Contributions of Retinoids to the Generation and Repair of the Pulmonary Alveolus*

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COPD is associated with a progressive, irreversible decline in pulmonary function. This is caused in large part by emphysema, the ongoing inflammatory destruction of the alveoli and increased airspace size, loss of lung elastic recoil, and hyperinflation. The discovery of novel drugs that can reduce the rate of lung destruction and airflow limitation, or even stop or reverse the underlying processes remains a “holy grail” for the therapy of this disorder. Recent reports that retinoic acid markedly ameliorates the emphysema induced in rats by administration of elastase have sparked considerable interest in retinoids and other alveolar morphogens as potential therapeutic agents. This review focuses on mechanisms involved in mammalian alveolar formation, which occurs mainly after birth. Roles of endogenous retinoids on pulmonary structural cell (epithelial and mesenchymal cells) differentiation and lung development are discussed. Also discussed are lung development and structural studies in mice that are genetically altered with respect to the expression of subtypes of retinoid receptors. Effects of retinoids and other growth factors on elastin gene expression and alveolar formation, and the potential pitfalls of drugs that interfere with these processes as therapeutic agents are addressed.

(CHEST 2002; 121:206S–208S)

Key words: alveolar development; branching; emphysema; fibroblast; growth factors; lung development; retinoic acid

Abbreviations: ATRA = all-trans retinoic acid; LIF = lipid-laden interstitial fibroblast; RA = retinoic acid; RAR = retinoic acid receptor; RXR = retinoid X-receptor

During the last 4 years, there has been a renewed interest in strategies directed at the restoration of the alveolar surface area and the respiratory and mechanical function of the lung parenchyma. Retinoids have received considerable attention as alveolar morphogens and potential therapeutic agents, after retinoic acid was shown to ameliorate the emphysema that was observed in rats after an intratracheal instillation of elastase.1 To better define the potential contributions of retinoids, I will summarize the work in my laboratory and by other investigators that defines how retinoids alter alveolar structure and the expression of a few selected gene products.

Alveolar architecture is dependent on the anatomy of the conducting airways that are primarily formed prior to birth in mice, rats, and humans. Airway branching, elongation, and cellular differentiation are all influenced by retinoids. However, this discussion will be limited to the process of alveolar formation that occurs largely after birth in these species. Alveolar septa are comprised of epithelial and endothelial cells, fibroblasts, and a few immune and neuroendocrine cells.2 The effects of retinoids have been most extensively characterized for pulmonary epithelial and mesenchymal cells. In the alveolus, the fibroblast is a major contributor to the formation of the extracellular matrix, which provides tensile strength and elasticity to the gas exchange surface. Two populations of pulmonary interstitial fibroblast have been identified morphologically, but they share many similar protein synthetic characteristics.3 The most obvious morphologic difference is the abundant lipid droplets in the lipid-laden interstitial fibroblast (LIF) subpopulation during early postnatal life, which are particularly abundant in mice and rats. During the second postnatal week, they may comprise up to 25% of alveolar cells. The lipids are primarily neutral lipids that are acquired, much like adipocytes, but they also contain retinyl esters. Retinyl esters are most abundant during late gestation in the rat, and their content decreases after birth.4 This decrease in endogenous LIF retinyl esters is accompanied by an increase in the retinol and retinoic acid (RA) contents of these cells. We hypothesized that the endogenous LIF retinoids may influence the expression of genes in these cells and influence their ability to produce extracellular matrix proteins. To evaluate this hypothesis, we have used several strategies. First we have examined the effects of exogenous all-trans RA (ATRA) and 9-cis-RA on gene expression by cultured LIF. Second we have interrupted the conversion of endogenous retinyl esters to RA and examined changes in the expression of elastin and collagen. Third, we studied mice with null deletions for various RA receptors (RAR) and retinoid X-receptors (RXR), and evaluated changes in alveolar architecture and gene expression.

Retinoids Influence Elastin Gene Expression in Cultured LIF and Lung Explants

Retinoids are chemical derivatives of all-trans retinol, commonly known as vitamin A. Vitamin A is acquired from the diet primarily as retinyl esters, linked to fatty acids such as palmitic acid, or as carotenoids that are dimers of retinal (the aldehyde oxidation product of retinol) iso-

COPD Symposium: Into the New Millennium

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to late gestational and early postnatal life (up to postnatal day 21 in the rat and 7 years in humans). The RA-mediated increase in tropoelastin messenger RNA results from an approximately twofold increase in elastin gene transcription, rather than an increase in tropoelastin messenger RNA stability. Retinoid-responsive elements within the first 2 kilobases upstream of the transcriptional start site are required for this effect, and multiple elements may be involved.

