

Atypical development of the tracheal basement membrane zone of infant rhesus monkeys exposed to ozone and allergen

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Evans, Michael J., Michelle V. Fanucchi, Gregory L. Baker, Laura S. Van Winkle, Lorraine M. Pantle, Susan J. Nishio, Edward S. Schelegle, Laurel J. Gershwin, Lisa A. Miller, Dallas M. Hyde, Philip L. Sannes, and Charles G. Plopper. Atypical development of the tracheal basement membrane zone of infant rhesus monkeys exposed to ozone and allergen. *Am J Physiol Lung Cell Mol Physiol* 285: L931–L939, 2003. First published June 27, 2003; 10.1152/ajplung.00175.2003.—Development of the basement membrane zone (BMZ) occurs postnatally in the rhesus monkey. The purpose of this study was to determine whether house dust mite allergen (HDMA) plus ozone altered this process. Rhesus monkeys were exposed to a regimen of HDMA and/or ozone or filtered air for 6 mo. To detect structural changes in the BMZ, we measured immunoreactivity of collagen I. To detect functional changes in the BMZ, we measured perlecan and fibroblast growth factor-2 (FGF-2). We also measured components of the FGF-2 ternary signaling complex [fibroblast growth factor receptor-1 (FGFR-1) and syndecan-4]. The width of the BMZ was irregular in the ozone groups, suggesting atypical development of the BMZ. Perlecan was also absent from the BMZ. In the absence of perlecan, FGF-2 was not bound to the BMZ. However, FGF-2 immunoreactivity was present in basal cells, the lateral intercellular space (LIS), and attenuated fibroblasts. FGFR-1 immunoreactivity was downregulated, and syndecan-4 immunoreactivity was upregulated in the basal cells. This suggests that FGF-2 in basal cells and LIS may be bound to the syndecan-4. We conclude that ozone and HDMA plus ozone effected incorporation of perlecan into the BMZ, resulting in atypical development of the BMZ. These changes are associated with specific alterations in the regulation of FGF-2, FGFR-1, and syndecan-4 in the airway epithelial-mesenchymal trophic unit, which may be associated with the developmental problems of lungs associated with exposure to ozone.

perlecan; fibroblast growth factor-2; fibroblast growth factor receptor-1; syndecan-4; epithelial-mesenchymal trophic unit

THE BASEMENT MEMBRANE ZONE (BMZ) is the central structure of the epithelial-mesenchymal trophic unit. The epithelial-mesenchymal trophic unit consists of opposing layers of epithelial and mesenchymal cells sepa-

rated by the BMZ (11, 13, 17). The primary trophic unit consists of a layer of basal cells, the BMZ, and the attenuated fibroblast sheath. Recognition of the attenuated fibroblast sheath as a distinct layer of resident fibroblasts is key to the concept of an epithelial-mesenchymal trophic unit. The BMZ has a number of functions in the epithelial-mesenchymal trophic unit. It is specialized for attachment of epithelium with the extracellular matrix; it also serves as a barrier, binds specific growth factors, hormones, and ions, and is involved with electrical charge and cell-cell communication (1, 8, 40). Binding and storage of growth factors is an important function of the BMZ. The exchange of information between the epithelium and fibroblasts in the epithelial-mesenchymal trophic unit occurs via the BMZ.

With transmission electron microscopy, the BMZ appears as three component layers: the lamina lucida, the lamina densa, and the lamina reticularis (LR). Together they make up the basal lamina. The LR is especially pronounced under the respiratory epithelium of large conducting airways, where it may be several micrometers thick. Collagen type I, III, and V form heterogeneous fibers that account for the thickness of the LR. The collagen fibers are arranged as a mat of large fibers oriented along the longitudinal axis of the airway. Smaller fibers are cross-linked with the larger fibers to complete this structure (14). The BMZ also has numerous pores (14, 19, 20). Heparan sulfate proteoglycans (perlecan) and chondroitin sulfate proteoglycans (bamacan) are an intrinsic part of the BMZ that are involved with most of its functions (37–39). Attenuated fibroblasts beneath the BMZ are thought to synthesize the collagen type I, III, and V components of the BMZ (11, 13, 17).

In previous studies, we found that development of the epithelial BMZ occurred postnatally in the rhesus monkey (10). The collagen BMZ increased in width from 1 to 6 mo, and perlecan was localized in the BMZ at all stages of development. Fibroblast growth factor-2

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(FGF-2) was strongly expressed in basal cells at 1–3 mo but not in the BMZ. However, by 6 mo the distribution of FGF-2 had changed; FGF-2 was now expressed throughout the BMZ and weakly in basal cells. Fibroblast growth factor receptor-1 (FGFR-1) was present in basal cells and the nuclei of columnar cells throughout this time period. In rhesus monkeys treated with house dust mite allergen (HDMA) during this period of development, we found significant thickening of the tracheal BMZ (12). Both perlecan and FGF-2 were evenly distributed throughout the thickened BMZ. We also found that all HDMA tracheal samples expressed thin focal areas of the BMZ ($<2.0\ \mu\text{m}$) associated with leukocyte trafficking. In these areas, the collagen BMZ was damaged and depleted of perlecan and FGF-2. However, in the adjacent basal cells, there was increased FGF-2 immunoreactivity. We concluded that basal cells and FGF-2 are involved with significant remodeling of the BMZ in the developing trachea of infant rhesus monkeys exposed to HDMA.

