Immunohistochemical expression of surfactant apoproteins in pneumonic ovine lungs

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Abstract: The present study was undertaken to investigate the immunohistochemical expression of pulmonary surfactant proteins (SP-A, SP-B, proSP-C) associated with proliferating cell nuclear antigen (PCNA), thyroid transcription factor-I (TTF-I), and lymphocytic phenotypes (CD3+ T and CD79αcy+ B cells) in ovine lungs with exudative and verminous pneumonia. Pneumonic lungs (n = 50) were classified as presenting fibrinous bronchopneumonia (n = 16), abscessing necrotic bronchopneumonia (n = 10), verminous pneumonia (n = 15), and interstitial pneumonia (n = 9). The lungs with fibrinous bronchopneumonia displayed consolidation of lobes with adhesion to surrounding tissues. The lungs with verminous pneumonia were characterized by lungworm sections in airways. Type II pneumocytes were highly proliferated in the pneumonic lungs and revealed intense immune positivity for the surfactant proteins. Proliferated type II cells, lymphocytes, and fibroblasts showed a strong immune reaction for PCNA. TTF-I positivity occurred in the ovoid nuclei of alveolar II epithelial cells. Lymphoid infiltrations revealed a positive reaction to the CD markers. In conclusion, the present study revealed that alveolar type II epithelial cells were severely proliferated in the sheep lungs with pneumonia and produced most of surfactant proteins compared to normal type II cells. Consequently, type II cells are very important in the restoration of damaged lungs, and surfactant apoproteins might play an important role in the host defense mechanisms against involved microorganisms.

Key words: Immunohistochemistry, pneumonic ovine lungs, surfactant proteins

1. Introduction

Pneumonia is an important lung disease arising from the protective inflammatory response of the airways and alveoli in the lungs to injury and infection, resulting in the damaging of parenchymal tissue (1). The disease causes heavy economic problems in ovine farms, due to either mortality or decreased growth, along with animal welfare implications. Pneumonia occurs in different types in sheep, one of which is acute fibrinous bronchopneumonia observed in sheep, and the other is the chronic nonprogressive pneumonia of lambs (2). The alveoli of the lungs are mainly covered by two types of alveolar epithelium named type I and II cells. Type I alveolar epithelial cells constitute approximately 96% of the alveolar surface and allow the exchange of gases between the alveoli and the vascular compartment. Type II cells play an important role in the repair of damaged parenchyma and in preventing the collapse of alveoli (3–6). Lung surfactants are surface-active substances produced by alveolar type II epithelial cells and nonciliated Clara cells. The main function of surfactant proteins is to reduce the surface tension at the air–liquid interface in the alveoli of the lungs. Pulmonary surfactant apoproteins are a mixture of lipids and proteins. To date, four types of surfactant apoproteins have been identified and named as SP-A, SP-B, SP-C, and SP-D regarding the chronological order of their detection (5,7–11).

Proliferating cell nuclear antigen (PCNA) is a well-characterized antigen produced in the nuclei of cells during DNA replication, and it has a central role in many cellular processes including DNA synthesis and damage repair. PCNA has often been used as a diagnostic and prognostic tool in different types of neoplasms (12). Thyroid transcription factor-I (TTF-I) is also found mainly in normal type II alveolar epithelial cells in the lungs and has important roles in epithelial morphogenesis and synthesis of pulmonary surfactant proteins and in the regulation of secretory products in Clara cells. Since TTF-I usually retains its tissue specificity, it has often been used in the identification of primary tumor cell lines in some thyroid and lung carcinomas (13).

Pulmonary surfactants have mainly been examined in neoplastic human (14,15) and ovine (16–18) lungs. However, there is no record of the observation of immunohistochemical expression of surfactant proteins (with the exception of SP-D) in ovine lungs with exudative and verminous pneumonia. The present study was undertaken to investigate the immunohistochemical...
expression of surfactant apoproteins (SP-A, SP-B, proSP-C) associated with PCNA and TTF-I. Additionally, the lymphocyte infiltration into the pneumonic lungs was evaluated by determination of lymphocytic phenotypes (CD3+ T and CD79αcy+ B cells).

