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# Adjuvant Effect of Respiratory Irritation on Pulmonary Allergic Sensitization: Time and Site Dependency

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**It has been suggested that airway irritation, by acting as an adjuvant, as well as producing damage, may be an important factor related to asthma. The present study examined the window of time following acute upper and lower airway irritant exposure to determine the period of increased risk of immunological sensitization. Brown Norway rats were exposed to 87 ppm $NO_2$ or 1000 ppm $NH_3$ for 1 hr. A 30-min ovalbumin (OVA) exposure of 18.14 $\mu$g/liter air was given at various times based upon the time course of irritant associated inflammatory response (either immediately prior to or 1 or 7 days after the irritant exposure). OVA-only, $NO_2$-only or $NH_3$-only controls, and saline controls were also studied. Weekly booster exposures of OVA (or saline) were given. Circulating OVA-specific IgE, IgA, and IgG levels were quantified periodically during the 6 weeks of the study. Bronchoalveolar lavage (BAL) was also performed to examine the inflammatory response to allergic and irritant challenge. Significant increases in OVA-specific IgE, IgG, and IgA antibody titers were seen in rats given the sensitizing OVA exposure within 1 day of the $NO_2$, but not $NH_3$ exposures. Enhancement of cellular infiltrate in BAL was noted in groups given the sensitizing OVA exposure within 1 day of the $NO_2$ or $NH_3$. It is concluded that the inflammatory and immunological response to antigen exposure can be modified by the site of respiratory tract irritation and the relative times of irritant and antigen exposure.** 

Respiratory tract allergies and asthma are caused by a variety of agents including molds, pollens, animal dander, insects, and other organic dusts. The occupational and environmental factors, other than the presence of the antigen, which may facilitate the development of respiratory allergies or asthma are unknown. An increase in hospital admissions for asthma on days of high ozone pollution has been reported (Ponka, 1991), suggesting that airway irritation may be an important factor related to asthma. Ozone exposure is known to cause airway hyperreactivity (Holtman *et al.,* 1983) and bronchial constriction which is in part mediated through mast cell degranulation (Kleeberger *et al.*, 1988; Fouke *et al.*, 1990). The exacerbation of existing asthma could occur through such a mechanism.

In the laboratory, protocols for the sensitization of animals with allergens by injection have been investigated. It is known that specific adjuvants not only boost the immune response to a substance, but may also shunt the response in favor of IgE or IgG. Alum [$Al(OH)_3$], administered in conjunction with optimal antigen doses, enhances IgE antibody production, while use of Freund's adjuvant and higher antigen levels tends to promote IgG production (Bertrand *et al.,* 1989, 1991). The shift to IgE production can also be enhanced by pretreatment with the antibiotics cyclophosphamide or cyclosporin (Graziano *et al.,* 1981; Swey-Shen *et al.,* 1992).

Respiratory tract irritants may act as immunological adjuvants during pulmonary sensitization. A study by Folkesson *et al.* (1991) of the effect of airway inflammation on passage of different-sized molecules from the lung to blood in a rat model found that inflammation caused an increased passage of even large molecules such as albumin from the lung into the blood. This may potentially increase the bioavailability of inhaled proteins to act as immunological sensitizers. Ozone exposure has been reported to increase immunoglobulin in the mouse lung (Osebold *et al.,* 1979). Ozone exposure with allergen inhalation increases the number of IgE-containing monocytic cells in the mouse lung, and these cells have a more disseminated distribution than that seen with sensitization by injection (Gershwin *et al.,* 1981). Airway irritants may have an adjuvant effect on the immune response to inhaled antigens by such mechanisms. Enhanced airway reactivity upon allergen challenge in a nonhuman primate model, when irritants were used in such an adjuvant-like way, has been noted (Biagini *et al.,* 1986). There has been one report of inhibition of IgE in mice when the antigen was administered after cessation of ozone exposure (Ozawa, 1985). The immunological and nonimmunological effects of $NO_2$ have been reviewed (Moldéus, 1993).

The purpose of the present study was to examine site and time dependency of acute irritation of the respiratory tract on pulmonary allergic sensitization. Site selective irritation was accomplished using the upper respiratory tract irritant,



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ammonia, and the lower respiratory irritant, $NO_2$. Irritant exposures consisted of a single 1-hr exposure. A total of six weekly 30-min antigen (or saline) exposures were administered. Antigen exposure was initiated at various periods during the respiratory tract inflammatory response to a single acute irritant gas exposure to examine the time dependency of the irritant-induced immunological adjuvant effect. The exposure paradigm is one that is similar to an occupational setting in which a worker, who works with a allergic pulmonary sensitizer, would receive a high brief exposure to a respiratory tract irritant (e.g., due to a spill or leak).

