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Lipid-derived free radical production in superantigen-induced interstitial pneumonia

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Abstract

We studied the free radical generation involved in the development of interstitial pneumonia (IP) in an animal model of autoimmune disease. We observed an electron spin resonance (ESR) spectrum of α -(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) radical adducts detected in the lipid extract of lungs in autoimmune-prone mice after intratracheal instillation of staphylococcal enterotoxin B. The POBN adducts detected by ESR were paralleled by infiltration of macrophages and neutrophils in the bronchoalveolar lavage fluid. To further investigate the mechanism of free radical generation, mice were pretreated with the macrophage toxicant gadolinium chloride, which significantly suppressed the radical generation. Free radical generation was also decreased by pretreatment with the xanthine oxidase (XO) inhibitor allopurinol, the iron chelator Desferal, and the inducible nitric oxide synthase (iNOS) inhibitor 1400W. Histopathologically, these drugs significantly reduced both the cell infiltration to alveolar septal walls and the synthesis of pulmonary collagen fibers. Experiments with NADPH oxidase knockout mice showed that NADPH oxidase did not contribute to lipid radical generation. These results suggest that lipid-derived carbon-centered free radical production is important in the manifestation of IP and that a macrophage toxicant, an XO inhibitor, an iron chelator, and an iNOS inhibitor protect against both radical generation and the manifestation of IP.

Keywords

autoimmune disease; interstitial pneumonia; superantigen; lipid-derived carbon-centered free radical; ESR spin trapping

INTRODUCTION

The lung is frequently involved in autoimmune disease, and complications involving the lung include bronchiolitis, interstitial pneumonia (IP), and pulmonary vasculitis [1–4]. The pathogenesis and mechanisms underlying the development of pulmonary manifestations associated with autoimmune disease remain unknown, although it appears that genetic,

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infectious, environmental, and hormonal factors are all involved in complex, interrelated ways [5].

In recent years, there has been considerable interest in the role of superantigens in the etiopathogenesis of several autoimmune diseases, including rheumatoid arthritis, insulin-dependent diabetes mellitus, and systemic vasculitides [6–10]. Superantigens, such as staphylococcal enterotoxin B (SEB), are extremely potent polyclonal mitogens that stimulate a large proportion of T cells both in humans and in animals such as mice. Superantigens bind to major histocompatibility complex molecules outside of their peptide-binding grooves and interact only with V β domains of T-cell receptors, resulting in the stimulation of up to 20% of the entire T-cell population, in contrast to processed antigenic peptides [11]. We previously reported that an intratracheal instillation of SEB induced severe IP in autoimmune-prone mice [12]. We also showed that SEB-reactive T cells in the bronchoalveolar space triggered the development of IP in this model [13]. Furthermore, we observed increases in macrophages, neutrophils, and lymphocytes in the bronchoalveolar lavage (BAL) fluid.

There have been many studies reporting that both reactive oxygen species (ROS) and reactive nitrogen species are involved in the pathogenesis of several autoimmune diseases. It is suggested that nitric oxide (NO) and ROS can function as mediators of tissue damage in autoimmune disease [14]. We have shown that the overproduction of NO and superoxide were implicated in the pathogenesis of SEB-induced IP in the autoimmune-prone model [15]. In addition, increased lipid peroxidation is also reported in systemic autoimmune diseases [16]. However, the potential of these reactive species to elicit autoimmune response and their contribution to disease pathogenesis remains unclear.

Here we hypothesized that the alveolar epithelial cell-injury that characterizes IP associated with autoimmune disease may result from enhanced lipid peroxidation. We used the electron spin resonance (ESR) in vivo spin-trap technique to determine that free radical generation occurs in mice with SEB-induced IP. It was found that the macrophage toxicant gadolinium chloride (GdCl₃), the xanthine oxidase (XO) inhibitor allopurinol, the iron chelator Desferal, and the iNOS inhibitor 1400W all had a protective effect on lipid radical production and the development of IP in this model. These results show for the first time that lipid-derived free radical production is important in the disease manifestation of IP and that macrophage toxicants, XO inhibitors, iron chelators, or iNOS inhibitors may be potential therapeutic agents in treating IP.

