American Journal of Pathology, Vol. 149, No. 5, November 1996

Commentary

Alveolar Type II Cells at the Crossroad of Inflammation, Fibrogenesis, and Neoplasia

Umberto Saffiotti

From the Laboratory of Experimental Pathology, National Cancer Institute, Bethesda, Maryland.

In a paper in this issue of the *Journal*, Driscoll and coworkers[1] report that α-quartz dust particles instilled in rat lungs induced expression of macrophage inflammatory protein-2 (MIP-2) mRNA in epithelial cells of the terminal bronchioles and alveolar ducts as well as in alveolar type II cells and macrophages. MIP-2 is a chemokine of the C-X-C group known for its neutrophil recruiting activity. To determine whether quartz particles can induce this effect directly in target epithelial cells, the authors used cell culture systems. They report that when treated with quartz *in vitro*, rat alveolar type II cells in primary culture and the RLE-6TN cell line of alveolar type II origin (previously established by Driscoll et al[2]) showed increased mRNA expression of MIP-2 and of the related neutrophil recruiting chemokine, cytokine-induced neutrophil chemoattractant, but not of MIP-1α. These findings were confirmed by the observed neutrophil recruitment by quartz-treated cells and its inhibition by rabbit antimurine MIP-2 antiserum. Increased chemokine expression in RLE-6TN cells was also induced by crocidolite fibers, but not by MMVF-10 glass fibers nor by nontoxic anatase particles. As the authors point out, for the first time their results indicate that an inflammatory agent induces a chemokine response in the peripheral lung epithelium and that quartz, TNF-α, and endotoxin can induce chemokine responses by direct action on alveolar epithelial cells in culture.

These novel observations contribute to our growing awareness of the important multiple roles of peripheral lung epithelia and especially of the alveolar type II cells. The role of type II cells as stem cells for the alveolar epithelium and as the site of production of pulmonary surfactant has been well documented for many years,[3] but recent studies revealed an increasing number of other functions for these cells that bring them to the forefront of pulmonary pathology mechanisms.

Alveolar type II cells, in the rat model, are being recognized as key participants in the mechanisms of inflammatory reactions and fibrogenesis, as is exemplified by their reactions to quartz particles in the pathogenesis of silicosis. They are also identified as the cells of origin of many peripheral lung adenocarcinomas, including those induced by quartz and associated with inflammation and fibrosis. Alveolar type II cells appear therefore at the crossroad of inflammation, fibrogenesis, and carcinogenesis, and invite new studies of molecular mechanisms linking these basic pathological reactions.

Fibrogenesis develops progressively following quartz exposure, probably as a response to complex pathways. An active role of alveolar type II cells in fibrogenesis, mediated by production and secretion of transforming growth factor-β1 (TGF-β1), was indicated by recent studies in the rat silicosis model in our laboratory.[4-6] Antibodies to TGF-β1/latency associated peptide, which mark the site of production of TGF-β1, were intensely localized in alveolar type II cells adjacent to silicotic granulomas, whereas antibodies to mature TGF-β1 were localized in the fibroblasts of the granulomas and on the collagenous stroma.

As a cellular model, we used the FRLE cell line derived from fetal rat lung alveolar cells,[7] which shows epithelial morphology and localization of surfactant protein C by immunogold electron-microscopy.[8] Haralson and coworkers demonstrated that this cell line produces extracellular matrix, including col-

Accepted for publication September 9, 1996.

Address reprint requests to Dr. Umberto Saffiotti, Laboratory of Experimental Pathology, National Cancer Institute, Building 41, Room C-105, Bethesda, MD 20892-5055.

lagen types I, III, IV, and V,[7] and that collagen biosynthesis in these cells can be stimulated by retinoic acid and epidermal growth factor[9] and by TGF-β1.[10] Work in our laboratory showed that FRLE cells express mRNA for TGF-β1, TGF-β2, and TGF-β receptor type II and are weakly immunostained by antibodies to TGF-β1/latency associated peptide.[8] Exposure to quartz increased the secretion rate of TGF-β1 in these cells.[5] Because TGF-β1 is a multifunctional peptide capable of stimulating collagen formation,[10–12] TGF-β1 production by alveolar type II cells and its enhancement by quartz further elucidate the role of these cells in pulmonary fibrogenesis.

