Proliferation of type II pneumocytes in the lung biopsy specimens reflecting alveolar damage

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Abstract

Proliferation of type II pneumocytes may be the most sensitive pathological indicator of alveolitis, and the density of type II pneumocytes reflecting the severity of the recent alveolar damage. Twenty-nine patients were divided into three groups by the severity of ground-glass opacities on the CT scans: the severe (acute), the moderate (subacute) and the mild (chronic) groups. We compared the density of type II pneumocytes in the transbronchial lung biopsy specimens and cell analysis of bronchoalveolar lavage (BAL) fluid with the ground-glass opacities. Clinical and laboratory findings and BAL fluid analysis also corresponded well with this grouping. Type II pneumocytes were selectively stained with an antibody against Thomsen–Friedenreich and the density of the type II pneumocytes was expressed as the number per 1 mm alveolar septal length. The densities of the type II pneumocytes in the severe, the moderate, the mild, and the control groups were $21.2 \pm 11$, $14.4 \pm 7.2$, $11.0 \pm 3.3$ and $7.5 \pm 0.9$ mm, respectively. There were significant differences between the acute group and the other three groups, and between the control and chronic groups. The density of type II pneumocytes is a useful index for evaluating alveolar damage even in mild alveolitis.

Keywords interstitial pneumonia; pneumocytes; alveolitis; lung biopsy; bronchoscopy.

INTRODUCTION

It is difficult to evaluate recent alveolar damage in diffuse interstitial lung diseases, especially in idiopathic pulmonary fibrosis (IPF), in which slight but continuous alveolar injury persists. Chest CT findings, analysis of bronchoalveolar lavage fluid (BALF), respiratory function tests, and measurements of lactate dehydrogenase and KL-6 in serum have been used clinically as ways of assessing alveolar damage (1–4). However, these findings are not sensitive enough to detect mild alveolar injury.

Diffuse interstitial lung diseases have been diagnosed on the basis of pathological findings in the specimens obtained by open lung biopsy or by video-assisted thoracoscopic lung biopsy (5), and their activity levels also have been evaluated pathologically (6). One shortfall of such lung biopsies is that they cannot usually be repeated for reevaluation. Although transbronchial lung biopsy (TBLB) via a fiberoptic bronchoscope is a repeatable method for pathological evaluation, its usefulness is limited because the samples are too small to allow us to assess the interstitial changes throughout the lung as a whole. If TBLB specimens do actually give a reflection of the severity of the alveolar damage present, TBLB could prove especially valuable in forming the treatment of diffuse interstitial lung diseases.

Proliferation of type II pneumocytes is one of the most sensitive pathological findings in cases of alveolar injury (7,8). In the reparative phase, type II pneumocytes proliferate, replacing sloughed type I pneumocytes. In fact, proliferation or hyperplasia of type II pneumocytes develops approximately 3–7 days after injury in cases of diffuse alveolar damage (9). A similar repair process is observed in chronic fibrosing lung diseases (10,11).

In this study, we examined the proliferation of type II pneumocytes in specimens obtained by TBLB from patients with interstitial lung diseases and assessed its usefulness in the assessment of alveolar damage.
**MATERIALS AND METHODS**