MATERIALS AND METHODS

Materials

2, 2'-Dipyridyl, α -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN) was obtained from Alexis (San Francisco, CA). Staphylococcus enterotoxin B (SEB), gadolinium chloride, deferoxamine mesylate (Desferal), and allopurinol were purchased from Sigma Chemical Co. (St. Louis, MO). N-(3-aminomethyl)benzylacetamidine (1400W) was from Calbiochem-Novabiochem (San Diego, CA). Pentobarbital (Abbott Laboratories, North Chicago, IL) and Diff-Quik stain kits (Dade Behring Inc, Newark, DE) were used as received.

Animals and treatments

MRL/mpj mice and the NADPH oxidase (Nox) 2 knockout (Nox2 $-/-$) mice were obtained from The Jackson Laboratory. Age-matched mice of the C57BL/6 strain that possessed normal Nox activity served as control animals for Nox deficient experiments. MRL/mpj mice were fed a vitamin E-deficient diet (Harlan Teklad, Madison, WI) for two weeks in order to avoid vitamin E-derived ESR signals. Nox2 $-/-$ mice and C57BL/6 mice were fed a regular diet, not

a vitamin E-deficient diet, since the lipid lung extracts did not show vitamin E-derived ESR signals. All mice were females 12 weeks of age. MRL/mpj mice weighed 35 ± 5 g, and Nox2^{-/-} and control mice weighed 20 ± 4 g. All mice were group-housed in a temperature-controlled room at 23–24°C with a 12/12-h light/dark cycle; all were allowed free access to food and water. The studies adhered to the National Institutes of Health guidelines for the care and handling of experimental animals. All animal studies were approved by the National Institute of Environmental Health Sciences review board.

The control group received phosphate-buffered saline (PBS) (40 μ l) intratracheally. The SEB group received 10 μ g/mouse of SEB dissolved in PBS (40 μ l) intratracheally. All experiments were carried out under anesthesia with 4% isoflurane (Baxter, Deerfield, IL). The macrophage toxicant GdCl₃ (60 mg/kg) and the iNOS inhibitor 1400W (5 mg/kg) were given intraperitoneally once a day, beginning the day before the intratracheal instillation of SEB and continuing throughout the course of the experiment. The iron chelator Desferal (100 mg/kg) and the XO inhibitor allopurinol (30 mg/kg) were given intraperitoneally twice a day at the same points.

ESR studies

POBN was injected intraperitoneally (6 mmol/kg) under anesthesia with pentobarbital (30 mg/kg) 3 d after the intratracheal instillation of SEB. Animals were killed 30 minutes after administration of POBN, and the lipid phase of the lung tissue was extracted.

Extraction was performed as previously described with some modification [17]. Briefly, the lung tissue was homogenized in a mixture containing 2.5 ml of 2:1 chloroform:methanol, 0.5 ml of 30 mM 2, 2'-dipyridyl, 2 ml of phenol, and 2 ml of deionized water using a homogenizer (OMNI International, Marietta, GA); samples were kept on ice during the procedure. For the lipid extraction, 16 ml of 2:1 chloroform:methanol were added, and the mixture was shaken and then centrifuged at 2500 rpm for 15 minutes (Beckman Coulter, Inc., Fullerton, CA). The chloroform layer was then isolated and dried by passing through a sodium sulfate column, and the solvent was evaporated by bubbling with nitrogen gas to a final volume of 0.5 ml.

ESR spectra were immediately recorded at room temperature using a quartz flat cell in a Bruker EMX EPR spectrometer equipped with a super-high Q cavity. Spectra were recorded on an IBM-compatible computer interfaced with spectrometer instrument settings of 9.8 GHz, 20 mW microwave power, 100 kHz modulation frequency, 1310 ms conversion time, and 655 ms time constant. Simulations of ESR spectra were obtained using the computer program WINSIM developed in this laboratory [18]. The relative intensity of the signal was quantified by the WinEPR program provided by Bruker (Bruker Bio Spin Co., Billerica, MA).

