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Effects of rolipram on cyclic AMP levels in alveolar macrophages and lipopolysaccharide-induced inflammation in mouse lung

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1 Our previous work demonstrated that bacterial lipopolysaccharide (LPS), administered by aerosol, induced tumour necrosis factor (TNF-α) synthesis leading to the infiltration of neutrophils into mice lungs. The treatment of animals with prostaglandin E_2 or dibutyryl cyclic AMP impaired both processes. In this study, the target cell for LPS and the modulation by cyclic AMP of TNF-α production and neutrophil recruitment were investigated.

2 One hour after inhalation of 2 ml of 0.3 mg ml^{-1} LPS, TNF-α levels measured by an ELISA method increased in the bronchoalveolar lavage fluid (BALF) of BALB/c mice, reaching a maximal level 3 h after inhalation. The immunocytochemistry assay demonstrated that 1 h after inhalation, 21.2% of alveolar macrophages collected in the BALF were immunopositive for TNF-α.

3 When mice were pretreated, i.p., with 20 mg kg^{-1} rolipram, a selective inhibitor of phosphodiesterase type 4, TNF-α levels in the BALF were significantly reduced and only 7.3% of alveolar macrophages were immunopositive for TNF-α.

4 Alveolar macrophages from rolipram-treated mice collected 30 min after inhalation of LPS had a significant increase in the intracellular concentrations of cyclic AMP. This was accompanied by a marked reduction of TNF-α levels in the BALF that were associated with a suppression of TNF-α mRNA expression.

5 Systemic treatment with 20 mg kg^{-1} rolipram almost completely inhibited the LPS-induced neutrophil recruitment, whereas it did not significantly reduce the recruitment induced by rmTNF-α.

6 Our results indicate that alveolar macrophages may be the target cells for both the induction and control of the lung inflammatory response to LPS. They also suggest that systemic treatment with cyclic AMP-elevating agents may be useful to control local inflammation resulting from inhalation of bacterial endotoxin.

Keywords: Lung inflammation; alveolar macrophages; neutrophils; TNF-α; cyclic AMP; LPS; rolipram

Introduction

The host response to the bacterial endotoxin lipopolysaccharide (LPS) is not fully understood in part due to redundant complex cellular reactions triggered after contact with the endotoxin. LPS stimulates macrophages to produce different mediators and particularly tumour necrosis factor-α (TNF-α) which is considered to be a primary inflammatory mediator (Beutler *et al*., 1985; Ulich *et al*., 1991). At the lung level, it has been demonstrated that the release of TNF-α favours the sequestration and migration of neutrophils which play a critical role in the pathogenesis of lung inflammation (Ulich *et al*., 1991; 1993; Denis *et al*., 1994). Using a mouse model to mimic the local inflammation caused by bacterial particles present in the inhaled air, we observed that the inhalation of aerosols of LPS led to a significant production of TNF-α detected in the bronchoalveolar lavage fluid (BALF), followed by the infiltration of neutrophils (Moraes *et al*., 1996). These results are in line with those obtained after aerosolization or intratracheal administration of LPS, by aerosol or intratracheally, in rats or guinea-pigs (Kips *et al*., 1993; Tang *et al*., 1995; Howell *et al*., 1995), showing a neutrophil infiltration into pulmonary spaces probably due to the LPS-induced TNF-α formation.

A number of *in vitro* studies have shown that adenosine 3′:5′-cyclic monophosphate (cyclic AMP) elevating-agents such as prostaglandin E_2 (PGE_2) can also down regulate the LPS-induced TNF-α synthesis at the transcriptional level (Spengler *et al*., 1989; Tannenbaun & Hamilton, 1989; Prabhakar *et al*., 1994; Verghese *et al*., 1995; Zhong *et al*., 1995). In a rat model of lung inflammation (Turner *et al*., 1993) or in mice treated with an intraperitoneal injection of LPS (Pettipher *et al*., 1996), it has been demonstrated that rolipram, a selective inhibitor of phosphodiesterase type 4 (PDE 4), the isoform of the enzyme present in inflammatory cells and in lung tissues, inhibited the TNF-α production. Zardaverine, an inhibitor of PDE 3 and 4 has also been shown to suppress endotoxin-induced airway inflammation in rats (Kips *et al*., 1993). We recently observed that local treatment of mice with dibutyryl cyclic AMP or PGE_2 reduced the TNF-α production and neutrophil recruitment induced by inhalation of aerosols of LPS (Moraes *et al*., 1996). These results indicated that the local inflammation induced by LPS could be modulated by systemic treatment with drugs capable of increasing the intracellular cyclic AMP concentration. The present study was designed to support this assumption, to investigate the target cell at the origin of the lung inflammatory reaction induced by aerosols of LPS and at which stage, between inhalation of LPS and neutrophil recruitment, modulation by cyclic AMP would be playing a role.

