Primary Pulmonary B-Cell Lymphoma Diagnosed by $\kappa$-$\lambda$ Imaging of Broncho-Alveolar Lavage Fluid Lymphocytes

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A 69-year-old woman was examined due to abnormal pulmonary shadows on her chest roentgenogram. Although small lymphocyte proliferation was revealed in specimens by transbronchial lung biopsy (TBLB), immunoglobulin light chain restriction could not be seen. We attempted to verify the B-cell clonality by broncho-alveolar lavage (BAL) and a new sensitive method called $\kappa$-$\lambda$ imaging (KLI), which was available for the detection of monoclonal B cells. Therefore, B-cell monoclonality was found. Thus, the patient was diagnosed as having primary pulmonary lymphoma (PPL). PPL may be differentiated from benign lymphoproliferative disorders such as pseudolymphoma and lymphocytic interstitial pneumonia by KLI of BAL-derived lymphocytes.

Key words: non-Hodgkin’s lymphoma, B-cell monoclonality, lymphocytes in BALF, immunoglobulin light chain restriction

Introduction

Primary pulmonary lymphoma (PPL) is very rare. The diagnosis is usually made on the basis of pathological or immunohistochemical examination of specimens taken by transbronchial lung biopsy (TBLB). Furthermore, PPL must be differentiated from pseudolymphoma (PDL) and lymphocytic interstitial pneumonia (LIP). However, these three diseases can not always be easily distinguished from each other, even when investigated immunohistochemically; the clonality of proliferating cells can not always be verified at the TBLB-derived tissue level. Thus, open lung biopsy (OLB) or lobectomy is required in many cases to make an accurate diagnosis (1).

Recently, we have developed a new and more sensitive type of immunoglobulin $\kappa$, $\lambda$ light chain analysis called $\kappa$-$\lambda$ imaging (KLI) (2) to verify the clonality of B-cells. We applied KLI to analyze broncho-alveolar lavage fluid (BALF) cells and thus were able to diagnose the patient with abnormal pulmonary shadows on the chest roentgenogram as having PPL.

Case Report

A 69-year-old woman was admitted for assessment of abnormal pulmonary shadows on her chest roentgenogram in April 1990. Physical examination revealed no superficial lymphadenopathies or hepatosplenomegaly. Auscultation disclosed diminished breath sounds in the right lower lung field. The abnormal findings on the chest roentgenogram were a tumorous shadow in the right lower lung field and infiltrative (interstitial pneumonia [IP]-like) shadows bilaterally in the peripheral lung fields, as shown by the arrows A ($S^4$) and B ($S^3$), L ($S^5$) respectively in Fig. 1. These lesions were also confirmed by the chest CT (Fig. 2). Echography and the CT scan revealed that the abdomen was disease-free. Blood cell counts and biochemical analysis showed no abnormality, although the blood gas data were abnormal (PaO$_2$: 62.4 Torr, PaCO$_2$: 34.2 Torr, pH: 7.49, HCO$_3^-$: 25.9 mEq/L). Bone marrow biopsy revealed no morphologically abnormal cells. Nor was there any evidence of leukemic conversion on the peripheral blood smear.

Immunologic study ruled out autoimmune diseases including Sjögren’s syndrome or other immunological disturbances. H & E-stained specimens taken by TBLB from the site of the tumorous shadow ($S^4$) and the site of infiltration ($S^3$) demonstrated diffuse proliferation of small lymphocytes without follicular centers (Figs. 3a, b). These paraffin-embedded tissue sections were stained with anti-pan B-cell monoclonal antibody (CD20: MX-PanB; Kyowa Co. Ltd., Tokyo, Japan), but not with anti-immunoglobulin $\kappa$ or $\lambda$ light chain antibodies. Thus, PPL, PDL and LIP could not be differentiated immunohistochemically. Therefore, we tried to determine the clonality...
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Fig. 1. The chest roentgenogram on admission showing a tumorous shadow (arrow A: S\textsuperscript{4}) in the right field and bilateral infiltrative shadows (arrows B\textsubscript{R}: S\textsuperscript{3} and B\textsubscript{L}: S\textsuperscript{4}).

Fig. 2. The chest CT image showing a tumorous shadow (arrow A: S\textsuperscript{4}) in the right field and an infiltrative shadow (arrow B\textsubscript{L}: S\textsuperscript{4}) in the left field.

Fig. 3. Histological findings of the specimens (S\textsuperscript{3} and S\textsuperscript{4}) taken from B\textsuperscript{3} and B\textsuperscript{4} area (Fig. 3a and b respectively) showing diffuse proliferation of small lymphocytes without follicular center (HE stain, ×200).

Materials and Methods

BAL of areas B\textsuperscript{3} and B\textsuperscript{4} on the right side was repeated three times with 50 ml of saline. The sample cells were collected by centrifugation of the BALF at 700×G for 10 min. These cells were stained with fluorescein isothionate (FITC)-labeled polyclonal antibody specific for immunoglobulin κ or λ light chain (TAGO Inc., Burlingame, C.A., U.S.A.) and FITC-labeled monoclonal anti-pan T-cell antibody (Leu4; CD3). Stained cells were passed through a flow cytometer (FACScan; Becton-Dickinson Electronics, Mountain View, C.A., U.S.A.) and small round cells corresponding to normal lymphocytes were gated on to the scattergram and then their cell types were analyzed. Macrophages were excluded by gating out large cells.

