Inflamm. res. 53 (2004) 658–663
1023-3830/04/120658-06
DOI 10.1007/s00011-004-1308-7

Inflammation Research

Original Research Papers

Evidence that arachidonic acid derived from neutrophils and prostaglandin E_2 are associated with the induction of acute lung inflammation by lipopolysaccharide of *Escherichia coli*

T. C. Alba-Loureiro [1], E. F. Martins [2], C. K. Miyasaka [2], L. R. Lopes [1], R. G. Landgraf [3], S. Jancar [3], R. Curi [2] and P. Sannomiya [4]

[1] Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 1524, 05508-900 São Paulo, SP, Brazil, Fax: ++55 11 3091 7245, e-mail: tatica@fisio.icb.usp.br

[2] Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, SP, Brazil

[3] Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, SP, Brazil

[4] Research Division, Institute of Heart (INCOR), LIM-11, University of São Paulo Medical School, São Paulo, SP, Brazil

Received 19 October 2003; returned for revision 30 November 2003; accepted by N. Boughton-Smith 15 July 2004

Abstract. *Objective:* The involvement of arachidonic acid (AA) and PGE_2 during the *E. coli* lipopolysaccharide (LPS)-induced acute lung injury was investigated.
Material: Adult male Wistar rats were used. For in vitro studies, rat neutrophils, bronchoalveolar lavage (BAL) fluid, and lung vascular endothelium were used, as described below.
Treatment: Rats were given an intratracheal injection of LPS (750 µg).
Methods: Total and differential cell counts in BAL fluid; enzyme-linked immunoassay (ELISA) analyses of TNF-α, IL-1β, LTB_4 and PGE_2 in BAL, and immunohistochemical detection of ICAM-1 on lung vascular endothelium were performed six h after LPS challenge. Fatty acid composition of blood neutrophils and plasma was analyzed by HPLC.
Results: Rats instilled with LPS presented a sixty three-fold increase in the number of neutrophils in BAL (from 0.5×10^6 to 31.5×10^6 cells), accompanied by increased levels of TNF-α and IL-1β ($p < 0.001$), and a three-fold increase in ICAM-1 expression on vascular endothelium. The content of AA in blood neutrophils was reduced by 50%, whereas the level of PGE_2 in BAL was increased by 3.5 fold, without changes in the levels of LTB_4.
Conclusions: These findings suggest that AA and PGE_2 are associated with LPS challenge.

Key words: Neutrophils – Lipopolysaccharide – Acute lung injury – PGE_2 – Arachidonic acid

Correspondence to: T. C. Alba- Loureiro

Introduction

Experimental acute lung injury induced by lipopolysaccharide (LPS) shares similarities with Adult Respiratory Distress Syndrome – ARDS, considering that this disease is a consequence of the sepsis by gram-negative bacteria [1]. Lipopolysaccharide is a component of the gram-negative bacterial cell wall that is known to induce a global activation of inflammatory responses [2]. The LPS interacts with LPS-binding protein and CD14 [3–5]. This latter protein is the main LPS receptor [6], which presents LPS to toll-like receptor 4 (TLR4) [7, 8], and activates inflammatory genes expression through nuclear factor κB (NFκB) and mitogen-activated protein-kinase signalling [9, 10].

The lung injury is characterized by influx of neutrophils into the airways and the presence of proteins in BAL fluid [11]. LPS stimulates inflammatory cells to release cytokines, such as interleukin- 1β (IL-1β) and tumor necrosis factor α (TNF-α); chemokines, like cytokine-induced neutrophil chemoattractant – CINC, which plays an important role for expression of adhesion molecules [12–14]. However, the role of lipid mediators in acute lung injury remains to be investigated. There is evidence that lipid mediators are associated with neutrophil recruitment in lung injury. Leukotriene B_4 (LTB_4) is involved with the influx of neutrophils in acute lung injury by LPS in pigs [15], and LTB_4 and prostaglandin E_2 (PGE_2) are involved in immune complex-induced lung inflammation in mice [16].

The aim of this study was to investigate the participation of arachidonic acid and its metabolites – LTB_4 and PGE_2 – in the recruitment of neutrophils to lung after LPS-induced injury. For this purpose, the fatty acid composition was deter-

mined in blood neutrophils and plasma by using HPLC and the levels of LTB_4 and PGE_2 were determined in BAL fluid by ELISA.