The study demonstrates that systemic treatment of mice with rolipram increased the concentration of cyclic AMP within alveolar macrophages (AM), and inhibited the TNF-α mRNA expression in these cells and the alveolar neutrophil infiltration induced by an aerosol of LPS.

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Methods

Animals and experimental protocol

Male BALB/c mice weighing 25–30 g (Iffa-Credo, France) were used throughout this study. Mice were treated with saline (vehicle) or different concentrations of rolipram intraperitoneally (i.p.), 30 or 45 min before inhalation of LPS. For some experiments, animals lightly anaesthetized with ether, received directly into their muzzles 50 μl of a solution containing 0.5 μg of rmTNF-α in 0.2% bovine serum albumin (BSA). Control groups received saline or 0.2% BSA or inactive rmTNF-α obtained by boiling the solution for 1 h. The inhalation procedure has been done as described elsewhere (Moraes *et al*., 1996). Briefly, mice inhaled aerosols of LPS dissolved at 0.3 mg ml^{-1} in 2 ml saline for 10 min. After different time intervals, airspaces were washed with saline to provide 4 ml of bronchoalveolar lavage fluid and aliquots were used for leukocyte analysis and TNF-α assay.

Immunocytochemistry for TNF-α

Cytospin preparations of AM from BALF collected 1 h after inhalation of LPS were fixed in cold acetone for 10 min, and either immediately used for immunostaining or frozen at −20°C. Immunostaining was done by use of the standard alkaline phosphatase anti-alkaline phosphatase antibody complexes (APAAP kit system, Dakopatts, France) method. Briefly, the slides were incubated with rat anti-mouse TNF-α monoclonal antibody for 1 h, washed in Tris buffer at pH 7.6 and incubated with the rabbit anti-rat antibody for 45 min. This was followed by washing and by a third incubation with a rat APAAP complex for 45 min. All incubations were carried out in a moist, light-protected chamber at room temperature. After washing, the reaction was revealed with a substrate composed of naphtol AS-MX phosphate, fast red and levamisole (all from Sigma) in Tris buffer at pH 8.2. The percentage of alveolar macrophages expressing TNF-α (red-coloured cells) was determined by counting 350 to 500 cells with an optical microscope (Carl Zeiss, Germany). We observed that immunopositive AM from rolipram-treated mice were weakly stained as compared with those from non-treated animals. In order to demonstrate this difference, we used the following score: (+) for very weak positive cells and (+++++) for the strongly stained cells.

mRNA for TNF-α

Total RNAs were isolated from 10^5 alveolar macrophages as previously described (Chomczynski & Sacchi, 1987). RT-PCR was performed with specific primers for TNF-α (sense 259-282: CTTGTGGCAGGGGCCACCACGCTC; antisense 782-756: CTCAGCGCTGAGTTGGTCCCCCTTCTC), and for β-actin (sense 368-389: GCCCCTGAGGAG-CACCCTGTG; antisense 989-966: GGTACCACCAGA-CAACACTGTGTT) as control. The cDNAs were synthesized in 25 μl with 5 μg of total RNAs, 0.5 μg oligo (dT 12-18) as a primer, 0.5 u RNAsin (Promega) in the manufacturer's buffer, for 1 h at 42°C. Hot start PCR was performed on Peltier Thermal Cycler type 200 (MJ Research). For a 100 μl reaction, 4 μl of cDNA (pure or ten fold diluted), primers (100 mM each), dNTP (0.2 mM) each and Biotaq polymerase Bioprobe (2.5 u) in the manufacturer's buffer were used. The thermocycling protocol was as follows: 95°C for 3 min, then 30 cycles (actin) or 33 cycles (TNF-α) of: 95°C for 45 s, 62°C for 45 s and 72°C for 45 s, then a final incubation at 72°C for 7 min. Amplification products were resolved on a 1% agarose gel containing 0.5 μg ml^{-1} ethidium bromide, then transferred on a nylon membrane (Hybond N$^+$, Amersham) in 0.4 N NaOH overnight, then hybridized with the corresponding cDNA probes (p*vu*II-P*vu*II fragment for TNF-α, and B*am*HI-A*cc*I fragment for β-actin), labelled by random priming, with [α-^{32}P]-dCTP (3.000 Ci mmol^{-1}, Amersham). Washing was performed twice for 30 min in 2× saline sodium citrate (SSC), twice for 30 min in 0.5×SSC and quantification was achieved on phosphor-imager (Molecular Dynamics), comparing TNF-α and β-actin levels. We ensured that amplifications were performed in the exponential phase by varying dilutions and cycle number (from 24 to 39).

