Analyzing the Genes and Peptide Growth Factors Expressed in Lung Cells in Vivo Consequent to Asbestos Exposure and in Vitro

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Inhalation of fibrogenic particles causes injury to the bronchial–alveolar epithelium. Consequently, there is a rapid proliferative response as the epithelium recovers and interstitial mesenchymal cells divide and produce connective tissue. In our model of brief (5-hr) exposure to chrysotile asbestos (~1000 fibers/cc) in rats and mice, these events result in focal scarring at the bronchial–alveolar duct junctions in a histopathologic pattern identical to that seen in asbestos-exposed individuals. After 3 consecutive days of exposure, these lesions persist for at least 6 months postexposure. We postulate that cell proliferation and production of extracellular matrix is mediated in large part by three peptide growth factors, transforming growth factors alpha and beta (TGF-α and β), and platelet-derived growth factor (PDGF) A- and B-chains. To test this hypothesis in part, we have asked whether the genes that code for these growth factor proteins are activated at sites of asbestos-induced lung injury. If these genes were not activated, it would be reasonable to suspect that other potent growth factors and cytokines released during lung injury could be the primary mediators of fibroproliferative lung disease. In the studies reported here, we show, by in situ hybridization (ISH) and immunohistochemistry, that the four genes and their concomitant proteins are expressed within 24 hr in the bronchial–alveolar epithelium and underlying mesenchymal cells. RNase protection assay and ISH showed that the PDGF gene was upregulated during the first 6 hr of exposure and all the gene products remained above control levels for at least 2 weeks postexposure. TGF-α is a potent mitogen for epithelial cells, whereas the PDGF isoforms are potent growth factors for mesenchymal cells. TGF-β retards fibroblast growth but stimulates extracellular matrix synthesis. Further studies using gene knockouts, appropriate antibodies, or antisense technology will be necessary to prove whether any of the growth factors are playing a significant role in fibrogenic lung disease. In addition, we have carried out a series of studies using type II alveolar epithelial cells purified from adult mouse lungs and maintained for up to 8 weeks in serum-free culture. These cells exhibit high transepithelial resistance values and they release TGF-β1 and -β2. This cell type also has been cultured from TGF-α knockout mice, resulting in monolayers with increased transepithelial resistance. This combination of studies in vivo and in vitro will allow us to pursue the mechanisms through which growth factors mediate lung fibrosis. — Environ Health Perspect 105(Suppl 9):1165-1171 (1997)

Key words: asbestos, in situ hybridization, immunohistochemistry, platelet-derived growth factor, transforming growth factors alpha and beta, tumor necrosis factor alpha, alveolar epithelial cell

Introduction

We have been studying the pathogenesis of asbestos-induced lung disease as a paradigm for understanding the biochemical and molecular mechanisms of interstitial pulmonary fibrosis (1,2). If investigators can establish the interacting factors that mediate the fibroproliferative process, it might then be possible to develop effective therapeutic strategies where none now exist. Our approach to this long-standing problem is 3-fold: a) brief exposure (1–5 hr) of normal rats and mice to high concentrations (1000–5000 fibers/cc) of chrysotile asbestos fibers in order to induce a fibrogenic response and follow the temporal and anatomic patterns of selected growth factor genes and proteins; b) exposure of genetically defined and gene knockout mice to the asbestos aerosol in order to focus on the potential roles of specific growth factors; and c) long-term culture of alveolar epithelial cells from normal and genetically defined mice to test our working hypothesis that epithelial-derived growth factors contribute to the fibroproliferative process. Following is a summary of some of our ongoing work in these three approaches to understanding the mechanisms of interstitial pulmonary fibrosis.