Materials and methods

Animals

Male Wistar rats weighing 200 ± 20 g (about 2 months of age) were obtained from the Pharmacology Department of the Institute of Biomedical Sciences (São Paulo University, São Paulo, Brazil). The rats were maintained at 23 ± 2 °C under a cycle of 12 h light: 12 h darkness and the animals were allowed access to food and water *ad libitum*. The experimental procedure of this study was approved by the Animal Ethical Committee of the Institute of Biomedical Sciences.

LPS instillation

The animals were anesthetized by an ip injection of chloral hydrate (LabSynth, São Paulo, Brazil) (400 mg/Kg body weight) and the trachea exposed through a midline ventral incision of approximately 0.5 cm length in the neck. Saline (0.4 mL) containing 750 µg of *E. coli* lipopolysaccharide (Sigma Chemical Co., St. Louis, MO, USA) was instilled into the airways. Control animals received saline only by the same route. The incision was closed with cotton suture.

Bronchoalveolar lavage procedure

Bronchoalveolar lavage (BAL) was performed 6 h after intratracheal administration of LPS or saline. The animals were anesthetized as described above and the abdominal cavity was opened for blood collection and exsanguination from the abdominal aorta. The lungs were lavaged by instillation of 25 mL phosphate-buffered saline (PBS) at room temperature through a polyethylene tube (1 mm in diameter) inserted into the trachea. The BAL fluid was not used if the retrieved volume was less than 85% of the 25 mL instillated. Low recovered volumes indicate that substantial amount of cells still remains in the lung and so it does not reflect the inflammatory process in course. Total cell counts were determined by using an automatic hemacytometer (CC510; CELM, São Paulo, SP, Brazil). Differential cell counts were carried out on stained films under oil immersion microscopy. A total of 100 cells were counted and classified as neutrophils, eosinophils or mononuclear cells on the basis of normal morphological criteria.

Determination of TNF-α and IL-1β

The measurement of TNF-α and IL-1β was performed in samples of BAL fluid by ELISA using Duo-set available kits (R & D Systems Inc., Minneapolis, MN, USA). Firstly, 100 µL of an anti- TNF-α antibody or an anti-IL-1β antibody were added to the wells to cover the plate. After overnight incubation at 4 °C, the plate was washed 3 times with PBS containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, MO, USA), and 100 µL of each standard or 100 µL of BAL fluid were added to the wells. The plate was incubated for 2 h at room temperature, washed 3 times, and 100 µL of biotinylated anti-rat TNF-α or biotinylated anti-rat IL-1β were added to each well. After 2 hours at room temperature, the plate was washed, and 100 µL of streptoavidin-HRP were added to each well. The plate was covered to avoid placing the plate in direct light. After 20 min at room temperature, the plate was washed and 100 µL of 3,3′,5,5′-tetramethylbenzidine (TMB) (Calbiochem, San Diego, CA, USA) were added to each well. The plate was covered and incubated for 30 min at room temperature. The color development was stopped with 50 µL H_2SO_4 (2N) and the optical density determined at 450 nm. The sensitivity of the assays was of 15 pg/mL.

Immunohistochemistry for ICAM-1

The animals were anesthetized as described above and the trachea exposed and catheterized with a polyethylene tubing, 1 mm in diameter. The chest wall was opened and the lungs with occluded trachea were removed intact. Thereafter, 10 mL of tissue freezing medium (Leica Instruments Gmbh, Nussloch, Germany) diluted in H_2O (1:1, v/v) were instilled into the lungs. A portion of the inferior lobes was removed and frozen in liquid nitrogen. Serial 5 µm cryostat sections adhered to glass slides previously coated with organo-sylane (Sigma Chemical Co., St. Louis, MO, USA) were fixed in acetone for 10 min and H_2O_2/methanol solution (1:1, v/v) twice for 10 min. Tissue sections were incubated, for 1 h at 37 °C, with the primary antibody (mouse IgG_1 anti-rat ICAM-1, Seikagaku Co., Tokyo, Japan); diluted 1:1000 in PBS containing 1% bovine serum albumin. After washing the slides with PBS, sections were incubated, for 15 min at 37 °C, with biotinylated secondary antibody (anti-mouse IgG) (Vector Laboratories, Burlingame, CA, USA) followed by streptoavidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA, USA). Slides were washed, developed with 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO, USA) and hydrogen peroxide and counterstained with hematoxylin. The analysis of immunohistochemical reactions was based on its intensity by Image Analysis System and the results were processed using an Image Pro® Plus software, version 4.1.

