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Short Communication

Role of tumor necrosis factor-alpha in endotoxin-induced lung parenchymal hyporesponsiveness in mice

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Abstract

Although changes in airway responsiveness in pulmonary inflammation are commonly related to the action of infiltrated leukocytes, our previous report suggested a direct role of inflammatory cytokines in LPS-induced lung hyporesponsiveness. The aim of this study was to define if cytokines detected in the BALF (bronchoalveolar lavage fluid) of intratracheal LPS-treated mice could be, at least in part, responsible for 5-HT (5-hydroxytryptamine) lung hyporeactivity. Our results show that intratracheal instillation of LPS induced a time-dependent increase in IL-(interleukin-)1β, IL-6, and TNF (tumor necrosis factor)α in the BALF. Cytokine production was paralleled by 5-HT lung hyporesponsiveness, and intratracheal administration of TNFα proved to be very efficient in inhibiting 5-HT responsiveness. In addition, systemic treatment with rolipram, an inhibitor of TNFα production, was paralleled by a significant recovery of lung responsiveness. On the contrary, IL-1β and IL-6 were not demonstrated to play a relevant role in 5-HT hyporesponsiveness. It is concluded that TNFα could be a crucial mediator of LPS-induced lung hyporesponsiveness.

Keywords: Tumor necrosis factor; Lung parenchyma; Hyporesponsiveness; 5-hydroxytryptamine; Lipopolysaccharide; Inflammation

1. Introduction

Pulmonary inflammatory diseases are characterized by changes in airway responsiveness [1,2]. This phenomenon is related to the action of inflammatory mediators produced by infiltrated leukocytes [3,4]. Among inflammatory cytokines, TNFα is considered the main mediator of LPS-induced pulmonary inflammation, with alveolar leukocyte recruitment blocked by selective inhibition of TNFα activity [5,6]. In this regard, a correlation between blood eosinophilia and airways hyperresponsiveness to 5-HT was observed in sephadex particle-treated rats [7]. PMN influx into the bronchoalveolar lumen was also correlated with airway hyporeactivity [4,8]. However, PMN infiltration was also reported to be paralleled by bronchial hyporeactivity in LPS-treated guinea pigs [9].

We have recently reported that acute intratracheal LPS instillation induces a dramatic reduction in parenchymal strip responsiveness to 5-HT in mice [10]. The effect was not paralleled by leukocyte infiltration [11] and was inhibited by betamethasone or pentoxifylline pretreatment, suggesting a direct action of inflammatory cytokines in LPS-induced lung hyporesponsiveness.

The present study was undertaken to further elucidate the mechanism by which LPS causes 5-HT hyporeactivity. Our results suggest that among inflammatory cytokines detected in the BALF of LPS-treated mice, TNFα could be a crucial mediator of LPS-induced lung hyporeactivity.

2. Materials and methods

2.1. Animals and drugs

Female CD-1 mice weighing 25–30 g were obtained from Harlan Nossan. Animals were housed and acclimatised for 1 week under conditions of controlled temperature (20° ± 1), humidity (55% ± 10) and lighting (7 a.m.–7 p.m.); standard sterilised food and water were supplied *ad lib*. during acclimatisation and experiments. All the proce-

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Abbreviations: BALF, bronchoalveolar lavage fluid; 5-HT, 5-hydroxytryptamine; IL-, interleukin-; IL-1RA, interleukin-1 receptor antagonist; LPS, lipopolysaccharide; PMN, polymorphonuclear neutrophil; and TNFα, tumor necrosis factor-α.

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dures were performed in the animal facilities according to ethical guidelines for the conduct of animal research (Authorisation Italian Ministry of Health No. 271/95-B, D.Lvo 116/92; Italian Legislative Decree 116/92, Gazzetta Ufficiale della Repubblica Italiana No. 40, February 18, 1992; EEC Council Directive 86/609, OJL 358, 1, December 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

LPS (from *Escherichia coli* 055:B5) was obtained from Difco Laboratories. 5-HT, ketamine–xylazine, rolipram, and DMSO were from Sigma. Rolipram was solubilized in DMSO and diluted to appropriate concentration in saline. Murine recombinant TNFα, IL-1β, and IL-6 were purchased from PeproTech. IL-1RA was from Dompé S.p.A.

