Invited Review

Alterations in the alveolar epithelium after injury leading to pulmonary fibrosis

M. Kasper and G. Haroske
Institute of Pathology, Medical Faculty «Carl Gustav Carus», Technical University of Dresden, Dresden, Germany

Summary. This review discusses current knowledge of the involvement of the alveolar epithelium in tissue remodelling during fibrogenesis. The purpose of the present paper is to give an overview, including the authors' own results, of knowledge of ultrastructural alterations, proliferation kinetics and phenotypic changes of pneumocytes in experimental and clinical pathology of pulmonary fibrosis. After lung injury, the alveolar epithelial cells show ultrastructural alterations, hypertrophy and hyperplasia, and a modulation of a number of recent investigations underline the hypothesis that multiple pulmonary inflammatory reactions alone (e.g. without substantial damage to the epithelium) do not result in fibrosis (Evans et al., 1975; Adamson and Bowden, 1979; Brown et al., 1988, 1993; Nakashima et al., 1991). For example, the intratracheal application of human recombinant C5 in the hamster lung does not induce sufficient lung injury, except a focal accumulation of leukocytes (Hyde et al., 1991). Repeated exposition to reactive oxygen species, however, resulted in a classical fibrosis. In order to describe a common pathogenetic mechanism for pulmonary fibrosis, we have to distinguish between three stages of the disease process, independent of the causing agent:

Immediately after damage an inflammatory reaction occurs, the alveolitis which is regarded to be the determinant for the course of the disease: restoration of sufficient pulmonary histoarchitecture; or development of pulmonary fibrosis with loss of functional integrity of the whole organ. The reason for this may be the different equipment of inflammatory cells with enzymes capable of producing oxygen radicals and subsequent cytotoxic effects of the entire lung parenchyma, which includes epithelial, endothelial as well as interstitial cells. Oxidants do not only produce cell injury in a direct fashion. They can also activate metalloproteinases and other proteases (Abe et al., 1994; Agre et al., 1994). Then the phase of tissue regeneration follows. The alveolar epithelium consists of type I and type II epithelial cells in an almost balanced numerical proportion.

Type I cells cover approximately 90% of the alveolar surface, whereas type II pneumocytes represent the stem cell population or replicatory precursors of type I cells. Shortly after injury, the alveolar surface is denuded due to serious damage of the alveolar epithelium. This loss is characterised by fibrosis and inflammation of the pulmonary interstitium, leading finally to a degradation of alveolar histoarchitecture. The initiation of this process starts with the damage of endothelial and/or epithelial cells, caused by inhalation of dust and chemical agents or by intratracheal or intravenous administration of drugs (Fig. 1). Further, we distinguish a group of idiopathic interstitial pneumonias (cryptogenic fibrosing alveolitis), where no causative agent is known.

Key words: Alveolar epithelium, Pulmonary fibrosis, Epithelial, Fibroblast interaction, Immunohistochemistry

Introduction

Pulmonary fibrosis, also called fibrosing alveolitis, represents a disorder of the lower respiratory tract, which is characterised by fibrosis and inflammation of the pulmonary interstitium, leading finally to a destruction of alveolar histoarchitecture. The initiation of this process starts with the damage of endothelial and/or epithelial cells, caused by inhalation of dust and chemical agents or by intratracheal or intravenous administration of drugs (Fig. 1). Further, we distinguish a group of idiopathic interstitial pneumonias (cryptogenic fibrosing alveolitis), where no causative agent is known.

A number of recent investigations underline the hypothesis that multiple pulmonary inflammatory reactions alone (e.g. without substantial damage to the epithelium) do not result in fibrosis (Evans et al., 1975; Adamson and Bowden, 1979; Brown et al., 1988, 1993; Nakashima et al., 1991). For example, the intratracheal application of human recombinant C5 in the hamster lung does not induce sufficient lung injury, except a focal accumulation of leukocytes (Hyde et al., 1991). Repeated exposition to reactive oxygen species, however, resulted in a classical fibrosis. In order to describe a common pathogenetic mechanism for pulmonary fibrosis, we have to distinguish between three stages of the disease process, independent of the causing agent:

Immediately after damage an inflammatory reaction occurs, the alveolitis which is regarded to be the determinant for the course of the disease: restoration of sufficient pulmonary histoarchitecture; or development of pulmonary fibrosis with loss of functional integrity of the whole organ. The reason for this may be the different equipment of inflammatory cells with enzymes capable of producing oxygen radicals and subsequent cytotoxic effects of the entire lung parenchyma, which includes epithelial, endothelial as well as interstitial cells. Oxidants do not only produce cell injury in a direct fashion. They can also activate metalloproteinases and other proteases (Abe et al., 1994; Agre et al., 1994). Then the phase of tissue regeneration follows. The alveolar epithelium consists of type I and type II epithelial cells in an almost balanced numerical proportion.

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to the high vulnerability of flat type I epithelial cells to toxic agents. Over the course of several days type II pneumocyte proliferation results in alveolar re-epithelialization. A contribution of the bronchial epithelial cells is also discussed (Nettesheim and Szakat, 1972; Kawamoto and Fukuda, 1990). The immediate proliferative reaction of type II cells after injury may be the key event in the sufficient restoration of the alveolar epithelial surface. For this reason, agents which are toxic for both type I as well as type II cells or which inhibit type II cell proliferation preferentially induce the irreversible reaction leading to the pulmonary fibrosis.

Classical examples are the herbicide paraquat and the anti-neoplastic drug bleomycin. Both substances have a remarkable effect for inducing fibrotic lesions in humans as well as in animals (Adanson and Bowden, 1979; Brown et al., 1988; Hampson and Pond, 1988; Kawamoto and Fukuda, 1990).

The present review has been aimed at summarizing the great number of alveolar epithelial alteratations during fibrogenesis. This review is not intended to be a discussion of the general pathomechanisms of fibrotic disease. The endothelial changes, such as altered vascular permeability, oedema and swelling of basal membranes, and the modulation of extracellular matrix composition are the subject of many reviews in fibrosis research (Adanson and Bowden, 1983; Crouch, 1990; Simionescu, 1991; Fischer and Morgenroth, 1993; Kantak et al., 1993; Matthy et al., 1993; Ward et al., 1993; Zhang et al., 1993; Brown et al., 1994; Kovacs and DiPietro, 1994; Piguet and Vesin, 1994; Takahashi et al., 1994). Therefore, some areas are briefly touched on and some are not discussed (e.g. intraalveolar coagulation). Further, we wish to summarize several aspects of epithelial cell-interstitial interaction in the disease process.

