The Effects of Ozone on Immune Function

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A review of the literature reveals that ozone (O3) exposure can either suppress or enhance immune responsiveness. These disparate effects elicited by O3 exposure depend, in large part, on the experimental design used, the immune parameters examined as well as the animal species studied. Despite the apparent contradictions, a general pattern of response to O3 exposure can be recognized. Most studies indicate that continuous O3 exposure leads to an early (days 0–3) impairment of immune responsiveness followed, with continued exposures, by a form of adaptation to O3 that results in a re-establishment of the immune response. The effects of O3 exposure on the response to antigenic stimulation also depend on the time at which O3 exposure occurred. Whereas O3 exposure prior to immunization is without effect on the response to antigen, O3 exposure subsequent to immunization suppresses the response to antigen. Although most studies have focused on immune responses in the lung, numerous investigators have provided functional and anatomical evidence to support the hypothesis that O3 exposure can have profound effects on systemic immunity.

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Introduction

Broadly defined, the function of the immune system is to protect the body from damage by infectious microorganisms and neoplastic cells. The immune response is mediated by a variety of soluble factors and cells grouped according to their capacity to mediate innate (natural) or adaptive (acquired) immune responses. Innate immunity is primarily dependent on the phagocytic effector mechanisms (including humoral factors such as complement, lysozyme, etc.), which are neither specific for particular infectious agents nor improved by repeated encounters with the same agent. Acquired immunity is specific for the inducing agent and is marked by an enhanced response upon repeated encounters with that agent, thus displaying memory. These systems do not function independently since innate and acquired immune mechanisms can interact; specific antibodies enhance the phagocytic ingestion process and cell-mediated immune responses increase the intracellular microbicidal and tumoricidal capabilities of the phagocytes. Both innate and acquired immune responses and the cells that carry out these processes are regulated by soluble factors such as cytokines and various autoantigens.

The multiple interlocking mechanisms involved in the complex sequence of events that lead to the expression of the immune response can present a plethora of targets for air pollutants and xenobiotic compounds. Recognition that environmental agents can impair immune function has lead to the emerging field of immunotoxicology (1,2). As with any new area of scientific endeavor, uncertainties abound as the field is defining itself and gaining recognition. This is especially true when many questions remain unanswered in the parent discipline immunology. Against this background the literature on the effects of ozone on immune function is reviewed.

Ozone and the Immune Response

A survey of the literature on the effects of O3 exposure on innate and acquired immunity reveals that the responses range from no effect to impaired and/or enhanced effects. Several factors related to the experimental systems used are responsible for this ambiguous situation. First, many studies have examined a diverse range of immunologic parameters with varying sensitivities to O3. Second, there are differences in responses to O3 among animal species. Third, it is becoming increasingly apparent that the O3 concentrations employed and the exposure protocols utilized (i.e., continuous versus intermittent) heavily influence the responses measured. Finally, the time at which O3 exposure occurs, relative to the time at which responsiveness is measured, appears to be critical.

To unravel the variables and to determine the trends that emerge, it is helpful to group the approaches that have been used to investigate the effect of O3 on the immune system into several categories. These include: a) measurement of lymphoid organ weights and cellular composition; b) determination of the functional capacity of lymphocytes in the absence of antigenic stimulation; c) measurement of the immune response following antigenic stimulation; d) assessment of the phagocytic capacity of alveolar macrophages; and e) measurement of the host response to infectious agents. Because multiple variables determine the response of the immune apparatus to experimental O3 exposure, attention is given herein to the exposure conditions utilized. Thereafter, the studies performed in our laboratories in an attempt to validate and gain insights into the mechanisms of the divergent effects of O3 on the immune system will be presented.
Effect of Ozone on Lymphoid Organ Weight and Cell Numbers

The effect of O3 exposure on lymphoid organ weights and/or cellularity have been examined in several studies. Although there is no clearcut conclusion, a general pattern of response to O3 is reflected in the changes in lymphoid tissues that can be correlated with changes in immune parameters.

Continuous 0.3 to 0.8 ppm O3 exposure for 20 to 24 hr/day elicits decreases in murine spleen, thymus, and mediastinal lymph node (MLN) weights after 1 to 3 days of exposure (3, 4). The changes in MLN weight were found to be dose dependent over a range of 0.3 to 0.7 ppm O3 (4).

With prolonged exposures (7–14 days), spleen, thymus, and MLN weights returned to control levels (3, 4). Concomitant with the return of lymphoid organ weights to control levels is an increase in the number of T-cells in these organs. Thus, after an initial decrease, T-cells increase in lymphoid organs after continued O3 exposure (5–8).

The increase in T-cell numbers is not paralleled by a change in B-cell numbers. IgG- and IgM-secreting cells in murine bronchus-associated lymphoid tissue (BALT) (9) and B-cell numbers in the lung as a whole did not change with prolonged O3 exposures (7). Likewise, exposure of rats to 0.13 ppm O3 for 1 week increased T/B cell ratios in the MLN, with the increases lasting for at least 5 days after cessation of exposure (10).

Based on the studies cited above, it is apparent that a pattern in the response to O3 can be recognized. Subsequent to an initial decrease in lymphoid organ weights and cellularity after 1 to 7 days of exposure, continued exposure of 1 to 3 weeks revealed that the organ weights not only stop decreasing, but actually return to control levels and, in some cases, exceed control levels (4). Most of these changes in lymphoid organ weights can be correlated to changes in T-cell numbers and prolifera-

Effect of Ozone on Immune Function in the Absence of Antigenic Stimulation

Longitudinal studies on the effects of O3 exposure on lymphoid organ cell numbers provide information on cellular traffic and cell numbers but provide few insights into the functional capacity of the lymphocytes. A number of studies have investigated the effect of O3 exposure on the blastogenic response of lymphocytes to nonspecific mitogens. These assays measure nonspecific clonal expansion of the lymphocyte populations, a critical step during the amplification of the immune response. Thus, MLN cells stimulated with concanavalin A (ConA) during the course of continuous 0.7 ppm O3 exposure (20 hr/day, 7 days/week for 28 consecutive days) showed little change in responsiveness during the first week; however, by day 14, an enhanced reactivity was observed that continued to increase through day 28 (4,8).

Short-term exposure studies have shown that the rat splenic cell responses to T-cell mitogens phytohemagglutinin (PHA) and ConA and a B-cell mitogen (Escherichia coli LPS) were significantly enhanced by exposure to 1 ppm O3 (8 hr/day for 7 days) (14). In contrast, intermittent exposure to 2 ppm O3 (8 hr/day for 4 days, 2–4 days in ambient air followed by another day of O3 exposure) was without effect while long-term exposure of mice to 0.1 ppm O3 (5 hr/day, 5 days/week for 103 days) suppressed the splenic cell responses to T-cell mitogens (PHA and ConA) but not to a B-cell mitogen [Salmonella typhosa LPS; (15)].

Natural killer (NK) cell activity has also been used as an index of O3-induced effects on the functional integrity of the immune surveillance system. One such study demonstrated that continuous exposure of rats to 1 ppm O3 for 10 consecutive days results in a significant decrease in pulmonary NK cell activity on days 1, 5, and 7 of exposure, with activity recovering by day 10 (16). In another study, continuous exposure of rats to O3 (0.2 ppm, 0.4 ppm, and 0.8 ppm) enhanced lung NK cell activity on day 7 at the two lower concentra-

The above-mentioned studies on the effects of O3 exposure on mitogen responses and NK cell activity used exposures to O3 in great excess of that found in ambient air. To investigate the effects of acute and chronic exposure to O3 at near ambient concentrations, Selgrade et al. (18) used an experimental protocol designed to mimic diurnal urban O3 exposure patterns. Rats were exposed to a background level of 0.06 ppm for a period of 13 hr, a broad exposure spike rising from 0.06 ppm to 0.25 ppm and returning to 0.06 ppm over 9 hr, and a 2-hr downtime. The exposures to this profile were 5 days/week; weekend exposures were to the background. After 1, 3, 13, 52, or 78 weeks of exposure, blood was drawn and the spleens removed. Spleen cells were assessed for NK cell activity and responses to T-cell mitogens (PHA and ConA) and a B-cell mitogen (Salmonella typhimurium glycoprotein). Peripheral blood leukocytes were also assessed for responses to T-cell mitogens. The data show that O3 exposure had no effect on NK cell activity, nor were there any O3-related changes in mitogen responses in splenic or blood leukocytes. There were also no effects of a single 3-hr exposure to 1 ppm of O3 on spleen cell responses to the mitogens immediately after exposure or at 24, 48, and 72 hr thereafter.