## MATERIALS AND METHODS

***Animals.*** Male Brown Norway rats (200–225 g, 9–10 weeks old) were purchased from Harlan Laboratories (Indianapolis, IN). The animals were housed in polycarbonate cages containing wire bottom inserts to prevent exposure to feces and bedding. Cages were held in laminar flow hoods. Food and water were provided *ad libitum.* All rats were examined by a veterinarian upon arrival into the facility. Additional Brown Norway rats from the same shipment were also housed along with the study animals. At the end of the study these rats were euthanized for serological and pathological examination to ensure that there was no infection present.

***Irritant exposures.*** $NO_2$ was generated by diluting flow from a stock cylinder containing 1000 ppm $NO_2$ with filtered room air. The final chamber concentration was continually monitored using a Miran infrared analyzer (Foxboro Co., Foxboro, MA). The infrared analyzer was calibrated using the Saltzman method for $NO_2$ (Saltzman, 1954). Ammonia atmospheres were generated by dripping ammonia water (Aldrich, Milwaukee, WI) into an evaporation flask at a controlled rate using a syringe pump. Dilution air was flushed through the flask and into the plastic exposure chamber. The infrared analyzer, used for continuous monitoring of ammonia levels in the exposure chamber, was calibrated by injecting known amounts of ammonia water into a closed loop calibration system. Animals were given a single 1-hr irritant exposure. Ammonia and nitrogen dioxide chamber concentrations were 1000 and 87 ppm, respectively.

***Antigen exposures.*** Ovalbumin (OVA, Sigma Chemical, St. Louis, MO) was used as the antigen/allergen. A 1% solution of OVA in saline was generated using an Ultra-Neb 99 ultrasonic nebulizer (DeVilbiss, Somerset PA). Filtered air was passed through the nebulizer and then the aerosolized OVA was further diluted with filtered air before entering the exposure chamber. The size distribution of the aerosol was determined using a TSI Model 3300 aerodynamic particle analyzer (TSI Incorporated, St. Paul, MN). The aerosol size distribution was bimodal with an aerodynamic mass median diameter of 8.51 $\mu$m. Ninety-five percent of the particles generated in the aerosol cloud were $<2$ $\mu$m in diameter. Chamber OVA aerosol concentrations were determined by collecting samples in an impinger containing saline. Subsequent protein analysis employed the Coomassie blue dye reagent (Bio-Rad Laboratories, Hercules, CA). The rats were exposed to $18.14 \pm 2.2$ $\mu$g/liter OVA, 30 min per week for 6 weeks.

***Exposure groups.*** For each irritant gas ($NO_2$ or $NH_3$) there were three control groups and three exposure groups. The control groups consisted of: (i) a saline control group which was exposed to an aerosol of saline for 30 min per week for 6 weeks; (ii) an irritant exposure group that was exposed once to the irritant gas for 1 hr, as described above, followed by weekly aerosol exposures to saline; and (iii) an OVA (in saline) control group exposed as described above. The three exposure groups contained animals that were exposed to the irritant gas on (i) the day the first OVA exposure was started ($OVA–NO_2$); (ii) 1 day prior to the start of the first OVA exposure ($NO_2$–1d-OVA); and (iii) 7 days prior to the start of the OVA exposure ($NO_2$–7d-OVA). A total of six weekly 30-min OVA exposures were given to all groups. Preliminary studies in our laboratory showed that the inflammatory responses to $NO_2$ in the lung and $NH_3$ in the nose were maximal at 24 to 48 hr after exposure and had resolved by Day 7. The exposure groups were chosen so that the antigen would be present throughout the inflammatory response ($OVA–NO_2$), during the resorptive phase of the inflammatory response ($NO_2$–1d-OVA), or after the inflammatory response had resolved ($NO_2$–7d-OVA). Blood was collected from the heart of anesthetized animals periodically throughout the study for examination of antibody profile. Half of each group of 6 or 7 rats were euthanized after the third exposure and the rest after the sixth exposure. A few rats were lost during the blood drawing procedure.

