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Research Contract Final Report to the State of California Air Resources Board

Studies on air pollution: Effects of nitrogen dioxide on airway caliber and reactivity in asthmatic subjects; Effects of nitrogen dioxide on lung lymphocytes and macrophage products in healthy subjects; Nasal and bronchial effects of sulfur dioxide in asthmatic subjects.

Contract number: A6-200-33

Contract period: June 26, 1987 - November 26, 1988

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Date of the Report : December 13, 1988

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Abstract

We performed three studies of the effects of NO_2 and SO_2 on airway function in human subjects. In the first study, we found in 9 exercising asthmatic subjects that a 30 min exposure to 0.3 ppm nitrogen dioxide did not alter specific airway resistance, maximal expiratory flow, or the slope of phase III on the single breath test of nitrogen distribution and had no effect on airway hyperresponsiveness to sulfur dioxide. In the second study, we found that repeated exposure of 5 healthy subjects to nitrogen dioxide (0.60 ppm x 2 h on 4 different days in a 6 day period) was associated neither with any significant change in pulmonary function nor in the levels of secretory products of lung macrophages (interleukin 1, tumor necrosis factor) in bronchoalveolar lavage fluid. Analysis of the numbers and types of lymphocytes in venous blood and bronchoalveolar lavage fluid revealed no change apart from a small, possibly artifactual increase in natural killer cells in bronchoalveolar lavage fluid after NO_2 exposure. Our third study examined whether brief exposures to moderately high concentrations of SO_2 caused acute increases in nasal symptoms and nasal resistance in 8 subjects with a history of both asthma and allergic rhinitis and with demonstrable bronchial hyperreactivity to SO_2. In this group of subjects we did not find a greater change in nasal resistance and nasal symptoms after 10 min of nasal inhalation of a concentration of $S0_2$ more than twice that required to provoke symptomatic bronchoconstriction when inhaled by mouth than we found after a similar inhalation of conditioned room air.

Acknowledgments

This report was submitted in fulfillment of State of California Air Resources Board Contract Number A6-200-33 by the Cardiovascular Research Institute and the University of California, San Francisco, under the sponsorship of the State of California Air Resources Board. The work was completed as of August 19, 1988.

Disclaimer

The statements and conclusions in this report are those of the contractor and not necessarily those of the State of California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

Glossary

B-Cell - Named for its origin, the bone marrow, these lymphocytes produce specific antibodies in response to an antigen.

BAL - Bronchoalveolar lavage fluid. Obtained by bronchoscopy, it is composed of cells and fluid from both the airway and alveolar surfaces.

ELISA - Enzyme-linked immunosorbent assay. A method used in Project 2 to measure Interleukin-1 and tumor necrosis factor.

FEV1.0 - Forced expiratory volume in one second. The amount of air exhaled during the first second of an FVC maneuver.

FVC - Forced vital capacity. The amount of air that can be exhaled after a maximal inspiration.

IgE - Immunoglobulin E. Primary immunoglobulin involved in allergic reactions.

IL-1 - Interleukin-1. A hormone-like mediator produced by macrophages (and other cells), which is released in response to various stimuli and stimulates proliferation of lymphocytes.

Lymphocytes - Primary cells of the body's immune system.

mRNA - Messenger ribonucleic acid. The molecule that translates genes into proteins.

NK-Cells - Natural killer cells are lymphocytes that are tumorocidal.

PC8uMeth - The provocative concentration of inhaled methacholine, in mg per ml, that caused an 8 L x cm H2O/L/sec increase in specific airways resistance.

PD8uSO2 - Provocative dose of sulfur dioxide, in parts per million, that caused an 8 L x cm H2O/ L/sec increase in specific airways resistance.

Rhinomanometry - Measurement of flows and pressures in the nose.

SBD - Single breath distribution test. A pulmonary function test used as a sensitive measure of small airway obstruction.

SRaw - Specific airways resistance, the product of two measurements, airways resistance and thoracic gas volume, as measured in a body plethysmograph. The resulting units are liters x centimeters of water per liter per second.

T-Cell - Named for its origin, the thymus, this type of lymphocyte is involved in antigen presentation

TNF - Tumor necrosis factor. Produced primarily by macrophages, TNF is involved in promoting inflammation.

Summary and Conclusions

The effects of controlled exposures to the common, widespread air pollutants, nitrogen dioxide (NO_2) and sulfur dioxide (SO_2), on physiologic tests of pulmonary function and on symptoms of respiratory distress have been well studied in healthy adult subjects. For SO_2, the acute effects on pulmonary function and symptoms have also been well studied in people with asthma; asthma has been found to be associated with a marked increase in sensitivity to the airway effects of SO_2. For NO_2, the evidence on responsiveness of asthmatic subjects is conflicting. Some studies have shown changes in airway caliber and reactivity after brief exposure to concentrations often exceeded in urban and indoor atmospheres while others have shown no such effect. In the studies performed in fulfillment of this contract, we first examined the question whether a 30 min exposure of exercising asthmatic subjects to 0.3 ppm NO_2 (a duration and concentration reported by some investigators to alter airway function in people with asthma) would be associated with alteration in the single breath test of gas distribution, a test predominantly determined by the function of peripheral airways, and/or with a change in airway reactivity to another common urban air pollutant, SO_2. Our findings showed no evidence for an effect of NO_2 on either the single breath test or on bronchial reactivity to SO_2. Our results thus do not corroborate prior reports of demonstrable changes in bronchial reactivity in asthmatic subjects after brief exposures to low concentrations of nitrogen dioxide.

The second study performed in this contract period also found little effect of low level exposures to $N0_2$ on the functions examined. This study differed from the first in that the subjects were healthy, the exposures were repeated (to 0.6 ppm x 2 h on 4 separate days over a 6 day period), and the functions studied were markers of immune function rather than of physiologic function of the lungs. These markers included the concentrations of secretory products of macrophages, interleukin-1 (IL-1) and tumor necrosis factor (TNF), and the numbers of subtypes of lymphocytes in bronchoalveolar lavage fluid. In 5 subjects, we found no differences in the concentrations of IL-1 and TNF or in lymphocyte numbers in bronchoalveolar lavage fluid obtained in the baseline state and shortly after the final exposure to NO_2, with the exception of a small, possibly artifactual increase in lymphocytes classified as "natural killer cells." Thus, we have found no evidence that repeated exposure to a low level of NO_2 either stimulates the cell presumed to be involved in the initiation of immune responses (the macrophage) or alters the cells involved in humoral and cell-mediated immunity (lymphocytes). Although we found no change in what we believe to be sensitive markers, our results do not exclude the possibility of some alteration in immune function. It is possible, for example, that the function of some lymphocyte subtypes may have been altered even though their numbers were unaffected. Our results also do not exclude, of course, the possibility that longer exposures to greater concentrations of $N0_2$ would alter immune function of the lungs. A complete assessment of all immune functions after exposures of different durations to a range of NO_2 concentrations would be an enormous undertaking. Our results, showing no change in sensitive markers of cells critical to immune responsiveness after four exposures to levels of NO_2 higher than those achieved in urban atmospheres indicate that in the absence of compelling new data, such an undertaking would be unlikely to show important effects.

In the third study performed in this contract period, we examined whether brief exposures to moderately high concentrations of sulfur dioxide (SO_2) caused acute

increases in nasal symptoms and nasal resistance in subjects with chronic rhinitis and demonstrable bronchomotor responsiveness to SO_2. We studied 8 subjects with a history of both asthma and allergic rhinitis. Each subject developed symptoms of dyspnea or wheezing and an increase in specific airway resistance of at least 8 L x cm H_2O/L/s after breathing 1 or 2 ppm of SO_2 by mouthpiece at 20 L/min., and did not develop these changes after breathing room air under the same conditions. No subject, however, developed more nasal symptoms or a greater increase in nasal airway resistance after breathing SO_2 through the nose than they did after breathing room air. To insure that we did not miss an effect, we exposed the nose to a concentration of SO_2 that was double the concentration that caused bronchoconstriction when delivered through a mouthpiece to the lower airways. We conclude that brief exposure to SO_2 at a concentration of 4 ppm or less is unlikely to cause significant nasal dysfunction in subjects with both allergic rhinitis and asthma, and that responsiveness to SO_2 is not uniform throughout the respiratory tract.

In summary, our conclusions are:

1) Relatively young people with mild to moderate asthma who are exposed while exercising for short periods of time to low levels of nitrogen dioxide are unlikely to develop significant bronchospasm or greater sensitivity to a subsequent exposure to sulfur dioxide.

2) In healthy relatively young adult subjects, repeated, (4 times) over a 6-day period, exposure to nitrogen dioxide in a concentration that exceeds that in urban air is not associated with altered numbers of subsets of pulmonary lymphocytes or with an increase in the concentrations of markers of macrophage activation, interleukin-1 or tumor necrosis factor in bronchoalveolar lavage fluid.

3) Brief exposure to 4 ppm or less of sulfur dioxide does not cause worsening of nasal obstruction or nasal symptoms in people with allergic rhinitis and asthma, even in subjects selected because they demonstrate bronchial responsiveness to SO_2.

Recommendations

In these studies, we have examined the effects of NO_2 on airway caliber and airway responsiveness in subjects with asthma, a condition known to be associated with increased sensitivity to SO_2; we have examined the effects of SO_2 on nasal symptoms and airway caliber in subjects with a history of both rhinitis and asthma, conditions that we hypothesized would be associated with increased nasal sensitivity to SO_2; and we have examined the effects of repeated exposure to 0.6 ppm of NO_2 on what we believe to be sensitive markers of immune function of the lungs.

In the first study, our subjects were people with mild asthma, who were able to withhold their drugs for several hours before each experiment, and who were capable of mild to moderate exercise. We felt that this group would be likely to be exposed to pollutants in the environment in a similar way to that used in our protocol. We found no effects attributable to NO_2 even when we used levels of SO_2 that are infrequently found in the environment, (1.0-4.0 ppm). We cannot be sure that our negative results would extend to more severe asthmatics, or to people with chronic obstructive pulmonary disease. It might be important to study a group of sicker patients, but research is more difficult in this group because medications cannot be withheld, and these sicker patients would be less likely to be exercising in a polluted atmosphere without medication.

In the third study, our subjects were people with allergic rhinitis who also had mild asthma. Because we used such high (4.0 ppm) levels of SO_2 in a particularly sensitive subgroup, we feel it is unlikely that we missed an important health effect. Taken together with our studies reported in CARB Contract #A5-163-33, our results do not support a need for further studies of the effects of SO_2 pollution on people with allergic rhinitis.

