Effects of endothelin ET<sub>A</sub> receptor antagonism on granulocyte and lymphocyte accumulation in LPS-induced inflammation

André L. F. Sampaio,* Giles A. Rae,† and Maria das Gracas M. O. Henriques*‡

*Department of Applied Pharmacology, FarManguinhos, Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil; and †Department of Pharmacology, CCB, Universidade Federal de Santa Catarina, Florianópolis, Brazil

Abstract: Endothelin peptides play active roles in different aspects of inflammation. This study investigates the contribution of endogenous endothelins to lipopolysaccharide (LPS) pulmonary inflammation by assessing the influence of ET<sub>A</sub> receptor antagonism on leukocyte accumulation, granulocyte adhesion molecule expression, and chemokine/cytokine modulation. Local pretreatment with BQ-123 or A-127722 (150 pmol), two selective and chemically unrelated endothelin ET<sub>A</sub> receptor antagonists, inhibits neutrophil and eosinophil accumulation in LPS-induced pleurisy at 24 h but not neutrophil migration at 4 h. The effect of endothelin antagonism on neutrophil accumulation at 24 h was concomitant with inhibition of eosinophil and CD4 and CD8 T lymphocyte influx. It is surprising that the ET<sub>A</sub> receptor blockade did not inhibit the accumulation of γδ T lymphocytes, cells that are important for granulocyte recruitment in this model. Blockade of ET<sub>A</sub> receptors did not influence the expression of adhesion molecules (CD11b, CD49d) on granulocytes but abrogated the increase in tumor necrosis factor α levels 4 h after LPS stimulation and also markedly inhibited increases in levels of interleukin-6 and keratinocyte-derived chemokine/CXC chemokine ligand 1 but not eotaxin/chemokine ligand 11. Thus, acting via ET<sub>A</sub> receptors, endogenous endothelins play an important role in early cytokine/chemokine production and on granulocyte and lymphocyte mobilization in LPS-induced pleurisy. J. Leukoc. Biol. 76: 210–216; 2004.

Key Words: eosinophil · neutrophil · lung inflammation · γδ T cell · cytokine · chemokine

INTRODUCTION

Endothelins are a family of structurally related peptides comprised of endothelin-1, endothelin-2, and endothelin-3, produced by various cell types in response to diverse stimuli. Endothelins are believed to exert important autocrine and paracrine roles in many tissues and systems, mediated through activation of specific ET<sub>A</sub> and ET<sub>B</sub> receptors (for reviews, see refs. [1, 2]). In addition to their widely recognized vascular effects, endothelins also trigger and/or modulate inflammatory reactions, but their roles in these processes are still incompletely understood.

Some evidences suggest a participation of endothelins in allergic inflammation. Endothelin levels are increased in the plasma and bronchoalveolar lavage fluid of sensitized animals challenged with antigen [3, 4]. The antagonism of endothelin ET<sub>A</sub> receptors inhibits eosinophil recruitment in allergic models of lung inflammation [4–6], and the blockade of ET<sub>A</sub>/ET<sub>B</sub> receptors inhibits hyper-responsiveness in mice [7] and antigen-induced, nociceptive responses in mice [8]. In addition, in an allergic pleurisy model, endogenous endothelins participate in lymphocyte accumulation and interleukin (IL)-5 production via ET<sub>A</sub> receptors [5]. Moreover, endothelin-1 stimulation leads to a neutrophil and lymphocyte influx into the pleural cavity, and this phenomena is dependent of ET<sub>A</sub> receptor activation [6].

Endothelin peptides also seem to participate in inflammation triggered by bacterial endotoxins [for review, see ref. [9]]. Thus, elevated levels of endothelins in plasma were described in animals injected with endotoxin [10, 11] and also in patients with septic shock [12, 13]. Increased endothelin plasma levels are also correlated with increased plasma tumor necrosis factor α (TNF-α) levels and severity of sepsis, suggesting a role for these peptides in the pathophysiology of this disorder [14, 15]. Lungs isolated from rabbits stimulated with lipopolysaccharide (LPS) display increased endothelin-1 levels and edema [16]. The fact that lung edema can be prevented by pretreatment with a selective ET<sub>A</sub> receptor antagonist, LU135252, suggests the vascular actions of endothelin-1 are important during pulmonary inflammation induced by LPS. Reinforcing these findings, we have described that treatment with another specific ET<sub>A</sub> receptor antagonist, BQ-123, inhibits the recruitment of neutrophils and eosinophils in LPS lung inflammation [6].

