COMPARISON OF ACUTE OZONE-INDUCED NASAL AND PULMONARY INFLAMMATORY RESPONSES

PRINCIPAL INVESTIGATORS

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Abstract — The present study was designed to compare the effects of acute ozone exposure in the nose and lungs of rats. Rats were exposed to 0.0, 0.12, 0.80, or 1.5 ppm O_3 for 6 h and were sacrificed immediately, 3, 18, 42, or 66 h after exposure. Cellular inflammatory responses were assessed by quantitating polymorphonuclear neutrophils (PMN) recovered by nasal lavage (NL) and bronchoalveolar lavage (BAL) and morphometric quantitation of PMN within the nasal mucosa and pulmonary centriacinar region. Rats exposed to 0.12 ppm O_3 had a transient nasal PMN response 18 h after exposure but no increase in pulmonary PMN. Rats exposed to 0.8 ppm O_3 had a marked increase in nasal PMN immediately after exposure but the number of PMN within the nasal cavity decreased as the number of pulmonary PMN increased with time after exposure. Rats exposed to 1.5 ppm O_3 had an increase in pulmonary PMN beginning 3 h post-exposure, but no increase in nasal PMN at any time. Our results suggest that at high O_3 concentrations, the acute nasal inflammatory response is attenuated by a simultaneous, competing, inflammatory response within the lung.

The centriacinar pulmonary lesion induced by acute and chronic inhalation of O_3 has been extensively characterized in several laboratory animal species, including nonhuman primates. Recently, it has been shown that both short- and long-term exposure to ambient levels of O_3 can induce significant epithelial lesions in the nasal cavity of monkeys.[1] Short-term alterations included an influx of neutrophils into the nasal mucosal epithelium. Humans may have a similar acute response to O_3, since subjects exposed briefly to 0.5 ppm O_3 had increased numbers of nasal exudate PMN for at least 22 h after exposure.[2] Since PMN influx is an acute cellular response to epithelial injury, changes in the number of inflammatory cells recovered by lavage may be a useful indicator of acute O_3 toxicity. Although both upper and lower respiratory tract epithelia are damaged by O_3, there is only one brief report in the literature[3] of a study of simultaneous lesions induced in the nose and lungs of animals exposed to O_3. Our study was designed to characterize the acute inflammatory response within the nasal cavities and lungs of rats exposed to a range of O_3 concentrations.

METHODS

Rats were exposed for 6 h to 0.0 (air controls), 0.12, 0.8, or 1.5 ppm O_3 (equivalent sea level concentrations; 0.0, 0.10, 0.66, 1.2 ppm) in whole-body inhalation chambers. Groups of rats (n = 6) from each exposure group were euthanized immediately after (0 h), or 3, 18, 42, or 66 h following the end of exposure. The nasal cavity and right lung of each rat were lavaged with saline, and then fixed and prepared for light microscopy.[4] Total and differential cell counts were performed on cells recovered by bronchoalveolar and nasal lavage. The anatomic sites that were sampled for morphometric analysis are depicted in Figure 1. The neutrophilic response in the nasal mucosal epithelium was estimated by quantitating the number of pavementing neutrophils (i.e., neutrophils in contact with the luminal surface of an endothelial cell) within blood

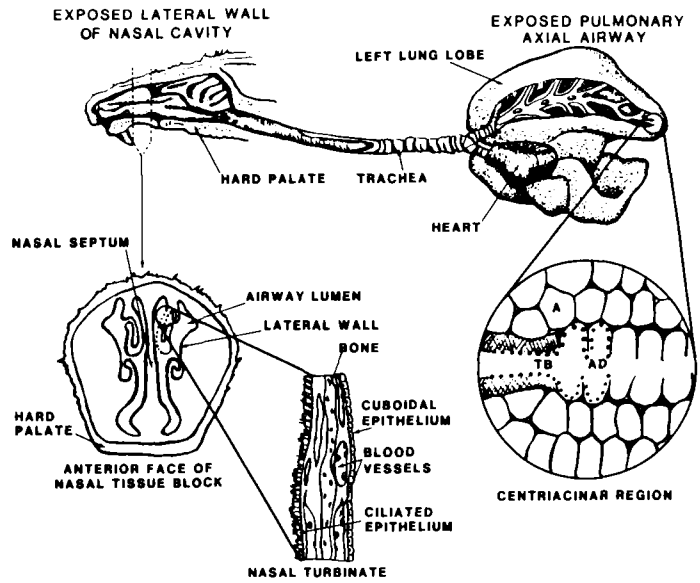

Figure 1. Sites sampled in the nasal cavity and centriacinar region of the rat. Dotted lines represent epithelial locations from which morphometric measurements were made. TB = terminal bronchiolar lumen. AD = alveolar duct lumen. A = alveolar lumen.

vessels of the region of nasal turbinate that parallels the nasal septum. Terminal bronchioles from centriacinar regions were analyzed for infiltrating PMN by quantitating the number of intramural PMN nuclear profiles per unit length of alveolar and bronchiolar basal lamina.