### 2. Morphological characteristics

The alveolar surface of the lung is lined by two distinct epithelial cells. The thin alveolar type I cells, which realize an effective gas exchange, cover the pulmonary capillaries, whilst cuboidal type II pneumocytes are found in the «corner» of the alveolus. Type II cells are also in close proximity to endothelial cells, alveolar macrophages, fibroblasts and other

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### Table 1. Ultrastructural alterations of the alveolar epithelial cells.

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>DAMAGE</th>
<th>EXPERIMENTAL MODEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adamson and Bowden, 1974</td>
<td>Damage of type I cells, presence of intermediate cells, proliferation of type II cells which lacks LB but contains electron-dense inclusions</td>
<td>Oxygen treatment, mice</td>
</tr>
<tr>
<td>Evans et al., 1975</td>
<td>Transformation of type II into type I cells, evidence of an intermediate cell</td>
<td>NO₂ exposition, rat</td>
</tr>
<tr>
<td>Kawanami, 1982</td>
<td>Type II cells with increased number of foot processes, giant LB, irregular microvilli, deep recesses between adjacent cells, type I with numerous microvilli and hemidesmosomes</td>
<td>Pulmonary fibrosis, human</td>
</tr>
<tr>
<td>Fukuda et al., 1987</td>
<td>Alveolar damage denudation</td>
<td>Intraalveolar fibrosis, human</td>
</tr>
<tr>
<td>Smian and Smith, 1988</td>
<td>Type II cells with atypical large mitochondria</td>
<td>Propanolol treatment, rat</td>
</tr>
<tr>
<td>Lopez et al., 1988</td>
<td>Type I cells normal, type I cells with depletion of LB</td>
<td>Hydroxysulfide treatment, rat</td>
</tr>
<tr>
<td>Hampson and Pond, 1988</td>
<td>Degenerated, focally detached type II cells with dispersed nuclear chromatin, decrease of LB and secretory granules, swollen mitochondria, reduced size of microvilli</td>
<td>Paraquat model, dog</td>
</tr>
<tr>
<td>Birkun and Zagorulko, 1990</td>
<td>Type II cells with vacuolization of LB, destruction of mitochondria, irregular nuclei, presence of immature forms of type II cells with fewer organelles</td>
<td>Pulmonary fibrosis, human</td>
</tr>
<tr>
<td>Kaup et al., 1990</td>
<td>Type II cell necrosis, increased and enlarged LB, fatty changed glycogen deposits</td>
<td>Alveolar fibrosis, horse</td>
</tr>
<tr>
<td>Harris et al., 1991</td>
<td>Type I cell hyper trophy, increased organelles in type I cells</td>
<td>Hyperxia, rat</td>
</tr>
<tr>
<td>Nakashima et al., 1991</td>
<td>Lipid inclusions in type II cells</td>
<td>Oxidant injury, hamster</td>
</tr>
<tr>
<td>Palazzo et al., 1992</td>
<td>No changes</td>
<td>Hypoxic injury, hamster</td>
</tr>
<tr>
<td>Kyono et al., 1992</td>
<td>Proliferation of type II cells intermediate stage type II cells, ballooned cytoplasm of type I cells with large nucleolus and appearance of residual bodies and vacuoles, self-repairing type II cells</td>
<td>Cobalt exposition</td>
</tr>
<tr>
<td>Tayo et al., 1993</td>
<td>Type II cell hyper trophy, increased inclusions</td>
<td>Radiation (³²⁵⁹ Pu O₂), mouse</td>
</tr>
<tr>
<td>Fischer and Morgenroth, 1993</td>
<td>Type II cells with detached flat processes, more pinocytotic vesicles, Type II cells plump in snap</td>
<td>Pulmonary fibrosis, human</td>
</tr>
<tr>
<td>Adamson et al., 1993</td>
<td>Type I cell destruction</td>
<td>Iron-poisoned mice</td>
</tr>
<tr>
<td>Woods et al., 1993</td>
<td>Type II cell necrosis, type II cell hyperplasia</td>
<td>3-methyl indole treatment, rat</td>
</tr>
<tr>
<td>Hammar, 1994</td>
<td>Intracellular tubular inclusions in type II cells</td>
<td>Idiopathic pulmonary fibrosis, human</td>
</tr>
<tr>
<td>Penney et al., 1994</td>
<td>Disruption of type II cells, degeneration and sloughing, enlarged LB, atypical coalescence of LB, alveolar flooding</td>
<td>X-ray irradiation, mouse</td>
</tr>
</tbody>
</table>
interstitial cells, which implicates their immediate involvement in inflammatory processes at the alveolar level. A third pneumocyte type, the alveolar brush cell (Meyrick and Reid, 1968) is present in the alveolar lining of some species (Dornans, 1983; Gomi et al., 1991; Kasper et al., 1994a).

Alveolar and bronchial injury induced by toxic agents leads to necrosis of the epithelium, which includes a lot of ultrastructural alterations (Table 1).

Very often, type I alveolar epithelial cells show detached flat processes and blebbing as a typical feature of denudation, accumulation of pinocytotic vesicles, increased number of apical microvilli, cytoplasmic swelling and the occurrence of electron-lucent material. In some cases, increased organelles are found and interpreted as a «self-repairing» feature of type I cells (Kyono et al., 1992). The second prominent feature of epithelial injury is the occurrence of intermediate cells with type II pneumocyte characteristics but a lack of lamellar bodies (Adamson and Bowden, 1974). These cells probably represent either a transitory stage between type II and type I cells or immature stem cells of the alveolar and bronchial epithelium (Ten Have-Opbroek et al., 1991).

Certain toxic agents such as bleomycin, X-ray irradiation, paraquat and oxidants specially destroy or alter type II cells (Table 1).

As a result the type II cells are hyperplastic and hypertrophic (Miller and HooK, 1990). There is a cuboidal transformation of pneumocytes accompanied by increased number of foot processes (see also below), vacuolization, alterations of mitochondria, development of atypical lamellar body configuration and the presence of glycogen as well as lipids. Proteinaceous precipitate in alveoli indicates «alveolar flooding» after severe epithelial injury (Penney et al., 1994).

The «end-stage» fibrotic lung (honeycomb lung) is characterized by intraalveolar fibrosis and alveolar obliteration. The remaining alveoli are lined by metaplastic epithelia which can show histological and ultrastructural evidence of squamous differentiation.

