Keratinocyte Growth Factor Is a Growth Factor for Type II Pneumocytes In Vivo

Thomas R. Ulich,* Eunhee S. Yi,* Ken Longmuir,‡ Songmei Yin,* Rebecca Biltz,§ Charles F. Morris,§ Regina M. Housley,§ and Glenn F. Pierce§

**Department of Pathology, University of California, San Diego School of Medicine, San Diego, California 92013; ‡Department of Physiology, University of California, Irvine School of Medicine, Irvine, California 92717; and §Departments of Molecular Biology, Laboratory Animal Resources, and Experimental Pathology, Amgen, Inc., Thousand Oaks, California 91320*

Abstract

Keratinocyte growth factor (KGF) administered as a single intratracheal injection causes a prominent dose-dependent proliferation of type II alveolar epithelial cells in the lungs of adult rats. The increase in mitotically active alveolar cells histologically appears as a micropapillary epithelial cell hyperplasia after 2 d and peaks after 3 d in the form of monolayers of cuboidal epithelial cells lining alveolar septae. Proliferating cell nuclear antigen immunohistochemistry confirmed the profound proliferative response induced by KGF. The hyperplastic alveolar lining cells contain immunoreactive surfactant protein B and are ultrastructurally noted to contain lamellar inclusions characteristic of surfactant-producing type II pneumocytes. Mild focal bronchiolar epithelial hyperplasia is noted but is much less striking than the proliferation of type II pneumocytes. Large airways are unaffected by KGF. Daily intravenous injection of KGF is also able to cause pneumocyte proliferation. The normal adult rat lung constitutively expresses both KGF and KGF receptor mRNA, suggesting that endogenous KGF may be implicated in the paracrine regulation of the growth of pneumocytes. In conclusion, KGF rapidly and specifically induces proliferation and differentiation of type II pneumocytes in the normal adult lung. (*J. Clin. Invest.* 1994. 93:1298–1306.)

Key words: keratinocyte growth factor • type II pneumocytes • lung

Introduction

The alveolar epithelial lining of the lung is composed of type I and II pneumocytes. Type I pneumocytes are flat metabolically relatively inactive cells that cover > 95% of the alveolar air space with a thin layer of cytoplasm. Type I pneumocytes are therefore suited to the function of oxygen exchange between air and the blood of the alveolar capillary bed. Type II pneumocytes are cuboidal metabolically active cells that are also known as the corner cells of the alveolus because of their characteristic location in the corners of alveoli in healthy lungs. Type II pneumocytes synthesize and secrete surfactant. Surfactant is composed of lipids, proteins, and carbohydrates, and stabilizes the patency of the alveolar space by decreasing the surface tension of the alveolus. Type II pneumocytes are ultrastructurally seen to contain lamellar inclusions that represent intracellular surfactant. After lung injury, type II pneumocytes proliferate and eventually can come to line the alveolar septae as rows of columnar cells. During the course of healing, type II pneumocytes differentiate into type I pneumocytes to allow the reconstitution of normal alveolar parenchymal architecture. Type II pneumocyte hyperplasia persists during chronic pneumonitis.

A number of defined growth factors as well as undefined activities in bronchoalveolar lavage fluid and in conditioned media from cultured cells have been observed to stimulate DNA synthesis in type II pneumocytes in vitro (1, 2). However, DNA synthesis as measured by [^{3}H]thymidine uptake in vitro in primary type II pneumocyte cultures is not generally accompanied by a proportionate increase in cell number (1, 3). Several members of the fibroblast growth factor (FGF)[1] family, including acidic and basic FGF, stimulate DNA synthesis in type II pneumocytes in vitro (1–3).

Type II pneumocytes are prominent in fetal lungs and most likely play an important role in the morphogenesis of the lungs as well as in the production of surfactant necessary for the successful transition of the alveolar space from an environment of amniotic fluid to air (1). Glucocorticoids, estrogen, thyroid hormones, bombesin, and epidermal growth factor may enhance the maturation of fetal lung and promote fetal surfactant production in vivo, whereas androgens, transforming growth factor beta, and insulin may inhibit fetal lung maturation (1, 4).

Type II pneumocyte hyperplasia and hypertrophy in the adult can be induced in vivo in a large number of experimental models of lung injury. Exposure to oxygen, ozone, silica, and asbestos, for example, cause type II pneumocyte hyperplasia accompanied by an increase in type II pneumocyte size and lamellar body content (1, 5). The endogenous mediators that initiate and regulate type II pneumocyte proliferation in vivo in the adult lung remain unknown.

The purpose of this study is to report that the intratracheal administration of keratinocyte growth factor (KGF) causes a striking proliferation of type II pneumocytes in vivo. KGF, also

Address correspondence to Dr. Thomas R. Ulich, Amgen Inc., 1840 DeHavilland Drive, Thousand Oaks, CA 91320.

Received for publication 17 June 1993 and in revised form 9 December 1993.

J. Clin. Invest.

0021-9738/94/03/1298/09 $2.00
Volume 93, March 1994, 1298–1306

1. *Abbreviations used in this paper:* FGF, fibroblast growth factor; KGF, keratinocyte growth factor; PCNA, proliferating cell nuclear antigen.

known as FGF-7, is an 18.9-kD member of the FGF family that was originally purified from a lung fibroblast line and, unlike other FGF molecules that can also stimulate mesenchymal cells, appears to be a specific epithelial growth factor secreted by stromal cells to act in a paracrine fashion (6). KGF has recently been reported by Panos et al. (7) to be a growth factor for alveolar type II cells in vitro. Although a number of growth factors are known to promote the proliferation of cultured pneumocytes in vitro and can be considered as putative growth factors for pneumocytes in vivo, we are unaware of any reported growth factors that cause significant type II pneumocyte hyperplasia in vivo in adult animals.