Several studies have also investigated the effect of O3 exposure on human peripheral blood lymphocytes. Exposure of subjects to 0.4 ppm O3 for 4 hr impaired the ability of B-cells to form rosettes with human erythrocytes, whereas the ability of T-cells to form rosettes with sheep erythrocytes was unaffected (19). Another study examined the effects of a single 2-hr exposure to 0.6 ppm O3 on the mitogenic response of peripheral lymphocytes following stimulation with PHA, ConA, pokeweed mitogen (PWM), and Candida albicans. The mitogenic response to PHA was significantly reduced at 2 and 4 weeks following O3 exposure, with normal responses observed at 2 months. In contrast, no significant changes in lymphocyte responses to ConA, PWM, and C. albicans were observed (20). Finally, subjects were exposed for 2 hr to 0.12 and 0.4 ppm O3 during moderate exercise, and PHA-induced peripheral T-cell proliferation was assessed before exposure and 24 and 72 hr after exposure (21). In the absence of O3, exercise enhanced proliferation as compared

Environmental Health Perspectives
Effect of Ozone on Immune Function with Antigenic Stimulation

A handful of studies have examined the effect of O₃ on immune responses to antigenic stimuli. In general, these studies have used a Pandora's box of O₃ exposure regimens and varied the time and site (aerosol, systemic, or a combination of both) of antigenic stimulation. A common thread through most of these studies is that when the antigenic stimulation was performed during O₃ exposure, enhanced allergic responses were observed (22–26). However, when O₃ exposure preceded OA stimulation, IgE antibody production was suppressed (27).

In a series of studies, Fujimaki and colleagues (3,11,28) evaluated the systemic effects of short-term O₃ exposure on the humoral and cell-mediated immune response. In the first study, mice were continuously exposed to 0.8 ppm O₃ for 1, 3, 7, and 14 days. After each day of exposure, the animals were tested for the primary splenic antibody response to either sheep erythrocytes (SRBCs; T-dependent antigen) or DNP-Ficoll (T-independent antigen). The antibody response to SRBCs was suppressed following all exposure periods. In contrast, no suppression was observed in the primary antibody response to DNP-Ficoll (3). When the continuous 0.8 ppm O₃ exposure was extended to 56 days, suppression of the primary splenic antibody response to SRBCs, but not DNP-Ficoll, was still evident (11). Using the same exposure protocol (continuous 0.8 ppm O₃ for 1, 3, 7, and 14 days), the delayed-type hypersensitivity (DTH) response to SRBCs was also examined (28). Ozone exposure gradually depressed the DTH reaction from 1 day to 7 days of exposure and returned to control levels by day 14. In the same study, the timing between O₃ exposure and SRBC immunization for the DTH response was also examined. Continuous exposure to 0.8 ppm O₃ for 3 days immediately before immunization (with no post-immunization exposure) had no effect on the DTH reaction, whereas continuous 0.8 ppm O₃ exposure for 3 days following immunization significantly suppressed the DTH response (28). In total, it appears that O₃ exposure preferentially affects the immune repertoire of the T-cells rather than that of the B-cells.

The effect of O₃ exposure on DTH responses to Listeria monocytogenes antigen has also been investigated (10). For this, rats were continuously exposed to 0.75 ppm O₃ for 7 days immediately before or immediately after intratraheal infection with L. monocytogenes. The DTH response, tested at 7 and 14 days after infection (immunization), was not significantly influenced by exposure to O₃ for 1 week before infection. However, if O₃ exposure occurred during the Listeria infection, the DTH response was suppressed. Using the same exposure protocol, the proliferative responses of spleen cells to Listeria antigen was also examined. The effect of O₃ exposure on this expression of immunity closely paralleled the effect found with DTH to Listeria antigen [i.e., whereas exposure before immunization did not decrease blasticogenesis, the proliferative response was significantly suppressed in animals that were exposed to O₃ immediately after immunization (10)]. In a related study, mice were continuously exposed to 0.59 ppm of O₃ for 36 days. On the fifth day of exposure, the animals were immunized with tetanus toxoid and challenged with tetanus toxin on day 27. Compared with unexposed controls, O₃-exposed mice exhibited greater mortality and morbidity following the toxin challenge (29).

Effect of Ozone on Alveolar Macrophage Phagocytosis

Studies on the effects of O₃ exposure on innate immunity have focused on the phagocytic activity of alveolar macrophages (AMs). Acute exposures (2.5 ppm for 5 hr) decreased the in situ phagocytosis of inhaled Staphylococcus aureus in rat lungs (30). In a similar manner, exposure to 0.4 and 0.8 ppm O₃ ppm for 3 hr decreased phagocytosis of intratracheally administered Streptococcus zooepidemicus in murine lungs in a dose-dependent manner (31). Ex vivo studies on AM phagocytosis have found divergent effects of O₃ exposure. For example, when rats were continuously exposed to 0.8 ppm O₃ (for 3, 7, and 20 days), an enhancement in the ingestion of carbon-coated latex particles was observed, with the greatest increase following 3 days of exposure (32). On the other hand, rat AM phagocytosis of opsonized SRBCs was progressively suppressed from day 1 to day 3 of continuous 0.5 ppm O₃ exposure, with recovery of phagocytic activity by day 6 of exposure (33). When rabbits were given a single 2-hr exposure of 0.1 ppm O₃, AM phagocytosis was maximally decreased immediately after exposure and remained low throughout the ensuing day, albeit to a lesser extent; complete recovery of phagocytic activity occurred by day 7 post-exposure (34). Increasing the single 2-hr exposure dose of rabbits to 1.2 ppm O₃ resulted in a continued suppression of AM phagocytosis through post-exposure day 7. Intermittent exposure of rabbits (0.1 ppm O₃ for 2 hr/day for 14 days) resulted in a significant reduction of AM phagocytosis on day 3 and 7 with recovery of phagocytic potential by day 14.

To assess species differences in O₃-induced changes in AM phagocytosis, rats and mice were intermittently exposed to 0.4 ppm O₃ (12 hr/day for 7 days). After 1 day of exposure, AM phagocytosis of opsonized SRBCs was suppressed in mice and enhanced in rats. By days 3 and 7 post-exposure, rat AM phagocytosis returned to control levels, whereas mouse AM phagocytosis continued to be suppressed (35).

The effect of O₃ exposure on human AM-mediated phagocytosis has also been examined. For this, subjects were exposed for 6.6 hr to either 0.08 ppm or 0.10 ppm O₃ during moderate exercise (36). Bronchoalveolar lavage was performed 18 hr after exposure (36). Ozone exposure significantly decreased the ability of AMs to ingest Candida albicans yeast particles coated with serum (complement receptor-mediated phagocytosis). However, no such effect was observed with IgG-coated yeasts (Fc receptor-mediated phagocytosis) or unopsonized yeasts (non-specific receptor-mediated phagocytosis). Finally, a human study investigated the effect of a small 0.4 ppm O₃ exposure on peripheral blood leukocyte function. The capacity of polymorphonuclear neutrophils (PMNs) to phagocytize and kill Staphylococcus epidermidis was significantly suppressed at 72 hr after exposure and returned to normal levels 2 weeks after exposure (37).

The Effect of Ozone on Resistance to Infectious Agents

Recognition of the association between exposures to air pollutants and the development of acute respiratory illnesses has led to animal models that use microbiologic parameters to evaluate toxicity (38,39). Many studies on the effects of O₃ on host defenses have been performed with a rodent model that is often referred to as the "infectivity model" (39). In this model, rodents are...
challenged by aerosol inhalation with highly virulent Klebsiella pneumoniae or Streptococcus pyogenes following various O3 exposure protocols and observed for deaths over the subsequent 14-day period. The data on the effects of O3 exposure are expressed as increases in mortality. The earliest of these studies (40) found significant increases in mortality following K. pneumoniae challenge when mice were exposed to 1 ppm and 4 ppm O3 for 3 hr, 1 ppm O3 for 100 hr, and 1 ppm O3 for 4 hr/day, 5 days/week for 2 weeks. With the use of Streptococcus pyogenes, the sensitivity of the “infectivity model” increased in that significant differences in mortality were demonstrated when the infectious challenge was given simultaneously or 2 hr after exposure to 0.1 ppm O3 for 3 hr (41). Mice exposed 5 hr/day, 5 days/week for 103 days to 0.1 ppm O3 also exhibited increased susceptibility to death from streptococcus challenges (15). Other studies with the “infectivity model” involving O3 exposure focused on the relationship between O3 and NO2 exposure on resistance to streptococcal pneumonia (42-44). The “infectivity model” links interference with pulmonary antibacterial defenses to mortality following pulmonary challenges with virulent organisms. Another approach is to use minimally virulent organisms, such as S. aureus, that do not provoke injurious responses in the lungs (45). In this system, rodents are challenged by aerosol inhalation with staphylococci and intrapulmonary killing of the organism is assessed over a 4- or 5-hr period by standard microbiologic methods. Exposure to O3 can occur before, after, or before and after bacterial challenge. Under normal conditions, approximately 90% of the staphylococci are killed over the initial 4-hr period of infection. The effects of O3 exposure are assessed in terms of suppression of intrapulmonary bacterial killing.

Exposure of mice to 0.1 ppm, 0.5 ppm, and 1 ppm O3 for 3 hr immediately before staphylococcal challenge significantly suppressed the intrapulmonary killing of S. aureus in a dose-dependent manner (46,47). Similarly, exposure to increasing concentrations of O3 for 17 hr before or 4 hr after bacterial challenge also impaired the intrapulmonary killing of staphylococci in a dose-dependent manner (48,49).

More recent studies with the Streptococcus zooepidemicus infectivity model found that 0.4 ppm and 0.8 ppm O3 exposure of 5- and 9-week-old CD-1 mice for 3 hr impaired intrapulmonary bacterial killing, with the most severe effect elicited by the higher dose of O3 in the younger mice (50). When the study was performed in two different mouse strains, the results showed that C3H/Hej mice were more sensitive to the effects of O3 exposure on susceptibility to streptococci than C57BI/6 mice (31).