2. Materials and methods

2.1. Tissues and histopathology

Materials of the study were the lungs from sheep of various ages with pneumonia (n = 50) and healthy sheep (n = 5) slaughtered at a local abattoir. Following the gross examination, all lung specimens were fixed in 10% neutral buffered formalin solution, routinely processed, and embedded in paraffin. Sections of 4 µm in thickness were cut and stained with hematoxylin and eosin (H&E).

2.2. Immunohistochemistry

Serial lung sections were stained immunohistochemically using antisera against pulmonary surfactant apoproteins (SP-A, SP-B, proSP-C), PCNA, TTF-I, and CD3+ T and CD79αcy+ B lymphocytes (19). Details of the primary antibodies used in the study are shown in the Table. Sections of 4 µm in thickness were deparaffinized in xylene and rehydrated through graded alcohols to water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 20 min. The sections were incubated with Tris-buffered saline (TBS, pH 7.6) for 5 min and subsequently placed in citrate-buffered saline (pH 6.0) in a microwave oven for 20 min for antigen retrieval. After having been washed with TBS for 5 min, the sections were incubated with 5% normal goat (SP-A, SP-B, proSP-C, TTF-I) or rabbit (PCNA, CD79αcy) serum for 1 h at room temperature. The sections were then incubated with the primary antibodies. Having been washed with TBS three times for 5 min, respectively, the sections were incubated with biotinylated goat antirabbit IgG (SP-A, SP-B, proSP-C, TTF-I) and biotinylated rabbit antimouse IgG (PCNA, CD79αcy), at a dilution of 1/200 in TBS, for 60 min at room temperature (secondary antibodies supplied by Dako, Carpinteria, CA, USA). After further washing of the sections with TBS, they were treated with streptavidin peroxidase complex (Dako), at a dilution of 1/300, for 30 min at room temperature. Polyclonal rabbit antihuman CD3 antibody and the LSAB2 system (Dako) were used to differentiate T lymphocytes, following the manufacturer's instructions. Immunoreaction was obtained using 3,3-diaminobenzidine or 3-amino-9-ethylcarbazole as the chromogen. Mayer's hematoxylin was used to counterstain. All primary antibodies were removed from negative control sections, which were incubated with either TBS or diluted normal serum from the species in which the primary antibodies were raised. Mean numbers of immunopositive cells that exhibited specific immunolabeling for each antibody were assessed in three most representative fields with the 40× objective of a light microscope.

3. Results

3.1. Gross and histopathological findings

Pneumonic lungs revealed varying degrees of consolidation, fibrin collections, and adhesions in the ventral or dorsal parts of lung lobes. Based on histopathology, pneumonia was classified as fibrinous bronchopneumonia (n = 16), abscessing necrotic bronchopneumonia (n = 10), verminous bronchopneumonia (n = 15), or interstitial pneumonia (n = 9). The predominance of fibrinous bronchopneumonia was the presence of a mixture of macrophages and neutrophils with the accumulation of oat cells. There was also edema in alveoli, congestion, and hemorrhages as well as fibrin clots within the interlobular septa. Abscessing necrotic bronchopneumonia showed varying sizes of necrotic foci surrounded by connective tissue, neutrophilic accumulation within the airways, and, peripherally, gland-like type II cell proliferation. Verminous pneumonia revealed adult worm sections and eggs within airways with eosinophilic leukocytic infiltration. There were also large subpleural lymphoid foci

Table. Details of primary antibodies used for IHC.