Remodeling of the epithelial BMZ involves increased deposition of subepithelial collagen, resulting in thickening of the BMZ (4, 5, 12, 36). Thickening of the BMZ is thought to protect against airway narrowing and air trapping (26). It is not known how thickening affects the various functions of the BMZ, such as its role in FGF-2 signaling. Ozone is an oxidant gas thought to act synergistically with allergens in airway remodeling. Two fundamental characteristics have been defined for ozone effects on the lung. First, postnatal animals, before weaning, are less susceptible to acute pulmonary injury than are adults. Second, chronic exposure to oxidant gases retards postnatal maturation of the lung. The purpose of the present study was to determine whether combined cyclic inhalation of ozone plus HDMA affected development of the BMZ in infant rhesus monkeys during the first postnatal 6 mo. From our previous study, we know this is a time when the BMZ is undergoing active development (10) and that HDMA alters the normal pattern of development (12). To detect structural changes in BMZ development, we measured collagen I. To detect functional changes in the BMZ, we measured the distribution of perlecan and FGF-2. To detect functional changes in the adjacent basal cells and attenuated fibroblasts, we measured the expression of FGFR-1 and the cell surface proteoglycan syndecan-4, both components of the FGF-2 ternary signaling complex (2, 30, 48). We found that ozone depleted BMZ perlecan and caused structural changes in the BMZ collagen. These changes altered the expression of FGF-2, FGFR-1, and syndecan-4 in the epithelial-mesenchymal trophic unit.

MATERIALS AND METHODS

Animals and experimental protocol. All monkeys selected for these studies were California Regional Primate Research Center colony-born rhesus macaques (*Macaca mulatta*). Care and housing of animals complied with the provisions of the Institute of Laboratory Animal Resources and conforms to

practices established by the American Association for Accreditation of Laboratory Animal Care.

Twenty-four infant rhesus monkeys (30 days old) were exposed to 11 episodes of either filtered air, HDMA aerosol, ozone, or HDMA plus ozone (5 days each followed by 9 days of filtered air). Ozone was delivered for 8 h/day at 0.5 parts per million. Twelve of the monkeys (HDMA and HDMA + ozone groups) were sensitized to HDMA (*Dermatophagoides farinae*) at age 14 and 28 days by subcutaneous inoculation (SQ) of HDMA in alum and intraperitoneal injection of heat-killed *Bordetella pertussis* cells. HDMA sensitization was confirmed via skin testing with SQ HDMA on day 38 of the exposure protocol. Sensitized monkeys were exposed to HDMA aerosol for 2 h/day on days 3–5 of either filtered air (HDMA, $n = 6$) or ozone (HDMA + ozone, $n = 6$) exposure. Nonsensitized monkeys were exposed to either filtered air ($n = 6$) or ozone ($n = 6$). Details of the ozone and allergen exposure procedures are given in another paper (42). Immunoreactivity for collagen I, perlecan, and FGF-2 in the filtered air and HDMA groups were presented in a previous paper concerning remodeling of the BMZ and leukocyte trafficking (12). Data from this paper are included here for comparison with the ozone treatment groups of this study.

Preparation of animals. After the exposure protocol, monkeys were killed with an overdose of pentobarbital sodium after being sedated with Telazol (8 mg/kg intramuscularly) and anesthetized with Diprivan ($0.1\text{--}0.2\ \text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ intravenously). Monkeys were necropsied after exsanguination through the abdominal aorta, and the lungs were prepared for analysis as previously described (41). Tracheal samples were sliced perpendicular to the long axis of the airway into rings fixed in either 1.0 or 4.0% paraformaldehyde for 1 h and embedded in paraffin.

Immunohistochemistry. For routine histology, 5- μm sections were stained with hematoxylin and eosin. For immunohistochemistry, 5- μm sections were deparaffinized in xylene, hydrated in ethanol, and washed in PBS. For collagen, sections fixed in 1.0% paraformaldehyde (PFA) were treated with pepsin (1.0 mg pepsin/ml 3.0% acetic acid) at 37°C for 2 h, blocked with bovine serum albumin, and then treated with antibody to collagen I (1:250; rabbit anti-human polyclonal antibody; Biogenesis, Kingston, NH) overnight at 4°C. For perlecan, sections fixed in 1.0% PFA were treated with 0.1% Pronase in PBS for 30 min, rinsed in nanopure water followed by PBS, blocked with bovine serum albumin for 30 min, and incubated with an antibody to perlecan (1:2,000; mouse anti-human monoclonal antibody, clone 7B5; Zymed, San Francisco, CA) overnight at 4°C. For syndecan-4, sections fixed in 4.0% PFA were placed in citrate buffer and heated in a microwave oven at 95°C for 5 min and then placed in fresh buffer for 10 min at room temperature. Nonspecific binding was blocked with bovine serum albumin. The sections were treated with antibody to syndecan-4 (1:200; Santa Cruz Biotechnology) overnight at 4°C. For FGF-2, sections fixed in 1.0% PFA were treated with 0.1% H_2O_2 in methanol for 60 min, followed by 50 mg/ml of bovine testicular hyaluronidase in 0.05 M Tris buffer (pH 7.6) for 30 min. The sections were blocked with 5.0% horse serum for 30 min and incubated with an antibody to FGF-2 (1:750; mouse anti-human monoclonal antibody, clone bFM-2; Upstate Biotechnology, Lake Placid, NY) overnight at 4°C. For FGFR-1, sections fixed in 1.0% PFA were treated with 0.02% trypsin in PBS at room temperature for 30 min, washed, blocked with 25 μg of purified goat IgG in PBS for 60 min, and incubated with an antibody to FGFR-1 (1:500; rabbit anti-chicken polyclonal antibody; Upstate Biotechnology) overnight at 4°C. After immunohistochemistry, the sections were

washed in PBS, treated with the secondary antibody (1:1,000; Alexa Fluor 568; Molecular Probes, Eugene, OR) for 30 min, washed in PBS, and the coverslip was mounted in enzyme-linked fluorescence, fluorescent safe media (Molecular Probes). Fluorescence was visualized on an Olympus BH-2 fluorescent microscope.

