Modulation of Bronchial Epithelial Cell Barrier Function by *in vitro* Ozone Exposure

Xiao-Ying Yu, Nobuyoshi Takahashi, Thomas L. Croxton, and Ernst W. Spannhake

Department of Environmental Health Sciences, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD 21205 USA

The epithelial cells lining the small, peripheral airways function as important targets for the action of inspired ozone. Loss of epithelial barrier integrity in these regions is a common element in ozone-induced airway inflammation. To investigate the direct effect of ozone on epithelial barrier function, canine bronchial epithelial (CBE) cells grown with an air interface were exposed for 3 hr to 0.2, 0.5, or 0.8 ppm ozone or to air. Mannitol flux, used as an index of paracellular permeability, increased above air controls by 461%, 774%, and 1172% at the three ozone concentrations, respectively. Transcellular electrical resistance exhibited a dose-related decrease. The immediate effect of 0.8 ppm ozone on permeability was significantly inhibited by preincubation for 48 hr in the presence of 1 ng/ml vitamin E (33%) or 1 μM vitamin A (34%). Responses to 0.5 ppm or 0.8 ppm were inhibited by pretreatment of the cells with 0.1 μM of the actin polymerizing agent phalloidin (34% and 25% inhibition, respectively). The increases in permeability induced by 0.2 and 0.5 ppm ozone were attenuated by 54% and 22%, respectively, at 18 hr after exposure, whereas that to 0.8 ppm was further enhanced by 42% at this time. The effects of ozone are modulated by the availability of antioxidants to the cells and appear to be associated with cytoskeletal dysfunction in CBE cells. The data are consistent with a loss of barrier function linked to a direct oxidative effect of ozone on individual CBE cells and indicate that the reversible or progressive nature of this effect is dose dependent. *Key words: mannitol, paracellular flux, permeability, primary culture, transepithelial electrical resistance. Environ Health Perspect* 102: 1068–1072 (1994).

Ozone is a highly reactive oxidant pollutant that is extracted from inspired air with greater than 90% efficiency by its interaction with a variety of biological targets within the airways (*1*). One characteristic consequence of *in vivo* exposure to ozone is an enhancement of airway permeability through reduction of epithelial cell barrier integrity (*2,3*). The mechanisms through which ozone affects permeability are not well understood. Despite the observation that increased airway permeability is associated with the transit of inflammatory cells, principally neutrophils, into the airway lumen (*4–6*), a causal role for these cells is brought into question by the demonstration that ozone-induced permeability is not affected by neutrophil depletion (*7,8*) and that increased permeability and cellular influx can exhibit temporal separation (*9*). Alternatively, ozone may affect barrier integrity through direct action on epithelial cells by causing oxidation of membrane lipids or other cellular components (*10,11*), leading, in some cases, to the release of mediators (*12,13*). Some data suggest that perturbation of contractile cytoskeletal elements may also be involved in increasing paracellular movement through the cell layer (*14*).

The current studies were carried out to investigate the nature and mechanisms of the direct actions of ozone on epithelial cell barrier function in the absence of other resident airway cells and inflammatory cell activity. Using canine bronchial epithelial (CBE) cells grown in culture with an air interface, we measured paracellular mannitol flux and transepithelial resistance as markers of epithelial barrier integrity (*15*), determined the dose dependency of onset and recovery of the actions of ozone, and tested the modulatory effects of selected pharmacologic agents.

