Regulation of alveolar macrophage and type II cell DNA synthesis: effects of ozone inhalation

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Prokhorova, Svetlana, Naimish Patel, and Debra L. Laskin. Regulation of alveolar macrophage and type II cell DNA synthesis: effects of ozone inhalation. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L1200–L1207, 1998.—A characteristic reaction of the lung to inhaled ozone is an increase in the number of type II epithelial cells and alveolar macrophages (AMs). In the present study, we analyzed mechanisms regulating this response. Acute exposure of rats to ozone (2 parts/million, 3 h) induced expression of proliferating cell nuclear antigen, a marker of cellular proliferation, in both type II cells and AMs. This was maximum 48 h after ozone inhalation. Type II cells and AMs isolated from treated rats at this time also incorporated significantly more [3H]thymidine ([3H]Tdr) than cells from control animals. When type II cells and AMs were cocultured, a synergistic increase in [3H]Tdr incorporation by both cell types was observed. This appeared to be due to increased DNA synthesis by both cell types. Thus [3H]Tdr incorporation by type II cells and AMs cocultured with mitomycin C-treated AMs and type II cells, respectively, was elevated compared with cells cultured alone. Type II cells and AMs plated onto tissue culture inserts, as well as culture supernatants from these cells, were found to stimulate DNA synthesis in AMs and type II cells, respectively. In addition, crude membrane preparations from these cells exhibited growth-promoting activity. Thus the mitogenic effects of both cell types appeared to be mediated by soluble factors and membrane-associated molecules. Ozone inhalation resulted in an increase in the mitogenic activity of AMs treated with mitomycin C and plated on tissue culture inserts toward type II cells and of type II cell culture supernatants toward AMs. These data suggest that type II cell and AM proliferation contributes to the regulation of the number of cells in the lung under normal homeostatic conditions and after ozone-induced injury. Moreover, type II cells and AMs produce paracrine mediators that contribute to cellular proliferative responses.

Proliferating cell nuclear antigen expression; deoxyribonucleic acid synthesis; lung cells

ACUTE INHALATION OF OZONE results in damage to type I epithelial cells in centriacinar regions of the lung (15, 30). In response to this injury, type II cells begin to proliferate and subsequently differentiate into type I cells, eventually restoring the integrity of the alveolar epithelium (8, 10a, 15). The mechanisms regulating type II cell proliferation after lung injury are poorly understood. Macrophages are known to express growth factors, such as transforming growth factor-α, fibroblast growth factor, and hepatocyte growth factor (4, 27, 34), that stimulate DNA synthesis in adult type II cells (3, 4, 13, 18) and may contribute to growth regulation. This is supported by the finding that culture supernatants from alveolar macrophages (AMs) are mitogenic for type II cells (3, 4, 12), an activity that is augmented after exposure of the cells to pulmonary toxicants (4, 20).

Ozone exposure also results in increased numbers of macrophages in areas of tissue injury (17, 24, 30). Although this may be due to an influx of blood monocytes into the alveolar space, local proliferation of resident AMs may also contribute to this response. In this regard, increased AM labeling and mitotic indexes (10, 35), as well as colony-forming ability (2), have been described after ozone inhalation. Studies on the regulation of AM proliferation have been limited. Colony-stimulating factors, such as macrophage and granulocyte-macrophage colony-stimulating factors (M-CSF and GM-CSF, respectively), which are potent mitogens for AMs (14, 21), have been reported to be produced by type II cells (1, 5, 33). This observation suggests a potential role of these cells in macrophage growth. The present study was aimed at analyzing cellular mechanisms regulating AMs and type II cell proliferation in the lung under normal homeostatic conditions and after acute ozone exposure. We speculate that these cells release factors that contribute to growth in a paracrine manner.

MATERIALS AND METHODS

Animals and exposures. Female specific pathogen-free Sprague-Dawley rats (200–225 g) were obtained from Taconic (Germantown, NY). Animals were maintained on food and sterile water ad libitum and were housed in microisolation cages. Rats were exposed to ozone or ultrapure air in 5.5-ft³ airtight Plexiglas chambers for 3 h. Ozone was generated from oxygen gas via an ultraviolet-light ozone generator (Orec, Phoenix, AZ) and mixed with air. Ozone concentrations were monitored with an ozone monitor (model 1008 AH, Dathibi Environmental, Glendale, CA).

