemorrhage index when administered 30 min before but not 60 min after induction of the immune-complex reaction. Pretreatment of the rats with RO-0254094 and L-NAME also significantly reduced the intensity of lung haemorrhage, whereas chlorproma-zine had no effect. In another series of experiments, we measured the haemorrhage at 4 h after induction of lung inflammation. The haemorrhagic index at this time was low and was significantly inhibited by chlorpromazine pretreatment (nontreated: 1.02 ± 0.2 vs. CPZ-treated: 0.4 ± 0.1; $n = 4$).

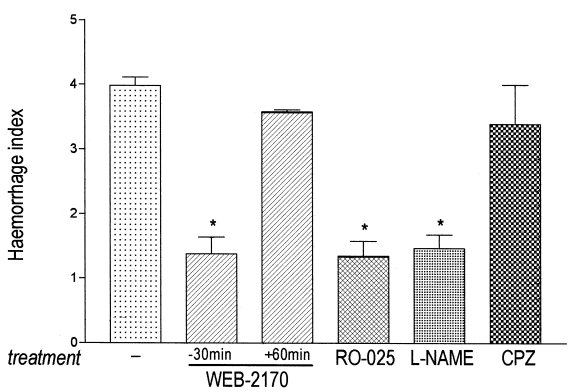

Fig. 3. Pharmacological modulation of the haemorrhagic lesions in rats with immune-complex pneumonitis. The animals were treated with WEB-2170 (5 mg/kg i.v.) 30 min before or 60 min after induction of the reaction. L-NAME (30 mg/kg i.v.) and Chlorpromazine (CPZ, 1.25 mg/kg i.p.) was also given 30 min before while RO-0254094 (RO-025, 1 mg/kg p.o.) was given 3 h before the reaction. The immune-complex reaction was induced by intrabronchial instillation of antibodies to ovalbumin (150 μg) followed by intravenous administration of ovalbumin (10 mg). The animals were killed 24 h after induction of the reaction and concentration of extravascular haemoglobin was measured in the lobe affected by the reaction subtracted of the concentration found in the contralateral nonaffected lobe (haemorrhage index). Data represent the mean ± S.E.M. of 5–11 animals. $* P < 0.05$ comparing treated with nontreated group.

3.3. Pharmacological modulation of the lung neutrophil infiltration

Lung neutrophil infiltration was evaluated by measuring the levels of myeloperoxidase in homogenates of lung tissue. A significant increase (7.6-fold) in myeloperoxidase activity was observed in pulmonary tissue obtained from rats 24 h after immune-complex-induced lung injury when compared to a control group that received an intrabronchial instillation of antibodies followed by i.v. saline (immune complex: 1.6 ± 0.15 vs. control group: 0.21 ± 0.04). A significant increase in myeloperoxidase activity was observed when the above control group was compared to a naive group (0.04 ± 0.01). It can be seen in Fig. 4 that the compounds that inhibited the lung haemorrhagic lesions, L-663,536, WEB-2170 and L-NAME also reduced the levels of myeloperoxidase activity in homogenates of lung tissue (66.1%, 62.4% and 77.7% of inhibition).

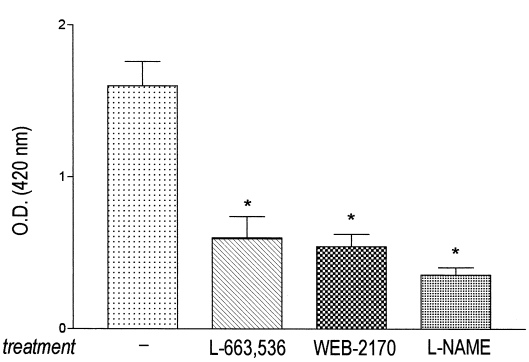

Fig. 4. MPO activity in the lung homogenates from rats with immune-complex pneumonitis. The immune-complex reaction was induced by intrabronchial instillation of antibodies to ovalbumin (150 μg) followed by intravenous administration of ovalbumin (10 mg). The MPO activity was assayed in homogenates of lung tissue from rats pretreated or not with L-663,536 (10 mg/kg p.o.), WEB-2170 (5 mg/kg i.v.) or L-NAME (30 mg/kg i.v.). Results are expressed as OD at 420 nm and represent the mean ± S.E.M. of 5–11 animals. $* P < 0.05$ comparing treated with non treated group.