Methods

Lewis rats, male, weighing ~ 250 g (n = 29), received a single intratracheal injection, as previously described by our laboratory (8), of varying doses (5 mg/kg with the exception of dose-response experiments) of recombinant human KGF. The KGF was purified to homogeneity, is endotoxin free, is 94% homologous to rat KGF (9), and stimulates human and rodent keratinocytes equally well. At 6 h and at 1–6 d after intratracheal injection the rats were killed. Control rats (n = 12) received an intratracheal injection of saline. Additional Lewis rats (n = 18) were killed up to 1 wk after daily intravenous or intraperitoneal injections of 5 mg/kg KGF. The lungs were inflated with Bouin's fixative via an intratracheal catheter, saggital sections of the lung were

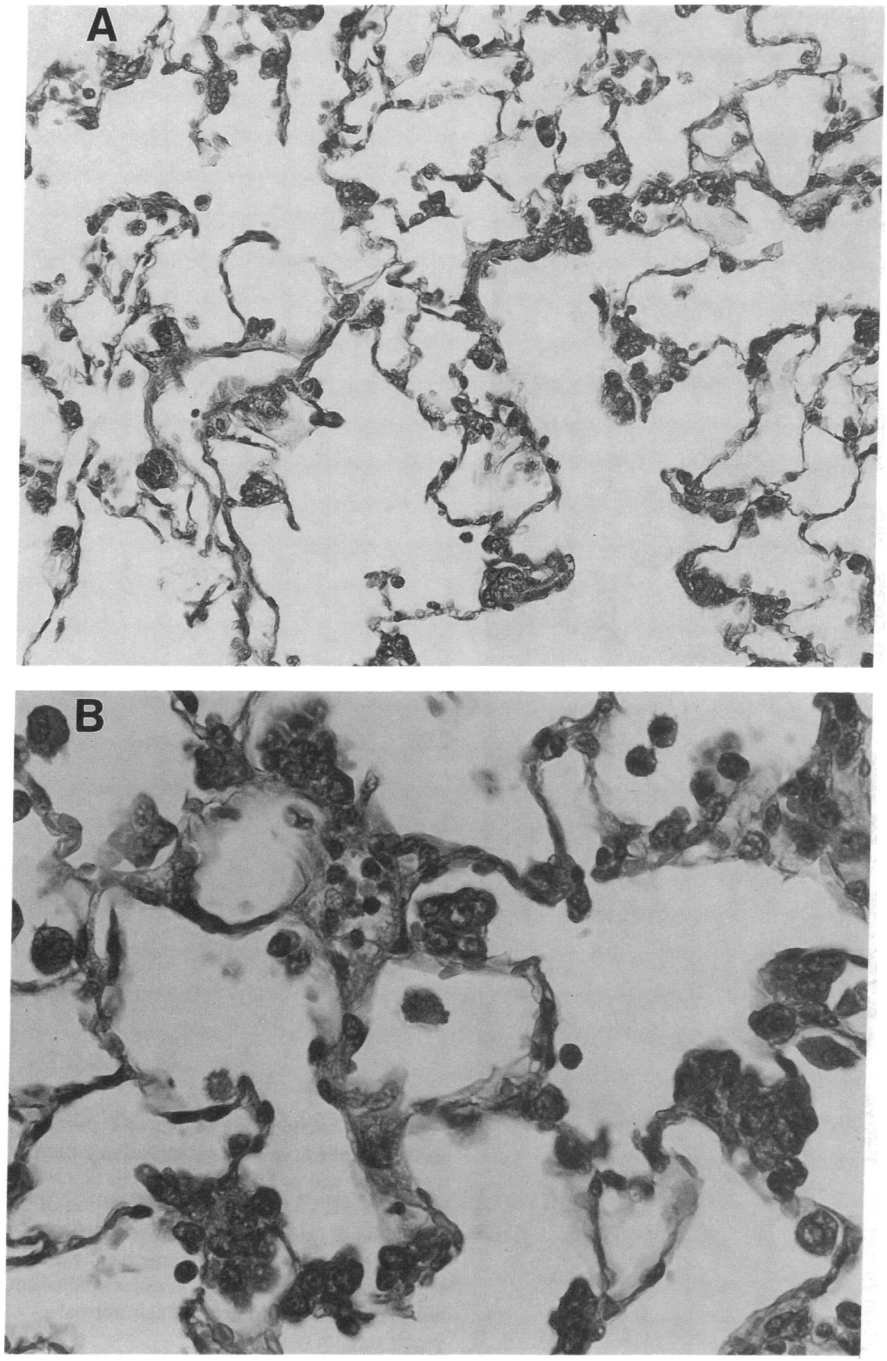

Figure 1. A multifocal knobby micropapillary proliferation of alveolar epithelial cells is noted along alveolar septae at 2 d after intratracheal injection of 5 mg/kg KGF (H&E; [*A*] ×200 and [*B*] ×400 original magnification).

paraffin embedded, and histologic sections were stained with hematoxylin and eosin (H&E). Indirect immunohistochemistry was performed on paraffin-embedded tissues pretreated for 5 min with 3% hydrogen peroxide in methanol using the avidin-biotin complex technique, horseradish peroxidase, and diaminobenzidene as a chromogen (10) for the demonstration of surfactant protein B and proliferating cell nuclear antigen. The rabbit anti-bovine surfactant protein B antiserum (a generous gift of Dr. Jeffrey Whitsett, Children's Hospital Medical Center, Cinncinati, OH) has been previously well characterized (11), as has the monoclonal anti-rat proliferating cell nuclear antigen (PCNA) clone PC10, which recognizes the 36-kD polymerase delta accessory protein (Dako Corp., Glostrup, Denmark).

Total RNA from lung and skin of normal adult Sprague Dawley rats was isolated as described by Chomczynski and Sacchi (12). RNase protection mapping of KGF receptor (KGFR) and KGF transcripts was performed with DNA probes cloned into the transcription vectors pGEM4Z and pSP72, respectively (Promega Biotec, Madison, WI). The rat KGF (9) antisense transcript corresponded to bases 132–336 (EMBL accession number X56551). The KGFR (13) antisense transcript corresponded to bases 1270–1417 (GenBank accession number M63503). This region of KGFR sequence was determined to be identical in mouse and rat (R. Biltz, unpublished data). The vectors were linearized and antisense transcript was synthesized in vitro using SP6 or T7 RNA polymerase and ^{32}Pr-UTP (800 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear, Boston, MA). The full-length RNA probe was purified from an 8% polyacrylamide/7 M urea gel. After the protocol of RPA II kit (1410; Ambion) triplicate samples of 50 μg of total cellular RNA were hybridized at 45°C overnight with 10^5 cpm of labeled antisense probe. RNase digestion was performed for 40 min with a 1:500 dilution of solution R (Ambion). The RNA/RNA hybrids were

