A Case of Hodgkin's Disease of Submandibular Lymph Nodes: Differential diagnosis from anaplastic large cell lymphoma by immunohistochemistry

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A case of Hodgkin's disease, mixed cellularity type, similarly to the histological findings of anaplastic large cell lymphoma (ALCL) is presented. A 64-year-old man with a painless mobile swelling of left submandibular lymph nodes was referred to Nagasaki University Dental Hospital. CT, MRI and ultrasonic examination identified three enlarged lymph nodes adjacent to the submandibular gland. Histologically, proliferation of lymphoid cells with clusters of epithelioid cells, plasma cells and a few eosinophils were observed. Giant cells with one or more bizarre nuclei including distinct nucleoli were scattered or cohesively arranged in the lymph nodes. The giant cells were regarded as Hodgkin or Reed-Sternberg cells. However, cohesive arrangement and immunopositivity for EMA of the giant cells resemble the findings of ALCL. In order to discriminate between Hodgkin's disease and ALCL, immunohistochemical examination using other antibodies was performed. Finally, the lesion was diagnosed as Hodgkin's disease because of immunoreactivity for CD 15, CD 30 and EBV-LMP 1, and strong reactivity for fascin, in addition to the presence of classical Reed-Sternberg cells.

***Key words*: Hodgkin's disease, submandibular lymph node, anaplastic large cell lymphoma**

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Introduction

Malignant lymphoma is divided into two main types, termed Hodgkin's disease and non-Hodgkin's lymphoma. Hodgkin's disease usually arises from lymph nodes. Most cases of Hodgkin's disease are not difficult to diagnose histologically because they include peculiar giant cells, namely Hodgkin cells and Reed-Sternberg cells, which are immunohistochemically positive for CD 30 (1-4). On the other hand, anaplastic large cell lymphoma (ALCL) is characterized by cohesive infiltration of large neoplastic cells in lymph nodes (5, 6), and belongs to non-Hodgkin's lymphoma. The large cells of ALCL are also positive for CD 30. Occasionally, histological features of tumor cells in ALCL resemble those of Hodgkin cells and Reed-Sternberg cells (4, 5, 7).

We report here a case of Hodgkin's disease with similar histological appearance to ALCL, and discuss differences between this disease and ALCL.

Case Report

In February of 1999, a 64-year-old man attended an otorhinolaryngology clinic because of painless swelling in the left submandibular area. The swelling did not improve despite administration of antibiotic agents. As the lesion was regarded as arising from a tooth, the patient consulted a dental clinic. However, no inflammatory changes were detected in the teeth or periodontal tissues. Therefore, on March 15 of 1999, he was referred to Nagasaki University Dental Hospital. Three mobile swellings covered with normal colored skin were present from the left submandibular to the infra-auricular area. CT, MRI and ultrasonic examination revealed three enlarged lymph nodes approximately 1, 2 and 3 cm in diameter, respectively adjacent to the submandibular gland (Fig. 1). No lymphadenopathy was observed in other sites. Serological examination showed high IgG titers against rubella, cytomegalovirus, mumps, measles, herpes simplex, varicella and Epstein-Barr virus, but infection with HTLV-I was not detected. Counts of blood cells were in the normal ranges, and biochemical examination displayed no unusual findings. Clinically, metastatic tumor was denied, and malignant lymphoma or lymphadenitis was suspected. On April 8 of 1999, the lymph nodes were removed.