Measurement of cyclic AMP

An aliquot of 360 μl of glacial ethanol was added to 40 μl of a suspension of freshly collected AM (2.5×10^5 cells) from BALF, 1 h after inhalation of LPS. The concentration of cyclic AMP of each sample was determined by the specific enzyme immunoassay method, as described previously (Pradelles *et al*., 1989).

Materials

Escherichia coli lipopolysaccharide (lot 55:B5) was purchased from Difco Lab. (Detroit, MI, U.S.A.); rolipram was a gift from Rhône Poulenc-Rorer (U.K.); sodium pentobarbitone was from Sanofi (Libourne, France). Murine recombinant TNF-α (*E.coli*-derived) was kindly provided by Dr G.R. Adolf from E. Boehringer Institute (Vienna, Austria); for the TNF-α assay the antibodies were from Endogen Inc. (U.S.A.) and for immunocytochemical assay from Dako (France).

Statistical analysis

Results are expressed as means ± s.e.mean. Data were statistically analysed by use of Student's unpaired t test and considered to be significant when $P < 0.05$.

Results

Inhibition of LPS-induced TNF-α and neutrophil infiltration into mice lung by systemic treatment with rolipram

An aerosol of 0.3 mg ml^{-1} LPS induced a time-dependent release of TNF-α in the BALF of BALB/c mice reaching the maximal level 3 h after inhalation. Systemic treatment with 20 mg kg^{-1} rolipram i.p. 45 min before inhalation reduced TNF-α levels detected in the BALF (Figure 1a). Three hours after the aerosol of LPS, 65 to 70% of the cells present in the BALF were neutrophils, expressed as number of cells ml^{-1} (Figure 1b). The data also show that the treatment with 20 mg kg^{-1} rolipram i.p. 45 min before inhalation of LPS led to a reduction of neutrophil infiltration into the alveolar spaces. In preliminary experiments, mice received increasing doses of rolipram, i.p. (5 to 50 mg kg^{-1}), 30 or 45 min before inhalation. The results demonstrated that treatment with 20 mg kg^{-1} rolipram was able to inhibit strongly both TNF-α and neutrophil recruitment. After the injection of rolipram, mice became lethargic during a time period of 15 to 20 min. For these reasons it was decided to work with the dose of 20 mg kg^{-1} administered 45 min before inhalation of LPS.

Effect of rolipram on neutrophil infiltration induced by aerosols of LPS or instillation of rmTNF-α

To investigate at what stage, between the administration of LPS into the airways and the consequent neutrophil infiltration, the modulation of the cyclic AMP level could be effective, mice were pretreated with 20 mg kg^{-1} rolipram i.p. and the inflammation was induced by a direct instillation of 0.5 μg rmTNF-α or by inhalation of 50 μg ml^{-1} LPS. This amount of LPS was used to induce an influx of neutrophils comparable to that induced by TNF-α. As seen in Figure 2a, rolipram blocked almost totally the neutrophil recruitment triggered by LPS, whereas there has no significant reduction when the influx was triggered by rmTNF-α (Figure 2b).