Results and Discussion

In Vivo Exposures

Inhaled asbestos fibers that pass through the airways deposit initially at the bronchial–alveolar duct (BAD) regions (1–3). Our previous studies (4,5) and several from other laboratories (6,7) show that the fibers induce proliferation of epithelial and mesenchymal cell populations. These proliferative events can be measured by incorporation of tritiated thymidine (4) or bromodeoxyuridine (6–8) into DNA, or by immunohistochemical staining of proliferating cell nuclear antigen (PCNA) (9). All the techniques show the same results, i.e., low background proliferative rates in the lungs (~1%), and 5–30-fold increases in percentages of dividing cells, depending upon the cell types, proximity to lung injury, and time after exposure. Inasmuch as cell proliferation is essential to repair asbestos-induced injury to the epithelium and to enlarge the interstitial mesenchymal cell population, which produces components of the extracellular matrix, investigators must define which genes and their products are controlling these events. We are attempting to establish whether specific growth factor genes and their proteins are expressed with temporal and anatomic features consistent with a role in the disease process. At the time of this writing, we have positive evidence for the participation of four growth factors in the fibroproliferative process: a) platelet-derived growth factor (PDGF) A- and B-chains, the most potent mesenchymal cell growth factors; b) transforming growth factors alpha and beta; TGF-α, tumor necrosis factor alpha.
factor yet described (10); b) transforming growth factor alpha (TGF-α), a powerful mitogen for epithelial and mesenchymal cells (11); c) TGF-β, a potent inducer of extracellular matrix proteins by mesenchymal cells and, at the same time, a blocker of mesenchymal and epithelial cell proliferation; (12) and (4) tumor necrosis factor alpha (TNF-α), a prominent mitogen for mesenchymal cells (13).

Platelet-derived Growth Factors. Using in situ hybridization (ISH) of the mRNAs coding for both the PDGF A- and B-chain proteins, we showed that the genes are expressed primarily in epithelial cells and macrophages in asbestos-exposed rats. In fact, expression was very rapid, with both Northern analysis and ISH demonstrating a strong signal immediately after a 5-hr exposure to chrysotile asbestos. The message for PDGF remained elevated for several weeks postexposure, but only in the BAD regions where fiber deposition, macrophage accumulation, early epithelial injury, and cell proliferation are manifested. There was no message detected at any time in unexposed rats or in control animals exposed to high concentrations of nonfibrogenic iron spheres.

Figure 1. (A) In situ hybridization for PDGF-B-chain gene expression on asbestos-exposed rat lung 48 hr postexposure. The sense-directed nonisotopic probe [see Liu et al. (11) for technical details] shows no hybridization and only the alveolar duct lesion is seen (arrowheads). (B) The antisense probe demonstrates clear hybridization with epithelial cells of the terminal bronchioles and alveolar ducts (arrowheads) as well as alveolar macrophages (arrows). (C) An antibody against human PDGF-B protein clearly stains the epithelium and interstitium of asbestos-induced fibroproliferative lesions 48 hr after a single 5-hr exposure. Bar = 20 mm.
Immunohistochemistry (IHC) was used to ask if the PDGF A- and B-chain peptides have been released concomitant with the pattern of gene expression. The answer appears at this time to be unequivocally affirmative. Figure 1 shows that the ISH and IHC of PDGF expression coincide and are localized to regions of early lung injury. At the time of this writing, these findings have been submitted for consideration. The preliminary data presented here support our hypothesis that PDGF is playing a role in the fibroproliferative process because of the timing and pattern of expression and the known biological activity of the factor. However, it is very clear that this sort of information is far from proof that PDGF is actually an essential actor in the complex pathogenic process. The same disclaimer can be made for any of the factors for which we have only descriptive information, i.e., they may be along only for the ride, as epiphenomena to the real mediators that have yet to be defined.

Transforming Growth Factor-α. This work has been published (11) and shows a temporal and anatomic distribution similar to that of PDGF except for two interesting differences. First, TGF-α message and protein could not be measured until 24 hr after the 5-hr asbestos exposure. Second, the TGF-α exhibited little interstitial message. Actually, some TGF-α-positive interstitial cells could be counted at 24 hr postexposure, but this was the only time point that significant numbers of stained cells could be measured in this compartment. Clara cells, type II cells, and macrophages exhibited the most predominant IHC reaction (11) (Figure 2).

Transforming Growth Factor-β. We previously published IHC studies on the distribution of TGF-β in the lungs of rats exposed to chrysotile asbestos for 3 hr (14). Lung fibroblasts possess three classes of receptors that bind the three main TGF-β isoforms (12,15). The new findings shown here demonstrate the expression of mRNA for TGF-β1 in the rats exposed to asbestos for 5 hr (Figure 3). Again, the distribution of gene expression follows that of the protein shown by IHC. The major difference is the intensity of the message in alveolar epithelial cells and Clara cells of bronchioles in exposed animals. Alveolar macrophages also showed a clear message by ISH. As with our studies on PDGF and TGF-α, there was no detectable TGF-β signal by IHC or ISH in sham or iron-exposed rats.