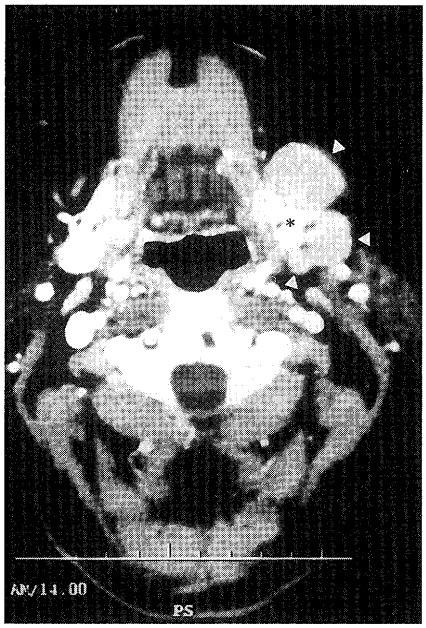

Fig. 1: CT revealed three enlarged lymph nodes (arrowheads) on the border of the left submandibular gland (asterisk).

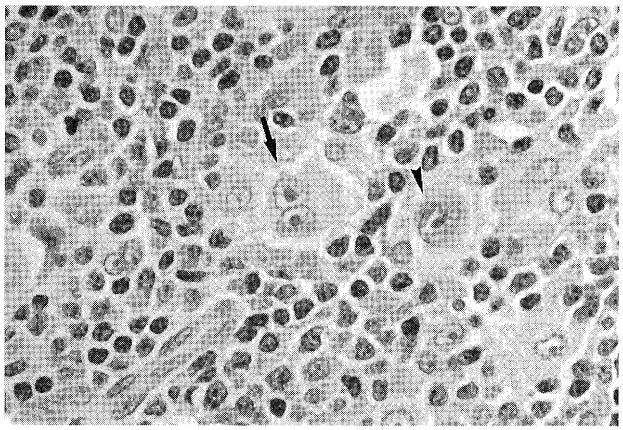

Fig. 2: Giant cells with mirror image nuclei (arrow) and bizarre indented nucleus (arrowhead). The halo around the distinct nucleoli was prominent. (H&E, ×600).

Pathological Findings

Extirpated lymph nodes were covered with a normal capsule, and the cut surface was milky to gray-white in color. Histologically, thickening of the capsule and fibrosis were not observed. Enlarged lymph nodes consisted of hyperplasia of lymphocytes accompanied with clusters or islands of epithelioid cells. Plasma cells and a few eosinophils were intermingled in the lymphoid tissue. These cells had no atypical nuclei. However, atypical giant cells with bizarre nuclei were scattered or cohesively infiltrated, and mitotic figures were increased near the giant cells. The giant cells had one or more nuclei including large distinct amphophilic nucleoli with a perinucleolar halo. Some of these had a symmetrical arrangement, i. e. mirror image nuclei. These giant cells were regarded as Reed-Sternberg cells (Fig. 2). Therefore, a possible histological diagnosis was Hodgkin's disease. However, it was necessary to distinguish the lesion from ALCL because cohesive clusters of giant cells were seen.

We performed immunohistochemical characterization of the giant cells. For immunohistochemical study, Envision+ system (Dako) was applied to formalin-fixed, paraffin-embedded sections. The antibodies used in the immunohistochemical examination were directed against CD 45 (Dako, 1:200), CD 20 (Dako, 1:100), CD 45 RO (Dako, 1:50), CD 30 (Dako, 1:40), CD 15 (Dako, 1:50), fascin (Dako, 1:100), BLA.36 (Dako, 1:50), ALK 1 (p80) (Dako, 1:50), Keratin-wide (Dako, 1:350), EMA (Dako, 1:100), S-100 protein (Dako, 1:400) and LMP 1 (Novocastra, 1:200). Moreover, in situ hybridization for EBER was performed in order to detect latent infection with Epstein-Barr virus according to the method described in our previous study with slight modification (8). For determination of Hodgkin's disease, antibodies to CD 30, CD 15, fascin (9) and BLA.36 (10) were applied.

