Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 498 (2004) 279–286

www.elsevier.com/locate/ejphar

Inhibition of interleukin-1β reduces mouse lung inflammation induced by exposure to cigarette smoke

Paulo Castro[a], Alexandre Legora-Machado[a], Larissa Cardilo-Reis[a], Samuel Valença[b], Luis Cristóvão Porto[b], Christoph Walker[c], Claudia Zuany-Amorim[d], Vera Lucia Gonçalves Koatz[a,*]

[a]*Departamento de Bioquímica Médica, ICB, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 21941-590, Brazil*
[b]*Depto de Embriologia e Histologia, UERJ, Rio de Janeiro, Brazil*
[c]*Novartis Research Centre, Horsham, England, United Kingdom*
[d]*Aventis Pharma, Vitry-sur-Seine, France*

Received in revised form 1 July 2004; accepted 6 July 2004
Available online 20 August 2004

Abstract

We examined nuclear factor κB activation, release of inflammatory mediators and cellular infiltration in acute cigarette smoke inflammation models. One hour after exposure to one puff of cigarette smoke, alveolar macrophages from bronchoalveolar lavage (BAL) fluid of C57BL/6J mice showed an increased activity of nuclear factor κB-DNA binding but similar numbers as compared to that of BAL fluid from mice exposed to ambient air. Exposure to 1 cigarette/day for 1, 4 or 7 days led to an increase in interleukin-1β and monocyte chemoattractant protein-1 levels and to a progressive influx of nuclear factor κB-activated alveolar macrophages into the BAL fluid and lung tissue. Exposure to 2 cigarettes/day for 7 days led to a significant increase in interleukin-1β levels accompanied by a massive alveolar macrophage influx into the BAL fluid. Tumor necrosis factor-α levels and subsequent neutrophil influx were only detected after exposure to 4 or 8 cigarettes/day for 7 days. Treatment of mice with an antibody anti-interleukin-1β during cigarette smoke exposure for 7 days significantly reduced both interleukin-1β levels and alveolar macrophage influx. These data show that a single exposure to cigarette smoke rapidly activates alveolar macrophages, inducing the production of interleukin-1β, which may play an important role in triggering chronic cigarette smoke-mediated lung inflammation.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Cigarette smoke; Lung, mouse; Inflammation

1. Introduction

The reduced capacity of people work during their productive life has transformed cigarette smoking into a worldwide public health problem. Cigarette smoke-related disorders are closely associated with vascular alterations in coronary arteries, suppression of gastric wound repair and reduction of lung respiratory capacity (Shah and Helfant, 1988; Sherman, 1991; Shin et al., 2002; Gajalakshmi et al., 2003).

Cigarette smoke is a complex mixture of over 4700 components (Rahman and MacNee, 1996) and contains high concentrations of active oxygen species in the gas phase and tar (Pryor et al., 1983; Repine et al., 1997). Free radicals activate the transcription of nuclear factor κB, which in turn leads to the expression of many genes encoding mediators of the inflammatory process, such as interleukins and chemokines (Baldwin, 1996; Blackwell and Christman, 1997). Analysis of samples from cigarette smokers showed that peripheral blood monocytes had high nuclear factor κB activation (Van den Berg et al., 2001) and altered levels of tumor necrosis factor-α, interleukins 1β, 6 and 8 in bronchoalveolar lavage (BAL) fluids (McCrea et al., 1994; Ryder et al., 2002). Cigarette smoke has been correlated with an increased susceptibility to respiratory infections, the mechanisms of which are not completely understood. The

* Corresponding author. Tel.: +55 21 2562 6758; fax: +55 21 2270 8647.
E-mail address: koatz@bioqmed.ufrj.br (V.L.G. Koatz).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.07.047

strategic position of alveolar macrophages in the air-tissue interface indicates that these cells are involved in the control of the pulmonary responses to injurious processes (Sibille and Reynolds, 1990; Goncalves-de-Moraes et al., 1998). Analysis of alveolar macrophages from cigarette smokers demonstrated a number of morphological and functional alterations (Harris et al., 1970; Hoidal and Niewoehner, 1982), suggesting that the continual exposure to cigarette smoke may stimulate these cells to work abnormally, contributing to the development of pulmonary chronic diseases. Once activated, alveolar macrophages can release different inflammatory mediators into the lung environment, such as interleukin-1β (Friedlander et al., 1994). This cytokine is a potent inflammatory mediator, stimulating chemokine production, recruiting leukocytes to the site of injury and inducing the synthesis of tumor necrosis factor-α and interleukin-6 (Barnes and Page, 1998; Hashimoto et al., 2000). Interleukin-1β up-regulates metalloproteinases (Sasaki et al., 2000) and fibroblast proliferation (Dinarello et al., 1989; Raines et al., 1989), features that are closely associated with the chronic inflammation and structural changes observed in the lungs of patients (Sasaki et al., 2000).

Chronic lung inflammation may be triggered by the first exposure to cigarette smoke. To test this hypothesis, we examined the effect of a short-term exposure to cigarette smoke on nuclear factor κB-DNA binding, production of mediators and cellular infiltration into the lungs. Furthermore, we investigated the effect of treatment with a neutralizing antibody anti-interleukin-1β on the cigarette smoke-induced lung inflammation in mice.

