Effect of Ozone Exposure on Alveolar Macrophage-Mediated Immunosuppressive Activity in Rats

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Ozone (O₃), a major component of photochemical air pollution, is a strong oxidizing agent and highly toxic. Resident alveolar macrophages (AM) play an important immunomodulatory role in the lung via suppression of lymphocyte proliferation, thus limiting the magnitude and duration of local immune responses. Nitric oxide (NO) plays a crucial role in the immunosuppressive activity of AM. However, during immunoinflammatory responses, microenvironmental changes within the alveoli inhibit this AM function, permitting full expression of local T-cell-mediated immune responses. We hypothesize that similar changes in AM function may occur during inflammation induced by exposure to inorganic air pollutants, such as O₃. In order to test this hypothesis, in the present study, we investigated (1) whether O₃ exposure of rats might affect the immunosuppressive activity and NO production of bronchoalveolar lavage cells (BAL cells) and (2) whether changes in the microenvironment of the alveoli induced by O₃ exposure can affect the immunosuppressive activity and NO production of AM. AM-mediated immunosuppressive activity was measured as inhibition of concanavalin A (Con A)-induced proliferation of lymph node cells (LNC). Bronchoalveolar lavage was used to sample the alveolar microenvironment, and the resulting fluid (BALF) was tested for capacity to modulate AM activity in the cultures. BALF and BAL cells from rats exposed to 1 ppm O₃ or filtered air for 3 days were used. The present results demonstrate that BAL cells isolated from O₃-exposed rats suppressed Con A-induced LNC proliferation and produced NO in the same manner as BAL cells (AM) from air-exposed rats. AM-mediated suppressive activity of LNC proliferation and NO production were markedly inhibited by BALF from O₃-exposed but not from air-exposed rats. These results suggested that AM-mediated immunosuppressive activity in vivo may be inhibited by the O₃-induced release of soluble factors which inhibit NO production by AM.

The tissues of the lung are constantly contact with incoming antigens from the atmospheric environment during gas exchange. Precise regulation of the response of local T-cells to this stimulation is required in order to prevent excessive immune responses. Alveolar macrophages (AM) function poorly as accessory cells for T-cell activation and as antigen-presenting cells (APC) (Bilyk and Holt, 1993; Holt, 1990). In many situations, AM suppress the antigen- or mitogen-induced proliferation of T-cells (Bilyk and Holt, 1993; Strickland et al., 1993, 1994; Upham et al., 1995). AM also suppress the antigen-presenting activity of lung dendritic cells (DC) in situ (Holt et al., 1993). These AM-mediated immunosuppressive activities have been proposed to play an important role in maintaining immunological homeostasis in the normal lung (Bilyk and Holt, 1993; Holt et al., 1993; Strickland et al., 1993, 1994; Upham et al., 1995). During lung inflammation induced by exposure to bacterial lipopolysaccharide (LPS), soluble factors released into the lung microenvironment have been shown to inhibit the AM-mediated suppressive activity of T-cell proliferation (Bilyk and Holt, 1993). Several mediators have been suggested as potential causes of the macrophage-mediated suppression of T-cell proliferation, including nitric oxide (NO) and tumor necrosis factor-α (TNF-α) (Kawabe et al., 1992; Metzger et al., 1980; Schleifer and Mansfield, 1993). However, in relation to the present model, it has been shown that AM-mediated suppression of lymphocyte proliferation in the rat can be almost entirely inhibited by the use of agents which block enzyme-inducible NO synthase (Kawabe et al., 1992; Upham et al., 1995), indicating that the principle immunosuppressive agent is NO. It is significant to note in this context that the loss of immunosuppressive activity of AM

2 Abbreviations used: AM, alveolar macrophages; APC, antigen-presenting cells; DC, dendritic cells; LPS, lipopolysaccharide; NO, nitric oxide; TNF-α, tumor necrosis factor-α; O₃, ozone; IgE, immunoglobulin E; BAL, bronchoalveolar lavage cells; LNC, lymph node cells; Con A, concanavalin A; BALF, bronchoalveolar lavage fluid; PBS, phosphate-buffered saline; FBS, fetal bovine serum; 2-ME, 2-mercaptoethanol; H₂PO₄, phosphoric acid; NaNO₂, sodium nitrite; ELISA, enzyme-linked immunosorbent assay; BrdU, 5-bromo-2'-deoxyuridine; RT-PCR, reverse transcriptase PCR; 100 μg/ml penicillin, 100 U/ml streptomycin, and 5 × 10⁻² M 2-ME; Air-BALF, BALF from air-exposed rats; O₃-BALF, BALF from O₃-exposed rats; NO₂, nitrite; LCM, lung-conditioned medium; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFN-γ, interferon-γ; IL, interleukin; PG, prostaglandin; TGF-β, transforming growth factor-β; SP-A, surfactant-associated protein A.
induced by soluble factors released from LPS-exposed lung tissue (Bilyk and Holt, 1993) was associated with loss of capacity to produce NO (Bilyk and Holt, 1995).