The effect of O3 exposure on chronic respiratory infections has been assessed using Mycobacterium tuberculosis and L. monocytogenes as challenge organisms. Mice exposed to 1.5 ppm O3 (4 hr/day, 5 days/week for 2 months) exhibited no increased mortality to intravenously administered M. tuberculosis (51). On the other hand, exposure of mice to 1 ppm O3 (4 hr/day, 5 days/week for up to 8 weeks) beginning at 1 or 2 weeks after inhalation challenge with M. tuberculosis significantly enhanced the number of bacteria recovered from the lungs (52). With L. monocytogenes, rats were exposed continuously to 0.13 ppm, 0.25 ppm, 0.5 ppm, 0.75 ppm, 1.0 ppm, and 2.0 ppm O3 for 1 week and thereafter intratracheally infected with Listeria. Exposure to 1 ppm O3 significantly increased mortality through dysfunctions in the intrapulmonary elimination of the bacteria (10).

Only a few studies have investigated the effects of O3 exposure on the outcome of viral respiratory infection. Fairchild (53) demonstrated that exposure to 0.6 ppm O3 for 3 hr after aerosol infection of mice with mouse-adapted influenza A2/Japan 305/57 virus inhibited viral replication in the nose but did not alter influenza virus growth in the lungs of the same animals. Continuous 1.5 ppm O3 exposure also had no effect on the proliferation of virus in the lungs of mice infected by aerosol inhalation with a sublethal dose of influenza A/PR8/34 virus (54). In another study (55), mice were exposed to 1 ppm O3 3 hr/day for 5 days and intranasally infected with mouse-adapted influenza virus A/Hong Kong/68 immediately after the first, second, third, fourth, and fifth of the five daily exposures. A 2-fold increase in the incidence of mortality was observed in mice infected after the second exposure, with no effects on percentage mortality in mice infected on the first, third, fourth, or fifth exposure. When the exposure concentration was lowered to 0.5 ppm, there were no effects on mortality in mice after the second exposure. Five daily 3-hr exposures to 1 ppm O3 had no effect on virus titers in the lungs of mice infected after either the second or fifth day of exposure.

Wolcott et al. (56) observed a protective effect of O3 exposure to influenza virus infection. Mice were continuously exposed to 0.5 ppm O3 for 2 weeks before and after aerosol infection with influenza A virus (WSN strain). Four groups were included in the study. The control mice were exposed to air for 2 weeks, virus infected and reexposed to air for 2 weeks. The experimental groups were exposed to O3 either before, after, or before and after virus infection. It was found that all animal groups exposed to O3 had a significant decrease in mortality, which correlated with a less widespread infection of the lung parenchyma. The reduced mortality was independent of peak pulmonary virus titers, pulmonary interferon titers, and pulmonary and serum-neutralizing antibodies.

Other Studies
Ozone exposure may also induce aberrant immune responses through modification of “self” determinants in the lungs by ways such as cross-linking of antigen (57), denaturation of protein (58), or by other means (59). Indeed, an autoimmune response has been attributed to O3 exposure (60).

The effect of in vitro O3 exposure on the immune responsiveness of isolated cells has also been investigated. These experiments have demonstrated that acute exposure to O3 reduces AM phagocytosis (61) in a dose-dependent manner (62). In addition, PGE2 production by AMs was increased by O3 exposure, and superoxide release from stimulated cells decreased (62). Exposure of peripheral mononuclear cells from healthy individuals to various concentrations of O3 revealed a general decrease in immune parameters such as NK activity (63), responsiveness to mitogens, expression of activation markers on monocytes and lymphocytes (64,65), and antibody production (66). These are important observations because they demonstrate similar responses to O3 between human cells exposed in vitro and animals cells exposed in vivo. Confirming the validity of the animal models, these studies demonstrate that the response to O3 of human cells exposed in vitro is similar to that of animal cells exposed in vivo.

Studies Performed
The studies performed in our laboratories were designed to clarify some of the issues regarding the effects of O3 exposure on the innate and acquired immune system. The first goal was to investigate the modulating effect of O3 exposure on AM phagocytosis and to determine whether an adaptive phase followed prolonged exposure. The second goal was to explore whether changes
in the local (lung) immune responses were mirrored by changes in the systemic immune responses. The third goal was to gain some insight into the mechanisms of the systemic effects and, finally, we attempted to elucidate the mechanisms by which \( O_3 \) exposure reduced the pathogenesis of experimental influenza virus infection.

**Suppression and Recovery of the Alveolar Macrophage Phagocytic System**

To investigate the effect of \( O_3 \) exposure on AM phagocytosis and to determine whether prolonged \( O_3 \) exposure results in an adaptive response, mice were exposed to 0.5 ppm \( O_3 \) for 23 hr/day for 14 consecutive days. At 1, 3, 7, and 14 days of exposure, AM phagocytosis of opsonized SRBCs was assessed in vivo (67). Figure 1A shows that 1 day of \( O_3 \) exposure suppressed AM phagocytosis. This impairment of phagocytic activity was further exacerbated at day 3 but began to recover at 7 days and returned to control levels by 2 weeks.

Using another particle to probe the functional capacity of the AM phagocytic system in situ (45), mice were challenged by aerosol inhalation with \( S. aureus \) and intrapulmonary bacterial killing was assessed over a 4-hr period. The data (Figure 1B) reflect the results obtained with the ex vivo assay on AM phagocytosis of opsonized SRBCs (Figure 1A); that is, an initial suppression of intrapulmonary staphylococcal killing was followed by recovery of bactericidal activity by day 14 (67).

**Modulation of the Immune Response**

In nonimmunized mice, continuous 0.7 ppm \( O_3 \) exposure results in an initial reduction of the number of cells recovered from MLN, which was followed by an increase and maintenance above basal levels as the \( O_3 \) exposure continued (4,8). To determine whether the splenic events were comparable to the changes in the MLN, mice were exposed 23 hr/day for 14 consecutive days to 0.8 ppm \( O_3 \). After 1, 3, 7, and 14 days of exposure, the number of cells in the MLN and the spleen/body weight ratios were determined. Then, to test the functional activity of these two cell populations, the proliferative response of MLN and splenic lymphocytes to PHA stimulation was measured (68). The data (Figure 2A) demonstrate that continuous \( O_3 \) exposure causes a reversible decline in MLN cells followed by hypertrophy. Spleen weights were decreased with respect to body weight after 1 day of \( O_3 \) exposure (Figure 2B). This effect was still evident at day 3 but returned to control values following 7 days of exposure. With continuous exposure, the spleens were larger in size relative to body weight than in control animals.

Cells from both MLN and splenic sources obtained after 1 day of \( O_3 \) exposure had reduced responsiveness to PHA mitogen (Figure 3A). However, this effect was ablated following prolonged exposure. In a similar manner, the NK cell activity of splenic lymphocytes was significantly reduced following 1 and 3 days exposure but was restored upon continued exposure (Figure 3B).

Another set of experiments was designed to investigate the effect of \( O_3 \) exposure on antigen-stimulated immune responsiveness. For this, an immunization protocol was adopted that maximizes the local (pulmonary) immune response. Briefly, mice were primed with an ip injection of OA and 2 weeks later boosted by aerosol inhalation of OA. One week after the aerosol boost (i.e., 3 weeks after the intraperitoneal priming injection), the MLN and splenic lymphocytes were assayed for proliferative responses to OA antigen, and the bronchoalveolar lavage fluid was assayed for specific IgG and IgA antibody to OA. Ozone exposure was incorporated into this immunization protocol to the extent that groups of animals were exposed to 0.8 ppm of \( O_3 \) for 23 hr/day for 1, 3, 7, or 14 days prior to assay as shown schematically in Table 1.

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**Figure 1.** Alveolar macrophage Fc-receptor-mediated phagocytosis (A) and intrapulmonary bacterial killing of *Staphylococcus aureus* (B) from mice exposed continuously to 0.5 ppm ozone. \( * = p<0.05 \). From Gilmour et al. (67).

**Figure 2.** Number of cells recovered from the mediastinal lymph nodes (A) and spleen/body weight ratios (B) from mice exposed continuously to 0.8 ppm ozone. \( * = p<0.05 \). From Gilmour and Jakab (68).

**Figure 3.** Proliferative responses of mediastinal lymph node and splenic lymphocytes to phytohemagglutinin stimulation (A) and natural killer cell activity of splenocytes (B) from mice exposed continuously to 0.8 ppm ozone. \( * = p<0.05 \). From Gilmour and Jakab (68).
Table 1. Ozone exposure protocol.

| Day  | 0   | 7   | 14  | 17  | 20  | 21  |
|------|-----|-----|-----|-----|-----|-----|
| 1 day exposure | Prime | Boost |      |      |      |      |
| 3 day exposure | Ozone | Assay |      |      |      |      |
| 7 day exposure | Ozone | Assay |      |      |      |      |
| 14 day exposure | Ozone | Assay |      |      |      |      |

Figure 4A shows that the MLN cell responses to OA antigen were not significantly different from controls for 1, 3, and 7 days of O₃ exposure. However, following 14 days of exposure to O₃ the MLN lymphocyte responses to OA antigen were significantly enhanced. Splenocyte responses to OA antigen were similarly unaffected by 1 and 3 days of exposure, but were significantly lower than control values at the 7- and 14-day time points.