2. Material and methods

2.1. Animals

C57BL/6J or DBA-2 male mice (20–25 g; FIOCRUZ, Rio de Janeiro, Brazil) were put in the smoking chamber, consisting of conic tubes attached to a glass chamber of 1 l. The tubes are opened to allow the muzzles to be in contact with the interior of the chamber. Through a tube adapted to the upper part of the chamber, a volume of 100 ml of cigarette smoke corresponding to one puff is drawn from a commercial filtered cigarette with a syringe and injected into the chamber. Mice were exposed for 60 s to cigarette smoke and then the chamber was opened to permit exchange with ambient air, for 60 s. This procedure was repeated according to the cigarette smoke exposure regimen used. All the procedures involving animals were done in accordance with international guidelines.

2.2. Cigarette smoke exposure

In a set of experiments, groups of seven mice were exposed to one puff of cigarette smoke and BAL fluid was collected 1 h after the exposure. Control mice were exposed to ambient air. In another set of experiments, mice were exposed to the smoke from one, two, four or eight cigarettes daily, receiving puffs in the morning and in the afternoon for 1, 4 or 7 days. Twenty-four hours after the last cigarette smoke exposure, BAL fluid was collected.

2.3. BAL fluid

At the indicated time points, animals were terminally anesthetized with pentobarbital sodium (60 mg/kg i.p.). Tracheas were cannulated and BAL fluid was obtained by injecting buffered saline (PBS) three consecutive times to a final volume of 1.5 ml. The fluid was withdrawn and stored on ice. Total cell number was determined in a Zi Coulter (Beckman Coulter, USA). Differential cell counts were performed on cytospin preparations (Shandon, USA) stained with Diff-Quik (Baxter Dade, Dudingen, Switzerland). At least 200 cells/BAL fluid were counted, using standard morphological criteria. Results are expressed as number of cell populations per milliliter. The remaining BAL fluid was centrifuged (400×*g* for 10 min) and the supernatant was collected and stored at −20 °C for interleukin-1β, tumor necrosis factor-α and monocyte chemoattractant protein-1 assays. For the nuclear factor κB assay, alveolar macrophages were separated and nuclear extracts were prepared immediately.

2.4. Quantification of lung tissue macrophages

For histopathological studies of lung tissue, mice were exposed to 1 cigarette/day or to ambient air for 1, 4 and 7 days. Twenty-four hours after the last exposure, mice were anesthetized with pentobarbital sodium (60 mg/kg i.p.) and exsanguinated. Lungs were distented with 10% phosphate-buffered formalin (pH 7.4) at a constant pressure of 2.45 kPa. Both the main bronchi and the parenchyma of the right caudal lobe were dissected out and embedded in paraffin for 24 h. Sections (5 μm thick) were cut from each sample. Histopathological assessment by light microscopy was performed in a blind fashion using randomized sections. Macrophages were counted in Giemsa-stained sections, in 30 fields of 26,000 μm^2 (10 random fields in 3 different sections-30 μm apart) in each lung, under 40× magnification, using an Olympus BH-2 microscope equipped with an eyepiece in a graticule.

2.5. Electrophoretic mobility shift assay

Nuclear extracts from alveolar macrophages and the electrophoretic mobility shift assay were performed as described by Munoz et al. (1994). As exposure to smoke from one or two cigarettes yielded to 97–100% of alveolar macrophages in BAL fluid, we prepared nuclear extracts without any further purification (i.e., separation from neutrophils). Nuclear extracts from culture cells 70Z/3

stimulated with 15 μg/ml lipopolysaccharide (*E. coli* serotype 055:B5—Sigma, Chemical Co. USA) for 1 h were used as a positive control (Sen and Baltimore, 1986). The binding reaction between nuclear factor κB consensus sequence 5′ AGTTTGATGAGTCAGCCG 3′ and 3′ CGGCTGACTCAT-CAAACT 5′ with nuclear protein (4 μg) was performed in a final volume of 10 μl in 8 mM HEPES, 10% glycerol, 20 mM KCl, 4 mM $MgCl_2$, 1.0 μg polydl-dC (pH 7.0). The oligonucleotides (DNAgency, NY, USA) were 5′-end labeled with T4 polynucleotide kinase and [γ-^{32}P]dATP (>5000 Ci/mmol; Amersham International Biosciences, England) and 50,000 cpm of relevant double-stranded oligonucleotides were used per reaction. Binding was allowed to proceed for 30 min at room temperature. The complexes were separated by gel eletrophoresis on a 6% polyacrylamide gel with 0.5× Tris buffer pH 7.0 containing borate and EDTA (TBE) at 180 V for 2 h at room temperature. Specificity was determined by addition of 50-fold excess unlabeled oligonucleotide. The gels were then dried and quantification was achieved on phosphorimager (Molecular Dynamics).

2.6. Cytokines

All of the cytokines in supernatant from BAL fluid were quantified with a specific enzyme-linked immunosorbent assay (ELISA), using rat anti-mouse monoclonal and polyclonal antibodies, with a detection limit of 10 pg/ml, according to manufacturer's instructions (R&D Systems, UK). Mouse recombinant cytokine standards were used in every assay.

2.7. Anti-interleukin-1β treatment

Mice were exposed to ambient air or to 1 cigarette/day for 7 days, as described above. On days 0, 4 and 7, animals received intravenously (i.v.) 5 or 20 μg IgG goat anti-mouse interleukin-1β antibody, or its matched isotype control (R&D Systems, UK) or saline. BAL fluid was collected 24 h after the last exposure and used for cellular analysis and cytokine determination.