Methods

Tissues used as sources of epithelial cells in these studies were obtained fresh from dogs sacrificed by other investigators according to research protocols approved by the Animal Care and Use Committees of the Johns Hopkins Medical Institutions; the animals were not sacrificed for the purpose of their use in the studies presented here. Lobar airways (second to fifth generation) were dissected free of parenchyma, and bronchial epithelial cells were prepared and cultured as previously described (*16*). In brief, following dissociation by 20–24 hr digestion with 1 mg/ml pronase (Calbiochem, San Diego, California) at 4°C, cells were washed and cultured at an initial density of 3–5 × 10⁵ cells/cm² in plastic dishes coated with human placental collagen (Sigma, St. Louis, Missouri) with supplemented Dulbecco's Modified Eagle's Medium/Ham's F12 (DMEM/F12; Gibco, Grand Island, New York). Cells were incubated at 37°C in a 95% air, 5% CO_2 atmosphere, and the medium was changed daily until cells reached 80% confluence. CBE cells were then detached by treatment with trypsin/EDTA (5%; Gibco), washed, and subcultured at a density of 2 × 10⁴ to 3 × 10⁵ cells/well in 24-well culture dishes on porous polycarbonate inserts (6.5 mm diameter, 0.4 μm pores; Costar, Cambridge, Massachusetts) coated with Vitrogen 100 (1:4 in ethanol; Celtrix, Palo Alto, California) with supplemented Ham's F12 medium above and below the filters. The media on the apical (0.1 ml) and basal (0.5 ml) sides of the cells were changed daily for 3–4 days, after which the apical medium was removed and the confluent cells were grown for an additional 2–3 days before the initiation of experimental protocols with an air interface at their apical surfaces. Some cultures that accumulated fluid above the cells, indicating a loss of barrier integrity, were excluded from the studies. Each experiment was conducted with cellular preparations derived from three or more dogs.

Confluent CBE cell cultures were exposed for 3 hr at 37°C in matched 6-l plexiglas chambers perfused at a flow rate of 1 l/min with 5% CO_2 in air (control) or 5% CO_2 in air containing ozone. Ozone was generated by passing the inflow to one chamber past a high-intensity UV mercury vapor lamp. The ozone concentration in that exposure chamber was continuously monitored with a Mast model 724 meter and was regulated by a computer-controlled feedback system which varied the UV lamp intensity. The inflow to each chamber was bubbled through water to achieve >97% relative humidity. The ozone chamber was allowed to equilibrate for at least 1 hr at the selected ozone concentration before adding the cells. The timing of the 3-hr exposure period was begun when the chamber concentration was reestablished after adding the cells (generally 30 min).

We used paracellular flux of ¹⁴C-mannitol as an index of the permeability of the CBE cell barrier (*15,16*). After exposure, the culture plate inserts were placed in a new 24-well plate prefilled with 0.35 ml/well DMEM/F12 medium containing 0.5 μCi/ml of ¹⁴C-mannitol (55 mCi/mmol, 0.1 mCi/ml; NEN Research Products, Boston, Massachusetts) and 0.2 ml of warm (unlabeled) medium was layered over the surface of CBE cells.

Paracellular flux in the basolateral to apical direction was calculated over the 2-hr incubation period based on the radioactivity of 20-μl samples removed from the apical side without replacement at 0, 0.5, 1.0, and 2.0 hr. Radioactivity was determined by liquid scintillation spectrometry (Beckman, Fullerton, California). Paracellular flux of mannitol (nmol/cm^2/hr) was calculated from the simplified formula:

$$F = (C \cdot V_{total}/V_{sample})(\alpha/SA)/(area \cdot t),$$

where F is the average flux across the cell layer between time zero and each time point (t), C is the radioactivity in the apical sample, V_{total} is the volume of solution in the apical chamber at the time of sampling, V_{sample} is the volume of the sample taken, α is a conversion constant that equals 4.55 × 10^{-4} nCi/dpm, SA is the specific activity of the ^{14}C-mannitol, and area is the surface area of the filter (0.33 cm^2). Cumulative flux data were expressed as nmol mannitol/cm^2 at each measurement time. We did not attempt to eliminate the diffusion gradient by adding unlabeled mannitol nor to compensate for variations in hydrostatic pressure caused by sampling from the apical chamber without replacement. Thus, the absolute values of flux reported may exhibit slight bias.

Immediately after exposure, medium was returned to the apical surfaces of the cultures, and 2 hr later, measurements of electrical resistance (RT) across confluent subcultured CBE layers were made with a "chopstick"-style electrode (World Precision Instruments, Inc., Sarasota, Florida). Known current pulses (+10 μA and -10 μA) were passed through the filter and Ohm's law was used to calculate the electrical resistance, expressed as ohm-cm^2 (Ω-cm^2), from the resulting transepithelial voltages. Data were corrected for the typical resistance of blank filters (30 Ω-cm^2).

