Acta Med. Nagasaki 43 : 62−66

Evaluation of Bronchoalveolar Lavage as a Diagnostic Procedure for Primary Pulmonary B-cell Lymphoma

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We evaluated retrospectively the role of bronchoalveolar lavage (BAL) in the diagnosis of primary pulmonary B-cell lymphoma in four patients. Histological examination of transbronchial lung biopsy specimens showed nonspecific infiltration of small lymphocytes. Examination of BAL fluid (BALF) samples showed lymphocytosis in all samples with dominant B-cell in two patients and T-cell in the remaining patients. In two patients only, there was a increase in B-cell bearing IgM light-chain or M-protein in BALF samples. our results suggest that the diagnostic value of BAL in primary pulmonary B-cell lymphoma is limited and that new molecular biological techniques should be adapted for analysis of BALF samples.

Introduction

Various lymphoproliferative diseases containing reactive and tumorous lesions may involve the lung (1), and it is often difficult to accurately distinguish such lesions by conventional radiography or small biopsy specimens such as transbronchial lung biopsy (TBLB). A definitive diagnosis in the early stages of the disease is quite important in these cases since certain pulmonary lymphoproliferative disorders, especially lymphomas of the small lymphocytic type, frequently show malignant features during long the clinical course (2). A commonly used diagnostic procedure in pulmonary medicine is bronchoalveolar lavage (BAL). Using a fiberoptic bronchoscope, BAL is a useful method for the diagnosis of various pulmonary diseases (3). In addition, recent advances in immunological or molecular biological techniques have made BAL a more useful diagnostic procedure in several other diseases such as pulmonary lymphoma (4-7). In the present study, we analyzed samples of bronchoalveolar lavage fluid (BALF) obtained from four patients with primary pulmonary lymphomas, and retrospectively examined the diagnostic value and limitations of BAL in these cases.

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Patients and Methods

Case 1

A 75-year-old asymptomatic nonsmoker male was admitted with infiltrate in the left lower lobe on chest roentgenogram in 1992. A small amount of pleural effusion with pleural thickening in the right lower region posteriorly was also found on computed tomography. Transbronchial lung biopsy (TBLB) was obtained from the affected area of the lung, where histological examination of the specimen showed infiltration of small lymphocytes. Laboratory examination showed a marked increase in serum IgM and IgM-kappa monoclonal gammopathy. A proportion of lymphocytes in pleural effusion samples were B-cells surfaced with monoclonal IgM-kappa light chain on it's surface. The pleural biopsy specimen was histologically similar to the TBLB specimen.

Case 2

A 43-year-old nonsmoker female was admitted to our unit for further investigation of asymptomatic pulmonary infiltrates that had progressively increased in size during the last four years. The infiltrates were in the anteromedial segment of both lower lobes and were detected on a routine chest roentgenogram in 1988 (8). She had autoimmune hemolytic anemia with increased haptoglobin

and positive direct antiglobulin test (Coomb's test), and serum immunoglobulin levels were normal. Histological examination of TBLB specimens from both lung lesions showed non-specific infiltration of small lymphocytes. The patient underwent diagnostic segmentectomy of the right pulmonary lesion, where histological findings were compatible with those of pseudolymphoma as previously described by Saltzstein (9). Gene analysis of the surgical specimens showed Ig heavy-chain and kappa-chain genes rearrangement.

Case 3

A 75-year-old nonsmoker male had slowly spreading infiltrates without lymphadenopathy for approximately five years in the right upper lobe on the chest roentgenogram (4). TBLB was obtained from the right upper lobe in 1985 and histological examination was suggestive of a benign pulmonary lymphoid tumor with infiltration of small lymphocytes with germinal centers. The serum IgM, however, increased gradually together with a spread of the infiltrate. IgM-lambda monoclonal gammopathy was detected in the serum and BALF by immunoelectrophoresis.

Case 4

A 62-year-old asymptomatic nonsmoker male was admitted for further examination of infiltrates in the left upper lobe on the chest roentgenogram in 1985. Histological examination of the TBLB specimen showed infiltration of small lymphocytes with germinal follicles, and immunoperoxidase staining of the tissue showed IgM-kappa monoclonality.

Analyses of BALF and Gene

In all patients, BAL of the radiographically involved areas was performed as previously described (10). Supernatants obtained after centrifugation of BALF were used for measurement of protein fractions and immunoglobulins, and immunoelectrophoresis. TBLB specimens were stained with hematoxylin-eosin, and the frozen sections were stained by using the immunoperoxidase method.