Asthma and allergic rhinitis appear to be increasing in many countries (Leikauf et al., 1995; Wardlaw, 1995). Previous reports have shown that the prevalence rate of asthma (Koren, 1995; Leikauf et al., 1995) and allergic rhinitis (Ishizaki et al., 1987; Kaneko et al., 1980) in air-polluted areas is higher than that in nonpolluted areas. Controversial epidemiological results, however, have also been reported (Nicolai and von-Mutius, 1996, 1997). Therefore, the relationship between concentration and/or composition of air pollutants and prevalence of allergic disorders remained to be elucidated. Ozone (O$_3$), a major component of photochemical air pollution, is a strong oxidizing agent and highly toxic. Lung inflammation is caused by exposure to air pollutants, such as O$_3$ (Chen et al., 1995; Laskin et al., 1994; Peden et al., 1995; Pendino et al., 1996). A contributory role of O$_3$ to the development of asthma was suggested by the results of epidemiological studies (Balmes, 1993; Bascom, 1996; Koren, 1995; Leikauf et al., 1995). Therefore, it is necessary to determine whether O$_3$ might affect cellular mechanisms which are known to play a role in the induction of asthma and allergic rhinitis.

Immunoglobulin E (IgE) antibody production and airway hyperresponsiveness are important factors in respiratory allergic diseases. It has been reported that O$_3$ exposure enhances the activity of IgE-containing cells in lung (Gershwin et al., 1981) and also airway hyperresponsiveness to chemical mediators released by antigen–antibody reactions (Kobayashi, 1996). There are many reports on the nature of the mechanisms of O$_3$-induced airway hyperresponsiveness. The mechanism of enhancement of IgE production by O$_3$ exposure, however, remains to be elucidated. In this context, it has been shown that abolition of the AM-mediated immunosuppressive pathway in vivo by in situ depletion of AM enhances the functions of DC and T-cells in the lung (Holt et al., 1993; Strickland et al., 1993), both of which play an important role in IgE production. In addition, animals lacking functional AM display markedly enhanced IgE responses to aerosolized antigen (Thepen et al., 1992).

Therefore, in the present studies, we investigated (1) whether O$_3$ exposure of rats might affect the immunosuppressive activity and NO production of bronchoalveolar lavage cells (BAL cells) and (2) whether changes in the microenvironment of alveoli induced by O$_3$ exposure can affect the immunosuppressive activity and NO production of AM. Rats were exposed to O$_3$ or filtered air, and AM-mediated immunosuppressive activity was measured as inhibition of the proliferation of lymph node cells (LNC) induced by concanavalin A (Con A). Bronchoalveolar lavage fluid (BALF) was used as a sample of the microenvironment of alveoli. Our present results suggest that AM-mediated immunosuppressive activity is inhibited by the O$_3$-induced changes in the microenvironment of alveoli.

METHODS

Reagents. The following reagents were obtained: RPMI 1640 medium, Dulbecco’s calcium- and magnesium-free phosphate-buffered saline (PBS (−)), fetal bovine serum (FBS), and pentobarbital sodium from Dai nippon Pharmaceutical Co. (Osaka, Japan); 2-mercaptoethanol (2-ME) from Wako Pure Chemical Industries (Osaka, Japan); penicillin-streptomycin solution and Con A from Sigma (St. Louis, MO); trypan blue from Cibico Laboratories (NY); phosphoric acid (H$_3$PO$_4$), sulfanilamide, N-(1-naphthyl)ethylene diamine dihydrochloride and sodium nitrite (NaNO$_2$) from Nacalai Tesque, Inc. (Kyoto, Japan); and cell-proliferation enzyme-linked immunosorbent assay (ELISA) and the 5-bromo-2'-deoxyuridine (BrdU) (colorimetric) Kit from Boehringer Mannheim (Tokyo, Japan).

