

NK Cell Lymphoma

Kazuo Oshimi

Department of Hematology, Juntendo University School of Medicine, Tokyo, Japan

Abstract

Natural killer (NK) cells are lymphocytes with large granular lymphocyte morphology, CD3-CD56+ phenotype, non-MHC-restricted cytotoxicity, and germ-line configuration T-cell receptor genes. Two types of lymphomas originating from NK cells have been described; blastic NK-cell lymphoma, and nasal-type NK-cell lymphoma. Because recent reports indicate that blastic NK-cell lymphoma originates from the precursors of plasmacytoid dendritic cells, I will focus mainly on nasal-type NK-cell lymphoma, and discuss its pathogenesis, diagnostic problems, treatment strategy, and outcome. Nasal-type NK-cell lymphoma develops mostly in the nasal cavity and rarely in other sites, such as the skin and intestinal tract. Epstein-Barr virus (EBV) is found in lymphoma cells of almost all the patients, and is considered to be the etiologic agent. Indeed, EBV easily infects NK cells in the absence of CD21 antigen, or EBV receptor, on the surface of NK cells. Further, various types of oncogenes and suppressor oncogenes are found to be involved in its pathogenesis. Based on the data obtained from paraffin-embedded specimens, it is difficult to determine whether the lymphoma cells are of T-cell or NK-cell lineage, because immunohistochemical staining of cytoplasmic CD3 is positive both in T and NK cells, and CD56 is positive in a part of T cells. The presence of CD5 antigen indicates T-cell lineage. When the disease is limited, radiation therapy is effective, but not satisfactory. A new trial to use simultaneously both radiation and chemotherapy has started in Japan. In advanced stages, a combination chemotherapy including L-asparaginase seems to be promising, and high-dose chemotherapy with autologous or allogeneic stem cell support is under investigation. A recent report described the expression of short-length P-glycoprotein (P-gp), but not full-length P-gp in NK cells, and this mini-P-gp is unable to extrude daunorubicin. These findings may change the treatment strategy. Finally, I will present the results on interim analysis of 166 cases of nasal-type NK-cell lymphoma collected in Japan between 1994 and 1998.

1. Definition of Natural Killer (NK) Cells

Four types of cytotoxic lymphocytes have been described; major histocompatibility complex (MHC)-restricted cytotoxic T lymphocytes (CTL), MHC-unrestricted CTL or NK-like T cells, NK cells, and NKT cells. As shown in Table 1, these lymphocytes can be differentiated by the presence or absence of various surface antigens, cytoplasmic CD3, T-cell receptor gene rearrangements, large granular lymphocyte (LGL) morphology, MHC-unrestricted cytotoxicity, and NK receptors. NK cells are lymphocytes that can lyse, without prior sensitization, various types of target cells including tumor cells and virus-infected cells. When all the following criteria are fulfilled, lymphocytes are defined to be NK cells: LGL morphology; CD3- and CD56+ phenotype; MHC-unrestricted cytotoxicity; and germ-line

configuration T-cell receptor (TCR) genes [1,2]. Because the developmental pathway of NK cells is not fully understood, immature or precursor NK cells are not well defined, and this makes some confusion in making the diagnosis of NK-cell neoplasms when proliferating cells seem to be immature.

2. NK-cell Lymphomas and Their Putative Normal Counterparts

In the new WHO Classification [3], three diseases are included in NK-cell leukemia or lymphoma: aggressive NK-cell leukemia; extranodal NK/T-cell lymphoma, nasal type; and blastic NK-cell lymphoma. In addition, myeloid/NK cell precursor acute leukemia [4] originates probably from precursor NK cells.

NK cells develop from CD34+ hematopoietic stem

Table 1.

Characteristics of Cytotoxic Lymphocytes.

		MHC- restricted CTL	MHC- unrestricted CTL	NK	NKT
Surface marker	CD2	+	+	+	+
	CD3	+	+	-	+
	CD5	+	+	-	?
	CD7	+	+	+	?
	CD16	-	-	+/-	-
	CD56	-	+	+	-
	TCR	+	+	-	+
	Cytoplasmic CD3	+	+	+	+
	TCR gene rearrangement	+	+	-	+
LGL morphology	-	+	+	?	
MHC-unrestricted cytotoxicity	-	+	+	+	
NK receptors	-/+	?	+	+	