2.2. LPS-induced cytokine production

Mice were anaesthetized by i.p. injection of ketamine–xylazine (50 and 10 mg/kg, respectively) and a midline incision was then performed above the sternum. The trachea was exposed by blunt dissection to allow the instillation of 50 μL of saline solution (vehicle) or saline containing LPS (10 μg/mouse), as previously described [10]. At different times (1, 4, 8, and 24 hr) after LPS treatment, the animals were killed by ether hyperanaesthesia. The BALF was obtained by gently washing the lung cavities with repeated 600-μL saline lavages up to a total volume of 3 mL and was put into an ice-cold conical plastic tube, as previously described [11]. The samples were centrifuged ($400 \times g$) at 4° for 10 min and TNFα, IL-1β, and IL-6 were measured in the supernatants with ELISA kits (Amersham International plc).

2.3. Parenchymal strip reactivity

Mice were killed (with pentobarbital sodium 60 mg/kg i.p.) 4 hr after LPS (10 μg/mouse), TNFα (0.5 μg/mouse), IL-1β (0.5 μg/mouse), or IL-6 (0.5 μg/mouse) treatment. The heart and the lung were isolated and placed in a Petri dish containing a modified oxygenated Krebs solution (6.9 g/L of NaCl; 0.35 g/L of KCl; 0.21 g/L of $CaCl_2$; 0.11 g/L of $MgSO_4 \cdot 7H_2O$; 0.14 g/L of KH_2PO_4; 2.2 g/L of glucose; 2.1 g/L of $NaHCO_3$). The distal margin (1 mm) of the left lobe was removed and then a strip 4–5 mm wide was cut. Parenchymal strips did not include significant airway smooth muscle, as previously demonstrated by histological examination [10]. The lung strip was suspended in a 10-mL organ bath that contained modified Krebs solution at 37°. The solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 and the temperature was maintained at 37° with a constant temperature-circulating unit. Isotonic contractions were recorded with an isotonic transducer (mod. 7006 U. Basile) and a 2-channel recorder (Gemini 7070 U. Basile). Tissues were allowed to stabilize for 1 hr; during this time the bath fluid was changed every 15 min. 5-HT was dissolved in modified Krebs and tested over a range of concentrations from 10^{-9} M to 10^{-3} M. Volumes of 0.1 mL for each agonist concentration were added to the organ bath at 3-min intervals, without washing out, to produce a cumulative concentration–response curve. The responses of the organs to each concentration of the agonist were calculated graphically as mm of contraction and then converted into percentage of the maximal response to 5-HT of the respective control group, as previously described [10]. Rolipram (30 mg/kg i.p.) and IL-1RA (0.5 μg/mice intratracheal) were administered 45 min before or simultaneously with LPS intratracheal treatment, respectively.

2.4. Statistics

All data are means ± SEM. Statistical analysis was performed by ANOVA for repeated measures (SAS/STAT 6.12) in order to test the effects of treatments, concentrations, and treatment–concentration interaction. The preplanned *t*-test was applied to compare similarly defined treatment groups. The significance threshold was set at $P < 0.05$.

3. Results and discussion

3.1. Effects of LPS intratracheal instillation on cytokines production in the BALF

Since IL-1β and TNFα are considered main mediators of pulmonary inflammation [12], we determined the production of these cytokines in the BALF of animals intratracheally instilled with LPS. The production of IL-6, a critical factor in leukocyte recruitment [13], was also taken into consideration. We observed that intratracheal instillation of LPS induced a time-dependent, significant increase in cytokine production, reaching maximal levels 4 hr after LPS treatment (2319 ± 236, 1771 ± 140, and 148 ± 25 pg/mL for TNFα, IL-6, and IL-1β, respectively; mean ± SEM of four animals for group) and declining at longer time points (e.g. 24 hr). Spontaneous cytokine production was 488 ± 17, 438 ± 54, and 51 ± 20 pg/ml for TNFα, IL-6, and IL-1β, respectively. Our data show that LPS-induced parenchymal lung hyporesponsiveness is paralleled by inflammatory cytokine production. In fact, we previously reported that the maximal lung hyporeactivity to 5-HT was reached 4 hr and decreased 24 hr after LPS treatment [10], further suggesting that LPS-induced lung hyporesponsiveness could be mediated by a direct effect of inflammatory cytokines. In agreement with our hypothesis, cytokine production and lung hyporesponsiveness was not related to PMN influx, since the accumulation of PMNs into the BALF was maximal only 12–24 hr from LPS instillation [11].