The results of immunohistochemical examination are listed in Table 1. CD 30 and CD 15 were localized in the cytoplasm of giant cells in the present case. CD 30-positive cells dispersed or showed cohesive arrangement in the lymph nodes (Figs. 3 A and 3 B). The giant cells displayed intense cytoplasmic immunoreactivity for fascin and BLA.36. Surrounding dendritic cells and some lymphocytes also reacted with fascin and BLA.36, respectively (Fig. 4). The giant cells were negative for CD 45, CD 20 and CD 45 RO, whereas circumferential small lymphocytes were homogeneously positive for CD 45, and showed heterogeneous immunoreactivity for CD 20 and CD 45 RO. We detected no ALK 1 in the giant cells in this case. Keratin-wide, EMA and S-100 protein were employed to exclude metastasis of malignant epithelial tumor and malignant melanoma. All giant cells were negative for keratin and S-100 protein, but a small number of the cells reacted positively for EMA in the Golgi area (Fig. 5). Both immunohistochemistry for LMP 1 and in situ hybridization for EBER revealed intense reaction in the giant cells and some lymphocytes (Fig. 6).

Consequently, we made final histological diagnosis as Hodgkin's disease, mixed cellularity type.

Table 1: Immunohistochemistry of atypical giant cells

Antibody	Reactivity	Antibody	Reactivity
CD 45/LCA	−	ALK 1(p80)	−
CD 20/L 26	−	Keratin-wide	−
CD 45 RO/UCHL 1	−	EMA	+*
CD 30/BerH 2	+	S-100 protein	−
CD 15	+	LMP 1	+
Fascin	+		
BLA.36	+	EBER-ISH**	+

Abbreviations: BLA.36, B lymphocyte antigen, 36 kD; ALK 1, anaplastic lymphoma kinase 1; EMA, epithelial membrane antigen; LMP 1, latent membrane protein 1; EBER, Epstein-Barr virus encoded small RNAs

*A few giant cells were positive. **in situ hybridization for EBER

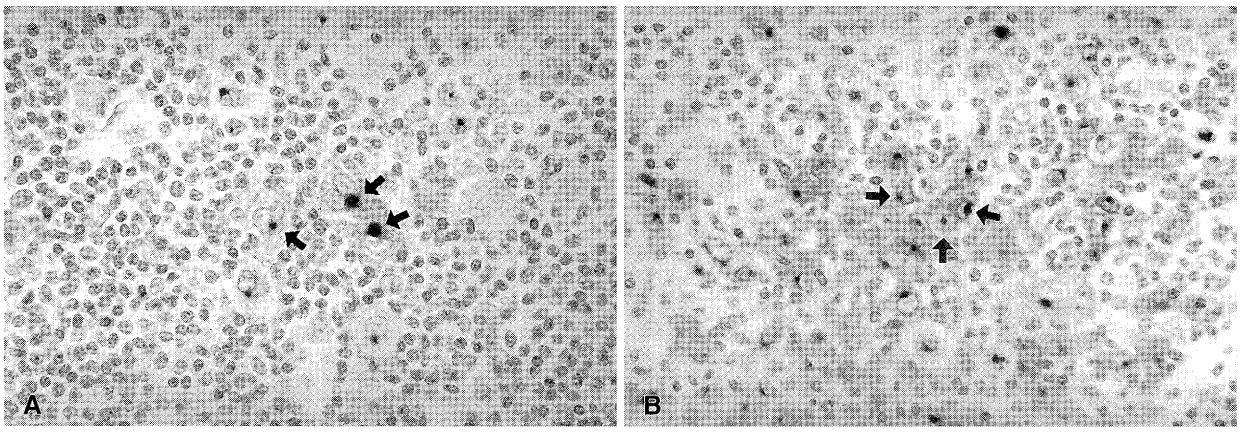

Fig. 3: (A) Scattered giant cells reacting with CD 30. Immunoreactivity was found in the juxtanuclear (Golgi) area (arrows, ×350). (B) Cohesive infiltration of CD 30-positive cells. CD 30 was localized in Golgi area (arrows, ×350).