### 3. Cell kinetics of the alveolar epithelial cells

The study of cell proliferation in the lung has given some insight into the functions of various cell types in the normal lung, as well as into the differentiation process of the lung throughout growth and in disease. Cell kinetics have also shed light on the process of lung fibrosis following several kinds of lung injury. For previous reviews, the studies of Evans and Shami (1989) and Shami and Evans (1992) should be mentioned.

The cell kinetic studies have not been performed as extensively as they have in other organs, because the techniques are difficult to apply to the very heterogeneously structured lung tissue.

However, in the last few years precise data have also been gathered about the proliferation behaviour of alveolar epithelium.

The type II pneumocytes are the only cells among alveolar epithelia which are capable of cell division and proliferation. They act as progenitor cells for the alveolar epithelium, proliferating to replenish themselves and to differentiate into the type I cells. This conclusion was first drawn from studies in lung injury (Evans et al., 1973, 1978), and has since repeatedly been confirmed (Kauffman, 1980; Evans, 1982).

The papers agree that a proportion between 0.3% and 6.5% of the type II cells are in distinct phases of the cell cycle (mostly in S-phase) in the lungs of different species (Kauffman, 1972; Adamson et al., 1977; Witschi and Morse, 1985; Tryka et al., 1986). In aging animals the proliferation indices decline with time. Therefore, in healthy lungs most of the type II proliferation is associated with growth processes (Ulich et al., 1994).

Considering the fact that about 60% of all alveolar epithelia are type II pneumocytes (Pinkerton et al., 1992), the actual number of proliferating cells in the healthy lung is very low. In fact, mitotic figures in type II cells are only found by chance. All cycle data of type II pneumocytes, published so far as labelling indices, concern distinct cycle states and/or the duration of cycle phases only. Most of the indices refer to the S-phase (e.g. thymidine labelling index= TLI) as well as to the mitosis. Data about the Ki-67 index (growth fraction) in lungs are still lacking. The phase durations of S (7.3 to 9 hrs), G2 (1.0 to 2.0 hrs), and M (0.8 hrs) are known (Kauffman, 1980). Hitherto, there has not been an approach to estimate the total proliferative activity or the proliferative output of the type II pneumocytes.

The mechanisms responsible for proliferative activity (PA) are the speed of the cycle (which is inversely proportional to the generation time T) and the proportion of cells that is committed to the cycle (growth fraction G). The proliferative activity gives a figure of the average number of cells produced per cell of the population and per unit of time.

\[
PA = \frac{G}{T}
\]

In steady state conditions the growth fraction G is also proportional to the cycle phase duration (e.g. Ts for S-phase duration) and to the generation time (Brugal, 1994), e.g.

\[
TLI = \frac{(G*Ts)}{T}
\]

Thus, the proliferative activity is given by

\[
PA = \frac{TLI}{Ts}
\]

By using the averaged figures of labelling indices for the S-phase and their mean duration, mentioned above, the proliferative activity is 0.625% per hour.

That means that about 0.9% of all type I pneumocytes per hour can be restored at the basal rate of proliferation, if all «excess» type II cells are allowed to differentiate. In a survey about cell turnover in the lung, Bowden (1983) gave an estimation of the mean turnover of type II cells between 28-35 days. In terms of proliferative activity this figure would mean a percentage of 0.14% of cells produced in one hour.

In lung injuries, following increased NO2 concentrations in the breathed air, the proliferative
Alveolar epithelium and pulmonary fibrosis

Table 2. Properties of the alveolar epithelium.

<table>
<thead>
<tr>
<th></th>
<th>PNEUMOCYTES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
</tr>
<tr>
<td>Production of cytokines</td>
<td>-</td>
</tr>
<tr>
<td>Secretion of proteases</td>
<td>*/+#</td>
</tr>
<tr>
<td>Antigen-presenting function</td>
<td>-</td>
</tr>
<tr>
<td>Target for leucocytic alvolar macrophage adhesion</td>
<td>+</td>
</tr>
<tr>
<td>Bacterial adhesion</td>
<td>+</td>
</tr>
<tr>
<td>Secretion of surfactant protein</td>
<td>-</td>
</tr>
<tr>
<td>Recycling of surfactant protein</td>
<td>-</td>
</tr>
<tr>
<td>Transporting epithelium</td>
<td>+</td>
</tr>
<tr>
<td>Production of ECM components</td>
<td>*/+</td>
</tr>
<tr>
<td>Gas exchange/Air-blood barrier</td>
<td>+</td>
</tr>
<tr>
<td>Regenerating capacity</td>
<td>-</td>
</tr>
<tr>
<td>Antioxidant capacity</td>
<td>*/+</td>
</tr>
<tr>
<td>Lectin binding</td>
<td>+</td>
</tr>
<tr>
<td>Synthesis of complement components</td>
<td>-</td>
</tr>
<tr>
<td>Other enzyme activities**:</td>
<td></td>
</tr>
<tr>
<td>Carbonic anhydrase II</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>-</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>-</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>-</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin-K-dependent carboxylase</td>
<td>-</td>
</tr>
<tr>
<td>Carboxypeptidase M</td>
<td>-</td>
</tr>
<tr>
<td>Heat-stable alkaline phosphatase</td>
<td>+</td>
</tr>
<tr>
<td>Protein disulphide isomerase</td>
<td>+</td>
</tr>
<tr>
<td>Carbonyl reductase</td>
<td>-</td>
</tr>
<tr>
<td>Secretory phospholipase A₂</td>
<td>-</td>
</tr>
</tbody>
</table>

#: presence of cathepsin D in lysosomes of human type I alveolar epithelial cells (Kasper, unpublished data); *: metalloproteinases, cathepsins H, E, pepsinogen II; **: for further information see Simon et al., 1982; Fischbein et al., 1993; Nauwen et al., 1990; Neagost et al., 1993; Matsubara et al., 1994.

activity of the type II cells had reached a maximum after 2 to 3 days and then decreased to near control values by the fifth day (Evans et al., 1978). The labelling indices were increased to threefold of the basal rate (Barth et al., 1994). If the AgNOR counts published in the same paper are regarded as a rough estimator of the generation time, a reduction in Ts of about 50% can be speculated. Consequently, the proliferative activity of the type II cells would amount to 3.75% per hour. Theoretically, at maximum proliferation a loss of half of the type I cell population could be compensated by the type II cell proliferation in about 9.25 hours.