Serum antibody titers to OA antigen were high in all animals and were unaffected by any period of O₃ exposure (68). Pulmonary IgG and IgA antibody titers to OA antigen (Figure 4B) were lower in bronchoalveolar lavage fluid from animals exposed to O₃ for 7 and 14 days. At the earlier time points, IgA titers remained unchanged; IgG levels were at first suppressed (day 1) and then returned to control values (day 3) before decreasing again at the later time points.

Finally, all the experiments detailed above with exposure to 0.8 ppm O₃ were also performed with continuous exposure to 0.5 ppm O₃. The results of these studies showed similar trends in responses, however, the magnitude of these responses was not as large as that observed with 0.8 ppm O₃ exposure (MI Gilmour and GJ Jakab, unpublished observations).

**Impairment of Peritoneal Macrophage Phagocytosis: The Role of Prostanoids**

During the course of studies on the effect of continuous 0.5 ppm O₃ exposure on AM Fc-receptor-mediated phagocytosis, it was also observed that the exposure impaired peritoneal macrophage (PM) phagocytosis. Due to its high reactivity, it seemed unlikely that O₃ could directly activate the phagocyte function. Rather, as has been suggested by others, the systemic effects of oxidant gases are likely indirect, due to mediators produced upon oxidant exposure (69). One such class of mediators may be the prostaglandins, potent immunomodulatory autacoids found in high concentrations in the airways (70,71) and serum of animals (72) and man (73) following acute O₃ exposure. Furthermore, exogenously added prostaglandin E₂ (PGE₂) inhibits macrophage phagocytosis (74). To test the hypothesis that PGE₂ plays a role in mediating the O₃-induced suppression of macrophage phagocytosis, mice were continuously exposed to 0.5 ppm O₃ and the effect of the cyclooxygenase inhibitor indomethacin on AM and PM Fc-receptor-mediated phagocytosis was assessed.

Figure 5A shows that the time course for O₃-induced suppression of PM phagocytosis paralleled that of the AMs in that maximal suppression occurred at 3 days of exposure, which progressively abated as exposure continued. PGE₂ levels in BAL fluid during the course of O₃ exposure are presented in Figure 5B. The data show that the time course of PGE₂ content in BAL fluid is strikingly similar to the time course of O₃ induced suppression of AM and PM phagocytosis. Treatment of mice with indomethacin for 3 days before and during O₃ exposure ablated the O₃-induced increases in bronchoalveolar lavage fluid (BAL) PGE₂ levels (Figure 6) and suppression of PM phagocytosis (Figure 7). Indomethacin also significantly reduced the effect on AM phagocytosis on the third day of exposure (Figure 7). The effect of indomethacin on O₃-induced suppression of PM phagocytosis were mimicked by pretreatment of mice with the active enantiomer of the cyclooxygenase inhibitor naproxen (74). Thus, prostanoids seem to play a key role in the...
Although with viral local and systemic increases in phagocytic activity induced by O₃.

The observations that prostanoids play a significant role in the suppression of AM phagocytic function has been recently confirmed with S. zoopuis as shown by the reduced susceptibility of mice to O₃-exposed infection by indomethacin treatment (50).

**Reduction of Influenza Virus Pathogenesis**

Although O₃ exposure has been shown to increase susceptibility to infectious bacterial challenges, the opposite has been observed with viral infections; ozone exposure actually diminishes the severity of influenza virus infection, as evidenced by decreased mortality and increased survival time (56).

Mortality from experimental influenza pneumonia is due to extensive damage of alveolar tissue resulting in impaired gas exchange (75). The antiviral immune response is known to participate in influenza-induced lung injury since immunodeficient (76-80) and immunosuppressed (81-83) mice develop much less cellular infiltration and histopathology than their immunocompetent counterparts. Also, adoptive transfer of specific immunity to immunosuppressed animals during the course of influenza virus infection reestablishes lung injury and increases pneumonic deaths (84). Because the integrity of the antiviral immune response is required for the tissue destructive phase of viral pneumonia, we tested the hypothesis that O₃ exposure reduced the pathogenesis of influenza pneumonia through suppression of the antiviral immune response.

Mice were infected with a nonlethal dose of influenza A (PR8/34 strain) and continuously exposed to 0.5 ppm O₃ (23 h/day for 14 consecutive days). On the ninth day of infection, at the height of the virus-induced pathologic changes (85,86), morphometric analysis of lung tissue, lung wet/dry weight ratios, and lung wash albumin concentrations showed that O₃ exposure was associated with a significant reduction in these parameters of lung injury (Figure 8). Because viral-induced pathologic changes are infectious dose related (87,88), the effect of O₃ exposure on viral proliferation and antigen accumulation in the lungs was quantitated. Figure 9 shows that neither virus proliferation nor the accumulation of viral antigen was affected by O₃ exposure.

Since immunosuppression during the course of viral infection reduces the number of lymphocytes retrieved from the lungs, which, in turn, is related to reduced immunopathologic changes (86,89), the effect of O₃ exposure on the recovery of two major classes of lymphocytes from lung tissue was phenotypically quantitated. In the presence of viral infection, both the T- and B-cell populations are significantly reduced by exposure to O₃ (Figure 10) as were the antiviral serum antibody responses (85). These data support the notion of the immunosuppressive nature of O₃ in that immunopathologic mechanisms are involved in the virus-induced lung injury, which are mitigated by O₃ exposure.

With lethal doses of virus, immunosuppressed mice still succumb to influenza virus infection. However, the deaths are not the early pneumonic deaths but occur later and are due to the extrapulmonary spread of the virus (90,91). The effects of continuous 0.5 ppm O₃ exposure on a severe influenza A/PR/34 infection were investigated. Twenty-five percent of the unexposed control mice died between day 8 and day 12 of infection, with no deaths occurring thereafter. In contrast, in O₃-exposed mice, 5% died between day 8 and day 12, with another 35% succumbing between day 12 and day 20 of the infection (GJ Jakab, unpublished observations).

**Discussion**

Before reaching any conclusions on the effects of in vivo exposure to O₃ on the immune system, several related issues warrant discussion. These issues broadly encompass inhalation toxicology, immunotoxicology, host factors, and experimental design.
Considerations on the Inhalation Toxicology of Ozone

Concepts of inhalation toxicology dictate that the animals exposed to \( O_3 \) and unexposed animals are treated in an identical manner (i.e., the control animals are exposed to filtered air in an identical exposure chamber so that the only difference between the experimental and control group is the \( O_3 \) exposure). Assuming that this practice is followed, several issues arise with the longer term continuous \( O_3 \) exposure studies that may influence the outcome of an immunologic parameter under investigation. For example, continuous \( O_3 \) exposure leads to a loss of appetite in rodents as reflected by a slower weight gain in young animals (92) and, depending on the \( O_3 \) concentrations, weight loss in older mice (GJ Jakab, unpublished observations). Malnutrition is known to affect the integrity of the immune system (93) and, to a lesser extent, undernutrition also blunts the immune response (94). In addition to \( O_3 \) exposure retarding growth due to loss of appetite, the food consumed by control and exposed animals is not the same. Ozone exposure oxidizes the food thereby altering its nutritional value (95). Variations in diet composition are known to affect the immune response (93,94,96).

Because of this, animals exposed to \( O_3 \) for longer periods of time have a nutritional disadvantage that may act in concert with \( O_3 \) exposure to suppress immunity. During long-term continuous \( O_3 \) exposure, little can be done about the loss of appetite except to match the food intake of the control animals to that of the exposed animals. To minimize the effects of ingesting \( O_3 \)-oxidized food, it may be prudent to provide \( O_3 \)-treated food for the control animals (85,86).

In addition to the nutritional aspect, the health status of the experimental animals also merits consideration. Animal husbandry has greatly improved over the past few decades to the point that reputable suppliers are capable of providing animals for research purposes that are free of intercurrent diseases. However, endogenous infections of rodents are pervasive (97), and assessment of the health status of the animals requires constant vigilance by suppliers and in the research setting since intercurrent infections are known to have profound effects on toxicity and research testing (98,99). For example, one of the most common infections of rodent colonies is Sendai (parainfluenza 1) virus (100). Sendai virus infection suppresses AM phagocytosis (101,102) and the primary antibody response of splenocytes to SRBCs (103) reduces T-cell mitogenesis (104,105), increases splenic NK cell activity (106,107), alters the \( in vitro \) response to heterologous erythrocytes (108), and alters immune cell function in aging mice (109). Other endogenous rodent infectious agents of concern to toxicologists include pneumonia virus of mice, mouse hepatitis virus, rat coronavirus, minute virus of mice, Kilham rat virus, Tooland’s virus, and Mycoplasma \( pu\)lmonis (98,110). Because of the possibility that intercurrent infections confound studies, the validity of observed results may be at risk if the health status of animals is not defined.