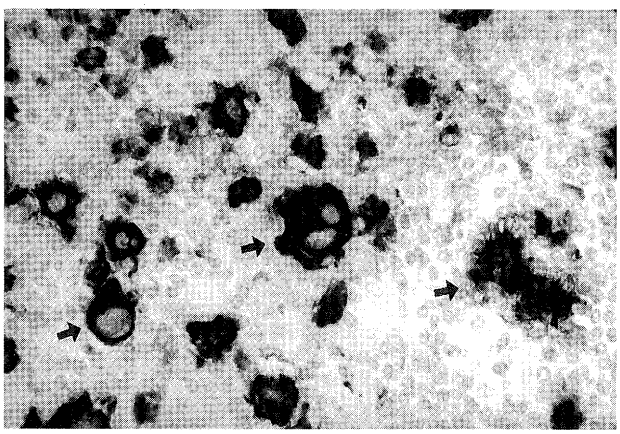

Fig. 4: Immunostaining for fascin. Neoplastic giant cells displayed intense immunoreactivity (arrows). Dendritic cells were also positive. (×350)

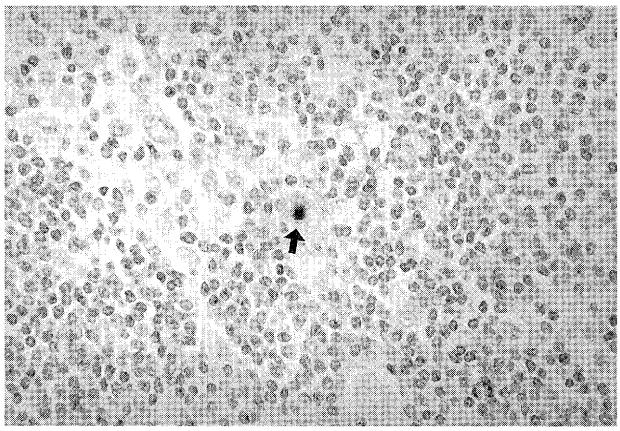

Fig. 5: A neoplastic giant cells was positive for EMA in the Golgi area. (arrow, ×350)

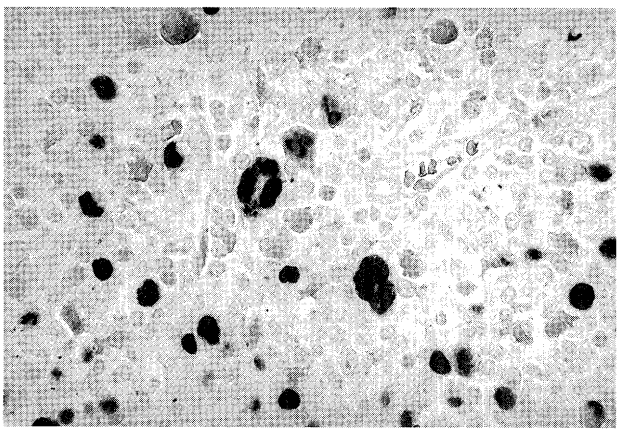

Fig. 6: In situ hybridization for EBER revealed intense signals in giant cells. (×600)

Management

The patient was referred to Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University School of Medicine. Combination chemotherapy with cyclophosphamide, adriamycin and vincristine was applied during May and August in 1999. His condition improved and a complete remission was confirmed. He has been followed up as an outpatient since leaving the hospital.

Discussion

Histologically, Hodgkin's disease is characterized by the presence of Hodgkin cells and Reed-Sternberg cells. Therefore, it is easy to make a diagnosis of Hodgkin's disease if typical Reed-Sternberg cells are present. In 1985, Stein *et al.* (5) advocated Ki-1-positive large cell lymphoma (anaplastic large cell lymphoma) belonging to non-Hodgkin's type. Ki-1 is categorized as CD 30, which is an antibody against a Hodgkin's disease-derived cell line. Most cases of ALCL originate from lymphocytes with cytotoxic potential (11). As neoplastic large cells of ALCL are morphologically similar to Hodgkin cells and Reed-Sternberg cells, a differential diagnosis from ALCL is recommended when Hodgkin's disease is confirmed (4, 5, 7).