Animals and ozone exposure. Male, specific pathogen-free Wistar strain rats (8–12 weeks old) were obtained from Japan Charles River Inc. (Yokohama, Japan) and were housed under conventional conditions until used. The rats were placed in stainless-steel cages and exposed to 1 ± 0.1 ppm O$_3$ or filtered air for 3 days. Food or water was provided to the animals during the exposure period. The conditions in the chamber (volume 1.16 m$^3$) were (temperature) 25 ± 1°C; (humidity) 55 ± 10%; (pressure) −5 mm H$_2$O relative to atmospheric pressure; (total air flow rate) 90–100 m$^3$/h. The concentrations of O$_3$ were continuously monitored with an O$_3$ analyzer (Model 8410, Monitor Lab. Inc., U.S.A.) using a chemiluminescence method.

Preparation of bronchoalveolar lavage fluid and bronchoalveolar lavage cells. Air- or O$_3$-exposed rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and exsanguinated from the aorta abdominals. BALF and BAL cells were obtained via in situ BAL. An incision was made between the cartilaginous rings of the trachea, and a cannula was inserted into the trachea and secured with suture. Lungs and trachea were lavaged with 37°C RPMI 1640 medium (10 ml/300 g body weight). The lavage was done 10 times by slowly instilling and withdrawing the instillation with the same solution. The alveolar lavage fluid was then sterilized by filtration with MILLEX-HA (0.45 μm; Millipore, Bedford, MA). Ten percent heat-inactivated FBS, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 5 × 10$^{-5}$ M 2-ME were added to each filtrate and this final solution was designated the BALF. There was no significant difference in pH of the BALF from O$_3$- or air-exposed rats. In order to prepare BAL cells, the lungs and trachea were lavaged twice with 10 ml 37°C PBS (−). BAL cells were collected by centrifugation and resuspended in RPMI 1640 medium plus 10% heat-inactivated FBS, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 5 × 10$^{-5}$ M 2-ME (R10). The numbers of viable cells were determined using the trypan blue exclusion method.

Preparation of lymph node cells. LNC were derived from a pool of superficial, facial, and posterior mediastinal lymph nodes in air-exposed rats. The lymph nodes were minced with a set of fine-tipped scissors and then pushed through a sterile wire mesh in order to disperse LNC from tissue, and the resulting cells were suspended in warm PBS (−). The suspension was rapidly filtered through a nylon mesh to sieve a mass of residual tissue (200 mesh, Ake Science, Chiba, Japan). Cells were collected by centrifugation at 400g for 10 min and resuspended in R10. The numbers of viable cells were determined using the trypan blue exclusion method.

Differential bronchoalveolar lavage cell counts. The lavage fluid was centrifuged at 400g for 10 min and resuspended in R10, and total cells were counted with a hemacytometer. Cell viability was assayed by the trypan blue exclusion method. Differential cell counts were determined by Diff-Quik (International Reagents Co., Kobe, Japan) stain after cyto spin preparation.

Protocol of the effect of O$_3$ exposure on BAL cell-mediated suppression. BAL cells were prepared from rats exposed to air or 1 ppm O$_3$ for 3 days (Air-BALF and O$_3$-BALF, respectively). All LNC were prepared from rats exposed to air for 3 days. Aliquots of 4 × 10$^6$ LNC were placed (in triplicate) into wells of a 96-well flat-bottom plate (NuncIon, Denmark) in 190 μl R10 containing 10 μg/ml Con A, and 10-μl aliquots of various numbers (0.075 to
20% vs LNC) of BAL cells in R10 were added to each well. These cell cultures were incubated for 72 h in a humidified atmosphere at 37°C in a 5% CO₂ incubator. Cell proliferation was measured by incorporation of BrdU added 24 h before the measurement. After incubation, culture supernatant was collected for measurement of nitrite content as an indicator of NO production.

**Protocol of the effect of BALF on AM-mediated suppression.** BALF were prepared from rats exposed to air or 1 ppm O₃ for 3 days 24 h before the measurement. After incubation, culture supernatant was collected for measurement of nitrite content as an indicator of NO production.