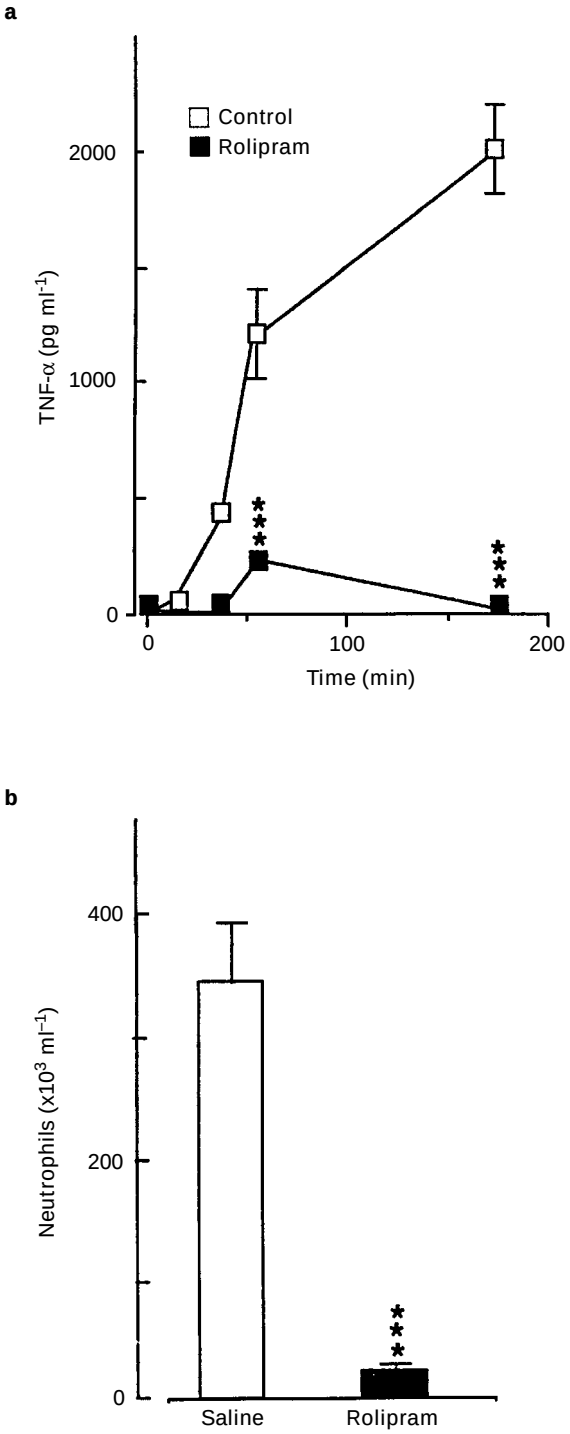

Figure 1 Effects of rolipram on the kinetics of TNF-α production and neutrophil infiltration, 3 h after inhalation of LPS. Each mouse received saline (control) or 20 mg kg^{-1} rolipram i.p. 45 min before inhalation of 0.3 mg ml^{-1} LPS. In (a), BALF were collected at different time intervals after inhalation and TNF-α measured by ELISA method; in (b), BALF were collected 3 h after inhalation and neutrophils counted. Results are expressed as mean ± s.e.mean (vertical lines) of 5–6 animals. ***Significant difference ($P<0.001$) between mice pretreated with rolipram and untreated (control).

Effect of rolipram on LPS-induced intracellular production of TNF-α by alveolar macrophages

To investigate the origin of TNF-α present in the BALF, AM were collected 1 h after inhalation of 0.3 mg ml^{-1} LPS and analysed by immunocytochemistry. Figure 3 shows a summary of the data obtained in 5 different experiments. Following the inhalation of LPS, 21.2 ± 3.4% of AM were strongly immunopositive for TNF-α (+ + + + +/5+), whereas AM from the animals that had inhaled saline were negative. Treatment of mice with 20 mg kg^{-1} rolipram i.p. 45 min before inhalation of LPS reduced by 65% this number. Moreover, the positive AM were less immunopositive (+ +/5+) than those from untreated animals.

Effect of rolipram on cyclic AMP concentrations of alveolar macrophages

The data suggested that the inhibition of neutrophil recruitment by the PDE 4 inhibitor was indeed due to inhibition of the TNF-α synthesis rather than to inhibition of the effect of TNF-α. To confirm this hypothesis, cyclic AMP concentrations were measured in freshly collected AM from mice pretreated with 20 mg kg^{-1} rolipram i.p., 30 min after inhalation of LPS, in parallel with the TNF-α determination in the BALF. As shown in Figure 4a, there was a significant augmentation in the cyclic AMP concentration inside AM from roplipram-treated mice when compared to untreated mice, and with a concomitant decrease in TNF-α levels in the BALF (Figure 4b).

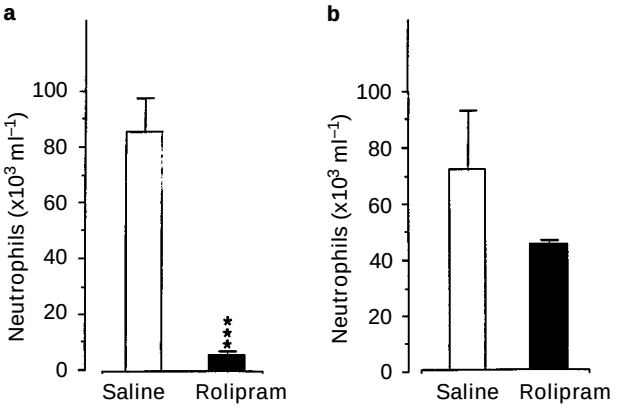

Figure 2 Effect of rolipram on neutrophil recruitment induced by LPS or TNF-α. Mice were pretreated with saline or with 20 mg kg^{-1} rolipram, i.p., 45 min, in (a) before inhalation of 50 μg ml^{-1} LPS, or in (b) before the intra nasal instillation of 0.5 μg rm TNF-α. BALF were collected after 3 h and neutrophils counted. Results are expressed as mean ± s.e.mean of 5–6 animals. ***Significant difference ($P<0.001$) between mice pretreated with rolipram and untreated (saline).

In vivo *effects of rolipram on the LPS-induced expression of TNF-α mRNA in alveolar macrophages*

Figure 5 shows that AM collected 1 h after inhalation of LPS were expressing mRNA for TNF-α, differently from AM obtained from animals that had inhaled saline. The treatment of mice with 20 mg kg^{-1} rolipram, i.p., led to a total suppression of LPS-induced expression of TNF-α mRNA. The same results were obtained with three other groups of mice. Under our experimental conditions, actin mRNA accumulation was not affected by LPS and/or rolipram treatments, demonstrating that these treatments did not specifically affect the global mRNA production.