Our results do not indicate a need for changing the existing ambient air quality standards for nitrogen dioxide or sulfur dioxide, but we have studied only a small population of people with mild asthma, and a few healthy people. Our studies were designed to be pilots, in which we would look for important health effects similar to those we have found previously with pollutant exposures (6).

Reports of Individual Projects

Project 1: Effects of nitrogen dioxide on airway caliber and reactivity in asthmatic subjects.

Purpose

To determine the whether a brief exposure to a low concentration of nitrogen dioxide (1) modifies airway caliber as assessed by specific airway resistance, maximal expiratory flow, and the slope of phase III on the single breath test of nitrogen distribution, and (2) potentiates airway reactivity to serially increasing concentrations of sulfur dioxide (SO_2) in adults with mild asthma.

Background

Short-term exposures to concentrations of nitrogen dioxide (NO_2) at and above those achieved in outdoor urban environments do not produce symptoms or alter the mechanical properties of the lungs of healthy adult subjects. (1-4) It is a point of controversy, however, as to whether similar concentrations of NO_2 affect the airway of people with asthma. That asthmatic subjects might be especially sensitive to NO_2 seems logical, for asthma is characterized by bronchial hyperreactivity, an increase in the responsiveness of the airways to a wide variety of stimuli (5), and asthmatic subjects have been shown to have greatly increased sensitivity to SO_2, another common atmospheric pollutant (6). Epidemiologic studies have supported the speculation that asthmatics might be sensitive to NO_2, for Ussetti and co-workers reported an association between excess asthmatic attacks and elevated levels of NO_2 (at approximately 0.50 ppm) in Barcelona, Spain (7). Some of the acute controlled exposure studies of asthmatics to NO_2 have supported this speculation. Orehek and co-workers reported that a 1 hour exposure of 20 resting asthmatic subjects to 0.1 ppm NO_2 caused a slight increase in specific airway resistance (SRaw) and in the bronchomotor response to carbachol in 13 subjects but affected neither SRaw nor reactivity to carbachol in 7 subjects (8). Ahmed and co-workers also reported that 0.10 ppm NO_2 increased reactivity to carbachol in asthmatics (9). Kleinman and associates reported a similar effect of 0.20 ppm NO_2 in two thirds of their asthmatic volunteers (10). Most recently, Bauer, Utell and their colleagues reported their study of the effects of a 30 minute exposure to 0.30 ppm NO_2 on the magnitude of exercise-induced bronchospasm and bronchial reactivity to eucapnic hyperpnea of cold, dry air in 15 asthmatic subjects (11). Their results showed that this low concentration of NO_2 had no effect on airway caliber when inhaled at rest but significantly potentiated the bronchospasm provoked by exercise. NO_2 inhalation plus exercise compared to control (air) exposure plus exercise produced significantly greater reductions in $FEV_{1.0}$ and partial expiratory flow rates at 60% of total lung capacity. One hour after NO_2 exposure, bronchial reactivity was increased, as shown by augmentation of the bronchomotor response to hyperpnea of cold air.

Other studies do not confirm these results. A study of 15 asthmatic subjects at the Health Effects Research Laboratory of the Environmental Protection Agency compared the effects of 1 hour resting exposures to air or to 0.1 ppm of NO_2. NO_2 did not alter SRaw, the resistive properties of the respiratory system, or bronchial reactivity to methacholine (12). A carefully controlled study of 29 asthmatic subjects exposed for 2

hours to air, 0.3 ppm, and 0.6 ppm NO_2 while performing intermittent light exercise similarly found no significant effect of the exposures (13). At most, small (and statistically insignificant) changes in forced expiratory flows but not in SRaw occurred after 0.3 ppm, but the changes were smaller after 0.6 ppm. Bronchial reactivity to cold air, measured one hour and one day after exposure, showed no significant variation attributable to NO_2 effects, except possibly one day after exposure to 0.6 ppm, and even this was a small and inconsistent effect.

From these studies, it seems unlikely that asthmatics are exquisitely more sensitive than healthy subjects to NO_2 as they are to SO_2. It nonetheless seems possible that brief (30-60 minute) exposure to low concentrations of NO_2 (0.1-0.4 ppm) may cause airway narrowing and increase airway responsiveness in people with asthma. Because of NO_2's poor solubility (14), these effects may be greatest in peripheral airways, sometimes called the "quiet zone" of the lung because they have so little influence on the usual tests of pulmonary function, such as thoracic gas volume, airways resistance, and FEV1.0 (15).

In this project, we measured the effects of 30 minute exposure to NO_2 on the usual tests of airway function (SRaw, FEV1.0, maximal expiratory flow rates), on a test more sensitive to changes in peripheral airway caliber, the single-breath test of oxygen distribution, (16) and on airway responsiveness to SO_2, another common pollutant to which asthmatics are already known to be sensitive.

Materials and Methods

Subject selection

Subjects were recruited by advertisements posted on campus or by personal telephone invitations to participants in prior studies. All were required to be life-long non-smokers, aged 21 to 45 years, and to have physician-diagnosed mild to moderate asthma. These volunteers underwent screening procedures that included: 1) medical history, 2) epidermal skin prick tests with antigen extracts characteristic for the Northern California region, 3) bronchial inhalation challenge with methacholine, 4) sulfur dioxide challenge if sensitivity to the gas was not known from prior studies. We screened 13 volunteers; 9 subjects who met the American Thoracic Society's definition of asthma (17) and who satisfied all screening requirements (see below), were accepted into the two-day study protocol. All 9 individuals used one or more anti-asthma medications regularly; they were asked to withhold their inhaled bronchodilator medications for at least 8 h, and oral bronchodilator therapy for at least 12 h before each experimental day. Caffeine-containing beverages were not consumed for at least 4 h prior to testing. No subject was using oral corticosteroids, cromolyn sodium, and non-steroidal anti-inflammatory drugs at the time of the study. All denied any symptoms of respiratory tract illness in the previous 4 weeks before being study.

On a screening day, bronchial hyperreactivity to methacholine was confirmed in each subject. Methacholine was delivered by mouthpiece from a nebulizer (DeVilbiss 646, Somerset, PA), which was equipped with a dose-metering device. We gave increasing concentrations of methacholine aerosol starting from 0.063 mg/ml until specific airway resistance (SRaw) increased 8 units or more from baseline. For each individual, we then constructed a dose-response curve plotting the log of the methacholine concentration versus the recorded SRaw. We determined by linear interpolation the concentration of methacholine required to to cause an increase in SRaw of 8 units above

baseline (the "provocative concentration," or PC8u Meth) form the log dose response curve. All subjects had a PC8u Meth of 0.5 mg/ml or less (see Table 1), in the range typical of asthmatic subjects previously studied in our laboratory.

The study protocol was approved by the Committee on Human Research of the University of California, San Francisco, and all subjects were informed of the risks of each procedure and gave informed written consent in order to participate in the study. All individuals were reimbursed for the time spent in the laboratory.

Skin tests

As part of the initial screening , each subject underwent skin prick-puncture tests using the Morrow Brown standardized disposable allergy prick test needles (Aller Guard, Topeka, KS) with 0.9% NaCl and histamine phosphate as negative and positive standards, respectively, and with extracts of antigens common to the Northern California region. The following allergenic extracts containing 50% glycerin and 0.4% phenol as preservatives were used in each subject: mixed grasses, mixed trees, mixed weeds, Alternaria, cat hair, dog hair, Hormodendrum, house dust and house dust mite.(Hollister-Stier, Spokane, WA). A second house dust extract (Berkeley Biologicals, Berkeley, CA) was also applied. Skin test reactions were read 15 min after application of the tests. The skin reactions were graded according to the manufacturer's specifications as follows: a wheal reaction of the same size as the prick histamine control was graded 3 plus and a reaction the same size as the negative (saline) control was indicated as zero. A wheal reaction greater than the positive control was called 4 plus. A positive skin reaction to any particular antigen was considered to be present if the wheal reaction caused by the antigen was equal or greater than that caused by the positive (histamine) control.

Pulmonary function studies

In each subject, airway caliber was inferred by measuring the following physiologic variables: 1) Airway resistance (Raw) and thoracic gas volume (TGV) were measured in a custom-made constant-volume, variable-pressure, whole-body plethysmograph that was interfaced to a minicomputer. Resistance was expressed as a resistance-volume product termed specific airway resistance (SRaw = Raw x TGV). Five measurements of each variable were recorded and the means computed; 2) Three reproducible (±5%) maximal expiratory flow-volume curves were recorded in the sitting position with nose clips in place using a dry sealed spirometer (Ohio 840 Spirometer, Ohio Medical Products, Houston, TX); from these curves forced vital capacity (FVC), forced expiratory volume in one second (FEV1.0), FEV1.0/FVC, and maximal expiratory flow rates at 25%-75% of vital capacity (FEF_{25-75}) were computed according to the American Thoracic Society's criteria; 3) Single breath distribution (SBD) test to determine the slope of phase III of exhaled N_2 during a slow vital capacity maneuver from total lung capacity to residual volume using a custom-made bag-in-box system serially interfaced with a dry sealed spirometer and a minicomputer (18, 19). The test was performed in the sitting position with nose clips in place and 3 reproducible maneuvers were obtained. The reported slope of phase III represents the average of 3 maneuvers.

In each subject, SRaw measurements always preceded the maximal expiratory flow-volume maneuvers and SBD test. Baseline SRaw measurements on both experimental days including SRaw values before administering the SO_2 dose-response curve, were

always within ± 2.5 SRaw units.