In current study, we attempt to analyze the mechanism(s) underlying the contribution of endogenous endothelin to LPS-induced neutrophil and eosinophil recruitment into the pleural cavity of mice.
MATERIALS AND METHODS

Animals

Male BALB/c mice (20–25 g) from the Fundação Oswaldo Cruz (FIOCRUZ) colony were lodged in a room with controlled temperature and lighting and free access to laboratory chow and tap water.

All experiments were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain [17] and approved by the Institutional Ethics Committee for Animal Care and Use (CEUA/FIOCRUZ License 0050/00).

Induction of pleurisy

Pleurisy was induced in mice as described by Henriques et al. [18]. Briefly, an adapted needle was inserted into the right side of the thoracic cavity to enable intrathoracic (i.t.) administration of BQ-123 (150 pmol/cavity), A-127722 (150 pmol/cavity), or sterile saline. Five minutes after treatment, the animals were similarly injected with Escherichia coli endotoxin (LPS; 250 ng/cavity). Control animals received an equal volume (50 μL/cavity) of pyrogen-free sterile saline (NaCl 0.9%).

Animals were killed by CO2 inhalation, 4 or 24 h after the stimulation, and their thoracic cavities were opened and flushed with 1 mL RPMI 1640 (containing heparin 20 IU/mL for flow cytometry experiments) or phosphate-buffered saline (PBS; containing 1 mg/mL EDTA for chemokine/cytokine determinations). Total leukocyte counts were performed in an automated particle counter (Z1, Coulter, Miami, FL). Differential leukocyte counts were performed on cytopsin smears stained by May-Grünewald Giemsa under light microscopy (100×).

For cytokine determinations, 700 μL of exudate was centrifuged, and cell-free lavage fluid was collected and stored at -70°C until use. For flow cytometric analysis, recovered cells were pooled and processed as described below.

Pleural macrophage culture

Animals were killed by CO2 inhalation, and their thoracic cavities were opened and flushed with 1 mL sterile RPMI 1640 (containing heparin 20 IU/mL). Recovered cells were washed and suspended in RPMI 1640 containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 25 μg/mL gentamicin, 50 μM β-mercaptoethanol, 1 mM pyruvate, and nonessential amino acids. Cells were then plated (2.5×106 cells/well) in a flat-bottom 96-well plate, cultured for 1 h in a controlled atmosphere (CO2 5% at 37°C), nonadherent cells were washed by gentle flushing with RPMI 1640, and adherent macrophages were stimulated for 4 h or 24 h with LPS (30 ng/ml) in the presence or not of BQ-123 (10 μM; ref. [19]), A-127722 (10 nM; ref. [20]), or dexamethasone (50 nM). After stimulation, the plate was centrifuged (1500 rpm, 10 min), and cell-free supernatant was collected and stored at -70°C until use for cytokine/chemokine determinations.

Immunofluorescent staining and flow cytometric analysis

Samples of 106 cells, obtained from pooled, pleural washes (n=10/pool), were suspended and incubated in PBS containing 0.1% sodium azide, 10% rat serum (PBS-S), and Fc receptor for immunoglobulin G (IgG) II block monoclonal antibody (mAb; CD16/CD32) for 30 min at 4°C to avoid unspecific background staining. After the blocking step, cells were labeled with the appropriate concentration of fluorescein isothiocyanate (FITC)-conjugated mAb (CD11b, CD49d), diluted in PBS-S for another 30 min at 4°C. After labeling, cells were washed and suspended for data acquisition in a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). Forward- and side-scatters (FSC and SSC, respectively) were set to exclude erythrocytes and dead cells, and at least 106 cells were analyzed per sample. Granulocytes were gated based on the FSC/GSSC distribution using CellQuest® software (Becton Dickinson). Determination of positive and negative populations was performed based on the control staining with an irrelevant IgG isotype labeled with FITC.