<table>
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<th>Antibody</th>
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<th>Incubation conditions</th>
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<td>Polyclonal rabbit antihisheep SP-B*</td>
<td>1/2000</td>
<td>Overnight, 4 °C</td>
<td>Chemicon (AB3780)</td>
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<tr>
<td>Polyclonal rabbit antihuman proSP-C*</td>
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<td>Overnight, 4 °C</td>
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<tr>
<td>Polyclonal rabbit antirat TTF-I*</td>
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<td>Overnight, 4 °C</td>
<td>Seven Hills Bioreagents (WRAB-TTF I)</td>
</tr>
<tr>
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<td>Overnight, 4 °C</td>
<td>Chemicon (PC10)</td>
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<tr>
<td>Polyclonal rabbit antihuman CD3*</td>
<td>1/150</td>
<td>Room temperature</td>
<td>Dako (N1580)</td>
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<tr>
<td>Monoclonal mouse antihuman CD79αcy*</td>
<td>1/25</td>
<td>Overnight, 4 °C</td>
<td>Dako (M7051)</td>
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*Antibody has been shown to cross-react with sheep according to the manufacturer’s datasheet.
with germinal centers containing mineralized material and neutrophilic exudation in airways. The mineralized material was identified as a remnant of dead parasites by using H&E-stained sections. Interstitial pneumonia was characterized by interalveolar septal thickening with proliferated type II cells and mononuclear cell infiltration. Healthy lungs did not show any lesions.

3.2. Immunohistochemistry

3.2.1. Surfactant proteins (SP-A, SP-B, proSP-C)
In fibrinous bronchopneumonia, type II cells proliferated, glandular in appearance, around the necrotic areas and showed positive labeling for SP-A (Figure 1) and SP-B. The number of immunopositive type II cells for the markers at three most representative areas varied between 70 and 100 cells. SP-A immunolabeled hyperplastic type II cells were recognized by the presence of numerous cuboidal nuclei, which formed a continuous alveolar lining, or gland-like proliferations around the necrotic foci and neutrophilic collections. Extracellular staining was seen to be high in SP-A-immunostained sections compared to SP-B. In less affected lungs, the number of proliferated type II cells was lower than seen in chronically affected sheep. Alveolar macrophages also showed a positive cytoplasmic reaction to both SP-A and SP-B. In cases of acute bronchopneumonia showing septal hyperemia, fibrinous exudation, and edema in the lung parenchyma, alveolar type II cell hyperplasia was found only in alveoli and the rate of the cells was 30–40. Type II cells reacting to the SP-A and SP-B antibodies were easily distinguished from the oat cells, which did not show a positive labeling for SPs. Proliferated type II cells infrequently revealed mitosis in that cytoplasmic granules were also positive for SP-A and SP-B. In a few lungs with typical signs of pneumatic pasteurellosis, the mean number of type II cells was 40–50. However, in alveoli densely occluded by oat cells, type II cells were not seen, or only a few cells were scarcely found.

In hyperplastic type II cells, positive reaction for SP-B was evidently detected in the large cytoplasmic granules of complete cytoplasm, which were most probably lamellar bodies and caused expansion of the cytoplasm and hypertrophy of the cells. In normal lungs, positive staining occurred only at the apical cytoplasm of the cells. Nonciliated Clara cells positively stained with both SP-A and SP-B were noticeably frequent in terminal bronchioles compared to healthy lungs (Figure 2). ProSP-C labeling was detected in the perinuclear area of the alveolar type II epithelial cells. No extracellular reaction was detected in any cases. SP-C positive staining openly disclosed type II cells without positive reaction of Clara cells. In healthy or unaffected areas of pneumatic lungs, type II cells were mainly found at alveolar septa and within alveoli, which were found to protrude into the lumen. As in SP-B stained sections, proliferated type II cells showed cytoplasmic distension and intense reaction for proSP-C.

In abscessing necrotic bronchopneumonia, SP-A and SP-B stainings confirmed that numerous type II cells proliferated at the margin of the fibrotic capsule surrounding the necrotic areas (Figure 3). The number of alveolar type II cells was over 100 in the high-power field (HPF). Although type II cells were difficult to identify among inflammatory cells and fibrocytes in H&E-stained sections, immunolabeling openly disclosed the hyperplastic type II cells in all parts of the lungs. Immune reaction products were large granules distributed within the cytoplasm of the cells. In some areas, hyperplasia of type II cells was so severe that all microscopic fields consisted of the cells. However, in some areas where the fibroblastic reaction was predominant, the number of type II cells was fewer than in other regions. As expected, immunostainings using proSP-C identified numerous proliferated type II cells (Figure 4). Type II cells were rarely desquamated into the alveoli and mixed with alveolar

Figure 1. SP-A immunopositive reaction in the cytoplasm of proliferated type II cells in a lung with fibrinous bronchopneumonia. IHC. Bar: 51 µm.