During the last 3 days of prenatal life, rat lungs contain an abundant supply of retinyl esters, which is equal in amount to that in the liver, the major reservoir of retinyl esters in the adult. Dietethylumbelliferyl phosphate inhibits retinyl ester hydrolysis in organ cultures of lungs obtained from fetal rats, whose mothers are given a vitamin A-sufficient diet. This results in a decrease in elastin, but not collagen gene expression. Similar decrements in elastin gene expression are observed after the addition of citral, an inhibitor of retinoldehyde oxidation to RA. When cultured LIF were supplemented with retinol, elastin gene expression was increased, in a fashion similar to that observed after ATRA supplementation. These data indicate that endogenous retinoids increase elastin gene expression during late gestation in the rat.

**Retinoids Influence Alveolar Formation In Vivo**

Since interrupting the flux of retinyl esters to RA reduced elastin gene expression in lung explants, we posited that a similar process may occur in vivo. To address this hypothesis, we studied mice bearing specific RAR and RXR gene deletions. Studies using cultured LIF indicate that the levels of RAR-β and RAR-γ increase at birth, at the same time that these cells contain their maximal amount of RA. The increase in RAR-γ was transient, decreasing after postnatal day 2 (when endogenous LIF RA also decreases), suggesting that RA may signal transiently through RAR-γ to influence cellular function in the early postnatal period. To examine this issue further, we studied mice bearing a null deletion for the RAR-γ gene. Because RARs heterodimerize with RXRs prior to the binding of the heterodimeric complex to DNA, we also examined mice heterozygous for the RXR-α gene deletion. Homozygous, null RXR-α mice die in utero from cardiac malformations. Mice bearing the null deletion of RAR-γ and heterozygote for the RXR-α deletion (RAR-γ−/−, RXR-α+/−) demonstrate a decrease in elastin gene expression in the LIF at postnatal day 10 and a decrease in elastic tissue in the lung at postnatal day 28. The decrement in elastic fibers was limited to the alveolar septa and did not occur in the airway or vascular walls. Morphometric analysis of alveolar architecture demonstrated that the compound deletion of RAR-γ and RXR-α resulted in approximately twofold fewer alveoli and a similar decrease in alveolar surface area. Mice bearing only the RAR-γ gene deletion demonstrated a decrease in alveolar number and surface area that was intermediate between that of the wild-type and the RAR-γ−/−, RXR-α−/− mice. This decrement was accompanied by changes in the physiologic properties of the distal lungs of these mice. The static compliance of the lung was increased by the RAR-γ−/− gene deletion. Preliminary data indicate that the decrease in alveolar surface area is accompanied by a decrease in the diffusion of carbon monoxide in mice bearing the RAR-γ gene deletion. In summary, RAR-γ−/− mice demonstrate biochemical, anatomic and physiologic characteristics of emphysema at postnatal week 4. The time course of the development of the emphysematous phenotype is similar to that observed in the tight-skinned mouse, another genetic model of congenital emphysema resulting from a duplication within the coding region of the fibrillin-1 gene. These data indicate that retinoids are involved in alveolar formation and that elastin is one of the target genes.

**Future Considerations for Evaluating a Role for Treatment of Emphysema With Retinoids**

While elastin gene expression is required for alveolar formation, our data do not establish this as the only or the primary source of the abnormalities observed by interrupting RA-mediated signaling during alveolar development. It is likely that other genes are also influenced by retinoids, and alterations of elastin expression may be secondary to a more generalized effect on cellular proliferation or differentiation. Among the other genes that may be targets of retinoid action may secondarily alter elastin gene expression are the growth factors, transforming growth factor-β and platelet-derived growth factor-α and its receptor. Other potential targets are the nuclear receptors for homeodomain proteins, such as Hox B5 and GATA family members such as GATA-4, GATA-5, and GATA-6. Our future goals include identifying better markers of the pulmonary myofibroblast phenotype, identifying mechanisms that regulate LIF proliferation, migration, and differentiation, and to evaluate the interstitial myofibroblast phenotype during emphysema.

Other important questions that must be addressed to establish the clinical utility of retinoids in the treatment of emphysema include the following: (1) can selective retinoids be targeted to specific cellular events to achieve the desired effects without toxicity, and (2) how can the duration of administration of potentially deleterious compounds be limited in a slowly progressive disease?

**References**


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