Protocol for gas exposures

Sulfur dioxide: While seated with nose clips in place each subject inhaled partially humidified filtered air at room temperature through a standard pulmonary function laboratory mouthpiece at 20 L/min to which doubling concentrations of SO_2 (0.25 to 4 ppm) were added. Inspired air temperature and humidity were measured with a dew-point hygrometer (EG&G Model 911, Dew-All; EG&G, Waltham, MA) with the probe placed at the inspiratory port of a Koegel two-way valve (Ewald Koegel Co., San Antonio, TX). The average temperature was 23 ± 1.0 °C (mean±SD), and average dew point was 14 ± 0.5 °C. Inspired air flow was measured with a #3 Fleisch pneumotachygraph and a differential pressure transducer (MP-45; Validyne Corp., Northridge, CA). The flow signal was then amplified and integrated (CD-19 amplifier and FV156 integrator; Validyne). The resulting minute volume was recorded continuously on a ultraviolet recorder (Visicorder 1858; Honeywell, Denver, CO). The inspired volume was corrected to BTPS before reporting. Minute ventilation was kept constant by having the subject breathe in time to a metronome and inhaling a constant tidal volume (1.5 L) as displayed to the subject on an electronic bar graph. In addition, we continuously measured expired carbon dioxide (CO_2) concentration by sampling exhaled gas at the mouth with an infrared CO_2 analyzer (Model LB-1, Beckman Instruments, Palo Alto, CA). Exhaled carbon dioxide (CO_2) concentration was maintained near baseline value (4%-5%) by adding a metered flow of 100% CO_2 to the inspired air stream. Different concentrations of SO_2 were prepared by mixing flow from a tank of SO_2 (500 ppm, Liquid Carbonic, Chicago, IL) in a glass mixing chamber with filtered, partially humidified room temperature air delivered from a compressed air source and passed through a bubble humidifier and a HEPA filter (ALFCO, Carpinteria, CA). The concentration of SO_2 delivered to the subject was measured continuously at the inspiratory limb of the Koegel two-way valve in the mouthpiece assembly with a pulsed fluorescent SO_2 analyzer (Series 43, Thermo Electron Corp., Hopkinton, MA) calibrated with a gravimetrically determined concentration of SO_2 (0.80 ppm, Scott-Marrin, Riverside, CA) traceable to the National Bureau of Standards. All tubing in contact with the gas mixture was made of Teflon. Each concentration of SO_2 was inhaled for 4 min. Five measurements of SRaw were obtained at 30 second intervals beginning 2 min after each exposure, and the mean SRaw value was then computed. The concentration of SO_2 was increased stepwise in doubling increments from 0.25 to 4 ppm until SRaw increased by at least 8 units or until the highest concentration was reached. We then constructed dose-response curves plotting the concentration of SO_2 versus the recorded SRaw. We then determined by linear interpolation the concentration of SO_2 required to cause an increase in SRaw of 8 units above baseline (the "provocative dose" or $PD_{8u}SO_2$).

Nitrogen dioxide: Exposure to NO_2 was conducted in a controlled 8x8x10 ft. stainless steel modular walk-in environmental room (Vista Scientific Corp., Ivyland, PA). Detailed description of the exposure facility can be found in our previous report to the CARB (20). Ambient conditions in the room, monitored throughout the the protocol, were maintained at relative humidity of 55 ± 4% (mean ± SD), and temperature of 22 ± 0.7 °C. Nitrogen dioxide was supplied from a gas cylinder containing 239-520 ppm NO_2 in air (Scott-Marrin, Riverside CA, and Liquid Carbonic, Chicago, IL) directly into the purified air supply duct and into the exposure room through a perforated ceiling. Teflon tubing was

used to transport NO_2 from the cylinder to the exposure room and back to the NO_2 analyzer. Nitrogen dioxide concentration in the exposure room was monitored continuously using a chemiluminescent NO-NO_2-NO_X analyzer (Series 14, Thermo-Electron Corp., Hopkinton, MA). The NO_2 analyzer was calibrated in situ by the State of California Air Resources Board. Zero and span calibration of the NO_2 analyzer were checked periodically with an ultrapure air cylinder (NO_X < 0.001 ppm, Scott-Marin, Riverside, CA) as the zero, and a span cylinder containing 0.82 ppm of NO_2 in air traceable to the National Bureau of Standards (Scott-Marin, Riverside, CA). The mean 30 min nitrogen dioxide exposure level was 0.30 ± 0.01 ppm (mean ± SD).

Symptom score

Each subject completed a brief questionnaire on symptoms possibly attributable to gas exposure before and immediately following exposure. The symptoms scored were: breathlessness, chest tightness, wheezing, cough, secretions (including sputum), eye irritation, throat irritation, taste, and a miscellaneous category. The severity of each symptom was rated subjectively by each individual from nil (none) to 10 (incapacitating). Thus the highest possible total score was 90.

Experimental design

All subjects who satisfied the screening criteria were randomly assigned to one of the two possible exposure sequences of filtered air (sham exposure) and NO_2 (0.30 ppm). Both air and NO_2 exposures were of 30 minute duration; during the first 20 min of exposure, each subject exercised on a stationary cycloergometer (Gould Godard BV, Bilthoven, the Netherlands). Bicycle workloads were selected to approximately triple the normal resting minute ventilation (60-80 watts) simulating a light to moderate level of outdoor activity. At the end of the exercise, the subject rested quietly for 10 min and then left the exposure chamber. For each subject, the two exposures were carried out in a double-blinded fashion in that neither the subject nor the technician who performed the pulmonary physiologic tests outlined above and administered the SO_2 dose-response curve knew the test atmosphere. Exposures were separated by at least one week and were performed at the same time of the day.

The sequence of pulmonary physiological measurements and gas exposure on each of the two exposure days was as follows:
Time 0: Symptom score and measurements of SRaw, FEV1.0, FVC, and single breath test for oxygen distribution were obtained as described above.
Time 30 min: Gas exposure as outlined above.
Time 60 min: End of exposure. Symptom score was obtained as at Time 0.
Time 65 min: The pulmonary physiological tests were repeated as at Time 0, except for the single breath test for oxygen distribution.
Time 120 min: The pulmonary physiological tests were repeated as at Time 0.
Time 150 min: Sulfur dioxide dose-response curve was performed according to the protocol outlined above.

Upon completion of the SO_2 dose-response curve, each subject was given an inhaled beta-agonist (albuterol, Ventolin, Glaxco Inc., Research Triangle Park, NC) in order to reverse bronchoconstriction.

Data analysis

The results are expressed as mean and standard deviation where appropriate. Group means for each pulmonary function variable and $PD_{8u}SO_2$ were compared before and after each gas exposure using two-tailed paired Student's t test. Symptom scores were converted to rank scores in order to compare pre- and post-exposure scores by the Wilcoxon signed rank test. Statistical significance was set at $p < 0.05$.

Results

The anthropometric, methacholine bronchoprovocation challenge, and bronchodilator therapy data on 9 subjects with asthma who participated in the study are summarized in Table 1. There were 5 men and 4 women, 23 to 34 years of age. The PC_{8u}Meth ranged from 0.02 mg/ml to 1.0 mg/ml (median: 0.12 mg/ml) which is within the "asthmatic range" established in our laboratory (PC_{8u}Meth of less than 2.0 mg/ml). The random assignment of the exposure sequence resulted in 4 individuals receiving first air and 5 received NO_2 first. Most subjects were able to identify which gas they were exposed to each day when asked at the end of the study, because of the slight odor of nitrogen dioxide.

The baseline pulmonary physiologic data and those obtained following either air or nitrogen dioxide exposure are summarized in Table 2. None of the respiratory variables showed evidence of untoward change attributable to NO_2 exposure. Specifically, mean SRaw increased from 7.1 ± 2.2 L x cm H_2O/L/s to 9.5 ± 2.6 L x cm H_2O/L/s 5 min after completion of the air exposure, and from 7.1 ± 2.2 L x cm H_2O/L/s to 9.7 ± 3.5 L x cm H_2O/L/s 5 min after completion of the NO_2 exposure (p = not significant). FEV1.0/FVC did not change significantly after NO2 exposure. Mean SBD was similar before and 60 min after completion of the air exposure.

No statistically significant changes attributable to NO_2 exposure were found in the mean total scores derived from pre- and post-exposure symptom questionnaires for air and NO_2 (Table 2). Furthermore, subject symptoms such as occasional chest tightness or wheezing, were evenly distributed between the air and NO_2 exposures.

The relationship between air and NO_2 exposures and subsequent SO_2-induced bronchoconstriction in 9 subjects with asthma is summarized in Table 2. The graphical presentation of the individual SO_2 dose-response curves following air (open symbols) and NO_2 (closed symbols) exposures is shown in Figure 1. In each individual, baseline SRaw before SO_2 administration was similar 90 min after completion either the air or NO_2 exposure. All 9 subjects developed acute bronchoconstriction following exposure to SO_2 at a dose of 4 ppm or less. Mean $PD_{8u}SO_2$ was 1.25 ± 0.7 ppm (range: 0.53 - 2.5 ppm) after air exposure, and 1.31 ± 0.75 ppm (range: 0.29 - 2.8 ppm) after NO_2 exposure (p = not significant). In other words, the dose of SO_2 needed to cause the same degree of bronchoconstriction was similar after the air and NO_2 exposures.

Discussion

Our data indicate that in subjects with mild to moderate asthma a 30-min exposure to 0.3 ppm NO_2 was not associated with subsequent potentiation of SO_2-induced bronchoconstriction in these subjects. Furthermore, the short-term exposure to NO_2 was not associated with any greater change in airway caliber than that observed after a similar exposure to purified air.

Our study was intentionally designed to closely resemble those that have demonstrated an effect of NO_2 on bronchial reactivity in that we used the concentration (0.3 ppm) and the duration of exposure (30 min with moderate exercise) used in earlier

"positive" studies. Our study differed in that we analyzed NO_2's effect on bronchial reactivity to SO_2 rather than to aerosolized drugs, for we felt that people with asthma are far more likely to encounter SO_2 under real world conditions.

Our study also differed in that we measured the effects of NO_2 exposure on a test of peripheral airway function, the single breath oxygen test (18), for some experimental studies have suggested that NO_2, as a poorly soluble gas, might cause greater injury to small peripheral airways (2mm or less in diameter) than to large, central airways (14).

Our failure to show either a change in bronchial reactivity to SO_2 or a change in airway caliber after exposure to NO_2 does not necessarily indicate that no changes occur. The number of subjects we studied was small and our observations only permit the statement that there was less than a 5% chance of missing a 100% change in the provocative dose of SO_2. It must also be emphasized that our findings should not be extrapolated to the general asthmatic population including people with corticosteroid-dependent asthma, and patients in the older age groups.

Our conclusion that short-term NO_2 exposure of people with mild to moderate asthma is not associated with any significant change in airway caliber is consistent with previous reports by Hackney and his group (1), and Hazucha and his colleagues (12). The reasons for the differences between these studies and previous observations demonstrating an increase in bronchial hyperreactivity after short-term NO_2 exposure are not clear but may be related to differences in subject selection, study design, and data analysis.

In conclusion, people with mild to moderate asthma who are exposed for a short periods of time to 0.3 ppm or less of nitrogen dioxide are unlikely to develop significant bronchoconstriction or aggravate their asthma if subsequently exposed to sulfur dioxide.

Table 1. Subject Characteristics

Subject	Sex	Age	Ht(cm)	Wt(kg)	*PC8u Meth	†Medications
1	F	24	168	56	0.02	B
2	F	26	168	57	0.12	B,T,A
3	M	33	169	100	0.12	B,T
4	F	23	155	59	0.02	B,T,A
5	M	28	183	77	0.50	B,A
6	M	34	175	66	0.50	B
7	M	28	168	70	0.12	B,A
8	F	29	168	57	0.06	B,T
9	M	34	180	64	1.00	B

* The concentration of inhaled methacholine, in mg per ml, that caused an 8 L x cm H2O/L/sec increase in specific airways resistance.