Vitamin E (tocopherol acetate, Sigma), vitamin A (retinol acetate, Sigma), phalloidin (Sigma), and cytochalasin B (Sigma) were prepared as stock solutions in ethanol at concentrations of 10^{-3} M. Sodium meclofenamate (a gift from Warner-Lambert, Ann Arbor, Michigan) and MK886 (a gift from Merck-Frosst, Quebec, Canada) were prepared as stock solutions in Hanks' Balanced Salt Solution (HBSS) at concentrations of 10^{-4} M. L-659,989 (a gift from Merck, Sharpe and Dohme, Rahway, New Jersey) was dissolved in ethanol, suspended in 0.5% methyl cellulose at a concentration of 1 mg/ml, and brought to a stock concentration of 10^{-3} M in HBSS. All stock solutions were aliquoted and stored at -70°C before use.

All data are presented as the means ± SEM. Differences between means for group data were tested for significance by nonpaired Student's *t*-test. One-way analysis of normal variance (ANOVA) was employed for multiple comparisons. Fisher's protected least significant difference (PLSD) multiple *t*-test was used to identify significant differences between means. Statistical analyses were carried out using StatView 4.0 statisical software (Abacus Concepts, Berkeley, California); *p*-values <0.05 were considered significant.

Results

Dose-related effects of ozone on barrier integrity.
Figure 1 depicts the dose-related effects of 3-hr exposures of CBE cells to 0, 0.2, 0.5, or 0.8 ppm ozone on the cumulative paracellular flux of mannitol during a 2 hr-incubation period immediately following the end of exposure. Using the control (air-exposed) data from these experiments and assuming neglible changes in mannitol concentrations in the first 30 min of measurement, a baseline permeability coefficient of 1.7 × 10^{-6} cm/sec was calculated for the CBE cell preparation. At 0.2, 0.5, and 0.8 ppm, significant increases in cumulative flux of the tracer were elicited, reaching levels at the end of the 2-hr period that were 461%, 774%, and 1172%, respectively, above controls (p<0.05). Paracellular permeability induced by air exposure (control) resulted in movement of less than 1.5% of the mannitol from the basolateral chamber to the apical chamber during 2-hr incubation period. In parallel with these increases in mannitol flux across the cell layer, ozone exposure resulted in dose-related decreases in transcellular electrical resistance (R_T). Two hours after exposure, 0.5 ppm ozone decreased R_T by 41% (1285 versus 760 Ω-cm^2, p<0.05) and 0.8 ppm ozone decreased R_T by 78% (1285 versus 289 Ω-cm^2, p<0.05) (Fig. 2).

Role of mediators in modulation of increased permeability.
Experiments with CBE cells exposed to 1.0 ppm ozone for 1 hr demonstrated that pretreatment of the cells for 30 min with the cyclooxygenase inhibitor sodium meclofenamate (4 μM), the 5-lipoxygenase inhibitor MK 886 (1 μM), or the platelet-activating factor antagonist L-659,989 (0.3 μM) was without significant effect on the permeability-enhancing effects of ozone exposure. Values for mannitol flux (nmol mannitol/cm^2/hr) in ozone-exposed cultures in the absence and presence of each of these agents were, respectively: meclofenamate, 0.44 ± 0.03 versus 0.46 ± 0.06 (n = 5); MK886, 0.44 ± 0.03 versus 0.50 ± 0.04 (n = 5); L-659,989, 0.47 ± 0.03 versus 0.53 ± 0.01 (n = 6). To investigate further if stable mediators formed in or released into the medium by the cells were crucial in mediating the permeability change, medium below the cells was exchanged immediately after exposure. Flux across unexposed CBE cell cultures was not affected by incubation with medium removed from air-exposed or ozone-exposed cells (0.021 ± 0.005 nmol/cm^2/hr versus 0.015 ± 0.006 nmol/cm^2/hr, respectively, n = 15). Likewise, ozone-induced permeability over the 18-hr period after exposure was not different in cultures in which fresh medium was immediately substituted compared to those in which medium from the exposure period was allowed to remain (0.68 ± 0.13 nmol/cm^2/hr versus 0.77 ± 0.14 nmol/cm^2/hr, respectively, n = 9).