cells. CD34+ stem cells give rise to common lymphoid progenitors, and then to common T/NK progenitor cells [5]. Myeloid/NK cell precursor acute leukemia probably originates from common T/NK progenitor cells [4]. Common T/NK progenitor cells lose capacity to develop into NK cells when TCR gene rearrangements are initiated. A molecular switch controlling NK-cell development is not fully understood, but the earliest NK marker to appear on the NK-cell membrane is NKR-P1 (CD161). Blastic NK-cell lymphoma has been considered to originate from the cells of a developmental stage prior to expressing CD161, because blastic NK-cell lymphoma does not express this antigen [6]. However, recent reports clearly indicate that the normal counterpart of blastic NK-cell lymphoma is the precursor cells of plasmacytoid dendritic cells [7,8]. Here I will briefly discuss blastic NK-cell lymphoma. With further maturation in normal NK cell developmental pathway, CD56 appears on the cell surface, followed by CD94. According to Spits et al. [5], CD161 single positive cells are called pre-NK cells, CD161+CD56+ cells immature NK cells, and CD161+CD56+CD94+ cells mature NK cells. Nasal-type NK lymphoma and aggressive NK-cell leukemia originate from mature NK cells, because these lymphoma or leukemia cells consistently express CD94 antigen [6].

In the WHO Classification [3], the term extranodal NK/T-cell lymphoma, nasal type is used for T cell-lineage or NK cell-lineage lymphoma presenting in the nasal cavity or extranasal sites with characteristic morphological features. This term, however, includes both T-cell and NK-cell lymphomas. The reason why the WHO Classification includes these two different types is because, I think, it is difficult to discriminate T-cell and NK-cell lymphoma based on paraffin-embedded specimens, as will be discussed later.

Aggressive NK-cell leukemia is a rare disease seen mainly in young adults with presenting features of

fever, hepatosplenomegaly, lymphadenopathy, and pancytopenia [9]. Leukemic cells are LGL, and sometimes looking immature with conspicuous nucleoli and fine chromatin patterns. Their surface phenotype is generally CD3-CD4-CD8-CD16-CD56+CD57-. The disease progresses rapidly, and is refractory to combination chemotherapy. It is often difficult to differentiate it from leukemic phase of nasal-type NK-cell lymphoma.

Recently described NK-like T-cell lymphomas are defined to be lymphomas of NK-like T-cell (MHC-unrestricted CTL) origin, and are characterized by an extranodal presentation, LGL morphology, CD3+CD4-CD8+CD16+/-CD56+CD57- phenotype, and an aggressive clinical course [10].

Here, I will discuss mainly on nasal-type NK-cell lymphoma with special reference to its pathogenesis, diagnostic problems, and treatment. I will present also the results on interim analysis of 166 cases of nasal-type NK-cell lymphoma collected in Japan between 1994 and 1998.

4. Nasal-type NK-cell Lymphoma

4.1. Clinical Features

Nasal type NK-cell lymphoma is characterized by an extranodal presentation, angiocentric and angi-destructive proliferation of lymphoma cells, LGL morphology, CD2+CD3-CD16-/+ CD56+CD57- phenotype, and an aggressive clinical course. The lymphoma is common in Asia and Latin America, but remains rare in North America and Europe. It develops in middle aged persons, and males are more involved than females. Nasal cavity is the main presenting site, and less often the skin, intestinal tract and various other organs are involved at presentation. Because of its predilection for vessels, massive necrosis of the tissue is often one of the presenting features, and it is sometimes difficult to make a diagnosis with repeated biopsies.

NK-cell lymphoma may develop from extra-nasal sites, such as the skin and intestinal tract. These cases also exhibit relentless progressive course, without showing remission by chemotherapy. In rare cases, however, the clinical course is indolent. We have recently experienced two such cases, and these unusual cases will be presented.

4.2. Pathogenesis

Nasal NK-cell lymphoma is almost always associated with Epstein-Barr virus (EBV). With in situ hybridization technique, EBV-encoded small RNA EBER is found in the tumor cells, and Southern blot analysis detects monoclonal proliferation of EBV. These findings indicate that EBV infection has been established at an early stage of tumorigenesis, and strongly suggests its etiologic role. Our recent experiments clearly show that EBV easily infects NK cells, and the expression of EBER in infected cells is confirmed by RT-PCR. Interestingly, this infection occurs in the absence of CD21 antigen, or

EBV receptor, on the NK cell surface.