† B, inhaled Beta-agonist; T, theophylline preparation; A, antihistamine.

Table 2. Subject Data

AIR

Subject	*SRaw		FEV1.0 /FVC		**SBD		†Symptoms		‡PD8uSO2
	Baseline		5 min	Baseline		5 min	Baseline		
1	5.7	8.9	3.4/4.0	3.2/3.9	2.6	2.4	0	15	0.6
2	6.1	6.2	2.6/3.6	2.6/3.6	1.8	1.9	3	0	2.3
3	5.6	10.0	3.1/4.0	3.1/3.9	1.8	1.4	8	15	2.5
4	6.0	9.5	3.6/4.3	3.5/4.4	1.3	1.4	5	3	1.2
5	4.8	6.3	4.8/6.5	5.0/6.3	1.0	0.9	6	7	1.2
6	6.8	13.3	3.5/5.0	2.8/4.6	1.6	1.3	12	16	1.1
7	8.6	8.5	3.8/5.3	3.8/5.4	1.8	1.8	7	4	1.1
8	12.0	13.8	2.2/3.1	2.3/3.1	N.D	N.D	1	6	0.5
9	7.9	9.3	2.4/4.7	2.3/4.7	4.2	4.9	3	2	0.7
Mean	7.1	9.5			2.0	2.0	Sum 45	68	1.25
S.D.	±2.2	±2.6			±1.0	±1.3			±0.70

* Specific Airways Resistance (L x cm H2O/L/sec)

** Single breath distribution test. Normal slope of phase III= < 2 (% N_2/Liter).

† Reported symptom scores are based on a scale of 0-90, with 90 representing maximum.symptoms.

‡ Provocative dose of sulfur dioxide that caused an 8 unit increase in specific airways resistance. Reported values were obtained by interpolation from the dose response curves shown for each subject in Figure 1.

5 min and 60 min are time after end of exposure.

N.D. means that the test was not done.

Table 2. continued

NITROGEN DIOXIDE

Subject	*SRaw		$FEV_{1.0}$/FVC		**SBD		†Symptoms		‡PD_{8uSO2}
	Baseline		5 min	Baseline		5 min	Baseline		60 min
	Baseline		5 min						
1	7.2	7.8	3.4/4.0	3.3/4.0	2.6	2.1	3	5	0.3
2	4.9	5.3	2.7/3.6	2.7/3.7	2.4	1.8	0	8	1.6
3	6.0	8.2	3.0/3.9	3.1/3.9	1.7	1.8	3	8	2.8
4	5.5	9.1	3.4/4.3	3.5/4.3	1.8	1.6	2	5	1.3
5	6.0	6.3	5.0/6.5	5.2/6.6	1.1	1.0	4	7	1.9
6	4.7	12.0	3.9/5.3	3.2/5.0	1.5	1.3	2	7	1.4
7	9.4	8.9	3.4/5.2	3.4/5.1	2.3	1.8	6	5	1.0
8	10.2	16.4	2.2/3.2	2.2/3.2	(2.7)	(2.0)	19	9	1.0
9	9.7	13.0	2.1/4.6	2.1/4.5	2.6	3.1	1	1	0.5
Mean	7.1	9.7			2.0	1.8	Sum 40	55	1.3
S.D.	±2.2	±3.5			±0.5	±0.6			±0.7

* Specific Airways Resistance (L x cm H2O/L/sec)

** Single breath distribution test. Normal slope of phase III= < 2 (% N_2/Liter).

† Reported symptom scores are based on a scale of 0-90, with 90 representing maximum.symptoms.

‡ Provocative dose of sulfur dioxide that caused an 8 unit increase in specific airways resistance. Reported values were obtained by interpolation from the dose response curves shown for each subject in Figure 1.

5 min and 60 min are time after end of exposure.

N.D. means that the test was not done.
Numbers in parentheses were not included in the mean values nor in the statistical analysis because the test was not done after air exposure.

Figure 1. Individual Dose Response Curves

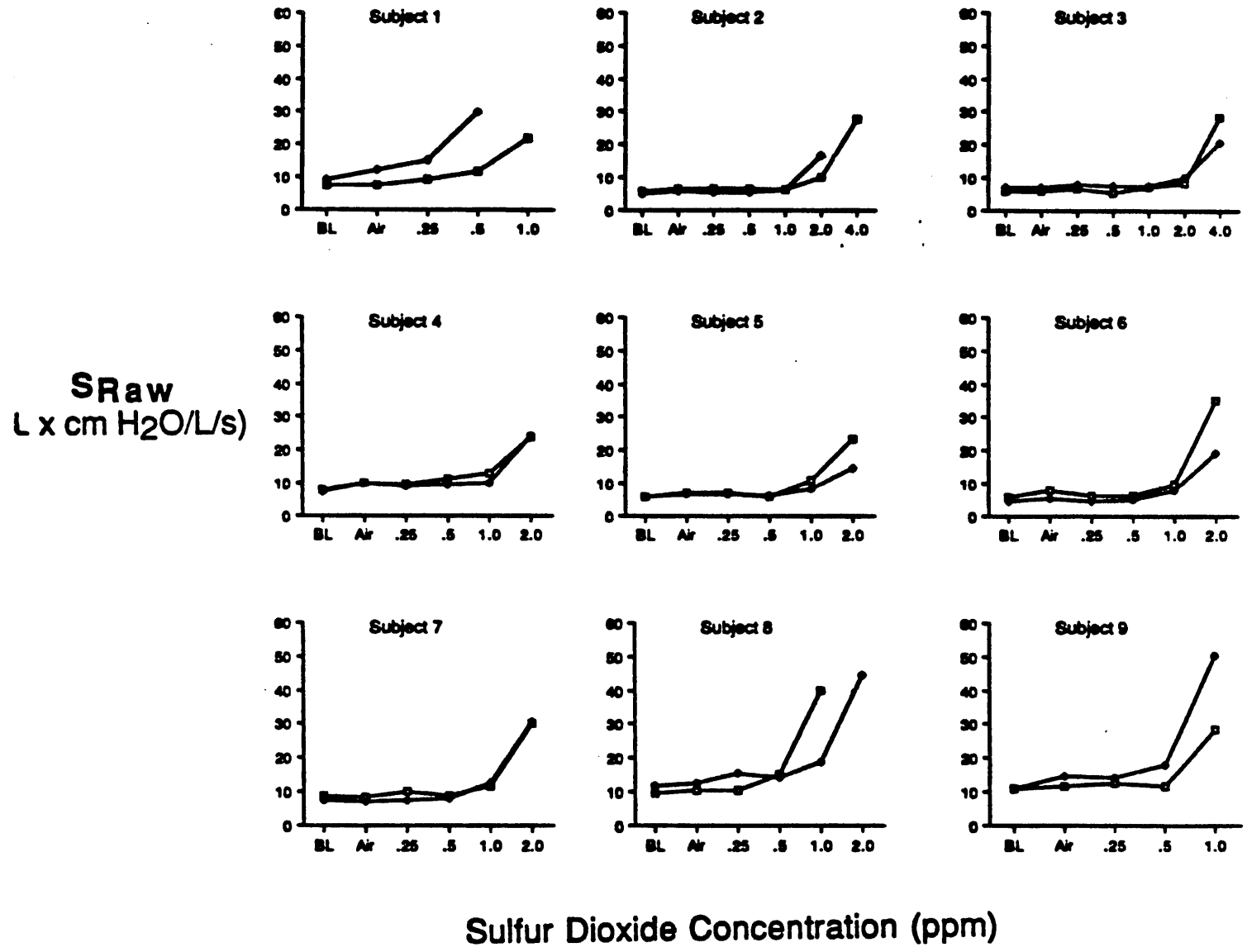

Figure 1. Dose response curves to SO2 after exposures to air (open symbols), and NO2 (closed symbols), in each of the 9 subjects

Project 2: Effects of nitrogen dioxide on lymphocyte subtypes and macrophage products in bronchoalveolar lavage fluid from healthy subjects.

Purpose

To determine the effects of repeated exposure to 0.60 ppm nitrogen dioxide on pulmonary function, on pulmonary lymphocyte phenotypes, and on the concentrations of interleukin 1 and tumor necrosis factor in bronchoalveolar lavage fluid from healthy volunteers.

Background

Both animal and epidemiologic studies suggest a possible effect of NO_2 on immune function (1). The finding that 3 months of daily intermittent exposure to 0.5 ppm NO_2 increases the susceptibility of mice to bacterial pneumonia (2, 3)) can be attributed to depression of function of alveolar macrophages (4). This finding alone suggests that NO_2 may alter immune responsiveness, for the macrophage is a pivotal cell in activating the immune system; it serves as an antigen-presenting cell, initiating the production of antibodies, the activation of cell-mediated immunity, and the institution of immunologic memory by lymphocytes. Direct evidence that NO_2 affects immune function not just by depressing macrophages but also by altering or suppressing the function of lymphocytes has been provided by Dr. Richters' research group. They have found that six weeks of exposure to 0.35 ppm NO_2 alters spleen weight and spleen cell populations in mice (5), and Fujimaki's group found that four weeks' exposure to 0.4 ppm NO_2 suppressed the primary antibody response to splenic lymphocytes. (6) Dr. Richters' more recent (and currently on-going) work involves use of flow-cytometry characterization of the numbers of different subtypes of lymphocytes in the spleens of NO_2-exposed and non-exposed mice. Flow cytometry is a powerful tool, for it identifies lymphocytes by specific surface markers now known to be associated with different functions. This technique thus supplants the need to do a large number of assays of the many different lymphocyte functions, and can guide the selection of assays to be used in future studies. Dr. Richters' laboratory, for example, is seeking a correlation between a reduction in the number of "killer' (NK or tumorocidal) lymphocytes identified by flow cytometry and a reduction in the ability of splenic lymphocytes of the NO_2-exposed mice to alter melanoma cells so that they will not implant and grow in the lungs after intravenous infusion (7, 8). In other words, Dr. Richters and his co-workers are examining the idea that the decreased ability of NO_2-exposed mice to defend against tumor metastasis to the lungs is due to a reduction in the number of NK lymphocytes. The survey of splenic lymphocytes by flow-cytometry may identify other immune functions that are also depressed.

