CLINICOPATHOLOGIC CHARACTERISTICS OF THE LUNGS OF PATIENTS WITH HUMAN T CELL LYMPHOTROPIC VIRUS TYPE 1–ASSOCIATED MYELOPATHY

KATSUNORI SUGISAKI, TOMIYASU TSUDA, TOSHIHIDE KUMAMOTO, AND SIN’ICHIRO AKIZUKI

Third Department of Internal Medicine, Oita Medical University, Oita, Japan; Department of Pathology, Shin-Beppu Hospital, Oita, Japan

Abstract. Lung autopsy specimens were evaluated histologically in the six patients with human T cell lymphotropic virus type 1 (HTLV-1)–associated myelopathy (HAM). The results revealed two histologic changes. First, lymphoid infiltrates were distributed widely in peribronchiolar and perivascular regions, subpleural regions, and the alveolus. Lymphoid infiltrates were also observed in bronchial mucosal glands in relatively large bronchi, in which the acinar epithelium was sometimes degenerated. Second, chronic inflammatory changes, such as smooth muscle hypertrophy, fibrosis, or squamous cell metaplasia, were increased significantly in the membranous bronchioles of HAM patients compared with specimens from lung cancer control patients. Such histologic changes were subclinical in most cases, but one case had an abnormal chest shadow, and two cases had recurrent pneumonia. In HAM patients, high levels of HTLV-1-specific cytotoxic T lymphocytes are believed to attack the HTLV-1-bearing cells in the lung, resulting in inflammatory reactions.

Human T cell lymphotropic virus type I (HTLV-1), the causative agent of adult T cell leukemia/lymphoma (ATL), causes either HTLV-1-associated myelopathy (HAM) or tropical spastic paraparesis (TSP). In the central nervous system, T lymphoid interstitial infiltrates were observed around small vessels and myelin fibers, and were especially prominent in the corticospinal tracts of both lower limbs. These infiltrates were detected in the joints of extremities and the ocular system, in addition to the nervous system. The lymphocyte population also increased in bronchoalveolar lavage fluids. Furthermore, lung disorders such as diffuse panbronchiolitis (DPB) or lymphocytic interstitial pneumonia (LIP) develop in a small number of HTLV-1 carriers. To investigate what kind of change progresses in the lung of HTLV-1 carriers, we evaluated histologically the lungs of six autopsied HAM patients.

SUBJECTS AND METHODS

Subjects. Six patients were diagnosed as having HAM by neurologic examination and high HTLV-1 titers in both serum and cerebrospinal fluid (CSF). The profiles of six patients are shown in Table 1. As shown in Figure 1, patient 3 had diffuse nodular infiltrates throughout both lung fields on a chest radiograph. Patients 2 and 3 had repeated bouts of pneumonia during their clinical courses. Patient 2 died of sepsis and disseminated intravascular coagulation syndrome due to pneumonia by multidrug-resistant Staphylococcus aureus.

The other five patients died of nonpulmonary diseases. Patients 1, 4, and 6 developed HAM 2–6 years following blood transfusions. Patient 4 underwent bronchoalveolar lavage, which showed an increase in both total cell number (7

![Figure 1. Chest radiograph of patient 3, showing a diffuse reticulonodular shadow in the bilateral lung fields.](image-url)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Cause of death</th>
<th>Smoking history</th>
<th>History of blood transfusion</th>
<th>Chest radiograph findings</th>
<th>Serum anti-HTLV-1 titer</th>
<th>CSF anti-HTLV-1 titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>F</td>
<td>Heart failure</td>
<td>0</td>
<td>+</td>
<td>NP</td>
<td>1,280</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>M</td>
<td>Sepsis</td>
<td>Unknown</td>
<td>–</td>
<td>NP</td>
<td>64</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>F</td>
<td>Liver cirrhosis</td>
<td>0</td>
<td>–</td>
<td>Bilateral nodular shadow</td>
<td>2,560</td>
<td>512</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>M</td>
<td>Renal failure</td>
<td>0</td>
<td>+</td>
<td>NP</td>
<td>20,430</td>
<td>512</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>M</td>
<td>Bladder carcinoma</td>
<td>23</td>
<td>–</td>
<td>NP</td>
<td>5,120</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>63</td>
<td>F</td>
<td>Liver cirrhosis</td>
<td>0</td>
<td>+</td>
<td>NP</td>
<td>256</td>
<td>128</td>
</tr>
</tbody>
</table>