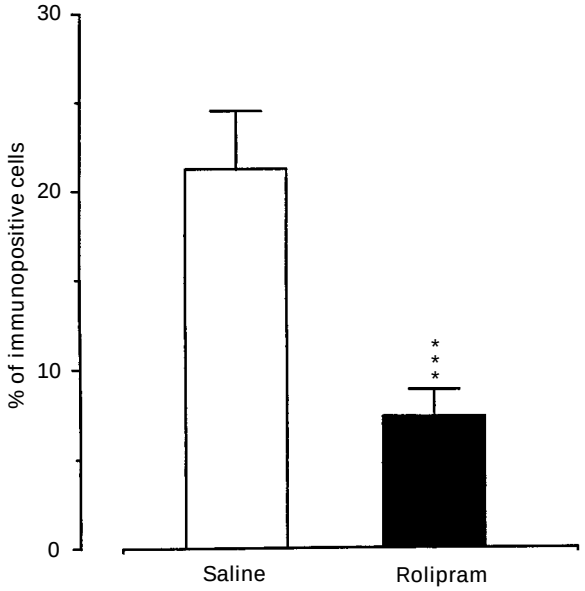

Figure 3 Inhibition by rolipram of LPS-induced TNF-α positive cells in the BALF. Mice were pretreated with saline or 20 mg kg^{-1} rolipram, i.p., 45 min before the inhalation of 0.3 mg ml^{-1} LPS. AM were collected 1 h after inhalation of LPS and used for immunocytochemistry as described under Methods. Results represent the % of immunopositive cells ±s.e.mean. ***Significant difference ($P<0.001$) between pretreated with rolipram and untreated (saline).

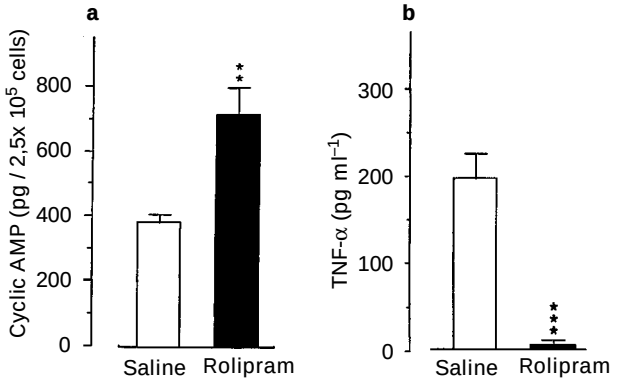

Figure 4 Effect of rolipram on the cyclic AMP content of freshly collected alveolar macrophages and TNF-α production in the BALF. Mice were pretreated with saline or 20 mg kg^{-1} rolipram, i.p., 45 min before the inhalation of 0.3 mg ml^{-1} LPS. In (a), AM freshly collected 30 min after the inhalation were used to assay for cyclic AMP by EIA method and in (b), BALF were used to measure for TNF-α by ELISA method. Results are expressed as mean±s.e.mean of 5–6 animals. ** $P<0.01$, *** $P<0.001$, significance of difference between rolipram pretreated and untreated (saline) mice.

Discussion

This study demonstrates that the local acute inflammation induced by LPS into mice lungs can be blocked by systemic treatment with cyclic AMP-elevating agents. Furthermore, it indicates that this occurs via the suppression of the *in vivo* expression of TNF-α mRNA in alveolar macrophages.

The respiratory tract is continuously exposed to organisms and particles from inhaled air. In the distal part of the airways, AM and neutrophils play a vital role in the neutralization and clearance of particles or microorganisms that have not been retained in the upper respiratory tract. The strategic position of AM at the air-tissue interface suggests that these cells are implicated in the control of the pulmonary responses to injurious processes (for review see Fels & Cohn, 1986; Thepen *et al*., 1989; Sibille & Reynolds, 1990). In our studies, we developed an animal model to mimic the environmental conditions in which the respiratory tract is continuously exposed to aerosols of LPS. This procedure minimizes the toxic effects of LPS administered through a systemic route, particularly on endothelial cells (Meyrick, 1987), and provides a model to study the acute respiratory distress syndrome (ARDS) caused by a direct pulmonary insult.