Antibody specificity. The antibodies for collagen type I and V and FGFR-1 had negligible cross-reactivity with other collagens or noncollagen matrix proteins (per supplier). The antibody for collagen type III has no cross-reactivity with other collagens (16). The antibody for FGF-2 has no cross-reactivity with FGF-1 or heparin-binding growth factor-1 (25). The antibody for perlecan may cross-react with the short arm of laminin A and B chains (28). The antibody for syndecan-4 is specific for a peptide mapping within an internal region of syndecan-4.

Semiquantitation. The intensity of immunoreactivity for perlecan, FGF-2, FGFR-1, and syndecan-4 expression was graded on a scale of 0–3 for each animal (23). A scale of 0 indicates no staining beyond background, 1 indicates isolated areas of staining in the BMZ or cells, 2 indicates staining in 25–75% of the BMZ or cells, and 3 indicates strong staining in most of the BMZ or cells. Analysis of the tissues was performed in a blinded fashion. The mean and SD of perlecan, FGF-2, FGFR-1, and syndecan-4 expression for each group of animals were then determined.

Quantitation of BMZ width. The width of the BMZ was measured morphometrically to quantitate the immunohistochemical results. In human biopsy samples, it was demonstrated that 31–45 measurements, at least 20 μm apart and covering 1,000 μm of BMZ, are necessary to give an accuracy of $\pm 15.0\%$ (45). In this study, eight micrographs were taken equidistant apart around the circumference of the tracheas. The width of the BMZ was measured at four points, 50 μm apart, on each micrograph. A total of 1,600 μm of BMZ were sampled in each tracheal ring in this manner. The average width of the BMZ was determined from these measurements for each animal. To estimate the proportion of the BMZ that was thin, the percentage of measurements that was $<2.0 \mu\text{m}$ was determined for each animal. The mean and SD of BMZ width and the percentage of the measurements $<2.0 \mu\text{m}$ were then determined for each group of animals (10).

Statistics. The differences between treatment groups were compared with Mann-Whitney's rank sum test, with significance set at $P < 0.05$.

RESULTS

Collagen I. The width of the BMZ in the filtered air group was $4.3 \pm 0.7 \mu\text{m}$. It increased in the HDMA group to $6.3 \pm 0.8 \mu\text{m}$. The width of the BMZ in the ozone group was $5.7 \pm 1.2 \mu\text{m}$ and in the HDMA plus ozone group was $4.7 \pm 1.1 \mu\text{m}$. The width of the BMZ was irregular in each group but more so in the ozone and HDMA plus ozone groups (Fig. 1, A and B). Thin regions of the BMZ ($<2.0 \mu\text{m}$) made up 4.7 ± 3.3 and $4.7 \pm 4.3\%$ of the measurements in the filtered air and HDMA groups. In the ozone and HDMA plus ozone groups, thin regions of the BMZ made up 7.8 ± 3.8 and $16.7 \pm 6.1\%$ of the measurements (Fig. 2). It is not clear whether these thin areas represent regions of the BMZ damaged by leukocyte trafficking (12) or regions of the BMZ that had developed abnormally (10).

Perlecan. Immunoreactivity was evenly distributed throughout the epithelial BMZ, the walls of blood vessels, and smooth muscle bundles in both the filtered air