Reagents. Elastase was purchased from Worthington (Freehold, NJ) as an aqueous suspension. DNase I, purified rat IgG, and mitomycin C were from Sigma (St. Louis, MO). Fetal bovine serum (FBS) containing <0.3 mg/ml of endotoxin was obtained from Atlanta Biologicals (Norcross, GA) and heat inactivated at 56°C for 45 min before use. Cell isolation. AMs were obtained from perfused lung by lavage as previously described (11, 25). Cells were >95% viable as determined by trypsin blue dye exclusion. In some experiments to eliminate contaminating neutrophils, lavage cells were layered onto Ficoll-Paque (density 1.077 g/ml; Pharmacia, Piscataway, NJ) and centrifuged at 1,000 g at 4°C for 20 min. AMs were collected at the gradient interface. Cells were cultured in DME-containing 10% FBS, 100 U/ml of penicillin, 100 mg/ml of streptomycin, and 0.25 mg/ml of fungizone (complete DME). Type II cells were isolated from lavaged lung by elastase digestion, followed by selection of nonadherent cells on rat IgG-coated plastic petri dishes (7). Cell purity assessed by modified Papanicolaou staining (6) was >85% and viability was >90% as determined by trypan
Cells were plated in 24-well dishes (2.5–3 × 10⁵ cells/well) in complete DMEM. After 24 h of culture, nonadherent cells were removed by aspiration, and the cells were refed with fresh medium.

Preparation of cell culture supernatants. AMs or type II cells plated in 24-well dishes (2 × 10⁵ cells/well) were incubated at 37°C for 1 and 24 h, respectively. The medium was then replaced with 0.5 ml of DMEM without FBS. After an additional 48-h incubation, supernatants were collected and concentrated with Centriplus-10 (Amicon, Beverly, MA) for type II cells or Centriplus-3 for AMs. Supernatants were then filtered through a 0.22-mm filter and stored in aliquots at −20°C until analysis.

Isolation of cell membranes. Crude membrane preparations were prepared as previously described (31). Type II cells (4 × 10⁶) cultured for 48 h or freshly isolated AMs were suspended in buffer containing 10 mM Tris·HCl, 1 mM MgCl₂, and 1 mM phenylmethylsulfonyl fluoride (pH 7.4). After incubation on ice for 1 h, the cells were homogenized, mixed with sucrose (final concentration of 0.3 M), and centrifuged at 500 g at 4°C for 15 min to remove nuclei. The supernatants were then centrifuged at 100,000 g at 4°C for 60 min, and the membrane fractions were collected. Membranes were resuspended in 100 ml of complete DMEM and stored at −70°C until analysis.

Measurement of DNA synthesis. Type II cells (2 × 10⁶ cells/well) and AMs (2 × 10⁵ cells/well) plated in 24-well dishes alone or in combination were incubated in 0.5 ml of complete DMEM in the presence of 1 mCi/ml of [³H]thymidine ([³H]TdR; specific activity 2 Ci/mmol; NEN Research Products, Wilmington, DE). After 48 h, the cells were harvested onto glass fiber filters with a PHD cell harvester (Cambridge Technology, Watertown, MA) and counted for radioactivity.

Autoradiography. Type II cells were plated on eight-well culture chamber slides (1 × 10⁶ cells/well; Nunc, Naperville, IL) in 0.25 ml of complete DMEM. After incubation for 24 h, the medium was replaced, and the cells were cultured with or without freshly isolated AMs (1 × 10⁵ cells/well) and 1 mCi/ml of [³H]TdR. Forty-eight hours later, the cells were washed, fixed with 2.5% glutaraldehyde, and air-dried. The slides were covered with NBT2 emulsion (Eastman Kodak, Rochester, NY) and exposed for 1 wk in the dark.

Immunocytochemical localization of proliferating cell nuclear antigen. The lungs were perfused and then fixed by intratracheal instillation of 10% buffered Formalin at 25 cmH₂O pressure. Sections (6 µm) prepared from paraffin-embedded lungs were preincubated in 0.01 M citrate buffer, pH 6.0, for 10 min at 95°C and then blocked with 10% normal goat serum for 10 min. The sections were then incubated with a 1:50 dilution of mouse IgG2a monoclonal antibody against proliferating cell nuclear antigen (PCNA; Zymed Laboratories, South San Francisco, CA) or nonimmune mouse IgG2a for 1 h. A Histostain kit (Zymed) was used to visualize antibody binding.