Neutrophil separation

After 6 h of LPS instillation, the animals were anesthetized as described above, blood was collected from the abdominal aorta, using syringes with heparin, and the polymorphonuclear leukocytes (PMN) isolated by Ficoll-dextran sedimentation. Briefly, blood samples diluted (1:1) in sterile PBS were layered on an equal volume of Ficoll-Hystopaque. After centrifugation (400 g, 45 min, room temperature), the superior mononuclear-rich layer was discarded and red blood cells were separated from the neutrophil-rich pellet by the addition of 2 mL dextran (6%) for 1 h at 37 °C. Cells were then washed and the remaining erythrocytes removed by hypotonic lysis. Neutrophils accounted for 95% of the cells, with 5% of mononuclear cells contamination.

HPLC equipment and chromatographic conditions

The HPLC used was purchased from Shimadzu (Kyoto, Japan). The system consists of two pumps (model LC-10AD), autoinjector (model SIL-10^A), G-ODS shim-pack pre-column (2 cm × 4.6 mm × 5 µm) and analytical shim-pack column CLC-C8 (25 cm × 4.6 mm × 5 µm). The separation of fatty acids was carried out using an acetonitrile (EM Science-Merck, Darmstadt, Germany) gradient (77–90%) at 1.0 ml/min. The results were processed using a Class LC-10^A software, version 1.4. The fatty acids used as standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA): linoleic (C18:2), linolenic (C18:3), arachidonic (C20:4), eicosapentaenoic (C 20:5), docosa-hexaenoic (C22:5), and margaric (C17:0) acids.

Lipid extraction and saponification

Total lipids of plasma and blood neutrophils were extracted using the Folch method [17] and saponified afterwards. Margaric acid (5 mg/mL) was added to the samples (250 µg) to estimate the loss during all process. After homogenization for 30 s with chloroform and methanol (2:1 v/v) (EM Science-Merck, Darmstadt, Germany), the samples were centrifuged and filtered twice. The procedure was repeated with aqueous solution, and after the extraction, the aqueous phase was discharged. The organic phase was then evaporated in a speed-vac Sc110 (Savant). The lipids were saponified using 1.4 mL of an alkaline methanol solution (1 mol/mL NaOH in 90% methanol) at 37 °C, for 2 h, in a shaking water bath. Afterwards, the alkaline solution was acid-

ified to pH 3.0 with HCl solution 1 mol/mL. Fatty acids were then extracted twice with 1 mL hexane (EM Science-Merck, Darmstadt, Germany). The samples were dried and kept under a nitrogen atmosphere and protected from light at –20 °C until to fatty acids determination [18, 19].

Extraction and quantification of eicosanoids

The cell-free BAL fluid was acidified with HCl 1 N to pH 3.4–3.6 and passed slowly through an octadecylsilyl silica column (Sep Pak C 18 column), pre-washed with 10 mL ethanol and 10 mL water. After washing the column with 10 mL water and 1 mL ethanol (35%), the eicosanoids were eluted from the column with 2 mL absolute ethanol and the samples dried under a stream of nitrogen. PGE_2 and LTB_4 concentrations were determined by using ELISA kits (Cayman Chemical Co., Ann Arbor, MI, USA). The sensitivity of the PGE_2 assay was of 15 pg/mL and of the LTB_4 assay was of 4 pg/mL.

Statistical analysis

Data are expressed as means ± SEM. Statistical comparisons were made by the Student's t-test or one-way analysis of variance followed by the Tukey-Kramer multiple comparisons test. Data were considered statistically significant if p values were lower than 0.05.

Results

The intratracheal instillation of LPS significantly increased the cell number in BAL fluid (Table 1). Compared to saline, the total cell number was five times higher in the LPS-instilled rats. Neutrophil counts were elevated, number of mononuclear cells decreased, and number of eosinophils remained unchanged in LPS-treated group.

LPS-instilled rats showed higher levels of both TNF-α and of IL-1β in BAL as compared to saline (Fig. 1) and three fold increase in the immunohistochemical detection of ICAM-1 in lung vascular endothelium (Fig. 2). Representative photomicrographs are shown in Fig. 3.

Neutrophils from rats exposed to LPS exhibited a 50% reduction in the content of arachidonic acid, without significant changes in other fatty acids analyzed, incluing linoleic, linolenic, eicosapentaenoic and docosa-hexaenoic acids (Fig. 4). The content of these fatty acids and of arachidonic acid in the plasma did not change after exposure of the animals to LPS (Fig. 4).