2.8. Statistical analysis

Comparisons were made by analysis of variance (ANOVA). Post-hoc tests, Dunnett's T3 and Tukey, were also used to identify differences between values. Results are expressed as means±S.E.M. and values of $P<0.05$ were considered statistically significant.

3. Results

3.1. Cigarette smoke-induced cellular infiltration

In a first set of experiments, the ability of cigarette smoke to induce lung inflammation was investigated in C57Bl/6J mice, after 1 or 7 days of exposure. As shown in Fig. 1A, exposure to cigarette smoke from 1 cigarette/day induced a time-dependent influx of alveolar macrophages into the BAL fluid of C57Bl/6J mice when compared with that of control animals. The number of alveolar macrophages was elevated in BAL fluid already 1 day after exposure to cigarette smoke. Even higher numbers of alveolar macrophages were found after 7 days of cigarette smoke exposure. The number of alveolar macrophages in control mice was similar after 1 or 7 days of exposure to ambient air and for this reason the value obtained after 7 days was used as control. Similar to the finding in BAL fluid, a significant increase in the number of alveolar macrophages was also found in the pulmonary tissue from C57Bl/6J mice, 7 days after cigarette smoke exposure (Fig. 1B). The effect of cigarette smoke in another mouse strain (DBA-2) was also investigated. In this case, however, exposure to smoke from one cigarette for 7, 30 or 60 days did not change the number of alveolar macrophages in BAL fluid compared with those of mice exposed to ambient air (data not shown). With respect to the neutrophil accumulation, our data showed no significant increase in the number of neutrophils in BAL fluid or lung following exposure to cigarette smoke over the entire duration of the experiment. To investigate if the absence of neutrophils was due to the amount of cigarette smoke used, C57Bl/6J mice were exposed daily to 1, 2, 4 or 8 cigarettes/day, for 7 days. As shown in Fig. 2, exposure to 2 cigarettes/day led to a massive influx of macrophages into the lungs, detected in the BAL fluid that was accompanied by a discrete increase in lymphocyte num-

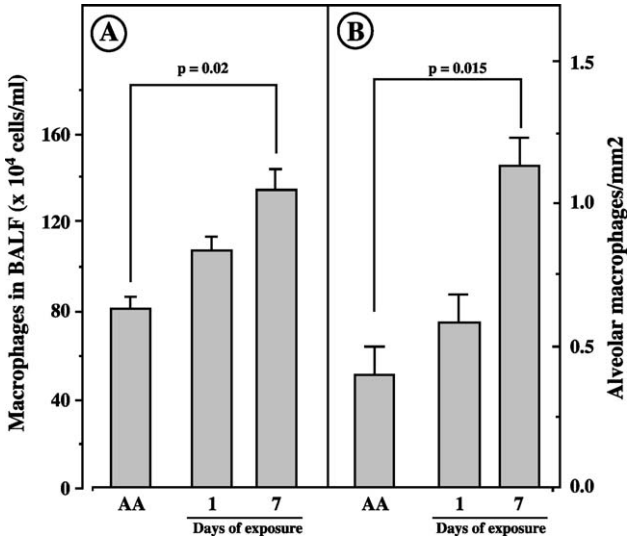

Fig. 1. Alveolar macrophage recruitment into the BAL fluid and lung tissue after cigarette smoke exposure-C57Bl/6J mice (n=7) were daily exposed to ambient air (AA) or to smoke from one commercial filtered cigarette for 1 or 7 consecutive days. (A) BAL fluids were collected 24 h after the last exposure; (B) lungs of C57Bl/6J were removed 24 h after the last exposure to ambient air or cigarette smoke, and alveolar macrophages were counted as described in Section 2. Control values were taken from the group exposed to ambient air for 7 days. Data are expressed as means±S.E.M.

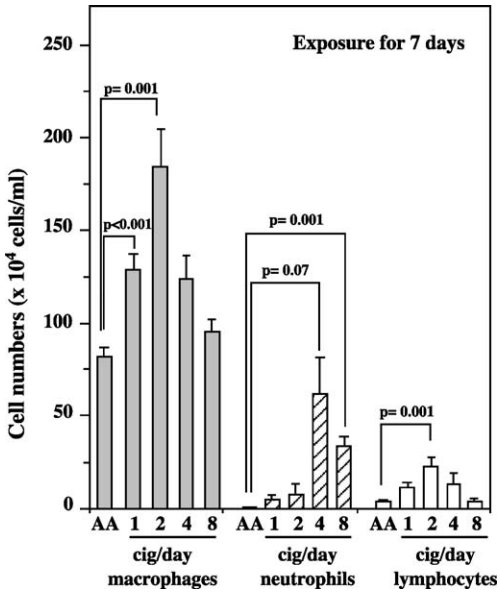

Fig. 2. Cellular influx into the BAL fluid of C57BI/6J mice after exposure to increasing amounts of cigarette smoke—groups of seven C57BI/6J mice were daily exposed to ambient air (AA) or to smoke from one, two, four or eight cigarettes for 7 consecutive days. BAL fluids were collected 24 h after the last exposure. At least 200 cells/BAL fluid were counted, using standard morphological criteria. The number of each cell population per milliliter is expressed as means±S.E.M.