However, few data are available about the real durations of the cycle phases following several lung injuries. It has been shown that in vivo the duration of S- or G2/M-phase were not affected by an increased proliferation after pneumectomy. On the other hand, in vitro the same proliferation type was linked to a two-fold to ten-fold prolongation of S- and G2/M-phase, respectively (Uhal, 1994). Furthermore, the actual proportion of type II cells which differentiate into type I cells as well as the time needed for the differentiation process are largely unknown. The same holds true for the possible variations in the differentiation starting from damaged type II cells.

The mechanisms for growth control and cycle control in the type II cells have not been studied extensively so far. Voelker and Mason (1989) summarized those factors known to stimulate type II cell proliferation and those that do not. Among the growth factors, the EGF, IGF, and FGF were found to have stimulating effects, whereas the PDGF, TGF-β, and TNF-α could not be shown to stimulate growth. In more recent papers, the IGF-binding protein-2, the IGF-II, and the type 2 IGF receptor are reported to also control the transition of lung alveolar epithelia in and out of the cell cycle (Mouhieddine et al., 1994). The TGF-β is included in these regulatory effects (Cazals et al., 1994), probably due to a downstream effect on the E2F transcription factor (Schwarz et al., 1995). For some special forms of silica-induced lung injury the PDGF also seems to play a role in the positive cycle control (Melloni et al., 1994). Keratinocyte growth factor, an 18.9-kD member of the fibroblast growth factor family produced by lung fibroblasts, causes a prominent dose-dependent proliferation of type II cells in vivo (Ulich et al., 1994).

Like other cells, too, the type II pneumocytes have been shown to be regulated throughout their cycle by cyclins (and their appropriate genes). Wu et al. (1995) reported an up-regulation of the p34cdc2 kinase activity following hyperoxic lung injury. This paper also showed that cyclin D2 and cdc2 gene expression played an important functional role in determining the proliferative and non-proliferative phenotypes of the type II cells.

Fig. 1. Principal influence of exogenous and endogenous factors on the pulmonary parenchyma.
### Table 3. Immunohistochemical findings in bleomycin- and radiation-induced pulmonary fibrosis of rats (R) and mini pigs (P) and in human (H) fibrosis (after radiotherapy) with special emphasis on alveolar epithelial changes.

<table>
<thead>
<tr>
<th>MARKER</th>
<th>NORMAL TISSUE</th>
<th>FIBROSIS</th>
<th>SPECIES</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin</td>
<td>Type II: mainly CK18,B Type I: mainly CK7,19 Type III: CK18</td>
<td>No cell type-specific CK pattern Loss of type III-cells</td>
<td>R</td>
<td>Kasper et al., 1993a,b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P, H</td>
<td>Kasper et al., 1994a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Lectins</td>
<td>Type II: MPA Type I: LEL, ECL Type I: BPL SBA</td>
<td>Loss of MPA-binding LEL binding to type II cells Denudation marker</td>
<td>R</td>
<td>Kasper et al., 1993b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>Kasper et al., 1994t</td>
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<td></td>
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<td>R</td>
<td>Kasper et al., 1994c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td>Kasper et al., 1993u</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>Kasper and Hughes, unpublished</td>
</tr>
<tr>
<td>Secretory proteins</td>
<td>Type II: SP-A, SP-D</td>
<td>SP-D and SP-A enhanced intra and extracellularly</td>
<td>R, P</td>
<td>Kasper et al., 1994b,c</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Type I: Pdi Type II: CA II</td>
<td>PDI enhanced in all cell types CA II enhanced and in type I cells</td>
<td>R</td>
<td>Kasper et al., 1995a</td>
</tr>
<tr>
<td>Adhesion molecules</td>
<td>Type II: basolaterally</td>
<td>Epithelium, cytoplasmic in type I cells</td>
<td>H, R</td>
<td>Kasper et al., 1995c,d</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Type II: basolaterally</td>
<td>Epithelium, membranous</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Ep-CAM</td>
<td>Type II: basolaterally</td>
<td>Epithelium, cytoplasmic, downregulation of epithelial isoforms</td>
<td>R</td>
<td>Kasper et al., 1995b</td>
</tr>
<tr>
<td>CD44s and v</td>
<td>Type II: basolaterally</td>
<td>Enhanced in type I' cells</td>
<td>R</td>
<td>Kasper, unpublished</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Type I: positive</td>
<td>ICAM-1 shedding by type I cells</td>
<td>R</td>
<td>Kasper et al., 1995e</td>
</tr>
<tr>
<td>P cadherin</td>
<td>Type I: positive</td>
<td>Focally negative</td>
<td>P, R</td>
<td>Kasper and Müller, 1994</td>
</tr>
</tbody>
</table>

PDI: β subunit of poly 4-hydroxilase; CAII: carboxydrase II; DBA: Dolichos bifolius lectin; MPA: Maclura pomifera lectin; SBA: soybean lectin; BPL: Bauhinia purpurea lectin; ECL: Erythrina cristagalli lectin; LEL: Lycopersicum esculentum lectin; SP-D: surfactant protein D; SP-A: surfactant protein A.

### Table 4. Survey on further characteristic changes in the phenotype of alveolar epithelial cells during fibrosis.

<table>
<thead>
<tr>
<th>NORMAL TISSUE</th>
<th>FIBROSIS</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines</td>
<td>Metaplastic epithelium positive</td>
<td>Iyonaga et al., 1994</td>
</tr>
<tr>
<td>MCP-1(1)monocyte chemoattractant protein</td>
<td>Type II cells positive mRNA in type II cells Enhanced protein level</td>
<td>Payne et al., 1993, Nasn et al., 1991, Pique et al., 1993, Maddes et al., 1994, Khalil et al., 1991, Conn et al., 1994, Blau et al., 1994</td>
</tr>
<tr>
<td>TNFα</td>
<td>Type II: negative</td>
<td></td>
</tr>
<tr>
<td>TGFC</td>
<td>Type II: positive</td>
<td></td>
</tr>
<tr>
<td>TGFβ</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>TGFβ1</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>In vitro positive</td>
<td></td>
</tr>
<tr>
<td>Factors of the coagulation cascade</td>
<td>Further enhancement</td>
<td>Marshall et al., 1991; Gross et al., 1992</td>
</tr>
<tr>
<td>Plasminogen activator</td>
<td>Type II: positive</td>
<td></td>
</tr>
<tr>
<td>Tissue factor</td>
<td>Type II: positive</td>
<td></td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>Cytokeratin CK18</td>
<td></td>
</tr>
<tr>
<td>Adhesion molecules</td>
<td>Selectively in type II</td>
<td></td>
</tr>
<tr>
<td>α4, β6 integrin</td>
<td>Neoeexpression in the alveolar epithelium</td>
<td>Papadopoulos et al., 1993</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Type I: positive</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>Endothelin 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neoeexpression in type II cells</td>
<td>Giud et al., 1993</td>
</tr>
</tbody>
</table>