Bronchoalveolar lavage fluid

BAL fluid was obtained from the lungs of mice that had received an intratracheal instillation of SEB 3 d previously. The mice were deeply anesthetized and killed by exsanguination of the abdominal aorta. After the chest was opened, a 20-gauge catheter was inserted into the trachea and tied with 5-0 silk. The lungs from each animal were lavaged three times through the catheter with 1 ml of chilled PBS. The cells were pelleted by centrifugation and counted using a hemocytometer. Differential counts were performed on a Diff-Quick-stained preparation.

Histopathology

Lung tissue was removed 7 d after intratracheal instillation of SEB and fixed-inflated to 20 cm H₂O pressure with 7% formalin. After fixation, all lung lobes were cut sagittally through the center. Tissue sections were stained with hematoxylin-eosin and Masson-Trichrome stain.

Statistical analysis

Statistical significance of the difference was determined by unpaired Student's *t* test. Data are expressed as mean $\pm$ SEM. Differences between groups were considered statistically significant at the level of $p < 0.01$.

RESULTS

Lung free radical generation by SEB in autoimmune-prone mice

We detected POBN radical adducts in organic extracts of lungs from mice intratracheally instilled with SEB. The greatest advantage of the extraction method is that the radical adduct is transferred from a sample with a high dielectric constant, the lung tissue, to a solvent with lower dielectric constant, the chloroform solution. Only non-polar radical adducts that are soluble in chloroform such as the POBN lipid-derived radicals can be detected with organic extraction by ESR [19]. To determine the involvement of lipid radical generation in the pathogenesis of IP in SEB-instilled MRL/mpj mice, we evaluated the ESR signal of POBN radical adducts in lipid extracts of the lung 3 d after intratracheal instillation of SEB. As shown in Fig. 1A, the six-line ESR spectrum was detected in the SEB-treated and POBN-injected mouse. The instillation of PBS instead of SEB resulted in a much weaker ESR signal (Fig. 1). Without the spin trap, neither SEB nor PBS instillation yielded a detectable spectrum (data not shown). The differences between SEB-instilled mice and PBS-instilled mice on day 3 were statistically significant (Fig. 1E, $p < 0.01$). When the ESR spectrum was simulated using a computer program developed in this laboratory, the hyperfine coupling constants for the POBN radical adducts were $a^N = 14.93 \pm 0.04$ G and $a^H_\beta = 2.39 \pm 0.08$ G ($n = 8$), similar to those previously reported for the POBN radical adducts of carbon-centered, lipid-derived radicals [17,20].

Hydroxyl radical can initiate lipid peroxidation by hydrogen abstraction. We also administered the hydroxyl radical scavenger dimethyl sulfoxide (DMSO) to investigate whether hydroxyl radical is produced in SEB-induced IP. Hydroxyl radical is converted into the methyl radical via its reaction with DMSO, and the methyl radical is detected as a POBN adduct. POBN lipid radical did not appear to increase in the presence of DMSO, and the six-line signal was not changed in the presence of ^{13}C -labeled DMSO (data not shown). This result indicates that POBN adducts induced by SEB reflect lipid-derived, carbon-centered radicals as a result of enhanced lipid peroxidation, which is supported by previous *in vivo* studies [17,20]. Since the lungs have relatively high concentrations of α -tocopherol per gram of tissue, MRL/mpj mice of all experiments were fed a vitamin E-deficient diet for two weeks to avoid the ESR detection of stable vitamin E radical in the lipid extracts of lungs. To exclude the possibility that an animal fed vitamin E deficient diet may respond differently to SEB than one with a normal level of this antioxidant, experiments were conducted to determine whether histopathological or cell count changes occurred *in vivo* in the lungs and BALF of MRL/mpj mice fed for 2 weeks a vitamin E deficient diet. No statistically significant differences in comparison to the control group fed a vitamin E sufficient diet were found in BALF total cell count or the number of alveolar macrophages, neutrophils or lymphocytes (data not shown). Subsequently, no differences in the lung histopathology or the growth rate of mice were observed until two weeks after feeding. These results indicate that the radicals detected in MRL/mpj mice resulted from the SEB induced IP and not from the vitamin E deficiency since there is not sufficient evidence to suggest alternative interpretations.

