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NK AND NK-RELATED NEOPLASMS

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ABSTRACT

Owing to the immunological progress in recent years, differentiation and maturation process of NK cells or NK-related cells has been made clear. Also, their neoplastic counterparts, namely NK cell related lymphoma/leukemia have been characterized. In this paper, we will report NK cell neoplasms based on the new classification of malignant lymphomas. Among these, nasal lymphoma is one of the most cleary defined entities, followed by NK cell leukemia/lymphoma, and blastic/blastoid NK cell leukemia/lymphoma.

INTRODUCTION

In recent years, various monoclonal antibodies have been developed against natural killer (NK) cell or NK-related cells. Of these antibodies, NKH1[1,2] and Leu19[3,4] detect neural adhesion molecule, a relatively specific marker for NK cells. A monoclonal antibody (123C3)[5] recognizing CD56 in paraffin embedded tissue sections, has become commercially available. In addition, monoclonal antibodies against various cytotoxic molecules playing some major role in apoptosis, such as perforin,[4,6-9] granzyme B,[7,10] and TIA-1[11] have been developed. Studies using these antibodies have elucidated various characteristics of lymphomas containing these cytotoxic molecules, such as cytotoxic T cell lymphoma[9] or NK cell or NK-related lymphoma.[3,12] These progress has resulted in characterization of nasal lymphoma[4,12] as a distinct type of NK cell neoplasm accompanying infection with Epstein-Barr virus (EBV). The other NK cell-related neoplasms are mostly extranodal lymphomas affecting skin,[1] muscle, and the gastrointestinal tracts. Sporadic cases of aggressive NK cell leukemia[3,13-15] have been reported, especially in Asian countries including Japan. Like nasal lymphoma, this disease is also considered to be related to EBV infection.

In the REAL classification of malignant lymphomas, NK cell neoplasia was included within the categories of large granular lymphocyte leukemia and angiocentric lymphoma. Recently, however, Jaffe[16] classified NK cell neoplasm into three categories, namely precursor NK cell leukemia, nasal and nasal-type NK cell lymphoma, and aggressive NK cell leukemia. In the new WHO classification, which will be reported in one year or so, NK-related neoplasms will be categorized as NK cell leukemia, extranodal NK/T cell lymphoma, nasal and nasal type.

In this paper, we will describe NK-related neoplasms as, nasal/nasal type NK/T cell lymphoma (focusing on nasal lymphoma), aggressive NK cell leukemia/lymphoma and blastic/blastoid NK cell leukemia/lymphoma.

NASAL/NASAL TYPE NK/T CELL LYMPHOMA

Lymphoproliferative disorders occuring in the sinonasal regions have been called various names such as lethal midline granuloma or polymorphic reticulosis. It has been made clear that these lymphoproliferative disorders are the T cell related lymphomas. Recent studies have further suggested that these cases are natural killer (NK) cell derived lymphomas.[4,17-19] This malignancy occurs at a relatively high frequency among people in Southeast Asia, including those in Japan and China. Although the reason is totally unknown, NK or NK cell related lymphomas are concentrated in some unusual extranodal sites including sino-nasal tissue. This lymphoma also appears to be associated with Epstein-Barr virus (EBV) infection.[4] It is therefore believed that this lymphoma can be categorized as a distinct, unique entity. We have encountered 16 patients with nasal lymphoma and investigated morphologically and immunohistochemically, focusing on perforin expression[4,6,7] in the neoplastic cells and try to elucidate the lineage derivation of these neoplastic cells.

In table 1, clinical and histological characteristics of these cases were shown. The age of the patients ranged from 27 to 89, with the average of 49 years, and the median age was 47 years. Males were 13 and females 3. Prognosis of the patients was generally poor, with the shortest 5 months and the longest 10 years. According to Nakamura et al.,[20] overall survival was estimated 49% at 5 years and correlated with clinical stage. Morphologic features revealed that only two of the cases were diffuse, large cell type and the remaining were diffuse, mixed cell type. In the patients with diffuse, mixed cell lymphoma, the proliferating cells consisted of round, large cells and medium-sized cells with small cleaved-like nuclei. Widespread necrosis was observed in most of the cases, however, angioinvasive or angiodestructive lesion was observed in only half of the cases (Fig.1). Our present study revealed that all of the cases were positive for polyclonal CD3, except the one that was poorly fixed, on paraffin sections (Table 2). On frozen tissue sections, most of the cases (14 cases) were CD3 (Leu4)–, CD4 (leu3)–, CD5 (Leu1)–, CD8 (Leu 2)–, CD16 (Leu11b)–, CD56 (Leu19)+, and CD57 (Leu7)– pheno-