Modulation of the effects of ozone on permeability.
To investigate the potential protective effects of lipid-soluble antioxidants on the permeability changes induced by ozone, cultures grown for 2 days before exposure in the presence of vitamins E or A, followed by removal of the supplemented medium before exposure, were compared to those grown in normal medium.
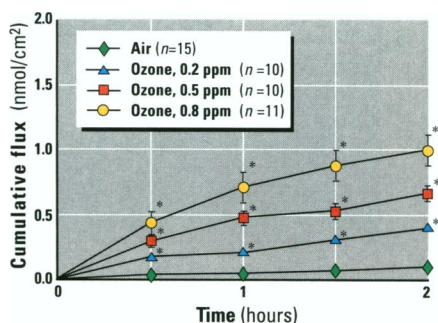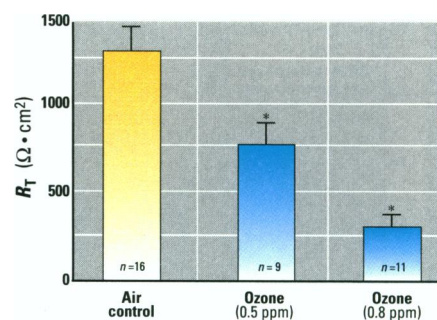

Figure 1. The dose-related effect of 3-hr exposures to 0.2, 0.5, and 0.8 ppm ozone on the cumulative flux of mannitol during a 2-hr incubation period immediately after the end of exposure. The control exposure was to air under identical conditions. *p <0.05, compared to air (ANOVA; Fisher's protected least significant difference); n = number of cultures from three to four dogs.

Figure 2. The dose-related effect of 3-hr exposures to 0.5 and 0.8 ppm ozone on transcellular electrical resistance (R_T). Measurements were made 2 hr after the end of the 3-hr ozone exposure period. *p <0.05, compared to air-exposed control (unpaired t); n = number of cultures from three dogs.

Due to potential effects of vitamin A on cell growth/differentiation, a preliminary experiment was carried out to determine the effect of the presence of vitamin A on the time-course of CBE growth to confluence. Using electrical resistance as an index of the development of barrier integrity, CBE cells subcultured in the presence or absence of 0.1 µM vitamin A exhibited identical growth patterns over the 7-day study period (data not shown). In addition, baseline electrical resistance and paracellular flux were not different in vitamin A-pretreated and control CBE cell preparations before exposure (912 ± 101 versus 1074 ± 48 Ω-cm^2 and 6.1 ± 0.8 versus 7.6 ± 1.4 pmol/cm^2/hr, control and treated, respectively). In response to 0.8 ppm ozone for 3 hr, cells preincubated with 1 ng/ml vitamin E demonstrated a significant 33% decrease in the ozone-induced change in permeability immediately after exposure (Fig. 3). A similar (34%) decrease in the effect of ozone was observed in cells preincubated with 0.1 µM vitamin A (Fig. 3). Nonetheless, significant increases in permeability were still induced by ozone, in comparison to air controls, in cultures pretreated with either antioxidant.

To determine if the permeability-enhancing effects of ozone were related to the loss of barrier integrity due to changes in cytoskeletal elements within the CBE cells, cultures were treated with the known actin-polymerizing agent, phalloidin, at a concentration determined in previous studies of CBE cells (16) to provide maximal protection against the microfilament disruptive action of cytochalasin B. As shown in Figure 4, the increase in permeability induced by exposures to 0.5 ppm or 0.8 ppm ozone was significantly inhibited, 34% and 25%, respectively, by 20 min of pretreatment with 0.1 µM phalloidin. The actions of 1 µM cytochalasin B on cells unexposed to air or ozone were likewise inhibited 37% by phalloidin.