Gene analysis of tumors in the case 2 was performed by using the method reported by Fishleder et al. (11). DNA isolated from the tumor tissue was digested by the restriction-endonuclease enzyme *Hind III, Bam HI/Hind III, or Eco* RI, and size-fractionated by agarose gel electrophoresis. Digests transferred by Southern blotting were hybridized with 32P-labeled JH, Jκ, Cβ, and Jr1 probes (8).

Results

As stated above, the four TBLB specimens stained with hematoxylin-eosin showed infiltration of small lymphocytes without atypical lymphocytes and/or with germinal centers. However, these histological features were not diagnostic for lymphoma. Although M-protein was found in the sera of two patients (cases 1 and 3), monoclonality in the biopsy specimens was not defined (Table 1). In case 4, only the TBLB specimen was diagnostic, where

Table 1. Serological and Pathological Analyses

Patient	Immunoglobulin (per albumin)		Immuno-electrophoresis	Histological finding of TBLB	Immuno-peroxidase stain	Gene rearrangement
Case 1	IgG	0.313	M-protein (IgM-kappa)	small mature lymphocytes infiltrate	Polyclonal	N.D.
	IgA	0.053				
	IgM	0.765				
Case 2	IgG	0.374	Normal	diffuse small lymphocytes infiltrate	Polyclonal	IgH and kappa light chain rearrangement
	IgA	0.069				
	IgM	0.025				
Case 3	IgG	0.367	M-protein (IgM-lambda)	small mature lymphocytes infiltrate	IgG-lambda (scattered)	N.D.
	IgA	0.142				
	IgM	0.292				
Case 4	IgG	0.337	Normal	small mature lymphocytes infiltrate	Monoclonal (IgM-kappa)	N.D.
	IgA	0.225				
	IgM	0.029				

TBLB, transbronchial lung biopsy; N.D., not done

IgM-kappa positive B-cells were diffusely stained in the frozen sections.

Lymphocyte population in BALF in all but case 4 was markedly increased, where the major population of lymphocytes was B-cell in cases 1 and 2, and T-cell in cases 3 and 4 (Table 2). In case 1 with B-cell dominance and case 3 with T-cell dominance in BALF, M-protein with IgM-kappa and IgM-lambda was detected in the supernatants of BALF. The ratio of IgM to albumin in BALF in each patient (1.815 and 0.765) was much higher than that in serum (1.414 and 0.292). In another B-cell dominant BALF (case 2), rearrangements of Ig heavy-chain and kappa-chain genes were noted in the surgical specimen (Fig. 1). Consequently, primary pulmonary B-cell lymphoma was diagnosed in two patients by BALF analysis, in one patient by immunostaining of the TBLB specimen, and in the other patient by gene analysis of the surgical specimen.

Our four patients had very slow progressive pulmonary lesions with a range of approximately two to more than ten years, and a poor response to chemotherapy.

Table 2. Analysis of Bronchoalveolar Lavage Fluid

	CASE 1	CASE 2	CASE 3	CASE 4
Total cell number (x 10^7)	2.676	4.572	0.957	1.31
Lymphocyte (%)	42.3	47.9	78	16
Lymphocyte subset (%)				
CD3	9.6	5.2	87.2	79.7
CD4	11.2	1.8	37	37
CD8	5.1	3.4	55.4	31.3
CD21	84.5	88.9	2	2
Surface marker ($^{\kappa}$-B/$^{\lambda}$-B)	16.6	12.3	Not detected	Not detected
Immunoglobulin per albumin				
IgG	0.624	0.333	0.371	0.571
IgA	0.052	0.057	0.126	0.571
IgM	1.815	0.007	1.414	0.029
Immunoelectrophoresis	M-protein (IgM-kappa)	Normal	M-protein (IgM-lambda)	Normal