**Measurement of cell proliferation.** Cell proliferation was measured by a sandwich-type enzyme immunoassay using a monoclonal anti-BrdU antibody. This technique is based on the incorporation of pyrimidine analogue BrdU instead of thymidine into the DNA of proliferating cells. BrdU incorporated into DNA is measured by a sandwich-type enzyme immunoassay using a monoclonal anti-BrdU antibody. This technique is at least as sensitive as the traditional counting of [³H]thymidine (Porstmann et al., 1985). Cell proliferation was measured by adding 20 μl of 100 μM BrdU to each well 24 h before the measurement. After incubation, cells were fixed and DNA denatured by adding FixDenat (200 μl/well) and incubating the plate for 10 min, and the culture medium was removed. Then the cells were fixed and DNA denatured by adding FixDenat (200 μl/well) and incubating the plate for 30 min at room temperature. FixDenat solution was decanted from wells and residual solution was removed thoroughly by tapping the plate on clean paper towel, anti-BrdU-peroxidase (100 μl/well) added, and the plate incubated for 90 min at room temperature. Excess antibody conjugate was removed by decantation and wells were rinsed three times with washing solution (PBS) (200 μl/well). Washing solution was decanted from wells and residual solution was removed thoroughly by tapping the plate on clean paper towel, substrate (tetramethylbenzidine) (100 μl/well) was added, and the plate was incubated for 30 min at room temperature. Substrate reaction was stopped by adding 1 M sulfuric acid (25 μl/well) and mixing. Absorbance of the samples was measured in an ELISA reader (ImmunoReader NJ-2000, Inter Med, Tokyo, Japan) at a wavelength of 450 nm (reference wavelength: 620 nm).

**Measurement of NO production.** LNC were cultured with Con A in R10, Air-BALF, or O₃-BALF in the presence or the absence of AM as described in the protocols. Culture supernatant was collected to measure NO production. NO was measured as nitrite (NO₃⁻) in the culture supernatant using the Griess reagent. Culture supernatant (50 μl) was mixed with 50 μl of 1% sulfanilamide, 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, and 2.5% H₃PO₄ in the wells of a 96-well flat-bottom plate and incubated for 10 min at room temperature. Absorbance was measured in an ELISA reader at a wavelength of 540 nm. The values measured were compared with the standard curves, which were established with known amounts of NaNO₃ in R10.

**RESULTS**

**Differential Bronchoalveolar Lavage Cell Counts**

Table 1 shows that the number of AM collected after exposure to O₃ for 3 days increased by approximately 50% over the number in control rats. The differential counts of BAL cells revealed that cells in O₃-BALF consisted of 72 ± 6% AM, 22 ± 5% neutrophils, 4 ± 1% eosinophils, and 2 ± 1% lymphocytes, whereas BAL cells from control rats were >99% AM.

**Effect of O₃ Exposure on BAL Cell-Mediated Suppression of LNC Proliferation**

Graded numbers of BAL cells were added to LNC cultures. Figure 1 shows that Con A-induced LNC proliferation was suppressed by addition of BAL cells from both air- and O₃-exposed rats. This suppression depended on the number of BAL cells added to the LNC cultures. BAL cells, at numbers equivalent to between 5 and 20% of the LNC, almost completely suppressed Con A-induced LNC proliferation. BAL cells from O₃-exposed rats also suppressed Con A-induced LNC proliferation in the same manner as BAL cells from air-exposed rats.

**Effect of O₃ Exposure on NO Production by BAL Cells**

To confirm that suppression of Con A-induced LNC proliferation in this system is associated with release of NO into the

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**TABLE 1**

| Differential Bronchoalveolar Lavage Cell Counts |
|-----------------|-----------------|------------------|
|                 | Air             | Ozone            |
| Alveolar macrophages | >99% (2.28 X 10⁶/µl) | 72 ± 6% (3.42 ± 0.29 X 10⁶/µl) |
| Neutrophil        | 22 ± 5% (1.04 ± 0.24 X 10⁶/µl) | 4 ± 1% (0.18 ± 0.04 X 10⁶/µl) |
| Eosinophil        | 4 ± 1% (0.18 ± 0.04 X 10⁶/µl) | 2 ± 1% (0.07 ± 0.04 X 10⁶/µl) |

**Note.** Differential bronchoalveolar lavage cell counts. Results are the mean ± SE of data from four individual rats. * and * represent percentages of total cells and cell number, respectively.
cultures, we measured the NO\textsuperscript{2} content in the supernatants of the cultures. Figure 2 shows NO production in the LNC:BAL cell cocultures. The NO production was increased by addition of BAL cells from both air- and O\textsubscript{3}-exposed rats. The level of NO production depended on the number of BAL cells added to the LNC cultures.