Figure 2. SP-A positive labeled nonciliated Clara cells in terminal bronchioles. IHC. Bar: 51 µm.
macrophages. However, macrophages did not show a positive reaction for the marker. Clara cells did not show a positive immunolabeling to proSP-C.

In verminous pneumonia, where lungs contained worms or eggs in airways, proliferation of type II pneumocytes was generally moderate (Figure 5). Immunolabeled cells for SP-A and SP-B were often seen to desquamate into the alveolar lumen and mixed with worms and infiltrative cells. Severe hyperplasia of type II cells immunopositive to SP-A and SP-B were evident around the parasitic foci, although a few type II cells were detected in the alveoli and interalveolar septum. In some areas with thickened alveolar septa, the number of type II pneumocytes and surfactant protein expression increased parallelly to the thickening of the septum. Even though alveoli around the inflammatory foci could not be easily observed because of severe fibrosis and inflammatory cells, high amounts of extracellular surfactant protein for SP markers and glandular hyperplasia of type II pneumocytes were detected. In particularly, surfactant protein expression at inflammatory foci was seen as focal aggregations of large brownish granules, or rarely within the cytoplasm of type II cells. Moreover, in some areas type II cells were difficult to identify because of severe extracellular surfactant protein reaction to the marker.

In interstitial pneumonia, immune-stained sections revealed that thickening of the alveolar septa was predominantly due to the hyperplasia of type II pneumocytes. Intense reactivity to both SP-A and SP-B was present in both extracellular surfaces and the cytoplasm of type II cells. In some areas that contained few neutrophils and thickened alveolar septa, type II cells highly proliferated and secreted high amounts of surfactant. The mean number of proliferated type II cells in these areas was over 100 cells in the HPF. Unlike exudative pneumonia, type II cells did not show a glandular pattern of hyperplasia and randomly proliferated in the parenchyma. Interestingly, even though the cells were highly proliferated, extracellular expression of SP-A or SP-B was rarely noticed. ProSP-C positive staining confirmed that type II cells were mixed with inflammatory cells and fibrocytes at the thickened alveolar septa. ProSP-C positive cells were also seen to increase in the margin of interlobular septa and large airways.

Healthy lungs revealed a small number of immunopositive type II cells to the SPs, with 2 or 3 cells in the HPF (Figure 6). Positive cells were commonly located at the corners of alveoli. Immunoreaction products were mainly seen to be small granules. The granules in the sections stained with SP-A and SP-B were located at the apical surface of the cells, but in proSP-C-stained sections, immunoreaction products were seen next to the nuclei. At high magnification (100× objective), the number of granules was smaller than that seen in the pneumatic lungs. Nonciliated Clara cells of terminal bronchioles were immunopositive against SP-A and SP-B.
3.2.2. Proliferating cell nuclear antigen
PCNA nuclear positive reaction was predominantly detected in ovoid nuclei of type II cells that proliferated in a glandular appearance at the margin of fibrotic tissue surrounding necroses (Figure 7). The number of immunopositive cells varied among the pneumonic lungs and a high number of the cells were seen in lungs, resulting in fibrosis. In fibrinous and necrotic bronchopneumonia, nuclear positive cells were mainly found at the margin of necrotic areas, with over 100 cells in the HPF. PCNA positive immunolabeling was also found in the germinal centers of bronchus-associated lymphoid tissue (BALT) and at the epithelial lining of bronchi and bronchioles. In healthy lungs, few cells showed a positive reaction in alveolar septa, with 3–5 cells in the HPF. In terminal bronchioles, few lining epithelial cells showed nuclear positivity to PCNA. However, these cells could not be identified as either Clara or ciliated epithelial cells. PCNA positive stained spindle cells were identified as a fibroblastic lineage, which was confirmed in H&E-stained sections. Additionally, a few cells showing mitosis at the alveolar septa revealed positivity to PCNA.