***Serum ovalbumin-specific IgE, IgG, and IgA measurements.*** Blood was collected via cardiac puncture at intervals 2 days prior to the weekly OVA exposure and at euthanization, 1 day after antigen exposure in the $NO_2$ study. Blood was drawn 1 day after the fourth or sixth OVA exposure in the $NH_3$ study. Sera were diluted to 1/10, 1/100, and 1/1000 with phosphate-buffered saline containing 5% horse serum albumin (HOSA/PBS). The duplicates of each diluted serum (100 $\mu$l) was then added to a 96-well plate (Cat. No. 76-381-04, ICN Pharmaceuticals Inc., Costa Messa, CA) which had been previously coated with 200 $\mu$l of 10 $\mu$g OVA/ml carbonate coating buffer and blocked with 5% HOSA/coating buffer. Blank wells containing the HOSA/PBS carrier were also run to assess nonspecific binding. All ELISA buffers were prepared according to Voller and Bidwell (1986). Plates were washed three times after each step with PBS/Tween buffer. The plates were incubated overnight at 4°C. Sheep IgG anti-rat IgE (100 $\mu$l, 1/500 dilution in HOSA/PBS; Cat. No. 64-325-1, ICN Pharmaceuticals Inc.) followed by horseradish peroxidase-bound donkey IgG anti-sheep IgG (100 $\mu$l, 1/1000 dilution in HOSA/PBS; Cat. No. 67-253-1, ICN Pharmaceuticals, Inc.) were incubated in the plate for 2 hr at room temperature. The plate was developed using the substrate tetramethylbenzidine (TMB, Sigma Chemical Co.) and read at either 450 or 630 nm. Higher dilutions than those shown under Results were also evaluated and were consistent with the significant changes reported.

Both OVA-specific IgG and IgA measurements were made using the same protocol but with the appropriate detection antibodies (Sigma Chemical) for each assay. Sera for IgG and IgA measurements were diluted from 1/100 to 1/10,000. Goat IgG anti-rat IgG (Cat. No. R2129, 1/500 dilution), goat IgG anti-rat IgA (Cat. No. R9630, 1/500 dilution), and peroxidase-labeled rabbit IgG anti-goat IgG (Cat. No. A3540, 1/14,000 dilution) were the detection antibodies used for these measurements. All immunoassays employed in this study were improved from the first $NO_2$ study to the $NH_3$ study allowing for increased sensitivity.

***Lavage procedure.*** Rats were euthanized using an overdose of sodium pentobarbital (50 mg) 1 day after either the fourth or sixth OVA exposure. The trachea was cannulated and bronchioalveolar lavage (BAL) was performed repeatedly with 5 ml PBS until 50 ml lavagate was collected. Protein content in the first 5 ml lavage supernant fluid was measured as a marker of edema. The upper respiratory tract (UTR) was lavaged by turning the tracheal canulae toward the head and flushing the UTR with 3 ml PBS while the rats head was slanted downward with the mouth held closed. The entire UTR lavagate was recovered from the nares. All lavagates were placed on ice until analysis.

***Lavage cell content analysis.*** Cell differential analysis was performed using a Coulter counter equipped with a chanalyzer (Coulter Corp., Hiealeah, FL). Granulocytic cells were further differentiated in some cases by evaluating microscope slide BAL cell preparations stained with a Wright stain.

***Cellular eosinophil peroxidase activity.*** The method employed to measure cellular peroxidase activity from BAL cells was performed by a modified method of Tagari *et al.* (1993). BAL cells were incubated for 10 min, 37°C in 3 mM KBr/Hepes + $Ca^{2+}$, $Mg^{2+}$ buffer. The reaction was terminated by centrifugation and the supernant fluid was stored at −70°C until analysis. Peroxidase activity was measured using the substrate *o*-phenylenediamine (OPD) at 450 nm.