### 4. Phenotypic alterations of the alveolar epithelium: immunohistochemical analysis

Many studies have demonstrated that the alveolar epithelial cells, particularly the type II pneumocytes, play an important regulatory role in the remodelling process after diffuse lung injury. The equivalent of functional activation or inactivation of alveolar epithelial cells is a changing phenotype, which can be characterized by biochemical, molecular biological and immunocytochemical techniques. The use of «immunotargeting» (Funkhouser and Peterson, 1989) has already provided valuable insights into alveolar epithelial differentiation during development (Williams and Dobbs, 1990).

Different properties can be given to the different...
alveolar epithelial cells types (Table 2). The availability of antibodies to various antigens of type I and II cells should facilitate more profound investigations concerning alveolar epithelial function, differentiation and behaviour after injury.

Immunohistochemical marker analysis revealed modulation in the expression patterns of structural proteins, enzymes, secretory products, membrane receptors and adhesion molecules of alveolar epithelial cells (compare Tables 3, 4). Furthermore, different lectin binding to sugar residues of glycoproteins and glycolipids in lung epithelial cells were found employing lectin histochemistry (Schulte and Spicer, 1986; Spicer and Schulte, 1992; Kasper and Singh, 1995).

This report deals with important morphological and biochemical events that occur after exposure of epithelial cells to fibrosis-inducing agents. With respect to the time of injury, the earliest immunohistochemically detectable alteration of alveolar epithelial cells could be observed when searching for the cytokeratin patterns.

**Cytokeratins**

Intermediate filament proteins of the cytokeratin type are an established marker of epithelial cells (Moll, 1994) and can also be used to distinguish different alveolar epithelial subpopulations (Kasper et al., 1993a).

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Fig. 2. Paraffin sections of normal adult (a) and irradiated (b) mini pig lung. Immuno-peroxidase demonstration of cytokeratin 16 (monoclonal antibody KS18.04). Note the selective labelling of type II cells in a and the presence of the antigen in type I-like cells, possibly indicating incomplete differentiation of type II pneumocytes into type I cells. ABC technique with haemalaun counterstain. x 150
Irradiation or exposition of lungs to bleomycin resulted in expression of cytokeratin epitopes usually not present in the alveolar epithelium (Fig. 2; Woodcock-Mitchell et al., 1986; Kasper et al., 1993c). A possible explanation for the early induction of altered cytokeratin epitopes is a modulated signal transduction over AP-1-regulated transcription of cytokeratin genes (Pankov et al., 1994; Wu et al., 1994) that occurs after early release of reactive oxygen species in radiation-induced fibrosis. Irradiation may also directly induce the synthesis of inflammatory gene products (Morgan and Breit, 1995).

Adhesion molecules

Adhesion molecules are a group of cell surface glycoproteins that have been extensively investigated in numerous biological systems (Kemler, 1992; Watanabe et al., 1992; Bosman et al., 1993; Grunwald, 1993; Juhasz et al., 1993; Nathke et al., 1993; Nikkari et al., 1993; Bernstein and Liotta, 1994). For the pulmonary cells only sparse data exist (reviewed in Pilewski and

Fig. 3. Frozen sections of normal (a, c) and irradiated (b, d; 4 weeks after irradiation) rat lung. Double immunofluorescence demonstration of cytokeratin (a,b: monoclonal antibody MNF116) and E-cadherin (c,d: polyclonal antiseraum against rat E-cadherin kindly provided by Prof. Dr. Kemler, Freiburg, Germany). Note the increased E-cadherin expression of the alveolar epithelium in the irradiated sample (d). x 300
Fig. 4. Immunogold labelling of iOAM-1 in a pre-embedding technique of human fibrotic (a,b) and normal mouse (c) lung. Note the presence of gold particles at the surface of type I alveolar epithelial cells only. Type II pneumocytes (II) are devoid of staining. a, x10,500; b, c, x 31,500.
Albelda, 1993). Adhesion molecules of epithelial cells are members of the immunoglobulin supergene family (CAMs) such as ICAM-1, N-CAM and CEA or belong to the cadherins, particularly E-cadherin, or to the family of CD44s and CD44v isoforms. Further adhesion molecules include SAMs (cell substratum adhesion molecules) such as fibronectin and laminin and, last but not least, the integrins. The heterogeneity of epithelial adhesion molecules is reflected by different modes of biological action.

E-cadherin

E-cadherin, a Ca++- dependent 120kd transmembrane glycoprotein, is enriched in the adherens junctions of epithelial cells (Birchmeier et al., 1993) and mediates homotypic cell-cell interactions. The alveolar epithelial E-cadherin can be localized in type I-type I cell, in type II-type II cell and in type II-type I cell junctions (Böhm et al., 1994; Kasper et al., 1995c). Immunohistochemically, a predominantly basolateral E-

Fig. 5. Consecutive serial paraffin sections of rat lung after bleomycin exposition. ICAM-1 is focally present at the alveolar epithelial surface (a). Compare the corresponding labelling of type I cells with the type I cell-specific monoclonal antibody MEP-1 (b). Arrowheads in a indicate loss of ICAM-1 immunoreactivity. Arrow in b indicates alveolar area lacking epithelial cell surface. x 150
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cadherin has been observed in human lungs, which was abolished or changed to a cytoplasmic pattern in cases of severely injured specimens (Kasper et al., 1995c). In rat lungs, increased E-cadherin can be observed on alveolar epithelial type I cells (Fig. 3; Kasper et al., 1995d). The disturbance of E-cadherin distribution in pathologically altered pneumocytes reflects the involvement of epithelial adhesion molecules in remodelling processes after tissue injury.

The search for molecules whose expression changes during fibrogenesis can lead to the discovery of those molecules which predominantly play a role in malignant transformation and the development of metastases.