3.2.3. Thyroid transcription factor-I
TTF-I positive reactions predominantly occurred in the ovoid nuclei of type II cells. Nonciliated and ciliated lining cells of terminal bronchioles from both affected and healthy lungs revealed nuclear expression for TTF-1, with a strong immunolabeling of type II cells in the lungs of young animals. Even though TTF-I positive reaction was found in all alveolar type II cells, a diffuse and strong reaction for the marker was detected in the nuclei of proliferated epithelial cells at the margin of fibrotic tissue in lungs with abscessing necrotic bronchopneumonia. Healthy and unaffected areas of the pneumonic lungs expressed nuclear reaction to TTF-1.

3.2.4. Lymphocytic phenotyping (CD3+ T and CD79αcy+B cells)
The number of CD3+ T cells in all of the pneumonic lungs was higher than the number of CD79αcy+B cells. In verminous bronchopneumonia, CD3+ T and CD79αcy+B cells were mainly seen in lymphocytic cuffing of airways. Abscessing necrotic bronchopneumonia often displayed positively stained lymphocytes for the CD markers in the pneumonic parenchyma. CD3+ T cells often infiltrated into the thickened alveolar septa by fibrosis and the number of infiltrated CD3+ T cells increased in the lungs showing lymphoid cuffing of airways. CD3+ T and CD79αcy+B cell infiltrations were also seen in subpleural lymphoid foci containing central calcified masses and in mononuclear infiltrations of vessels. Although CD3+ T and CD79αcy+B cells infiltrated into the peribronchial areas and alveolar septa, a high number of the cells was detected in the peribronchial lymphoid tissues (Figure 8). No positive cells for the CD markers were found in healthy lungs.

4. Discussion
Pneumonia is an important cause of loss in the sheep industry and many forms of the disease have been reported with clinical signs and mortality (1). The disease begins with a severe inflammatory response in the wall of alveoli and subsequent parenchymal destruction and extensive fibrohyperplasia of the alveolar airspace ensues, consisting of fibroblasts, capillaries, and connective tissue products (1,20). In the present research pneumonic lungs revealed marked fibrosis, alveolar epithelization, and mononuclear cell infiltration around the airways. In particularly, in lungs with fibrinous bronchopneumonia, alveoli and bronchioles were occluded by fibrinous exudation and were repaired by connective tissue. However, proliferative or reparative characteristics of alveolar type II cells in pneumonic lungs have not been investigated to date. Moreover, little information is available regarding the repair of damaged

Figure 6. Immunopositive reaction of type II cells for SP-B in a healthy sheep lung. IHC. Bar: 51 μm.

Figure 7. Widespread positive reaction for PCNA of proliferated type II cells around the necrotic foci in a pneumonic lung. IHC. Bar: 543 μm.
parenchyma caused by different infectious agents and worms in ovine lungs with pneumonia. Therefore, this research was undertaken to investigate the proliferation of type II cells in various types of ovine pneumonia by using surfactant proteins as a marker for type II cells and it was found that type II cells proliferated highly in pneumonic lungs regardless of the injurious agent. Grubor et al. (21) found that hyperplasia of alveolar type II cells was the most striking histological finding in *Mannheimia haemolytica*-infected lambs and that cell-associated SP-D expression decreased with the progression of inflammation and with the increased presence of proliferative alveolar epithelium. Alveolar injury begins with damage or desquamation of type I epithelium, followed by type II cell hyperplasia and eventually transformation of type II cells into type I epithelium. Thus, type II cell hyperplasia represents a nonspecific reparative response to various forms of lung injury and plays important roles in lung development and restoration of the lung parenchyma (22). In this work, identification of type II cells depended on the presence of cuboidal nuclei and cytoplasmic granules supposed to be osmophilic lamellar bodies in the SP-immunostained sections, as reported by others (3,6). Hyperplasia of alveolar type II epithelial cells was higher in cases of pneumonia caused by bacterial agents than those of verminous pneumonia. Therefore, it is reasonable that hyperplastic type II cells developed as a response to alveolar injury in a variety of pulmonary disorders, especially in the setting of inflammation and interstitial fibrosis and during the healing phase of parenchymal injury. Shilo et al. (23) reported that type II cell hyperplasia is most evident in severe alveolar damage, but it is also detectable in organizing or interstitial pneumonia. It is also well known that type II cells serve as stem cells for the replacement of type I cells that are damaged as a consequence of pulmonary diseases or chemical-induced injury (3). Likewise, type I cells appear to be incapable of dividing and, moreover, these cells are quite sensitive to injurious agents (3). Our study also found that type II cell hyperplasia was invariably higher in chronically affected lungs compared to those of acute stage.