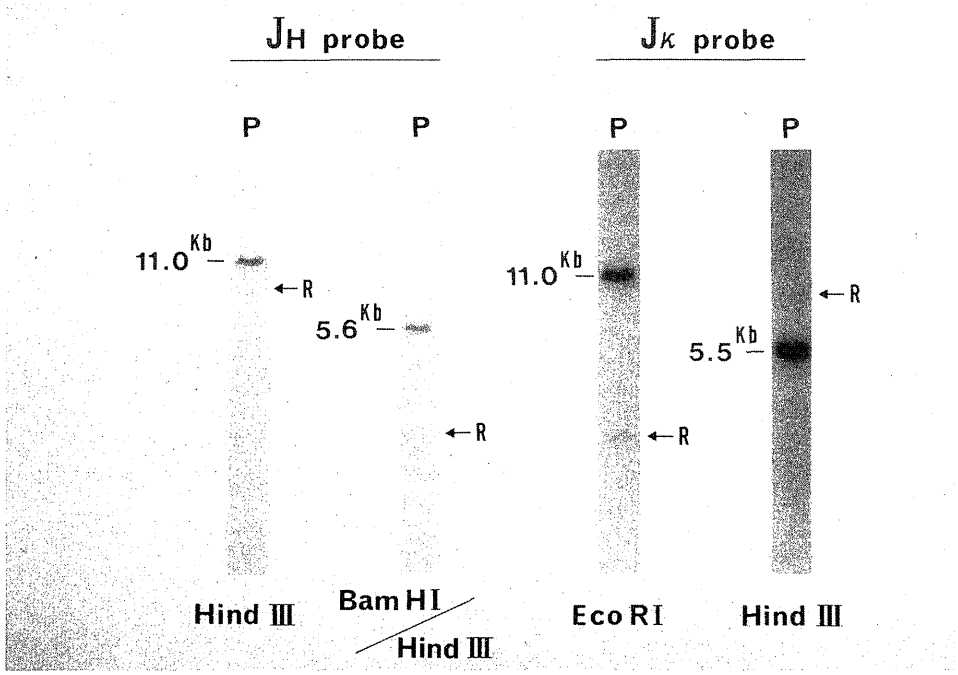

Figure 1. Southern blot analysis of DNA from surgical tumor specimen in the case 2.
—, germline band position; R, rearranged band position. (publication permitted by Lancet Ltd., London, UK).

Discussion

Pulmonary lymphoproliferative disorders include a variety of diseases ranging from benign to malignant (1). Primary pulmonary lymphoma is relatively rare with less than one percent of all lymphomas (12), and its diagnosis is often difficult. Histopathological examination of TBLB specimens allows relatively easy diagnosis of certain T-cell lymphomas or Hodgkin's lymphoma due to their characteristic morphological features such as cleaved, convolutes or Sterunberg-Reed cells. On the other hand, TBLB specimens of B-cell lymphoma, especially small lymphocytic type (13) or lymphocytic type (14), are often difficult to diagnose even after a thorough histopathological and immunohistochemical examination, and surgical specimens by thoracotomy may be required to establish a firm diagnosis.

BAL is a non-invasive and may be a useful diagnostic procedure in lymphoproliferative disorders. For example, detection of lymphoid cells in BALF with specific morphologic features is quite diagnostic for Hodgkin's lymphoma (5-6) or plasma cell dyscrasia (15). However, the presence of lymphoma cells with scanty atypism may make the diagnosis based on morphological changes difficult because of the degenerative changes affecting the cells during aspiration. Therefore, a false positive or negative diagnosis may be made. Thus, lymphoid cells in BALF seem to have a limited value in the diagnosis of lymphoproliferative disorders. At the present, to confirm pulmonary B-cell lymphoma by BALF, the following finding should be detected in BALF: monoclonality such as monoclonal gammopathy, dominant B-cell population, or gene rearrangement in lymphocytes.

Detection of monoclonal proliferation of lymphoid cells is important in differentiating malignant lymphomas from benign lymphoproliferative disorders (16). On the other hand, immunoperoxidase staining of lymphoid cells in BALF is a useful method for detecting monoclonality. In two of our patients, monoclonal lymphoid cells were detected in BALF by immunoperoxidase staining of IgM-kappa or lambda, though monoclonality on TBLB specimens could not be detected. The major population of BALF lymphocytes in these patients were CD21 positive B-cells, which were considered to represent lymphoma cells. Immunoperoxidase staining of BALF lymphoid cells is thought to be a useful method especially in B-cell dominant lymphoma.

On the other hand, monoclonality of BALF lymphocytes could not be detected in the two T-cell dominant lymphomas. Accordingly, lymphocytes in BALF from these patients were considered reactive lymphoid cells. In one T-cell dominant case (case 3), immunoelectrophoresis of the BALF supernatant was useful in detecting monoclonality. Monoclonal gammopathy in BALF is considered to be produced by a small population of lymphoma cells that have originated from B-cell. For this reason, the presence of a much higher ratio of IgM to albumin in BALF than in serum supports a local production of the immunoglobulin (2). Immunoelectrophoresis and measurement of immunoglobulins and protein fraction in BALF supernatant may be useful in T-cell dominant lymphomas.

Using the polymerase chain reaction technique, several studies have detected gene rearrangement in lymphocytes from BALF of patients with pulmonary lymphoma (17, 18). Accordingly, in suspicious pulmonary lymphoma, we recommend that cells and supernatant of BALF should be stored separately in a deep freezer for further gene or protein analysis. In conclusion, BAL is a safe and non-invasive method for the diagnosis of certain lung diseases, however, in primary pulmonary B-cell lymphoma, the method should be considered investigational at this stage. Immunological and molecular biological techniques should be extensively adapted to analyze BALF samples.

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