CEA

A further example of altered CAM expression is carcinoembryonic antigen (CEA), which is a cell surface glycoprotein of colon mucosal cells. It has a long history as a tumor marker (Majuri et al., 1994; Rapellino et al., 1994). CEA is a member of the CAM family and can mediate intercellular adhesion by homophilic and heterophilic interaction. The overexpression of CEA may lead to disruption of the normal tissue integrity (Benchimol et al., 1989). In lungs, CEA can be detected in bronchial epithelial cells and pneumocytes (Shiota et al., 1989). Alveolar epithelial cells of fibrotic lungs exhibited enhanced immunoreactivity for CEA, and patients with idiopathic interstitial fibrosis had increased CEA plasma values (Abbona et al., 1993; Harari et al., 1994).

ICAM-1

An example of a leukocytic CAM with importance for epithelial adhesion is ICAM-1 (Albeida, 991; Kelley and Singer, 1993). The ligand of ICAM-1 is LFA-1, a leukocyte specific integrin.

ICAM-1 is expressed in alveolar epithelial cells, mainly in type I cells (Christensen et al., 1993; Paine et al., 1994). The majority of isolated type II pneumocytes of humans express ICAM-1 (Cunningham et al., 1994; Guzman et al., 1994). In frozen as well as paraffin sections of human lung tissue it was not possible to distinguish type II cells from type I cells (Cunningham et al., 1994). In preembedding experiments, however, we were not able to detect ICAM-1 at the surface of type II pneumocytes (Fig. 4; Kasper et al., 1995e). During inflammation, the levels of «epithelial» ICAM-1 decrease dramatically (Kasper et al., 1995e). Fibrotic lesions in advanced stages of bleomycin-induced disease were characterized by ICAM-1 negative alveolar epithelial cells (Fig. 5). ICAM-1 can be shed in soluble form and has been detected in the bronchoalveolar fluid.

Fig. 6. Lowicryl HM20 embedded lung tissue from a patient with pulmonary fibrosis. Postembedding immunogold technique employing CD44v6-specific monoclonal antibody and goat anti mouse lgG coupled with 18 nm gold. Note the weak but selective immunostaining for CD44v6 at the basolateral cell processes (arrowheads; «microfoldings» according to Kawanami et al., 1982) of type II pneumocytes II; LB: lamellar bodies). Inset at the left upper corner shows the corresponding immunoperoxidase staining at light microscopic level. x 31,500 (inset x 600)
of patients with lung disease (Shiubo et al., 1992). ICAM-1 mediates adhesive interactions with different types of activated leukocytes. The possible role of ICAM-1 in fibrogenesis remains unclear. Perhaps the loss of ICAM-1 from the alveolar epithelium reduces the ability to interact with leukocytes and alveolar macrophages, thus preventing retention of intraalveolar inflammation.

CD44

CD44 is a family of cell surface glycoproteins also termed hyaladherins (Underhill, 1989), belonging to the group of adhesion molecules. The standard form of CD44 (CD44s) functions as a receptor for hyaluronic acid as well as collagen and fibronectin (Shiota et al., 1989; Aruffe et al., 1990; Lesley et al., 1993). Furthermore, variant molecules of CD44 (CD44v) have been described, whose primary mRNA transcripts undergo alternative splicing of variant exons. The epithelial form containing by variant exons v6-v10-encoded proteins is commonly expressed on normal epithelial cells (Shiota et al., 1989; Sceaton et al., 1992; Lesley et al., 1993). CD44 variants containing exons v6- and v9-encoded epitopes are selectively found in type II pneumocytes of mouse, rat and human lungs with a strong basolateral distribution pattern (Fig. 6; Kasper et al., 1995b) and in basal cells of the bronchial epithelium (Terpe et al., 1993, 1994). CD44s were observed in a smaller number of type II pneumocytes and at the surface of alveolar macrophages (Underhill et al., 1993) and in some interstitial cells. The examination of fibrotic lungs in humans and mini pigs revealed downregulation of CD44v6 and CD44v9 in the alveolar epithelium (Kasper et al., 1995b). First preliminary experiments using a post embedding technique showed a selective localization of CD44 in foot processes of type II pneumocytes (see also chapter 5; Fig. 6).

Integrins

The integrins are a family of transmembrane glyco-

Fig. 7. Paraffin section of fibrotic rat lung (bleomycin-induced). Double immunofluorescence demonstration of cathepsin B (a; polyclonal rabbit antibody) and cytokeratin (b; monoclonal antibody MNF 116) in alveolar epithelial cells (arrow, thin arrow shows a cytokeratin-positive cell with four small cathepsin B-positive granules; small arrow-heads indicate cytokeratin-negative alveolar macrophages). Note some autofluorescence of erythrocytes, particularly in a. x 300
proteins involved in cell-cell or cell-matrix interaction (Kishimoto and Rothelien, 1994). They are heterodimers consisting of $\alpha$ and $\beta$ subunits. Most integrins bind extracellular matrix ligand such as collagen, laminin and fibronectin but also bind adhesion molecules (ICAMS or VCAM-1) of other cells. Pneumoocytes consistently express $\alpha 1$, $\alpha 2$, $\alpha 3$ and $B1$ chains (Papadopoulos et al., 1993), which are downregulated in malignancy (Roussel et al., 1994). In pneumocytes of fibrotic samples neo-expression of $\alpha 4$, $\alpha 6$ and $B4$ chains has been reported (Papadopoulos et al., 1993).

**Enzymes**

Alveolar epithelial cells have been studied for a variety of enzyme activities (for detailed information see Simon, 1991). These activities are connected with the diverse biological functions of the alveolar epithelium and are mainly localized in type II pneumocytes (Table 2; further reviewed in Sannes, 1991). Some of the enzyme activities are also present in bronchial epithelial Clara cells and in the alveolar macrophages, but are excluded from the present discussion which deals with the epithelial changes after injury.

The first «marker enzymes» which were described for type I alveolar epithelial cells were the heat-stable alkaline phosphatase (Nouwen et al., 1990) and the carboxypeptidase M (Nagae et al., 1993). The function of both enzymes in type I cells and their involvement in pulmonary disease still has to be clarified. To date, no enzyme activities have been reported that are to some extent a specific marker for epithelial injury or the early activation of epithelial cells during fibrogenesis.