**Materials**

Twenty-nine TBLB specimens were obtained from 29 patients among 536 who underwent fiberoptic bronchoscopy in Shinshu University Hospital between Jan. 1996 and Dec. 1997. All 29 patients showed interstitial opacities on chest CT scans, and TBLB and BAL were performed on the same day. The 29 patients were divided into three groups: seven patients showing bilateral marked ground-glass opacities that had developed over a 1-week period [severe (acute) group], 14 exhibiting partial ground-glass opacities that had developed over several weeks [moderate (subacute) group], and eight with slight reticulonodular and ground-glass opacities that had been gradually progressing for several years [mild (chronic) group]. The acute group comprised four with hypersensitive pneumonia, one bronchiolitis obliterans organizing pneumonia (BOOP), one eosinophilic pneumonia, and one collagen vascular disease. The subacute group comprised five BOOP, five collagen vascular disease, two eosinophilic pneumonia and two hypersensitive pneumonia. The chronic group comprised five idiopathic pulmonary fibrosis and three collagen vascular disease. We also examined five TBLB specimens (biopsy control group) and five necropsy specimens (necropsy control group) as controls. The five patients in the biopsy control group, who had no CT findings, were biopsied for the diagnosis of possible sarcoidosis because of uveitis. And, in fact, none of their findings including findings from histology of TBLB specimens and BALF analysis were indicative of sarcoidosis. The five necropsy specimens were obtained from patients without lung disease.

**Light microscopic study**

Biopsy and necropsy tissues were fixed in 20% buffered formalin for 24 h. Each specimen was dehydrated through graded alcohols, cleared in xylene, and embedded in paraffin. Three-micron serial sections were stained with hematoxylin and eosin (HE), and with antibodies against Thomsen-Friedenreich (TF) (DAKO, Glostrup, Denmark) after sialidase digestion.

Sialidase digestion pretreatment (from Arthrobactor ureafaciens, Nakarai Chemicals, Kyoto, Japan) (1 unit/ml in 0.05 M phosphate buffer, pH 7.0) was carried out at 37°C for 4 h before immunostaining for TF antigen. The sections were incubated in 0.3% hydrogen peroxide in methanol for 30 min to remove endogenous peroxidase activity. They were then incubated in phosphate buffered saline (PBS) containing 1% bovine serum albumin and monoclonal antibodies (anti-TF I:100) at 4°C for 18 h. After rinsing in PBS, the sections were incubated in solutions containing horseradish peroxidase-conjugated goat anti-mouse IgG antibody (diluted to I:100 in PBS).

After further rinsing in PBS, antibody binding sites were visualized using a solution containing 0.02% 3,3′-diaminobenzidine (Wako Pure Chemical, Tokyo, Japan) and 0.005% hydrogen peroxide in phosphate buffer. Counterstaining was carried out using hematoxylin.

**Density of type II pneumocytes**

After staining with an anti-TF antibody, five areas of 0.58 × 0.44 mm in each slide were randomly selected and photographed using a digital camera (SONY DKC 5000, SONY, Tokyo, Japan). Type II pneumocytes were counted, and the total length of the alveolar septum was measured using NIH-Image 1.60 (pcc software (National Institutes of Health, U.S.A.) obtained from sippy.nimh.nih.gov via anonymous FTP. The number per 1 mm of alveolar septal length was taken as the density of the type II pneumocytes.

**Statistic analysis**

Data are expressed as the mean ± sd. Differences were taken to be statistically significant when the probability value was less than 0.05 (unpaired t-test).

**RESULTS**

The clinical data for the patients in the four biopsy groups are summarized in Table 1. There were no significant differences in age, pulmonary function tests, or blood gas analysis. C-reactive protein and the number of white blood cells also showed no significant differences among the groups, and did not rise to a level indicating bacterial pneumonia. The serum level of lactate dehydrogenase was high in all three lung-disease groups, but not in the biopsy control group, and that in the severe group was significantly higher than that in the moderate group. The interval between the onset of respiratory symptoms and the performance of fiberoptic bronchoscopy was significantly shorter for the severe and moderate groups than for the mild group.

The results of the BALF analysis are summarized in Table 2. Both the number and percentage of lymphocytes were significantly higher in the three lung-disease groups than in the biopsy control group. The number of lymphocytes was also significantly higher in the severe group than in the mild group. The number of lymphocytes expressing HLA-DR was significantly greater in the severe group than in the other three groups. Total protein and albumin were both greater significantly in the severe group than in the mild group. Lactate dehydrogenase was significantly elevated in the three lung-disease groups by comparison with the biopsy control group.