Hahon and Castranova[4] reported that rat alveolar type II cells were active producers of interferon in response to a viral inducer, which suggests their importance for antiviral activity, stimulation of phagocytosis, and modulation of immune responses. Martinez et al[5] recently showed by immunohistochemistry and *in situ* hybridization that hyperplastic alveolar type II cells in the proximity of human lung carcinomas have intense expression of peptidyl-glycine α-amidating monooxygenase, an enzyme necessary for the biosynthesis of active amidated peptide hormones and possibly involved in mitogenesis and carcinogenesis.

Because of the role of alveolar type II cells as the cells of origin of peripheral lung adenocarcinomas, the mechanisms of carcinogenesis involving this cell type are of great interest. The relative frequencies of different histological types of lung cancer in the general population in the United States have shifted significantly in recent decades, with a relative decline in the frequency of bronchogenic squamous cell carcinomas and an increasingly higher proportion of adenocarcinomas, which have now become the prevalent histological type.[15] The major external etiological factor for lung cancer is cigarette smoke. It has been suggested that the shift in the histological types of lung cancer may have been caused by the substantial lowering of tar levels in the smoke of the cigarette types prevalently smoked in the United States, which probably mostly affected squamous cell carcinogenesis in the larger airways. The smoke of current cigarette types still contains carcinogenic tobacco-specific *N*-nitrosamines, and other agents (including particulates) that may be more prone to induce peripheral lung adenocarcinomas. Other lung carcinogens include radiation and certain occupational exposures to organic materials, metals, asbestos fibers, and quartz dust. They may combine their effects with cigarette smoke and with each other. Some synergisms have been demonstrated, but much remains to be done to clarify the complex etiological mechanisms of lung cancers. In the case of quartz, the second most common mineral in the Earth's crust, exposure is ubiquitous and quartz particles are present in the lungs of the general population[16]; this is a condition that should be considered in evaluating epidemiological evidence for silicosis-associated lung cancer, because there is no unexposed control population. As we learn more about the biological activities induced by quartz particles at the cellular and molecular level, we wonder whether the ubiquitous quartz particles in peripheral lung tissues of the general population may not exert subtle changes that affect the pathogenesis of lung diseases.

The interest in the pathogenetic activity of crystalline silicas has widened to include their carcinogenic mechanisms. Since 1984, experimental and epidemiological evidence has indicated the carcinogenicity of quartz particles in the lung and the association of silicosis with lung cancer.[17–20] The main targets of quartz-induced lung carcinogenesis in rats (the choice experimental animal) are the alveolar type II cells, which show a progressive development of hyperplasia, adenomatoid hyperplasia, adenomas, and carcinomas with a prevalence of adenocarcinomas, many of which are closely associated with silicotic fibrosis.[20]

The cells of origin of human adenocarcinomas are not defined in many cases. There are clearly alveologenic carcinomas, but others have bronchiolar or bronchial origin. TenHave-Opbroek and coworkers[21] have shown the presence of cells with features of alveolar type II cells (including surfactant protein SP-A) in carcinomas arising from bronchioles and bronchi in a canine carcinogenesis model, where these cells are localized in areas with bronchioloalveolar morphology and in the basal epithelial layer of the tumors, which suggests a stem cell role for these cells in bronchial and bronchiolar neoplasms.

Considering the role of alveolar type II cells as progenitor cells that replace injured type I cells in the alveolar lining (the body's widest barrier with the external environment), it is interesting that reciprocal epithelial-fibroblast interactions have been detected in the repair process of alveolar epithelium and in the onset of alveolar type II hyperplasia[22] and corresponding culture models.[23] We can understand that type II cells evolved as cells ready for reparative proliferation and that external factors reaching the alveoli may inflict genetic damage to proliferating type II cells leading to their neoplastic transformation.