Considerations of the Immunotoxicology of Ozone

Undoubtedly, one of the overriding factors involved in the divergent responses to \( O_3 \) exposure on innate and acquired immune systems are the types of experimental methods used among the various studies. For example, a close examination of the methods used to determine the effects of \( O_3 \) exposure on AM phagocytosis reveals the following techniques: a) visual counting of the ingestion of 3.5 \( \mu \)m diameter carbon-coated latex microspheres by adherent AMs (32); b) visual counting of the ingestion of latex particles of unknown size by rabbit AMs in suspension (34); c) scintillation counting of Fc-receptor-mediated phagocytosis of \( 91 \)Cr-labelled SRBCs by mouse and rat AMs seeded in 96-well microtiter plates (35); and d) visual counting of Fc-receptor-mediated ingestion by adherent rat AMs (33) and mouse AMs (67,74). With the use of the 3.5 \( \mu \)m carbon-coated latex particles, it was observed that continuous exposure to 0.8 ppm \( O_3 \) for 3 days enhanced Sprague-Dawley rat AM phagocytosis (32). In contrast, the opposite was observed when the challenge particles were sensitized SRBCs in that continuous 0.5 ppm \( O_3 \) exposure suppressed Wistar rat AM phagocytosis (33). These data point to vast differences in sensitivities to \( O_3 \) exposure between the two assay systems, the response between the two strains of rats, or the 0.3 ppm difference in the \( O_3 \) exposure concentration.

In a manner similar to that exemplified with the effects of \( O_3 \) exposure on AM phagocytosis, questions arise about the relative sensitivities of the assays that measure lymphocyte function. Uptake of radiolabeled thymidine following stimulation with lectins or microbial products is a common method for assessing the ability of T- and B-cells to proliferate, a step required for the amplification of the immune response. These mitogens have specific binding sites which may not be present on all cell types (111). For example, PHA binds to the T-cell receptor and CD2 antigens of most T-cells (111). Concanavalin A binds to all T-cells (CD3) and is more potent than PHA (112), while differences in the binding sites of PWM may exist between different species of animals. Although the binding of these mitogens to specific glycoproteins on the cell surface are integral to subsequent proliferation, these binding sites are many and varied on any given cell population. Thus, depending upon the mitogen employed and the species from which the cells are derived, immune defects in any population of cells may be exaggerated or underestimated during a proliferative event.

Interpretation of experimental results becomes more complex when examining specific immune responses toward antigens. Often the generation of antibody against T-cell-dependent antigens (e.g., SRBCs) are compared to antibody production against T-cell-independent antigens (e.g., DNP-Ficoll) as indicators of T- and B-cell competence respectively. Even though these regimens are optimized to provide maximal immune stimulation, the relative immunogenicity of the test antigens and the avidity and specificity of the antibodies generated may differ between assay systems. Thus, problems of antigen potency and antibody specificity may arise not only in the immunization stages \( in vitro \) but also in the detection of antibody during immunosassay.

Insofar as there are shortfalls in conventional testing of immune function and in comparing the relative sensitivity of xenobiotic agents on the different arms of the immune response, Luster et al. (113,114) have demonstrated concordance between a chemical effect on the ability of mice to form antibody to SRBCs and its potential to cause an immunotoxic effect in any of a number of other assays measuring immune function. Changes in lymphocyte surface markers and, to a lesser extent, cell proliferation to mitogens and NK activity also showed good correlation with immunotoxicity; however, B-cell proliferation to LPS and the mixed lymphocyte reaction (graft rejection) did not.

In studying the possible immunomodulatory effects of \( O_3 \) and other air pollutants, some attempts have been made to examine the effect of \( O_3 \) on pulmonary immune responses such as local antibody production (22,23,68), NK activity (16), and lymphocyte function in the draining lymph nodes (68). Although these experiments are technically feasible, their interpretation can be challenging because of the constant trafficking of serum factors and immune
cells from the circulation to the lung and possible enhancement of these features during inflammation. For example, the observation of decreased lymphocyte sensitivity to antigen in the spleen following prolonged O₃ exposure and a contrasting increase in MLN lymphocyte activity (Figure 4A) could be explained by local inflammation and cellular recruitment to the site of oxidant damage (i.e., a simple matter of immune circulation). Until the cellular trafficking between the systemic immune compartment and that of the lung during immune stimulation are more clearly understood, these complicating factors will remain.

Considerations of Host Factors

A number of studies have investigated the age-dependent response to O₃ exposure on nonimmunologic bioparameters of the lung (115–119). In toto, these studies show that, depending on the point, the age of the animals can play a significant role in the outcome of the effects of O₃ exposure. With respect to immunologic parameters, it should be kept in mind that the immune response is known to wane with age (120).

In addition to age, qualitative difference in nonimmunologic responses to O₃ among animal species have also been documented (121–127). Also, it is becoming increasingly clear that pulmonary responses to O₃ exposure is subject to genetic influence (128–132), and it is also known that the immune response is under genetic control (133,134). While the wide diversity of receptor sites on antibody and T-cell receptors occur by gene rearrangement of the immunoglobulin superfamily, a more innate and stable genetic influence exists over host susceptibility in mice to intracellular infections such as Listeria, Mycobacteria, and Salmonella spp. (135). In many cases, the host response, which governs protection from these organisms, is the DTH reaction, involving both T-cells and subsequent activation of macrophages. Genetic differences have also been recently observed in the ability of two different strains of mice to resist streptococcal challenge (59). It is also becoming clear that some mouse strains produce high or low titers of circulating antibody following identical immunization procedures (136). Thus, in addition to species differences, strain differences may also play a role in the immunotoxicology of O₃ at this time, it is not known to what extent the combination of variables that include age, animal species, and genetic constitution may influence the effect of O₃ exposure on immune responsiveness.

Considerations of the Ozone Exposure Protocols

Investigations of the effects of O₃ on immunologic end-points that do not involve antigen stimulation have primarily used the following protocols: a) continuous (or intermittent) exposure on consecutive days with tests performed during the exposure protocol; b) a short (hourly) exposure with tests performed immediately after exposure or at intervals thereafter; and c) continuous (or intermittent) exposure for several days or weeks with tests performed immediately after exposure or at intervals thereafter.

Using continuous O₃ exposure with assays performed during a 2- to 4-week exposure period, four general response patterns emerge from the literature (Figure 11). In the first (Figure 11A), an initial suppression followed by recovery has been observed with spleen weights (3), spleen/body weight ratios (68), thymus weight (4), AM phagocytosis (33,34,67,74), PM phagocytosis (74), pulmonary NK and splenic NK cell activity (16,68), MLN proliferative response (68), and AM-dependent intrapulmonary bacterial killing (67). The second response pattern (Figure 11B), an initial suppression followed by an increased response, has been observed with MLN cell numbers (4,68). The third response pattern (Figure 11C), an initial absence of a response followed by increased activity, has been observed with MLN (8) and splenic (14) proliferative responses. Finally, in Figure 11D, a sustained response of thymic atrophy has also been observed (11).

The second exposure protocol consists of short (hourly) O₃ exposures with the tests performed immediately after exposure or at various times thereafter. The data show that immediately after exposure, AM phagocytosis is suppressed (30,31,34) and then followed by reestablishment of phagocytosis as the interval between exposure and assay was lengthened.

The third exposure protocol consists of continuous O₃ exposure for several days (or weeks) followed by tests immediately after exposure or at various intervals thereafter. Using this exposure protocol, T/B-lymphocyte ratios remained elevated for at least 5 days after exposure (10). Perhaps more studies were not performed with this O₃ exposure protocol because of a lack of an effect. A nonresponse situation may be predicted by the observations that continuous O₃ exposure, after a period of initial suppression, is followed by reestablishment.

Figure 11. Relative responses of the immune apparatus (lymphoid organ weights, proliferative responses to mitogen stimulation, NK cell activity, alveolar macrophage phagocytosis, and intrapulmonary staphylococcal killing (see

|    | Relative Percent of Control | Relative Percent of Control | Relative Percent of Control | Relative Percent of Control |
|----|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| A  |                             |                             |                             |                             |
| B  |                             |                             |                             |                             |
| C  |                             |                             |                             |                             |
| D  |                             |                             |                             |                             |
of the response, as exemplified by the O₃ exposure response pattern depicted in Figure 11A. This is supported by our range-finding studies that showed that continuous 0.5 ppm O₃ exposure for 10 consecutive days had no effect on AM-dependent intrapulmonary bacterial killing of S. aureus, either immediately after cessation of exposure or at 1 and 5 days thereafter (GJ Jakab, unpublished observations).

Superimposed on the three exposure patterns are the O₃ concentrations and whether the exposures are continuous or intermittent. In general, it appears that the O₃ concentrations modulate the intensity and the duration of the responses. Intermittent O₃ exposures can be viewed as alternating cycles of O₃-induced injury and repair that appear to blunt the intensity of the responses.

When the effects of O₃ exposure on antigen-stimulated responses are examined, the number of possibilities in exposure protocols is vastly increased. The simplest of these protocols is to expose animals to O₃ either before or after immunization. The data show that continuous O₃ exposure immediately before immunization had no effect on the DTH response to SRBCs (28), the DTH response to Listeria antigen, or the proliferative response of MLN to Listeria antigen (10), whereas O₃ exposure after immunization suppressed the responses. Using primary and secondary immunizing regimens, we have investigated the timing between immunization and O₃ exposure and found that as the interval was increased beyond 3 days, no effect was observed (68).