Exposure of Defined Mouse Strains
Strain Differences

In the studies we are beginning to execute on genetically defined transgenic and gene knockout mice, it has become apparent that all strains do not respond in a similar fashion to inhaled agents. We exposed four different strains of mice to the same dose of chrysotile asbestos (as shown by lung digestion and fiber counts) and measured a continuum of severity. The C57 strain exhibited the most severe lesions and the 129 strain was essentially protected from the fibrogenic events of a brief asbestos exposure.

Figure 2. Immunohistochemistry of TGF-α distribution shows vivid staining, mainly in epithelial cells of bronchiolar alveolar duct junctions. Bar = 20 mm. Abbreviations: TB, terminal bronchioles; AD, alveolar ducts.

Figure 3. In situ hybridization of the mRNA coding for TGF-β1 is clearly demonstrated after asbestos exposure in the bronchiolar-alveolar duct epithelium (arrowheads) and alveolar macrophages (arrows). Bar = 20 μm. Abbreviations: TB, terminal bronchioles; AD, alveolar ducts.
The SJL and C57 × SJL hybrid strains responded to a degree between that of the C57 and 129 strain, but clearly more like the C57 strains. This finding has raised the important issue of what mechanism(s) protects the 129-strain mice from asbestos-induced lung fibrosis. Could there be different levels of growth factor gene expression or protein production that mediate the disease process? This straightforward question currently is being tested in our laboratory, and preliminary data follow.

Figure 4 shows the dramatic difference in the fibroproliferative lesions that develop at the BAC regions of the C57-strain versus 129-strain mice. The lesions of the C57 strains are the same as those we have measured extensively in rats. The responses in the 129-strain mice were significantly reduced, so much so that an experienced pathologist, blinded as to the identity of the mice, included most of the 129-strain mice in the control (unexposed) category; this never occurred in any C57-strain mice.

Consistent with the hypothesis that a mitogenic factor could be playing a role in this response, we counted the numbers of cells incorporating bromodeoxyuridine (BrdU) during the development of the lesions. The C57 strains, as expected, had the typical approximately 10-fold increases in numbers of BrdU-positive cells, while the 129 strain mice exhibited significantly fewer dividing cells through 2 weeks postexposure. At the time of this writing, IHC staining for TGF-β1 was more prominent in the C57 strain than in the 129-strain mouse. Whether this potentially important finding is due simply to more cells being present in the C57 strain, or is due to an actual increase in synthesis of TGF-β is under rigorous study. Since the macrophages of the exposed 129-strain mice exhibit little evidence of TGF-β staining, it is our current hypothesis that this potent factor may be playing a central role in the developing lesions. The molecular biology studies to test this hypothesis are under way.

Tumor Necrosis Factor-α Receptor Knockout Mice. TNF-α is a potent cytokine with numerous effects on the immune system and on multiple cell types (16). The technology to knock out specific genes of interest has raised new possibilities for developing an understanding of disease processes (17). There are apparently two separate membrane receptors for TNF-α, with molecular weights of 55 and 75 kDa. Mice bred on a genetic background of C57Bl/6 and 129 had the gene coding for these receptors knocked out. The mice appear perfectly normal and live a normal life span. In preliminary studies presented here, we are asking whether the receptors are necessary for the development of early asbestos-induced fibroproliferative lesions described previously. The following results are most provocative and point toward TNF-α as a central factor in mediating the disease process.

As of this writing, one inhalation experiment has been carried out and two essential sets of data have been collected. Four groups of experimental animals were exposed to chrysotile asbestos fibers for 5 hr. Four animals in each group were sacrificed immediately after exposure and at 48 hr postexposure. The animals were injected with BrdU 5 hr before sacrifice to establish the percentages of dividing cells in the developing lesions. The four groups of mice were unexposed wild-type C57Bl/6 strain and three exposed groups, C57Bl/6, an F2 hybrid of a cross between C57Bl/6 and 129 mouse strains upon which the receptor
knockout was made, and C57Bl/6-129 TNF-α-receptor knockouts. In the first analysis, histopathologic review showed that the exposed wild-type and C57 x 129 hybrids exhibited the typical asbestos-induced lesions, just as in the C57 x SJL hybrids reported above. In sharp contrast, the blinded histopathologist could not distinguish the four asbestos-exposed receptor knockout mice from the unexposed controls. Consistent with this finding, levels of BrdU incorporation into dividing cells were approximately 10-fold over normal in the asbestos-exposed C57-strain mice and hybrid mice, whereas the unexposed and knockout animals exhibited normal low levels of proliferation.