To elucidate the biological activities and molecular mechanisms of alveolar type II cells, adequate cell culture models are needed. Work on these cells has been done mostly in primary cultures, because these cells usually do not survive repeated passage. To obtain continuous growth and preservation of rat alveolar type II cells, corresponding to the *in vivo* model, a few investigators have established cell lines by spontaneous immortalization and clonal selection of alveolar cells from adult rats[2,24,25] or from fetal rats[7]; others have induced immortalization in newborn rat lung alveolar type II cells by transfection with the oncogene adenovirus 12SE1A.[26] Pilot studies in our laboratory use alveolar cells from young adult rats transfected with either SV40-T antigen or with an adenovirus E1a gene (U. Saffiotti and N. Ahmed, unpublished results). Alveolar type II-derived cell lines are needed to study their neoplastic transformation mechanisms by direct exposure to carcinogens under the control of molecular pathways corresponding to their multiple functions. The transforming activity of quartz, which was reported in nonepithelial cell systems such as the Syrian hamster embryo cells[27] and the mouse embryo BALB/3T3/A31-1-1 cell line,[28] was recently also observed in the FRLE cell line by *in vitro* exposure to quartz at a highly toxic dose[8] and at lower, less toxic doses (Y. Mao and U. Saffiotti, unpublished observations). The cell lines developed by Driscoll et al[2] could prove useful models for such investigations.

Whereas quartz induced both pulmonary fibrosis and adjacent carcinogenesis in rats, it failed to elicit a fibrogenic response in hamster lungs in which quartz particles were phagocytosed and stored in macrophages with no further fibrogenic reaction and neither epithelial hyperplasia nor neoplasia.[20] In contrast, Williams and Knapton[29] in our laboratory have recently shown that hamster livers develop marked fibrosis and cirrhosis when treated with quartz, which implicates organ-specific rather than species-specific factors as determinants of the cellular reaction.

The role of signaling molecules and the corresponding gene expression are becoming critical markers for many cell types. Of particular concern in the present context is the possible interplay of the different biological activities that are being discovered in alveolar type II cells. It is hoped that the cellular models for type II cells will make it possible to elucidate the molecular mechanisms responsible for the role of alveolar type II cells in inflammatory, fibrogenic, and carcinogenic pathways and their relationships.

References

1. Driscoll KE, Howard BW, Carter JM, Asquith T, Johnston C, Detilleux P, Kunkel SL, Isfort RJ: α Quartz-induced chemokine expression by rat lung epithelial cells: effects of *in vivo* and *in vitro* particle exposure. Am J Pathol 1996, 149:1627–1638
2. Driscoll KE, Iype PT, Kumari HL, Carter JM, Aardema MJ, Crosby LL, Isfort RJ, Cody D, Chestnut MH, LeBeuf RA: Establishment of immortalized alveolar type II cell lines from adult rat lungs. *In Vitro* Cell & Devel Biol 1995, 31:516–527
3. Mason RJ, Williams MC: Alveolar type II cells. The Lung: Scientific Foundations. Edited by RG Crystal, JB West, PJ Barnes, NS Cherniack, and ER Weibel. New York, Raven Press, 1991, pp 235–246
4. Williams AO, Flanders KC, Saffiotti U: Immunohistochemical localization of transforming growth factor-β1 in rats with experimental silicosis and alveolar type II epithelial hyperplasia and lung cancer. Am J Pathol 1993, 142:1831–1840
5. Williams AO, Knapton AD, Saffiotti U: Growth factors and gene expression in silica-induced fibrogenesis and carcinogenesis. Appl Occup Environ Hyg 1995, 10:1089–1098
6. Williams AO, Saffiotti U: Transforming growth factor β1, ras and p53 in silica-induced fibrogenesis and carcinogenesis. Scand J Work Environ Health 1995, 21(Suppl 2):30–34
7. Leheup BP, Federspiel SJ, Guerry-Force ML, Wetherall NT, Commers PA, DiMari SJ, Haralson MA: Extracellular matrix biosynthesis by cultured fetal rat lung epithelial cells. I. Characterization of the clone and the major genetic types of collagen produced. Lab Invest 1989, 60:791–807
8. Williams AO, Knapton AD, Ifon ET, Saffiotti U: Transforming growth factor β expression and transformation of rat lung epithelial cells by crystalline silica (quartz). Int J Cancer 1996, 65:639–649
9. Federspiel SJ, DiMari SJ, Guerry-Force ML, Haralson MA: Extracellular matrix biosynthesis by cultured fetal rat lung epithelial cells. II. Effects of acute exposure to epidermal growth factor and retinoic acid on collagen biosynthesis. Lab Invest 1990, 63:455–466
10. DiMari SJ, Howe AM, Haralson MA: Effects of transforming growth factor-β on collagen synthesis by fetal rat lung epithelial cells. Am J Respir Cell Mol Biol 1991, 4:455–462
11. Roberts AB, Sporn MB: 26: The transforming growth factor-β's. Peptide Growth Factors and Their Receptors. Edited by MB Sporn and AB Roberts. New York, Springer-Verlag, 1990, pp 419–472
12. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci AS: Transforming growth factor type β: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci USA 1986, 83:4167–4171