The levels of LTB_4 in BAL fluid were not affected by LPS instillation. However, LPS instillation caused a significant increase of PGE_2 levels in the BAL fluid (Fig. 5).

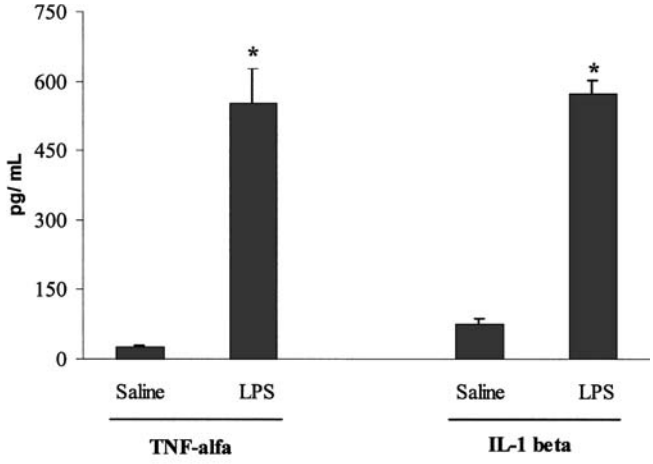

Fig. 1. Levels of TNF-α and IL-1β in the bronchoalveolar lavage fluid obtained 6 h after LPS instillation of the rats. Values are presented as means ± SEM from 4 animals in each group. *$P < 0.001$ as compared to saline.

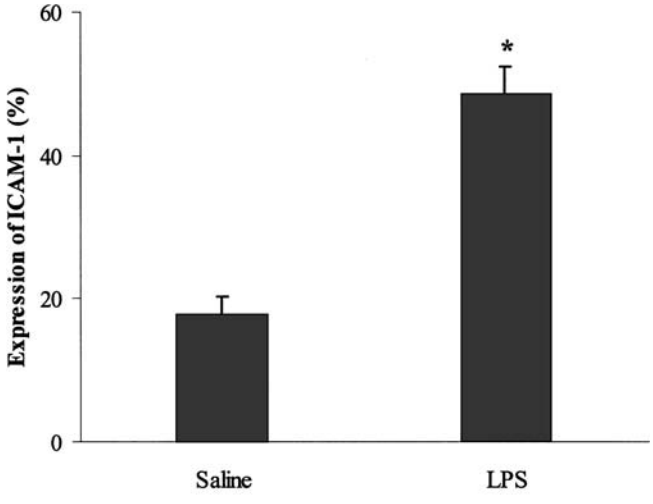

Fig. 2. Immuno-histochemical detection of ICAM-1 on lung vascular endothelium. Values are presented as means ± SEM from 5 rats in each group. The analysis was performed in 5 vessels per animal by using the software Image Pro-plus, 4.1. *$P < 0.001$ as compared to saline.

Table 1. Cell composition of bronchoalveolar lavage fluid of rats exposed to lipopolysaccharide.

Treatments	n	Number of cells (× 10^6)			
		Total	Neutrophils	Mononuclear cells	Eosinophils
Saline	5	6.2 ± 0.3	0.5 ± 0.6	5.7 ± 0.6	0
LPS	8	33.3 ± 4.0[a]	31.5 ± 4.4[a]	1.8 ± 0.1[b]	0

Values are presented as means ± SEM. [a]$P < 0.01$ and [b]$P < 0.05$ as compared to saline.