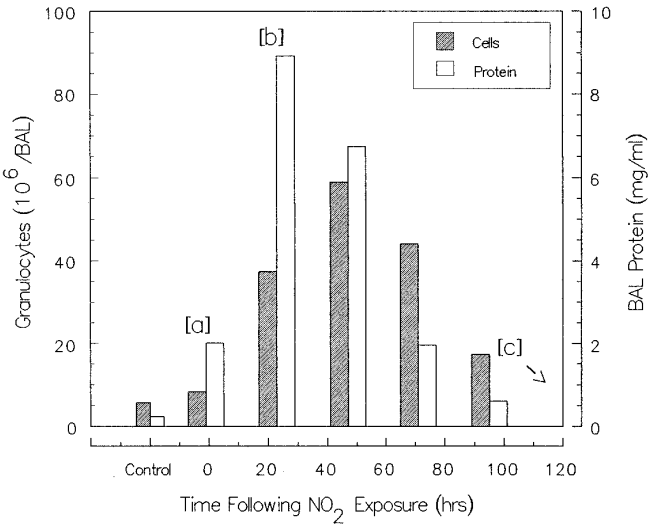


**FIG. 1.** BAL inflammation markers following a 1-hr exposure to 87 ppm $NO_2$. BAL supernatant fluid protein and inflammatory cell numbers were measured for up to 96 hr after exposure as markers of edema and inflammation, respectively. BAL protein peaked at 24 hr while inflammatory cell content at the 48-hr time point. The 0 time point represents the time point immediately after the exposure. $n = 2$/time point. The points during the inflammatory response when the initial OVA exposure for the OVA–$NO_2$, OVA–1d-$NO_2$, and OVA–7d-$NO_2$ experimental groups are identified as a, b, and c, respectively.

***Statistics.*** The data are presented as means ± SEM. Significant differences ($p < 0.05$) were determined by one-way analysis of variance. Correlation coefficients were tested by using a *t* test.

## RESULTS

*Preliminary study.* The time course of the pulmonary inflammatory response to 1 hr exposure to 87 ppm $NO_2$ is shown in Fig. 1. Similar exposures using other rat strains and guinea pigs have been performed in this lab with qualitatively identical results. The pulmonary inflammatory profile following acute $NO_2$ exposure to rats has also been previously reported (Lehnert *et al.*, 1994). A similar time profile was obtained for the nasal inflammatory response to $NH_3$ (data not shown). These profiles were then used to determine when the initial exposures to OVA were to be given. The inflammatory cell response in the lung (neutrophil influx) to the lower lung irritant, $NO_2$ peaked by the second day, while the edematous response peaked at 24 hr after exposure. Nasal lavage from animals exposed to $NO_2$ for 1 hr showed no signs of nasal inflammation. The nasal inflammatory response to ammonia showed a similar time course, peaking on Day 1 following exposure. BAL samples showed no increase in inflammatory cells following ammonia exposure. The sensitizing OVA exposures were given (i) immediately prior to irritant exposure, (ii) at the peak of the irritant response (1 day following irritant exposure), (iii) after resolution of the irritant response (7 days following irritant exposure), or (iv) to naive animals, as described under Materials and Methods.

*OVA/$NO_2$ studies.* Figure 2a displays the OVA-specific IgE levels in the sera. At the 3-week time point, circulating antigen-specific IgE was approximately 10-fold higher in groups in which the sensitizing exposure was present during the pulmonary inflammatory response to $NO_2$ (i.e., OVA–$NO_2$ and $NO_2$–1d-OVA) than that in the OVA control group. When the sensitizing dose was given after resolution of this inflammatory response, there was a tendency toward inhibition of the IgE response, but this was not statistically significant. The altered IgE production by acute lower lung irritation was short-lived; by the sixth week IgE levels were progressing toward the levels seen in rats exposed to OVA only. The OVA-specific IgG and IgA levels differed from those of IgE (Figs. 2b and 2c). They continued to increase throughout the study. The enhancement of IgG and IgA production due to $NO_2$-induced lower lung irritation was more evident at the 6-week time point.

Lavage was performed 1 day after the fourth or sixth OVA exposure. A significant increase in granulocytes (neutrophils and eosinophils) and eosinophil cellular peroxidase was noted after the sixth weekly exposure to OVA. Table 1 is the BAL cell differential from the rats after 6 weeks of exposure. The BAL alveolar macrophage and granulocyte (i.e., neutrophils and eosinophils) counts are presented in Table 2. There was a significant attenuation of the eosinophilic inflammation in the group receiving the initial sensitizing OVA exposure 7 days after acute $NO_2$ inhalation ($p < 0.05$) (Fig. 3). A significantly greater content of granulocytes was noted in the animals which received their initial sensitizing OVA exposure at the peak of the $NO_2$ inflammatory response (Table 2). The enhanced response was seen mainly in the neutrophil fraction. No difference in BAL alveolar macrophage numbers was seen between any of the paired treatment and control groups.