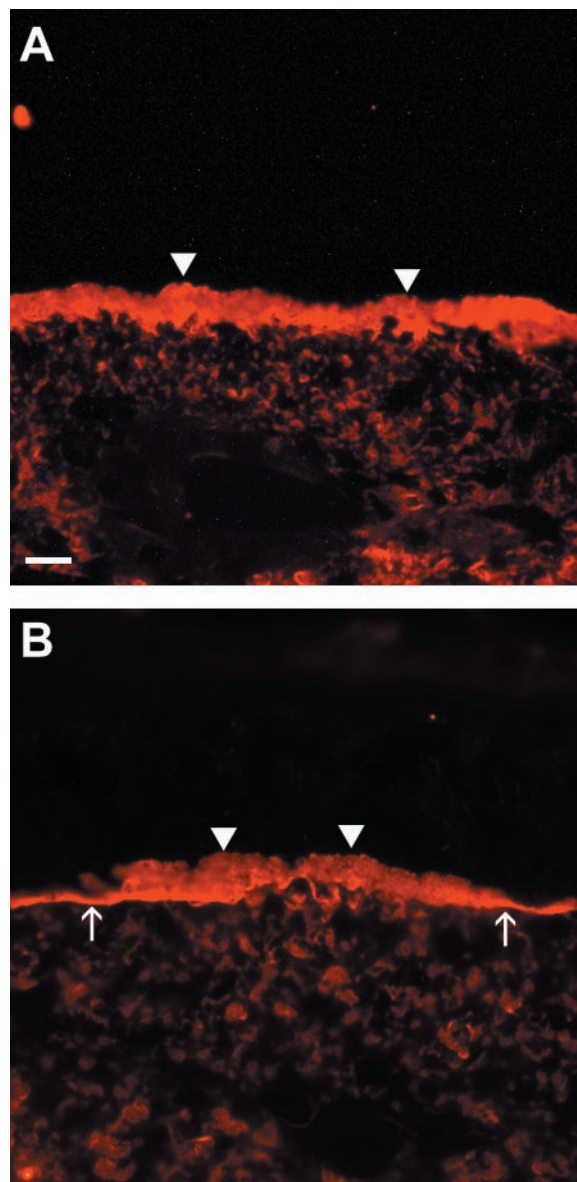


Fig. 1. A: collagen I immunoreactivity in the basement membrane zone (BMZ; arrowheads) of a house dust mite allergen (HDMA)-treated monkey. Both of the surfaces are irregular. The mean width of the BMZ was $6.3 \pm 0.8 \mu\text{m}$. The percentage of the BMZ $<2.0 \mu\text{m}$ in width was 4.7 ± 3.3 . B: collagen I immunoreactivity of an HDMA + O_3 -treated monkey. Some areas of the BMZ appear normal (arrowheads) and other areas are thin ($<2.0 \mu\text{m}$) and incomplete (arrows). It is not clear whether these irregular areas represent regions of the BMZ damaged by leukocyte trafficking (12) or regions of the BMZ that had developed abnormally (10). The mean width of the BMZ was $4.7 \pm 1.1 \mu\text{m}$. The percentage of the BMZ $<2.0 \mu\text{m}$ was 16.7 ± 6.1 . Bar = 10 μm .

and HDMA groups (Fig. 3A). The relative immunoreactivity was strong in the BMZ. In contrast, perlecan immunoreactivity was uniformly weak or absent from the epithelial BMZ of the ozone and HDMA plus ozone groups (Fig. 3B). However, in the walls of blood vessels and smooth muscle bundles, it remained high. The decrease in the relative intensity of perlecan immunoreactivity in the epithelial BMZ of the O_3 treatment groups, but not in the walls of blood vessels, suggests

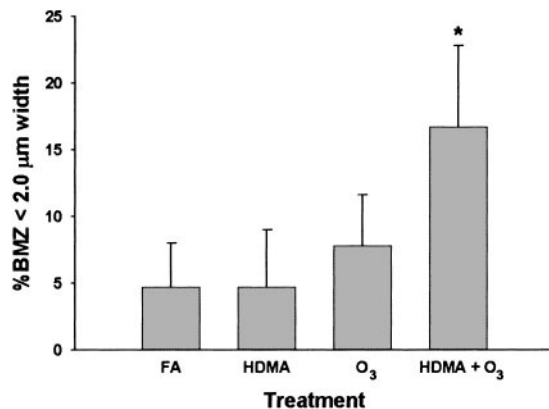


Fig. 2. Graphic representation of the percentage of the BMZ that was $<2.0\ \mu\text{m}$ in width. There was a significant increase in the number of animals in the HDMA + O₃ group with BMZ measurements that were $<2.0\ \mu\text{m}$ in width. * $P < 0.05$ vs. the filtered air (FA) group.

that perlecan was not being incorporated into the epithelial BMZ during development.

FGF-2. Immunoreactivity for FGF-2 in the epithelial BMZ mirrored that of perlecan in each treatment group. It was evenly distributed throughout the epithelial BMZ and the walls of blood vessels but not in smooth muscle bundles in the filtered air and HDMA groups (Fig. 4A). The relative immunoreactivity was strong in the epithelial BMZ but was less in the walls of blood vessels and smooth muscle bundles. In contrast, FGF-2 immunoreactivity was uniformly weak or absent from the epithelial BMZ of the ozone and HDMA plus ozone groups (Fig. 4B), whereas in the walls of blood vessels and smooth muscle bundles, it remained the same. The decrease in the relative intensity of FGF-2 immunoreactivity in the epithelial BMZ of the ozone and HDMA plus ozone groups suggests that FGF-2 was not being stored in the epithelial BMZ during development due to a lack of perlecan (Fig. 5). However, FGF-2 immunoreactivity was present in basal cells and the lateral intercellular space on the epithelial side of the BMZ. On the mesenchymal side of the BMZ, it was present in attenuated fibroblasts and other cells in the extracellular matrix of both ozone treatment groups. This is in contrast to the filtered air and HDMA groups in which FGF-2 immunoreactivity was relatively weak on both sides of the BMZ (Fig. 6).

Syndecan-4. Weak to moderate syndecan-4 immunoreactivity was expressed on the surface and cytoplasm of basal cells and occasional ciliated cells on the epithelial side of the BMZ in the filtered air and HDMA groups (Fig. 7A). Weak immunoreactivity was present in some attenuated fibroblasts on the mesenchymal side of the BMZ. However, in the ozone and HDMA plus ozone groups, syndecan-4 immunoreactivity was strong in the basal cells and lateral intercellular space on the epithelial side of the BMZ (Fig. 7B). Immunoreactivity in attenuated fibroblasts on the mesenchymal side remained the same. The relative intensity of basal cell syndecan-4 immunoreactivity was significantly

more in the ozone and ozone plus HDMA groups than the filtered air or HDMA groups, suggesting that it had been upregulated in the ozone exposure groups. Syndecan-4 immunoreactivity mirrored that of FGF-2 in the epithelium, suggesting that FGF-2 normally bound to BMZ perlecan is now bound to basal cell-associated syndecan-4.