After sialidase digestion, TF antibody stained the apical surface of type II pneumocytes in the normal lung.
Although the alveolar surface was slightly stained in the peripheral lung showing alveolitis, this staining sequence enabled us to recognize and to count type II pneumocytes more easily than HE staining. The density in the necropsy control group was $6.6 \pm 0.7 / \text{mm}$. The values obtained for the density of type II pneumocytes in the severe (Fig. 2), moderate, mild, and biopsy control groups were $21.2 \pm 7.1$, $14.4 \pm 7.2$, $11.0 \pm 3.3$ and $7.5 \pm 0.9 / \text{mm}$, respectively (Fig. 3). There was no significant difference between the biopsy and necropsy control groups. The density was significantly higher in the severe and mild groups than that in the control group. The density in the severe group was also significantly higher than that in the moderate group or the mild group. The moderate group did not show a significant difference statistically from the biopsy control ($P=0.0516$), and there was no significant difference between the moderate and the mild groups ($P=0.228$).

**DISCUSSION**

The density of the type II pneumocytes, which was expressed as the number per 1 mm alveolar septal length, is a useful pathological index for evaluating alveolar damage even in mild (chronic) interstitial lung diseases. It corresponded well with the severity of ground-glass opacities on the chest CT scans.

Proliferation of type II pneumocytes is one of the most sensitive pathological indicators of alveolitis after various lung injuries. Proliferation of type II pneumocytes is observed in the reparative phase after alveolar damage (12), and alveolar lining-cell hyperplasia develops approximately 3–7 days after such injury (9). However, this pathological finding is subjective, with different pathologists gaining different impressions, and there are no criteria to guide us in grading their proliferation. The density of type II pneumocytes as calculated here is a useful index of their proliferation, which itself indicates recent alveolar injury.

The density of type II pneumocytes in TBLB specimens may directly reflect the severity of recent alveolar damage. The pathological features of interstitial pneumonia are mainly the presence of cellular infiltrates, epithelial necrosis and proliferation, capillary and epithelial pericytes, air space cellular exudates, granulomas, necrosis, organizing and scarring and honeycombing (12). However, proliferation of type II pneumocytes may be the most sensitive among these findings, and it could detect even mild lung injury in the present study.

Counting the number of type II pneumocytes per 1 mm alveolar septal length enabled us to easily detect a slight proliferation of type II pneumocytes even in idiopathic pulmonary fibrosis and chronic interstitial pneumonia in collagen vascular disease. Immunostaining with an antibody against TF antigen after sialidase digestion

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**Table 1.** Clinical data

<table>
<thead>
<tr>
<th></th>
<th>M/F</th>
<th>Age (years)</th>
<th>S/Non-S</th>
<th>Interval (weeks)</th>
<th>CRP (mg/dl)</th>
<th>WBC ($\times 10^3 / \text{mm}^3$)</th>
<th>S-LDH (UI/L)</th>
<th>%FVC</th>
<th>%FEV1</th>
<th>DLCO</th>
<th>P_{O2}</th>
<th>P_{CO2}</th>
<th>PO2</th>
<th>PCO2</th>
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<tbody>
<tr>
<td>Severe</td>
<td>1/3</td>
<td>61.8±18.7</td>
<td>6/6</td>
<td>2/2</td>
<td>8.2±14.5</td>
<td>6.7±7.4</td>
<td>73±9.4</td>
<td>75±10.9</td>
<td>81±5.12</td>
<td>900±14.3</td>
<td>289±1.7</td>
<td>364±1.6</td>
<td>398±1.6</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>4/10</td>
<td>59.5±12.1</td>
<td>6/8</td>
<td>2/2</td>
<td>8.2±14.4</td>
<td>6.7±7.4</td>
<td>73±9.4</td>
<td>75±10.9</td>
<td>81±5.12</td>
<td>900±14.3</td>
<td>289±1.7</td>
<td>364±1.6</td>
<td>398±1.6</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>2/6</td>
<td>62.8±12.2</td>
<td>3/3</td>
<td>3/3</td>
<td>8.2±14.4</td>
<td>6.7±7.4</td>
<td>73±9.4</td>
<td>75±10.9</td>
<td>81±5.12</td>
<td>900±14.3</td>
<td>289±1.7</td>
<td>364±1.6</td>
<td>398±1.6</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3/2</td>
<td>55.8±12.8</td>
<td>3/2</td>
<td>3/3</td>
<td>8.2±14.4</td>
<td>6.7±7.4</td>
<td>73±9.4</td>
<td>75±10.9</td>
<td>81±5.12</td>
<td>900±14.3</td>
<td>289±1.7</td>
<td>364±1.6</td>
<td>398±1.6</td>
<td></td>
</tr>
</tbody>
</table>