Cytokine measurement

The TNF-α, keratinocyte-derived chemokine (KC)/CXCL1 chemokine ligand 1 (CXCL1), eotaxin/chemokine ligand 11 (CCL11), IL-6, and IL-12 levels in pleural lavage fluid or culture supernatant were measured by capture enzyme-linked immunosorbent assay (ELISA) using antibodies from R&D Systems (Minneapolis, MN). Cytokine levels were determined based on a standard curve, obtained using recombinant cytokine.

Materials

Antibodies were purchased from PharMingen (San Diego, CA) or R&D Systems. BQ-123 [cyclo(DTrp-DAsp-Pro-DVal-Leu)] was purchased from American Peptide Co. (Sunnyvale, CA). FBS (Clone I) was purchased from HyClone (Logan, UT). E. coli LPS (serotype O55:B5) and all other best-grade reagents were purchased from Sigma Chemical Co. (St. Louis, MO). A-127722 (2R*,3R*,4S*)-1-(N,N-dihydroacetamido)-4-(1,3-benzoxazol-5-yl)-2-(4-methoxyphenyl)pyrrolidine-3-carboxylic acid was kindly provided by Dr. Terry J. Oppenorth (Abbott Laboratories, Abbott Park, IL).

Statistical analysis

Results are presented as mean ± SEM and were statistically evaluated by one-way ANOVA followed by the Student’s-Newman-Keuls i-test. The significance level was set at P ≤ 0.05.

RESULTS

Inhibition by BQ-123 and A-127722 of LPS-induced leukocyte infiltration

As shown in Figure 1, i.t. stimulation with LPS leads to pronounced leukocyte recruitment at 24 h (from 2.00±0.14 to 4.34±0.42×106 leukocytes/cavity), which is characterized by a mixed accumulation of neutrophils and eosinophils (Fig. 1, C and D, respectively). Confirming our previous results, we have once again found that treatment with the selective ETα receptor antagonist, BQ-123 (150 pmol/cavity), 5 min before LPS, significantly reduced the infiltration of neutrophils and eosinophils measured 24 h after injection (P<0.05; Fig. 1). Reinforcing these findings, treatment with A-127722 (150 pmol/cavity), a chemically unrelated, nonpeptidic ETα receptor antagonist, also inhibited the 24-h eosinophil and neutrophil accumulation triggered by LPS but failed to change neutrophil accumulation at 4 h (Table 1).

Lymphocyte accumulation triggered by LPS: effects of BQ-123

Flow cytometry analysis of cells, recovered from the pleural cavity of LPS-injected animals, revealed an increase in the T lymphocyte (CD3+) population (P<0.05; Fig. 2A). This T cell accumulation was mainly a result of an increase in CD4+ and CD8+ T lymphocytes (Fig. 2A). LPS stimulation also triggered a significant increase in the number of γδ+ T cells (Fig. 2B).

In situ treatment with BQ-123 completely prevented the accumulation of CD3+ cells induced by LPS (Fig. 2A). Regarding the specific T cell populations, treatment with BQ-123 significantly inhibited the accumulation of CD4+ (P<0.05) and CD8+ T cells (P<0.05; Fig. 2A). However, the ETα receptor antagonist BQ-123 failed to inhibit the increase in γδ+ T cell numbers (from 1.65±0.50 to 1.42±0.098×107/cavity in BQ-123-treated animals; Fig. 2B).

Effect of in vivo BQ-123 treatment on adhesion molecule expression on lymphocytes and granulocytes recruited by LPS

We next analyzed the effect of endothelin ETα receptor blockade on expression of adhesion molecules on granulocytes. As
To understand the effects of endothelin ET<sub>A</sub> receptor antagonism on cytokine/chemokine production, we investigated the effect of specific endothelin ET<sub>A</sub> receptor antagonists BQ-123 and A-127722 on KC/CXCL1 and IL-6 production by pleural macrophages stimulated in vitro with LPS. As illustrated in Figure 3A, analyses of CD11b expression on saline granulocytes revealed a variable degree of CD11b expression in these cells (Fig. 3E); however, the majority of LPS-recruited granulocytes demonstrated a high degree of CD11b expression (Fig. 3F). Prior treatment with BQ-123 failed to influence the change in profile of granulocyte CD11b expression induced by LPS (Fig. 3G). The few granulocytes recovered from saline-injected animals showed a low degree of CD49d expression, discounting the background staining. In LPS-injected animals, despite the higher number granulocytes, the degree of CD49d expression was not increased in these cells relative to that detected in the control group (Fig. 3B). Moreover, in vivo treatment with BQ-123 failed to change the profile of CD49d expression on granulocytes recruited by LPS stimulation (Fig. 3C).