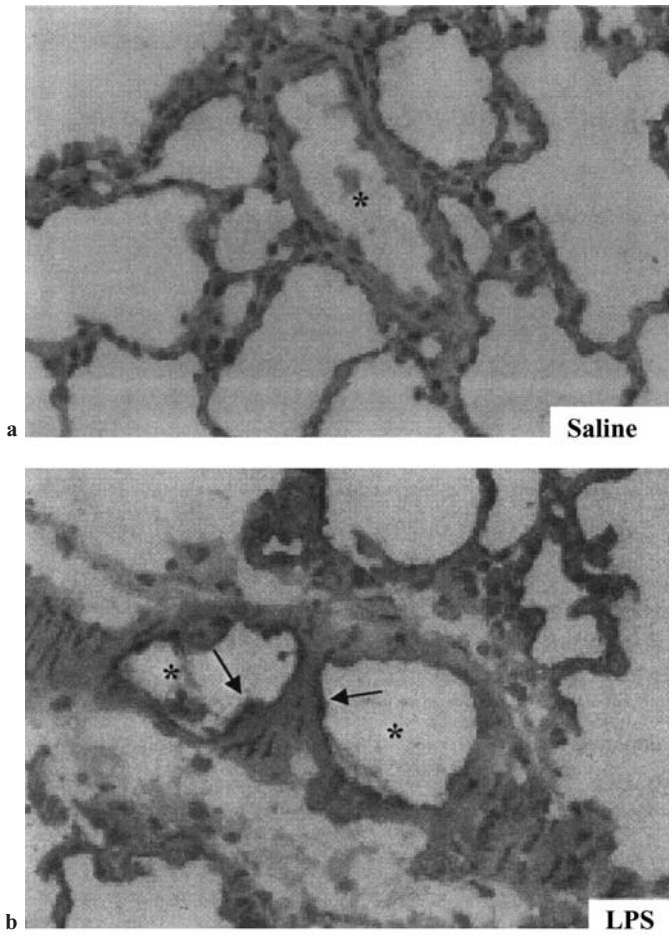

Fig. 3. Microphotographs of the lung tissue after instillation of saline (**a**) or lipopolysaccharide (**b**). Lung sections (5 µm) were stained (*arrowheads*) for the detection of ICAM-1. * Indicates a pulmonary vessel (Original magnification ×1500).

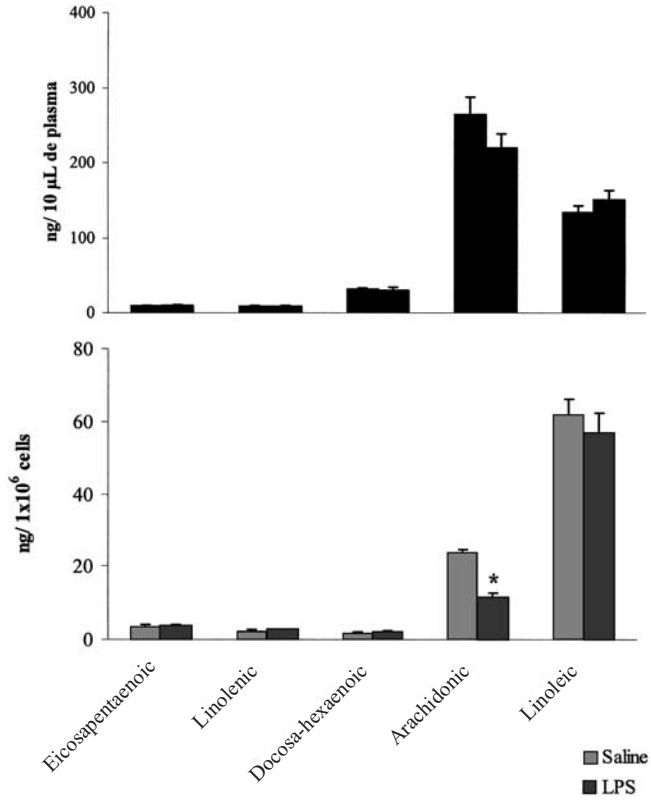

Fig. 4. Fatty acid composition of rat plasma (A) and blood neutrophils (B) obtained 6 h after LPS instillation of the rats. Fatty acids were extracted, saponified, and the composition determined by HPLC. Values are presented as means ± SEM from 4 (plasma samples) or 5 (neutrophils) animals in each group. * $P < 0.05$ vs saline instilled rats.

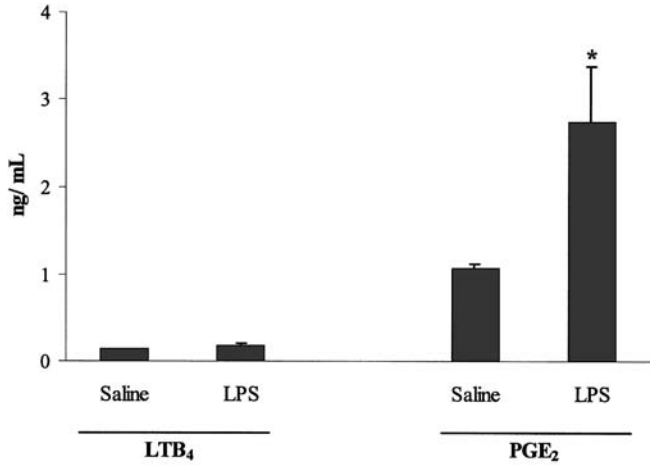

Fig. 5. Levels of LTB_4 and PGE_2 in the bronchoalveolar lavage fluid obtained 6 h after LPS instillation of the rats. Values are presented as means ± SEM from 4 animals in each group. * $P < 0.05$ as compared to saline.