The effect of GdCl_3 on the lung free radical generation by SEB in autoimmune-prone mice

We administrated GdCl_3 , an inhibitor of macrophage activation [21], to examine the role of phagocytes in lipid-derived carbon-centered free radical production in the lung. There was a significant increase in total cells of BAL fluid on day 3 in mice injected with SEB ($74.5 \times$

$10^4/\text{ml}$) as compared to the control mice ($6.6 \times 10^4/\text{ml}$) (Fig. 2A, $p < 0.01$). When GdCl_3 was administrated to mice once a day, the numbers of total cells, macrophages, neutrophils, and lymphocytes in BAL fluid from SEB-instilled mice decreased significantly (Fig. 2A, $p < 0.01$). The production of free radicals in the lung decreased by 68.5% with GdCl_3 , whereas the hyperfine coupling constants ($a^N = 14.95 \pm 0.06 \text{ G}$ and $a^H_\beta = 2.39 \pm 0.05 \text{ G}$) remained unchanged (Fig. 2B, C).

To study cell infiltration and the synthesis of pulmonary collagen fibers, we performed additional histological experiments on day 7 (Fig. 3). We found that the instillation of SEB led to cell infiltration into the alveolar septal walls and increased synthesis of pulmonary collagen fibers. PBS administration alone caused no histological changes (Fig. 3A, D). Pretreatment with GdCl_3 significantly decreased thickening in the alveolar septa with inflammatory cells and decreased fibroblasts induced by SEB (Fig. 3C, F).

Free radical generation in the lung of NADPH oxidase knockout mice treated with SEB

To evaluate Nox activity, we investigated lipid radical formation in the SEB-treated lung of Nox2 $-/-$ mice, which lack a critical membrane-bound subunit of this major source of ROS in activated phagocytes. The instillation of SEB into the lung markedly increased the ESR signal intensity of POBN radical adducts in the lungs of wild-type mice 3 d after the instillation. In the knockout mice, the ESR spectrum was not different from the wild-type mice after instillation (Fig. 4A). There was no significant change in the numbers of total cells, macrophages or neutrophils in BAL fluid in Nox wild-type and Nox2 $-/-$ mice (Fig. 4B). All these results suggest that Nox, one enzyme which produces superoxide radical, has no involvement in IP.

The effect of allopurinol, Desferal, and 1400W on lung free radical generation by SEB

XO produces superoxide and hydrogen peroxide from molecular oxygen, and the reaction between ferrous ion and hydrogen peroxide generates hydroxyl radical. To test for the involvement of XO, we administrated an XO inhibitor, allopurinol, and an iron chelator, Desferal. Pretreatment with allopurinol twice a day for 3 d (30 mg/kg, intraperitoneally) or Desferal twice a day for 3 d (100 mg/kg, intraperitoneally) resulted in a marked reduction of the ESR signal of POBN adduct in the lung by 55 – 60% ($p < 0.01$) in each case (Fig. 5).

In addition, we administrated an iNOS inhibitor, 1400W, to evaluate the involvement of NO. Pretreatment with 1400W once a day for 3 d (5 mg/kg, intraperitoneally) inhibited the production of lipid radical in the lung by almost 50% (Fig. 5).

The effect of allopurinol, Desferal, and 1400W on bronchial alveolar lavage cells by SEB

The numbers of total cells, neutrophils, and lymphocytes in BAL fluid from SEB-instilled mice were significantly decreased with allopurinol pretreatment (Fig. 6, $p < 0.01$, $n = 8$). A significant decrease in the count of total cells, macrophages, neutrophils, and lymphocytes in BAL fluid was observed after pretreatment with Desferal or 1400W (Fig. 6, $p < 0.01$). Allopurinol did not have a statistically significant effect on macrophage cell count.