4. Discussion

We have previously reported that immune-complex deposition in the lung of rats induce haemorrhagic lesions which are mediated by PAF and products of 5-lipoxygenase, and suggested that it was leukotriene B_4 that was responsible for this phenomenon (Tavares de Lima et al., 1992). The results presented here showing that RO-0254094, a leukotriene B_4 receptor antagonist (Cohen et al., 1994; Fretland and Penning, 1996) inhibited the haemorrhage following immune-complex deposition in the lungs confirmed this suggestion. In a previous paper we showed that following induction of immune-complex-mediated inflammation in the lungs leukotriene B_4 is released into the bronchoalveolar lavage and that the peak release occurs in the first hour (Tavares de Lima et al., 1989). In sequence, we showed that release of this eicosanoid is inhibited by two PAF receptor antagonists, BN-52021 and WEB-2086 (Tavares de Lima et al., 1992). In the present work we showed that another PAF receptor antagonist, WEB-2170 also reduced the leukotriene B_4 levels. Thus, release of leukotriene B_4 in this model seems to be dependent on previous release of PAF.

Although we did not measure the PAF levels in bronchoalveolar lavage fluid, the observation that PAF antagonist WEB-2170, administered 30 min before the lung injury was able to prevent the pulmonary haemorrhage indicate that PAF release occurred soon after the injury and that this mediator could profoundly affect the course of the reaction leading to tissue injury. We do not know if PAF continues to be released, but if it does, it certainly does not have a relevant role any more since treatment with the antagonist, 1 h after the reaction was initiated, had no effect on the haemorrhage.

Although these results suggest that PAF plays a pivotal role in the pathogenesis of immune-complex-induced lung damage this is not the case in mice. In the same model of lung injury adapted to mice, we found that a PAF receptor antagonist did not affect the lung haemorrhage. However, inhibition of leukotriene synthesis and antagonism of leukotriene B_4 receptor markedly reduced the lesions (Steil et al., submitted). Thus, it seems that leukotriene B_4 is the final effector molecule of immune-complex-induced lung injury, PAF being relevant in rats because in this animal species the release of leukotriene B_4 is dependent on PAF.

Warren et al. (1989) showed the involvement of TNF in the acute lung injury induced by the deposition of immune complexes. We showed here that TNF levels in the bronchoalveolar lavage of rats were maximal 4 h after induction of lung injury and that the release of TNF is not dependent on leukotriene B_4 or NO in this model. Although Rossi et al. (1992) found no evidence for the involvement of leukotriene B_4 in immune-complex reaction in the skin, it might be argued that differences in microenvironmental characteristics of the lung and skin would account for these discrepancies. Indeed, some reports have indicated that cytokines, such as TNF and interleukin-1 are relevant for acute lung injury but not in skin injury (Warren, 1991b; Mulligan and Ward, 1992).

Chlorpromazine is a neuroleptic compound, which has been reported to inhibit the production of TNF in various systems. We showed here that at a dose of 1.25 mg/kg (i.p.) of chlorpromazine inhibited TNF release in the bronchoalveolar lavage of rats stimulated with immune complexes. We found here that inhibition of TNF production by chlorpromazine did not affect the development of lung haemorrhage measured 24 h, but it had an inhibitory effect on the haemorrhage measured 4 h after the reaction. This is in agreement with the finding of Warren et al. (1989) who were able to block the lung haemorrhage at 4 h by neutralising the effects of TNF in vivo using anti-TNF antibodies. Thus, it is possible that the early haemorrhage is dependent on TNF, whereas later TNF is no longer relevant for the haemorrhage. Release of TNF like that of leukotriene B_4 was also dependent on previous release of PAF. This kind of interaction was not observed with leukotriene B_4 or NO whose inhibition did not affect the release of TNF.