Table 1 Clinical and histological characteristics of the cases

Case	sex/age	classification	necrosis	angioinvasion	prognosis
1	M/64	diffuse, large	+	–	2yr (CR)
2	M/52	diffuse, large	+/–	+	6mo (dead)
3	M/32	diffuse, mixed	+	–	unknown
4	M/27	diffuse, mixed	+	–	3.5yr (CR)
5	M/37	diffuse, mixed	+	–	2yr (CR)
6	M/47	diffuse, mixed	+	–	unknown
7	M/43	diffuse, mixed	–	–	1.5yr (dead)
8	M/50	diffuse, mixed	+	+	10yr (dead)
9	F/89	diffuse, mixed	+	–	unknown
10	M/53	diffuse, mixed	+	–	unknown
11	M/33	diffuse, mixed	+	+	5mo (dead)
12	F/36	diffuse, mixed	+	+	7mo (PR)
13	F/47	diffuse, mixed	+	–	1yr (CR)
14	M/64	diffuse, mixed	+	+	6mo (CR)
15	M/44	diffuse, mixed	+	–	4yr (dead)
16	M/59	diffuse, mixed	–	+	5yr (dead)

CR: complete remission; PR: partial remission

types. The remaining two cases (Case 15 and 16), in contrast, showed CD3 (Leu4)+, CD4 (Leu3)–, CD5 (Leu1)+, CD8 (Leu2)–, CD16 (Leu11b)–, CD56 (Leu19)+, CD57 (Leu 7)– phenotypes (Table 3). All the cases were positive for granzyme B and perforin, on paraffin and frozen tissue sections, respectively. In situ hybridization using the EBER-1 probe revealed that positive reactivity was observed in most of the neoplastic cells in all the cases (Fig.2).

Immunoelectron microscopic studies with polyclonal CD3 revealed that positive reactivity was localized in the perinuclear space but was not on the surface membranes of the neoplastic cells. Immunoelectron microscopic studies with perforin revealed that positive reactivity was expressed as granules in the cytoplasm of the neoplastic cells. Simultaneous staining for perforin and Leu4 (CD3) using immunoelectron microscopy on the samples that were Leu19 (CD56)+,

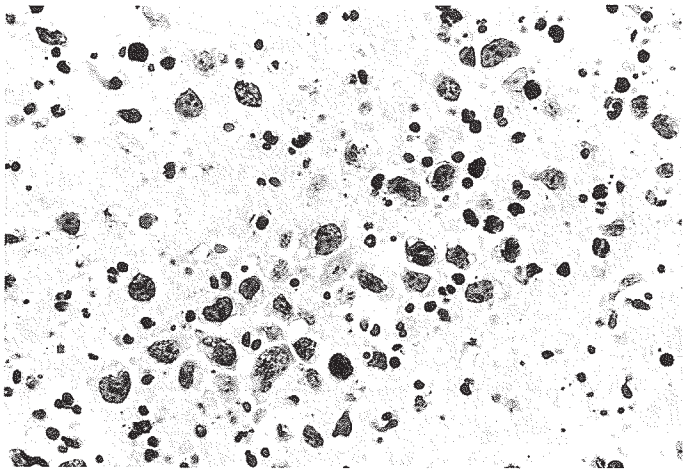

Fig.1 H.E.section of nasal lymphoma reveales extensive necrosis and apoptosis around the neoplastic cells.

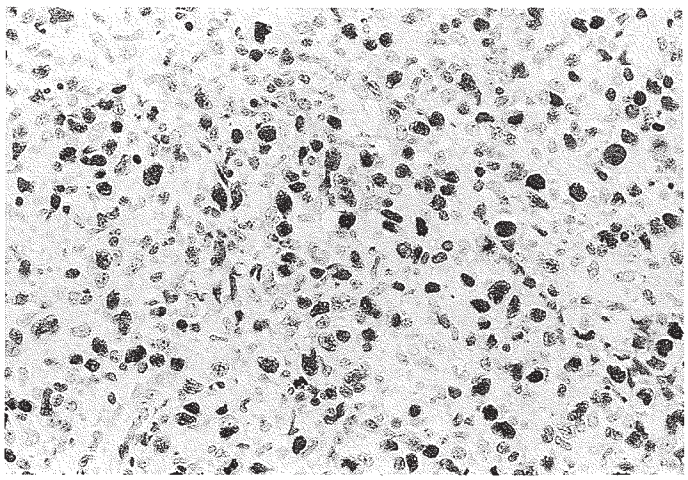

Fig.2 In situ hybridization for EBER-1 reveales positive signals in the nucleus of the neoplastic cells.