In addition to the conventional chromosomal analysis, various types of procedures including comparative genomic hybridization (CGH), loss of heterozygosity (LOH), and fluorescence in-situ hybridization (FISH) have been applied to demonstrate genetic abnormalities. The results indicate that complexed chromosomal abnormalities are often seen [11], and frequent deletions at 6q and 13q are found [12,13]. DNA gains are also frequent [13].

Various types of suppressor oncogenes are inactivated in NK-cell lymphoma. These include p53, p73, p16INK4A, p15INK4B and p14ARF [14,15]. Studies of oncogenes from our laboratory show normal K-, H-, and N-Ras genes, and c-myc and n-myc genes. However, Mdm-2 gene is strongly expressed. Relationship between EBV infection, chromosomal abnormalities, and suppressor oncogene and oncogene abnormalities are to be clarified.

4.3. Diagnostic Problems

In patients with nasal lymphoma, 3/4 of them have NK- or T-cell lymphoma, and the remaining have B-cell lymphoma. The incidence of T-cell lymphoma seems to be much lower than that of NK-cell lymphoma. However, because cytoplasmic CD3 is positive both in T and NK cells, and a part of T cells are also CD56+, it is difficult to differentiate NK- and T-cell lineage based on paraffin-embedded specimens. CD5 positivity strongly suggests T-cell lineage [16]. The demonstration of surface CD3 by flow cytometry, and that of monoclonal TCR gene bands by Southern blot analysis are helpful in their differentiation, and these two procedures should be routinely employed. LGL morphology demonstrated by Giemsa stain of imprint smears also suggests NK-cell lineage, but NK-like T cells also have such morphology.

4.4. Treatment and Prognosis

Because of a rare incidence of the disease, prospective treatment study has not been reported, and a standard treatment protocol is not established. Seventy to ninety percent of the cases have stage I or II disease. In localized diffuse large B-cell lymphoma, three courses of CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) followed by radiation therapy give excellent results. However, in localized nasal NK-cell lymphoma, this protocol seems to be discouraging. Response of the primary tumors to chemotherapy is minimal or transient if present, and early metastasis is common. Compared with combination chemotherapy, radiation therapy seems to be much more effective. To cure the disease, at least 50 Gy of dose to the involved area will be required [17-19]. Although bone marrow involvement at diagnosis is uncommon, radiation therapy alone is not satisfactory, possibly because of the presence of unidentified metastatic lesions in some patients. The addition of chemotherapy, however, does not appear to modify significantly the survival of patients, probably due to early dissemination of the disease before the be-

ginning of chemotherapy. In Japan, therefore, a new trial to use simultaneously both radiation and chemotherapy has started.

In advanced disease, conventional chemotherapy regimens such as CHOP are discouraging, and overall survival in 5 years is approximately 10% [20,21]. This poor outcome has been partially explained by the presence of P-glycoprotein (P-gp) on the NK-cell membrane, that extrudes various cytotoxic agents such as vinca alkaloids and anthracyclines. A recent report, however, indicates that NK cells express 70-80 kD short-length P-gp, but not 170 kD full-length P-gp, and this mini-Pgp demonstrates a restricted substrate profile, being unable to extrude daunorubicin [22]. Indeed, our study supports this notion, and doxorubicin as well as daunorubicin is not transported from the cells. These findings may change the treatment strategy for advanced-stage NK-cell lymphoma.

L-asparagine synthetase is an enzyme that makes L-asparagine from L-aspartic acid and L-glutamine, and cells that can upregulate this enzyme has been reported to be resistant to L-asparaginase. Normal and abnormally expanded NK cells do not have L-asparagine synthetase, and are shown to be sensitive to L-asparaginase in in vitro study. The integration of L-asparaginase to combination chemotherapy may be promising [23].

High-dose chemotherapy with autologous or allogeneic stem cell support is an alternative way of treatment. Although some reports describe successful results, the number of cases is limited, and its true significance is to be evaluated.

4.5. Nasal-type NK-cell Lymphoma in Japan

Between 1994 and 1998, we have collected 166 cases of nasal-type NK-cell lymphoma, with 136 cases having nasal lymphoma and 30 cases extra-nasal lymphoma. Males are predominant. Stage I/II disease is found in 70% of the patients with nasal NK-cell lymphoma, and in 39% of the patients with extra-nasal NK-cell lymphoma. EBV is found in lymphoma cells of all the nasal NK-cell lymphoma patients tested, and in those of 86% extra-nasal NK-cell lymphoma patients. At present, their follow-up data are being collected, including the outcome. Hopefully, the results of stem cell transplantation, and overall survival will be presented at the meeting.