Statistics. All experiments were repeated at least three times. Data were analyzed by Student’s t-test or one-way ANOVA and Fisher’s least significance difference test. A P value of <0.05 was considered to be significant.
RESULTS

Effects of ozone inhalation on PCNA expression and AM and type II cell number. Acute exposure of rats to ozone resulted in a time-dependent increase in expression of PCNA in type II cells, which was localized immunohistochemically in lung tissue sections (Fig. 1). This was evident 12 h after ozone exposure (Fig. 1C) and reached a maximum at 48 h (Fig. 1E). At this time, cells were hypertrophic and cuboidal in shape, whereas by 72 h postexposure, they appeared more squamous (Fig. 1F). The type II cell proliferative response was limited to proximal alveolar regions of the lung, which represent areas most susceptible to ozone-induced injury (15, 30). AMs were also found to express PCNA after ozone exposure (Fig. 1); however, only limited numbers of these cells could be identified in the tissue, most likely due to their loss during the fixation procedure. No PCNA expression was observed in either type II cells or AMs in the lungs of air-exposed animals (Fig. 1B). Ozone inhalation was also associated with an increase in the number of AMs recovered by lung lavage, a response that persisted for at least 96 h (Table 1). In contrast, similar numbers of type II cells (15–18 × 10^6 cells/animal) were isolated from air- and ozone-exposed animals.

DNA synthesis in isolated AMs and type II cell cultures. We next examined the effects of ozone inhalation on DNA synthesis in AMs and type II cells by quantifying [3H]TdR uptake. We found that both AMs and type II cells isolated 48 h after ozone exposure incorporated significantly more [3H]TdR than did cells from air-exposed rats (Fig. 2). Furthermore, [3H]TdR uptake by cocultures of AMs and type II cells was synergistically increased. Uptake of [3H]TdR was greater in cocultures containing AMs from ozone-exposed animals compared with air-exposed animals. In contrast, a similar increase in [3H]TdR incorporation was observed in cocultures containing type II cells from air- and ozone-exposed rats (Fig. 2). Elimination of neutrophils from the AM population by Ficoll gradient centrifugation had no effect on [3H]TdR uptake by AMs cultured alone or with type II cells (data not shown).

Effects of AMs on type II cell DNA synthesis. To analyze the mitogenic effects of AMs on type II cells, we pretreated AMs with the DNA synthesis inhibitor mitomycin C before their coculture with type II cells. This treatment inhibited [3H]TdR uptake by AMs (Fig. 3), allowing us to evaluate type II cell DNA synthesis in the cocultures. In the presence of mitomycin C-treated AMs, [3H]TdR uptake by type II cells was increased compared with type II cells cultured alone (Fig. 3A). However, this increase was less than that observed in cocultures of untreated AMs and type II cells (compare Figs. 2 and 3A). Ozone inhalation resulted in an increased capacity of mitomycin C-treated AMs to stimulate [3H]TdR uptake by type II cells from control animals. However, the response of type II cells from ozone-exposed rats to AMs from treated and untreated animals was similar. The results of these experiments were confirmed by autoradiography that allowed visual identification of cells actively synthesizing DNA (Fig. 3B). As observed in our [3H]TdR uptake studies, type II cell DNA synthesis was increased after ozone inhalation and upregulated in response to AMs (Fig. 3). After mitomycin C treatment, no labeling was observed in AMs (data not shown).

We next determined whether cell-cell contact was required for AM-induced mitogenesis. In these experiments, AMs were separated from type II cells by plating them on tissue culture inserts (1-mm pore size; Falcon, Franklin Lakes, NJ). Under these conditions, AMs retained their capacity to stimulate type II cell DNA synthesis (Fig. 4A). However, the level of type II cell [3H]TdR incorporation was less than that observed in cocultures of type II cells and AMs without inserts (compare Figs. 2 and 4A). In accord with our experiments with mitomycin C-treated cells, AMs isolated from ozone-treated rats were more effective in stimulating control type II cell [3H]TdR uptake than were AMs from control rats. These results suggest that the mitogenic response of type II cells is mediated, at least in part, by soluble factors released from AMs. To further