Discussion

LPS induces a local inflammatory reaction characterized by a pronounced leukocyte infiltration [20, 21] which is due to a massive and acute neutrophil accumulation, followed by a late mononuclear cell and eosinophil influx [22]. The present study showed, like previous studies, that intratracheal instillation of LPS (injection or aerosol) causes acute inflammation characterized by increased number of neutrophils in the alveolar space [23–26]. Leukocyte recruitment has generally been described as a multistep cascade involving endothelial and leukocyte adhesion molecules, like selectins, integrins and immunoglobulins, but in LPS-induced lung inflammation, the sequestration of neutrophils is also entirely dependent on CD14 and TLR_4 [27].

The inflammatory process in the airways is accompanied by a marked release of TNF-α and IL-1β [11, 28, 29]. Results presented herein, as compared to saline, LPS-instilled rats presented increased levels of TNF-α and IL-1β in the BAL fluid. These cytokines amplify the lung inflammatory response by stimulating the release of chemoattractant factors by alveolar macrophages and airways epithelial cells, and the expression of adhesion molecules by leukocytes and endothelial cells [30]. Airway instillation of LPS into rat lungs induces neutrophil accumulation, which is known to be ICAM-1 dependent [14, 31, 32]. Furthermore, increases in whole lung ICAM-1 message and protein require the availability of TNF-α or IL-1β [31]. In the present study, sharply elevated levels of both cytokines were accompanied by a

three fold increase in the immunohistochemical detection of ICAM-1 on lung vessels. Other adhesion molecules, in particular E-selectin and CD11/CD18 (β_2 integrins) are also involved in pulmonary sequestration of neutrophils during the course of LPS-induced acute lung injury [14, 33, 34]. After arriving in the lungs these activated leukocytes induce pulmonary injury through the release of multiple cytotoxic substances, including reactive oxygen species, and proteolytic enzymes. In addition, neutrophils release growth factors, cytokines, chemokines, and eicosanoids, which may enhance the inflammatory response [35].

In order to evaluate the role of lipidic mediators in lung inflammation associated with bacterial LPS, fatty acids composition was determined in blood neutrophils and plasma. The fact that neutrophil-derived arachidonic acid might be a source of lipid mediators during the course of LPS-induced inflammation is supported by the following observations: i) levels of linoleic acid, a precursor of arachidonic acid synthesis, were higher than the ω-3 fatty acids, including eicosapentaenoic, linolenic and docosa-hexaenoic acids, in both neutrophils and plasma, derived either from LPS or saline treated rats; ii) content of linoleic acid, however, did not change after LPS instillation; iii) whereas the content of arachidonic acid in plasma did not differ between LPS and saline treated rats, there was a 50% reduction in the content of neutrophil-derived arachidonic acid after exposure of the animals to LPS. In this condition, levels of PGE_2 in BAL fluid were sharply elevated. In contrast, levels of LTB_4, a 5-lipoxygenase-derived lipid mediator, did not change as compared to saline instillated animals. Other mediators, particularly the chemokine cytokine-induced neutrophil chemoattractant (CINC) is involved with the recruitment of neutrophils into the lungs during LPS-induced inflammation in rats [13, 36].

Since LPS induces the synthesis of cyclooxygenase-2 in rat blood leukocytes [37], activated neutrophils recruited to the lungs after LPS instillation might be an additional source for the local production of PGE_2 as do alveolar macrophages and other cells. Similarly, human neutrophils stimulated in vitro with variable pro-inflammatory agents, including LPS, express the inducible form of the enzyme and synthesize PGE_2 and thromboxane A_2 [38]. It has been showed that PGE_2 inhibits LPS-induced TNF-α generation by human blood monocytes [39], and blocks both neutrophil recruitment and TNF-α production in a mouse model of acute lung injury induced by inhalation of LPS [40].

Data presented suggest that during the course of LPS-induced acute lung injury, the recruitment of neutrophils is associated with the release of TNF-α, IL-1β, and the expression of ICAM-1 in lung vessels. Evidence is presented herein that neutrophil-derived arachidonic acid and PGE_2 may be involved in the pathogenesis of lung inflammation induced by bacterial LPS.

Acknowledgements. This study was supported by FAPESP, CNPq and CAPES.