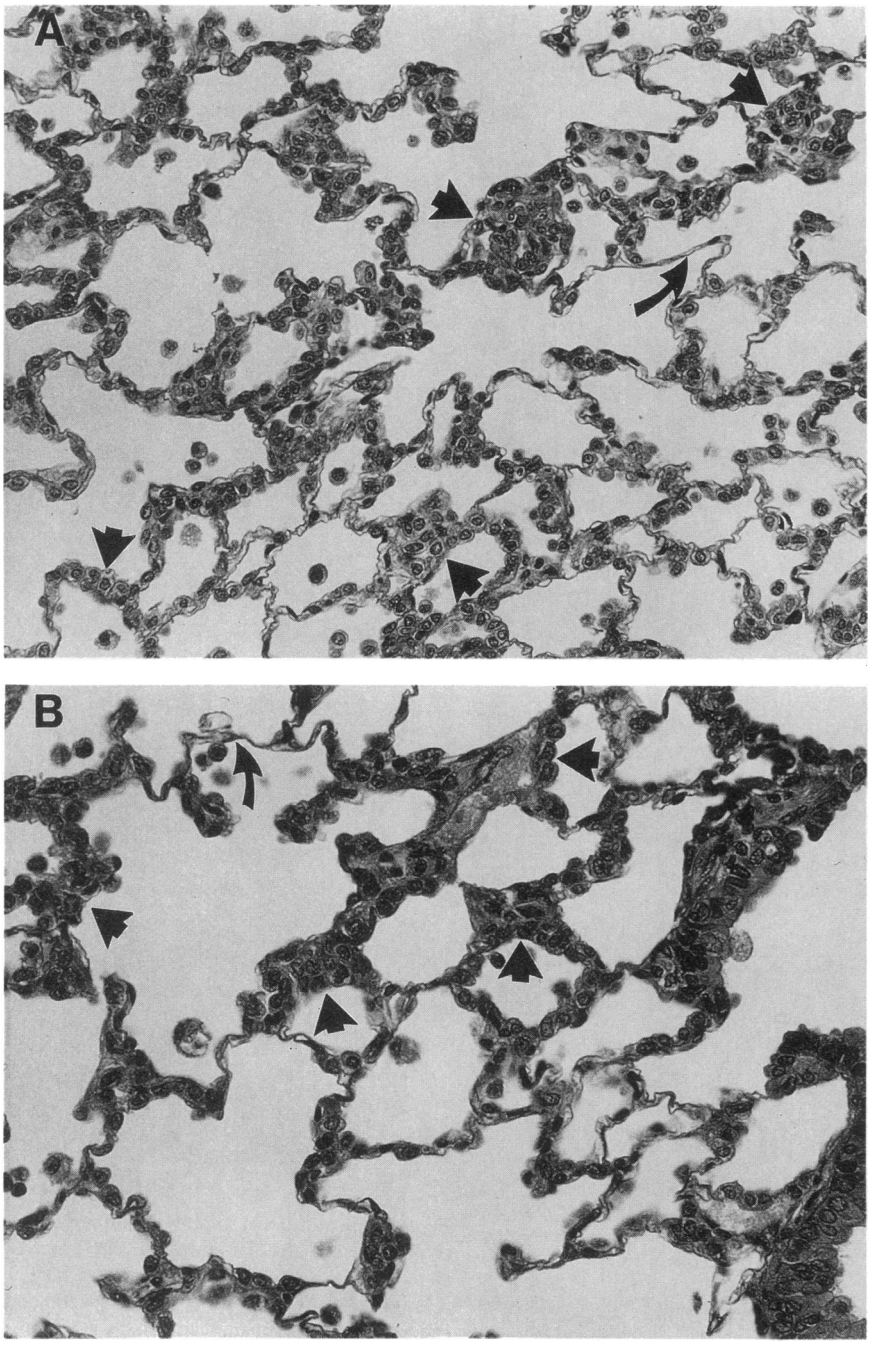

Figure 2. A proliferation of cuboidal alveolar epithelial cells lines alveolar septae in a diffuse monolayer and as focal nests of cells (*short arrow*) at 3 d after intratracheal injection of 5 mg/kg KGF (H&E; [*A*] ×200 and [*B*] ×400 original magnification). In comparison with the septae lined by hyperplastic alveolar epithelium, note a remaining thin hypocellular normal alveolar septum (*long arrow*).

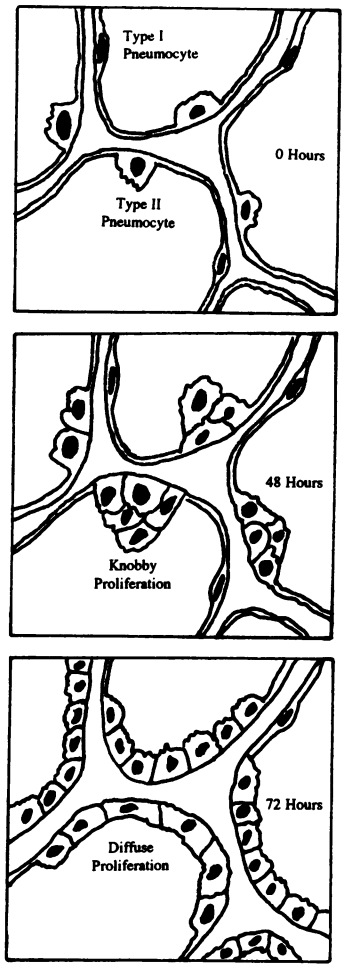

Figure 3. A schematic diagram shows the progression of type II pneumocyte hyperplasia in the lung after a single intratracheal injection of KGF.

precipitated, resuspended, and separated on an 8% polyacrylamide/7 M urea gel. Signal from protected fragments was quantified on a phosphorimager (Molecular Dynamics, Inc., Sunnyvale, CA) and averaged over the triplicate sample points. To quantify the amount of message in various tissue samples, unlabeled sense RNA corresponding to the labeled antisense probe was synthesized, purified over a G50 spin column, quantified by OD_{260nm}, and used as standards in the hybridization assay. The equivalent picograms of message per 50 μg of total RNA was calculated by comparison of each tissue RNA-protected band signal to one derived from hybridization to the known standard of sense RNA. The values obtained were then normalized using the ratio of the probe size to the full-length message.