KLI was performed using the κ, λ-histograms obtained from the above cytometric analysis and the Kolmogolov-Smirnov two-sample statistical test. The KLI procedure was described previously in detail (2). The results of KLI are expressed as δ-curves (Fig. 4). The criteria for δ-curves are as follows (shown on the left side of Fig. 4): a δ-curve with a maximum point more than 1,000 implies that κ-monoclonal B-cells are present, while a curve with a minimum point less than 0 indicates that λ-monoclonal B-cells are present. When there are monoclonal B-
cells, the curve shows a characteristic shape, which resembles the M-peak seen in protein electrophoresis as seen in multiple myeloma.

**Results**

BALF cells from the B\(^4\) area linked with the tumorous lesion consisted predominantly of T-cells (CD3\(^+\) 89.9%) and a minor population of B-cells (surface immunoglobulin positive B cell 8.9%). However, these B-cells produced a convex M-peak-like profile on the δ-curve (Fig. 4, right). In other words, κ-monoclonal B-cells were thought to be present among the B\(^4\) derived BALF cells. On the other hand, BALF cells from the B\(^3\) area linked with the infiltrative lesion were also composed of many T-cells (78.0%) and a small number of B-cells (19.6%). Similarly, these B-cells demonstrated a monoclonal pattern (Fig. 4, center). Both B\(^3\) - and B\(^4\)-derived BALF B-cells were judged to be small lymphocytes according to the gating procedure.

Consequently, the patient was diagnosed as having PPL of the diffuse, small lymphocytic (NCI-sponsored Working Formulation) (3) and B-cell type. Moreover, the clinical stage was judged to be IV according to the Ann Arbor classification (4) because the disease was disseminated in both lungs.

**Discussion**

Until now, the final diagnosis of PPL was decided chiefly by OLB or lobectomy (1). However, it has remained difficult to differentiate PPL from LIP morphologically, because their patterns of lymphocytic proliferation are similar.

Recently, advances in immunohistochemistry have made it possible to investigate the clonality of a B-cell population at the tissue level. Immunoglobulin light chain restriction, as seen mainly in frozen-tissue section, can indicate the monoclonal proliferation of B-cells. This would suggest the existence of B-cell tumors such as B-cell lymphoma or plasmacytomas. On the other hand, mosaicism of κ- and λ-chain stained cells implies that the B-cell population is polyclonal. This would be characteristic of non-malignant B-cell proliferative disorders such as PDL or LIP (1, 5-8). The volume of tissue taken by OLB or lobectomy is enough to verify the clonality immunohistochemically, but that provided by TBLB is too small. Thus, OLB or lobectomy is currently thought to be indispensable for the clinical diagnosis of pulmonary lymphoproliferative disorders. However, these procedures are so cumbersome they can not always be performed in every institution.

Recently, the subsets of BALF lymphocytes were investigated in various pulmonary diseases (9–11). However, BALF analysis has not yet been applied in general to diagnose pulmonary lymphomas because of several limitations including the fact that only a paucity of lymphocytes can be collected by BAL.

The κ-λ analyses such as Ault’s D-value assessment (12) and our KLI require a much lower absolute cell number than that required for DNA analysis; while being too few for DNA analysis, the number of cells collected from the usual BALF volume (150 ml) is sufficient for KLI. Furthermore, KLI has the advantage that only small cells, i.e. small lymphocytes or small-cleaved lymphocytes, are usually analyzed by using the

![Fig. 4. Schematic normal and monoclonal κ-, λ-patterns of δ-curves and the recorded δ-curves for B\(^3\)- and B\(^4\)-areas (center and right, respectively). Both maximum δ(n) values are greater than 1,000. Abscissa n shows fluorescence intensity. KLI: κ-λ imaging, BALF: bronchoalveolar lavage fluid.](image-url)
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cytometric cell-gating technique. Thus, KLI is thought to be suitable for investigating primary pulmonary lympho-proliferative disorders such as PPL, PDL and LIP in which small lymphocytes proliferate. Moreover, KLI is almost as sensitive as the usual DNA analysis by Southern blot hybridization (13), because KLI can detect as little as 3–7% of monoclonal small B-cells among normal lymphocytes (2).

In the present case, we were unable to verify the clonality at the tissue level, because the specimens obtained by TBLB were not prepared for freezing, but for paraffin-embedding. However, when BALF cells obtained from both B4 and B3 areas were analyzed by KLI, the monoclonality of κ-type was disclosed in both BALF B-cells which constituted only a minor population among BALF lymphocytes. These results suggest that both the tumorous and IP-like lesions were composed of B lymphoma cells.

KLI has a disadvantage that it can not be used to study B-cells which do not express slg. However, most infiltrative B-cells found in pulmonary lymphoproliferative disorders are small and usually do express Ig. Thus, KLI can be used to verify the clonality of these B-cells. If the differential diagnosis of PLL, PDL and LIP can not be made from TBLB and if OLB or lobectomy can not be done, the combination of BAL and KLI should allow one to make the diagnosis.

References