**Effect of ET<sub>A</sub> receptor blockade on cytokine and chemokine production by pleural macrophages**

To understand the effects of endothelin ET<sub>A</sub> receptor antagonism on cytokine/chemokine production, we investigated the effect of specific endothelin ET<sub>A</sub> receptor antagonists BQ-123 and A-127722 on KC/CXCL1 and IL-6 production by pleural macrophages stimulated in vitro with LPS. As illustrated in Figure 4, in vitro stimulation with LPS triggered an important IL-6 and KC/CXCL1 production by pleural macrophages at 4 and 24 h. Treatment in vitro with BQ-123 or A-127722 significantly inhibited IL-6 but not KC/CXCL1 production by pleural macrophages, whereas dexamethasone blocked the production of IL-6 and KC/CXCL1 (Fig. 4).

**Influence of ET<sub>A</sub> receptor blockade on cytokine and chemokine levels in LPS inflammation**

The observation that ET<sub>A</sub> receptor antagonists affect cytokine and chemokine production by pleural macrophages in vitro led us to investigate the possible effect of endothelin blockade on cytokine/chemokine levels in LPS inflammation. We observed that i.t. LPS injection increases TNF-α levels in pleural lavage fluid at 4 h but not at 24 h. This increase in TNF-α levels at 4 h was abolished by prior in situ treatment with BQ-123 (Fig. 5). In accordance with the increase of TNF-α at 4 h, we analyzed the effect of BQ-123 on cytokine and chemokine levels at 4 h. LPS stimulation also induced elevations of KC/CXCL1 and IL-6 levels in the pleural lavage fluid 4 h after injection, which were attenuated substantially by BQ-123 (Fig. 6). However, although LPS also raised eotaxin/CCL11 levels, this effect was not influenced by pretreatment with the ET<sub>A</sub>.

**TABLE 1. Influence of Treatment with the Selective Endothelin ET<sub>A</sub> Receptor Antagonist A-127722 (150 pmol/Cavity, 5 min Beforehand) on Leukocyte Accumulation into the Mouse Pleural Cavity Induced by i.t. Injection of LPS**

<table>
<thead>
<tr>
<th>Time</th>
<th>Cells (×10&lt;sup&gt;6&lt;/sup&gt;/cavity)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total leukocytes</th>
<th>Mononuclear cells</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL + SAL</td>
<td>0.96 ± 0.13</td>
<td>0.94 ± 0.13</td>
<td>0.00 ± 0.00</td>
<td>0.02 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>4 h</td>
<td>LPS + SAL</td>
<td>1.64 ± 0.15*</td>
<td>0.61 ± 0.06</td>
<td>0.97 ± 0.12*</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>LPS + A-127722</td>
<td>1.41 ± 0.27</td>
<td>0.57 ± 0.12</td>
<td>0.73 ± 0.11</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>SAL + A-127722</td>
<td>1.25 ± 0.15</td>
<td>1.24 ± 0.15</td>
<td>0.01 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>24 h</td>
<td>LPS + SAL</td>
<td>2.16 ± 0.18*</td>
<td>1.35 ± 0.14</td>
<td>0.61 ± 0.09*</td>
<td>0.20 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>LPS + A-127722</td>
<td>1.58 ± 0.15</td>
<td>1.16 ± 0.11</td>
<td>0.32 ± 0.05</td>
<td>0.01 ± 0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values were assessed at 4 h or 24 h after stimulation with LPS (250 ng/cavity) and represent the mean ± SEM of at least six animals. * † P ≤ 0.05 when compared with the control, saline (SAL)-injected group or when compared with the LPS-stimulated, saline-treated group, respectively (one-way ANOVA followed by Student’s-Newman-Keuls t-test).
antagonist. Finally, LPS (with or without BQ-123) did not change IL-12 levels in the pleural lavage fluid.