*OVA/$NH_3$ studies.* Acute exposure (1000 ppm, 1 hr) to ammonia did not significantly alter the OVA-specific IgG, IgA, and IgE profiles over the 6-week period (Table 3). The same general trends in the antibody titers over time of the OVA-only exposed group were observed. Ovalbumin-specific IgG and IgA titers increased throughout the 6-week observation period. Ovalbumin-specific IgE titers, in comparison, were more variable at the sixth week with titers that had increased, decreased or remained the same from the third week titer in individual animals.

The alveolar macrophage and granulocyte (neutrophils and eosinophils) cell counts from BAL are presented in Table 2. Complete differentials were not performed due to a cytology processing error. The counts from the granulocyte size range of the cell counter were corrected for noise from nongranulocytes. BAL granulocytes were sig-

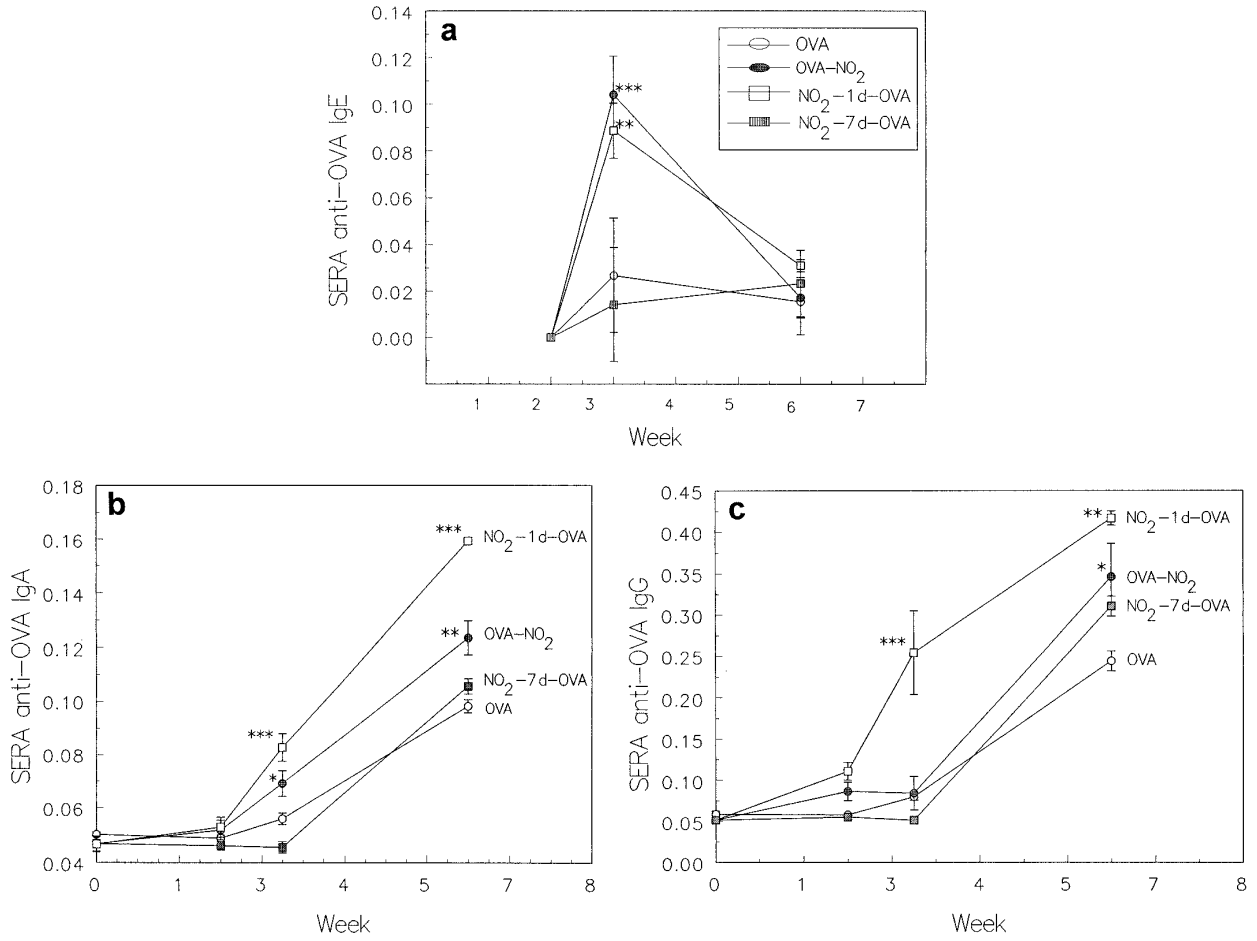


**FIG. 2.** Sera anti-OVA IgE (a), IgA (b), and IgG (c) profiles of rats given an initial OVA-sensitizing exposure at various time points during the acute pulmonary inflammatory response to $NO_2$ as described under Materials and Methods. $n = 3$ to 6/group (6 weeks has 3/group). Each point is an average ± SEM. ******Significantly different from OVA (only) exposures, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