References

[1] Brigham KL, Meyrick B. Endotoxin and lung injury. Am Rev Respir Dis 1986; 133: 913–27.
[2] Wagner JG, Roth RA. Neutrophil migration during endotoxemia. J Leukoc Biol 1999; 66: 10–24.
[3] Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM. The monocytes differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. J Immunol 1988; 141: 547–52.
[4] Haziot A, Tsuberi B-Z, Goyert SM. Neutrophil CD14: biochemical property and role in the secretion of tumor necrosis factor-α in response to lipopolysaccharide. J Immunol 1993; 150: 5556–65.
[5] Luchi M, Munford RS. Binding, internalization, and deacylation of bacterial lipopolysaccharides by human neutrophils. J Immunol 1993; 959–69.
[6] Haziot A, Ferrero E, Kontgen F, Hijiya N, Yamamoto S, Silver J et al. Resistance to endotoxin shock and reduced dissemination of Gram-negative bacteria in CD14-deficient mice. Immunity 1996; 4: 407–14.
[7] Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000; 406: 782–7.
[8] Medzhitov R. Toll-like receptors and innate immunity. Nature Rev Immunol 2001; 1: 135–45.
[9] Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem 1999; 274: 10689–92.
[10] Beutler B. Endotoxin, toll-like receptor 4, and the afferent limb of innate immunity. Curr Opin Microbiol 2000; 3(1): 23–8.
[11] Ulich TR, Watson LR, Yin S, Guo K, Wang P, Thang H et al. The intratracheal administration of endotoxin, and cytokines. I. Caracterization of LPS-induced IL-1 and TNF mRNA expression and the LPS, IL-1 and TNF-induced inflammatory infiltrate. Am J Pathol 1991; 138: 1485–96.
[12] Ulich TR, Yin S, Remick DG, Russell D, Eisenberg SP, Kohno T. Intratracheal administration of endotoxin, and cytokines. IV. The soluble TNF receptor type I inhibits acute inflammation. Am J Pathol 1993; 142: 1335–8.
[13] Ulich TR, Howard SC, Remick DG, Wittwer A, Yi ES, Yin S et al. Intratracheal administration of endotoxin and cytokines. VI. Antiserum to CINC inhibits acute inflammation. Am J Physiol 1995; 268: L245–50.
[14] Tang WW, Yi ES, Remick DG, Wittwer A, Yin S, Qi M, Ulich TR. Intratracheal injection of endotoxin and cytokines. IX Contribution of CD11a/ICAM-1 to neutrophil emigration. Am J Physiol 1995; 269: L653–9.
[15] Vandermeer TJ, Menconi MJ, O'Sullivan BP, Larkin VA, Wang H, Sofia M et al. Acute lung injury in endotoxemic pigs: role of leukotriene B_4. J Appl Physiol 1995; 78(3): 1121–31.
[16] Steil AA, Tavares de Lima W, Jancar S. Modulation by lipid mediators of immune complex-induced lung inflammation in mice. Eur J Pharmacol 1998; 361: 93–9.
[17] Folch J, Lees M, Sloanne Stanley GH. A simple method for isolation and purification of total lipid from animal tissue. J Biol Chem 1957; 226: 497–509.
[18] Beyer ES, Jensen LNJ. Overestimation of the cholesterol content of eggs. Agric Food Chem 1989; 37: 917.
[19] Hamilton S, Hamilton RJ, Sewell PA. Extration of lipids and derivate formation In: Hamilton RJ, Hamilton S, editors. Lipid analysis – a practical approach. Ed. Chapter 2. p.14, IRL press at Oxford University Press, 1992.
[20] Issekutz AC, Megyeri P, Issekutz TB. Role of macrophage products in endotoxin-induced polymorphonuclear leukocyte accumulation during inflammation. Lab Invest 1987; 56(1): 49–59.
[21] Bozza PT, Castro-Faria-Neto HC, Pires AL, Silva PMR, Martins MA, Cordeiro RSB. Endotoxin induces eosinophil accumulation in the rat pleural cavity. Braz J Med Biol Res 1991; 24(9): 957–60.
[22] Bozza PT, Castro-Faria-Neto HC, Martins MA, Larangeira AP, Perales JE, Silva PMR et al. Pharmacological modulation of lipopolysaccharide-induced pleural eosinophilia in the rat: a role for a newly generated protein. Eur J Pharmacol 1993; 248(1): 41–7.