Leu4 (CD3)+ light microscopically revealed that the perforin-positive cells were different from the Leu4 (CD3)– positive cells. These latter cells were mainly small lymphoid cells with positive reactivity for Leu4 (CD3) mostly on their surface membranes. From these results, we con-

Table 2 Immunohistochemical findings on paraffin tissue sections

Case	CD3	MT1 (CD43)	UCHL-1 (CD45RO)	Leu7 (CD57)	LMP-1	L26 (CD20)	EBER-1	grazB	123C3 (CD56)
1	+	–	+	–	+	–	+	+	+
2	+	–	+	–	+	–	+	+	+
3	+	+	+	–	–	–	+	+	+
4	+	+	–	–	–	–	+	+	+
5	+	+	–	–	+	–	+	+	+
6	ND	ND	ND	ND	ND	ND	+	+	–
7	+	+	+	–	–	–	+	+	–
8	+	ND	ND	ND	ND	–	+	+	+
9	+	+	+	–	+	–	+	+	+
10	+	+	–	–	+	–	+	+	+
11	+	+	+/–	–	–	–	+	+	+
12	+	+	+	–	–	–	+	+	+
13	+	–	+	–	+	–	+	+	+
14	+	–	–	–	–	–	+	+	+
15	+	+	+	–	+	–	+	+	+
16	+	–	–	–	–	–	+	+	–

grazB: granzyme B

Table 3 Immunohistochemical findings on frozen tissue sections

Case	Leu1 (CD5)	Leu2 (CD8)	Leu3 (CD4)	Leu4 (CD3)	Leu5b (CD2)	Leu7 (CD57)	Leu9 (CD7)	Leu11b (CD16)	Leu19 (CD56)	βF1	TCRδ1	PF
1	–	–	–	–	–	–	–	–	+	–	–	+
2	–	–	–	–	+	–	–	–	+	–	–	+
3	–	–	–	–	–	–	–	–	+	–	–	+
4	–	–	–	–	–	–	+	–	+	–	–	+
5	–	–	+	–	+	–	+	–	+	–	–	+
6	–	–	–	–	+	–	+	–	+	–	–	+
7	–	–	–	–	–	–	–	–	+	–	–	+
8	–	+	–	–	–	–	–	–	+	–	–	+
9	–	–	–	–	–	–	–	–	+	–	–	+
10	–	–	–	–	–	–	–	–	+	–	–	+
11	+/–	–	–	+/–	–	–	+	–	+	–	–	+
12	–	–	–	–	+	–	–	–	+	–	–	+
13	–	–	–	–	+	–	+	–	+	–	–	+
14	–	–	–	–	–	–	–	–	+	–	–	+
15	+	–	+	+	+	–	+	–	+	+	–	+
16	+	–	+	+	–	–	–	–	+	+	–	+

PF: perforin

sider that these two cases we tentatively evaluated positive for T cell markers, such as Leu4 or βF1, on light microscopy, are actually negative for them.