nificantly higher when the sensitizing dose was given during the $NH_3$ inflammatory response (OVA–I-$NH_3$, $p = 0.013$; $NH_3$–1d-OVA, $p < 0.05$). The granulocytes represent a combination of neutrophils and eosinophils. Even though the complete cell differential between these two is not available, there was no difference seen in cellular EPO levels in the groups (data not shown). This suggests that, similar to the $NO_2$ effect, the increased inflammation in the $NH_3$ groups was due to an increase in neutrophil content.

**TABLE 1**
**BAL Cell Differentials in OVA + $NO_2$ Exposure and Control[a]**

| Group ($n = 3$) | Alveolar macrophage | Lymphocyte | Neutrophil | Eosinophil | Other[b] |
|---|---|---|---|---|---|
| Saline | 97.7 ± 0.9 | 0.8 ± 0.3 | 0.2 ± 0.2 | 0.7 ± 0.4 | 0.5 ± 0.3 |
| Saline–$NO_2$ | 96.7 ± 0.3 | 2.2 ± 0.2 | 0.3 ± 0.2 | 0.2 ± 0.2 | 0.7 ± 0.2 |
| OVA | 68.8 ± 6.4 | 4.7 ± 1.2 | 9.5 ± 5.0 | 13.8 ± 6.9 | 3.2 ± 0.6 |
| OVA–$NO_2$ | 74.7 ± 3.4 | 6.0 ± 2.3 | 10.3 ± 4.9 | 4.8 ± 0.9 | 4.2 ± 1.1 |
| OVA–1d-$NO_2$ | 57.7 ± 7.6 | 6.3 ± 1.4 | 15.0 ± 3.8 | 11.8 ± 1.6 | 9.3 ± 4.5 |
| OVA–7d-$NO_2$ | 93.2 ± 1.0 | 3.2 ± 0.2 | 2.3 ± 0.6 | 0.5 ± 0.3 | 0.8 ± 0.2 |

[a] 200 cells counted/differential; all values are average percentages ± SEM.
[b] Other includes pycnotic cells.

**TABLE 2**
**BAL Granulocytes and Alveolar Macrophages after Sixth OVA Exposure[a]**

| | $NO_2$ | | $NH_3$ | |
|---|---|---|---|---|
| Group ($n$ = 3 or 4) | Granulocytes[b] | Alveolar macrophages | Granulocytes[b] | Alveolar macrophages |
| Saline | 0.75 ± 0.38 | 9.78 ± 3.21 | 0.24 ± 0.08 | 7.76 ± 1.81 |
| Saline–gas | 0.84 ± 0.29 | 10.95 ± 1.92 | 0.38 ± 0.08 | 9.11 ± 0.58 |
| OVA | 1.25 ± 0.16 | 6.48 ± 0.5 | 0.50 ± 0.11 | 10.1 ± 2.04 |
| OVA–gas | 1.88 ± 0.94 | 7.48 ± 0.58 | 6.92 ± 2.52* | 9.44 ± 0.99 |
| OVA–1d-gas | 4.56 ± 1.08* | 8.41 ± 0.85 | 4.69 ± 1.67* | 10.13 ± 1.58 |
| OVA–7d-gas | 0.39 ± 0.04* | 9.55 ± 0.40 | 1.25 ± 0.61 | 8.59 ± 0.65 |

[a] Cells ± SEM × $10^6$/BAL.
[b] Granulocytes: neutrophils and eosinophils.
* Significantly different from OVA group, $p < 0.05$.

## DISCUSSION

Several studies (Osebold *et al.*, 1979; Gershwin *et al.*, 1981; Biagini *et al.*, 1986) have demonstrated that respiratory irritants when administered in conjunction with an antigen produce an enhanced IgE immune response. The objective of this study was to determine the adjuvant effect of respiratory irritants with respect to both area of the respiratory tract affected and length of time after irritation that this effect can be observed. Nitrogen dioxide and $NH_3$ were employed to produce an inflammatory response in the lower lung and upper airways, respectively.