Thus, it appears that TNF-α is playing a key role in early fibroproliferative responses to inhaled asbestos. Whether this factor is important in subsequent events and the mechanisms through which TNF-α exerts its influence in the process are the subjects of our ongoing studies. These experiments offer exciting new possibilities in our search for the growth factors that are mediating fibroproliferative lung disease.

Long-term Culture of Alveolar Epithelial Cells

In vitro models of the lung are powerful tools that allow investigators to specifically isolate cause and effect relationships between particle-induced injury, elaboration of growth factors, and cellular responses. For example, in our laboratory we demonstrated that asbestos stimulates the upregulation of PDGF-α receptors on cultured primary rat lung fibroblasts (18) and that asbestos-stimulated macrophages assayed in vitro elaborate elevated levels of PDGF (19). Using more complex in vitro models, we demonstrated that intact rat epithelial monolayers exclude growth factors, whereas injured monolayers allow translocation of PDGF to underlying cocultured fibroblasts, resulting in fibroblast proliferation (20). Numerous other laboratories also have utilized in vitro models to understand how inorganic particles interact with cells. Lesur et al. (21) demonstrated that silica dust directly stimulates DNA synthesis in cultured fetal alveolar epithelial cells, and Walker et al. (22) determined that the proliferation of asbestos-transformed mesothelial cells is at least partially regulated through a TGF-α-mediated autocrine feedback mechanism.

Recently, we added to the rapid progress in this field. We developed an in vitro model of the alveolar epithelium using isolated mouse type II cells. Cells are cultured on a porous membrane support in serum-free medium for up to 8 weeks. It has been demonstrated previously that cultured type II cells organize into continuous monolayers with tight junctions connecting their lateral edges. The integrity of the monolayers and the strength of the junctions within the monolayers can be assessed by measuring electrical resistance across the monolayer. This is achieved by passing low-level currents across the monolayers, then measuring the ability of the monolayer to

Figure 5. TGF-β1 and TGF-β2 production as measured by ELISA by alveolar epithelial cells and alveolar macrophages isolated from C57Bl/6 mice, cultured alone or co-cultured. Data represents means ± SE. *Significantly elevated over media, p < 0.005. **Significantly elevated over AEC alone, p < 0.0001. Abbreviations: AEC, alveolar epithelial cells; AMa, alveolar macrophages.

Figure 6. Particles were added to 14-day mouse alveolar epithelial monolayers. Transepithelial resistances were measured and compared to unexposed control monolayers. Only the highest concentration chrysotile asbestos significantly reduced resistances. Latex had no effect on resistances and iron beads appear to stabilize monolayers, preventing the normal decrease observed in controls. Data represent means ± standard errors, n = 3. *Multiply by 10 for iron concentration. **Latex and asbestos concentration, 1.6 μg/cm²; iron concentration, 16 μg/cm². **Significantly different from unexposed controls, p ≤ 0.05. Significantly different between iron and asbestos, p ≤ 0.05.
block these currents. This is an excellent model system for investigating aspects of the fibroproliferative response including a) maintenance and repair of the barrier integrity of the monolayer after lung injury, b) production and compartmentalization of growth factors by epithelial cells after injury, c) compartmentalization or translocation of particles across the monolayer over time, and d) compartmentalization, migration and cell–cell interactions of macrophages and fibroblasts in co-culture with epithelial cells.