The present study found that SP-A positive reaction was generally extracellular within the alveoli or in the cytoplasm of type II cells from healthy lungs and those with pneumonia. However, extracellular SP-A immunopositive reaction was stronger in pneumonic lungs compared to healthy lungs. Release of most SP-A into the alveoli by constitutive secretion, independently of lamellar bodies, might explain the strong extracellular positivity for SP-A in pneumonic lungs, as stated by others (5,9,24). In accordance with our results, it was reported that SP-A content in inflamed lungs was higher than that seen in healthy lungs (5) and that lamellar bodies of type II cells contain small amounts of SP-A (6,25). It was also reported that most of the SP-A expressed into the alveolar surface is again taken up by the alveolar type II epithelial cells, catabolized, and transported into osmophilic lamellar bodies for recycling of the protein (26). Thus, cytoplasmic SP-A immunolabeling of the alveolar type II cells is most probably due to the redirection of captured SP-A to the lamellar bodies. SP-A immunopositive reaction was also found in the cytoplasm of some alveolar macrophages, in accordance with the results reported by Balis et al. (22). Aggregation of SP-A may lead to the activation

Figure 8. Numerous CD3+ T (A) and a few CD79αcy+ B (B) lymphocytes in a lung chronically affected by pneumonia. IHC. Bar: 51 µm.
of macrophages and the stimulation of phagocytosis. Likewise, SP-A in humans has been reported to induce macrophage and neutrophil uptake and the killing of pathogens in the lungs (24). Many alveolar type II cells showed twin nuclei with a single cytoplasm and they protruded into the alveolar lumen, which was confirmed by PCNA staining. SP-A immunopositive twin cells demonstrated propagation capacity and mitotic activity of the cells. Additionally, SP-A positivity was detected in the Clara cells of terminal bronchioles. SP-A is the most abundant and best-characterized protein of the pulmonary surfactants produced by Clara cells (5,7,24,27).

It was reported that mature SP-B is found exclusively in the osmophilic lamellar bodies of the alveolar type II cells, where it is stored and expressed into the alveolar lumen to form tubular myelin (5,8). In this research, mature SP-B immunopositive reaction occurred especially in the apical surface area of the proliferated type II cells, and immunoreaction products appeared as fine granules, as shown by others (11,28). Although some authors (10,25,27) reported that SP-A is the major protein of the surfactant system, lamellar bodies particularly contain SP-B and SP-C. This supports our results, with more strong immunopositivity of lamellar bodies in the cytoplasm of type II cells for SP-B than that seen for SP-A. Immunolabeling for SP-B in the hyperplastic type II cells was evidently stronger than that seen in normal type II cells, consistent with the results reported by Balis et al. (22). Strong immunostaining of hyperplastic type II cells in the areas resulting in fibrosis facilitated their identification among the inflammatory infiltrates. The apical cytoplasm of the proliferated type II cells was seen to expand because of the multiple lamellar bodies positively labeled for mature SP-B. The strong immunoreactivity for SP-B and enhanced cytoplasmic rate of the proliferated type II cells were most probably due to the storage of the protein in lamellar bodies before secretion into the alveolar lumen. Similar to our results, Miller and Hook (3) reported that hypertrophy of type II cells is caused by an increase in the number or size of cytoplasmic lamellar bodies. SP-B positive staining was also observed in the cytoplasm of Clara cells of the terminal bronchioles. Like type II cells, Clara cells constitute a major hyperplastic population and progenitors of ciliated epithelium following bronchial injury (21).