The involvement of NO in the lesions associated with this model was first reported by Mulligan et al. (1991). Our results are in agreement with these findings since L-NAME reduced the extent of haemorrhage measured 24 h after the injury indicating that immune-complex-induced lung injury can stimulate the production of NO which contributes to the haemorrhagic lesion.

The mechanism by which NO induces pulmonary damage is not completely understood but one possibility is by generating peroxynitrites which can directly damage vascular tissue (Beckman et al., 1990). Recent data using pulmonary cells in vitro indicate that the pathological effects of NO may be regulated by its reaction with superoxide, which determines peroxynitrite production (Gow et al., 1998). However, there are several contradictory data concerning the role of NO in tissue damage in acute and chronic inflammation (for review see Evans, 1995). Since this type of pulmonary damage is induced by neutrophils, it is possible that NO contributes to tissue damage by stimulating neutrophil migration. In fact, some data indicate that NO inhibitors, are able to reduce neutrophils migration in vivo and in vitro (Belenky et al., 1993; Menezes-de-Lima et al., 1997).

It is well established that tissue injury induced by immune complexes correlates positively with recruitment, activation and release of toxic products from neutrophils (reviewed by Mulligan and Ward, 1994). We have found a significant influx of polimorphonuclear leukocytes in the bronchoalveolar lavage of rats 6 and 24 h after induction of immune-complex pneumonitis (Tavares de Lima et al., 1992). Neutrophils also infiltrate the pulmonary parenchyma as shown here by the increased myeloperoxidase activity found in rats submitted to immune-complex reaction. This phenomenon, like the haemorrhage, seems

to be mediated by PAF, leukotriene B_4 and NO. Thus, these findings suggest that the regulatory effects on the haemorrhage are associated with a significant reduction in neutrophil migration. This is in agreement with others studies indicating that a decrease in neutrophil migration to the lung results in a lower level of haemorrhage (Mulligan et al., 1992, 1993). However, Mulligan et al. (1992) observed that L-NAME prevented the haemorrhage in this model without causing any alteration in the lung myeloperoxidase activity. These divergent observations may be explained by the fact that these authors measured lung myeloperoxidase activity 4 h after inducing the reaction, whereas in our study, the haemorrhage was measured after 24 h. In a limited number of experiments we measured the lung myeloperoxidase activity and haemorrhage at 4 h and at this time L-NAME has no effect on myeloperoxidase even though the haemorrhagic lesions were significantly reduced (data not shown). These data reinforce the concept that during the inflammatory reaction caused by immune-complex deposition, changes in the physiology of the lung microenvironment result in the release of various mediators.

In conclusion, our results show that immune-complex formation in rat lungs induces release of PAF, which further stimulates release of TNF and leukotriene B_4. These mediators together with NO mediate neutrophil infiltration into the lungs and development of haemorrhagic lesions. Inhibition of the haemorrhagic lesions was parallel with inhibition of neutrophil infiltration supporting the well-established concept that tissue lesions induced by immune complexes are dependent on activated neutrophils.

We propose that the immune complexes that are formed in the experimental model described here bind to the alveolar macrophages which are known to express the low affinity receptors for IgG immune complexes, FcγRII and III (reviewed by Ravetch and Kinet, 1991). Activation of these receptors would stimulate the production of PAF which in turn would induce generation of leukotriene B_4 and synthesis of TNF, possibly in an autocrine fashion. These and other products released by local cells would then promote migration of neutrophils and their accumulation at the inflammatory site. PAF and TNF are known to induce priming of neutrophils and endothelial cells (Warren et al., 1989, 1990b; Vercellotti et al., 1990). Primed neutrophils additionally stimulated by the immune complexes via FcγRIII, would generate NO and reactive oxygen intermediates releasing them together with lysosomal enzymes and cationic proteins, which would finally lead to endothelial damage.

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