This model represents several major advances over previous models. One major advance is the isolation and culture of highly purified alveolar type II cells from mice (23). Since its original description, substantial improvements have been made with mouse type II cell cultures in our laboratory. To this end, our second major advance is the long-term culture of mouse alveolar epithelial cells (24). Other models of the alveolar epithelium are hampered by rapid differentiation of cultured cells to a type I-like phenotype that leads to rapid attrition of the cells. These monolayers lose their functional barrier integrity within 2 weeks of isolation, restricting usefulness of the cultures in the investigation of chronic diseases such as particle-induced pulmonary fibrosis. Although cultured type II cells from mice also differentiate to type I-like cells, the process is much slower, as determined by morphologic evaluation of the cells by osmium tetroxide, tannic acid staining, and analysis of light and scanning electron micrographs. Gradually the cells appear to differentiate to flattened type I-like cells until, by 8 weeks, 100% of the cells appear differentiated. Monolayers formed by mouse type II cells exhibit transepithelial resistances that are approximately 50% higher than those previously reported for monolayers of rat alveolar epithelial cells (25). This is an average maximum resistance of 4.0 KΩ·cm² across our monolayers. The cultures have been maintained up to 8 weeks, with resistances greater than 2 KΩ·cm² through culture week 5. Furthermore, long-term mouse alveolar epithelial cultures are maintained in serum-free media, allowing researchers to study the specific effects of growth factors and other soluble peptides without the complications of high background levels from serum. For example, a number of molecules upregulate TGF-β, which stimulates matrix production by fibroblasts (12). In our laboratory, Riva et al. (26) demonstrated that TGF-β1 and -β2, quantified by ELISA of cell-conditioned serum-free medium, are endogenously produced in early mouse type II cell cultures (Figure 5), and the production of TGF-β by these cells is stimulated when co-cultured with alveolar macrophages. These data indicate that type II cells may be an important source of TGF-β and that macrophages, which increase in number dramatically at the sites of particle-induced injury (1,2,7), may stimulate the production of TGF-β by type II cells.

One powerful feature of this model is that it allows researchers to use cells isolated from genetically defined strains and transgenic mice. For example, in our laboratory, type II cells from TGF-α knockout mice were cultured in serum-free medium and monitored over 4 weeks. Cells from knockout mice attach more rapidly, are 55% larger, and are proportionately less numerous. In addition, transepithelial resistances are consistently elevated, averaging 40% higher than controls, suggesting a possible role for TGF-α in the increased permeability of the alveolar epithelium observed after lung injury.

Similar systems can be used during in vitro investigation of particle-induced fibroproliferative disorders. For example, particles were added to 14-day mouse alveolar epithelial monolayers after peak transepithelial resistances had formed. Resistances were monitored over the following week and compared to control monolayers (Figure 6). Although latex particles (nonmineral controls) had no effect on resistances, iron beads (mineral controls) appeared to stabilize monolayers relative to unexposed controls, and only the highest concentration of chrysotile asbestos that we used significantly reduced transepithelial resistances. Further experiments are underway to establish the mechanisms through which asbestos increases permeability of alveolar epithelial cells and influences expression of peptide growth factors and cytokines. These new in vitro models of the lung will allow investigators to isolate direct effects of particles on lung cells, to establish the specific functions of growth factors and other peptides during injury and repair, and finally, to determine the roles of these factors in the progression of chronic lung diseases.

REFERENCES

1. Brody AR. Asbestos exposure as a model of inflammation-induced interstitial pulmonary fibrosis. In: Inflammation: Basic Principles and Clinical Correlates (Gallin JI, Goldstein IM, Snyderman R, eds). New York:Raven Press, 1992:1033-1049.
2. Brody AR. Asbestos-induced lung disease. Environ Health Perspect 100:21–30 (1993).
3. Brody AR, Roe MW. Deposition pattern of inorganic particles at the alveolar level in the lungs of rats and mice. Am Rev Respir Dis 128:724–729 (1983).
4. Brody AR, Overby LH. Incorporation of tritiated thymidine by epithelial and interstitial cells in bronchiolar-alveolar regions of asbestos-exposed rats. Am J Pathol 134:133–144 (1989).
5. Brody AR. Asbestos-induced proliferation of bronchial and pulmonary parenchymal cells. In: Asbestos-Related Cancer (Sluyser M, ed). Amsterdam:Ellis Horwood, 1991:191–206.
6. Quinlan TR, BéruBe KA, Marsh JP, Janssen YMw, Taisi P, Leslie KO, Hemenway D, O'Shaughnessy PT, Vacek P, Mossman BT. Patterns of inflammation, cell proliferation, and related gene expression in lung after inhalation of chrysotile asbestos. Am J Pathol 147:729–739 (1995).
7. Coin PG, Osornio-Vargas A, Roggli, VL, Brody AR. Pulmonary fibrogenesis after three consecutive inhalation exposures to chrysotile asbestos. Am J Respir Crit Care Med 154:1511–1519 (1996).
8. Gardner SU, Brody AR. Incorporation of bromodeoxyuridine as a method to quantify cell proliferation in bronchiolar-alveolar duct regions of asbestos-exposed mice. Inhal Toxicol 7:215–224 (1995).
9. Mishra A, Liu J-Y, Brody AR, Morris GF. Inhaled asbestos fibers induce p53 expression in the rat lung. Am J Respir Cell Mol Biol 16:479–485 (1997).
10. Ross R, Raines EW, Bowen-Pope DF. The biology of platelet-derived growth factor. Cell 46:155–169 (1986).
11. Liu J-Y, Morris GF, Lei W-H, Corti M, Brody AR. Up-regulated expression of transforming growth factor-α in the bronchiolar-alveolar duct regions of asbestos-exposed rats. Am J Pathol 149:205–217 (1996).
12. Roberts AB, Anzano MA, Wakefield LM, Roche NS, Stern...
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DF, Sporn MB. Type β transforming growth factor: a bifunctional regulator of cellular growth. Proc Natl Acad Sci USA 82:119–123 (1985).