Evidence has accumulated that proteases, which are also produced by the alveolar epithelium may be responsible for proteolysis of the surrounding extracellular matrix. Tissue degradation and remodelling in lung injury during early and late stages of pulmonary fibrosis are regulated by the action of proteolytic enzymes, such as metalloproteinases (e.g. collagenases, gelatinases, stromelysin); serine proteases (e.g. urokinase-type plasminogen activator (uPA)); aspartyl proteases (e.g. cathepsin D, E; pepsinogen II) and cysteine proteinases (e.g. cathepsin B, S, H, L).

Two aspartatic proteinases, pepsinogen II and cathepsin E, were identified immunocytochemically in type II pneumocytes and bronchiolar Clara cells, respectively, and epithelial hyperplasia in interstitial lung diseases was accompanied by enhanced expression of both proteinases (Bosi et al., 1993).

The cysteine proteinase cathepsin B is involved in extracellular matrix processing of epithelial lung tumor cells (Inoue et al., 1994; Spiess et al., 1994; Werle et al., 1994) and in vivo degradation of rat lung collagen (Cardozo et al., 1992). Cathepsin H has been found in normal type II pneumocytes and alveolar macrophages (Ishii et al., 1991).

Lung macrophages are the source of a number of potent proteases that are capable of degrading structural elements of the lung (Chapman et al., 1994). Under pathological conditions cathepsin B was found in myofibroblasts and in the regenerating alveolar epithelium (Fig. 7; own unpublished data). Therefore, epithelial protease involvement in the pathogenesis of pulmonary fibrosis may be considered (for further discussion see chapter 5).

Alveolar epithelial type II pneumocytes express both the serine protease uPA and tissue factor: important components of the coagulation and fibrinolytic cascade which are involved in the intra-alveolar fibrin clearance during acute and inflammatory lung diseases (Marshall et al., 1991; Gross et al., 1992; Simon et al., 1992). Furthermore, alveolar epithelial cells also express cell surface receptors for uPA (Gross and Schultz, 1992). uPA and its receptor are biologically relevant components of a proteolytic system involved in the regulation of cellular interaction and cellular migration during tissue repair (Blasi et al., 1993). Epithelial cell-mediated fibrinolysis may be necessary to avoid that intra-alveolar fibrin, lying at the alveolar surface, prevent the normal reepithelialization of denuded basal membranes (Simon et al., 1992).

Metalloproteinases, specifically directed to ECM components, play a key role in ECM degradation and are found in several pulmonary cell types, particularly polymorphonuclear leukocytes and macrophages (Weiss and Peppe, 1986; Welgus et al., 1990). They contribute to the lesions of the ECM observed in acute lung injury (D'Ortho et al., 1994). In the meantime, there are also reports on epithelial localization of such a type of enzyme activity (Arden et al., 1993; Dunsmore et al., 1995; Pardo et al., 1995). Collagenerases and tissue inhibitor of metalloproteinases (TIMP) were additionally found in lung fibroblasts and with increased activity in homogenates of fibrotic lung (Pardo et al., 1992). After hyperoxic exposure of murine lungs, increased metallothionin levels were found in inflammatory cells (Piedboeuf et al., 1994).

**Cytokines**

The key role of cytokines for acute and chronic inflammation in the pathogenesis of pulmonary fibrosis is widely accepted (Gauldie et al., 1993; Piguet, 1993). Inflammatory cells, including alveolar macrophages and endothelial cells, participate in the disease process through the release of a variety of cytokines such as interleukins, the tumor necrosis factor (TNF), monocyte chemotactic peptide monocyte chemo-attractant protein (MCP-1), transforming growth factor (TGFβ, TGFβ), platelet-derived growth factor (PDGF), granulocyte macrophage colony stimulating factor (GM-CSF) and others (reviewed in Gauldie et al., 1993).

In recent studies, evidence for the synthesis and secretion of cytokines by alveolar epithelial cells come from in vivo and in vitro experiments (Table 4). However, most schematics exclude the alveolar epithelium from a constitutive cytokine expression and
interaction (Kelley, 1990).

If the cytokines, such as, for example, TNFα, TGFβ or MCP-1, are present in normal as well as regenerating epithelial cells, then their function must be augmented to include a role in epithelial wound healing and in local regulation of inflammatory responses within the alveolar space. In case of MCP-1, alveolar epithelial cells may be responsible for the initiation and maintenance of inflammatory processes in the lung by recruiting and activating the circulating monocytes through the secretion of MCP-1 (Paine et al., 1993). Interestingly, MCP-1 was secreted towards the apical (alveolar) compartment of type II cells, indicating the major role of this cytokine in the activation of alveolar macrophage (Paine et al., 1993). For TNFα, which is produced by type II pneumocytes, contribution to the surfactant synthesis via paracrine exposure of pneumocytes to low concentrations of the cytokine has been suggested (Arias-Diaz et al., 1994). The TNF gene is a typical target for the AP-1- and NFKB-controlled transcription after irradiation (Morgan and Breit, 1995).

TGFβ is a potent chemoattractant for macrophages and fibroblasts and induces transcription of other cytokines and TGFβ itself (Laiho and Keski-Oja, 1992;

![Image](0x0)
Border and Noble, 1994). All observations suggest that TGFβ, as a key cytokine, initiates and terminates tissue repair in pulmonary fibrosis (Kovacs and DiPietro, 1994). In patients with advanced idiopathic pulmonary fibrosis prominent staining of hyperplastic type II pneumocytes for TGFβ was found (Khalil et al., 1991). The enhanced production of TGFβ by type II pneumocytes is regarded in terms of inhibition of proliferation and in promoting epithelial differentiation (Khalil and Greenberg, 1991).

TGFα, a cell surface-associated mitogen and a chemotactic factor, is also present in alveolar type II pneumocytes and its expression is upregulated together with the EGF receptor following bleomycin-induced injury of the rat (Madtes et al., 1994). This activity contributes to the proliferative processes and inflammatory cell migration during fibrogenesis.

**Modulation of other lung specific antigens**

Injury to epithelial cells was also determined by the biochemical assessment of type I cell-specific antigens in the alveolar fluid (Kasper et al., 1995; McElroy et al., 1995). In addition, changing glycoconjugates patterns of alveolar epithelial cells can be a sensitive indicator of cell damage or activation as has been evaluated by carbohydrate histochemistry (Kasper and Singh, 1995).