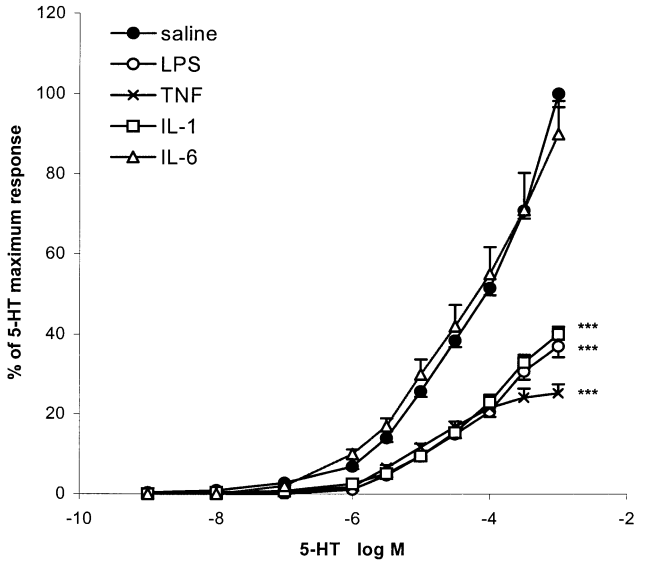

Fig. 1. Effect of IL-1β, IL-6, and TNFα on lung 5-HT responsiveness. Mice were intratracheally treated with vehicle (saline solution; ● N = 20), LPS (○ N = 15) TNFα (× N = 5), IL-1β (□ N = 5), or IL-6 (△ N = 5). Parenchymal lung strip responsiveness to 5-HT was determined 4 hr after intratracheal LPS (10 μg/mouse) treatment. Concentration–response curves are expressed as percent of the maximal contraction obtained in the control group (saline-treated). Each point represents the mean and vertical lines SEM. ***$P < 0.001$ vs control group.

3.2. Role of TNFα, IL-1β, and IL-6 in LPS-induced parenchymal lung strip hyporesponsiveness

To investigate the role of LPS-induced cytokine production in lung parenchymal 5-HT hyporesponsiveness, we determined the effect of intratracheal instillation of murine TNFα, IL-1β, or IL-6 on lung contractility. The effect of these cytokines was studied at a dose (0.5 μg/mouse) reported to induce pulmonary inflammation [12,14]. As shown in Fig. 1, TNFα and IL-1β treatment induced a dramatic decrease in 5-HT lung responsiveness, the effect being comparable to LPS-induced hyporesponsiveness. The maximal response to 5-HT was decreased by 62%, 75%, and 61% in LPS, TNFα, and IL-1β-treated groups, respectively. On the contrary, IL-6 treatment did not significantly change 5-HT lung responsiveness (Fig. 1). To further characterize the role of TNFα and IL-1β in 5-HT hyporeactivity, we investigated the effect of rolipram, an inhibitor of TNFα production [5], and IL-1RA, the physiologic receptor antagonist of IL-1β, on LPS-induced lung hyporesponsiveness. As shown in Fig. 2 (panel A), rolipram (30 mg/kg i.p.) significantly reduced LPS-induced 5-HT hyporesponsiveness, the maximal response being decreased to 5-HT by 71% and 24% in vehicle- and rolipram-pretreated groups, respectively. This effect was paralleled by a significant reduction in TNFα production in the BALF (Fig. 2, panel B). Our data indicate that TNFα could be a crucial mediator of LPS-induced lung hyporesponsiveness. In agreement with our hypothesis, we previously reported that pentoxifylline, another specific inhibitor of TNFα production, significantly reduced LPS-induced parenchymal hyporesponsiveness [10]. On the other hand, IL-1RA (0.5 μg/mouse) did not inhibit LPS-induced lung hyporeactivity, the maximal response being comparable to that evoked by 5-HT in the two experimental groups (25.4 ± 3.01 and 27 ± 5.08 mm of contraction in LPS and LPS + IL-1RA groups, respectively, mean ± SEM of six animals per group). The effect of IL-1RA was studied at a dose (0.5 μg/mouse intratracheally) supposed to completely antagonise the bio-