Effect of BALF on AM-Mediated Suppression of LNC Proliferation

In R10, AM suppressed LNC proliferation induced by Con A (Fig. 3). AM-mediated suppression of LNC proliferation was inhibited by O\textsubscript{3}-BALF, but not by Air-BALF. Figure 3 also shows that Con A-induced proliferation of LNC was directly suppressed by O\textsubscript{3}-BALF by approximately 40%. Air-BALF, however, had no effect on Con A-induced proliferation of LNC. In the presence of O\textsubscript{3}-BALF, the addition of AM to the coculture resulted in no further suppression of LNC proliferation.

Effect of BALF on NO Production by AM

We examined whether changes in the microenvironment of the alveoli by O\textsubscript{3} exposure potentially affected NO production by AM. Figure 4 shows that in vitro NO production by AM from air-exposed rats was markedly inhibited by O\textsubscript{3}-BALF. Air-BALF did not inhibit NO production by AM.

DISCUSSION

In this study, we demonstrated that O\textsubscript{3}-induced changes in the microenvironment of alveoli affected AM-mediated immunosuppressive activity. Suppression of Con A-induced LNC proliferation depended on the number of BAL cells added to the LNC cultures (Fig. 1). NO production was directly related to the number of BAL cells present (Fig. 2). Recently, several mediators have been suggested as potential causes of macrophage-mediated suppression of lymphocyte proliferation, including NO (Kawabe et al., 1992; Metzger et al., 1980; Schleifer and Mansfield, 1993; Upham et al., 1995).
O sub 3 inhibits suppressive activity of AM

The present results are consistent with a major role for NO in AM-mediated suppressive activity. It should also be noted that O sub 3 exposure increased the number of neutrophils in BALF (Table 1). It has been also shown that high-density cells which look like monocytes are increased in BAL by O sub 3 exposure (Mochitate and Miura, 1989). It is accordingly possible that such changes in the BAL cell population may affect the overall level of suppressive activity and NO production in the lung. Cytokines and mediators released from neutrophils and other types of cells also could affect suppressive activity and NO production of AM. To our knowledge, however, there are no reports in the literature which compare the suppressive activity and NO production of AM with those of neutrophils or monocytes. Figures 1 and 2 also show that BAL cells from O sub 3-exposed rats suppressed Con A-induced LNC proliferation and produced NO in the same manner as AM from air-exposed rats, but direct change in the BAL cells induced by O sub 3 exposure did not have major effects on suppressive activity and NO production. Figure 1 and Table 1 also suggested that AM from O sub 3-exposed rats might be more suppressive than those from air-exposed rats, since the absolute number of AM in under-loading BAL cells from O sub 3-exposed rats was less than that of air-exposed rats.

Air-BALF did not inhibit the AM-mediated suppression of LNC proliferation (Fig. 3). Figure 3 also shows that Con A-induced proliferation of LNC was directly suppressed by O sub 3-BALF by approximately 40%, but not by Air-BALF. However, in the presence of O sub 3-BALF, no further suppression of LNC proliferation was observed by addition of AM to the cocultures, whereas normal AM in the absence of O sub 3-BALF cause complete suppression of proliferation (Fig. 3). Therefore, our results suggested that AM-mediated suppressive activity of Con A-induced LNC proliferation was inhibited by O sub 3-BALF completely. It has been reported that in the presence of NO synthase inhibitor, N-O-monomethylarginine acetate, NO sub 3 levels were completely reduced, and in accordance with this, the suppression of T-cell proliferation was almost completely inhibited (Upham et al., 1995). Figures 1 and 2 also show that NO production was inhibited under 10 μM NO sub 3 in the culture medium, proliferation of LNC was not suppressed. Figure 4 shows that NO production was inhibited by O sub 3-BALF under 7 μM NO sub 3 in the culture medium. These results also supported the finding that AM-mediated suppressive activity was inhibited by O sub 3-BALF completely.

It is important to identify the factor in O sub 3-BALF which inhibits AM-mediated immunosuppressive activity and also Con A-induced LNC proliferation. In this regard, it has been shown that LPS-stimulated lung-conditioned medium (LCM) inhibits the AM-mediated suppressive activity of T-cell proliferation (Bilyk and Holt, 1993). This activity of LCM is blocked by an antibody specific for granulocyte/macrophage colony-stimulating factor (GM-CSF) and is reproduced by recombinant GM-CSF, which inhibits NO production (Bilyk and Holt, 1995). O sub 3 is a potent respiratory irritant known to induce pulmonary inflammation or lung injury in humans and experimental animals (Balms, 1993; Chen et al., 1995; Koren, 1995; Laskin et al., 1994; Peden et al., 1995; Pendino et al., 1996) and O sub 3 exposure increases GM-CSF in BALF (Balms et al., 1996; Scannell et al., 1996). Therefore, GM-CSF could be one of the factors underlying the inhibitory effect of O sub 3-BALF on AM-mediated immunosuppressive activity.