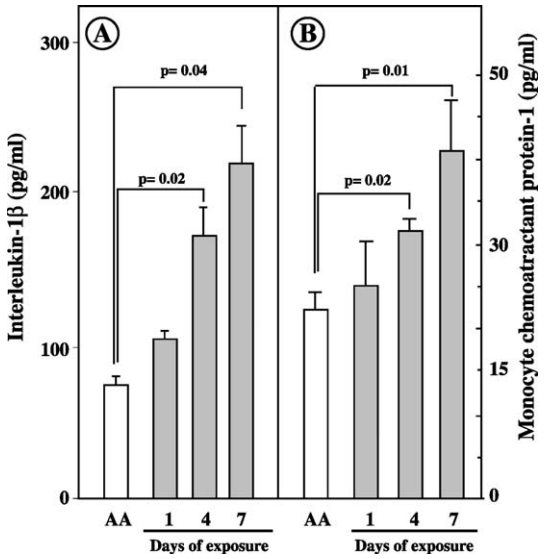

Fig. 4. Increased levels of interleukin-1β and monocyte chemoattractant protein-1 levels in the protein-1 in the BAL fluid of C57BI/6J after cigarette smoke exposure-C57BI/6J mice (n=7) were daily exposed to ambient air (AA) or to smoke from one commercial filtered cigarette. Interleukin-1β (A) and monocyte chemoattractant protein-1 (B) were measured by specific ELISA assay as described in Section 2. Control values were taken from the group exposed to ambient air for 7 days. Data are expressed as means±S.E.M.

bers. Neutrophils were largely recruited after exposure to 4 or 8 cigarettes/day.

3.2. Cigarette smoke-induced nuclear factor κB-DNA binding activity

To investigate if cigarette smoke could activate alveolar macrophages after the first exposure, mice were exposed to one puff of cigarette smoke and BAL fluid was analyzed 1 h after exposure. Similar numbers of alveolar macrophages were found in cigarette smoke-exposed mice and control animals; however, the nuclear factor κB mobility shift assay on nuclear extracts clearly showed that a single exposure to cigarette smoke was enough to activate nuclear factor κB-DNA binding in alveolar macrophages (Fig. 3). A similar activation of nuclear factor κB was found in infiltrating alveolar macrophages obtained 24 h after exposure to 1 and 2 cigarettes/day over 7 days.

3.3. Cigarette smoke-induced production of inflammatory mediators

The observed cigarette smoke-induced activation of nuclear factor κB could lead to the release of inflammatory mediators, which in turn could induce the influx of alveolar macrophages into pulmonary tissue. The alveolar macrophage-derived cytokines, interleukin-1β and tumor necrosis factor-α, and the chemokine monocyte chemoattractant protein-1 were analyzed in the BAL fluid of mice exposed to 1 cigarette/day for 1, 4 or 7 days. As shown in Fig. 4, a time-dependent and significant increase in the levels of both interleukin-1β and monocyte chemoattractant protein-1 was

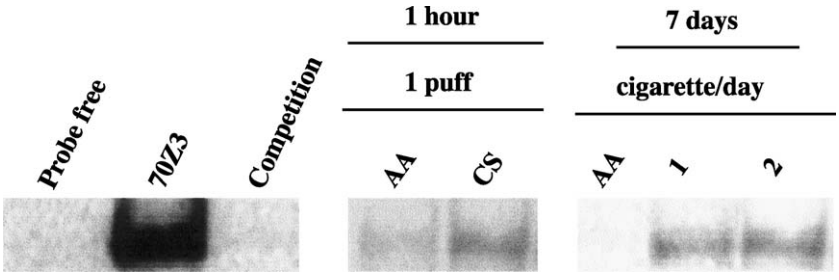

Fig. 3. Electrophoretic mobility shift assay showing the effect of cigarette smoke on nuclear factor κB activation in alveolar macrophages from C57BI/6J mice-Figure shows: on the left side the negative (nuclear extract-free nuclear factor κB probe without protein extracts and after cold competition) and the positive control with nuclear factor κB-DNA binding from 70Z/3 cells after incubation with 15 μg/ml lipopolysaccharide for 1 h (see Section 2); in the middle, nuclear factor κB DNA-binding in nuclear extracts of alveolar macrophages 1 h after a single exposure to one puff of cigarette smoke and, on the right side, binding after exposure to 1 or 2 cigarettes/day over 7 days. The results are representative of two independent experiments.

found in cigarette smoke-exposed animals compared to mice exposed to ambient air. Control values were similar after 1, 4 or 7 days of exposure to ambient air and for this reason values from 7 days were used as control. The observation that tumor necrosis factor-α was not detectable after exposure to 1 cigarette/day led us to investigate this cytokine in the BAL fluid of mice exposed to higher amounts of cigarette smoke. Fig. 5 shows that levels of tumor necrosis factor-α increased when mice were exposed to smoke from four or eight cigarettes, concomitant with the influx of neutrophils into the BAL fluid (Fig. 2).