Previous studies (Moraes *et al*., 1996) indicated that neutrophil infiltration into mice airways after inhalation of aerosols of LPS was dependent on protein synthesis. The systemic treatment of mice with two different anti-TNF-α antibodies reduced by approximately 70% the neutrophil number in the alveolar spaces, indicating that this cytokine was the main factor involved in the neutrophil recruitment triggered by aerosols of LPS. In fact, we now demonstrate that freshly collected AM were strongly immunopositive for TNF-α, with a marked expression of TNF-α mRNA, in the absence of an *in vitro* stimulation. Our data are in agreement with other results based on studies with freshly collected AM from patients with ARDS (Tran van Nhieu *et al*., 1993), macrophages present in the BALF after intratracheal instillations of LPS in rats (Xing *et al*., 1994; Tang *et al*., 1995) or human and murine macrophages cultured *in vitro* and stimulated by LPS (Spengler *et al*., 1989; Taffet *et al*., 1989; Greten *et al*., 1996). Neutrophils also produced TNF-α (Xing *et al*., 1994), but the fact that only a few of them were present in the BALF 1 h after LPS (Moraes *et al*., 1996), suggests a minor contribution of these cells to the total amount of TNF-α.

One of the most important effects of TNF-α is to induce profound changes of the vascular endothelium, as identified in pathologies such as ARDS (Neumann *et al*., 1996). Increased TNF-α concentrations in alveolar fluids and in blood of patients including those with ARDS (Tran van Nhieu *et al*., 1993), leads to the expression of pro-inflammatory cytokines such as interleukin-8 (IL-8; Cassatela *et al*., 1993) or KC (Huang *et al*., 1992) and endothelial adhesion molecules (Godin *et al*., 1993; Albelda *et al*., 1994; Tang *et al*., 1995)

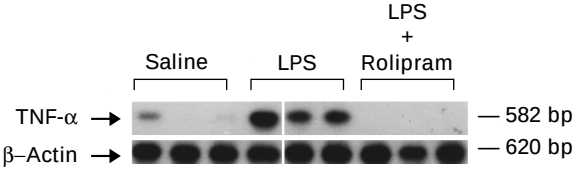

Figure 5 *In vivo* effects of rolipram on the LPS-induced TNF-α mRNA transcripts in alveolar macrophages. Mice were pretreated as in Figure 3 and AM of BALF collected 1 h after the inhalation were analysed to quantify the TNF-α mRNA expression. The data represent one of three experiments.

that may explain the massive neutrophil infiltration in animal models or in man. Therefore, strategies leading to control the TNF-α production by AM might be of value for reducing the neutrophil recruitment during lung inflammation. Previously, we observed that treatment of mice with PGE_2 or dibutytryl cyclic AMP impaired the lung inflammation induced by aerosols of LPS. These cyclic AMP-elevating agents can selectively suppress TNF-α production by activated macrophages, contributing negatively to regulate the inflammatory process triggered by LPS (Kunkel *et al*., 1988; Spengler *et al*., 1989; Tannenbaum & Hamilton, 1989). The effect of cyclic AMP on inflammation has also been investigated through the use of inhibitors of phosphodiesterase 4 (Raeburn *et al*., 1994; Prabhakar *et al*., 1994; Souness *et al*., 1995; Greten *et al*., 1996; Pettipher *et al*., 1996). We found that the systemic treatment of mice with rolipram was effective in increasing the intracellular amount of cyclic AMP within AM with simultaneous suppression of the LPS-induced expression of TNF-α mRNA and neutrophil infiltration. Rolipram was much less effective in inhibiting the neutrophil recruitment directly induced by the rmTNF-α, indicating that the neutrophil infiltration is controlled by modulation of cyclic AMP concentrations at a stage leading to the production of TNF-α. The data obtained with rolipram are in accordance with other results (Tannenbaun & Hamilton, 1989; Howell *et al*., 1995; Pettipher *et al*., 1996) indicating the essential role of cyclic AMP for TNF-α suppression. It is of note that treatment with rolipram inhibited almost completely the LPS-induced neutrophil influx, whereas the treatment with anti-TNF-α antibodies reduced it by 70% (Moraes *et al*., 1996). This difference may be due to an effect of rolipram at different sites, such as on the PDE 4 isoenzymes ubiquitously distributed in the airways, on the expression of endothelial adhesion molecules or on the release of corticosterone (Tanenbaun & Hamilton, 1989; Howell *et al*., 1995; Gozzard *et al*., 1996; Pettipher *et al*., 1996). Although we cannot rule out other effects of rolipram, our data indicate that systemic treatment with cyclic AMP-elevating agents can act by down regulating some essential functions of AM, showing a potential therapeutic advantage to limit inflammation caused by bacterial LPS.

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References

ALBELDA, S.M., SMITH, C.W. & WARD, P.A. (1994). Adhesion molecules and inflammatory injury. *FASEB J.,* **8,** 504 – 512.

BEUTLER, B., MILSAREK, I.W. & CERAMI, A.C. (1985). Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science,* **229,** 869 – 871.

CASSATELLA, M.A., MEDA, L., BONORA, S., CESKA, M. & CONSTANTIN, G. (1993). Interleukin 10 (IL-10) inhibits the release of pro-inflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1 beta in mediating the production of IL-8 triggered by lipopolysaccharide. *J. Exp. Med.,* **178,** 2207 – 2211.