13. Piguet PF, Ribaux C, Karpuz V, Grau GE, Kapanci Y. Expression and localization of tumor necrosis factor α and its mRNA in idiopathic pulmonary fibrosis. Am J Pathol 143:651–655 (1993).

14. Perdue TD, Brody AR. Distribution of transforming growth factor-β1, fibronectin, and smooth muscle actin in asbestos-induced pulmonary fibrosis in rats. J Histochem Cytochem 42:1–10 (1994).

15. Kalter VG, Brody AR. Receptors for transforming growth factor-β on rat lung fibroblasts have higher affinity or TGF-β1 than for TGF-β2. Am J Respir Cell Mol Biol 4:397–407 (1991).

16. Piguet PF, Collart MA, Grau GE, Sappino AP, Vassalli P. Requirement of tumor necrosis factor for development of silica-induced pulmonary fibrosis. Nature 344:245–247 (1990).

17. Harvey M, Vogel H, Morris D, Bradley A, Bernstein A, Donehower LA. A mutant p53 transgene accelerates tumor development in heterozygous but not nullizygous p53-deficient mice. Nat Genet 9:305–311 (1995).

18. Bonner JC, Goodell AL, Coin PG, Brody AR. Chrysotile asbestos upregulates gene expression and production of alpha-receptors for platelet-derived growth factor (PDGF-AA) on rat lung fibroblasts. J Clin Invest 92:425–430 (1993).

19. Bonner JC, Brody AR. Asbestos-induced alveolar injury: evidence for macrophage-derived PDGF as a mediator of the fibrogenic response. Chest 99 (3):54s–55s (1991).

20. Mangum JB, Everitt JI, Bonner JC, Moore LR, Brody AR. Co-culture of primary pulmonary cells to model alveolar injury and translocation of proteins. In Vitro: Cell Dev Biol 26:1135–1143 (1990).

21. Lesur O, Cantin AM, Tanswell AK, Melloni B, Beaulieu J-F, Bégin R. Silica exposure induces cytotoxicity and proliferative activity of type II pneumocytes. Exp Lung Res 18:173–190 (1992).

22. Walker C, Everitt J, Ferriola PC, Stewart W, Mangum J, Bermudez E. Autocrine growth stimulation by transforming growth factor α in asbestos-transformed rat mesothelial cells. Cancer Res 55:530–536 (1995).

23. Corti M, Brody AR, Harrison JH. Isolation and primary culture of murine alveolar type II cells. Am J Respir Cell Mol Biol 14:309–315 (1996).

24. Corti M, Brody AR. The influences of sera and substrate on transepithelial resistances and morphology of cultured mouse ATII cells. Am J Respir Crit Care Med 153 (4):A510 (1996).

25. Cheek JM, Kim K-J, Crandal ED. Tight monolayers of rat alveolar epithelial cells: bioelectric properties and active sodium transport. Am J Physiol 256:C688–C693 (1989).

26. Riva CM, Brody AR, Corti M. Inhibition of TGFβ1 and stimulation of TGFβ2 production in co-cultures of alveolar type II cells and alveolar macrophages isolated from butylated hydroxytoluene exposed C57B1/6 mice. Am J Respir Crit Care Med 153 (4):A850 (1996).