FGFR-1. In both the filtered air and HDMA groups, FGFR-1 immunoreactivity was expressed on the surface and cytoplasm of basal cells (Fig. 8A). It was also expressed in ciliated and goblet cell nuclei, cilia, and in some basal cell nuclei similar to that seen in developing BMZ (10). In contrast, FGFR-1 immunoreactivity was reduced in the basal cells and columnar cell nuclei

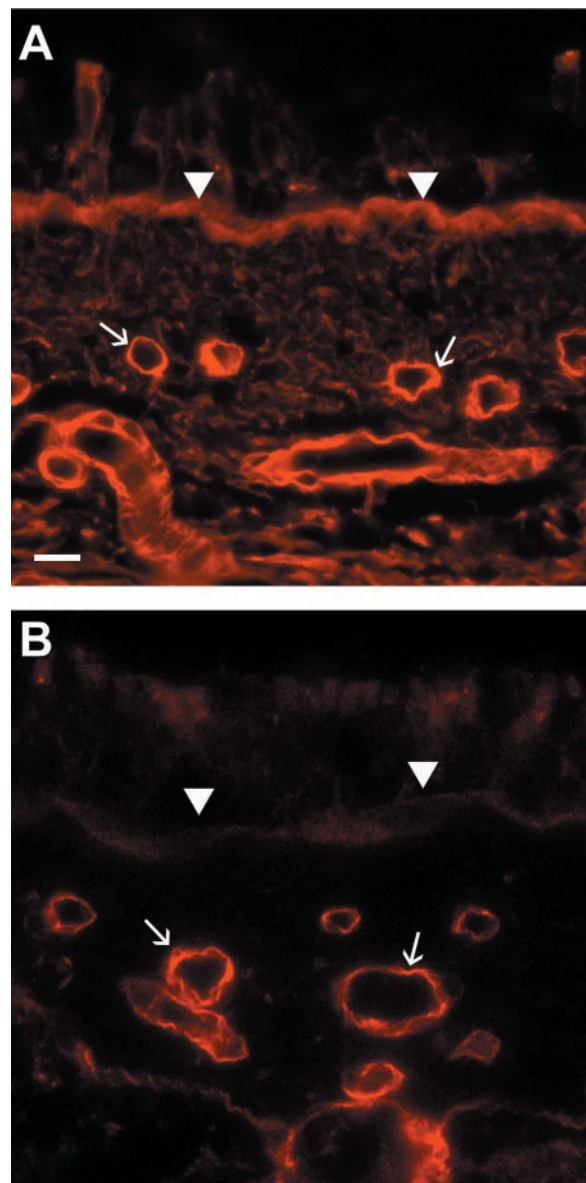


Fig. 3. A: perlecan immunoreactivity in the BMZ of an HDMA-treated monkey. There is strong immunoreactivity in the epithelial BMZ (arrowheads) and in the walls of blood vessels (arrows). B: in the epithelial BMZ of an HDMA + O₃-treated monkey (arrowheads), there is very little perlecan immunoreactivity; however, in the walls of blood vessels (arrows), it remained strong. Bar = $10\ \mu\text{m}$.

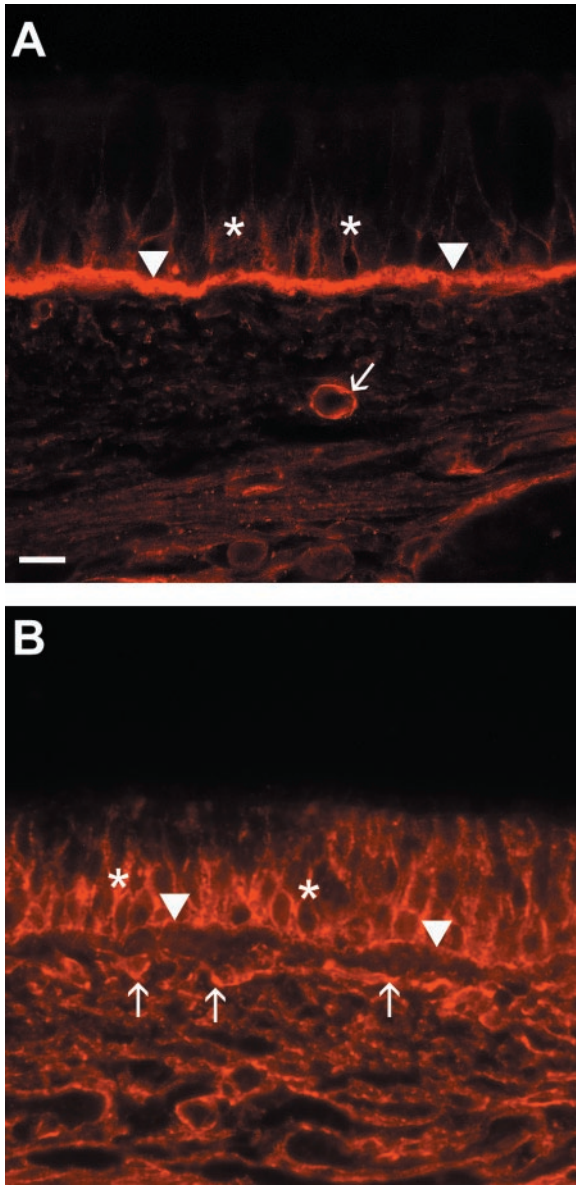


Fig. 4. *A*: fibroblast growth factor-2 (FGF-2) immunoreactivity in the BMZ of an FA-treated monkey. There is strong immunoreactivity in the epithelial BMZ (arrowheads). In the walls of blood vessels (arrow), it is less, and in the lateral intercellular space between basal cells and columnar cells (*), it is faint. *B*: in contrast, FGF-2 immunoreactivity was uniformly weak or absent from the epithelial BMZ (arrowheads) of O_3 -treated monkeys, whereas in the walls of blood vessels, it remained the same. Strong FGF-2 immunoreactivity was now present in basal cells (*) and the lateral intercellular space of the epithelium. On the mesenchymal side of the BMZ, strong immunoreactivity was present in attenuated fibroblasts (arrows) and other cells in the extracellular matrix. Bar = 10 μ m.