Table 1. Effect of ozone inhalation on number of macrophages recovered by lavage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Cells, × 10^6</th>
<th>%Macrophage</th>
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<tbody>
<tr>
<td>Air</td>
<td>0.92 ± 0.08</td>
<td>&gt;99</td>
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<tr>
<td>Ozone</td>
<td></td>
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<tr>
<td>12 h</td>
<td>0.94 ± 0.06</td>
<td>88.2</td>
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<tr>
<td>24 h</td>
<td>1.13 ± 0.08</td>
<td>87.9</td>
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<tr>
<td>48 h</td>
<td>1.56 ± 0.07</td>
<td>92.0</td>
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<tr>
<td>96 h</td>
<td>1.72 ± 0.19</td>
<td>&gt;99</td>
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Values are means ± SE of 4–5 experiments. Cells were isolated by lavage from control animals and animals 12, 24, 48, or 96 h after ozone exposure and counted with a hemocytometer. Percentage of cells that were macrophages (%Macrophage) was quantified in cytospin slides stained with Giemsa. *Significantly different from air-exposed animals (P < 0.05).
analyze this possibility, we examined the effects of AM culture supernatants on type II cell $[^3H]$Tdr uptake. Culture supernatants from AMs diluted 1:8 (Fig. 5A) and 1:4 (data not shown) were found to contain mitogenic activity for type II cells. However, this activity was limited to type II cells from control animals. Furthermore, AM culture supernatants from air- and ozone-exposed animals were equally effective in stimulating type II cell $[^3H]$Tdr uptake.

To determine whether cell-cell contact potentiated macrophage-mediated mitogenesis, we cultured type II cells in the presence of crude membrane fractions prepared from AMs. The addition of AM membranes to type II cells resulted in a 25–35% stimulation of DNA synthesis in type II cells from both air- and ozone-exposed animals (Fig. 6A). Ozone inhalation had no

Fig. 4. Mitogenic activity of type II cells and AMs plated on tissue culture inserts (ins). Type II cells (A) and AMs (B) isolated 48 h after exposure of rats to air (open bars) or O$_3$ (solid bars) were cultured with $[^3H]$Tdr in absence and presence of AMs or type II cells from air- and O$_3$-exposed rats plated on tissue culture inserts. After 48 h, tissue culture inserts were removed and $[^3H]$Tdr uptake was quantified. Values are means ± SE of triplicate samples from 1 of 3 similar experiments. Significant difference compared with: *cells cultured alone; †type II cells cocultured with AMs from air-exposed rats (Student's t-test).

Fig. 3. Effects of mitomycin C (mit) on mitogenic activity of type II cells or AMs. Type II cells (A and B) and AMs (C) isolated 48 h after exposure of rats to air (open bars) or O$_3$ (solid bars) were cultured with $[^3H]$Tdr in absence and presence of mitomycin C-treated AMs or type II cells from air- and O$_3$-exposed rats. Cells were cultured with mitomycin C (25 µg/ml, 20 min, 37°C) and washed before cultivation. A and C: $[^3H]$Tdr uptake. Values are means ± SE of 4 experiments. $[^3H]$Tdr uptake by mitomycin C-treated AMs from air- and O$_3$-exposed animals was 100 ± 70 and 500 ± 130 cpm, respectively. $[^3H]$Tdr uptake by mitomycin C-treated type II cells from air- and O$_3$-exposed animals was 300 ± 50 and 300 ± 100 cpm, respectively. Significant difference compared with: *untreated cells cultured alone; †type II cells cocultured with AMs from air-exposed rats. Data were analyzed by paired Student's t-test. B: autoradiography of 10 uniform fields randomly selected for counting. Each value is mean ± SD of no. of labeled nuclei per field for duplicate samples from 1 of 3 similar experiments.
When they were plated onto tissue culture inserts (Fig. 4B). We also found that culture supernatants from type II cells stimulated DNA synthesis in AMs (Fig. 5B). These effects were dose dependent over a range of 1:16 to 1:2 dilutions of culture supernatants (Fig. 7). Culture supernatants from type II cells from ozone-exposed animals were more mitogenic for control AMs than type II cell supernatants from control animals (Fig. 7A). In contrast, these culture supernatants induced similar increases in [3H]Tdr uptake in AMs from ozone-treated rats (Fig. 7B). Crude preparations of type II cell supernatants induced increases in [3H]Tdr uptake in AMs (Fig. 7C).