* Based on pack-years (the number of packs smoked per day times the number of years smoked).
† The findings of a chest radiograph taken in stable conditions. NP = no particular findings.
‡ CSF = cerebrospinal fluid.
Figure 2. Photomicrograph of a lung section of patient 4, showing small lymphocyte infiltrates in the wall of the small vessel (hematoxylin and eosin stained, magnification × 190, bar = 100 μ).

and percentage of lymphocytes (67%). The CD4+ /CD8+ ratio was 2.35, which was slightly higher than the average of six healthy controls (1.48 ± 0.84).

**Tissue preparation and staining.** A total of 12 tissue specimens, two specimens from each lobe, and two specimens from right main bronchus were obtained from each patient, avoiding zones of dependent congestion. Paraffin-embedded sections were stained with hematoxylin and eosin and elastica van Gieson stain. In a few cases with obvious infectious findings, Grocott, methenamine silver, and Ziehl-Nielsen acid-fast staining were performed in addition to routine staining.

**Number of infiltrated lymphocytes/mm².** We counted the number of lymphocyte per unit area. First, the areas including the bronchioles, small vessels, alveolus, pleura, and bronchial mucosal glands were measured on a computer screen after being captured by the computerized picture analyzing system (Cosmozone 1S; Nikon, Tokyo, Japan). The number of infiltrated lymphocyte was then counted on the same screen. The number of infiltrated lymphocytes/mm² was calculated in more than 10 regions and averaged. These values were classified into five categories: − = 0–500; ± = 501–1,000; + = 1,001–2,001; ++ = 2,001–3,000; and +++ = 3,001 lymphocytes/mm².

**Morphologic evaluation of membranous bronchioles.** We evaluated all membranous bronchioles observed in the tissues using the six morphologic variables representing chronic inflammation: 1) inflammatory cell infiltration, 2) smooth muscle hypertrophy, 3) fibrosis, 4) pigment deposition on bronchiolar walls, 5) squamous cell metaplasia of the epithelium, and 6) goblet cell metaplasia of the epithelium. All noncartilaginous airways less than 2 mm in internal diameter were identified under the microscope and defined as membranous bronchioles (MBs) when they were nonalveolated. The internal diameter from basement membrane to basement membrane was measured. All bronchioles observed in the tissues were evaluated by the method of Wright and others using a panel of photographs. A subjective grading from 0 (normal) to 3 (most abnormal) was made for each of the six morphologic variables. For each patient, the sum of the graded variables for all MBs was calculated and expressed as a percentage of the maximal score. As controls, we evaluated resected lungs from 37 patients who had solitary lung tumors and normal forced expiratory volume in 1 sec by the same method. The mean age was 63 years in the HAM group, and 60 years in the lung tumor group.

**RESULTS**

As shown in Figure 2, in patient 4, numerous lymphocytes were collected in various lung tissues, including bronchioles, small vessels, pleura, and alveolus. Similar lymphoid infiltrates were also seen in patients 1, 2, 3, and 6. Patient 2 had findings of lobar pneumonia, intra-alveolar exudate, and massive infiltration of neutrophils in both lower lobes and the middle lobe, in addition to lymphocyte infiltration. In patient 3, a small number of neutrophils were observed in the lumen of the bronchioles, mainly in the lower left lobe, in addition to a lymphoid infiltrate. The number of infiltrated lymphocytes/mm² was calculated and classified into five categories. As summarized in Table 2, lymphoid infiltrates were observed most frequently around the bronchiole, and also around the small blood vessels, alveolar septum, and visceral pleura. Furthermore, as shown in Figure 3, a marked lymphoid infiltrate was observed frequently in the stroma of bronchial mucosal glands in the large bronchus, in which acinar epithelium was sometimes degenerated only in the lesion with a severe lymphoid infiltrate. Malignant lymphoid infiltration was never seen in any tissue specimen.