Results

KGF was injected intratracheally at doses of 0.1, 1.0, 5.0, and 10.0 mg/kg, and the lungs at 3 d after injection were histologically examined in a blinded fashion by two pathologists (T. R. Ulich and E. S. Yi). KGF at 0.1 mg/kg did not cause histologically discernable alveolar epithelial cell hyperplasia. KGF at 1.0 mg/kg caused a mild but definite increase in alveolar epithelial cells. A substantial increase in alveolar epithelial cell hyperplasia was noted at 5.0 and 10.0 mg/kg as compared with 1.0 mg/kg. A slight increase in pneumocyte hyperplasia was noted between 5.0 and 10.0 μg/kg. In all subsequent studies to be described, KGF was injected at 5.0 mg/kg.

KGF does not cause a histologically discernable increase in alveolar epithelial cells at 6 or 24 h after intratracheal injection.

At 2 d a knobby micropapillary overgrowth of alveolar septal epithelial cells is noted within the lungs of KGF-treated rats (Fig. 1, *A* and *B*). At 3 d a diffuse low-cuboidal to cuboidal growth of alveolar epithelial cells is noted lining entire alveoli in large segments of the lung (Fig. 2, *A* and *B*). Some areas of the lung, however, retain a normal histology. Mitotic figures are present within pneumocytes in the areas of hyperplasia, demonstrating the occurrence of cellular proliferation (data not shown). The histologic appearance of the hyperplastic alveolar epithelium in the rat on day 3 is very similar to the appearance of reactive type II pneumocyte hyperplasia in human lungs. Pneumocyte hyperplasia and proliferation is not noted in control rats injected intratracheally with either saline or equivalent protein amounts of bovine serum albumin, confirming that the hyperplasia is not a nonspecific reaction to a foreign protein. The pneumocyte hyperplasia is not a response to inflammation since no inflammatory cells were noted either histologically or in cytocentrifuge preparations of bronchoalveolar lavage samples taken at daily intervals after the intratracheal injection of KGF. Hyperplastic alveolar epithelium is recognizable in decreasing amounts in the lungs of KGF-treated rats on days 4 and 5 after intratracheal injection. On day 6 the lungs of KGF-treated and control rats are indistinguishable. The histologic appearance of the pneumocyte hyper-

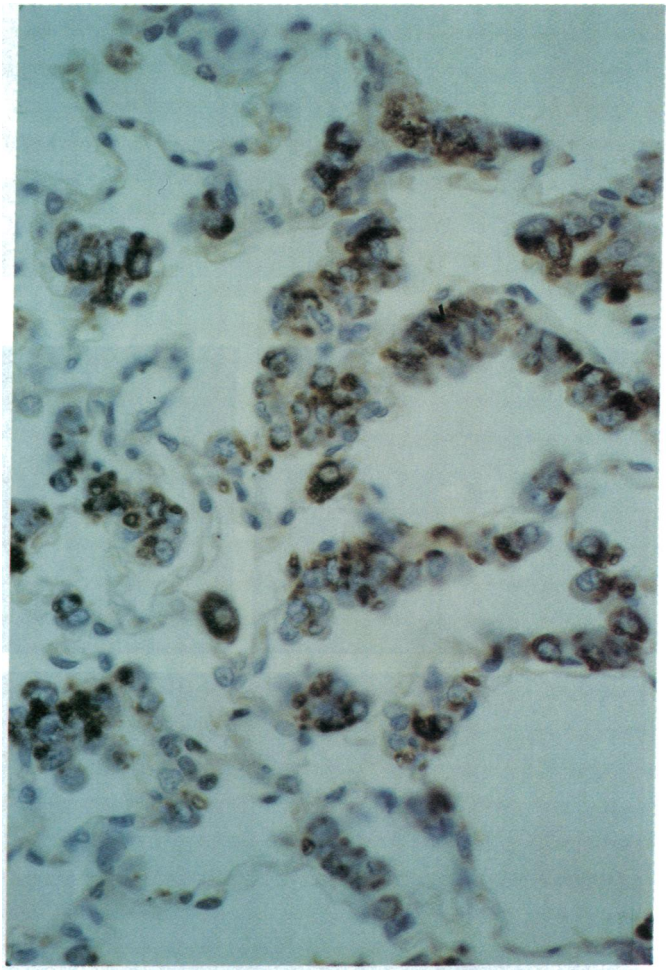

Figure 4. Immunoreactive surfactant protein B is demonstrable in hyperplastic alveolar lining cells 3 d after intratracheal injection of 5 mg/kg KGF (avidin-biotin complex indirect immunoperoxidase).