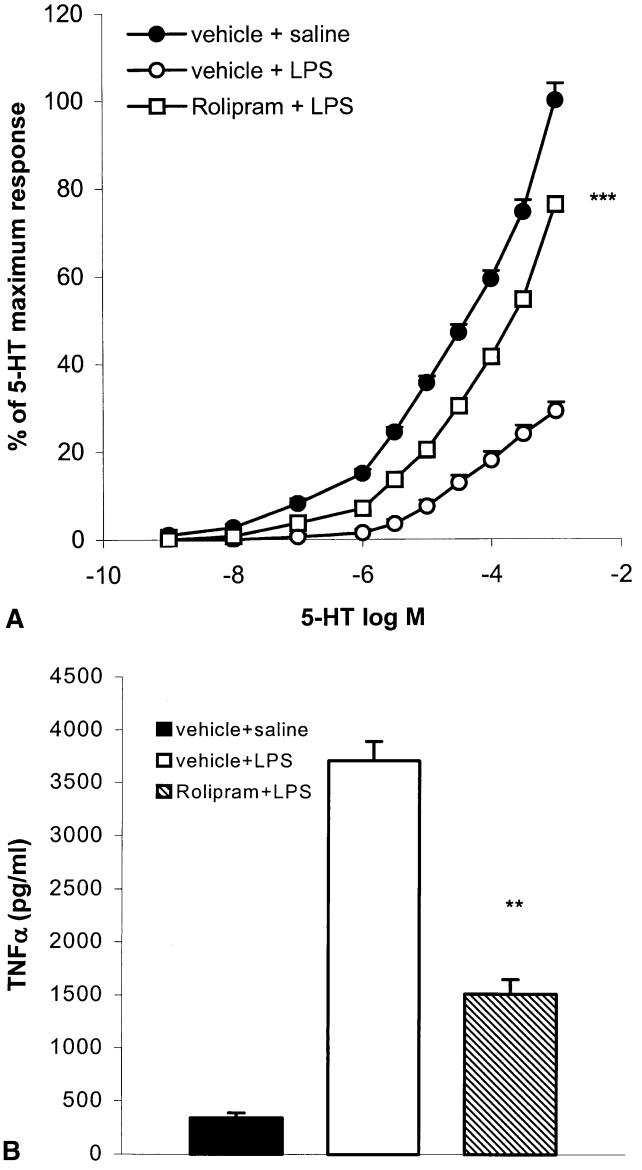

Fig. 2. Effect of rolipram on LPS-induced lung hyporesponsiveness and TNFα production in the BALF. Mice were pretreated with rolipram (30 mg/kg i.p.) 30 min before intratracheal LPS instillation and killed 4 hr after LPS treatment. Panel A: 5-HT concentration–response curves are expressed as percent of the maximal contraction obtained in the control (vehicle pretreated) group (N = 6 for each group). Each point represents the mean and vertical lines SEM. ***$P < 0.001$ vs LPS-alone group. Panel B: TNFα levels in the BALF (N = 4 for each group) are expressed as mean ± SEM. **$P < 0.01$ vs LPS-alone group.

logical activity of IL-1β detected in the BALF [15]. Our results suggest that IL-1β could play a minor role in LPS-induced 5-HT hyporesponsiveness.

In summary, we show that LPS-induced cytokine production in the BALF is paralleled by 5-HT parenchymal hyporesponsiveness. On investigating the role of single cytokines on lung responsiveness, TNFα proved to inhibit 5-HT responsiveness, and inhibition of TNFα production was paralleled by a significant recovery of lung reactivity. Taken together, these data suggest that TNFα could be a key mediator of LPS-induced lung hyporesponsiveness, although the involvement of other mediators cannot be ruled out. To prove more definitely that TNFα is the crucial mediator of LPS-induced lung hyporesponsiveness, the effect of anti-TNFα antibodies could also be taken into consideration.

Since TNFα is also considered the key mediator of LPS-induced PMN recruitment in the lung, our results suggest that treatment with selective inhibitors of TNFα may be useful to concomitantly control some changes in lung functions induced by bacterial infection.

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