in the ozone and HDMA plus ozone groups (Fig. 8B). However, it was still strong in the cilia. The relative intensity of FGFR-1 immunoreactivity was less in the ozone and ozone plus HDMA groups compared with the filtered air and HDMA groups, suggesting that it had been downregulated in the ozone exposure groups. Immunoreactivity for FGFR-1 was inversely related to that of syndecan-4 in the epithelium (Fig. 9). Immuno-

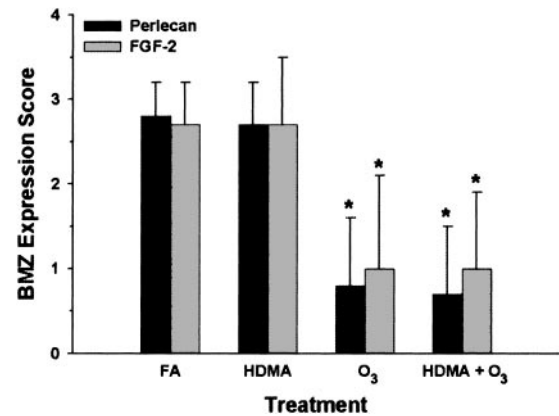


Fig. 5. Graphic representation of perlecan and FGF-2 immunoreactivity expression scores (means $\pm$ SD). In the ozone treatment groups, there was a significant decrease in the perlecan and FGF-2 scores. These findings suggest that perlecan was not being incorporated into the BMZ during development in the ozone groups. In the absence of perlecan, FGF-2 was not stored in the BMZ. * $P < 0.05$ vs. the FA group.

reactivity was not present in attenuated fibroblasts in any of the treatment groups.

DISCUSSION

In contrast to the previous study (12), the tracheal BMZ was significantly different in monkeys exposed to either ozone or HDMA plus ozone during this same developmental period. The collagen BMZ was irregular and thin in many areas, and perlecan was depleted or severely reduced in BMZ. These changes were present throughout the entire BMZ, and not only with areas of leukocyte trafficking, as seen in animals exposed to HDMA (12). Only the epithelial BMZ was depleted of perlecan; the BMZ around blood vessels and smooth muscle cells was not affected. This suggests that cells associated with synthesis of the epithelial BMZ perlecan are the main cells affected by the ozone. Examples of perlecan depletion in the BMZ have been reported in perlecan knockout mice (6), lung tumors (squamous

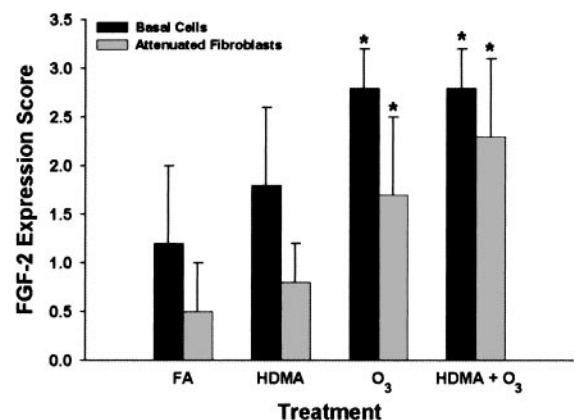


Fig. 6. Graphic representation of FGF-2 immunoreactivity expression scores in the basal cells and attenuated fibroblast compartments (means $\pm$ SD). In the ozone treatment groups, there was a significant increase in expression scores in both the basal cell and attenuated fibroblast compartments. * $P < 0.05$ vs. the FA group.

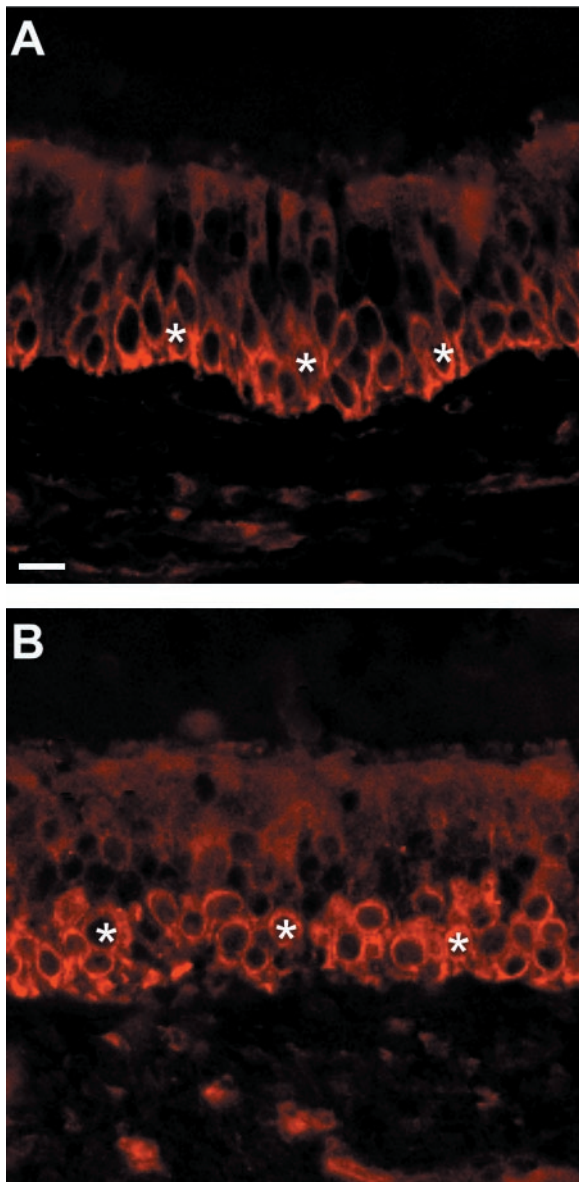


Fig. 7. *A*: weak syndecan-4 immunoreactivity was expressed on the surface and cytoplasm of basal cells and lateral intercellular space (*) of FA-treated monkeys. *B*: in contrast, syndecan-4 immunoreactivity was strong in the basal cells and lateral intercellular space (*) of HDMA + O₃-treated monkeys. Weak immunoreactivity was expressed by attenuated fibroblasts in all treatment groups. Bar = 10 μ m.