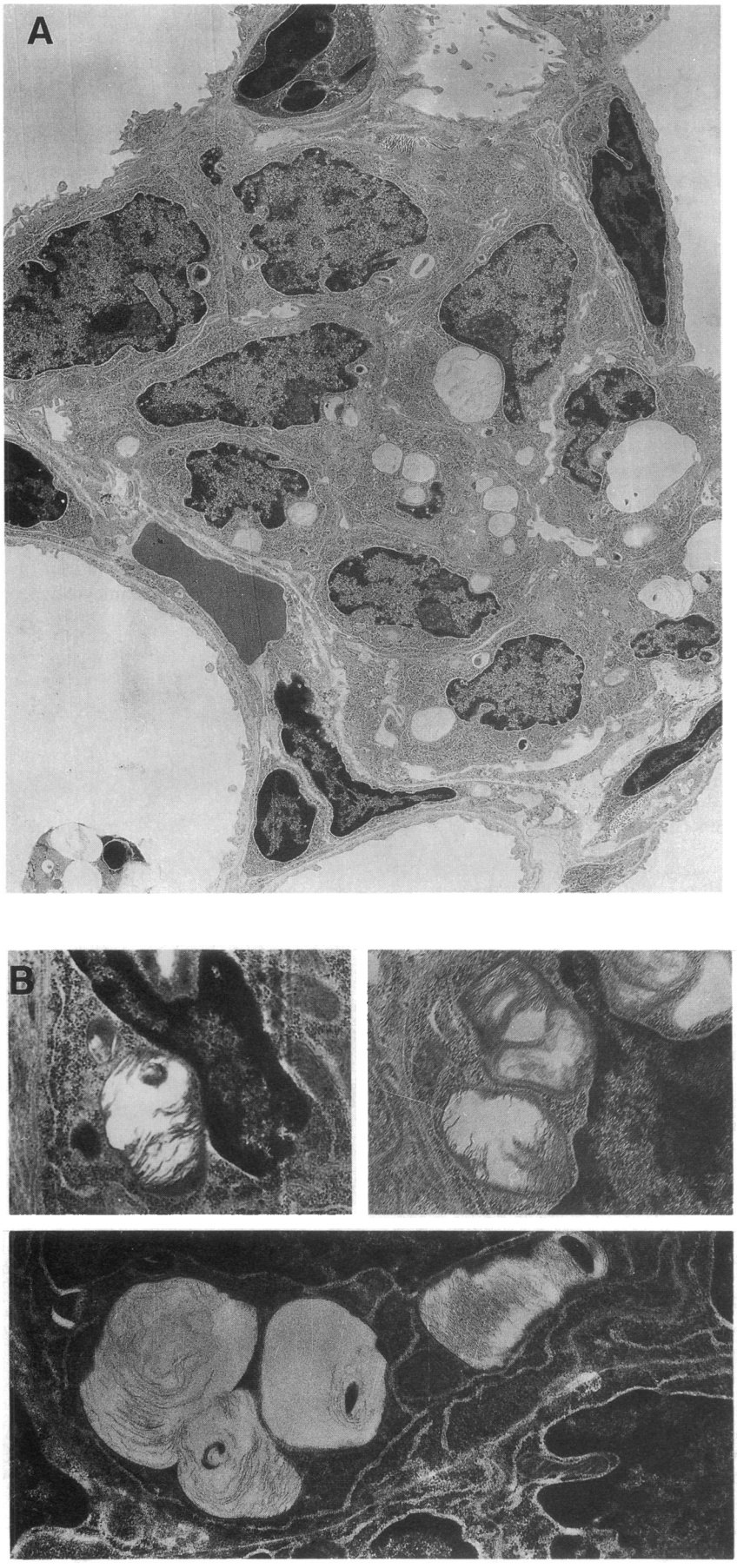

Figure 5. An electron micrograph shows a group of hyperplastic alveolar epithelial cells containing cytoplasmic lamellar inclusions at 3 d after intratracheal injection of KGF (×3,300). The lamellar structure of the inclusions is appreciated at higher magnification (×14,700).

plasia can be conceptually understood as an initial proliferation of numerous individual microanatomically separated type II cells resulting in multifocal micropapillary excresences followed 1 d later by the migration of type II cells to form a monolayer lining the alveolar septae (Fig. 3).

The hyperplastic alveolar epithelium contained abundant immunoreactive surfactant protein B on day 3 as demonstrated by immunohistochemistry (Fig. 4). The immunoreactivity was most prominent along the membranes of intracytoplasmic vacuolar structures. Surfactant immunoreactivity was also, as expected, shown within the cytoplasm of some alveolar macrophages that are known to phagocytose surfactant. The lung of a KGF-treated rat was ultrastructurally examined 3 d after intratracheal injection. The hyperplastic alveolar epithelial cells lining the alveolar septae almost invariably contained one or more lamellar inclusions of varying size and shape (Fig. 5, *A* and *B*).

The bronchiolar epithelium of KGF-treated rats is focally hyperplastic on day 3, but the hyperplasia is not nearly as prominent as the alveolar cell hyperplasia. Hyperplasia was appreciated as pseudostratification of the epithelial lining of smaller distal bronchi that are normally lined by a single layer of epithelium, by tufting or micropapillary growth of bronchiolar epithelium, and by an increase in mitotic figures within the bronchiolar epithelium (Fig. 6, *A* and *B*). Large airways did not demonstrate morphologically recognizable hyperplasia. KGF was not noted to exhibit any effects on goblet cells, pulmonary stromal cells, connective tissue, or vessels.

PCNA expression was prominently detected in type II pneumocytes at 24 h after the intratracheal injection of KGF