3.4. Inhibition of alveolar macrophage influx and interleukin-1β production by treatment with anti-interleukin-1β antibody

To further investigate the potential role of interleukin-1β in regulating the influx of alveolar macrophages, cigarette smoke-exposed mice were treated with 5 or 20 μg immunoglobulin G goat anti-mouse interleukin-1β antibodies on days 0, 4 and 7. Control groups received isotonic saline or 20 μg isotype control antibody. As shown in Fig. 6, the exposure of mice to 1 cigarette/day over 7 days induced significant increases in alveolar macrophage numbers and interleukin-1β levels in the BAL fluid that were significantly blocked by treatment with anti-interleukin-1β antibody. Analysis of lung homogenates also showed a

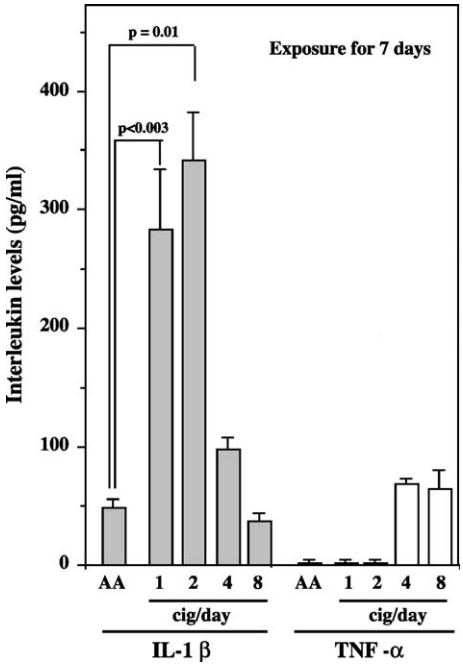

Fig. 5. Dose-dependent levels of interleukin-1β and tumor necrosis factor-α in the BAL fluid of C57Bl/6J after exposure to increasing amounts of cigarette smoke—groups of seven C57Bl/6J mice were daily exposed to ambient air (AA) or to smoke from 1, 2, 4 or 8 cigarettes/day for 7 consecutive days. BAL fluids were collected 24 h after the last exposure. Interleukin-1β (IL-1β—□) and tumor necrosis factor-α (TNF-α—□) were measured by specific ELISA assay as described in Section 2. Data are expressed as means±S.E.M.

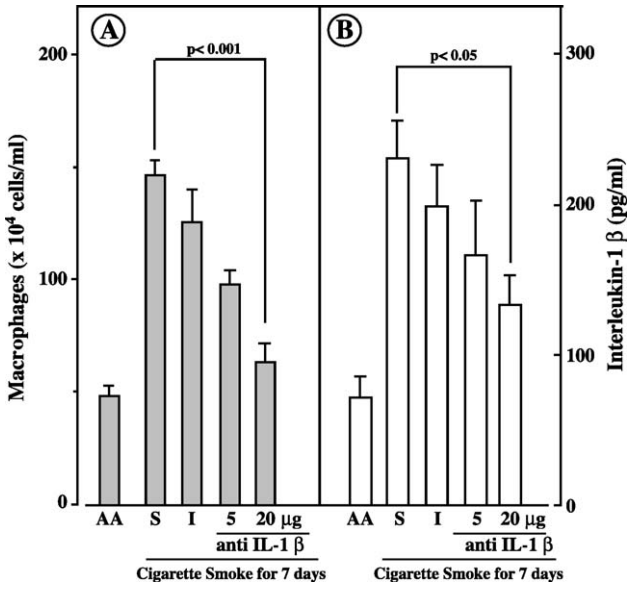

Fig. 6. Anti-interleukin-1β treatment reduces alveolar macrophage recruitment and interleukin-1β production induced by cigarette smoke exposure. C57Bl/6J mice (n=7) were daily exposed to ambient air (AA) or to smoke from one commercial filtered cigarette for 7 consecutive days. On days 0, 4 and 7, animals received i.v. 5 or 20 μg IgG goat anti-mouse interleukin-1β antibody, or 20 μg of its matched isotype control (I) or saline (S). BAL fluid was collected 24 h after the last exposure and used for cell analysis and cytokine determination. Alveolar macrophages (A) and interleukin-1β (IL-1β) levels (B) were determined as described in Section 2. Control values were taken from the group exposed to ambient air for 7 days. Data are expressed as means±S.E.M.

reduction in interleukin-1β levels from 225.6±22.1 pg/ml in mice exposed to cigarette smoke (saline- or isotype-treated) to 146.5±16.7 and 110.4±14 pg/ml after treatment with 5 and 20 μg anti-interleukin-1β antibody, respectively.

4. Discussion

In this study, we have shown that murine alveolar macrophages are rapidly activated following the first contact with cigarette smoke and that the inflammatory reaction triggered by cigarette smoke is dependent on interleukin-1β synthesis.

The data presented in this study show that C57Bl/6J mice reacted to small amounts of cigarette smoke soon after exposure. The susceptibility to inflammatory agents is associated with the cytokine profile produced in different mouse strains, as well as the sensitivity to oxidants and to anti-elastase activity (Gardi et al., 1994; Morokata et al., 1999; Leikauf et al., 2002). This could explain the different chronic inflammatory responses seen in DBA-2 and C57Bl/6J mice. However, DBA-2 and C57Bl/6J mice develop a similar pattern of lung alterations after 7 months of exposure to 3 cigarettes/day (Cavarra et al., 2001), suggesting that the induction of airway inflammation and the development of emphysema in different mouse strains may be time- and cigarette smoke-exposure dependent. Different responses to

cigarette smoke are also found among smokers (Gajalakshmi et al., 2003), and there is a clear relationship between the smoking habit and mortality due to respiratory diseases. However, why only 18–20% of smokers develop emphysema, which leads to chronic obstructive pulmonary disease (Sherman, 1991; Repine et al., 1997), remains to be elucidated.