RESULTS

Only neutrophils and epithelial cells were recovered by nasal lavage. There were no significant differences in the number of epithelial cells recovered from any experimental group. Cells recovered by BAL were predominately macrophages and neutrophils. Compared to control animals, there was a significant increase in the number of alveolar macrophages recovered by BAL at 42 and 66 h after exposure to 0.8 and 1.5 ppm O_3.[5] The total number of PMN recovered by NL and BAL is presented in Figure 2 (A and B, respectively). The number of PMN recovered by NL and BAL

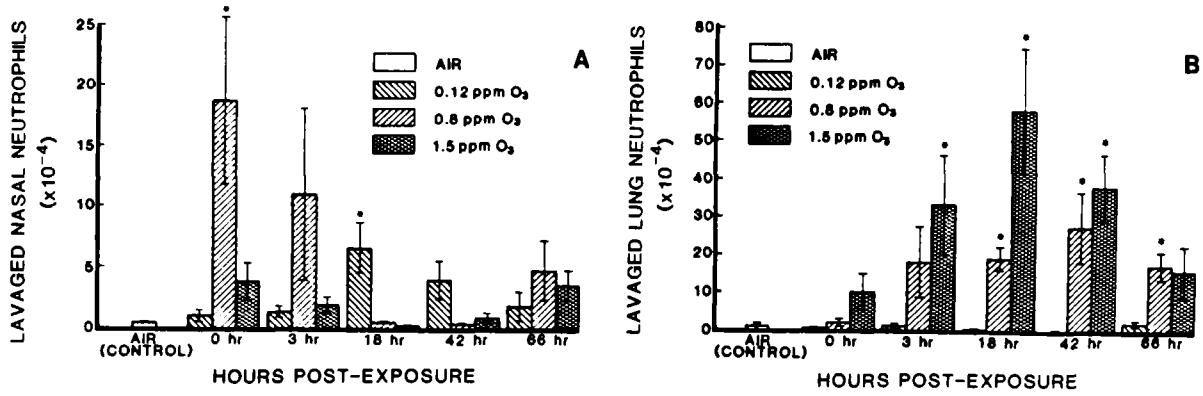

Figure 2. Mean number (± SEM) of neutrophils recovered by nasal lavage (A) and bronchoalveolar lavage (B) of rats 0, 3, 18, 42, or 66 h after a 6 h exposure to 0.0, 0.12, 0, 8, or 1.5 ppm O_3. * = significantly different than 0.0 ppm O_3-exposed (air) control group.

were affected by both the chamber O_3 concentration and the amount of time that had elapsed following exposure ($p \leq 0.05$).

There was a significant increase (15x) in the number of PMN recovered by NL 18 h after exposure to 0.12 ppm O_3, but no increase in PMN recovered by BAL at any time after exposure to this O_3 concentration (Fig. 2A and 2B). The mean number of PMN recovered by NL returned to control levels by 66 h after exposure. Compared to controls, rats exposed to 0.8 ppm O_3 had a 44X increase in PMN recovered by NL at the end of exposure, but no increase in the number of PMN in BAL fluid. Three hours after exposure, there was a decrease in the number of PMN in NL fluid, while there was a 15X increase in PMN recovered by BAL. Eighteen and 42 h after exposure, there were significantly more neutrophils in BAL fluid (16X and 23X greater than controls, respectively), while the number of PMN recovered by NL had returned to control levels. Sixty-six hours after exposure there was, again, an increase (11X) in the number of PMN in NL fluid, while the number of PMN recovered by BAL had decreased. Rats exposed to 1.5 ppm O_3 had significantly more neutrophils in BAL fluid than did air controls at 3, 18, and 42 h after exposure. However, there were no significant changes in the number of PMN recovered by NL at any time after exposure. The largest number of PMN recovered by NL were observed at the end of exposure and 66 h after exposure to 1.5 ppm O_3. These were the same times after exposure that the pulmonary PMN response was at its minimum. There was a significant ($p \leq 0.05$) interaction between the number of PMN recovered by BAL and the time after exposure on the number of PMN recovered by NL following O_3 exposure.