In the present study, we examined lymphocytes in both circulating blood and bronchial lavage fluid. The lung's immune system is closely integrated with systemic immune function but is nevertheless a distinct compartment. It is thus possible that changes in lymphocyte populations in the lung may not be reflected by similar changes in the circulation. The differentiation and activation phenotypes of lymphocytes found in bronchial lavage fluid differ from those of lymphocytes found in circulating blood; these differences are greater in patients with diseases such as sarcoidosis and hypersensitivity pneumonitis and in patients with depression of immunity predisposing to opportunistic

lung infections (e.g. AIDS). It is thus important to examine the lymphocytes obtained from the lungs and airways, especially when they are the sites of delivery of inhaled NO_2.

In this study we also examined markers of activation of another cell critical to immune function of the lung, the alveolar macrophage. Impairment of macrophage function is thought to be responsible for the increased susceptibility to bacterial infection caused in mice by exposure to 0.10 ppm of ozone for 3 h (4) and prolonged excessive stimulation of alveolar macrophages by inhaled inorganic particles is thought to be responsible for initiating the cellular infiltration and fibrosis of the lungs in silicosis, asbestosis, and other pneumoconioses (9).

The macrophage functions as a sentinel cell. Upon stimulation by a foreign material, it serves as an antigen-presenting cell and activates the lymphocytic arms of immune defense. It does this in part through the elaboration of soluble factors that stimulate activation, proliferation, and differentiation of other cells. Among the factors that have been identified, two that appear especially important are interleukin-1 (IL-1) and tumor necrosis factor (TNF). Interleukin-1 stimulates lymphocyte proliferation and contributes to the "acute phase responses" of fever, redistribution of certain amino acids and trace metals, and acceleration of hepatic production of certain plasma proteins (10). Tumor necrosis factor has several important pro-inflammatory effects, including stimulation of adherence of polymorphonuclear leukocytes to endothelial cells, enhancement of PMN leukocyte phagocytic activity, and a generalized increase in tissue catabolism (11, 12). Production and release of IL-1 and TNF by macrophages has been demonstrated in response to a number of stimuli that perturb the macrophage cell membrane, including mitogens, antigens, and bacteria (13). IL-1 and TNF constitute up to 5% of the total protein released by stimulated macrophages. Because alveolar macrophages are the major antigen processing cell in the lung and make up 90% or more of the cells recovered by bronchoalveolar lavage, we examined the effects of NO_2 on cytokine-production in the lungs and airway, as assessed both by direct measurement of IL-1 and TNF in BAL fluid (by ELISA assay) and by quantitation of the levels of messenger RNA for IL-1 in the recovered cells. Since TNF is post-transcriptionally regulated, its detection by mRNA probing would not be interpretable in the absence of a change in concentrations of the secreted product. mRNA for TNF was therefore not assessed.

This study was designed to examine the effects of repeated exposure of healthy subjects to 0.6 ppm nitrogen dioxide on markers of immune function in the lungs. The lung compartment was sampled by bronchoalveolar lavage. The markers analyzed for lung macrophage activation were the concentrations of two products secreted by macrophages to initiate immune reactions - interleukin-1 and tumor necrosis factor. The marker for changes in lymphocyte function was enumeration of different lymphocyte subtypes by flow cytometry, the method used in Richters' study of splenic lymphocytes.

The NO_2 exposure that we used in this study was chosen to simulate very severe but conceivable atmospheric conditions over a several day period. We thus exposed our subjects to 0.6 ppm of NO_2 for two hours with intermittent exercise on four separate days in a 6 day period. We performed bronchoalveolar lavage shortly after the final exposure. For purposes of comparison, we also performed bronchoalveolar lavage on another occasion, either two weeks before or more than two weeks after the NO_2 exposures.

Materials and Methods

Subject Selection

Subjects were recruited by advertisements posted on campus or by personal telephone invitations to participants in prior studies. All were required to be life-long non-smokers, aged 18-45 years, and to be healthy. The study protocol was approved by the Committee on Human Research of the University of California, San Francisco, and all subjects were informed of the risks of each procedure and gave informed written consent to participate in the study. All individuals were reimbursed for the time spent in the laboratory.

All subjects underwent 3 screening procedures as follows: 1) medical history, 2) bronchial inhalation challenge with methacholine, 3) epidermal skin prick tests with antigen extracts common to the Northern California region. We studied 5 volunteers who satisfied all screening requirements and agreed to participate in the five-day study protocol (Table 3). Caffeine-containing beverages were not consumed for at least 4 h before testing. No subject was taking any medication at the time of the study. All denied any symptoms of respiratory tract illness in the 4 weeks before being studied.

Methacholine Challenge

Methacholine was delivered by mouthpiece from a nebulizer (DeVilbiss 646), which was equipped with a dose-metering device. We gave doubling concentrations of methacholine aerosol starting from 0.063 mg/ml until specific airways resistance (SRaw) increased 8 units or more from baseline or until a concentration of 2 mg/ml was given. Because we have defined the "asthma range" to be well below this concentration, subjects who did not have an 8 unit increase in SRaw were accepted into the study.

Skin Tests

Each subject underwent skin prick-puncture tests as described in the Methods section of Part 1. Subjects who had 2 or more positive skin tests were excluded from the study.

Pulmonary Function Studies

Airway resistance, lung volumes and flows were measured as described in Part 1. Baseline measurements were similar on all days (see Table 4).

Nitrogen dioxide exposure

Exposure to 0.60 ppm NO_2 was conducted in the same environmental room used in Part 1. Ambient conditions in the room, monitored throughout the protocol, were maintained at a relative humidity of 55.6 ± 3.5% (mean ± SD),.and temperature of 21.0 ± 0.8°C

Nitrogen dioxide was supplied from a gas cylinder containing 250 or 500 ppm NO_2 in air (Scott-Marrin, Riverside Ca, and Liquid Carbonic, Chicago, IL) directly into the purified air supply duct and into the exposure room through a plenum. Teflon tubing was

used to transport NO_2 from the cylinder to the exposure room and back to the NO_2 analyzer. Nitrogen dioxide concentration in the exposure room was monitored continuously using a chemiluminescent $NO\text{-}NO_2\text{-}NO_x$ analyzer (See Methods, Part 1). The average daily NO_2 concentrations for each of the 5 subjects studied are summarized in Table 4. The mean (±S.D.) nitrogen dioxide concentration for all exposures was 0.59 ± 0.08 ppm.

Each exposure was of 2 hour duration, with the subject performing exercise on a stationary cycle ergometer (Gould Godard BV, Bilthoven, the Netherlands) for 15 of each 30 minutes at a workload calculated to increase resting minute ventilation by 3 to 4 fold, (50-80 watts), simulating a light to moderate level of outdoor activity.

Symptom Score

Each subject completed a brief questionnaire on symptoms possibly attributable to NO_2 exposure before and immediately following exposure. The symptoms scored were: breathlessness, chest tightness, wheezing, cough, secretions (including sputum), eye irritation, throat irritation, taste, and a miscellaneous category. The severity of each symptom was rated subjectively by each individual from nil (none) to 10 (incapacitating), with a total possible score of 90.

Bronchoalveolar lavage

Prior to bronchoscopy, each subject was given 0.8 - 1.0 mg of atropine sulfate and 8 - 10 mg of morphine sulfate by intramuscular injection. Supplemental oxygen was given by nasal cannula at a rate of 4 - 5 L/min throughout the procedure. The upper airway was anesthetized with topical application of 4% lidocaine hydrochloride (Abbott laboratories, North Chicago, IL); 1% preservative-free lidocaine hydrochloride (Elkins - Sinn, Inc., Cherry Hill, NJ) was applied to the glottis and subglottic airway through the bronchoscope as needed in order to inhibit cough. A fiberoptic bronchoscope (Model FB - 19D, O.D. 6.3 mm, Pentax Precision instrument Corp., Orangeburg, NY) was then passed into a subsegment of the right middle lobe and wedged into a subsegmental airway. Hank's balanced salt solution (HBSS) without calcium, magnesium, or phenol red (UCSF Cell Culture Facility; ph 7.36 - 7.44) warmed to 37°C in a water bath (Tek - Pro, Tek - Bath B6990, American Dade, Miami, FL) was instilled in four 50 ml boluses and suctioned immediately after each instillation into 80 ml mucous specimen traps (#3586, Davol Inc., Cranston, RI). The recovered bronchoalveolar lavage fluid was immediately placed on ice until processed.

Lymphocyte phenotyping

Before bronchoscopy, 10 ml of venous blood were drawn from an antecubital vein into a glass tube containing Ethylenediaminetetraacetic Acid (EDTA) (Vacutainer, Becton Dickinson, Rutherford, NJ) for complete peripheral blood cell count and white blood cell differential count. Mononuclear cells were separated in this sample by Ficoll-Hypaque density gradient centrifugation and were washed and resuspended in cold sterile saline and 10% fetal calf serum. This suspension was then processed for antibody staining as described below.

Bronchial lavage fluid was processed for lymphocyte analysis as follows: the sample volume was measured and centrifuged at 300 g x 10 min. The cell pellet was washed in phosphate buffered saline (PBS) and resuspended in cell culture medium and fetal calf serum. An aliquot of this suspension was taken for cell counting (Coulter counter) and for cytoprep for Diff-Quick staining and differential counting. To enhance lymphocyte enrichment of the cell suspension, adherent cells (especially alveolar macrophages) were removed by incubation on petri plates. This was done by placing the cell suspension on to petri plates pre-coated with fetal calf serum for one hour at 37°C, rinsing off non-adherent cells with warm cell culture medium, recentrifuging and resuspending these non-adherent cells in RPMI and 10% fetal calf serum, and incubating them on petri plates for 24 h at 37°C. The non-adherent cells were again removed by rinsing with warm RPMI, were counted, and were resuspended in RPMI at 20 x 10^6 cells/ml. This final suspension was then processed for antibody staining.

For antibody staining, 25 ul of cell suspension was added to each of 12 x 75 mm tubes. Fifteen microliters of a mixture of two monoclonal antibodies were added to the suspension and mixed. The tubes were incubated for 30 min on ice, washed twice, and held for analysis.

The monoclonal antibodies were supplied by Becton-Dickinson Immunocytometry Systems (Mt. View, CA). Simultaneous immunofluorescent studies were done for two cell markers by conjugating their respective antibodies with fluorescein isothiocyanate and

phycoerythrin. The antibodies employed were directed against the following cell surface markers:

Antibody Designation	Cell Type
Leu 4	T Cell
Leu 16	B Cell
Leu 3	Helper/Inducer cell
Leu 2	Suppressor/Cytotoxic cell
Leu 7	Large granular lymphocyte
Leu 11	Natural killer cell

Cytometric analysis was performed with a Becton-Dickinson FACS Analyzer interfaced to a data lister and Hewlett-Packard 9816 computer and printer. Ten thousand lymphocytes were analyzed for each pair of monoclonal antibodies.