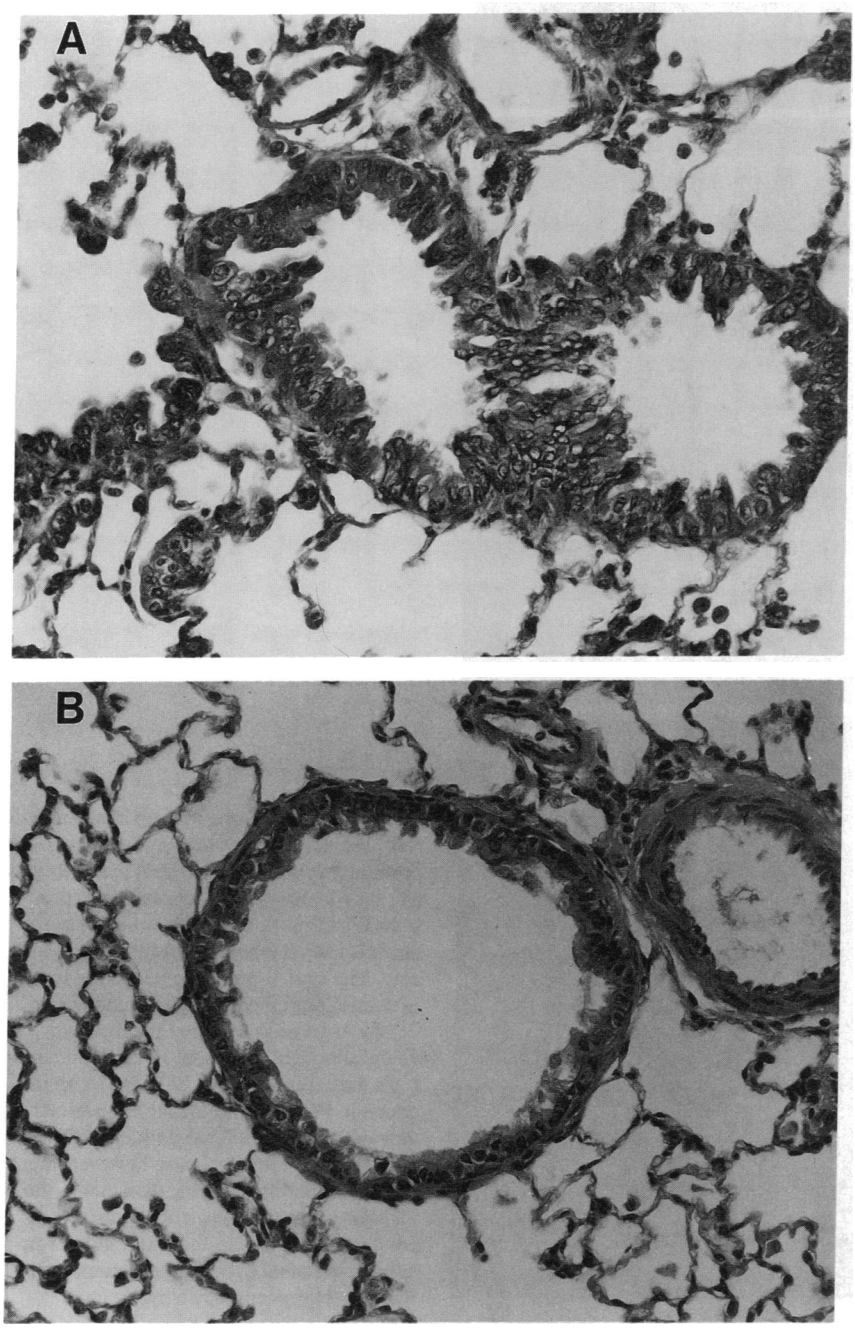

Figure 6. The bronchiolar epithelium of a 5 mg/kg KGF–treated rat shows epithelial hyperplasia with tufting (*A*) as compared with the bronchiolar epithelium of a control rat (*B*) (H&E).

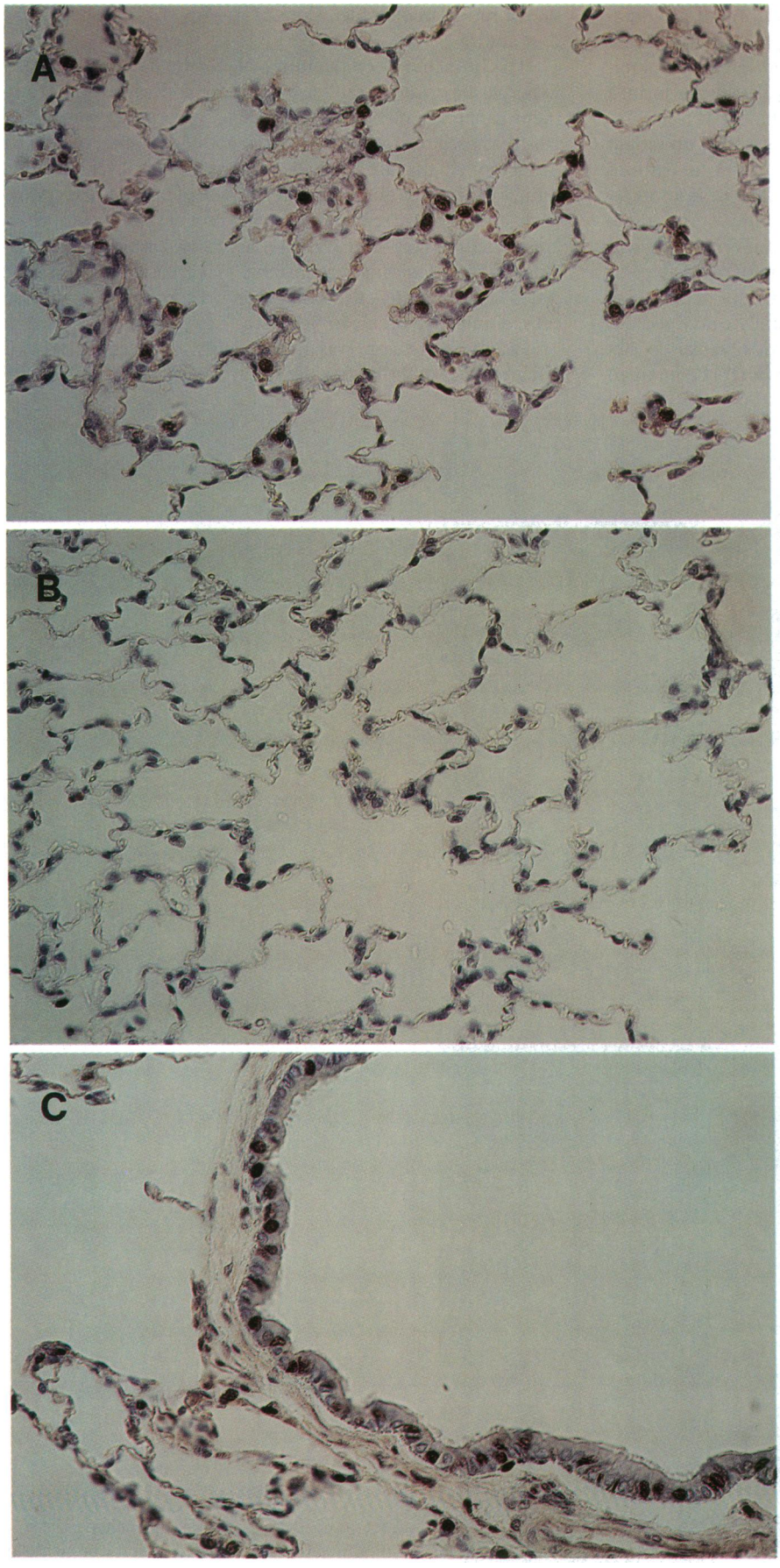

Figure 7. PCNA expression (avidin-biotin complex indirect immunoperoxidase) was prominently detected in type II pneumocytes (*A*) at 24 h after the intratracheal injection of KGF, a time at which pneumocyte hyperplasia is not yet morphologically recognizable. The type II pneumocytes in the lungs of control rats (*B*) do not demonstrate any PCNA expression in many microscopic fields, although occasional alveolar pneumocytes did express PCNA. KGF causes an increase in PCNA expression in bronchiolar airway epithelium (*C*) at 24 h. The PCNA expression in bronchioles is, however, much more focal and less striking than that in the alveolar pneumocytes, an observation that concurs with the very focal and mild bronchiolar epithelial hyperplasia as compared with alveolar pneumocyte hyperplasia.

(Fig. 7 *A*), a time at which pneumocyte hyperplasia is not yet morphologically recognizable. The type II pneumocytes in the lungs of control rats showed only very rare expression of PCNA (Fig. 7 *B*). The majority of the morphologically hyperplastic pneumocytes recognizable 2–3 d after intratracheal injection of KGF also demonstrated PCNA expression. KGF also caused an increase in PCNA expression in bronchiolar airways that was more focal and less striking than that in the alveolar pneumocytes (Fig. 7 *C*).