SP-C is restricted to only type II cells (28,29) and therefore has commonly been used in the identification of tumors caused by this cell lineage in sheep lungs (16–18). In the present report, strong immunolabeling of proSP-C was noted in hyperplastic type II cells of pneumatic lungs. While SP-A and SP-B immunopositivity indicated only alveolar type II cells and Clara cells, proSP-C positive reaction confirmed both hyperplastic and normal type II cells in fibrotic bundles and in alveoli of pneumatic and healthy lungs. Like SP-A and SP-B, proSP-C immunopositive type II cells were predominantly detected around the necrotic foci and neutrophil collections, along with the strong reaction of cells in glandular shapes at the margin of the fibrotic capsule. These results confirmed the hyperplastic and restorative capacity of type II pneumocytes in damaged lung tissue caused by injurious agents. Although immunoreaction products to SP-A and SP-B markers were seen predominantly in the apical surface of type II cells, SP-C immunopositivity was detected in the perinuclear areas of the cells. However, Clara cells did not show SP-C positive reaction, as reported earlier (16–18,29).

Immunolabeling to PCNA has often been used to elucidate the prognosis of various neoplasms in human (12) and sheep (17) lungs. PCNA immunolabeling in the present work was used to demonstrate the proliferative capacity of type II epithelial cells in pneumatic lungs. Nuclear positive reaction to PCNA was predominantly detected in the proliferated cells in glandular shapes at the margins of the fibrotic capsules surrounding the necrotic areas. The number of positive cells varied among the pneumatic lungs, with high numbers of PCNA immunopositive cells in lungs resulting in fibrosis. Immunopositive cells having round or ovoid nuclei around the necrotic areas were most likely proliferative type II cells. Likewise, strong expression of surfactant proteins in the same areas confirms type II cells. A PCNA-positive reaction was also present at the BALT, confirming the mitotic capacity of the cells. Additionally, PCNA positive cells having thin nuclei around the necrotic areas were also consistent with morphological characteristics of fibroblasts, indicating fibrotic repair of the damaged lungs.

It has been shown that TTF-I is a useful marker in the diagnosis of thyroid and lung tumors (13). Alveolar epithelial cells of pneumatic and healthy lungs revealed diffuse nuclear labeling for TTF-I. Regardless of the type and stage of pneumonia, TTF-I expression was intense in type II cells showing gland-like proliferations. Consequently, the marker might be considered as a useful tool in the identification of lung epithelium, as neoplastic cells. However, lining epithelial cells of airways, except for nonciliated Clara cells, did not show a positive reaction for TTF-I.

Lymphocytic aggregations in the peribronchial and peribroncholar tissues were constantly present in chronically affected pneumatic lungs, associated with fibrosis. Lymphocytic aggregations were clearly seen to increase around the airways and alveolar septum in lungs with verminous and exudative pneumonia compared to healthy lungs. In these spaces, the number of CD3+ T cells was predominantly high compared to CD79αcy+ B cells, as reported by Oros et al. (30). Such lymphocytic
proliferations may indicate a cell-mediated nonspecific immune response in ovine lungs with pneumonia. Although this work confirmed the predominance of CD3+ T cells in peribronchial and peribronchiolar lymphocyte proliferations, T cell subpopulations need to be determined in ovine pneumonia.

In conclusion, the present study revealed that type II cells are the main cells proliferating in lungs with inflammation and that the cells have very important roles in the reparative process. This research also found that necrotic parenchyma is replaced by fibrous tissue instead of regeneration. This research clearly revealed that in lungs with verminous pneumonia type II cell hyperplasia was less prominent as compared to exudative pneumonia. This result might show that lung parasites did not incite an inflammatory reaction in the absence of secondary infection in ovine lungs. It might be said also that increased secretion of the surfactant proteins with type II cell hyperplasia is not only for restoration of the damaged lungs but also might be for the killing of microbial agents.

References