In our study, 30 to 80% of the proliferating cells in all of the cases were positive for Leu19 (CD56), which is one of the markers of NK cells (Fig.3). In addition, our study also revealed perforin expression in the proliferating cells of all of the cases examined. Recent studies indicated that perforin is expressed by cytotoxic T cells or NK cells. The former cells have CD8-positive reactivity. In our present study, all of the cases were negative for CD8. This further indicated that these neoplasms were derived from NK cells. Immunoelectron microscopic studies also confirmed that perforin is localized in a granular fashion. In our present study, on paraffin tissue sections, 30 to 80% of the proliferating cells of all of the cases except one, which was poorly fixed, were all positive for polyclonal CD3. However, in frozen tissue sections, the proliferating cells of the 14 cases were negative for Leu4 (CD3). Our results were quite similar to Chan et al.,[18] indicating a 60% discordant rate of CD3 expression between paraffin and frozen tissue sections. Similar results were also reported by other researchers. Their explanation for that discordance in the cases with T/NK cell lymphomas is as follows. Activated adult NK cells can express CD3ε-chain transcript, which is recognized by polyclonal CD3 antibody. However, these cells can not be recognized by Leu4 (CD3) antibody, which recognizes a conformational determinant of CD3 γε or CD3 δε but can not recognize CD3ε only. Our immunoelectron microscopy studies also revealed that the neoplastic cells were positive for polyclonal CD3 in their cytoplasm but not on the surface membranes. This finding further suggests that these lymphomas were derived from NK cells. Although the number of cases in our study was small to draw definite conclusion in terms of lineage derivation, we believe that all of our cases were derived from adult type NK cells. Recently, it has been made clear that EBV plays a significant role in the occurrence of sino-nasal lymphoma. Our study also revealed that all the cases except one were positive for EBV by in situ hybridization. This finding further suggests that EBV plays an important role in the pathogenesis of nasal lymphoma. We therefore conclude that nasal lymphoma is a quite homogeneous entity and the neoplastic cells have a phenotype of activated NK cells. So far we experienced, we did not see any case with γδ phenotype.

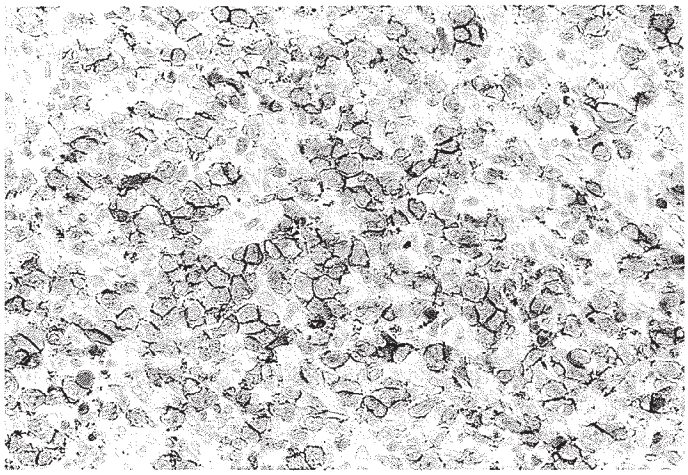

Fig.3 Immunostaining with 123C3 (CD56) reveales positive reactivity on the membranes of the neoplastic cells.

AGGRESSIVE NK CELL LEUKEMIA/LYMPHOMA

Previous reports have described clonal disorder of large granular lymphocytes. Among these, most represented CD3-positive large granular lymphocyte proliferation, which has an indolent clinical course but carries susceptibility to infection due to severe neutropenia, as well as associations with rheumatoid arthritis and splenomegaly. Reports of CD3-negative LGL leukemia are numerous,[3,13,14] most of which were Japanese cases. The CD3-negative LGL leukemia is considered to originate from NK cells. However, there have not been many cases reported with nodal type of this disease.

We experienced four cases with aggressive natural killer (NK) cell lymphoma. All cases followed an aggressive course with death occuring within about three months of initial presentation. In these cases, the neoplastic cells disseminated throughout systemic lymph nodes and invaded various tissues and organs. The lymphoma cells were large cells showing nuclear irregularity, and a pattern of sinusoidal invasion in lymph nodes. Apoptosis and coagulation necrosis both were frequently observed. Hemophagocytosis was observed in all cases. Neoplastic cells in paraffin embedded tissue specimens from these patients were immunoreactive for CD3 (CD3ε) in 4/4 cases, CD43 (MT1) in 0/4, CD45RO (UCHL-1) in 0/4, CD57 (Leu7) in 0/4, CD20 (L26) in 0/4, CD56 (123C3) in 4/4, granzyme B in 4/4, TIA-1 in 4/4, and EBER-1 in 4/4.