Reversibility of the ozone-induced permeability changes. To investigate the reversible nature of permeability changes induced by ozone, a series of experiments was carried out in which cultures exposed to air or ozone were randomly divided into two groups in which permeability was measured immediately after exposure or after being returned to the incubator for an 18-hr "recovery" period. The data from these experiments are presented in Figure 5. As previously observed, the increases in paracellular flux immediately after ozone exposure were dose related at 0.2, 0.5, and 0.8 ppm. At 18-hr after exposure, flux in cultures exposed to 0.2 ppm was significantly attenuated by 54%, compared to that immediately after exposure. In cultures exposed to 0.5 ppm, flux was not significantly different 18-hr after exposure, compared to that immediately after exposure. In contrast, the increase in flux in cultures exposed to 0.8 ppm was progressive and, at 18 hr, demonstrated a significant, 42% elevation above that measured immediately after exposure.

Discussion

The present study was carried out to examine the direct effects of ozone exposure on bronchial epithelial cell barrier function. Canine bronchial epithelial (CBE) cells, when grown to confluence on porous filters and conditioned with an air interface for 2–4 days before study, demonstrated minimal baseline permeability to mannitol, a marker of paracellular flux, after 3 hr of exposure to 5% CO_2 in air. A baseline value for the permeability coefficient of 1.7 × 10^{-6} cm/sec was calculated based on the flux during the first 30 min of exposure to air. This value is in good agreement with that reported by Stutts and colleagues in their measurements in intact segments of third- to fifth-generation canine bronchi

(17), and supports the utility of the CBE cell culture preparation as a surrogate for selected studies of airway permeability changes. Exposure of CBE cells to ozone at 0.2, 0.5, and 0.8 ppm resulted in a dose-related increase in permeability. Permeability data showed a high degree of reproducibility within CBE preparations from a single animal and somewhat increased variability between preparations from different animals, suggesting that interanimal variation with regard to ozone susceptibility may exist. The observed fourfold increase in permeability above air in response to 0.2 ppm ozone suggests that this exposure is well above the threshold of the effect of ozone on CBE barrier function and is in keeping with studies of other markers of epithelial cell activation, such as mediator release (12,13) or morphologic changes (18), which indicate responsiveness at levels in the 0.05–0.1 ppm range.

The relationship between measures of paracellular flux and R_T is well established (15), and thus R_T provides a convenient indicator of changes in the integrity of tight junctions. Although measurements of R_T following 0.2 ppm ozone were not made, those at 0.5 ppm and 0.8 ppm confirm the dose dependency of the effect of ozone on R_T in our system. Decreases in R_T can reflect enhanced ion movement by either transcellular or paracellular pathways. However, because the decrease in R_T demonstrated in the current study approached 80% at the higher ozone exposure and was associated with a substantial increase in mannitol permeability, it is most likely that changes in R_T reflect alterations in the paracellular pathway rather than a stimulation of ion transport. It is interesting to note that a previous study found a decrease in the *in vivo* tracheal potentials of C57BL/6J mice after acute ozone exposure that was apparently not
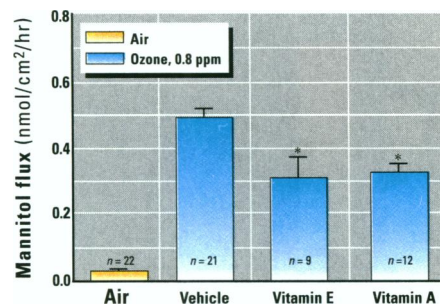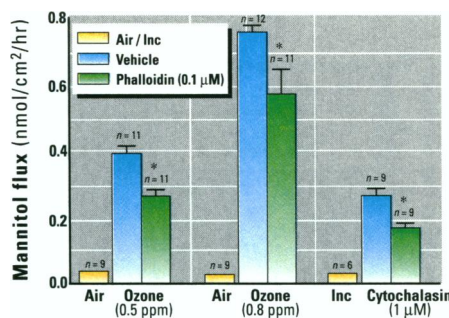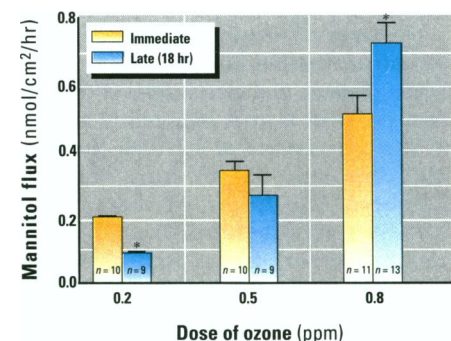

Figure 3. Influence of pretreatment of CBE cell cultures for 48 hr with vitamins E and A on the immediate increase in epithelial permeability induced by 0.8 ppm ozone. *p <0.05, compared to ozone in cells without pretreatment (unpaired t); n = number of cultures from three to four dogs.