Multiple studies indicate that repeated exposure to cigarette smoke may induce prolonged airway inflammation associated with cellular infiltration of macrophages (Harris et al., 1970; McCrea et al., 1994; Hautamaki et al., 1997; Ofulue et al., 1998; Ofulue and Ko, 1999), neutrophils (Dahmi et al., 2000; Churg et al., 2002) or eosinophils (Matsumoto et al., 1998). However, until now, the effector cell and the mechanism responsible for the inflammation have not been completely elucidated.

The dose-dependent massive influx of alveolar macrophages observed in our study could therefore be an essential trigger to the inflammatory process in smokers, and macrophage influx has indeed been correlated to the development of emphysema in animal models (Hautamaki et al., 1997; Ofulue et al., 1998; Ofulue and Ko, 1999). We were thus very interested to investigate whether a single exposure to cigarette smoke led to activation of alveolar macrophages with a subsequent release of pro-inflammatory cytokines. In this respect, activation of nuclear factor κB, a pivotal transcription factor in chronic inflammatory diseases (Barnes and Karin, 1997), is known to be intimately involved in the expression of different inflammatory mediators (Baldwin, 1996; Blackwell and Christman, 1997) associated with the induction of inflammatory responses in the airways. By using a specific DNA probe, we could indeed demonstrate a rapid activation of nuclear factor κB in macrophages 1 h after a single exposure to cigarette smoke, which was still detected after 7 days of daily exposure to increasing amounts of cigarette smoke. Similar results were obtained in studies showing that cigarette smoke rapidly up-regulates the expression of genes for mediators important to the pulmonary vasculature (Wright et al., 2002). Nuclear factor κB activation was also observed in mice or guinea pigs exposed to higher doses of cigarette smoke (Nishikawa et al., 1999; Churg et al., 2003). In the latter study, the effect was associated with presence of superoxide, the main reactive oxygen species present in cigarette smoke. Previous studies have demonstrated that activation of nuclear factor κB can be stimulated by oxygen radicals found in cigarette smoke (Pryor et al., 1983; Rahman and MacNee, 1996). Moreover, in peripheral blood mononuclear cells from healthy smokers, the activation of nuclear factor κB by cigarette smoke was suggested as a functional marker of oxidative stress (Van den Berg et al., 2001).

Interestingly, despite the significant activation of nuclear factor κB, not all cytokines known to be regulated by this transcription factor were found in measurable amounts in BAL fluid of mice exposed to cigarette smoke for 1 or 2 cigarettes/day. Our data show that exposure to cigarette smoke led to interleukin-1β and monocyte chemoattractant protein-1 production accompanied by alveolar macrophage influx, but did not lead to an increase in tumor necrosis factor-α levels, which may also explain the absence of neutrophils in the lungs of cigarette smoke-exposed animals. In a murine model using aerosols of lipopolysaccharide, we previously demonstrated (Goncalves-de-Moraes et al., 1998) the close interrelationship between production of tumor necrosis factor-α and neutrophil infiltration, showing that the blockage of tumor necrosis factor-α expression impaired neutrophil recruitment induced by lipopolysaccharide. In accordance with these findings, significant increases in tumor necrosis factor-α production with a parallel increase in neutrophil were only found after exposure to 4 and 8 cigarettes/day for 7 days. The influx of mononuclear or polymorphonuclear cells driven by cytokines, depending on the number of cigarettes "smoked", may explain the discrepancy observed in the literature. Our data also demonstrated that exposure to smoke from four commercial cigarettes for 1 day did not induce neutrophil influx observed after 4 days (data not shown), indicating that differences in cellular influx are also time dependent. A possible explanation for this may be an increase or persistence of toxic substances present in cigarette smoke, such as superoxide, in contact with lung parenchyma. The findings suggest that exposure to cigarette smoke activates nuclear factor κB, leading to the synthesis and release of interleukin-1β, which in turn stimulates the synthesis of tumor necrosis factor-α, as discussed in Barnes and Page (1998).

The finding of a parallel increase in interleukin-1β levels and alveolar macrophage infiltration in response to cigarette smoke was of particular interest as interleukin-1β exerts multiple functions which might be associated with the characteristic pathological changes in the airways of patients with chronic obstructive pulmonary diseases and emphysema. For example, in chronic lung inflammation, like that produced by cigarette smoke, there is tissue damage and subsequent fibrosis that may be associated with the up-regulation of macrophage metalloelastases known to be induced by interleukin-1β (Sasaki et al., 2000). Recently, it was suggested that the involvement of metalloelastases in cigarette smoke-induced lung inflammation (Churg et al., 2003) is mediated by the release of tumor necrosis factor-α from macrophages with subsequent neutrophil influx, which would partly explain why the release of interleukin-1β precedes that of tumor necrosis factor-α. Moreover, interleukin-1β mediates leukocyte extravasation through the up-regulation of adhesion molecules on both endothelial and respiratory epithelial cells (Bochner et al., 1991), as well as affecting the proliferation of fibroblasts through the production of platelet-derived growth factor (Raines et al., 1989), and the extracellular matrix components fibronectin and collagen (Dinarello et al., 1989). These effects are consistent with the role of interleukin-1β as an early, proximal mediator of the inflammatory response. Therefore,

inhibition of the synthesis of interleukin-1β or blocking the bioactivity of this cytokine is expected to have a broad spectrum of anti-inflammatory effects in the lung and to have an impact on the remodeling processes associated with long-term chronic lung injury. None of the currently used animal models replicates all of the pathological effects of chronic obstructive pulmonary disease; however, cigarette smoke exposure in a variety of experimental animals induces at least some changes in the lung that are similar in humans and animals species (Rylander, 1972; Cavarra et al., 2001; Churg et al., 2003). Our results showing that neutralizing interleukin-1β resulted in reduced macrophage infiltration in response to exposure of mice to cigarette smoke are in line with this hypothesis and suggest that strategies to inhibit interleukin-1β may have therapeutic benefit in chronic pulmonary diseases.