In the two cases where tissue was available for immunohistochemical study in frozen tissue sections, neoplastic cells showed reactivity for CD2 (Leu5b) in 0/2, CD3 (Leu4) in 0/2, CD4 (Leu3) in 0/2, CD5 (Leu1) in 0/2, CD7 (Leu 9) in 0/2, CD8 (Leu2) in 0/2, CD16 (Leu11b) in 1/2, βF1 in 0/2, TCRδ1 in 0/2, perforin in 2/2, and Fas ligand (Fas L) in 2/2. The natural killer cell lymphomas appear to represent a non-leukemic counterpart of aggressive natural killer cell leukemia, a relationship similar to that in adult T-cell leukemia/lymphoma. Chan et al.[15] described five cases of aggressive NK cell leukemia/lymphoma. According to their reports, all patients experienced a fulminant course, and died within six weeks of presentation. Hepatomegaly and blood and bone marrow involvement were characteristic, and occasinally splenomegaly and lymphadenopathy were noted. Few reports have described morphologic features of NK cell leukemia/lymphoma except to state that leukemic cells showed features of large granular lymphocytes, since most reported cases were leukemic rather than lymphomatous. In a previous reports by Kwong et al.,[22] angiocentric or angiodestructive features were pronounced. Our cases share some of the features of aggressive NK cell leukemia, since all included fulminant courses and bone marrow involvement. However, leukemic features were absent. We therefore believe that our cases do not completely fit the category of aggressive NK cell leukemia. Analogy with cases of adult T cell leukemia/lymphoma, known to be HTLV-1 related T cell neoplasm, illustrates how an entity can show features either lymphoma or leukemia. Therefore, in some cases with ATL neoplastic cells proliferate only in lymph nodes, while in others the neoplastic cells have a leukemic distribution. Similarly, NK cell neoplasia may be thought of as EBV-associated NK-cell type leukemia/lymphoma. Another type of NK cell neoplasia, namely angiocentric NK cell lymphoma, usually presents extranodally with lymph node involvement being rare. Again, however, most of our cases had nodal involvement, and the neoplastic cells in our cases showed in appearance of large cell lymphoma. This picture is rather different from findings in the usual angiocentric NK cell lymphoma, where cleaved cells are among the representative constituent. In this sense, as well as that of lacking angiocentricity, our cases can not be categorized in the angiocentric subtype. The neoplastic cells in our cases were large and had irregulary shaped nuclei; sometimes they were admixed with medium-sized cells. Previous reports[15] have mentioned that few NK cell lymphoma cases included nodal involvement, but our own showed nodal involvement in three of four patients. All cases with lymph node prolifera-

tion revealed a sinusoidal pattern of involvement. Widespread necrosis, prominent apoptosis and a large numbers of phagocytizing macrophages were observed. Because of this hemophagocytic feature, one of our cases previously was reported as malignant histiocytosis or histiocytic medullary reticulosis. Awareness of this aggressive lymphoma is important because of its fulminant course.

BLASTIC/BLASTOID NK CELL LEUKEMIA/LYMPHOMA

This disease mostly affects adults, and extranodal tissues such as skin are frequently the primary site.[12,23] It is often accompanied by leukemic change. Morphologically, necrosis is not pronounced, and the neoplastic cells have a nucleus with fine chromatin and show relatively monomorphic appearance, simulating lymphoblastic lymphoma. Phenotypically, the neoplastic cells are CD2+/–, sCD3 (surface CD3)+/–, cCD3 (cytoplasmic CD3)+, TdT+/–, CD56+. The neoplastic cells do not show TCR gene rearrangement, and are negative for EBER. From these findings, blastic NK cell leukemia/lymphoma appears to be a distinct clinicopathologic entity, characterized by cutaneous, nodal, and marrow involvement by blastic cells with immunophenotypic characteristics of NK cells.

Recently, Suzuki et al.[24] reported myeloid/natural killer cell precursor acute leukemia and they proposed as a distinct hematolymphoid disease entity. According to their report, striking extramedullary involvement was evident at initial presentation, with peripheral lymphadenopathy and/or mediastinal masses. The neoplastic cells expressed CD7, CD33, CD34, CD56, and frequently HLA-DR, but not other NK, T-cell, and B-cell markers. This disease appears earliest stage in the NK cell differentiation.

CONCLUSIONS

Nk cell leukemia/lymphomas have various clinical and morphological characteristics. Clinically these lymphomas have quite aggressive clinical course, and have propensity to extranodal spread, and are prevalent in Asian countries including Japan. Morphologically, involved sites are accompanied by widespread necrosis or apoptosis, and the neoplastic cells have occasionally show angioinvasive or angiodestructive features. Furthermore, these neoplastic cells mostly harbour Epstein-Barr virus (EB virus). Further clarification of these NK cell neoplasms will in turn contribute to the understanding of normal developmental pathway of NK cells.

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