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Effects of Ozone and Photochemical Oxidants on Interferon Production by Rabbit Alveolar Macrophages

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It has been known that air pollutants such as ozone (O_3) and/or nitrogen dioxide (NO_2) are causative or aggravating agents of respiratory infections induced by inhaled pathogenic microorganisms. A number of findings have confirmed that these gases injure the defense mechanisms against respiratory infection such as mucociliary clearance in the respiratory tract and bactericidal activity in the lung of animals (NAS 1977). However, the pathogenesis of decreased resistance against viral respiratory infection has not be well elucidated as that against bacterial infection inasmuch as the growth process of virus in the respiratory tract is more complicated than that of bacteria (FAIRCHILD 1977).

Alveolar macrophage is known to play a vital defensive role in respiratory virus infection. Although a limited number of experimental studies have been made to elucidate the deleterious effects of these gases on the function of alveolar macrophage from rabbit such as inhibition of phagocytic ability (GARDNER et al. 1969, ACTON et al. 1972, VASSALO et al. 1973), studies have not yet been made to demonstrate that interferon (IF) production by alveolar macrophage in response to virus infection is influenced by inhalation of O_3 and/or photochemical oxidants (Ox). IF is a substance which defends the host against viral infection by inactivating virus infectivity. IF is produced earlier than antiviral antibodies. It is therefore considered to be an important factor which influences the course of viral infection. In this study, the authors investigated the effects of exposure to either O_3 or Ox on the capacity of IF production by rabbit alveolar macrophages.

MATERIALS AND METHODS

Preparation of alveolar macrophages: Rabbits, weighing about 2.5kg, were exsanguinated by severing the carotid artery immediately after exposure to each gas. Alveolar macrophages were separated by bronchial lavage using the modified technique of MYRVIK et al. (MYRVIK et al. 1961, ACTON et al. 1968). Alveolar macrophages suspended in Hank's solution were washed by Eagle's Minimal Essential Medium (MEM) supplemented with 10% calf serum and this was then left standing for 2 hours in a glass dish containing the same medium. The glass adherent cells were mostly living macrophages containing a small number of polymorphonuclear leukocytes. Alveolar macrophages were cultured in Eagle's MEM supplemented with 10% calf serum

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by incubation in a humidified 5% CO_2 atmosphere at 37°C.

Interferon production: Interferon was produced in alveolar macrophages with Newcastle disease virus (NDV) as described elsewhere (SUGIYAMA et al. 1977). Alveolar macrophages from normal rabbits and rabbits exposed to O_3 or Ox were harvested by the procedure described in the materials and methods section and cultured in a glass dish as glass adherent cells. The culture of macrophages was inoculated with 20 moi (plaque forming units) of NDV per cell and then incubated at 37°C. After a given incubation period, 1 N HCl was added to adjust the pH of the culture fluid to 2.0 and stored for 24 hours at 4°C. The pH of the fluid was readjusted to 7.0 and then centrifuged at 25,000 rpm for 90 min. The supernatant was used as the sample for IF assay.

Interferon assay: IF samples were placed on a Falcon microplate and serial dilution was performed in this microplate. Then, RK-13 cells were added to the plate so that each well would contain $30X10^4$ cells/ml. They were incubated overnight. The culture fluid was discarded and washed one time with Eagle's MEM supplemented with 10% calf serum. Each culture plate was inoculated with 10 $TCID_{50}$ of vesticular stomatitis virus (VSV) in a total volume of 0.025 ml. After adsorption of VSV for 1 h , virus solution was discarded and washed one time with the foregoing MEM medium. Forty-eight hours later, the cytopathic effect (CPE) (HO et al. 1959) was observed. The IF titer was considered to be the reciprocal of the dilution which produced a 50% inhibition of CPE on RK-13 cells. According to this method, IF units are equivalent to the international units with L-cell.

Exposure of the animals to O_3 or Ox: Rabbits were placed in the exposure room (200ℓ) attached to the photochemical smog chamber (2 m^3) and then exposed for 3 hours to O_3 or Ox which were force-circulated from the smog chamber. As the air space was not sufficient enough to continue exposure for a long period, one hour exposure was repeated successively for three times, exchanging the gas totally at hourly intervals. Methods for O_3 or Ox generation have been described in detail previously (KUSUMOTO et al. 1976).

Briefly, O_3 generated by passing oxygen (O_2) through the discharge tube type generator was blown into the photochemical smog chamber, and after adjustment to the predetermined concentration, force-ventilation into the exposure room was made. The concentration of O_3 was kept constant (±10%) by supplementation as it decreased. One group of control rabbits was exposed to air flows having the same partial pressure of O_2 in air as O_3 exposure described above, and the other control group was kept in a filtered air atmosphere.

Ox was generated in the photochemical smog chamber by irradiating the diluted automobile exhaust gas (the concentration of carbon monoxide was approximately 50 ppm) with ultraviolet rays, to which nitrogen monoxide (NO) and propylene (PP) were added in order to increase the Ox concentration and then adjusted to 3 and 10 ppm, respectively. The concentration of Ox was measured as O_3 by a con-

tinuous chemiluminescent O_3 analyzer. Rabbits were individually placed in the exposure room, and when the predetermined concentration of Ox was attained in the smog chamber, force-ventilation into the exposure room was made. Exchanging the gas within the chamber completely one hour later, the generation of Ox and exposure were repeated in the same procedure as described above. One group of controls was exposed to the same concentration of non-irradiated automobile exhaust gas supplemented similarly by NO and PP, and the other control group was kept in a filtered air atmosphere.