Effect on the ability of AM membranes to stimulate type II cell DNA synthesis.

Effects of type II cells on AM DNA synthesis. In further studies, we analyzed the effects of type II cells on AM DNA synthesis. Initially, type II cells were treated with mitomycin C to block DNA synthesis. AMs cocultured with mitomycin C-treated type II cells were found to incorporate significantly more [3H]Tdr than AMs cultured alone (Fig. 3C). No major differences were noted between the AM mitogenic activity of type II cells isolated from air- and ozone-exposed animals. As observed with AMs, the effects of type II cells on AM DNA synthesis did not require cell-cell contact. Thus type II cells were also found to be mitogenic for AMs when they were plated onto tissue culture inserts (Fig. 4B). We also found that culture supernatants from type II cells stimulated DNA synthesis in AMs (Fig. 5B). These effects were dose dependent over a range of 1:16 to 1:2 dilutions of culture supernatants (Fig. 7). Culture supernatants from type II cells from ozone-exposed animals were more mitogenic for control AMs than type II cell supernatants from control animals (Fig. 7A). In contrast, these culture supernatants induced similar increases in [3H]Tdr uptake in AMs from ozone-treated rats (Fig. 7B). Crude preparations of type II cell supernatants induced increases in [3H]Tdr uptake in AMs (Fig. 7C).
membranes were also found to stimulate \[^{3}H\]TdR uptake by AMs from both air- and ozone-exposed animals (Fig. 6B). Membranes from ozone-treated rats were more mitogenic than membranes from air-exposed animals for control AMs.

**DISCUSSION**

In the present study, cellular mechanisms regulating increases in type II cell and AM numbers after ozone-mediated acute lung injury were investigated. The proliferative response of type II cells induced by ozone exposure has been reported to occur within 24 h, reaching a maximum by 48 h, and represents an attempt by the host to restore damaged alveolar epithelium (8, 30, 35). Consistent with this observation, we found that 12 h after acute ozone exposure, type II cells located in the proximal alveolar regions of the lung began to express PCNA, a marker of cellular proliferation (16). Moreover, by 48 h postexposure, the epithelium of these regions was, for the most part, composed of PCNA-positive cells. Our findings that PCNA staining remained elevated for 72 h after histological evidence that injury was resolved suggests that this may be a highly sensitive marker of ongoing tissue repair.

In accord with the increases in PCNA labeling observed in histological sections, type II cells isolated 48 h after ozone inhalation were found to incorporate more \[^{3}H\]TdR than cells from control animals. Despite morphological evidence of increased type II cell number, we did not isolate increased numbers of these cells from the lung after ozone exposure, which may be due to the relatively limited proportion of cells affected by ozone.

Inhalation of ozone also resulted in increased numbers of AMs recovered by lavage 48 h after exposure that was accompanied by expression of PCNA in situ and elevated DNA synthesis in vitro. These data are consistent with reports of a higher labeling index, mitotic index, and colony-forming ability of AMs from ozone-treated animals and support the concept that proliferation of these cells plays a role in augmenting AM number after ozone inhalation (2, 10, 35).

When type II cells and AMs from both air- and ozone-exposed animals were cocultured, \[^{3}H\]TdR uptake was synergistically increased. This appeared to be due to increased DNA synthesis by both cell types. Thus AMs and type II cells exhibited elevated \[^{3}H\]TdR uptake when cocultured with mitomycin C-treated type II cells and AMs, respectively. In addition, both AMs and type II cells were mitogenic when cocultured with cells plated on tissue culture inserts. These findings suggest that the proliferative responses of both AMs and type II cells in the lung are influenced by interactions between these two cell types.

AMs from ozone-treated rats were found to induce a greater increase in \[^{3}H\]TdR uptake by type II cells than AMs from control animals. These data are in accord with increased release of type II cell mitogenic activity from AMs described after silica exposure (19, 20) and suggest that activation of AMs for production of type II cell growth-promoting factors may be a common response to lung injury. Increased release of type II cell mitogenic activity from AMs was also observed after ozone inhalation when AMs were cultured on tissue culture inserts. In contrast, no major differences were noted in the mitogenic effects of AM culture supernatants from air- and ozone-exposed animals. It may be that the increased mitogenic activity of AMs after ozone exposure is due to the release of relatively unstable factors that act locally in vivo. A number of AM-derived growth factors have been described that stimulate type II cell DNA synthesis (3, 4, 10a, 12, 13, 18–20). Further studies are necessary to determine whether similar factors play a role in increased proliferation of type II cells after ozone-induced lung injury.