cell carcinoma and adenocarcinoma) (29), and atherosclerotic blood vessels (32). Also, in a previous study, total proteoglycans were shown to be decreased in rats chronically exposed to ozone (34). In perlecan knockouts, there is BMZ deterioration in blood vessels in areas of mechanical stress (such as the heart), which leads to bleeding and death of the animal (6). These studies demonstrate that perlecan depletion is associated with abnormal development of the BMZ, similar to what we found in monkeys exposed to either ozone or HDMA plus ozone.

FGF-2 is the main growth factor stored in the BMZ (10, 15, 40). FGF-2 is stored in the BMZ through

binding with perlecan, a heparan sulfate proteoglycan that is an intrinsic constituent of the BMZ. FGF-2 is stored in the BMZ of airway and alveolar epithelium, endothelium, and smooth muscle cells in the lungs of developing and adult rats (10, 33, 38). Presumably, it is stored in the BMZ for rapid cellular responses to changes in local environmental conditions, such as leukocyte trafficking or sloughing of columnar epithelium (3, 22, 30, 46). It can be released from perlecan in response to various conditions and become an important cytokine within the local microenvironment of the epithelial-mesenchymal trophic unit (3, 9, 27, 47). The significance of FGF-2 signaling in airway epithelium

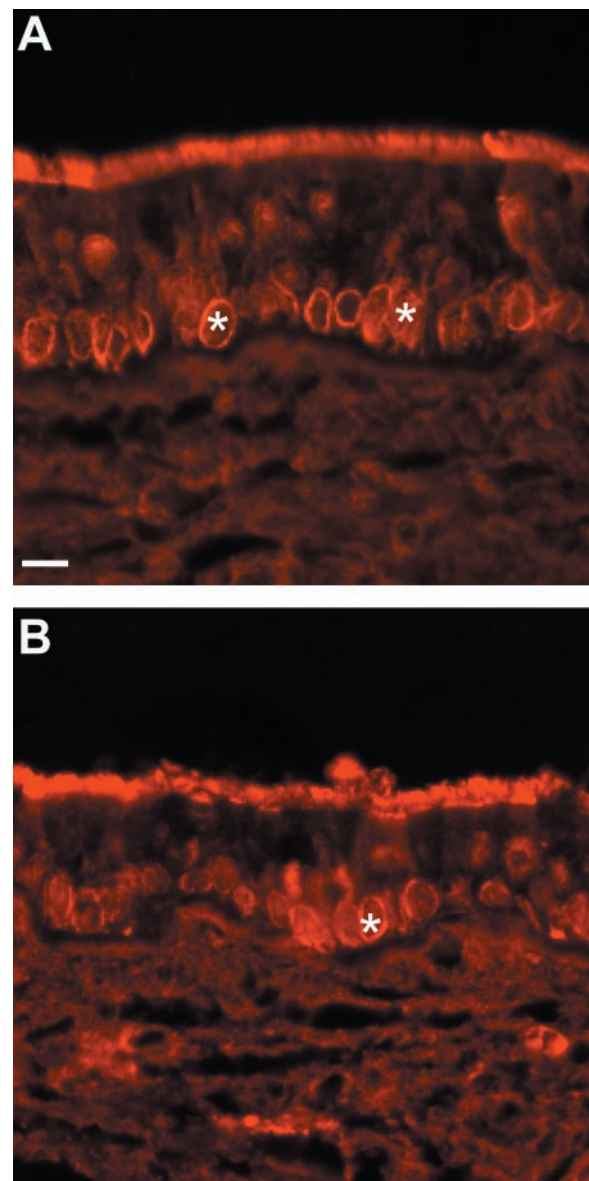


Fig. 8. *A*: strong fibroblast growth factor receptor-1 (FGFR-1) immunoreactivity was expressed on the surface and cytoplasm of basal cells (*) and cilia in FA-treated monkeys. Weak immunoreactivity was expressed in ciliated and goblet cell nuclei and in some basal cell nuclei. *B*: in contrast, FGFR-1 immunoreactivity was reduced to isolated groups of basal cells (*) in HDMA + O₃-treated monkeys. Bar = 10 μ m.