The effect of allopurinol, Desferal, and 1400W on histologic changes in the lungs after SEB instillation

When we investigated cell infiltration and pulmonary collagen fiber synthesis in the histopathological study at 7 d with pretreatment of allopurinol, Desferal, or 1400W, we found that the cell infiltration into the alveolar septal walls and the synthesis of pulmonary collagen fibers were diminished (Fig. 7). These results show that perhaps superoxide radical, hydroxyl radical, and NO participate in the development of IP.

DISCUSSION

In this study, we have demonstrated the *in vivo* detection of lipid-derived carbon-centered free radical in the SEB-induced IP model using the ESR spin-trapping method with POBN. SEB also increased the numbers of total cells, macrophages, neutrophils, and lymphocytes in the BAL fluid. These results suggest that the manifestation of IP is accompanied by lipid-derived carbon-centered free radical production. Although the ESR spin trapping technique can not prove the cell compartment of the POBN-trapped lipid-derived carbon-centered radicals, we assume that POBN have dissolved significantly in the cell membrane since the octanol-water partition coefficient of POBN is 0.15 [22,23]. We found that $GdCl_3$ significantly inhibited lipid radical formation and lung inflammation as indicated by the number of total cells in the BAL fluid and the manifestation of IP. SEB-induced lipid radical was unaffected by the genetic inactivation of Nox, whereas the radical generation was markedly decreased by the XO inhibitor allopurinol. Pretreatment with the iron chelator Desferal or the iNOS inhibitor 1400W significantly inhibited lipid radical generation, macrophage and neutrophil counts in the BAL fluid, and histological changes in the lung. These results suggest that activated alveolar macrophages, XO, and NO are potential sources of lipid radical and, perhaps, important in the pathogenesis of SEB-induced IP.

Alteration of membrane integrity by peroxidation is known to modify membrane fatty acid composition, disrupt permeability, decrease electrical resistance, and increase flip-flopping between monolayers and inactivated cross-linked proteins [24,25]. These collective effects of lipid peroxidation on cellular processes have been implicated as the underlying mechanism for numerous pathological conditions [26,27]. Others have shown that free radical-induced lipid peroxidation also has relevance to LPS-induced acute lung injury [28]. We found that the intratracheal instillation of SEB significantly enhanced free radicals in the lung, primarily in the form of lipid-derived carbon-centered free radicals detected as POBN radical adducts in the present study, and the free radical production was clearly correlated with an increase in alveolar macrophages and neutrophils (Fig. 2).

It is well known that phagocytes produce superoxide radicals via Nox, and several reports show that Nox has a crucial role in tissue damage [29,30]. In the pulmonary system, ROS generated by Nox play distinct physiological roles in airway and vascular remodeling [31]. Other studies suggest that excessive phagocyte-derived ROS also may play a role in lung injury during asthma and inflammatory events [32]. Our studies with Nox2 $-/-$ mice demonstrated that the generation of free radicals did not require oxidants generated by Nox.

On the other hand, we found that the production of lipid radical in the lung decreased and the histological change was ameliorated by the administration of allopurinol, an inhibitor of XO, the other enzyme which produces superoxide. XO and xanthine dehydrogenase (XDH) are interconvertible forms of the same enzyme, and XDH/XO is detected in several cells such as endothelial cells, epithelial cells, neutrophils, and macrophages [15,33–35]. Most investigators agree that XDH activity converts to an oxidase that produces superoxide and hydrogen peroxide [36]. This generation of ROS is thought to be the basis of XDH/XO involvement in various pathologic conditions such as influenza virus infection and neutrophil-mediated lung injury [37,38]. Our data show that XO activation may play a role in the radical generation by superoxide overproduction since an inhibitor of XO suppressed free radical generation in SEB induced IP.