Summary

From the above discussion, it is apparent that many known and unknown factors impinge on the effect of O₃ exposure on immunologic responsiveness. The extent to which nutritional factors, health status, age, species variability, genetic background, and immune assay sensitivity interact to play a role on the effect of O₃ exposure on the immune system is difficult to unravel from the available data. Mechanistic studies will undoubtedly provide insights into the causes of the observed responses. Questions that beg answers include: a) Is there a level of O₃ exposure below which immune responses are enhanced and above which there is a toxic effect that suppresses immune responses? b) What is the mechanism for adaptation? c) Given the fact that O₃ itself never reaches the systemic immune compartment, what is the mechanism of O₃ induced suppression of systemic immunity? d) Where, in the sequence of events that leads to an antigen-stimulated immune response, does O₃ have an effect? Is it antigen availability, antigen presentation or a step in the complex cascade of events in the afferent limb of the immune response which may result in altered effector function? As alluded to before, O₃ and other oxidant gases are inflammatory when inhaled and thus cause a variety of permeability changes in the lung that could cause an increase in reactive immune tissue (BALT) or increase the availability of antigen to immunoreactive areas.

Much information is available regarding the effects of O₃ exposure on immune function. However, this information has not paved major inroads toward an understanding of the mechanisms by which O₃ exposure enhances/impairs/modulates immune function. The necessary database from which specific mechanistic hypotheses can be tested is available. The results of these studies will undoubtedly lead to a more complete understanding of the mechanisms by which O₃ alters immune function.

Although it is expected that future experimental studies will unravel the mechanisms by which O₃ alters immune responsiveness, their relationship to human health effects will require serious consideration. The National Ambient Air Quality Standard for O₃ is 0.12 ppm for a maximum of 1 hr that is not to be exceeded more than once per year. The worst case situation for human environmental exposure is in urban areas with concentrations reported as high as 0.36 ppm O₃ (137). However, the ambient concentrations of O₃ do not reflect the high O₃ excursions documented in urban areas. Indeed, most experimental animal studies have had to use O₃ exposures in excess of the ambient and spike O₃ concentrations to demonstrate an immunotoxic effect. In this regard, the study of Selgrade et al. (18) found no effect on multiple immune parameters when rats were chronically exposed to O₃ in a way that simulated an urban profile. Finally, in addition to the O₃ concentrations, the inferences that can be drawn between human health effects and the battery of immune tests recommended in the National Toxicology Program guidelines for immunotoxicity evaluation in mice (113) need to be clarified.

REFERENCES

1. Dean JH, Luster MI, Munson AE, Amos H. Immunotoxicology and Immunopharmacology. New York: Raven Press, 1985.
2. National Research Council. Biologic Markers in Immunotoxicology. Washington: National Academy Press, 1992.
3. Fujimaki H, Ozawa M, Imai T, Shimizu F. Effect of short-term exposure to O₃ on antibody response in mice. Environ Res 35:490–496 (1984).
4. Dziedzic D, White HJ. Thymus and pulmonary lymph node response to acute and chronic ozone inhalation in the mouse. Environ Res 41:598–609 (1986).
5. Li FY, Richters A. Effects of 0.7 ppm ozone exposure on thymocytes: in vivo and in vitro studies. Inhalation Toxicol 3:61–71 (1991).
6. Li FY, Richters A. Ambient level ozone effects on subpopulations of thymocytes and spleen T lymphocytes. Arch Environ Health 46:57–63 (1991).
7. Bleavins MR, Dziedzic D. An immunofluorescence study of T and B lymphocytes in ozone-induced pulmonary lesions in the mouse. Toxicol Appl Pharmacol 105:93–102 (1990).
8. Dziedzic D, White HJ. T-cell activation in pulmonary lymph nodes of mice exposed to ozone. Environ Res 41:610–622 (1986).
9. Osebold JW, Owens SL, Zee YC, Dotson WM, LaBarre DD. Immunological alterations in the lungs of mice following ozone exposure: Changes in immunoglobulin levels and antibody-containing cells. Arch Environ Health 36:258–265 (1979).
10. Van Loveren H, Rombout PJ A, Wagenaar, SjSc, Walvoort HC, Vos JG. Effects of ozone on the defence to a respiratory Listeria monocytogenes infection in the rat. Toxicol Appl Pharmacol 94:374–393 (1988).
11. Fujimaki H. Impairment of humoral immune responses in mice exposed to nitrogen dioxide and ozone mixtures. Environ Res 48:211–217 (1989).
12. Hassett C, Mustafa MG, Coulson WF, Elastoff RM. Splenomegaly in mice following exposure to ambient levels of ozone. Toxicol Lett 26:139–144 (1985).
13. Dziedzic D, Wright ES, Sargent NE. Pulmonary response to ozone: reaction of bronchus-associated lymphoid tissue and lymph node lymphocytes in the rat. Environ Res 51:194–208 (1990).
14. Eskew ML, Scheuchenzuber WJ, Scholz RW, Reddy CC, Zarkower A. The effects of ozone inhalation on the immunologic response of selenium- and vitamin E-deprived rats. Environ Res 40:274–284 (1986).
15. Aruny C, Vana SC, Thomas PT, Bradof JN, Fenters JD. Effects of subchronic exposure to a mixture of O₃, SO₂, and (NH₄)₂SO₄ on host defenses of mice. J Toxicol Environ Health 12:55–71 (1983).

16. Burleson GR, Keyes LL, Stutzman JD. Immunosuppression of pulmonary natural killer activity by exposure to ozone. Immunopharmacol Immunotoxicol 11:715–735 (1989).

17. Van Loveren H, Kracig El, Rombout PJA, Blommaert FA, Vos JG. Effects of ozone, benzalkonium, and bis(tri-n-butyl)oxide on natural killer activity in the rat lung. Toxicol Appl Pharmacol 102:21–33 (1990).

18. Selgrade MK, Daniels MJ, Grose EC. Acute, subchronic, and chronic exposure to a simulated urban profile of ozone: effects on extrapulmonary natural killer cell activity and lymphocyte mitogenic responses. Inhalation Toxicol 2:375–389 (1990).

19. Savino A, Peterson ML, House D, Turner AG, Jeffries HE, Baker R. The effect of ozone on human and hamoral immunity: characterization of T and B lymphocytes by rosette formation. Environ Res 15:65–69 (1978).

20. Peterson ML, Smialowicz R, Harder S, Ketcham A, Smialewicz R, Keyes LL, Stutzman JR. The Aranyi of chronic of ozone: effects of single and repeated ozone exposures. Toxicol Appl Pharmacol 110:170–178 (1991).

21. Devlin RB, McDunnell WF, Mann R, Becker S, House DE, Schreinemachers D, Koren HS. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am J Respir Cell Mol Biol 4:72–81 (1991).

22. Peterson ML, Harder S, Rummo N, House D. Effects of ozone on leukocyte function in exposed human subjects. Environ Res 51:485–493 (1997).

23. Goldstein E, Jordan GJ, MacKenzie MR. Methods for evaluating the toxicological effects of gaseous and particulate contaminants on pulmonary microbial defense systems. Annu Rev Pharmacol Toxicol 16:447–463 (1976).

24. Gardner DE. The use of experimental airborne infections for monitoring altered host defenses. Environ Health Perspect 43:99–107 (1982).

25. Miller S, Ehrlich R. Susceptibility to respiratory infection of animals exposed to ozone. J. Susceptibility to Klebsiella pneumoniae. J Infect Dis 103:145–149 (1959).

26. Miller FJ, Illing JW, Gardner DE. Effect of urban ozone levels on laboratory-induced respiratory infections. Toxicol Letters 2:163–169 (1978).

27. Ehrlich R, Findlay JC, Gardner DE. Effects of repeated exposures to peak concentrations of nitrogen dioxide and ozone on resistance to streptococcal pneumonia. J Toxicol Environ Health 26:131–624 (1979).

28. Ehrlich R, Findlay JC, Fenters JD, Gardner DE. Health effects of short-term inhalation of nitrogen dioxide and ozone mixtures. Environ Res 14:223–231 (1977).

29. Graham JA, Gardner DE, Blommer EJ, House DE, Menache MG, Miller FJ. Influence of exposure patterns of nitrogen dioxide and modifications by ozone on susceptibility to bacterial infectious disease in mice. J Toxicol Environ Health 21:113–125 (1987).

30. Goldstein E, Lipterrt W, Warshauer D. Pulmonary alveolar macrophage: defender against bacterial infection of the lung. J Clin Invest 54:519–528 (1974).

31. Huber GL, LaForce FM. Comparative effects of ozone and oxygen on pulmonary antibacterial defense mechanisms. Antimicrob Agents Chemother 129–136 (1970).

32. Huber GL, LaForce FM, Johanson WG Jr. Experimental models and pulmonary antimicrobial defenses. In: Respiratory Defense Mechanisms, Part II (Brain JD, Proctor DF, Reid LM, eds). New York: Marcel Dekker, 1977:983–1022.

33. Osebold JW, Osebold JW, Zee YC. Immunoglobulin E-containing cells in mouse lung following allergen inhalation and ozone exposure. Int Arch Allergy Immunol 65:266–277 (1981).

34. Matsumura Y. The effects of ozone, nitrogen dioxide, and sulfur dioxide on the experimentally induced allergic respiratory disorder in guinea pigs I. The effects of ozone on the absorption and the retention of antigen in the lung. Am Rev Respir Dis 102:438–443 (1970).

35. Matsumura Y. The effects of ozone, nitrogen dioxide, and sulfur dioxide on the experimentally induced allergic respiratory disorder in guinea pigs. I. The effects of ozone on the absorption and the retention of antigen in the lung. Am Rev Respir Dis 102:438–443 (1970).