Figure 4. The effect of phalloidin pretreatment on the immediate increase in permeability to mannitol induced by 0.5 or 0.8 ppm ozone. Cytochalasin B was used as a positive control for the attenuating effects of phalloidin. Air-exposed cultures without pretreatment and unexposed, vehicle-pretreated cultures (incubator) served as controls. *p <0.05, compared to corresponding ozone or cytochalasin B in the absence of phalloidin (unpaired t); n = number of cultures from three to four dogs.

Figure 5. Comparison of the effects of 0.2, 0.5, and 0.8 ppm ozone on paracellular flux across CBE cell cultures immediately and 18 hr after exposure. The initial effect of 0.8 ppm was enhanced at the later time, that of 0.5 ppm was slightly attenuated, whereas that of 0.2 ppm was significantly attenuated. *p <0.05, compared to immediate effects under the same conditions (unpaired t); n = number of cultures from three to four dogs.

related to changes in active ion transport (*19*). It was speculated that ozone induced a decrease in RT of the mouse tracheal epithelium. The current data may provide an *in vitro* analogue of that response.

Exposure of primary human bronchial cells and bronchial cell lines to ozone has been shown to result in the release of a wide range of mediators including interleukins, fibronectin, and platelet activating factor and to stimulate release of arachidonic acid with subsequent synthesis of prostaglandins, leukotrienes, and arachidonate-derived aldehydes (*11–13*). Furthermore, it has been reported that the increased tracheal permeability observed *in vivo* after ozone exposure may decrease or be lost after excision of the tissues (*14*), suggesting that chemical mediators may be important in the permeability response. Our observation that inhibitors of eicosanoid synthesis and the platelet-activating factor antagonist L-659,989 did not attenuate the effects of exposures of CBE cells to 1 ppm ozone for 1 hr suggests that the release of stable mediators was not involved in the increase in permeability induced by ozone in this study. That premise was further supported by the observation that medium removed from ozone-exposed cells had no effect on paracellular flux in cultures of naive cells. Furthermore, it was found that replacement of the medium present during the exposure period with fresh medium did not affect the course of permeability change over the 18–hr period after exposure, suggesting that this change occurred independently of factors formed within, or released into, the medium during ozone exposure. Thus, it appears that significant increases in epithelial permeability may occur in response to the direct action of ozone even in the absence of detectable mediator release.

Ozone-induced injury is recognized to be associated with peroxidation of lipids within lung tissues, including those of the airway epithelial cell membranes (*11,18,20*). To determine if a similar mechanism mediated the increase in CBE permeability, cultures were grown for 2 days before exposure in the presence of lipid-soluble vitamins A and E. Vitamin E is well known as a biological free-radical scavenger capable of providing antioxidant protection against the effects of ozone (*20,21*). In addition to its other possible actions on cell proliferation and differentiation, vitamin A has been reported to be a potent inhibitor of nonenzymatic lipid peroxidation in rat brain mitochondria (*22*). Studies of rats undergoing oxidant challenge *in vivo* indicated significant incorporation of vitamin A into heart and brain membrane preparations and provided evidence that the resultant antioxidant

action of vitamin A was not mediated by secondary enhancement of antioxidant enzyme activities (*23*). In the present study, vitamins E and A were found to be essentially equipotent in attenuating the increase in permeability induced by 0.8 ppm ozone. Because the exposure took place in the absence of the antioxidants in the medium, it is reasonable to assume that their protective effects were not due to a reduction in the exposure dose, but rather to an action within the exposed cells themselves. We cannot rule out the possibility that the protective effects of preincubating the confluent cultures with vitamin A was associated with altered differentiation of the cells (*24*). However, baseline permeability and electrical resistance in vitamin A-preincubated cultures were not different from that in vehicle-exposed controls, suggesting that cellular development was similar in treated and untreated cultures. Thus, these data are consistent with the concept that the initiation of the permeability increase is mediated through an oxidative mechanism involving the CBE cell membrane.