CHOMCZYNSKI, P. & SACCHI, N. (1987). Single-step method of RNA isolation by acid quanidium thiocyanate-phenol-chloroforme extraction. *Anal. Biochem.,* **162,** 156 – 159.

DENIS, M., GUOJIAN, L., WIDMER, M. & CANIN, A. (1994). A mouse model of lung injury induced by microbial products: implications of tumor necrosis factor. *Am. J. Respir. Cell Mol. Biol.,* **10,** 658 – 664.

FELS, A.O.S. & COHN, Z.A. (1986). The alveolar macrophage. *J. Appl. Physiol.,* **60,** 353 – 369.

GODIN, C., CAPRANI, A., DUFAUX, J. & FLAUD, P. (1993). Interactions between neutrophils and endothelial cells. *J. Cell Sci.,* **106,** 441 – 452.

GOZZARD, N., HERD, C.M., BLAKE, S.M., HOLBROOK, M., HUGHES, B., HIGGS, G.A. & PAGE, C.P. (1996). Effects of theophylline and rolipram on antigen-induced airway responses in neonatally immunized rabbits. *Br. J. Pharmacol.,* **117,** 1405 – 1412.

GRETEN, T.F., SINHA, B., HASLBERGER, C., EIGLER, A. & ENDRES, S. (1996). Cicaprost and the type IV phosphodiesterase inhibitor, rolipram synergize in suppression of tumor necrosis factor-α synthesis. *Eur. J. Pharmacol.,* **299,** 229 – 233.

HOWELL, R.E., JENKINS, L.P. & HOWELL, D. (1995). Inhibition of lipopolysaccharide-induced pulmonary edema by isoenzyme-selective phosphodiesterase inhibitors in guinea pigs. *J. Pharmacol. Exp. Ther.,* **275,** 703 – 709.

HUANG, S., PAULAUSKIS, J.D., GODLESKI, J.J. & KOBZIK, L. (1992). Expression of macrophage inflammatory protein-2 and KC mRNA in pulmonary inflammation. *Am. J. Pathol.,* **141,** 981 – 988.

KIPS, J.C., JOOS, G.F., PELEMAN, R.A. & PAUWELS, R.A. (1993). The effect of zardaverine, an inhibitor of phosphodiesterase isoenzymes III and IV, on endotoxin-induced airway changes in rat. *Clin. Exp. Allergy,* **23,** 518 – 523.

KUNKEL, S., SPENGLER, M., MAY, A.M., SPENGLER, R., LARRICK, J. & REMICK, D. (1988). Prostaglandin E_2 regulates macrophage-derived tumor necrosis factor gene expression. *J. Biol. Chem.,* **263,** 5380 – 5384.

MEYRICK, B.O. (1987). Endotoxin-mediated pulmonary endothelial cell injury. *Fed. Proc.,* **45,** 19 – 24.

MORAES, V.L.G., VARGAFTIG, B.B., LEFORT, J., MEAGER, A. & CHIGNARD, M. (1996). Effect of cyclooxygenase inhibitors and modulators of cyclic AMP formation on lipopolysaccharide-induced neutrophil infiltration in mouse lung. *Br. J. Pharmacol.,* **117,** 1792 – 1796.

NEUMANN, B., MACHLEIDT, T., LIFKA, A., PFEFFER, K., VESTWEBER, D., MAK, T.W., HOLZMANN, B. & KRONKE, M. (1996). Crucial role of 55-kilodalton TNF receptor in TNF-induced adhesion molecule expression and leukocyte organ infiltration. *J. Immunol.,* **156,** 1587 – 1593.

PETTIPHER, E.R., LABASI, J.M., SALTER, E.D., STAM, E.J., CHENG, J.B. & GRIFFITHS, R.J. (1996). Regulation of tumor necrosis factor production by adrenal hormones *in vivo*: insights into the anti-inflammatory activity of rolipram. *Br. J. Pharmacol.,* **117,** 1530 – 1534.

PRABHAKAR, U., LIPSHUTZ, D., BARTUS, J.O'L., SLIVJAK, M.J., SMITH III, E.F., LEE, J.C. & ESSER, K.M. (1994). Characterization of cAMP-dependent inhibition of LPS-induced TNF-α production by rolipram, a specific phosphodiesterase IV (PDE IV) inhibitor. *Int. J. Immunol.,* **16,** 805 – 816.

PRADELLES, P., GRASSI, J., CHABARDES, D. & GUISO, N. (1989). Enzyme immunoassays of adenosine cyclic 3′ 5′-monophosphate using acetylcholinesterase. *Anal. Chem.,* **61,** 447 – 453.