Other studies have demonstrated that some cytokines or hypoxia upregulated XDH/XO generation at the translational and post-transcriptional levels [34,35,39,40]. Tumor necrosis factor- α (TNF- α), interleukin-1, and IFN- γ were shown to lead to increased XDH/XO activity in epithelial cells [34]. XO enzyme activity is also enhanced in endothelial cells by TNF- α and

chemotactic peptide [41]. These findings indicate that XDH/XO activity is regulated in a cell-specific manner and by inflammatory cytokines and physiologic events. However, we do not have data to confirm or reject these findings.

As alveolar macrophages play an essential role in the inflammatory response, we examined the effect of GdCl_3 , a macrophage inhibitor, on the generation of free radicals. The GdCl_3 pretreatment attenuated the number of alveolar macrophages after injection [42]. Some studies have shown that GdCl_3 depressed hepatic macrophage phagocytic activity and the production of reactive nitrogen and oxygen intermediates [43,44]. Several papers reported the inhibition of neutrophilic infiltration by GdCl_3 , which may be the result of enzyme blockage and factors released from macrophages that are related to neutrophil migration and adhesion [17,45]. Here, GdCl_3 pretreatment also indicated that the activation of alveolar macrophages was important for lipid radical generation, inflammation, and the development of IP. Although Nox was not responsible for the production of lipid-derived carbon-centered free radicals, nevertheless, proinflammatory cytokines, chemokines, and adhesion molecules released from phagocytes may play an important role in the free radical generation and IP by SEB.

Our experiment with Desferal pretreatment indicates that free iron plays a role in the formation of lipid radical in IP induced by SEB instillation. Desferal pretreatment significantly reduced the amplitude of the ESR signal generated after SEB instillation. Histologically, Desferal also decreased the alveolar septa thickened with inflammatory cells and fibroblasts in the lungs. Desferal pretreatment significantly reduced not only free radical generation but also total cell count in the BAL fluid. It has been reported that alterations in proinflammatory cytokines, adhesion molecules, and chemotactic gradients play an important role in the accumulation of neutrophils or lymphocytes in lung inflammation [46]. Desferal has been shown to interfere with the adhesion functions [47]. In addition, a previous report showed that iron regulated XO activity in the lung [48]. These findings suggest that Desferal-induced anti-inflammatory activities may be important in its protective effects, in addition to the inhibition of iron's catalytic production of hydroxyl radical.

The effect of iNOS inhibitors on SEB-induced POBN adduct formation was also tested, because we previously reported that SEB enhanced the NO production from macrophages and neutrophils in the lung, and the iNOS inhibitor tends to protect against the development of IP in this model [15]. Pretreatment with iNOS inhibitors significantly reduced the amplitude of the signal generated by SEB and decreased the thickened alveolar septa in inflammatory cells and fibroblasts in the lungs. These results suggest that NO also plays a role in the generation of lipid-derived carbon-centered free radicals in SEB-induced IP.

In conclusion, this is the first demonstration that superantigens generate lipid radicals. We found that SEB-induced lipid radical was generated through xanthine oxidase activation with iron and NO induction, but NADPH oxidase was not involved. Moreover, macrophage toxicants, xanthine oxidase inhibitors, iron chelators, or inducible nitric oxide synthase inhibitors may be potential therapeutic agents against alveolitis and fibrosis in interstitial pneumonia.

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Figure 1.

Staphylococcus enterotoxin B (SEB) induces free radical generation in the lung 3 d after intratracheal instillation. (A) Electron spin resonance (ESR) spectrum of α -(4-pyridyl-1-oxide)-*N*-tert-butyl nitron (POBN) radical adducts detected in lipid extracts of lungs 3 d after the intratracheal instillation of SEB. (B) ESR spectrum of treated with the intratracheal instillation of PBS. (C) Subtracting the spectrum of PBS from the spectrum of SEB. (D) Computer simulation of spectrum in C. (E) ESR signal intensity of radical adducts after instillation of SEB. The amplitude of the first peak on the left of the ESR signal was used for comparison and quantified by the Bruker WinESR program. * $p < 0.01$ versus control group. Data are expressed as mean $\pm$ SEM of eight animals in each group. Instrumental conditions: microwave power, 20 mW; modulation frequency, 100 kHz; conversion time, 1310 milliseconds; time constant, 655 milliseconds.