Figure 4 shows wall thickening around the membranous bronchioles of patient 1, which was seen frequently in our six HAM patients, in addition to inflammatory cell infiltration. Elastica van Gieson staining revealed the wall thickening was formed by the smooth muscle hypertrophy and fibrosis. We evaluated the pathology score of MBs in HAM patients or control patients with solitary lung tumors and the
TABLE 2
Number of infiltrated lymphocytes per unit area in the lung of patients with human T cell lymphotropic virus type 1–associated myelopathy*

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Peribronchial region</th>
<th>Perivascular region</th>
<th>Alveolar septum</th>
<th>Pleura</th>
<th>Bronchial mucosal glands</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>++</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>+++</td>
<td>±</td>
<td>±</td>
<td>−</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>+++</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>±</td>
<td>−</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>Not available</td>
</tr>
</tbody>
</table>

*− = 0–500; ± = 501–1,000; + = 1,001–2,000; +++ = 2,001–3,000; ++++ = ≥3,001 cells/mm².

results are summarized in Figure 5. Pathologic scores of all six morphologic variables increased significantly in HAM patients when compared with control patients. In particular, the score of inflammatory cell infiltration, smooth muscle hypertrophy, fibrosis in the wall of MBs, and of squamous cell metaplasia of epithelium increased markedly in HAM patients. The results of staining for special infectious pathogens were negative.

DISCUSSION

Human T cell lymphotropic virus type-1–associated myelopathy/tropical spastic paraparesis, is a neurologic disorder caused by HTLV-1, and recent evidence suggests that immunologic abnormalities are associated with this disorder. For example, lymphocyte populations are increased in bronchial alveolar lavage fluid (BALF), and the expression of soluble interleukin-2 receptors in the lungs is up-regulated. Histologic studies have not been conducted thoroughly in HAM patients with immunologic abnormalities in the lungs thus far due to difficulties in obtaining lung tissues from such patients. In the present investigation, we studied histologically not only peripheral lung tissues, but also the central bronchus and surrounding bronchial mucous glands using autopsied lung tissue specimens. The results of these studies revealed an extensive lymphocyte infiltration in lung tissues, in particular, lymphocyte infiltration in peribronchial and perivascular regions. Moreover, we compared lung tissues resected from HAM patients and lung cancer patients with solitary tumors using a morphologic method described by Wright and others that was intended to study chronic inflammatory changes in membranous bronchioles. Patients with HAM showed striking changes in bronchioles with regard to all parameters, indicating the possibility that advanced chronic inflammation occurred in the peripheral respiratory tract. Such histologic changes were subclinical in most patients, but chest radiographic findings revealed diffuse infiltration in bilateral lower lung fields of one patient, while intractable pneumonia occurred in two patients with HAM.

It has been well documented that a variety of respiratory tract infections occur frequently in patients with ATL. On the other hand, it has been demonstrated that tumor cells infiltrate into the respiratory tract directly, indicating an affinity of ATL cells for lung tissues. In addition, a number of ATL patients have been reported to have bronchiectasis, chronic bronchitis, and pulmonary fibrosis at the onset of the disease. On the other hand, the incidence of certain lung diseases has been reported to be high in carriers who have not developed ATL. Kimura and others have reported that antibody titers against HTLV-1 are higher in patients with diffuse DPB than in patients with other immunologic lung diseases. In addition, antibody titers against HTLV-1 are known to be high in patients with LIP. Diffuse panbronchiolitis is a typical disorder that causes chronic inflammatory changes in bronchioles, and is commonly seen in Japan. Patients with DPB often contract intractable respiratory infections. These facts suggest a possibility that immunologic abnormalities induced by chronic persistent HTLV-1 infection may lead to pulmonary lesions primarily involving the peripheral respiratory tract and alveolar septum.