Acknowledgments

We are in debt with Dr. Ronir R. Luiz and Dr. F. Rumjanek's research group for their helpful assistance. This work was supported by Brazilian agencies: CNPq, CAPES, FUJB and FAPERJ.

References

Baldwin Jr., A.S., 1996. The NF-κB and IκB proteins: new discoveries and insights. Annu. Rev. Immunol. 14, 648–649.

Barnes, P.J., Karin, M., 1997. Nuclear factor-κB—a pivotal transcription factor in chronic inflammatory diseases. N. Engl. J. Med. 336, 1066–1071.

Barnes, P., Page, C., 1998. Inflammatory mediators of asthma: an update. Pharmacol. Rev. 50, 515–596.

Blackwell, T.S., Christman, J.W., 1997. The role of nuclear factor κ-B in cytokine regulation. Am. J. Respir. Cell Mol. Biol. 17, 3–9.

Bochner, B.S., Luscinkas, F.W., Gimbrone, M.A., Newman, W., Sterbinsky, S.A., Derse-Anthony, C.P., Klunk, D., Schleimer, R.P., 1991. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1 activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. J. Exp. Med. 173, 1553–1557.

Cavarra, E., Bartalesi, B., Lucatteli, M., Fineschi, S., Lunghi, B., Gambelli, F., Ortiz, L.A., Martorana, P.A., Lungarella, G., 2001. Effects of cigarette smoke in mice with different levels of α1-proteinase inhibitor and sensitivity to oxidants. Am. J. Respir. Crit. Care Med. 164, 886–890.

Churg, A., Zay, K., Shay, S., Xie, C., Shapiro, S.D., Hendricks, R., Wright, J.L., 2002. Acute cigarette smoke-induced connective tissue breakdown requires both neutrophils and macrophage metalloelastase in mice. Am. J. Respir. Cell Mol. Biol. 27, 368–374.

Churg, A., Wang, R.D., Tai, H., Wang, X., Xie, C., Dai, J., Shapiro, S.D., Wright, J.L., 2003. Macrophage metalloelastase mediates acute inflammation via TNF-α release. Am. J. Respir. Crit. Care Med. 15 (167(8)), 1083–1089.

Dahmi, R., Gilks, B., Xie, C., Zay, K., Wright, J.L., Churg, A., 2000. Acute cigarette smoke-induced connective tissue breakdown is mediated by neutrophils and prevented by α1-antitrypsin. Am. J. Respir. Cell Mol. Biol. 22, 244–252.

Dinarello, C.A., Clark, B.D., Puren, A.J., Savage, N., Rosoff, P.M., 1989. The interleukin 1 receptor. Immunol. Today 10, 49–51.

Friedlander, M.A., Hilbert, C.M., Wu, Y.C., Finegan, C.K., Rich, E.A., 1994. Disparate cytochemical characteristicigarette smoke and production of cytokines and prostaglandin E2 by human mononuclear phagocytes from the blood, lung and peritoneal cavity. J. Lab. Clin. Med. 123, 574–584.

Gajalakshmi, V., Peto, R., Kanaka, T.S., Jha, P., 2003. Smoking and mortality from tuberculosis and other diseases in India: retrospective study of 43000 adult male deaths and 35000 controls. Lancet 362, 507–515.

Gardi, C., Cavarra, E., Calzoni, P., Marcolongo, P., de Santi, M.M., Martorana, P.A., Lungarella, G., 1994. Neutrophil lysosomal dysfunction in mutant C57BL/6J mice: interstrain variation in content of lysosomol elastase, cathepsin G, and their inhibitors. Biochem. J. 299, 237–245.

Goncalves-de-Moraes, V.L., Singer, M., Vargaftig, B.B., Chignard, M., 1998. Effects of rolipram on cyclic AMP levels in alveolar macrophages and lipopolysaccharide-induced inflammation in mouse lung. Br. J. Pharmacol. 123, 631–636.

Harris, J.O., Swenson, E.W., Johnson, J.E., 1970. Human alveolar macrophages: comparison of phagocytic ability, glucose utilization and ultrastructure in smokers and nonsmokers. J. Clin. Invest. 49, 2086–2096.

Hashimoto, S., Matsumoto, K., Gon, Y., Maruoka, S., Kujime, K., Hayashi, S., Takeshita, I., Horie, T., 2000. p38 MAP kinase regulates TNF alpha-, IL-1 alpha-, and PAF-induced RANTES and GM-CSF production by human bronchial epithelial cells. Clin. Exp. Allergy 30, 48–55.

Hautamaki, R.D., Kobayashi, D.K., Sêñior, R.M., Shapiro, S.D., 1997. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. Science 277, 2002–2004.