Figure 2.

Effect of GdCl₃ on cellular profiles in BAL fluid and lipid extract of lung tissue 3 d after intratracheal instillation of SEB. (A) Cellular profile of BAL fluid 3 d after SEB instillation. (B) ESR signal intensity of radical adducts in lipid extract of lungs 3 d after the instillation without and with GdCl₃ pretreatment. (C) The amplitude of the first peak on the left on the ESR signal was used for comparison and quantified by the Bruker WinESR program. * $p < 0.01$ versus SEB group. Data are expressed as mean $\pm$ SEM of eight animals in each group. Instrumental conditions: microwave power, 20 mW; modulation frequency, 100 kHz; conversion time, 1310 milliseconds; time constant, 655 milliseconds.

Figure 3.

Effect of GdCl_3 in interstitial pneumonia by SEB using histological studies. Lung specimens were obtained from PBS-instilled mice (A, D), SEB-instilled mice (B, E), and SEB-instilled mice pretreated with GdCl_3 (C, F) 7 d after intratracheal instillation of SEB. Lung tissue was stained with hematoxylin-eosin (A – C) and Masson-Trichrome (D – F). Original magnification, $\times 320$.

Figure 4.

The effect of NADPH oxidase deficiency on lung free radical generation induced by SEB. (A) Signal intensity of Nox wild-type mice or Nox2 $-/-$ mice 3 d after intratracheal instillation. Data are expressed as mean $\pm$ SEM of eight animals in each group. The amplitude of the first peak on the left of the ESR signal was used for comparisons and quantified with the Bruker WinEPR program. Instrumental conditions: microwave power, 20 mW; modulation frequency, 100 kHz; conversion time, 1310 milliseconds; time constant, 655 milliseconds. (B) Cellular profile of BAL fluid 3 d after SEB instillation. The total cell count was determined on a fresh fluid specimen using a hemocytometer. Differential cell count was assessed on cytologic preparations stained with Diff-Quick stain. Data are expressed as mean $\pm$ SEM of eight animals in each group.

Figure 5.

Effects of allopurinol, Desferal, and 1400W on free radical generation of lung tissue 3 d after intratracheal instillation of SEB. (A) ESR signal intensity of radical adducts in lipid extract of lungs 3 d after the instillation without and with allopurinol, Desferal, and 1400W pretreatment. (B) The amplitude of the first peak on the left of the ESR signal was used for comparison and quantified by the Bruker WinESR program. * $p < 0.01$ versus SEB group. Data are expressed as mean $\pm$ SEM of eight animals in each group. Instrumental conditions: microwave power, 20 mW; modulation frequency, 100 kHz; conversion time, 1310 milliseconds; time constant, 655 milliseconds.

Figure 6.

Effects of allopurinol, Desferal, and 1400W on the cellular profile of BAL fluid 3 d after SEB instillation. The total cell count was determined on a fresh fluid specimen using a hemocytometer. Differential cell count was assessed on cytologic preparations stained with Diff-Quick stain. * $p < 0.01$ versus SEB group. Data are expressed as mean $\pm$ SEM of eight animals in each group.

Figure 7. Effect of allopurinol, Desferal, and 1400W on the histological findings induced by SEB in the lung. Lung tissue shown was obtained from SEB-instilled mice (A, E), SEB-instilled mice pretreated with allopurinol (B, F), SEB-instilled mice pretreated with Desferal (C, G), and SEB-instilled mice pretreated with 1400W (D, H) at day 7. Lung specimens were stained with hematoxylin-eosin (A – D) and Masson-Trichrome (E – H). Original magnification, x 320.