It has been documented that other retrovirus infections, such as human immunodeficiency virus and simian im-

Figure 3. Photomicrograph of a bronchial mucosal gland in the lung of patient 4, showing extensive lymphoid infiltrates in the stroma and partially degenerated acinar epithelium (hematoxylin and eosin stained, magnification × 95, bar = 200 μ).
munodeficiency virus, induce pulmonary lesions. Based on clinical and pathologic studies on pulmonary complications in patients with acquired immunodeficiency syndrome (AIDS), LIP, nonspecific interstitial pneumonia, and lymphocytic bronchitis occur in AIDS patients in addition to infections caused by cytomegalovirus or Pneumocystis carinii, etc. that are associated with diminished immune functions. Histologic findings of nonspecific interstitial pneumonia include lymphocyte infiltration primarily in the bronchiole, areas surrounding small vessels, pleura, and other tissues rich in lymphatic vessels. These findings are consistent with ours, i.e., the sites of lymphocyte infiltration in HAM patients as shown in our present study. In addition, lymphocyte infiltration is also seen at similar sites in rhesus monkeys with simian immunodeficiency virus infection.

In the present study, we observed extensive lymphocyte infiltration in bronchial mucous glands present in relatively large bronchi in HAM patients. In addition, acinar epithelial cell degeneration was seen at the sites where lymphocytes were heavily infiltrated. Such severe inflammatory findings in bronchial mucous glands were seen in HAM patients without respiratory tract infections, suggesting that these changes were attributable to some immunologic abnormalities. In our previous report, we demonstrated that numerous B lymphocytes infiltrated into the bronchial mucous glands, in addition to T lymphocytes. Recently, SjoÈgren’s syndrome has been reported to occur as one of the complications in patients with HAM. In addition, the tax transgenic mouse presents an exocrine gland abnormality similar to that of SjoÈgren’s syndrome. Histologic studies of exocrine glands in patients with SjoÈgren’s syndrome have revealed that B lymphocyte counts increased as a progression of the disease, although T lymphocyte infiltration was predominantly seen at an early stage of the disease. Such inflammatory changes in exocrine glands are often seen in bronchial mucous glands, while insufficiency of respiratory tract mucous secretion may lead to chronic bronchitis symptoms. In the present study, we found chronic inflammation in bronchial mucous glands, and this finding is of interest in assessing a possible link between HTLV-1 infection and exocrine abnormalities.

Extensive lymphocyte infiltration, as well as chronic inflammatory changes in membranous bronchiole and bronchial mucous glands, were seen in lung tissues resected from...
patients with HAM. What is the mechanism for this phenomenon? It has been recently reported that peripheral blood and CSF collected from HAM patients contained high levels of CD8-positive HTLV-1-specific cytotoxic T cells. On the other hand, HTLV-1 proviral DNA was detected in cell extracts isolated from BALF collected from HAM patients, showing that HTLV-1 virus-infected cells were present in local pulmonary lesions. The HTLV-1-specific cytotoxic T cells attack HTLV-1-bearing cells, resulting in inflammatory reactions. Subsequently, a variety of cytokines are produced and inflammatory cells are further recruited to the lungs. Secondary infection may aggravate this process further.

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Authors’ addresses: Katsunori Sugisaki, Tomiyasu Tsuda, and To-shihide Kumamoto, Third Department of Internal Medicine, Oita University, Oita 879-5503, Japan. Sin’ichiro Akizuki, Deshihide Kumamoto, Third Department of Internal Medicine, Oita University, Oita 879-5503, Japan. Yoichi Hokkezu for providing lung tissues of HAM patients. We also thank Dr. Kenichi Matsuba for assistance in the pathologic evaluation of membranous bronchioles.

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