**DISCUSSION**

In this study, we demonstrated the participation of endothelins in granulocyte and lymphocyte accumulation during lung inflammation triggered by LPS by acting through ET₄ receptors coupled mechanisms. Blockade of endothelin ET₄ receptors was also able to modulate the TNF-α, IL-6, and KC/CXCL1 levels in LPS lung inflammation.

Several studies have implicated endothelin peptides with different aspects of inflammatory reactions [1, 9], but there are few existing reports concerning the role of these peptides on leukocyte recruitment. Most of such studies have detected the participation of endothelins in neutrophil, eosinophil, and macrophage accumulation triggered by antigen [4–6, 21]. We also demonstrated that the blockade of endogenous endothelins inhibits not only allergic eosinophilia but also leukocyte accumulation triggered by i.t. LPS stimulation [6]. The eosinophil and neutrophil accumulation induced by i.t. stimulation with LPS is also sensitive to inhibition by bosentan (a dual ETA/ETB receptor antagonist) or by BQ-123 (a selective ETA receptor antagonist), whereas selective blockade of ETB receptors (using BQ-788) is ineffective [6]. Moreover, although LPS challenge alone (or following bosentan injection) fails to increase pleural mononuclear cells numbers, treatment with BQ-123 or BQ-788 before LPS leads to significant increases and decreases in mononuclear cell counts, respectively. The current study extended those findings by showing that another chemically unrelated (nonpeptidic) and highly selective ETA receptor antagonist A-127722 [20] inhibited LPS-induced eosinophil and neutrophil recruitment in the same pattern of BQ-123 and bosentan [6]. Additional evidences for the participation of endothelins on leukocyte mobilization are given by the demonstration that i.t. stimulation with exogenous endothelin-1 leads to a neutrophil recruitment at 4 h with a concomitant influx of CD3⁺ lymphocytes [5].

In the allergic pleurisy model, the inhibition of eosinophil accumulation afforded by BQ-123 treatment seems to be a result of an impairment of CD4⁺ T lymphocyte recruitment. These effects of the ETA receptor antagonist were concomitant with a decrease of IL-5 levels in plasma and pleural wash fluid [6]. The CD4 T cells play a pivotal role in allergic disorders by the secretion of cytokines such as IL-5, which in combination with eotaxin/CCL11, accounts for eosinophil activation and mobilization into tissues and organs [22–25]. Conversely, eosinophil accumulation in LPS-induced pleurisy is highly dependent on γδ T cells and macrophages but not on CD4 or CD8 T lymphocytes [26]. It is interesting that we observed in the current study that inhibition of eosinophilia after BQ-123...
treatment was not accompanied by any inhibition in γδ T cells, although the numbers of CD4 and CD8 T cells were significantly decreased. Moreover, we observed an inhibition of in vitro IL-6 production by LPS-stimulated pleural macrophages after ETA receptor antagonism by BQ-123 or A-127722 in vitro, suggesting an autocrine effect of these peptides in cytokine/chemokine production by these cells. This suggestion is supported by our in vivo findings demonstrating the modula-

Fig. 4. Effects of selective endothelin ET₄ receptor antagonists BQ-123 and A-127722 on KC/CXCL1 and IL-6 production by pleural macrophages after LPS in vitro stimulation. Macrophages were recovered and stimulated with LPS in the presence or not of the ET₄ receptor antagonists or dexamethasone (Dexa), and the supernatant was harvested at 4 or 24 h and processed for ELISA as described in Materials and Methods. *, +, P < 0.05, when compared with medium (open bars) and LPS-stimulated, untreated groups (solid bars), respectively.

Fig. 5. Effects of selective endothelin ET₄ receptor antagonist BQ-123 on TNF-α levels in LPS inflammation. Pleural lavage fluid was harvested from mice and processed for ELISA as described in Materials and Methods. *, +, P < 0.05, when compared with saline-saline (SAL; open bars) and saline-treated, LPS-injected (solid bars) groups, respectively.

Fig. 6. Effects of the selective endothelin ETA receptor antagonist BQ-123 on KC/CXCL1, IL-6, IL-12, and eotaxin/CCL11 levels in LPS inflammation. Pleural lavage fluid was harvested from mice and processed for ELISA as described in Materials and Methods. *, +, P < 0.05, when compared with saline-saline (open bars) and saline-treated, LPS-injected (solid bars) groups, respectively.

lization of cytokine/chemokine production in LPS inflammation after ET₄ receptor antagonism. Several studies [19, 27-30] have demonstrated the modulation of macrophage/monocyte activity by endothelins as well as the capacity of these cells to produce endothelin-1.