The number of pavementing neutrophils within nasal turbinate blood vessels and the number of intramural pulmonary neutrophils in centriacinar terminal bronchiole-alveolar duct junctions were quantitated (Fig. 3A and B, respectively). Chamber O_3 concentration, time after exposure, and an interaction of these factors significantly ($p \leq 0.05$) affected nasal mucosal and pulmonary centriacinar PMN numbers. Compared to rats exposed only to air, rats exposed to 0.12 ppm O_3 for 6 h had significantly more pavementing nasal PMN 18 and 66 h after exposure, but no increase in the number of intramural pulmonary PMN at any time after exposure. Rats exposed to 0.8 ppm O_3 had significantly greater numbers of pavementing nasal PMN immediately, 18, and 66 h after exposure, but increased numbers of intramural pulmonary PMN at only 18 and 66 h after exposure. Compared to control animals, rats exposed to 1.5 ppm O_3 had significantly more intramural pulmonary PMN at all times after exposure. There were significantly less intramural PMN by 66 h post exposure than were present immediately after exposure. Sixty-six hours after exposure was the only time that there were significantly increased numbers of pavementing PMN within nasal turbinates of rats exposed to 1.5 ppm O_3.

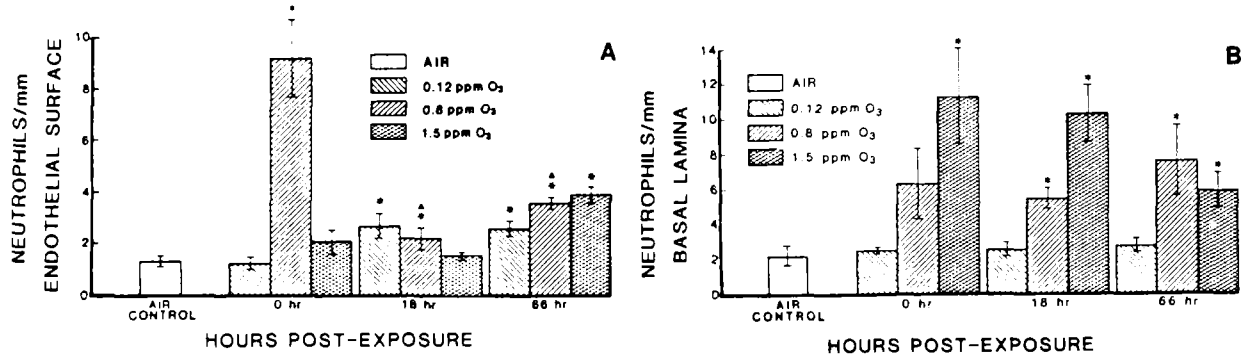

Figure 3. Morphometric quantitation of neutrophils within the nasal mucosa (A) and centriacinar region (B) of rats 0, 18, or 66 h after a 6 h exposure to 0.0, 0.12, 0.8, or 1.5 ppm O_3. Values are means ± SEM. * = Significantly different than 0.0 ppm O_3-exposed (air) control group. Δ = significantly less than 0 h value.

DISCUSSION

This study demonstrates that a single 6 h exposure to O_3, at a concentration equal to the current Air Quality Standard (0.12 ppm) or only slightly above peak excursion levels reached in several metropolitan areas (0.8 ppm), induces an acute inflammatory response within the nasal cavity of rats. The inflammatory lesion was restricted to the anterior portion of the nasal cavity and was characterized by an influx of neutrophils in the medial tips and lateral surfaces of the nasal and maxilloturbinates covered by nonciliated, cuboidal epithelia with scant epithelial mucosubstances. These data support the hypothesis that the observed attenuation of the acute nasal inflammatory response following exposure to 0.8 and 1.5 ppm O_3 was due to a competing inflammatory response in the lung.

We have previously shown that intranasal instillation of a potent PMN chemotaxinogen (endotoxin) induces a rapid (6 h maximum) influx of PMN into the nasal cavity of rats while simultaneous intranasal and intratracheal endotoxin instillation results in a massive influx of neutrophils in the lung, but a markedly attenuated PMN response in the nasal cavity.[6] These results are analogous to those in the present study. In one case, simultaneous, competing, inflammatory stimuli were produced through intranasal and intratracheal instillation of endotoxin, and in the present case by inhalation of an environmentally relevant pollutant, O_3. In both cases, the results were the same. The pulmonary stimulus induced an influx of PMN at the sites of injury and resulted in a functional sequestration of neutrophils that prevented or attenuated the nasal inflammatory response.

In summary, we have shown that the relative numbers of PMN recovered by NL and BAL accurately reflects the tissue PMN response at sites within the nose and lung injured by acute inhalation of O_3. Our results suggest that at high O_3 levels (0.8 and 1.5 ppm), the acute nasal inflammatory response is attenuated by a simultaneous, competing, inflammatory response within the centriacinar region of the lung. To our knowledge, this is the first study to examine the simultaneous effects of a range of O_3 concentrations of the upper and lower respiratory tract epithelium of rats. After acute exposures to ambient levels of O_3, alterations in cellular, and perhaps biochemical, parameters within the nose may provide sensitive indicators of O_3 exposure. In contrast, nasal cellular inflammatory responses after exposure to higher O_3 levels, may underestimate the effects of O_3 within the lung.

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