36. Ozawa M, Fujimaki H, Ima T, Honda Y, Watanabe N. Suppression of antibody production after exposure to ozone in mice. Int Archs Allergy Immunol 76:16–19 (1985).

37. Fujimaki H, Shiraisi F, Ashikawa T, Murakami M. Changes in delayed hypersensitivity reaction in mice exposed to O₃. Environ Res 43:186–190 (1987).

38. Campbell KL, Hilsenroth RH. Impaired resistance to toxin in toxoid-immunized mice exposed to ozone or nitrogen dioxide. Clin Toxicol 9:943–954 (1976).

39. Goldstein E, Bartlema HC, van der Ploeg M, van Duijn P, van der Stip JG, Lippert W. Effect of ozone on lysosomal enzymes of alveolar macrophages engaged in phagocytosis and killing of inhaled Staphylococcus aureus. J Infect Dis 138:299–305 (1978).

40. Gilmour MI, Park P, Selgrade MK. Ozone-enhanced pulmon-ary infection with Streptococcus pneumoniae in mice: the role of alveolar macrophage function and capillary virulence factors. Am Rev Respir Dis 147:753–760 (1993).

41. Christman CA, Schwartz LW. Enhanced phagocytosis by alveolar macrophages induced by short-term ozone insult. Environ Res 28:241–250 (1982).

42. Schultheiss AH, Gilmour MI, Jakab GJ. Altered Fc-receptor mediated phagocytosis in rat alveolar macrophages following continuous ozone exposure. Am Rev Respir Dis 139:A277 (1989).

43. Driscoll KE, Vollmuth TA, Schlesinger RB. Acute and subchronic ozone inhalation in the rabbit: Response of alveolar macrophages. J Toxicol Environ Health 21:27–43 (1987).

44. Oosting RS, Van Golde LMG, Verhoef J, Van Bree L. Species differences in impairment and recovery of alveolar macrophage functions following single and repeated ozone exposures. Toxicol Appl Pharmacol 110:170–178 (1991).

45. Loosli CG, Buckley RD, Hwang-Kow S-Y, Hertweck MS, Hardy JD, Serbinin R. Effect of air pollution on resistance of mice to airborne influenza A virus infection. In: Airborne Transmission and Airborne Infection (Hers JFP, Winkler KC, eds). Oosterhout: Utrecht, Netherlands, 1973:225–231.

46. Selgrade MK, Illing JW, Starnes DM, Stead AG, Menache MG, Stevens MA. Evaluation of effects of ozone exposure on influenza infection in mice using several indicators of susceptibility. Fundam Appl Toxicol 11:169–180 (1988).
56. Wolcoitz A, Zee YC, Osebold JW. Exposure to ozone reduces influenza disease severity and alters distribution of influenza viral antigens in murine lungs. Appl Environ Microbiol 44:723–731 (1982).

57. Buell GC, Mueller PK. Potential crosslinking agents in lung tissue. Arch Environ Health 10:213–219 (1965).

58. Scheel LD, Dobrogorski OJ, Mountain JT, Svirbely JL, Stokinger HE. Physiologic, biochemical, immunologic, and pathologic changes following ozone exposure. J Appl Physiol 14:67–80 (1959).

59. Stokinger HE, Scheel LD. Ozone toxicity: Immunotoxicological and tolerance-producing aspects. Arch Environ Health 4:327–334 (1962).

60. Atwal OS, Samaghi BS, Bhatnagar MK. A possible autoimmune parathyroiditis following ozone inhalation. Am J Pathol 80:53–61 (1975).

61. Wenzel DJ, Morgan DL. In vitro inhibition of alveolar macrophage phagocytosis by ozone. Absence of role for serum or mode of ozone administration. Toxicol Lett 18:57–61 (1983).

62. Becker S, Madden MC, Newman SL, Devlin RB, Koren HS. Modulation of human alveolar macrophage properties by ozone exposure in vitro. Toxicol Appl Pharmacol 110:403–415 (1991).

63. Harder SD, Harris DT, House D, Koren HS. Inhibition of human natural killer cell activity following in vitro exposure to ozone. Inhalation Toxicol 2:161–173 (1990).

64. Becker S, Jordan RL, Orlando GS, Koren HS. Ozone exposure downregulated human mitogen induced lymphocyte proliferation. J Environ Toxicol 1:53–64 (1988).

65. Becker S, Jordan RL, Orlando GS, Koren HS. In vitro ozone exposure inhibits mitogen-induced lymphocyte proliferation and IL-2 production. J Toxicol Environ Health 24:469–483 (1989).

66. Becker S, Quay J, Koren HS. Effect of ozone on immunoglobulin production by human B cells in vitro. J Toxicol Environ Health 34:353–366 (1991).

67. Gilmour MJ, Hmielecki RR, Stafford EA, Jakab GJ. Suppression and recovery of the alveolar macrophage phagocytic system during continuous exposure to 0.5 ppm ozone. Exp Lung Res 17:547–558 (1991).

68. Gilmour MJ, Jakab GJ. Modulation of immune function in mice exposed to 0.8 ppm ozone. Inhalation Toxicol 3:293–308 (1991).

69. Sanders VM, Fuchs BA, Pruet S, Kerkvliet NR, Kaminski NE. Symposium on indirect mechanisms of ozone modulation. Fundam Appl Toxicol 17:641–650 (1991).

70. Kleeberger SR, Kolbe J, Adkinson NF, Peters SP, Sannanhe EW. The role of mediators in the response of the canine peripheral lung to 1 ppm ozone. Am Rev Respir Dis 137:321–325 (1988).

71. Schlesinger RB, Driscoll KE, Gunnison AF, Zelikoff JT. Pulmonary arachidonic acid metabolism following acute exposure to ozone and nitrogen dioxide. J Toxicol Environ Health 31:275–290 (1990).

72. Doherty-Miller P, Ainsworth D, Lam HF, Amdur MO. Indomethacin and cromolyn sodium alter ozone-induced changes in lung function and plasma eosinophil concentrations in guinea pigs. Toxicol Appl Pharmacol 93:175–186 (1988).

73. Schlegle ES, Adams WC, Giri SN, Siefkin AD. Acute ozone exposure increases plasma prostaglandin F₁, in ozone-sensitive human subjects. Am Rev Respir Dis 140:211–216 (1989).

74. Canning BJ, Hmielecki RR, Sannahke EW, Jakab GJ. Ozone reduces murine alveolar and peritoneal macrophage phagocytosis: the role of prostanooids. Lung Cell Mol Physiol 5:1277–1282 (1991).

75. Sweet C, Smith H. Pathogenicity of influenza virus. Microbiol Rev 44:303–330 (1980).

76. Sullivan JL, Mayner KE, Barry DW, Ennis FA. Influenza virus infection in nude mice. J Infect Dis 133:91–94 (1976).

77. Wells MA, Albrecht F, Ennis FA. Recovery from viral respiratory infection. J Influenza pneumonia in normal and T-deficient mice. J Immunol 126:1036–1041 (1981).

78. Wyde PR, Cate TR. Cellular changes in lungs of mice infected with influenza virus: characterization of the cytotoxic responses. Infect Immun 22:423–429 (1978).

79. Wyde PR, Peavy DL, Cate TR. Morphological and cytochemical characterization of cells infiltrating mouse lungs after influenza virus infection. Infect Immun 21:140–146 (1978).

80. Wyde PR, Couch RB, Macker BD, Cate TR, Levey BM. Effect of high- and high-passage influenza virus infection in normal and nude mice. Infect Immun 15:221–229 (1977).

81. Berlin BS, Cochran KW. Delay of fatal pneumonia in X-irradiated mice inoculated with mouse-adapted influenza virus, PR8 strain. Radiat Res 31:342–351 (1967).

82. Singer SH, Noguchi P, Kirstein RL. Respiratory disease in cyclophosphamide-treated mice. II. Decreased virulence of PR8 influenza virus. Infect Immun 5:957–960 (1972).

83. Suzuki F, Ohya J, Ishida N. Effect of antilymphocyte serum on influenza virus infection in mice. Proc Soc Exp Biol Med 146:78–84 (1974).

84. Cate TR, Mold NG. Increased influenza pneumonia mortality of mice adoptively immunized with node and spleen cells sensitized by inactivated but not live virus. Infect Immun 11:908–914 (1975).

85. Jakab GJ, Hmielecki RR. Reduction of influenza virus pathogenesis by exposure to 0.5 ppm ozone. J Toxicol Environ Health 23:455–472 (1988).

86. Jakab GJ, Bassett DJP. Influenza virus infection, ozone exposure, and fibrogenesis. Am Rev Respir Dis 141:1307–1315 (1990).

87. Nayak DP, Kelley GW, Underdahl NR. The effect of varied inculous on the distribution and progression of influenza virus (S-15) in lungs of mice. Am J Vet Res 26:984–990 (1965).

88. Jakab GJ. Suppression of pulmonary antibacterial activity following Sendai virus infection in mice: dependence on virus dose. Arch Virol 48:385–390 (1975).

89. Jakab GJ, Warr GW. Lung defenses against viral and bacterial challenges during immunosuppression with cyclophosphamide in mice. Am Rev Respir Dis 123:524–528 (1981).

90. Balford G. Studies on the immune response and pathogenesis of Sendai virus infection of mice. III. The effects of cyclophosphamide. Immunology 28:871–883 (1975).