References

1. Hercend T, Schmidt RE. Characteristics and uses of natural killer cells. *Immunol Today*. 1988;9:291-293.
2. Trinchieri G. Biology of natural killer cells. *Adv Immunol*. 1989;47:187-376.
3. Tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC Press; 2001.
4. Suzuki R, Nakamura S. Malignancies of natural killer (NK) cell precursor: myeloid/NK cell precursor acute leukemia and blastic NK cell lymphoma/leukemia. *Leuk Res*. 1999;23:615-624.
5. Spits H, Blom B, Jaleco A-C, et al. Early stages in the development of human T, natural killer and thymic dendritic cells. *Immunol Rev*. 1998;165:75-86.
6. Mori KL, Egashira M, Oshimi K. Differentiation stage of

- natural killer cell-lineage lymphoproliferative disorders based on phenotypic analysis. *Br J Haematol.* 2001;115:225-228.
7. Chaperot L, Bendriss N, Manches O, et al. Identification of a leukemic counterpart of the plasmacytoid dendritic cells. *Blood.* 2001;97:3210-3217.
 8. Feuillard J, Jacob M-C, Valensi F, et al. Clinical and biologic features of CD4+CD56+ malignancies. *Blood.* 2002;99:1556-1563.
 9. Imamura N, Kusunoki Y, Kawa-Ha K, et al. Aggressive natural killer cell leukemia/lymphoma: report of four cases and review of the literature. *Br J Haematol.* 1990;75:49-59.
 10. Macon WR, Williams ME, Greer JP, et al. Natural killer-like T-cell lymphomas: aggressive lymphomas of T-large granular lymphocytes. *Blood.* 1996;87:1474-1483.
 11. Wong KF, Zhang YM, Chan JKC. Cytogenetic abnormalities in natural killer cell lymphoma/leukemia - Is there a consistent pattern? *Leuk Lymphoma.* 1999;34:241-250.
 12. Siu LLP, Wong K-F, Chan JKC, et al. Comparative genomic hybridization analysis of natural killer cell lymphoma/leukemia. *Am J Pathol.* 1999;155:1419-1425.
 13. Siu LLP, Chan V, Chan JKC, et al. Consistent patterns of allelic loss in natural killer cell lymphoma. *Am J Pathol.* 2000;157:1803-1809.
 14. Li T, Hongyo T, Syaifudin M, et al. Mutations in the p53 gene in nasal NK/T-cell lymphoma. *Lab Invest.* 2000;80:493-499.
 15. Sakajiri S, Kawamata N, Egashira M, et al. Molecular analysis of tumor suppressor genes, Rb, p53, p16INK4A, p15INK4B and p14ARF in natural killer cell neoplasms. *Jpn J Cancer Res.* 2001;92:1048-1056.
 16. Emile J-F, Boulland M-L, Haioun C, et al. CD5-CD56+ T-cell receptor silent peripheral T-cell lymphomas are natural killer cell lymphomas. *Blood.* 1996;87:1466-1473.
 17. Shikama N, Izuno I, Oguchi M, et al. Clinical stage IE primary lymphoma of the nasal cavity: Radiation therapy and chemotherapy. *Radiol.* 1997;204:467-470.
 18. Li Y-X, Coucke PA, Li J-Y, et al. Primary non-Hodgkin's lymphoma of the nasal cavity. *Cancer.* 1998;83:449-456.
 19. Kim GE, Cho JH, Yang WL, et al. Angiocentric lymphoma of the head and neck: Patterns of systemic failure after radiation treatment. *J Clin Oncol.* 2000;18:54-63.
 20. Liang R, Todd D, Chan TK, et al. Treatment outcome and prognostic factors for primary nasal lymphoma. *J Clin Oncol.* 1995;13:666-670.
 21. Cheung MMC, Chan JKC, Lau WH, et al. Primary non-Hodgkin's lymphoma of the nose and nasopharynx: Clinical features, tumor immunophenotype, and treatment outcome in 113 patients. *J Clin Oncol.* 1998;16:70-77.
 22. Trambas C, Wang Z, Cianfriglia M, et al. Evidence that natural killer cells express mini P-glycoproteins but not classic 170 kDa P-glycoprotein. *Br J Haematol.* 2001;114:177-184.
 23. Nagafuji K, Fujisaki T, Arima F, et al. L-asparaginase induced durable remission of relapsed nasal NK/T-cell lymphoma after autologous peripheral blood stem cell transplantation. *Int J Hematol.* 2001;74:447-450.