The fact that BQ-123 blocked LPS-induced increases in pleural levels of TNF-α and KC/CXCL1 at 4 h could in part account for the granulocyte inhibition triggered by BQ-123 treatment at 24 h. Indeed, it is noteworthy that eosinophil influx in LPS-induced pleurisy occurs via a CCR3-dependent mechanism but is independent of regulated on activated, normal T expressed and secreted and eotaxin/CCL11, and it was also described that eosinophil accumulation in this model is mediated by an unidentified, soluble, heat-stable protein, which seems to be produced by macrophages [31, 32]. In the current study, we observed that BQ-123 failed to affect the
increased eotaxin/CCL11 levels triggered by LPS, suggesting that BQ-123 could prevent the production of this unknown, unidentified, soluble, heat-stable protein. Allied to prior evidence that endothelin-1 stimulates IL-6 production in isolated human monocytes [33], our demonstrations that BQ-123 and A-127722 substantially reduced the production of IL-6 (but not of KC/CXCL1) by LPS-stimulated pleural macrophages in vitro and that the former ET₁ receptor antagonist also inhibited the enhancement of pleural IL-6 levels by LPS add further strength to this view. It is important, however, that dexamethasone enhancement of pleural IL-6 levels by LPS add further strength to this view. It is important, however, that dexamethasone enhanced the expression of CD49d and CD62L or activation markers (CD25 and CD69) on human or murine CD3⁺ cells (CD49d and CD62L) or activation markers (CD25 and CD69) on human or murine CD3⁺ cells (A. L. F. Sampaio and M. G. M. O. Henriques, unpublished observations). Nonetheless, considering that endothelins can up-regulate the expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin in endothelial cells in vitro or ex vivo [34–36], it seems plausible to hypothesize that leukocyte mobilization triggered by LPS might involve ET₁ receptor-mediated changes in adhesion molecule expression in endothelial cells lining the pleural cavity rather than on the leukocytes themselves.

Previous studies have established a relationship between TNF-α and endothelin levels during severe sepsis [14, 15]. However, in a predominantly neutrophilic model of lung inflammation, intratracheal instillation of LPS in rats does not induce synthesis of endothelin-1, although a substantial increase in TNF-α levels and neutrophil numbers is observed, suggesting that endothelin-1 production is not related to neutrophilic airway inflammation induced by LPS [37]. Conversely, our previous study [6] has shown that endothelin peptides participate in leukocyte accumulation in pleural inflammation dominated by eosinophils or involving neutrophils and eosinophils but strongly depends on the time elapsed after stimulation and on the nature of the inflammatory stimulus. Although endothelins do not mediate the acute/early neutrophil accumulation seen during LPS or allergic inflammation, they effectively contribute to LPS-induced neutrophil migration at a time that coincides with eosinophil accumulation and lymphocyte activation [3, 6]. Herein, we reinforced these results and demonstrated that BQ-123 treatment abrogated the raise in KC/CXCL1 and TNF-α levels triggered by LPS at 4 h and inhibited lymphocyte and neutrophil accumulation at 24 h. Moreover, the inhibition of TNF-α and KC/CXCL1 production by BQ-123 pretreatment suggests that the raised levels of these factors at 4 h and acute neutrophilia are dissociated events.

In conclusion, our results demonstrate an important role for endogenous endothelins, mediated via stimulation of ET₁ receptors, on early cytokine/chemokine production and on granulocyte and lymphocyte mobilization during the later stages of LPS-induced pleurisy. If this murine model of pulmonary inflammation bares any predictive value to the clinical situation, the present findings suggest that selective ET₁ receptor antagonists might be useful for the treatment of inflammatory lung diseases in humans.

ACKNOWLEDGMENTS

We thank Márcia V. Carvalho and Claudia Stutz for technical assistance in ELSA cytokine determinations and in vitro assays as well as Dr. Terry J. Opgenorth (Abbott Laboratories) for the kind gift of A-127722.

REFERENCES


Sampaio et al. Endothelins and LPS inflammation 215