91. Tashiro M, Tobita K, Seto JT, Rott R. Comparison of protective effects of serum antibody on respiratory and systemic infection of Sendai virus in mice. Arch Virol 107:85–96 (1989).

92. Larkin EC, Gollop WE, Rao GA. Diet consumption and growth are decreased in rats exposed to ozone. IRCS Med Sci 13:145 (1985).

93. Suskind RM. Malnutrition and the Immune Response. New York:Raven Press, 1977.

94. Gontzea, I. Nutrition and anti-infectious defense. Basel:S Karger, 1974.

95. Larkin EC, Rao GA. Reduced growth in rats exposed to ozone as compared to pair-fed controls breathing air. IRCS Med Sci 12:741–742 (1984).

96. Scrimshaw NS, Taylor CE, Gordon JE. Interaction of Nutrition and Infection. Geneva:World Health Organization, 1968.

97. National Research Council. Infectious Diseases of Mice and Rats. Washington:National Academy Press, 1991.

98. Hammers RJ Jr. Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing. Virginia: Hemispheres Publishing Corporation, 1986.

99. Bhart PN, Jacobo RO, Morse HC III, New AE. Viral and Mycoplasmal Infections of Laboratory Rodents. Effects on Biomedical Research. New York:Academic Press, 1986.

100. Parker JC, Whiteman MD, Richter CB. Susceptibility of inbred and outbred mouse strains to Sendai virus and prevalence of infection in laboratory rodents. Infect Immun 19:123–130 (1978).

101. Warr GA, Jakab GJ, Hearst JE. Alterations in lung macrophage immune receptor(s) activity associated with viral pneumonia. J Reticuloendothel Soc 26:357–366 (1979).

102. Jakab GJ, Warr GA, Sanna PL. Alveolar macrophage ingestion and phagosome-lysosome fusion defect associated with virus pneumonia. Infect Immun 7:960–968 (1980).
103. Hall WC, Lubet RA, Henry CJ, Collins MJ Jr. Sendai virus—
disease processes and research complications. In: Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing (Hamm TE Jr, ed). Washington: Hemisphere Publishing Corporation, 1986:25–52.

104. Garlinghouse L, Van Hooser G. Characterization of the suppressor factor in the lungs of Sendai virus-infected rats (Abstract). Lab Anim Sci 33:301 (1983).

105. Garlinghouse L, Van Hooser G. Studies on adjuvant-induced arthritis, tumor transplantability, and serologic response to bovine serum albumin in Sendai virus-infected rats. Am J Vet Res 39:297–300 (1978).

106. Anderson, M. Innate cytotoxicity of CBA mouse spleen cells to Sendai virus-infected L cells. Infect Immuno 20:608–612 (1978).

107. Anderson M, Pattison J, Heath R. The nature of the effector cells of cell-mediated immune responses to Sendai and Kunz virus infection in mice. Brit J Exp Pathol 60:608–612 (1978).

108. Weir E, Green D, Brownstein M. Influence of Sendai virus infection of regulation of the in vitro immune response in C57Bl/6J mice (Abstract). Lab Anim Sci 34:514 (1984).

109. Kay M. Long term subclinical effects of parainfluenza (Sendai) infection on immune cells of aging mice. Sci Exp Biol Med 158:326–331 (1978).

110. Hickman RL. Toxicology: complications caused by murine viruses and mycoplasmas. In: Viral and Mycoplasmal Infections of Laboratory Rodents. Effects on Biomedical Research. (Bhatt PN, Jacoby RO, Morse HC III, New AE, eds). New York: Academic Press, 1986:693–720.

111. Kanellopoulos JM, De Petriss S, Lea G, Crumpton MJ. The mitogenic lectin from Pseudomonas vulgaris does not recognize the T3 antigen of human T lymphocytes. Eur J Immunol 15:479–486 (1985).

112. Stobo JD, Paul WE. Functional heterogeneity of murine lymphoid cells. III. Differential responsiveness of T cells to phytohemagglutinin and concanavalin A as a probe for T cell subsets. J Immunol 110:326–375 (1973).

113. Luster MI, Munson AE, Thomas PT, Holkapple MP, Fenters JD, White KL Jr, Lauer LD, Gerholec DR, Rosenthal GJ, Dean JH. Development of a testing battery to assess chemical-induced immunotoxicity: evaluation in mice. National Toxicology Program’s guidelines for immunotoxicity evaluation in mice. Fundam Appl Toxicol 10:2–19 (1988).

114. Luster MI, Portier C, Paht DG, White KL Jr, Gennings C, Munson AE, Rosenthal GJ. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. Fundam Appl Toxicol 18:200–210 (1992).

115. Gunnison AF, Finkelson I, Weideman P, Su WY, Sobo M, Schlesinger RB. Age-dependent effect of ozone on pulmonary alveolar cell metabolism in rabbits and rats. Fundam Appl Toxicol 15:779–790 (1990).

116. Gunnison AF, Weideman PA, Sobo M, Koenig KL, Chen LC. Age-depending of responses to acute ozone exposure in rats. Fundam Appl Toxicol 18:360–369 (1992).

117. Elsayed N, Mustafa MG, Postlethwait EM. Age-dependent pulmonary response of rats to ozone exposure. J Toxicol Environ Health 9:835–848 (1982).

118. Tyson CA, Lunan KD, Stephens RJ. Age-related differences in GSH-shuttle enzymes in NO2- or O3-exposed rat lungs. Arch Environ Health 37:167–176 (1982).

119. Montgomery MR, Raska-Emery P, Balis JU. Age-related difference in pulmonary response to ozone. Biochim Biophys Acta 890:271–274 (1987).

120. Callard RE. Aging and the immune system. In: CRC Handbook of Immunology of Aging (Kay MB, Makinodan T, eds). Boca Raton, FL-CRC Press, 1981:103–122.

121. Cavender FL, Singh, B. Cockrell BY. The effects in rats and guinea pigs from six month exposures to sulfuric acid mist, ozone, and their combination. J Environ Pathol Toxicol 2:485–492 (1978).

122. Mustafa MG, Elsayed NM, Graham JA, Gardner DE. Effects of ozone exposure on lung metabolism: influence of animal age, species and exposure conditions. In: Biomedical Effects of O3 and Oxidants (Lee SD, Mustafa MG, Mehlan MA, eds). Princeton NJ: Princeton Scientific, 1983:57–73.

123. Mustafa MG, Elsayed NM, Quinn CL, Postlethwait EM, Gardner DE, Graham JA. Comparison of pulmonary biochemical effects of low-level ozone exposure on mice and rats. J Toxicol Environ Health 9:857–865 (1982).

124. Guth DJ, Warren DL, Last JA. Comparative sensitivity of measurements of lung damage made by bronchoalveolar lavage after short-term exposure of rats to ozone. Toxicology 40:131–143 (1986).

125. Harch GE, Slade R, Stead AG, Graham JA. Species comparison of acute inhalation toxicity of ozone and phosgene. J Toxicol Environ Health 19:43–53 (1986).

126. Sagai M, Arakawa K, Ichinose T, Shimojo N. Biochemical effects on combined gases of nitrogen dioxide and ozone. I. Species differences of lipid peroxides and phospholipids in lungs. Toxicology 46:251–265 (1987).

127. Brain JD, Beck BD, Warren AJ, Shaikh RA. Variations in Susceptibility to Inhaled Pollutants. Identification, Mechanisms and Policy Implications. Baltimore: The Johns Hopkins University Press, 1988.

128. Goldstein BD, Lai LY, Ross SR, Cuzzo-Spada R. Susceptibility of inbred mouse strains to ozone. Arch Environ Health 27:412–413 (1973).

129. Kleeberger SR, Bassett DFP, Jakab GJ, Levitt RC. A genetic model for evaluation of susceptibility to ozone-induced inflammation. Lung Cell Mol Physiol 2 258:L313–L320 (1990).

130. Kleeberger SR, Levitt RC. Animal models for studies on genetic predisposition of adverse effects of chemical exposure. In: Ecogenetics. Genetic Predisposition to the Toxic Effects of Chemical (Grandjean P, ed). London: Chapman and Hall, 1991:91–110.

131. Kleeberger SR, Levitt RC, Zhang LY. Susceptibility to ozone-induced inflammation. I. Genetic control of the response to subacute exposure. Am J Physiol 264:L15–L20 (1993).

132. Kleeberger SR, Levitt RC, Zhang LY. Susceptibility to ozone-induced inflammation. II. Separate loci control responses to acute and subacute exposures. Am J Physiol 264:L21–L26 (1993).

133. Clark EA, Harmon RC. Genetic control of natural cytotoxicity and hybrid resistance. Adv Cancer Res 31:227–285 (1980).

134. Sasaki T, Nishimura Y, Muto M, Ohna N. HLA-linked genes controlling immune response and disease susceptibility. Immunol Rev 70:51–75 (1983).

135. Skamene E. Genetic regulation of host resistance to bacterial infection. Rev Infect Dis 5:882–883 (1983).

136. Stewart GA, Holt PG. Immunogenicity and tolerogenicity of a major house dust mite allergen, DER P1, from Dermatophagoides farinae in mice and rats. Int Arch Allergy Appl Immunol 83:44–57 (1987).

137. Office of Technology Assessment. Catching Our Breath: Next Steps for Reducing Ozone. Washington: United States Congress, February 1989, Table 32.