The Brown Norway rat (BNR) model was chosen for this study for several reasons. BNR produces high levels of IgE antibodies (Imoka *et al.*, 1993). They can be repeatedly exposed to the antigen without use of antihistamines or bronchial dilators, since their lung is not a shock organ. Serial blood draws can also be performed to measure antibody levels.

The BNR response to antigen was not class specific. Ovalbumin-specific IgE, IgG, and IgA could be measured in sensitized rats. The immunoglobulin adjuvant effect of lower lung irritation on IgE antibody production was evident by the third week. The OVA-specific IgE levels in the rats given the initial OVA exposure, 1 week after the $NO_2$ exposure, appeared to be attenuated, suggesting that the response may be suppressed after severe pulmonary inflammation. This is

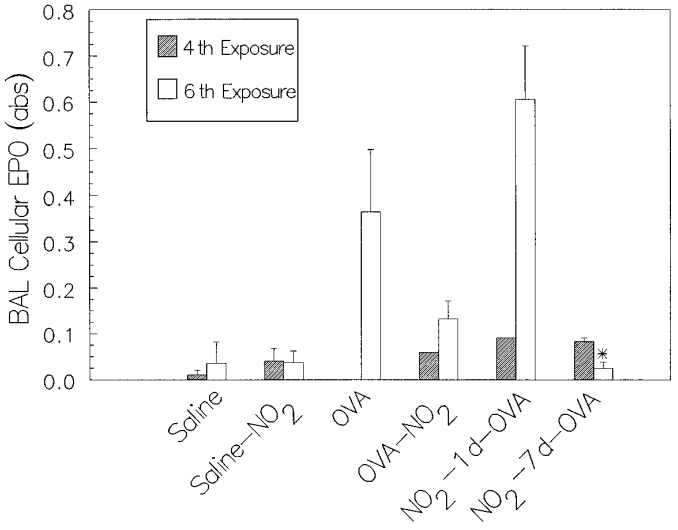


**FIG. 3.** BAL cellular EPO of rats given an initial OVA-sensitizing exposure at various time points during the acute pulmonary inflammatory response to $NO_2$, as described under Materials and Methods. $n$ = 3/group. Each point represents average ± SEM. *Significantly different from OVA (only) exposures, $p < 0.05$.

**TABLE 3**
**OVA-Specific Antibody Profile[a]**

| Group | IgE[b] | IgG[c] | IgA[b] |
|---|---|---|---|
| [1 day after 4th OVA exposure] ($n$ = 6 or 7/group) | | | |
| Saline | 0.098 ± 0.008 | 0.081 ± 0.006 | 0.103 ± 0.005 |
| $NH_3$ | 0.010 ± 0.005 | 0.080 ± 0.002 | 0.108 ± 0.012 |
| OVA-$NH_3$ | 0.332 ± 0.074 | 0.476 ± 0.152 | 0.316 ± 0.086 |
| $NH_3$-1d-OVA | 0.259 ± 0.032 | 0.479 ± 0.078 | 0.376 ± 0.049 |
| $NH_3$-7d-OVA | 0.692 ± 0.27 | 0.456 ± 0.127 | 0.361 ± 0.103 |
| OVA | 0.365 ± 0.068 | 0.337 ± 0.082 | 0.341 ± 0.059 |
| [1 day after 6th OVA exposure] ($n$ = 3 or 4/group) | | | |
| Saline | 0.083 ± 0.009 | 0.084 ± 0.002 | 0.071 ± 0.002 |
| $NH_3$ | 0.079 ± 0.029 | 0.069 ± 0.009 | 0.065 ± 0.002 |
| OVA–$NH_3$ | 1.111 ± 0.068 | 1.329 ± 0.050 | 0.920 ± 0.028 |
| $NH_3$–1d-OVA | 0.968 ± 0.094 | 1.309 ± 0.024 | 0.954 ± 0.033 |
| $NH_3$-7d-OVA | 0.691 ± 0.272 | 1.212 ± 0.055 | 0.955 ± 0.027 |
| OVA | 0.841 ± 0.300 | 1.146 ± 0.099 | 0.853 ± 0.024 |

[a] Values are absorbance at 450 nm ± SEM.
[b] Sera diluted $\frac{1}{100}$.
[c] Sera diluted $\frac{1}{1000}$.

consistent with the ozone-induced IgE suppression reported by Ozawa (1985). The IgE adjuvant effect of $NO_2$ was short-lived and by the sixth week had returned toward the values seen in rats exposed only to OVA. Repeated exposure to OVA in BNR has been reported to caused a tolerance by the fifth week of exposure (Sedgwick and Holt, 1983; McMenamin *et al.,* 1992). We did not observe such a phenomenon in the BNR exposed only to OVA. A possible explanation could be a large difference in exposure concentrations between those and the present study. Chamber OVA concentrations were not quantified in those studies.