The present study also provided novel data on the generation of AM mitogenic factors by type II cells. Thus type II cells from control animals were found to stimulate DNA synthesis in AMs. These findings suggest that type II cells may regulate the pool of AMs under normal physiological conditions. Ozone inhala-
tion was associated with an increased ability of AM culture supernatants to stimulate type II cell DNA synthesis. In contrast, treatment of rats with ozone did not increase or reduce the capacity of mitomycin C-treated type II cells or type II cells plated on tissue culture inserts to induce AM mitogenesis. It may be that unstable growth inhibitory factors are also released by type II cells after ozone inhalation. These may act in concert with mitogenic factors to regulate cellular proliferation in the lung during injury and repair. Interestingly, the increased ability of type II cell culture supernatants from ozone-treated rats to stimulate AMs was limited to AMs from control animals. Similarly, the greater mitogenic response of type II cells to AMs from ozone-exposed animals was only apparent in type II cells from control rats. This may be due to activation of AMs and type II cells in vivo after ozone treatment and their inability to be further stimulated in vitro. The nature of type II cell-derived mitogenic activity is unknown. Isolated type II cells have been shown to release factors that stimulate colony formation by bone marrow cells (1). In addition, GM-CSF has been reported to be expressed by epithelial cells in lung tissue (19). These data, together with the findings that M-CSF and GM-CSF stimulate DNA synthesis in AMs (14, 21), suggest that colony-stimulating factors may play a role in type II cell-induced mitogenesis.

Crude cell membrane fractions from both AMs and type II cells were found to induce proliferation. Thus cell-cell contact also appears to contribute to mitogenesis, although we cannot exclude the possibility that soluble factors associated with the cell membrane play a role in this activity. Whereas ozone inhalation had no effect on the ability of AM membranes to stimulate type II cell proliferation, a significant increase in the mitogenic response of naive AMs to type II cell membranes from ozone-treated rats was noted. This was reduced in AMs from ozone-treated rats, which may reflect downregulation of their responsiveness after in vivo activation. Our findings that AMs and type II cell membranes exert ozone-independent effects on proliferation provide support for their role in normal homeostatic regulation of lung cell number. The specific membrane-associated molecules responsible for mitogenesis have not been elucidated. Cell adhesion molecules such as Mac-1 and intercellular adhesion molecule-1 are upregulated after ozone inhalation (23, 32) and may contribute to the increased mitogenic effects of type II cells toward naive AMs. In this regard, Mac-1 and lymphocyte function-associated antigen-1 have been reported to trigger various functional responses in target cells (22, 23, 29). Moreover, in preliminary studies, we found that anti-intercellular adhesion molecule-1 antibody blocked DNA synthesis in cocultures of macrophages and type II cells. However, these data have to be interpreted with caution because this antibody also induced the production of nitric oxide by these cells, which is known to inhibit cellular proliferation (9, 26). Type II cell membranes also contain surfactant proteins such as surfactant protein A, which has been reported to stimulate AM secretion of colony-stimulating factors (1). Thus the growth-promoting effects of the type II cell membranes might be due to upregulation of endogenous mitogenic factors in AMs. It should also be noted that type II cell membrane mitogenic activity may be mediated, in part, by low numbers of contaminating AMs in the cultures.

In summary, we have shown that, in addition to the mitogenic effects of AMs on type II cells, type II cells stimulate DNA synthesis in AMs. In both cell types, these mitogenic effects appear to be mediated by soluble and membrane-associated factors. Ozone inhalation results in increased type II cell and AM PCNA labeling in situ, as well as [3H]TdR incorporation in vitro. Moreover, after ozone inhalation, the mitogenic activity of AMs toward type II cells and of type II cell culture supernatants toward AMs increased. These data suggest that interaction between AMs and type II cells is important in the regulation of cell proliferative responses under normal homeostatic conditions and in response to ozone-induced lung injury.

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