Quantification of Interleukin-1 and Tumor Necrosis Factor

Concentrations of IL-1 and TNF in a 35 ml aliquot of BAL fluid were directly quantitated by commercially available ELISA assays (Enzyme-linked immunosorbent assay) according to the manufacturer's instructions (IL-1: Cistron, Pine Brook, NJ; TNF: T cell Sciences, Cambridge, MA). The lower limit of detection for IL-1 is 25 pg/ml and for TNF, 40 pg/ml.

Quantitation of messenger RNA specific for IL-1 was performed as follows: total mRNA was recovered from washed cells following lysis in guanidine hydrochloride by established methods (14). Ten micrograms of total mRNA was subjected to electrophoresis, transferred to nylon membranes, and probed with ^{32}P-labeled oligonucleotide probes specific for human IL-1 beta and TNF (15).

Experimental Design

Each of the four 2 hour NO_2 exposures was conducted on a different day, within a 6-day period. The baseline and post-NO_2 bronchoscopies were performed at least 2 weeks apart. In 2 subjects, the baseline bronchoscopy was done first, and in the remaining 3 subjects, bronchoscopy after NO_2 exposure was done first. The sequence of pulmonary physiological measurements, gas exposure, and bronchoscopy was as follows:

Part I:

Baseline bronchoscopy as outlined above.

Part II:

Exposure days 1-3:

Time 0: Symptom score and measurements of SRaw, FEV1.0 , and FVC as described above.

Time 20-140 min: NO_2 exposure as outlined above.

Time 145 min: Symptom score and measurements of SRaw, FEV1.0, and FVC as at Time 0.

Exposure day 4:

Time 0: As on days 1-3.
Time 20-140 min: As on days 1-3.
Time 145 min: As on days 1-3.
Time 200 min: Measurements of SRaw, FEV1.0, and FVC.
Time 260 min: Repeated measurements as at time 200.
Time 265 min: Blood drawing for peripheral leukocyte count.
Time 270-445 min: Post-NO_2 bronchoscopy and recovery.

Data Analysis

The results are expressed as mean and standard deviation where appropriate. The changes in each of the pulmonary physiological parameters, lymphocyte counts, and macrophage products, from before to after exposure were compared using the two-tailed student's t test for paired data with the Bonferroni correction for multiple comparisons. Symptom scores were converted to rank scores in order to compare pre- and post-exposure scores by the Wilcoxon signed rank test. The level of statistical significance was set at $p<0.05$.

RESULTS

The anthropometric data on the 5 healthy subjects who participated in the study are summarized in table 3.

Respiratory symptoms and airway caliber

Mean total scores derived from pre- and post-NO_2 exposure symptom questionnaires are given in Table 4. No changes attributable to NO_2 exposure were found.

The baseline pulmonary physiologic data and those obtained following nitrogen dioxide exposure are summarized in Table 4. None of the respiratory physiologic variables measured showed evidence of untoward change attributable to NO_2 exposure.

Lymphocyte Phenotyping

Bronchoscopy and bronchoalveolar lavage was performed without difficulty in all 5 subjects on both occasions. Analysis of lymphocyte phenotypes in venous blood and in bronchoalveolar lavage fluid obtained under baseline conditions and after NO_2 exposure revealed few differences (tables 5 and 6). The total number of circulating lymphocytes was very slightly greater after NO_2 exposure (1792 ± 544 cells/mm^3 vs 1598 ± 549 cells/mm^3 under baseline conditions) and the proportions of different subsets were unaffected. In bronchoalveolar lavage fluid, the total number of lymphocytes and the proportions of T and B lymphocytes and of helper/inducer, suppressor/cytotoxic, and large granular lymphocytes were unaltered by NO_2 exposure.

A small but statistically significant increase in the proportion of natural killer cells

was found after NO_2 exposure (7.2 ± 3.1% vs. 4.2 ± 2.4% under baseline conditions; $p < 0.04$).

Markers of Macrophage Activation

ELISA values in BAL Fluid:

IL-1 concentrations were below the level of detection in all samples before and after NO_2 exposure. Concurrently run samples from patients with sarcoidosis and pulmonary infiltrates complicating AIDS had readily detectable levels (data not shown).

TNF was below detection levels in all samples before and after NO_2 exposure. It was detectable in one of seven AIDS patients with pulmonary infection (data not shown).

Messenger RNA (mRNA):

IL-1 RNA was not detected by Northern analysis in any of the samples from our subjects. It was detected in a concurrently run sample from a patient with active pulmonary sarcoidosis (data not shown).

Discussion:

Our data indicate that 2 h exposure to 0.6 ppm of NO_2 over 6 days is not associated with any significant changes in subjective respiratory symptoms, airway caliber, pulmonary lymphocyte subpopulations or alveolar macrophage products (interleukin-1 and tumor necrosis factor) in healthy, relatively young adult subjects.

The ambient exposure concentration to NO_2 in the present study (0.6 ppm) was a level which occurs in urban atmospheres only during heavy smog days. Since the minute ventilation was increased 3 to 4-fold by the exercise during exposure, our subjects were in fact exposed to relatively high levels of inhaled NO_2 . Nonetheless, no consistent change in respiratory symptoms or airway caliber could be detected indicating that ambient NO_2 concentrations are unlikely to cause disabling immediate effects in healthy, relatively young adults. Our findings support and extend the results reported by Folinsbee et al (16). They found no significant pulmonary function changes in a group of young men who were exposed to 0.62 ppm NO_2

Lymphocyte Phenotypes:

Our results showed little change in the total number of lymphocytes or in the proportions of different subtypes in venous blood and in bronchoalveolar lavage fluid obtained under baseline conditions and after NO_2 exposure. Lymphocytes are key cells of a closely integrated, complex immune system and are responsible for recognition of antigens and for mediating both antibody-mediated and cell-mediated responses. With the development of monoclonal antibodies to stable glycoprotein markers (differentiation antigens) on the surface of human lymphocytes, subpopulations of lymphocytes serving different functions can be identified. The broadest separation of lymphocyte compartments is made by designating one group as B cell (bursal or bone marrow

derived) and the other as T cell (thymus dependent). B cells are precursors of plasma cells, which synthesize and release immunoglobulins. T cells are responsible for reactions of cellular immunity, elaborating directly cytotoxic materials, and also serve to regulate immune responses. The "helper/inducer" subset of T cells provides necessary help and soluble signals for B cells to proliferate and differentiate into antibody producing cells. In contrast, the "suppressor/cytotoxic" subset exerts a suppressive influence on B cell activity in addition to serving a cytotoxic function on target cells. Some cells cannot be classified as T or B cells; these include large granular lymphocytes and killer lymphocytes. Natural killer cells appear important in tumor surveillance, for they mediate cytotoxic reactions without prior sensitization.

Abnormalities in the proportions of different lymphocyte subtypes have been demonstrated in bronchoalveolar lavage fluid of patients with diseases involving disturbances in immune function. Thus, the total number of T lymphocytes and the proportions of T helper/inducer cells is increased in bronchoalveolar lavage fluid from patients with diseases of increased immune activity, such as sarcoidosis (17, 18) and hypersensitivity pneumonitis (19). On the other end of the spectrum, a deficiency of helper/inducer T cells and an increased proportion of suppressor/cytotoxic cells has been found in bronchoalveolar lavage fluid as well as blood from patients with AIDS (20-22)

No such alterations in lymphocyte phenotypes were found in our subjects after NO_2 exposure (Tables 5 and 6). The single difference - a small increase in the numbers of natural killer lymphocytes - may have been artifactual, for these cells appear more likely than other lymphocytes to adhere to plastic or to fibronectin in the steps taken to enrich the proportion of mononuclear cells in the suspension analyzed by flow cytometry (23). In the single subject in whom we compared counts obtained in un-enriched samples (plastic adherence step not performed), we found a greater number of natural killer cells in both the baseline and NO_2 samples and the increase in the NK cells after NO_2 was no longer apparent. It is thus possible that NO_2's effect was not to stimulate an increase in NK cells but to reduce the adhesiveness of NK cells already present. Regardless of the reasons for our observed increase in NK cells, the increase is small and is unlikely to be of clinical importance. At most, the change observed would suggest increased immune surveillance, rather than an impairment of immune function.

Our analysis of lymphocytes in this study was entirely descriptive. The lack of change in the numbers of cells with various surface markers associated with different functions does not exclude the possibility of a change in function. Functional assessment of different lymphocyte subtypes was beyond the scope of this project.

Our analysis of IL-1 and TNF by both ELISA assay of bronchoalveolar lavage fluid and by quantitation of messenger RNA specific for IL-1 in cells recovered by lavage revealed no evidence of activation of macrophages by NO_2 exposure. Both cytokines were found in concurrently run lavage samples obtained from patients with sarcoidosis and with pulmonary infections complicating AIDS. Quantitation of mRNA for IL-1 is a highly sensitive test. Messenger RNA for IL-1 constitutes up to 0.5% of all mRNA in macrophages, so the lack of detectable mRNA for IL-1 in the cells obtained after NO_2 exposure make it very unlikely that the gene for IL-1 was activated by NO_2 exposure. Further study of the capacity of NO_2 to stimulate gene expression for IL-1 would require *in vitro* studies.

In conclusion, healthy, relatively young subjects who are exposed for a relatively prolonged period of time to 0.60 ppm or less of nitrogen dioxide are unlikely to develop significant acute respiratory impairment as judged by symptom scores, tests of airway caliber, and analysis of markers of immune function of the lung.