RESULTS

1. Effects of O_3 exposure on IF production by alveolar macrophages from rabbit.

IF production by alveolar macrophages from rabit was completely depressed immediately after cession of exposure to 5 ppm O_3 for 3 hours and was partially decreased at 1 ppm in the same conditions as shown in Table 1. However, the depressed IF production seemed to show a tendency towards recovery 24 hours after cession of exposurfe (Table 1)(WATANABE et al. 1973). On the other hand, in Dutch rabbits, weighing about 2kg, which were used to examine whether a strain difference in the sensitivity of alveolar macrophages against O_3 exposure existed or not, IF production by alveolar macrophages was depressed completely even at 3 ppm O_3 and slightly at 1 ppm (Table 1). In these experiments, it was shown that the reduction of IF production corresponded in grade to the increase of O_3 concentration.

2. Effects of Ox on IF production by alveolar macrophages from Dutch rabbit.

IF production by alveolar macrophages from Dutch rabbit completely decreased by exposure to average of 0.8 ppm Ox (max. 1.3 ppm) for 3 hours and also markedly decreased by exposure of nonirradiated automobile exhaust gas as done in one of the control experiments (Table 2). Similar results were observed by exposure to average of 0.5 ppm Ox (max. 1.1 ppm). Furthermore, by exposure at lower concentration of average of 0.3 ppm Ox (max. 0.7 ppm), IF production partially but significantly decreased (Table 2). These results suggested that the depressing action of Ox in IF production was stronger than O_3. The relationship between the level of reduction of IF production and Ox concentration was similar to the case of O_3 exposure.

DISCUSSION

A number of investigators have reported a reduction of mucociliary clearance rate and pulmonary bactericidal activity after exposure to O_3 or NO_2. As for the mechanism of reduction of pulmonary bactericidal activity, decrease of phagocytic ability and

TABLE 1

Effects of ozone exposure on interferon production by alveolar macrophage from rabbit

Experiment Number		Incubation time (hours after addition of inducer) 13	20	48
No. 1	Control		520*	310
	O_2 Control		140	30
	O_3		ND**	ND
No. 2	O_2 Control	490	810	1400
	O_3	100	100	80
No. 3	Control	390	290	290
	O_3	17	12	ND
No. 4	Control	20	180	250
	O_3	20	20	70

* Interferon titer (units). ** Not detected.
No. 1: From rabbit exposed to 5 ppm O_3 for 3 hours.
No. 2: From rabbit exposed to 1 ppm O_3 for 3 hours.
No. 3: From Dutch rabbit exposed to 1 ppm O_3 for 3 hours.
No. 4: From rabbit 24 hours after exposure to 5 ppm O_3 for 3 hours.

TABLE 2

Effects of oxidants or exhaust gas exposure on interferon production by alveolar macrophage from Dutch rabbit.

Experiment Number		Incubation time (hours after addition of inducer) 12	24	48
No. 1	Control	30*	40	20
	Exhaust gas	10	10	10
	Ox	ND**	ND	ND
No. 2	Control	230	230	240
	Exhaust gas	140	90	60
	Ox	40	70	40

* Interferon titer (units). ** Not detected.
No. 1: Exposed to Ox (maximum 1.3 ppm, average 0.8 ppm) for 3 hours.
No. 2: Exposed to Ox (maximum 0.7 ppm, average 0.3 ppm) for 3 hours.

increase of osmotic fragility of alveolar macrophage have been observed in rabbits exposed to O_3 or NO_2 (DOWELL et al. 1970). Concerning the effects on the physiological function of alveolar macrophage, VALAND et al. (1970) reported depression of IF production by alveolar macrophage from rabbits exposed to NO_2. According to IBRAHIM et al. (1976) exposure of mice to 0.8 ppm O_3 for a period of 11 days or more inhibited in vitro the capacity of tracheal epithelial cells to produce IF. In their report, however, O_3 did not seem to have any effect on the capacity of alveolar macrophages to produce IF in vitro. However, the effect of Ox on IF production has not yet been investigated.

The results obtained in this study demonstrated that the capacity of IF production by alveolar macrophage was depressed immediately after exposure to O_3 greater than 1 ppm or Ox exceeding average of 0.3 ppm (max. 0.7 ppm) for 3 hours. In these experiments, it was shown that depression in IF production corresponded in degree to elevation of gas concentration. This finding suggested that alveolar macrophages, existing in a state of single cell in the lung, were probably exposed directly to the inhaled gas in this experimental system. The results that depression of IF production in Dutch rabbit under the same O_3 concentration was greater in degree than that in rabbit suggest that sensitivity of alveolar macrophage to O_3 or presumably to other irritating gases is different among species.

It is interesting that IF production was depressed by exposure to Ox at a concentration lower than O_3. This finding seems to be responsible for the additive action of minor components contained in Ox to the major component of O_3. This finding suggests that deleterious action of Ox on alveolar macrophages is stronger than O_3. KUSUMOTO et al. (1976) previously reported that increase of leucocyte count and elevation of leucocyte index in the blood of mice were more strongly affected by Ox exposure than O_3 alone. Recently, the authors reported that the function of rabbit tonsillar lymphocytes was impaired after exposure to O_3 and/or Ox (SUGIYAMA et al. 1979). In these experiments, it was shown that Ox-induced decrease of IF production by lymphocytes was greater in degree than O_3-induced decrease similar to the results of this study. According to these findings, the real photochemical smog is considered to have more hazardous effects on health than photochemically synthesized Ox, because the real photochemical smog contains various species of aerosols other than Ox.

Epidemiological studies have presented suggestive evidences that the incidence of viral respiratory infections can be increased by exposure to elevated concentration of photochemical exidants (HAMMER et al. 1974).

The results obtained in this study suggest that the impaired ability of IF production in alveolar macrophages resulting from exposure to O_3 or Ox is a causative factor for the decrease of resistance against respiratory virus infection.

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