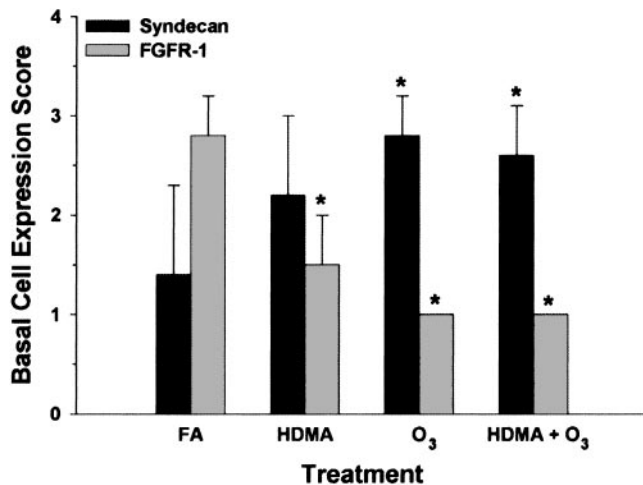


Fig. 9. Graphic representation syndecan-4 and FGFR-1 immunoreactivity expression scores in basal cells. Immunoreactivity for syndecan-4 was inversely related to that of FGFR-1 in the epithelium. There was a significant increase in syndecan-4 scores in the ozone treatment groups. Conversely, there was a significant decrease in FGFR-1 scores. * $P < 0.05$ vs. the FA group.

has not been determined. However, it may be associated with regulation of a number of molecules associated with airway remodeling, e.g., FGFs, epidermal growth factor, endothelin-1, and transforming growth factor- β (17).

In the BMZ depleted of perlecan, FGF-2 was not stored but instead was present around basal cells and lateral intercellular space in the epithelium and attenuated fibroblasts in the extracellular matrix. Here, it may be bound to cell surface proteoglycans, such as glypican or syndecan or FGF receptors. Basal cells express the cell surface proteoglycan, syndecan-4 (29), the most widespread member of the syndecan family (7, 48). Syndecan-4 expression is rapidly upregulated in injured tissues, and shedding of the syndecan-4 ectodomain into the surrounding extracellular matrix is stimulated. These ectodomains in the extracellular matrix exist as soluble regulatory macromolecules in the tissue. Syndecan-4 ectodomains in wound fluids bind to growth factors, proteases, and protease inhibitors (2, 31, 35). The results of the present study suggest that syndecan-4 was upregulated in ozone and HDMA plus ozone groups and possibly shed into the lateral intercellular space. In contrast, FGFR-1 was downregulated. Immunoreactivity for FGF-2 was colocalized with syndecan-4 immunoreactivity and basal cells. These findings suggest that syndecan-4 could act as a regulatory molecule to sequester and control FGF-2 signaling in the absence of perlecan.

In addition to atypical development of the BMZ, lack of BMZ perlecan probably also affected other developmental processes in the airway due to its regulatory role in FGF-2 signaling. FGF-2, an important cytokine in development that is stored in the BMZ through binding with perlecan, can be released from perlecan in response to various conditions and function as an important regulatory cytokine in the epithelial-mesen-

chymal trophic unit. FGF signal through a ternary complex that consists of FGF plus FGFR plus heparan sulfate proteoglycan (30). When the FGF ternary complex is formed, it initiates tyrosine kinase signaling associated with cell proliferation, migration, and differentiation. In airway epithelium, basal cells are the main cell type involved with FGF-2 ternary complex formation and signaling (Fig. 10). Basal cells express the cell surface receptors FGFR-1 (10, 21, 33) and syndecan-4. Syndecan-4, in combination with FGFR-1, has been shown to selectively regulate FGF-2 signaling (18, 43, 50). In the absence of perlecan, syndecan-4 was upregulated and FGFR-1 downregulated. The inverse relationship between these two molecules and the abundance of FGF-2 in the epithelium suggests that signaling through the FGF-2 ternary complex may have been negatively influenced by a lack of BMZ perlecan.

Altered regulation of FGF-2 signaling in animals exposed to ozone may be associated with the abnormal development of airways that has been reported. For example, ozone exposure reduces the postnatal morphogenesis of the gas exchange area (44), impairs bronchiolar formation (10, 49), and retards the differentiation of the mucociliary apparatus of proximal airways (24). Recently, we showed that the combined cyclic inhalation of ozone and HDMA, by HDMA-sensitized infant monkeys, resulted in a marked increase in serum IgE and histamine and airway eosinophilia (42). Furthermore, combined cyclic inhalation of ozone and HDMA resulted in alteration of airway structure that was associated with a significant increase in baseline airway resistance and reactivity. These results indicate that ozone can amplify the allergic and structural remodeling effects of HDMA sensitization and inhalation.

In conclusion, we found that exposure to ozone and HDMA plus ozone depleted the BMZ of perlecan and caused atypical development of the epithelial BMZ. This resulted in altered regulation of FGF-2, FGFR-1,

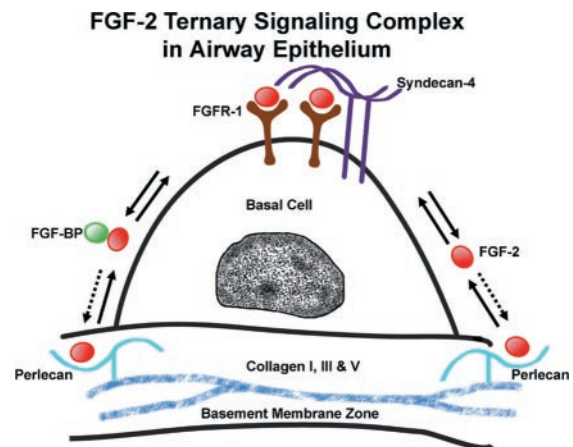


Fig. 10. Illustration of BMZ-bound FGF-2, extracellular signaling via diffusion or FGF-binding protein (FGF-BP), and formation of the FGF-2 ternary complex with basal cells of airway epithelium. [Adapted from Nugent and Iozzo (30).]

and syndecan-4 in the airway epithelial-mesenchymal trophic unit. We suggest that these alterations in FGF-2 regulation may be associated with the atypical development of the lung observed in the rhesus monkey after exposure to O₃ and may constitute an important mechanism that modulates responses to injury.

DISCLOSURES

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