Table 3. Subject Characteristics

Subject	Sex	Age	Ht (cm)	Wt (kg)
1	M	27	190	88
2	F	34	163	52
3	M	36	178	88
4	M	30	180	68
5	M	21	173	80

Table 4. Subject Data

Subject	Day	S_{Raw}		$FEV_{1.0}/FVC$		Symptoms	
		Before	After	Before	After	Before	After
1	1	2.3	1.4	5.7/6.8	5.8/7.0	10	13
	2	3.2	4.0	5.5/6.7	5.6/6.7	8	7
	3	4.4	4.3	5.4/6.6	5.3/6.6	6	7
	4	4.8	5.3	5.4/6.6	5.5/6.7	5	7
2	1	6.0	8.2	2.6/3.4	2.6/3.2	1	0
	2	6.4	7.6	2.6/3.4	2.6/3.0	0	0
	3	5.5	7.7	2.6/3.3	2.6/3.3	0	1
	4	5.6	8.0	2.6/3.5	2.7/3.5	2	0
3	1	3.9	3.7	5.3/6.4	5.3/6.4	0	0
	2	4.6	4.5	4.9/6.0	5.0/6.1	0	0
	3	4.8	4.7	5.4/6.5	5.4/6.3	0	0
	4	4.7	4.3	5.4/6.5	5.5/6.6	0	0
4	1	5.5	4.9	3.3/4.8	4.2/5.7	0	1
	2	5.4	5.6	4.0/5.6	4.1/5.6	0	1
	3	5.6	4.8	4.1/5.6	4.2/5.7	0	0
	4	4.6	4.8	4.2/5.6	4.3/5.7	0	1
5	1	4.8	4.7	4.6/5.6	4.5/5.4	0	2
	2	4.2	4.6	4.5/5.4	4.5/5.5	1	1
	3	4.6	4.4	4.4/5.4	4.6/5.4	0	2
	4	4.3	4.3	4.4/5.4	4.6/5.4	1	1

Table 5. Lymphocyte Phenotypes in Venous Blood

BASELINE

Subject	Total (per mm^3)	T	B	H/I	S/C	LGL	K
1	1890	63.7	14.3	46.0	32.8	8.5	15.9
2	920	75.2	10.2	53.5	17.0	11.1	5.1
3	2250	79.0	9.1	42.6	33.8	22.5	9.0
4	1150	72.5	5.3	47.8	19.4	9.4	8.2
5	1780	76.5	7.1	24.7	48.2	25.5	9.3
Mean	1598	73.4	9.2	42.9	30.2	15.4	9.5
S.D.	±549	±5.9	±3.4	±10.9	±12.6	±8.0	±4.0

AFTER NO_2 EXPOSURE

Subject	Total (per mm^3)	T	B	H/I	S/C	LGL	K
1	2170	70.6	11.1	48.7	32.8	7.5	13.8
2	1190	69.7	12.1	49.8	17.9	9.9	9.7
3	2450	77.6	11.5	42.3	34.2	18.8	3.0
4	1300	74.3	8.4	50.6	19.4	10.1	12.8
5	1850	73.6	8.4	25.9	48.1	27.0	11.1
Mean	1792	73.2	10.3	43.5	30.3	14.7	10.1
S.D.	±544	±3.1	±1.8	±10.3	±12.6	±8.1	±4.3

Table 5. Lymphocyte phenotypes in circulating blood under baseline conditions (above) and after NO_2 exposure (below). "Total" refers to the total number of lymphocytes per mm^3 of blood. All other values are percentages. T = T lymphocytes (Leu 4+), B = B lymphocytes (Leu 16+), H/I = Helper and Inducer cells (Leu 3+),
S/C = Suppressor and Cytotoxic cells (Leu 2+), LGL = large granular lymphocytes (Leu 7+), K = Natural killer cells (Leu 11+).

Table 6. Lymphocyte Phenotypes in Bronchoalveolar Lavage Fluid

BASELINE

Subject	Total (per mm^3)	T	B	H/I	S/C	LGL	K
1	3,000	72.2	1.1	41.2	27.7	6.3	5.5
2	20,526	67.5	2.3	25.6	35.0	6.1	7.6
3	4,167	70.7	1.9	46.1	24.8	9.1	2.2
4	13,760	66.2	1.2	22.4	22.9	3.4	3.9
5	34,500	87.1	1.4	71.1	14.5	13.4	1.7
Mean	15,190	72.7	1.6	41.3	25.0	7.7	4.2
S.D.	±12,976	±8.4	±0.5	±19.5	±7.4	±3.8	±2.4

AFTER NO_2 EXPOSURE

Subject	Total (per mm^3)	T	B	H/I	S/C	LGL	K
1	6,400	70.4	2.4	39.8	27.9	7.4	9.8
2	10,684	68.6	1.3	28.4	30.9	5.5	10.7
3	13,647	55.5	0.9	36.9	17.4	8.4	3.3
4	13,500	71.9	2.4	30.0	38.0	8.1	7.4
5	37,600	85.4	1.5	69.8	18.1	16.6	4.9
Mean	16,366	70.4	1.7	41.0	26.5	9.2	7.2
S.D.	±12,228	±10.6	±0.7	±16.8	±8.8	±4.3	±3.1

Table 6. Lymphocyte phenotypes in bronchoalveolar lavage fluid obtained under baseline conditions (above) and after NO_2 exposure (below). "Total" refers to the number of lymphocytes per milliliter of lavage fluid. All other values are percentages.
T = T lymphocytes (Leu 4+), B = B lymphocytes (Leu 16+), H/I = Helper and Inducer cells (Leu 3+), S/C = Suppressor and Cytotoxic cells (Leu 2+), LGL = large granular lymphocytes (Leu 7+), K = Natural killer cells (Leu 11+).

Project 3: Nasal and bronchial effects of sulfur dioxide in asthmatic subjects:

Purpose

To determine whether subjects with allergic rhinitis and asthma and who have demonstrable bronchomotor responsiveness to SO_2 also have nasal responsiveness to the gas.

Background

Sulfur dioxide (SO_2) has important effects on the respiratory tree. In healthy people, acute exposure to 5 ppm of sulfur dioxide for 10 minutes can cause bronchoconstriction, as shown by an increase in airways resistance (1). In people with asthma, symptomatic bronchoconstriction can occur at rest with oral inhalation of 1 ppm of sulfur dioxide (2) , and, during eucapnic hyperventilation or during moderate to heavy exercise, with inhalation of as little as 0.1 ppm (3,4).

In studies reported to the Board last year under Contract #A5-163-33, we found that nasal allergy alone was not associated with increased nasal responsiveness to SO_2. We therefore examined, in this study, whether bronchomotor responsiveness to the gas might be associated with increased nasal responsiveness. To do this, we studied asthmatic subjects with proven bronchial responsiveness to SO_2. We reasoned that if the mechanisms responsible for such responsiveness were also present in the nose, these subjects would be likely to develop greater nasal symptoms or resistance changes after nasally breathing SO_2 than after breathing conditioned air.

Materials and Methods

Subject Selection

We studied 8 subjects with a history of allergic rhinitis, including episodic nasal stuffiness, sneezing, and rhinorrhea requiring the use of anti-histamines and decongestants. Each had at least 2 positive skin reactions to a battery of 9 common allergens, and 6 had an abnormally high circulating IgE levels at the time we studied them (Table 7). All had documented asthma as well. Each subject developed an increase in specific airway resistance of at least 8 L x cm H20/L/s after inhaling methacholine at a concentration of 0.5 mg/ml or lower, and each subject also developed a similar increase in specific airway resistance after orally breathing 2 ppm or less of SO_2 at a minute ventilation of 20 L/min, but not after breathing conditioned room air at the same minute ventilation. These criteria helped ensure that our subjects were indeed responding to SO_2, and not to airway cooling and/or drying. Subject characteristics are presented in Table 7.

We excluded from the study people with a history suggestive of a viral respiratory infection in the 4 weeks before the study, and we studied subjects with seasonal allergic rhinitis during a season when they were least symptomatic. No subject used nasal preparations of steroids or cromolyn chronically. All subjects refrained from medication for 8-24 hr, and from tea or coffee for at least 4 hr, before each study. All were informed of the risks of each procedure and signed consent forms approved by the Committee on Human

Research of the University of California, San Francisco.

Skin testing

At least 1 week before the actual experiment began, each subject had skin prick tests to saline and histamine controls (Center Lab, Port Washington, NY) and to extracts of mixed weeds, mixed grasses, mixed trees, Alternaria, Hormodendrum, cat hair, house dust, and house dust mite which contained 50% glycerin and 0.4% phenol as preservatives (Hollister-Stier, Spokane, WA). Skin reactions were read 15 minutes after the tests were placed. Subjects were considered to have had a positive response to a particular antigen if they had no reaction to saline and if the wheal and flare caused by that antigen was greater than or equal to that caused by histamine alone.

Nasal airway resistance measurements

Nasal airway resistance was measured by posterior rhinomanometry (5, 6). Nasal air flow was measured through a Fleisch #1 pneumotachygraph inserted in the faceplate of an airtight diving mask which covered the eyes and nose. The pressure drop across the nose was measured as the difference between the pressure in the mask adjacent to the nares and the pressure in the oropharynx, sampled through a catheter placed approximately 5 cm into the mouth and held between the lips. Using 2 differential pressure transducers (Validyne MP45-16-871, Northridge CA), the transnasal pressure was displayed on the x-axis and flow on the y-axis of a calibrated image-retentive oscilloscope (Tektronix 5115). We measured the transnasal pressure during inspiration at a reference flow of 0.15 L/s. Nasal resistance was computed as transnasal pressure divided by 0.15 L/s.

Gas delivery

We generated a stream of filtered air having an average temperature of 24° C and an average dew point of 15°C (relative humidity, 55%) by passing air from a compressed air source through vapor filters, a bubble humidifier, and a high efficiency particle air filter. To deliver 4 ppm of sulfur dioxide, we added air containing 500 ppm SO_2 at a metered rate into the stream of air as it passed through a 3L glass mixing chamber. The gas was delivered to the inlet port of a nasal mask at a flow rate greater than 0.5 L/s, the maximum nasal inspiratory flow rate generated by any of our subjects during tidal breathing. Gas exited the mask through a 45 cm length of Teflon tubing and emptied into a laminar flow hood. The temperature, dew point, and SO_2 concentration of samples taken from a port in the inspiratory limb of the airstream 30 cm from the mask were monitored continuously with a digital humidity analyzer (EG and G Model 911 Dew-All) and a pulsed fluorescent sulfur dioxide analyzer (Thermo Electron Corp. Series 43, Walnut CA). All tubing to be in contact with SO_2 was constructed of Teflon, glass, or stainless steel. The nasal mask was constructed of rubber (Porter Instrument Co, Inc., Hatfield PA) and coated with a fluoropolymer (Fluoroglide, Norton Performance Plastics, New Jersey). We confirmed in preliminary trials that SO_2 concentrations in the mask reached 4 ppm within 1- 2 min of placing it over the subject's nose and remained at that level during 10 min of tidal breathing.by one of the investigators as a test of the stability of the system. All exposures in the actual study were 4 min in duration. Because expired water droplets interfered with our measurements of SO_2 , we chose to monitor SO_2 concentrations proximal to the mask during the experiments.

Symptom scores

Subjects were asked to rate on a scale of 0 (no symptoms) to 4 (the worst ever) the following symptoms: runny nose, stuffy nose, urge to sneeze, actual sneezing, itchy or scratchy throat, urge to cough, chest tightness, wheezing, and shortness of breath. Other symptoms (for example, itchy eyes) could be entered on the form and rated by the subject. Subjects rated symptoms before and immediately after each exposure. We generated 2 scores from the responses to this questionnaire. The total score was the mean of all symptoms (the sum of all scores divided by the number of symptoms rated, usually 10). The nasal score was the mean of the first 4 symptoms.