[23] Yi ES, Ulich TR. Endotoxin, interleukin-1, and tumor necrosis factor cause neutrophil-dependent microvascular leakage in post-capillary venules. Am J Pathol 1992; 140: 659–63.
[24] Yi ES, Remick DG, Lim Y, Tang W, Nadzienko CE, Bedoya A et al. The intratracheal administration of endotoxin: X. Dexamethasone downregulates neutrophil emigration and cytokine expression in vivo. Inflammation 1996; 20(2): 165–75.
[25] Boichot E, Sannomiya P, Escofier N, Germain N, Fortes ZB, Lagente V. Endotoxin-induced acute lung injury in rats. Role of insulin. Pulm Pharmacol Therap 1999; 12: 285–90.
[26] Escofier N, Boichot E, Germain N, Silva PMR, Martins MA, Lagente V. Effects of interleukin-10 and modulators of cyclic AMP formation on endotoxin-induced inflammation in rat lung. Fundam Clin Pharmacol 1999; 13: 96–101.
[27] Andonegui G, Goyert SM, Kubes P. Lipopolysaccharide-induced leukocyte-endothelial cell interaction: a role for CD14 versus Toll-Like receptor 4 within microvessels. J Immunol 2002; 169: 2111–9.
[28] Wesselius LJ, Smirnov IM, O'Brien-Ladner AR, Nelson ME. Synergism of intratracheally administered tumor necrosis factor with interleukin-1 in the induction of lung edema in rats. J Lab Clin Med 1995; 125: 618–25.
[29] O'Leary EC, Marder P, Zuckerman SH. Glucocorticoid effects in an endotoxin-induced rat pulmonary inflammation model: differential effects on neutrophil influx, integrin expression, and inflammatory mediators. Am J Respir Cell Mol Biol 1996; 15: 97–106.
[30] Striter RM, Kunkel SL. Acute lung injury: the role of cytokines in the elucitation of neutrophils. J Investig Med 1994; 42: 640–51.
[31] Beck-Schimmer B, Schimmer RC, Warner RL, Schmal H, Nordblom G, Flory CM et al. Expression of lung vascular and airway ICAM-1 after exposure to bacterial lipopolysaccharide. Am J Respir Cell Mol Biol 1997; 17: 344–52.
[32] Beck-Schimmer B, Madjpour C, Kneller S, Ziegler U, Pasch T, Wuthrich RP et al. Role of alveolar epithelial ICAM-1 in lipopolysaccharide-induced lung inflammation. Eur Respir J 2002; 19(6): 1142–50.
[33] Ulich TR, Howard SC, Remick DG, Yi ES, Collins T, Guo K et al. Intratracheal administration of endotoxin and cytokines. VIII. LPS induces E-selectin expression. Anti-E-selectin and soluble E-selectin inhibit acute inflammation. Inflammation 1994; 18: 389– 98.
[34] Kubo H, Doyle NA, Graham L, Bhagwan SD, Quinlan WM, Doerschuk CM. L- and P-selectin and CD11/CD18 in intracapillary neutrophil sequestration in rabbit lungs. Am J Respir Crit Care Med 1999; 159(1): 267–74.
[35] Lee WL, Downey GP. Neutrophil activation and acute lung injury. Curr Opin Crit Care 2001; 7: 1–7.
[36] Takano K, Nakagawa H. Contribuition of cytokine-induced neutrophil chemoattractant CINC-2 and CINC-3 to neutrophil recruitment in lipopolysaccharide-induced inflammation in rats. Inflamm Res 2001; 50(10): 503–8.
[37] Guiliano F, Warner TD. Origins of prostaglandin E2: involvements of cyclooxygenase (COX-1) and COX-2 in human and rat systems. J Pharmacol Exp Ther 2002; 303(3): 1001–6.
[38] Pouliot M, Gilbert C, Borgeat P, Poubelle PE, Bourgoin S, Grassi J et al. Expression and activity of prostaglandin endoperoxide synthase-2 in agonist-activated human neutrophils. FASEB J 1998; 12: 1109–23.
[39] Meja KK, Barnes PJ, Giembycz MA. Characterization of the prostanoid receptor (s) on human blood monocytes at which prostaglandin E_2 inhibits lipopolysaccharide-induced tumor necrosis factor-α. Br J Pharmacol 1997; 122: 149–57.
[40] Moraes VLG, Vargaftig BB, Lefort J, Meager A, Chignard M. Effect of ciclo-oxygenase inhibitors and modulators of cyclic AMP formation on lipopolysaccharide-induced neutrophil infiltration in mouse lung. Br J Pharmacol 1996; 177: 1792–6.