RAEBURN, D., UNDERWOOD, S.L., LEWIS, S.A., WOODMAN, V.A., BATTRAM, C.H., TOMKINSON, A., SHARMA, S., JORDAN, R., SOUNESS, J.E., WEBBER, S.E. & KARLSSON, J.A. (1994). Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor. *Br. J. Pharmacol.,* **113,** 1423 – 1431.

SIBILLE, Y. & REYNOLDS, H.Y. (1990). State of Art. Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am. Rev. J. Respir. Dis.,* **141,** 471 – 501.

SOUNESS, J.E., MASLEN, C., WEBBER, S., FOSTER, M., RAEBURN, D., PALFREYMAN, M.N., ASHTON, M.J. & KARLSSON, J.A. (1995). Suppression of eosinophil function by RP 73401, a potent and selective inhibitor of cyclic AMP-specific phosphodiesterase: comparison with rolipram. *Br. J. Pharmacol.,* **115,** 39 – 46.

SPENGLER, R.N., SPENGLER, M.L., LINCOLN, P., REMICK, D.G., STRIETER, R.M. & KUNKEL, S.L. (1989). Dynamics of dibutyryl cyclic AMP- and prostaglandin E_2-mediated suppression of lipopolysaccharide-induced tumor necrosis factor alpha gene expression. *Infect. Immun.,* **57,** 2837–2841.

TAFFET, S.M., SINGHEL, K.J., OVERHOLTZER, J.F. & SHURTLEFF, S.A. (1989). Regulation of tumor necrosis factor expression in a macrophage like cell line by lipopolysaccharide and cyclic AMP. *Cell. Immunol.,* **120,** 291–300.

TANG, W.W., YI, E.S., REMICK, D.G., WITTWER, A., YIN, S., QI, M. & ULICH, T.R. (1995). Intratracheal injection of endotoxin and cytokines. IX. Contribution of CD11a/ICAM-1 to neutrophil emigration. *Am. J. Physiol.,* **269,** L653–659.

TANNENBAUM, C.S. & HAMILTON, T.A. (1989). Lipopolysaccharide-induced gene expression in murine peritoneal macrophages is selectively suppressed by agents that elevate intracellular cAMP. *J. Immunol.,* **142,** 1274–1280.

THEPEN, T., N. VAN ROOIJEN & KRAAL, G. (1989). Alveolar macrophage elimination *in vivo* is associated with an increase in pulmonary immune response in mice. *J. Exp. Med.,* **170,** 499–509.

TRAN VAN NHIEU, J., MISSET, B., LEBARGY, F., CARLET, J. & BERNAUDIN, J.F. (1993). Expression of tumor necrosis factor-alpha gene in alveolar macrophages from patients with the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.,* **147,** 1585–1589.

TURNER, C., ESSER, K.M. & WHEELDON, E.B. (1993). Therapeutic intervention in a rat model of ARDS: IV. Phosphodiesterase IV inhibition. *Cir. Shock,* **39,** 237–245.

ULICH, T.R., WATSON, L.R., YIN, S., GUO, K. & DEL CASTILLO, J. (1991). The intratracheal administration of endotoxin and cytokines. I: characterization of LPS-induced TNF and IL-1 mRNA expression and the LPS-, TNF-, and IL-1-induced inflammatory infiltrate. *Am. J. Pathol.,* **138,** 1485–1496.

ULICH, T.R., YIN, S., REMICK, D.G., RUSSELL, D., EISENBERG, S.P. & KOHNO, T. (1993). Intratracheal administration of endotoxin and cytokines. IV. The soluble TNF receptor type 1 inhibits acute inflammation. *Am. J. Pathol.,* **142,** 1335–1338.

VERGHESE, M.W., MCCONNELL, R.T., STRICKLAND, A.B., GOODING, R.C., STIMPSON, S.A., YARNALL, D.P., TAYLOR, D. & FURDON, P.J. (1995). Differential regulation of human monocyte-derived TNF-α and IL-1β by type IV cAMP-phosphodiesterase (cAMP-PDE) inhibitors. *J. Pharmacol. Exp. Ther.,* **272,** 1313–1320.

XING, Z., JORDANA, M., KIRPALANI, H., DRISCOLL, K.E., SCHALL, T.J. & GAULDIE, J. (1994). Cytokine expression by neutrophils and macrophages in vivo: endotoxin induces tumor necrosis factor-α, macrophage inflammatory protein-2, interleukin-1β and interleukin-6 but not RANTES or transforming growth factor-B1 mRNA expression in acute lung inflammation. *Am. J. Respir. Cell Mol. Biol.,* **10,** 148–153.

ZHONG, W.W., BURKE, P.A., DROTAR, M.E., CHAVALI, S.R. & FORSE, R.A. (1995). Effects of prostaglandin E_2, cholera toxin and 8-bromo-cyclic AMP on lipopolysaccaride-induce gene expression of cytokines in human macrophages. *Immunology,* **84,** 446–452.

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