**5. Epithelial cell-fibroblast interactions in lung injury and repair**

The epithelial cell-fibroblast interaction starts to become evident through the early ultrastructural observations of direct cell to cell contacts between cuboidal epithelial type II pneumocytes and fibroblasts (Bluemink et al., 1976; Grant et al., 1982; Adamson and King, 1984). Contrarily, type I alveolar epithelial cells have a smooth basal cell surface lacking any contacts with interstitial cells. The morphological substrate of these contacts are foot processes (microfoldings; Kawanami et al., 1982), which penetrate and perforate the epithelial basement membrane. These cytoplasmic extensions of the type II cells crossing the basal membrane were most abundant in the fetal period (Grant et al., 1982; Riso, 1983; Adamson and King, 1986; Bluemink et al., 1986; Adamson, 1992; Lakritz et al., 1992; Young and Adamson, 1993). The number of foot processes varies with gestational age (Young et al.,

![Fig. 9. Schematic overview about cell-cell, cell-matrix interaction during fibrogenesis (CE: capillary endothelium; BM: basement membrane; ECM: extracellular matrix; F: fibroblast; MM: alveolar macrophage).](image-url)
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1991) and increases in number under condition of type II cell proliferation in human lung disease (Kawanami et al., 1982). From in vitro and in vivo experiments of lung injury, Adamson et al. (1990) suggested a reciprocal epithelial-fibroblast control system in the lung. Epithelial damage and successive denudation of basal membrane may promote fibroblast growth which is inhibited by type II cells under normal conditions (Hunter et al., 1989; Klein and Adamson, 1989; Adamson et al., 1991). However, suppression of fibroblast growth does not promote reepithelialization of injured lung explants (Bowder et al., 1994). In vitro co-culture experiments confirmed the complex interdependence of epithelial cells and fibroblasts and the modulation of metabolic properties by secreted factors derived from both cell types (Adamson et al., 1990; Young et al., 1991; Griffin et al., 1993). Fibroblast-derived factors have been shown to influence epithelial cell proliferation and differentiation (reviewed in Bowden et al., 1994). Conversely, alveolar epithelial cell-derived factors or cytokines act on fibroblasts (see above). This involvement of epithelial lung cells in intercellular signalling network has recently been augmented for TGFβ, hepatocyte growth factor, TGFβ, C3 complement component, IGF-1, and a factor that protects mesenchymal cells against apoptosis (Griffin et al., 1993; Williams et al., 1993; Mason et al., 1994; Rosenthal et al., 1994; Wen et al., 1994). Control mechanisms involving epithelial cell-fibroblast interactions may also depend on direct cell-ECM (Dunsmore and Rannels, 1995) or cell-cell interactions (for involvement of adhesion molecules see above).

The ECM of pulmonary tissue displays a great polymorphism (Guzowski et al., 1990; Sannes, 1991) and binds various biologically active molecules, such as TGFβ, GM-CSF and other cytokines (for review see Grimaud and Lortat-Jacob, 1994). Alveolar type II cells produce type IV collagen and other ECM-components which have diverse functions in regulating epithelial cell interaction with the ECM (Sage et al., 1983; Skinner et al., 1987; Maniscalco and Campbell, 1992; Rannels et al., 1992; Simon et al., 1993; Grimaud and Lortat-Jacob, 1994). The selective localization of CD44 adhesion molecule in foot processes of human type II pneumocytes as shown here for the first time (Fig. 6) can be a first step in the evaluation of specific ligands in the extracellular matrix.

At the present time, some evidence has been obtained to support the role of endogenous lectins in regulation of cell-cell and cell-matrix interactions (reviewed in Hughes, 1994). Galectin 3 is an endogenous carbohydrate binding protein with specificity to terminal β-galactose residues. Using immunocytochemistry, we showed that galectin 3 was localized to alveolar macrophages and to a lesser extent to bronchial epithelial cells. Shortly after irradiation-induced lung injury galectin 3 was expressed at the surface of the newly-formed type I alveolar epithelium and in part at the apical surface as well as in the cytoplasm of type II pneumocytes (Fig. 8; Kasper and Hughes, unpublished observation). Ligation of secreted lectin at the alveolar surface may promote spreading and migration of differentiating type I cells occurring during re-epithelialization.

Conclusion and outlook

There is general reason to believe that the development of pneumonitis and pulmonary fibrosis is caused by the disruption of the balance between various cell populations of the pulmonary parenchyma. Lung injury and repair during fibrogenesis mainly involve changes in the extracellular matrix protein composition. The (myo) fibroblasts of the alveolar wall are regarded as being the most efficient cells in the fibrotic process. Other inflammatory cells of the interstitium comprise the mast cells and lymphocytes (reviewed in Aldenborg et al., 1993; Ward et al., 1993a,b; Franko and Sharplin, 1994; Hamacher and Schgabeck, 1994). However, type I pneumocytes, endothelial cells, as well as alveolar macrophages are the primary target of injury. Subsequently, products of damaged type I and endothelial cells or signals derived from disturbed intercellular epithelial adhesion and factors released by alveolar macrophages such as cytokines, procoagulant factors and reactive oxygen species stimulate the type II pneumocyte to proliferate, to secrete various cytokines in autocrine and paracrine fashion, to release enzymes and other metabolic products and to contact the fibroblasts via epithelial foot processes (Fig. 9). Thus, these contacts may be, through a mechanism unknown so far, a parallel stimulus for subsequent proliferation and activation of fibroblasts, but may also represent a method of inhibiting epithelial proliferation and accelerating differentiation of type II pneumocytes, as occurs in fetal lung, and probably during repair. After lung injury and repair a transformation of proliferating type II pneumocytes into type I alveolar epithelial cells occurs (Evans et al., 1975). This transformation process involves an intermediate cell type which is cuboidal and lacks lamellar bodies. Interestingly, similar intermediate or precursor cells were seen during fetal development (Otto-Verberne and Ten Have-Oppbroek, 1987). Immunohistochemical stainings employing epithelial lung cell markers show a very similar expression pattern of alveolar epithelial remodelling during fetal development and repair. In lung fibrosis, however, the high number of cells expressing an intermediate differentiation pattern between type II and type I cells could also indicate a retarded (disturbed) differentiation process from the type II to the type I cell. Further studies are needed to explore whether there is a disturbance in epithelial transformation of type II into type I cells during fibrogenesis.
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Acknowledgements. This study was supported by the Bundesminister für Bildung, Wissenschaft, Forschung und Technologie (07NB03). The authors thank Prof. I.Y.R. Adamson (University of Manitoba, Winnipeg, Canada) for his critical reading of this review.

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