Note: Severe, severe (acute) group; moderate, moderate (subacute) group; mild, mild (chronic) group; control, biopsy control group.

* Significant difference from the mild group ($P<0.001$).

** Significant difference from the moderate group ($P<0.01$).

*** Significant difference from the control group ($P<0.05$).
selectively stained the apical surface of the type II pneumocytes (13), and use of this sequence enabled us to detect and count type II pneumocytes with ease under the light microscope. There was significant difference between the control and chronic groups in this study. However, the moderate group was not significantly different from the biopsy control because one case showed very low density of type II pneumocytes.

Earlier, we concluded that a reference range for type II pneumocyte density might be 6.5–8.5 per 1 mm of alveolar septal length for biopsy specimens ordinarily processed (14). In the present study, the densities in the biopsy and necropsy control groups were within this range. Their standard deviations were small, and tissue fixation did not seem to greatly alter the range of values obtained. Other ways of counting may be less reliable: for example, expression type II pneumocytes per square

<table>
<thead>
<tr>
<th>Table 2. Bronchoalveolar lavage data</th>
<th>Recovery Rate (%)</th>
<th>Total cell (x 10^6/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>43.7 ± 2.1*</td>
<td>394 ± 9.5**</td>
</tr>
<tr>
<td>Moderate</td>
<td>40.3 ± 16.7*</td>
<td>184 ± 32*</td>
</tr>
<tr>
<td>Mild</td>
<td>67.3 ± 31*</td>
<td>203 ± 81**</td>
</tr>
<tr>
<td>Control</td>
<td>53 ± 2.3</td>
<td>89 ± 4.9</td>
</tr>
</tbody>
</table>

Note: Severe: severe (acute) group; moderate: moderate (subacute) group; mild: mild (chronic) group; control: biopsy control group.

Recovery rate: amount of saline aspirated via bronchoscopy/150 ml. TP: total protein; Alb: albumin; LDH: lactate dehydrogenase.

Significant difference from the control group (P < 0.05).
expressing HLA-DR shows activation of lymphocytes. An increase in lymphocytes and percentage of lymphocytes in BALF indicates interstitial change. Both the serum (3) and decrease in the di¡using capacity of carbon monoxides indicate interstitial change. Anelevation of lactate dehydrogenase in BALF demonstrates increased permeability between alveoli and capillary as a result of alveolar septal damage. Although these findings demonstrate alveolar damage indirectly, the density of the type II pneumocytes showed recent alveolar damage directly and semi-quantitatively.

The usefulness of TBLB is thought to be limited and the specimen size obtained is thought to be too small for the evaluation of the activity of the interstitial pneumonia. The density of the type II pneumocytes may more precisely support the evaluation of the activity by CT, BALF or both examinations. An index of the density of type II pneumocytes permits sensitive detection of recent alveolar damage in combination with the above examinations which can evaluate the whole lung.

**REFERENCES**


14. Hayasaka M, Honda T, Kudo K, Sekiguchi M. Proliferation of type II pneumocytes and alteration in their apical surface

![](image)