The effects of systemic injection of KGF (5 mg/kg) on pulmonary epithelium also were investigated. A single intravenous injection of KGF did not cause alveolar epithelial cell hyperplasia. On the other hand, three daily injections of KGF caused patchy alveolar epithelial cell growth (data not shown) in a knobby pattern similar to, but less striking than, the proliferation noted in rats at 2 d after a single intratracheal injection of KGF. Alveolar epithelial cell hyperplasia within the central tissues of the pulmonary parenchyma was no longer appreciable after 1 wk of daily injections of KGF. The results suggest that the intratracheal injection of KGF is a more potent stimulus for type II pneumocyte hyperplasia and that a refractory state to the action of KGF develops after continued daily systemic injection. Although intraparenchymal alveolar epithelial cell proliferation in the above-described and illustrated fashion was not noted after 1 wk of daily injections of KGF, a proliferation of an often more glandular-appearing epithelium was focally noted localized immediately adjacent to the connective tissue of lobar septae, the peribronchial connective tissue sheath, or subpleurally. The cellular nature of the proliferation is not entirely clear but might be postulated to relate to the release of KGF that had become bound to the extracellular matrix (KGF is a heparin-binding protein) in the mentioned connective tissue structures after systemic administration.

The lungs of adult rats express mRNA for both KGF and KGFR as detected by RNAse protection assay (Figs. 8 and 9). The BALB/MK mouse keratinocyte cell line was used as a positive control. The amount of KGF and KGFR mRNA in lung and skin was compared to obtain a sense of the possible importance of KGF in the homeostasis of lung. An approximately equal amount of KGF mRNA per 50 μg total tissue RNA was present in the lung and skin, but an approximately threefold greater amount of KGFR mRNA was present in lung as compared with skin.

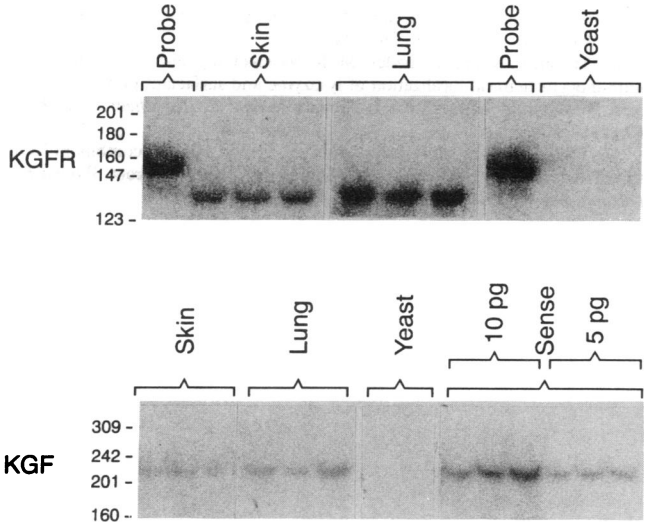

Figure 8. KGF and KGFR mRNA autoradiograms demonstrate the expression of KGF and its receptor in the adult rat lung, consistent with a role for endogenous KGF in the homeostasis of the alveolar epithelium.

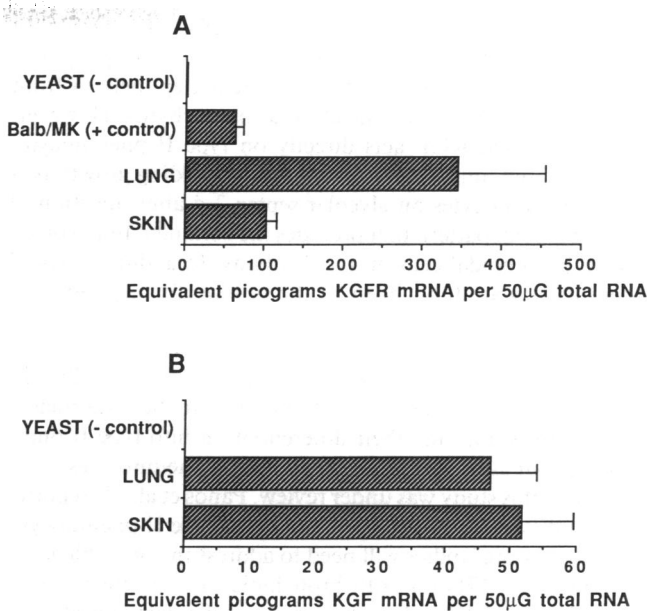

Figure 9. KGF and KGFR mRNA levels in rat lung and skin are presented as picograms±1 SD per 50 μg total organ RNA of triplicate samples. Note the high level of KGFR mRNA in the lung as compared with skin. Yeast total RNA served as a negative control and BALB/MK total RNA as a positive control for KGF receptor mRNA expression.

Discussion

KGF, a member of the FGF family, is an epithelial growth factor that is thought to be synthesized and secreted by stromal cells within epithelial organs (6, 14). The KGF receptor is a splice variant of the FGF receptor 2 and has high affinity for KGF and acidic FGF, but not basic FGF (13, 15–17). While acidic FGF can bind to other FGF receptors, KGF is thought to only bind to the KGF receptor that contains a unique 49–amino acid region in the COOH-terminal half of the third immunoglobulin loop (13, 15–17). In this study the finding of large amounts of KGF and KGFR mRNA in the adult rat lung is consistent with the hypothesis that KGF may play an important endogenous role in the maintenance of pulmonary epithelial integrity.

KGF administered intratracheally causes a striking hyperplasia of alveolar epithelial cells characterized first by micropapillary and then by linear type II pneumocyte hyperplasia (Figs. 1–4). The presence of lamellar cytoplasmic inclusions within the cells at the ultrastructural level is consistent with the proposal that the hyperplastic cells are type II pneumocytes. The lamellar inclusions did not appear as numerous or as osmiphilic, as those illustrated in some cases of experimental type II pneumocyte hyperplasia in the rat (1), but are very similar to the lamellar inclusions illustrated in normal rat lung (18). In addition to causing alveolar cell hyperplasia, KGF causes mild

focal bronchiolar epithelial cell hyperplasia. The possibility must be considered that the hyperplastic alveolar epithelium could represent a downgrowth of bronchial epithelium from terminal bronchioles into the alveolar parenchyma. However, we propose that KGF acts directly on type II pneumocytes because of the multifocal micropapillary budding growth pattern of pneumocytes on alveolar septae 2 d after injection of KGF, a growth pattern that precedes the confluent lining of the alveoli by cuboidal cells on the third day. In addition, type II pneumocytes are thought to be the mitotically responsive alveolar epithelial cell population and would be expected to be the alveolar cell type to respond to a potential endogenous mediator of alveolar cell growth such as KGF. Finally, the identification of lamellar inclusions within the hyperplastic cells suggests not only their differentiation into type II pneumocytes but also their origin from type II pneumocytes.