The IgG and IgA levels were also enhanced, and this augmentation was still very prominent by the sixth week. Levels of OVA-specific IgG and IgA in individual rats exposed weekly (30 min/week) only to OVA also continued to increase over the 6 weeks of the study. The *in vivo* serum half-life of IgG, IgA, and IgE is 23, 6, and 2.5 days, respectively (Kuby, 1992). The drop in OVA-specific IgE after 6 weeks when OVA–IgG and OVA–IgA were still rising can be only partly attributed to these half-life differences.

Specific antibody production was not significantly altered by upper airway inflammation. It is possible that the upper airways are much less involved in antigen processing. The amount of inflammation obtainable, as measured by lavagable granulocytes, was much less in the nose than in the lung. This may have been simply a matter of surface area affected. The sensitivity of the present study may not have been adequate to measure subtle changes in antibody production caused by upper airway irritation. There was a tendency toward elevation of specific IgG when the antigen was given within 1 day of $NH_3$ exposure. There was a significant increase in specific IgG production due to ammonia ($p < 0.05$), if the groups in which the rats were exposed to OVA within 1 day of $NH_3$ exposure were combined. Higher ammonia levels were not used to ensure that the exposure was confined to the upper airways. This study demonstrated that, for acute exposures, lower airway irritants were more potent than upper airway irritants in augmenting an antibody response. The present results were inconclusive, however, as to the ability of acute upper airway irritation to produce any effect on an antibody response.

BAL was a rather insensitive tool for the study of pulmonary allergic reactions in this animal model. More consistent BAL inflammatory changes were seen in animals euthanized after 6 weeks of exposure than those with 4 weeks of exposure. There was no correlation between the extent of allergic inflammation as determined by either BAL granulocyte count or EPO and levels of IgE. Allergic inflammation continued to increase without concomitant increases in OVA-specific IgE titers. The attenuation of the IgE response in the $NO_2$–7d-OVA group was evident in the inflammatory response also, suggesting that a period of depressed immune responsiveness may exist after a prominent acute pulmonary inflammation. Ozawa *et al.* (1985) reported a suppression of helper T cell function and specific IgE in mice exposed continuously to ozone for 1 week and then exposed to an antigen. It is possible that a similar phenomenon can occur from acute $NO_2$ exposure, without the need for continuous exposure or presence of the irritant at the time of antigen presentation to obtain this inhibition.

An increase in granulocytic infiltration was also seen in rats given the initial sensitizing antigen dose, during either an upper or lower airway inflammatory response. The irritant-induced increase in the severity of the allergic inflammatory response paralleled the initial-irritant-induced inflammatory response. The largest pulmonary allergic inflammatory response was seen in the group given the initial antigen exposure at the peak of the acute irritant response. This enhanced allergic inflammation was evident 6 weeks after this acute irritant exposure and was not accompanied by elevated antibody titers in the ammonia study. The allergic inflammatory response can thus be disassociated from the reaginic antibody response.

The effect of both upper and lower airway irritation has been reported to, at least, transiently increase bronchial hyperreactivity (Silbaugh *et al.,* 1981). As mentioned above, both the humoral immune and inflammatory response may also be altered by airway irritants and the degree of allergic inflammation does not always correspond to antibody titer. Preliminary work conducted in our laboratory (data not shown) and other reports in the literature have also demonstrated that in animals with high antibody titers and severe pulmonary inflammation, persistent airway hyperresponsiveness may be absent (Haczku *et al.,* 1994; Kips *et al.,* 1992).

In conclusion, the pulmonary sensitization response (inflammatory and humoral) may be, at least quantitatively, influenced by the area of the respiratory tract affected, with lower lung irritants being more potent adjuvants. The prominent effects observed when the sensitizing exposure was given 1 day after the irritant exposure suggest that the adjuvant effect may have a time dependency that, in turn, depends on the degree of lung inflammation.

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