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Dissociation between LPS-induced bronchial hyperreactivity and airway edema in the guinea-pig

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Abstract

The interactions between LPS-induced bronchial hyper-reactivity (BHR) and lung inflammation (LI) were investigated. LPS-induced LI was assessed with the augmented alveolo-capillary permeability (ACP) and with the increased migration of neutrophils into the broncho-alveolar lavage fluid. BHR was defined as the increase in the response to a standard dose of serotonin. Mepyramine and the PAF antagonist WEB 2170 blocked LPS-induced increase of ACP, whereas aspirin was inactive. By contrast, neither LPS-induced neutrophil attraction to airways, nor LPS-induced HBR were inhibited by these agents. Our results indicate that LPS-induced edema and BHR are dissociated.

Introduction

It is generally accepted that lung inflammation (LI) induces bronchial hyperreactivity (BHR) [1, 2], and indeed, intra-tracheal (i.t.) administration of *E. coli* endotoxin (LPS) to guinea-pigs is followed by hyperreactivity to 5HT [3]. Since LPS induces both BHR and LI, we now studied the modulation of LPS-induced BHR and LI by drugs to determine their underlying mechanisms and, particularly, whether BHR depends on the LPS-induced increased alveolo-capillary permeability (ACP) and/or cell accumulation.

Materials and methods

Male Hartley guinea-pigs (350–700 g), from Lebeau (Gambay, France), were anesthesized with sodium pentobarbitone (40 mg/kg; i.p.) and prepared for the recording of bronchial resistance to inflation on a Beckman Dynograph R 511 (Schiller Park, Il., USA) as described [4]. Spontaneous breathing was suppressed with pancuronium (2 mg, i.v.). A jugular vein was catheterized for drug injection and one carotid artery was canulated for blood sampling. The bronchial reactivity was assessed with intra-venous of 5HT (0.25–4 μg), or acetylcholine, (2.5–40 μg). The latter was used when pharmacological modulation with 5HT antagonists was performed. Experiments were initiated when three consecutive reproducible responses were obtained with the same dose of a bronchoconstrictor agent, which was used throughout the experiment.

Methods

1 mg of LPS W *E. coli* 055:B5 (Difco Laboratories, Detroit, Michigan, USA), in 0.1 ml of saline, was injected by the i.t. route at t = 0. Neutrophils and platelets were counted in blood before and 5, 10, 30, 60, 90, 120, 150 and 180 minutes after LPS administration. Bronchial reactivity to 5HT or

Table 1
Cell accumulation in guinea-pig BAL, measured 3 hours after intratracheal administration of LPS or saline.

	Macrophages	Neutrophils
Saline i.t.	73	28.8
LPS 1 mg i.t.	45.6	138

Results are expressed in 10^6 cells per milliliter of BAL fluid. ($n=8$ in each group).

acetylcholine, was evaluated every 20 minutes for three hours, using the dose of agonist selected initially.

Bronchoalveolar lavages (BAL) were performed as follows: a total of 50 ml of saline were injected i.t., starting with 5 ml, which were collected within a few seconds. Then, 10 ml volumes were injected and collected. Cells were counted with a Malassez haemocytometer in 0.05% trypan blue. Cell differentiation was performed in cytospin preparations (Hettish, Universal, RFA). Cell viability was judged by trypan blue exclusion.

ACP was evaluated following the rate of appearance of radiolabelled albumin in the blood after its i.t. administration. At $t=0$, ^{131}I-labelled albumin, 1 μCi, was administred i.t. just before LPS instillation. Blood samples (200 μl) were collected in citrated tubes from the carotid catheter, at $t=0$, 10, 30, 60, 90, 120, 150 and 180 minutes. Radioactivities of blood and plasma were counted in the well-type counter.

Mepyramine maleate, 1 mg/kg i.v. in saline (Rhône Poulenc S.A., Vitry sur Seine, France), the PAF antagonist WEB 2170, 1 mg/kg i.v. in saline (Boehringer Ingelheim, FRG, kindly provided by Dr H. Heuer), aspirin, 50 mg/kg i.v. in saline (Synthélabo, Paris, France) were administred half an hour before LPS. In order to ascertain that the injected antagonist was continously efficient, its effects were tested at the end of each experiment with the appropriate agonist.

Two way variance analysis or χ^2 test were used to compare groups during experiments. Statistical significance was admitted at $p<0.05$.

Results

Bronchial hyperreactivity (an increment of approximately three fold for the response to 5HT) was observed 3 hours after the i.t. administration of LPS ($F=20$, $p<0.005$; $n=6$), but was not detected after saline i.t. injection. Neither the PAF antagonist WEB 2170, aspirin, nor mepyramine prevented LPS-induced BHR. 1 mg i.t. LPS induced an 21% increase in ACP ($F=7.964$; $p<0.01$; $n=10$) compared to saline treated-animals. The increase of ACP was completely reversed by mepyramine i.v., or by WEB 2170 given i.v., whereas, by contrast, aspirin was ineffective.

Intra-tracheal administration of LPS is followed by an influx of neutrophils (Table 1) into the BAL fluid, after 3 hours ($\chi^2=65.341$; $p<0.001$; $n=8$). WEB 2170, mepyramine and aspirin did not prevent this influx of neutrophils when administred before LPS.

Discussion and conclusions

Our model of LPS-induced LI involves an increase of BHR, ACP and inflammatory cell invasion into the BAL fluid. LPS-induced increase of ACP was reduced to normal values of LPS-untreated animals by WEB 2170 or mepyramine, indicating involvement of PAF and histamine, respectively. Cell influx into BAL was not modified. BHR was not inhibited by these agents.

In conclusion, the BHR and cell accumulation induced by LPS is not mediated by histamine, PAF or cyclo-oxygenase products, the increased ACP induced by LPS appears to involve histamine and PAF but not cyclo-oxygenase products, the increased BHR induced by LPS can be dissociated from increased ACP.

References

[1] J. R. Snapper and K. L. Brigham. *Inflammation and airway reactivity*. Exp. Lung. Res. *6*, 83–89 (1984).

[2] G. Folkerts, P. A. Henricks, P. J. Slootweg and F. P. Nijkamp. *Endotoxin-induced inflammation and injury of the guinea-pig respiratory airways cause bronchial hyporeactivity*. Am. Rev. Resp. Dis. *137*, 1441–1448 (1988).

[3] D. Vincent, J. Lefort, J. Dry and B. B. Vargaftig. *Bacterial endotoxin induces platelet-dependent bronchial hypereactivity in guinea-pigs*. (Submitted).

[4] J. Lefort and B. B. Vargaftig, *Role of platelets in aspirin-sensitive bronchoconstriction in the guinea-pig; interactions with salicylic acid*. Br. J. Pharmacol. *63*, 35–42 (1978).