While this study was under review, Panos et al. (7) reported that KGF stimulates the proliferation of type II pneumocytes in vitro. Future studies will need to address the probable localization of KGFRs on both bronchiolar and alveolar epithelium. Since KGF binds to heparin, specific demonstration of the binding of KGF to its receptor in vivo will be technically difficult and the receptor may be best localized by in situ hybridization. The more substantial alveolar epithelial cell hyperplasia noted after intratracheal as compared with systemic injection of KGF suggests that the intratracheal administration of KGF allows direct access of a higher concentration of growth factor to the target epithelium. The molecular basis for the refractory state of the alveolar epithelium to KGF that appears to develop after continued systemic injection of KGF is unknown, but may be related to receptor downregulation. Some KGF-responsive epithelia do not develop a refractoriness to KGF after 1 wk of daily systemic injections (19).

The identification of KGF as a growth factor for type II pneumocytes raises the possibility that KGF will show therapeutic potential similar to or better than glucocorticoids as a stimulant of fetal lung maturation. KGF might also be clinically useful in stimulating bronchoalveolar repair after lung injury in the adult. The proliferative effect of KGF on type II pneumocytes suggests that KGF may play an important role in regulating the synthesis and secretion of surfactant. Finally, the histologic similarity of diffuse type II pneumocyte hyperplasia and the bronchoalveolar cell variant of lung carcinoma suggests that the possibility that dysregulation of KGF plays a role as a growth factor in pulmonary neoplasia should be investigated.

References

1. Panos, R. J. 1993. Cytokines and alveolar type II cells. *In* Cytokines of the Lung. Lung Biology in Health and Disease. J. Kelly, editor. Marcel Dekker, Inc., New York. 417–458.

2. Leslie, C. C., K. McCormick-Shannon, P. C. Robinson, and R. J. Mason. 1985. Stimulation of DNA synthesis in cultured rat alveolar type II cells. *Exp. Lung Res.* 8:53–66.

3. Leslie, C. C., K. McCormick-Shannon, and R. J. Mason. 1990. Heparin-binding growth factors stimulate DNA synthesis in rat alveolar type II cells. *Am. J. Respir. Cell Mol. Biol.* 2:99–106.

4. Ballard P. L. 1986. Hormones and lung maturation. *In* Monographs on Endocrinology. Springer Verlag, New York, Inc., New York. 1–354.

5. Balis, J. U., F. Patterson, E. M. Haller, S. A. Shelley, and M. R. Montgomery. 1988. Ozone-induced lamellar body responses in a rat model for alveolar injury and repair. *Am. J. Pathol.* 132:330–344.

6. Finch, P. W., J. S. Rubin, T. Miki, D. Ron, and S. A. Aaronson. 1989. Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. *Science (Wash. DC).* 245:752–755.

7. Panos, R. J., J. S. Rubin, S. A. Aaronson, and R. J. Mason. 1993. Keratinocyte growth factor and hepatocyte growth factor/scatter factor are heparin binding growth factors for alveolar type II cells in conditioned medium. *J. Clin. Invest.* 92:969–977.

8. Ulich, T. R., L. Watson, S. Yin, K. Guo, and J. del Castillo. 1991. The intratracheal administration of endotoxin and cytokines. I. Characterization of LPS-induced TNF and IL-1 mRNA expression and the LPS-, TNF-, and IL-1-induced inflammatory infiltrate. *Am. J. Pathol.* 138:1485–1496.

9. Yan, G., and F. Wang. 1991. Sequence of rat KGF (heparin binding growth factor type 7). *In Vitro Cell & Dev. Biol.* 27:437*a*. (Abstr.)

10. Carson, F. L. 1990. Histotechnology. American Society of Clinical Pathology Press, Chicago. 242–246.

11. Stahlman, M. T., M. E. Gray, and J. A. Whitsett. 1992. The ontogeny and distribution of surfactant protein B in human fetuses and newborns. *J. Histochem. Cytochem.* 40:1471–1480.

12. Chomczynski, P., and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156.

13. Miki, T., T. Fleming, D. Bottaro, J. Rubin, D. Ron, and S. A. Aaronson. 1991. Expression cDNA cloning of the KGF receptor by creation of a transforming autocrine loop. *Science (Wash. DC).* 251:72–75.

14. Rubin, J. S., H. Osada, P. W. Finch, W. G. Taylor, S. Rudikoff, and S. A. Aaronson. 1989. Purification and characterization of a newly identified growth factor specific for epithelial cells. *Proc. Natl. Acad. Sci. USA* 86:802–806.

15. Yayon, A., Y. Zimmer, S. Guo-Hong, A. Avivi, Y. Yarden, and D. Givol. 1992. A confined variable region confers ligand specificity on fibroblast growth factor receptors: implications for the origin of the immunoglobulin fold. *EMBO (Eur. Mol. Biol. Organ.) J* 11:1885–1890.

16. Dell, K. R., and L. T. Williams. 1992. A novel form of fibroblast growth factor receptor 2. *J. Biol. Chem.* 257:21225–21229.

17. Miki, T., D. P. Bottaro, T. P. Fleming, C. L. Smith, W. H. Burgress, A. M. Chan, and S. A. Aaronson. 1992. Determination of ligand binding specificity by alternative splicing: two distinct growth factor receptors encoded by a single gene. *Proc. Natl. Acad. Sci. USA.* 89:246–250.

18. Haller, E. M., S. A. Shelley, M. R. Montgomery, and J. U. Balis. 1992. Immunocytochemical localization of lysozyme and surfactant protein A in rat type II cells and extracellular surfactant forms. *J. Histochem. Cytochem.* 40:1491–1500.

19. Pierce, G. F., R. M. Housley, and C. F. Morris. 1993. Recombinant KGF stimulates epithelial elements in the skin and gastrointestinal tract. *Wound Repair and Regeneration.* 1:119. (Abstr.)