Hoidal, J.R., Niewoehner, D.E., 1982. Lung phagocyte recruitment and metabolic alterations induce by cigarette smoke in humans and hamsters. Am. Rev. Respir. Dis. 126, 548–552.

Leikauf, G.D., McDowell, S.A., Wesselkamper, S.C., Hardie, W.D., Leikauf, J.E., Korfhagen, T.R., Prows, D.R., 2002. Acute lung injury: functional genomicigarette smoke and genetic susceptibility. Chest 121 (3 Suppl), 70S–75S.

Matsumoto, K., Aizawa, H., Inoue, H., Koto, H., Takata, S., Shigyo, M., Nakano, H., Hara, N., 1998. Eosinophilic airway inflammation induced by repeated exposure to cigarette smoke. Eur. Respir. J. 12, 387–394.

McCrea, K.A., Ensor, J.E., Nall, K., Bleecker, E.R., Hasday, J.D., 1994. Altered cytokine regulation in the lungs of cigarette smokers. Am. J. Respir. Crit. Care Med. 150, 696–703.

Morokata, T., Ishikawa, J., Yamada, T., 1999. Differential susceptibility of C57BL:6 and DBA:2 mice to ovalbumin-induced pulmonary eosinphilia regulated by Th 1:Th2-type cytokines. Immunol. Lett. 70, 127–134.

Munoz, E., Courtois, G., Veschambre, P., Jalinot, P., Israel, A., 1994. Tax induces nuclear translocation of NFκB through dissociation of cytoplasmic complexes containing p105 or p100 but does not induce degradation of lκB alpha/Mad3. J. Virol. 68, 8035–8044.

Nishikawa, M., Kakemizu, N., Ito, T., Kudo, M., Kaneko, T., Suzuki, M., Udaka, N., Ikeda, H., Okubo, T., 1999. Superoxide mediates cigarette smoke-induced infiltration of neutrophils into the airways through nuclear factor-κB activation and IL-8 mRNA expression in guinea pigs in vivo. Am. J. Respir. Cell Mol. Biol. 20, 189–198.

Ofulue, A.F., Ko, M., Abboud, R., 1998. Time course of neutrophil and macrophage elastinolytic activities in cigarette smoke-induced emphysema. Am. J. Physiol. 275, L1134–L1144.

Ofulue, A.F., Ko, M., 1999. Effects of depletion of neutrophils or macrophages on development of cigarette smoke induced emphysema. Am. J. Physiol. 277, L97–L105.

Pryor, W.A., Tamura, M., Dooley, M.M., Premovic, P., Hales, B.J., Church, D.F., 1983. Reactive oxy-radicals from cigarette smoke and their physiological effects. In: Greenwald, R.A., Cohen, G. (Eds.), Oxy Radicals and Their Scavenger Systems, Cellular and Medical Aspects, vol. 11. Elsevier, New York, pp. 185–192.

Rahman, I., MacNee, W., 1996. Role of oxidants/antioxidants in smoking induced lung disease. Free Radic. Biol. Med. 21, 669–681.

Raines, E.W., Dower, S.K., Ross, R., 1989. Interleukin-1 mitogenic activity for fibroblasts and smooth muscle cells is due to PDGF-AA. Science 243, 393–396.

Repine, J.E., Bast, A., Lankhorst, L., The Oxidative Stress Study Group, 1997. Oxidative stress in chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 156, 341–357.

Ryder, M.I., Saghizadeh, M., Ding, Y., Nguyen, N., Soskolne, A., 2002. Effects of tobacco smoke on the secretion of interleukin-1β, tumor necrosis factor α, and transforming growth factor-β from peripheral blood mononuclear cells. Oral Microbiol. Immunol. 17, 331–336.

Rylander, R., 1972. Tobacco smoke toxicity: the experimental evaluation. Rev. Environ. Health 1, 53–74.

Sasaki, M., Kashima, M., Ito, T., Watanabe, A., Izumiyama, N., Sano, M., Kagaya, M., Shioya, T., Miura, M., 2000. Differential regulation of metalloproteinase production, proliferation and chemotaxis of human lung fibroblasts by PDGF, interleukin 1-beta and TNF-alpha. Mediat. Inflamm. 9, 155–160.

Sen, R., Baltimore, D., 1986. Inducibility of γ immunoglobulin enhancer-binding protein NFκB by a posttranslational mechanism. Cell 47, 921–928.

Shah, P.K., Helfant, R.H., 1988. Smoking and coronary artery diseases. Chest 94, 449–452.

Sherman, C.B., 1991. Health effects of cigarette smoking. Clin. Chest Med. 12, 643–658.

Shin, V.Y., Liu, E.S.L., Koo, M.W.L., Lou, J.C., So, W.H.L., Cho, C.H., 2002. Nicotine suppresses gastric wound repair via the inhibition of polyamine and K+ channel expression. Eur. J. Pharmacol. 444, 115–121.

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.

van den Berg, R., Haenen, G.R.M.M., van den Berg, H., Bast, A., 2001. Nuclear factor-κB activation is higher in peripheral blood mononuclear cells of male smokers. Environ. Toxicol. Pharmacol. 9, 147–151.

Wright, J.L., Tai, H., Dai, J., Churg, A., 2002. Cigarette smoke induces rapid changes in gene expression in pulmonary arteries. Lab. Invest. 82, 1391–1398.