ALCL reveals cohesive proliferation of large neoplastic cells, whereas Hodgkin's disease has a dispersed distribution of tumor cells (2, 5). Our case showed both arrangements of CD 30-positive tumor cells. Antibody against CD 15 (i.e. Leu M 1) is useful for diagnosis of Hodgkin's disease (12). Immunohistochemistry for CD 15 reveals cell membrane and juxtanuclear (Golgi) staining of most Reed-Sternberg cells and Hodgkin's cells. How-

ever, small numbers of ALCL cases show CD 15 expression in anaplastic large cells (4, 12). Moreover, ALCL occasionally exhibits immunoreactivity with EMA, and the incidence of EMA-positive Hodgkin's disease is lower than that of ALCL (4, 13). In our case, most of the giant cells were negative for EMA, but a few giant cells displayed localization of EMA in the Golgi area. ALK 1(p 80) was found as a chimeric protein associated with t(2; 5) in ALCL (13-16). However, ALCL without t(2; 5) exhibits no ALK 1-immunoreactivity (13). In the present case, no translocation was detected (unpublished data) and no immunoreactivity for ALK 1 was observed.

These immunohistochemical results emphasize the difficulty of differential diagnosis between Hodgkin' disease and ALCL. We depend on the histological appearance and fascin immunoreactivity for the diagnosis of such cases. Sometimes, multinucleated cells of ALCL resemble Reed-Sternberg cells, but there have been no histological reports of the perinucleolar halo in large neoplastic cells of ALCL (2, 4, 6). Moreover, the distribution of tumor cells differs from that of Hodgkin's disease (5). Neoplastic cells of ALCL often preferentially involve the sinus of lymph nodes (2, 5, 6). In the present case, we found typical "diagnostic" Reed-Sternberg cells, and clusters of the giant cells were not located in the sinus of the lymph nodes. Anti-fascin antibody reacts with Reed-Sternberg cells and their variants. Pinkus et al. (17) investigated immunolocalization of the protein in malignant lymphomas including Hodgkin's disease and ALCL, and reported that fascin might aid in distinguishing Hodgkin's disease from non-Hodgkin's lymphomas in difficult cases. They reported difference in immunoreactivity between Reed-Sternberg cells and neoplastic cells in ALCL, i.e. Reed-Sternberg cells exhibited intense reactivity for fascin, whereas anaplastic large cells showed weak immunoreactivity. Especially, null-cell type anaplastic large cells showed weak granular cytoplasmic staining. In our case, the giant cells regarded as Reed-Sternberg cells revealed diffuse intense immunoreactivity for fascin unlike anaplastic large cells.

The revised European-American classification of lymphoid neoplasms (REAL classification) includes Hodgkin-like ALCL as a provisional entity (2). This type of lymphoma has capsular thickening, nodular growth of tumor cells and sclerotic bands, and is usually negative for EBV. On the other hand, LMP 1 expression in Hodgkin's disease, mixed cellularity type, is observed in two thirds of all cases examined (18). In the present case, no fibrosis or sclerotic changes were detected, and most tumor cells were positive for LMP 1 and EBER. Consequently, we made a final diagnosis of Hodgkin's disease, mixed cellularity type.

The origins of Hodgkin cells and Reed-Sternberg cells are still unknown. The immunophenotype of tumor cells of Hodgkin's disease, mixed cellularity type, is CD 30+, CD 15+/-, CD 45-, B-cell and T-cell associated antigen-negative and EMA- (2). We found no immunoreactivity for CD 20 or CD 45 RO in tumor cells in the present case. Therefore, we could not determine whether they were derived from B- or T-cells.

In conclusion, to discriminate between Hodgkin's disease and ALCL, we applied the findings of immunoreactivity for CD 15 and CD 30, and strong immunoreactivity for fascin, in addition to the presence of classical Reed-Sternberg cells.

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