NO production from macrophages is regulated by various factors. Inducible NO synthesis in macrophages is up-regulated by interferon-γ (IFN-γ), TNF-α, interleukin (IL)-1β, and IL-2 and down-regulated by IL-4, IL-10, prostaglandin (PG) E 2, and transforming growth factor-β (TGF-β) (Al-Ramadi et al., 1992; Cunha et al., 1992; Gutierrez et al., 1995; Modolell et al., 1995; Schleifer and Mansfield, 1993; Warner et al., 1995; Bilyk and Holt, 1995). One or more of these cytokines may also be involved in the effects of O sub 3 in this system. Without LNC, AM from air- or O sub 3-exposed rats did not produce NO (data not shown). Some cytokines such as IFN-γ released from LNC could induce NO production by AM. Our preliminary study shows that O sub 3-BALF inhibited both IFN-γ production from LNC induced by Con A and NO production from AM induced by IFN-γ (data not shown).

It has also been shown that phospholipid (Bartmann et al., 1992) and pulmonary surfactant-associated protein A (SP-A) suppress phagocytosis-mediated lymphocyte proliferation. In contrast, Con A-activated lymphocyte proliferation is slightly augmented by SP-A (Boron et al., 1996). Dipalmityl-1-α-phosphatidylcholine and Suravanta as phospholipids suppress Con A-induced lymphocyte proliferation, which is reversed somewhat by addition of SP-A (Kremlev et al., 1994). It has been shown that TGF-β also decreased both Con A-induced T-cell proliferation and IL-2 mRNA expression (Ahmad et al., 1997). Lung inflammation, such as that which is O sub 3 exposure-induced, may alter the relative amounts of surfactant components and cytokines, such as TGF-β. These possibilities will be investigated in subsequent experiments.

It has been shown that the AM suppress the antigen-presenting activity of lung DC (Holt et al., 1993), antigen-induced proliferation of T-cells (Bilyk and Holt, 1993), as well as Con A-induced proliferation of T-cells. Moreover, in vivo elimination of AM increases IgE responses to inhaled antigen (Thelen et al., 1992). Therefore, inhibition of AM-mediated immunosuppressive activity by O sub 3 exposure might be linked with an enhancement in IgE and IgG production. The number of IgE-containing cells following allergen inhalation in O sub 3-exposed mouse lung was larger than that in air-exposed mouse (Gershwin et al., 1981). Conflicting results, however, also have been shown previously (Ozawa et al., 1985). This disparate observation depends on the experimental design used (duration and concentration of O sub 3 exposure, animal species, and so on). Therefore, systematic studies are necessary to clarify the relationship between IgE production and inhibition of AM-mediated suppressive activity by O sub 3 exposure.
Localization of AM, T-cells, and DC in peripheral lung is important in understanding how T-cell activation in the lung in vivo may be controlled by the microenvironment of the alveoli. Lung digestes contained significant numbers of Ia+ DC and T-cells, which were rarely found in BAL cells. A large population of T-cells was identified within the alveolar septa (Holt and Schon-Hegrad, 1987). The T-cell population is at least five times larger than the peripheral blood T-cell pool (Holt et al., 1985). A DC-like population and also Ia+, but differing from the airway DC were identified within the alveolar septal walls. Therefore, major populations of T-cells and DC present in rat lung were in close proximity to AM located in alveoli. These observations suggest that T-cell activation by interactions between DC and AM in the lung in vivo may be controlled by changes in the microenvironment in the alveoli.

In summary, AM-mediated immunosuppressive activity plays an important role in maintaining immunological homeostasis in the lung. Our present results suggest that AM-mediated immunosuppressive activity is inhibited by changes in the microenvironment of the alveoli induced by O₃ exposure. Therefore, the mechanisms underlying lung inflammation induced by O₃ exposure may involve not only damage of host tissue induced by the direct toxic effects of O₃ or lung stromal and/or mesenchymal cells, but also indirect tissue damage caused by disruption of control mechanisms which regulate the local activation of T-cells.

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