13. Hahon N, Castranova V: Interferon production in rat type II pneumocytes and alveolar macrophages. Exp Lung Res 1989, 15:429–445
14. Martinez A, Treston AM, Saldise L, Montuenga LM, Linnoila RI: Expression of peptidyl-glycine α-amidating mono-oxygenase (PAM) enzymes in morphological abnormalities adjacent to pulmonary tumors. Am J Pathol 1996 (in press)
15. Travis WD, Travis LB, Devesa SS: Lung cancer. Histology of Cancer Incidence and Prognosis: SEER Population-Based Data, 1973–1987. Edited by CP Percy, JL Young Jr, C Muir, L Ries, B Hankey, LH Sobin, and JW Berg. Cancer 1995, 75(Suppl 1):191–202
16. Churg A, Wright JT, Stevens B, Wiggs B: Mineral particles in the human bronchial mucosa and lung parenchyma. II. Cigarette smokers without emphysema. Exp Lung Res 1992, 18:687–714
17. Goldsmith DF, Winn DM, Shy CM (Eds): Silica, Silicosis, and Cancer. New York, Praeger, 1986
18. Goldsmith DF: Silica exposure and pulmonary cancer. Epidemiology of Lung Cancer. Edited by JM Samet. Marcel Dekker, New York, 1994, pp 245–298
19. International Agency for Research on Cancer: Silica. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon, International Agency for Research on Cancer 1987, 42:39–143
20. Saffiotti U, Williams AO, Daniel LN, Kaighn ME, Mao Y, Shi X: Carcinogenesis by crystalline silica: animal, cellular, and molecular studies. Silica and Silica-Induced Diseases. Edited by V. Castranova, V. Vallyathan, and WE Wallace. CRC Press, Boca Raton, 1996, pp 345–381
21. TenHave-Opbroek AAW, Hammond WG, Benfield JR, Teplitz RL, Dijkman JH: Expression of alveolar type II cell markers in acinar adenocarcinomas and adenoid cystic carcinomas arising from segmental bronchi. A study in a heterotopic bronchogenic carcinoma model in dogs. Am J Pathol 1993, 142:1251–1264
22. Adamson IYR, Hedgecock C, Bowden DH: Epithelial cell-fibroblast interactions in lung injury and repair. Am J Pathol 1990, 137:385–392
23. Adamson IYR, Young L, King GM: Reciprocal epithelial: fibroblast interactions in the control of fetal and adult rat lung cells in culture. Exp Lung Res 1991, 17:821–835
24. Douglas WHJ, Kaighn ME: Clonal isolation of differentiated rat lung cells. In Vitro 1974, 10:230–242
25. Li AP, Hahn FF, Zamora PO, Shimizu RW, Henderson RF, Brooks AL, Richards R: Characterization of a lung epithelial cell strain with potential applications in toxicological studies. Toxicology 1983, 27:257–272
26. Steele MP, Levine RA, Joyce-Brady M, Brody JS: A rat alveolar type II cell line developed by adenovirus 12SE1A gene transfer. Am J Respir Cell Mol Biol 1992, 6:50–56
27. Hesterberg TW, Barrett JC: Dependence of asbestos- and mineral dust-induced transformation of mammalian cells in culture on fiber dimension. Cancer Res 1984, 44:2170–2180
28. Saffiotti U, Ahmed N: Neoplastic transformation by quartz in the BALB/3T3/A31–1-1 cell line and the effects of associated minerals. Teratogen Carcinog Mutagen, 1995, 15:339–356
29. Williams AO, Knapton AD: Hepatic silicosis, cirrhosis, and liver tumors in mice and hamsters: Studies of transforming growth factor β expression. Hepatology 1996, 23:1268–1275.