Data analysis

The temperature and dew point of inspired room air and the changes in nasal symptoms, nasal resistance, and SRaw were compared by analysis of variance or the nonparametric equivalent. We considered a p value of < 0.05 as significant and, because we specifically sought to determine if SO_2 caused a greater increase in nasal resistance we used one-tailed tests for significance. Values are expressed as mean ± S.D.

Experimental design

Each subject returned to the laboratory for 5 visits at least 24 hr apart and at the same time of day. On the screening day, subjects performed eucapnic hyperpnea through a mouthpiece at 20 L/min, breathing 0, 0.5, 1, and 2 ppm SO_2 in succession during 4-min periods. Specific airway resistance was measured 2 min after the end of each 4-min period. Only subjects who developed an increase in SRaw of at least 8 L x cm H20/L/s after eucapnic hyperpnea with 2 ppm SO_2 or less, and who did not develop such an increase after eucapnic hyperpnea with filtered conditioned room air alone, were included in the study. On subsequent study days, these subjects 1) performed eucapnic hyperpnea with filtered room air 2) performed eucapnic hyperpnea with the concentration of SO_2 that provoked an increase of at least 8 SRaw units (PD_8USO_2). 3) tidally breathed through a nasal mask conditioned room air and 4) tidally breathed through a nasal mask a concentration of SO_2 that was twice the PD_8USO_2. Each exposure lasted 4 min and the order of the exposures was randomized. Subjects were not told which gas was delivered through the mouthpiece or nasal mask. Subjects scored their symptoms before and after each exposure. The timing of the resistance measurements for all exposures was as follows:

1-2 minutes after exposure:	Nasal resistance measurements
2-3 minutes after exposure:	Airway resistance measurements

Results

In this group of subjects, nasal symptoms and nasal resistance were no greater after breathing SO_2 than after breathing room air, irrespective of whether the gas was delivered through a mouthpiece or through a nasal mask (Table 8). In contrast, lower airway symptoms and SRaw were significantly greater after these subjects breathed SO_2 through a mouthpiece than after they breathed room air, confirming their bronchial responsiveness to

SO_2. This SO_2-induced increase in SRaw was not associated with a change in nasal resistance (Tables 8 and 9).

Discussion

Asthmatic subjects with documented bronchial responsiveness to SO_2 did not develop significantly greater increases in nasal symptoms or resistance after breathing SO_2 than after breathing room air. Even concentrations twice the PC8U, when delivered preferentially to the nose, did not increase nasal symptoms or resistance. Higher concentrations were selected for nasal delivery because of our findings in previous studies that the nose may be relatively insensitive to local effects of SO_2 and because flow in the nose is limited to less than 10 L/min. We could not increase SO_2 delivery to the nose by increasing flow, as is done for the lower airways.

Our results differ from those of a previous study,which reported that concentrations of SO_2 even lower than those we administered caused small (30-32%) but statistically significant increases in the nasal work of breathing of allergic asthmatic subjects (7). Some of the disparity in the conclusions of that study and our own may be at least partly explained by differences in exercise rate. Our subjects were exposed to SO_2 while resting and breathing tidally, whereas those in the earlier study were exposed while exercising. It is possible that the increases in nasal work of breathing found in the previous study were not due to a local effect of $S0_2$ on the nose, for the increases were similar whether $S0_2$ was delivered by mouthpiece, bypassing the nose, or by facemask, permitting nasal breathing. It is conceivable that exercise somehow alters nasal responsiveness to SO_2. Our study was not designed to examine such a possible interaction. Our results demonstrate, however, that the previously described increase in nasal work of breathing was probably not due to a reflex effect of irritation or contraction of the lower airways, for we did not observe an increase in nasal airway resistance when oral inhalation of $S0_2$ provoked a significant rise in specific airway resistance.

We have found it important to conduct a full control exposure when relying on measurements of nasal airflow and pressure, to control for the many factors influencing those measurements. Posture (8), facial encumbrances (9), exercise (10), recent or ongoing antigen exposure (11-13), P_{CO2} (14), bronchoconstriction (15), temperature and humidity of the inspired gas (16), and diurnal spontaneous nasal changes are all factors which have been reported to affect the nasal pressure-flow curve. Because these factors may not affect baseline measurements, but become important during the course of a study, comparing nasal function at the end of a stimulus to nasal function at baseline may not be sufficient to exclude many unforeseen variables. Comparing the changes during an experiment with the stimulus in question to the changes during an appropriate sham exposure is required to show which effects are due to the stimulus itself.

Technical differences aside, both our study and previous studies of the acute effects of SO_2 on nasal function concur that SO_2 rarely causes clinically important nasal symptoms, even when it causes statistically significant changes in nasal airflow and pressure.

Although this study did not examine the chronic effects of SO_2 or the effects of SO_2 in combination with other environmental or physical factors, it provides acute, laboratory controlled data to support the conclusions of an epidemiologic study which found that

symptoms of 5000 people with allergic rhinitis were provoked more during pollen season than during periods of high air pollution (17). We did not detect significant or important SO_2-induced increases in symptoms or nasal resistance, even though we delivered large concentrations of SO_2, at flow rates that maximize absorption of SO_2 by the nose, to subjects whose noses are likely to respond to environmental stimuli. This strongly suggests that concentrations of SO_2 that do not exceed current air quality controls (18) are per se unlikely to acutely aggravate the symptoms of a large population of people with chronic or recurring rhinorrhea, nasal congestion, or sneezing.

In addition to the epidemiological issues it raises, our study bears implications about basic mechanisms of airway responsiveness. It has focused on a group of subjects likely to be particularly sensitive to SO_2, with both symptoms of nasal allergy as well as demonstrated bronchial responsiveness to SO_2. From the results of our previous study in allergic rhinitics, it appears that the presence in the nasal mucosa of the mechanisms underlying nasal responsiveness to allergen are not sufficient to cause or to predispose to nasal responsiveness to SO_2. Our study does not shed light on the nature of the mechanism of SO_2 -induced bronchoconstriction, but the results of our study strongly suggest that the mechanism, or the expression of the mechanism, is confined to the tracheobronchial tree. A striking structural difference between the nasal and bronchial airways is the absence of airway smooth muscle in the nose. We cannot be certain that our nasal administration of SO_2 did not cause the local release of an airway smooth muscle constrictor. We can only observe that if such a material was released, it was not released in association with other mechanisms necessary for nasal congestion, sneezing, mucus hypersecretion, or other manifestations of antigen-induced responses in the nose.

Table 7. Subject Characteristics

Subject	Age	Sex	*PD8uSO_2 (ppm)	†PD8UMeth (mg/ml)	# positive skin tests	‡IgE (U/ml)
1	25	F	2	0.125	3	21
2	24	M	1	0.16	7	175
3	28	F	2	0.032	8	140
4	22	F	1	0.25	2	150
5	24	F	1	0.125	3	---
6	27	M	2	0.5	7	460
7	26	M	2	0.5	2	2980
8	23	F	1	0.125	5	470

*PD8u SO_2: the concentration of inhaled sulfur dioxide that caused an increase of 8 L x cm H_2O/L/s over the baseline specific airways resistance.

†PD8u Meth: the concentration of inhaled methacholine that caused an increase of 8 L x cm H_2O/L/s over the baseline specific airways resistance. In our laboratory, such an increase after breathing a concentration of methacholine of less than 2 mg/ml is considered an indication of airway hyperresponsiveness.

‡ Normal IgE value in our laboratory = < 40 U/ml

Table 8. Nasal Resistance*

Nasal Breathing:

Subject	Before air	After air	Change	Before SO_2	After SO_2	Change
1	0.73	1.26	0.53	1.66	1.23	-0.43
2	3.58	4.35	0.77	3.42	4.06	0.64
3	0.73	2.36	1.63	0.87	0.99	0.12
4	0.56	0.81	0.25	0.64	0.61	-0.03
5	2.39	2.41	0.02	2.22	2.18	-0.04
6	1.04	1.01	-0.03	2.06	1.65	-0.41
7	0.70	0.79	0.02	0.94	0.70	-0.24
8	2.11	2.67	0.56	3.23	1.65	-1.58
Mean	1.48	1.96	0.48	1.88	1.63	0.25
S.D.	±1.10	±1.23	±0.55	±1.06	±1.11	±0.64

Oral Breathing:

Subject	Before air	After air	Change	Before SO_2	After SO_2	Change
1	1.18	1.29	0.11	1.29	1.90	0.61
2	6.38	8.59	2.21	4.07	3.23	-0.84
3	0.39	0.51	0.12	0.63	0.66	0.03
4	1.00	1.21	0.21	0.79	1.02	0.23
5	1.75	2.09	0.34	3.23	2.93	-.30
6	1.08	0.81	-0.27	1.40	1.69	0.29
7	0.95	0.73	-0.22	1.97	1.88	-0.09
8	1.40	2.33	0.93	1.49	2.22	0.73
Mean	1.77	2.19	0.43	1.86	1.94	0.08
S.D.	±1.90	±2.66	±0.81	±1.20	±0.87	±0.51

*cm H2O/L/s

Table 9. Specific Airways Resistance (SRaw)*

Nasal Breathing:

Subject	Before air	After air	Change	Before SO_2	After SO_2	Change
1	4.86	5.52	0.66	6.41	6.27	-0.14
2	8.26	8.42	0.16	7.28	7.17	-0.11
3	8.70	9.88	1.18	9.39	13.35	3.96
4	7.17	6.21	-0.96	6.73	7.10	0.37
5	4.45	4.48	0.03	5.32	5.36	0.04
6	5.03	5.58	0.55	5.11	5.53	0.42
7	8.13	8.82	0.69	5.19	5.52	0.33
8	7.16	9.24	2.08	6.24	5.99	-0.25
Mean	6.72	7.27	0.55	6.46	7.04	0.58
S.D.	±1.70	±2.04	±0.88	±1.42	±2.64	±1.39

Oral Breathing:

Subject	Before air	After air	Change	Before SO_2	After SO_2	Change
1	4.72	4.47	-0.25	4.96	12.16	7.20
2	7.73	8.44	0.71	6.91	17.99	11.08
3	8.00	6.06	-1.94	8.32	41.89	33.57
4	6.53	7.18	0.65	7.69	14.31	6.62
5	6.91	7.12	0.21	7.56	29.14	21.58
6	5.60	6.12	0.52	5.71	18.65	12.94
7	4.67	5.33	0.66	6.85	20.40	13.55
8	8.21	8.12	-0.09	5.35	13.06	7.71
Mean	6.55	6.60	0.59	6.67	20.95	14.28
S.D.	±1.42	±1.36	±0.88	±1.21	±10.02	±9.18

*L x cm H_2O/L/s

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