The relationship between microfilament morphology and the maintenance of barrier integrity is well documented in studies of pulmonary endothelial permeability (*25*). Fluorescence microscopic examination of rat airway tissues has indicated that ozone induces alterations in the epithelial cell cytoskeleton similar to those induced by cytoskeletal-disruptive agents such as cytochalasin (*26*). Furthermore, synergism between the actions of ozone and these cytoskeletal agents with regard to tracheal permeability has been reported (*14*). To assess the importance of cytoskeletal changes in the ozone-induced increase in permeability observed in CBE cells, cultures were pretreated with phalloidin, an agent demonstrated to protect endothelial cells from the permeability-enhancing effects of inflammatory agents (*27*). Although phalloidin has been shown to bind to specific membrane proteins on liver cells (*28*), its principal action is generally considered to be the stabilization and enhanced formation of F-actin microfilaments associated with the cytoskeleton (*29*). Phalloidin, at a concentration of 0.1 µM, was able to inhibit the effects of 0.5 ppm ozone by 34% and those of the cytoskeletal-disruptive agent cytochalasin B by 37%. The protective effect of phalloidin against ozone-induced loss of CBE barrier integrity is in marked contrast to its action in the presence of polycation-induced permeability, where this stabilizing agent was without effect (*16*). The inhibitory effect of phalloidin on cytochalasin B in the present study was less than that previously reported by this laboratory (*16*), most like-

ly due to the fact that phalloidin was only administered to the basolateral side of the cells during the 30-min pretreatment period. These data support the hypothesis that perturbation of cytoskeletal elements contributes significantly to the loss of barrier integrity induced by the direct effect of ozone on bronchial epithelial cells.

The progression of airway injury, inflammation, and repair after ozone exposure *in vivo* has been well described in animal models (*30,31*). In the case of severe epithelial damage in rat airways, initial injury was followed by active proliferation in bronchiolar regions 2–3 days after exposure (*30*). Far less is known about the progression of changes involving barrier function that occur in airway epithelium under less severe exposure conditions and in the absence of the varied influence of differing inflammatory cell populations (*31*). When CBE cell preparations were returned to the incubator after exposure and evaluated 18 hr later, the progression of changes in permeability was dose related. At the lowest (0.2 ppm) ozone concentration, the immediate increase in permeability underwent significant reversal, suggesting the absence of permanent injury to the cells and a relatively rapid recovery process. At 0.5 ppm, the permeability at 18 hr was, on average, not different from that observed immediately after exposure. This suggests a stable level of barrier function loss and likely results from the establishment of a balance between cells recovering and those undergoing further loss of function. In contrast, at 0.8 ppm, the effects of ozone on barrier integrity were progressive and were consistent with an inability of repair processes to match those of further injury within the 18 hr period. Although cultures were not followed beyond 18 hr in the present study, we anticipate that at a later time, cultures exposed to 0.8 ppm ozone would also undergo reversal of the permeability increase.

In summary, the present study demonstrates a dose-related and reversible effect of ozone on bronchial epithelial barrier integrity in the absence of inflammatory cell activity. This effect appears not to be dependent on the release of transferable products into the medium below the cells. Preincubation of the cells with lipid-soluble vitamins E or A can significantly attenuate the loss of barrier integrity, presumably through their direct antioxidant activities within the CBE cell membranes. Experiments with the microfilament-stabilizing agent, phalloidin, suggest that the observed permeability changes involve perturbations of cytoskeletal elements. Finally, the reversible or progressive nature of the enhanced permeability is dose related and presumed to be time dependent. These

results indicate that a significant portion of the ozone-induced permeability observed *in vivo* may be due to the direct oxidant effects of this agent on the barrier function of epithelial cells in the peripheral airways.

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