Expression of Adult and Fetal Natural Killer Cell Markers in Sinonasal Lymphomas


The majority of sinonasal non-Hodgkin's lymphomas (NHLs) are thought to originate from T-cell lineage. However, they often express natural killer (NK)-cell markers so that their origin still remains obscure. In this study, cell type of sinonasal NHLs was characterized by immunohistochemical and Southern blot analyses. We examined nine patients with sinonasal NHL. Six patients with tonsillar or pharyngeal non-B-cell lymphomas served as a control group. Immunohistochemical study showed that all nine cases of sinonasal NHL were CD56-CD2+, whereas controls were CD56-CD2+. According to the rearrangement of T-cell receptors (TCRs) and expression of CD3 markers, the sinonasal NHL cases were classified into three groups: TCR-CD56(Leu-19)/CD3(Leu4) NHL (three patients), TCR-CD56-CD3+ NHL (five patients), and TCR-CD56-CD3+ NHL (one patient). In contrast, control patients' NHLs were TCR-CD56-CD3-. These results imply that eight cases of TCR-CD56+ sinonasal NHL are of NK-cell lineage. Among these eight cases, TCR-CD56-CD3+ cases (five of eight patients) were rather similar to the phenotype of fetal NK cells. From these results, the majority of sinonasal NHLs seem to originate from varying maturation stages of NK-cell lineage.

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IMMUNOHISTOCHEMICAL studies on sinonasal non-Hodgkin's lymphomas (NHLs),1-5 including the entity known as lethal midline granuloma and polymorphic reticulosis (PR), showed that these cells commonly expressed T-cell markers.6-10 Moreover, some patients had sinonasal NHL of the natural killer (NK)-cell phenotype.11,12

NK cells are identified as large granular lymphocytes that lack cell-surface CD3 and T-cell receptors (TCRs). They commonly express CD16 and CD56, and exhibit major histo-compatibility complex (MHC)-unrestricted cytotoxicity against target cells.13 Although definitive NK-cell markers have not been identified yet, CD56, which is recognized by monoclonal antibodies Leu-19 and NKH-1, is expressed on most NK cells.14,15 CD56 is also known as one of the family of cellular adhesion molecules associated with neural cells (N-CAM).16 However, CD56 (N-CAM) expression is rare in NHLs, except those that occur in the nasal or nasopharyngeal region.11 It is of interest that two groups have reported independently on patients with N-CAM (CD56)-positive malignant lymphomas characterized by a predilection for extranodal involvement and an aggressive clinical course.17,18 These findings suggest that cellular characteristics of sinonasal NHL are distinctively different from conventional nodal NHL.

The current study was performed to clarify the immunophenotypic and immunogenetic characteristics of sinonasal NHL.

MATERIALS AND METHODS

Patient and tissue preparations. Nine biopsy specimens diagnosed as sinonasal lymphoma were identified in the Department of Pathology, Fukuoka University. As a control group for the analysis of histology, immunohistochemistry, and immunogeneregulated genes, six patients with tonsils and/or pharynx involved by non-B-cell lymphoma, including two patients with adult T-cell leukemia/lymphoma (ATL), were also identified. A part of biopsy specimens was fixed in B5 fixative solution for paraffin embedding, and the remains were frozen in liquid nitrogen for immunophenotype characterization and Southern blot analysis. For routine histologic evaluation, hematoxylin-eosin staining was performed in the paraffin sections of tumor tissue from each patient. Imprint touch smears were also prepared for the cytology.

Immunohistochemical staining was applied to frozen sections of the biopsy specimens using an avidin-biotin complex alkaline phosphatase immunohistochemical method (Vectastain; Vector Labs, Burlingame, CA), and the following primary monoclonal antibodies: CD2 (T11; Coulter Clone [CC], Hialeah, FL), CD3 (Leu-4; Becton-Dickinson [BD], San Jose, CA), CD4 (OKT4; Ortho), CD7 (Leu-9; BD), CD8 (OKT8; Ortho, Raritan, NJ), CD16 (Leu-11; BD), CD19 (B4; CC), CD20 (B1; CC), CD25 (Tac; provided by Dr T. Uchiyama, Kyoto University, Kyoto, Japan), CD34 (HPCA-1; BD), CD56 (Leu-19; BD), terminal deoxynucleotidyl transferase (TdT; DAKO, Kyoto, Japan). Rabbit anti-CD3c antibody (DAKO) was applied to paraffin sections.

Southern blot analysis. High-molecular weight DNA was extracted from the tumor tissue by sodium dodecyl sulfate (SDS)-proteinase K digestion and extraction with phenol and chloroform, followed by ethanol precipitation. A total of 10 μg of DNA was digested with restriction enzymes (EcoRI, HindIII, or BamHI), according to the manufacturer’s recommendations. The digested DNA was electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose membrane according to the method described by Southern,19 and hybridized with a 32P-labeled complementary DNA probe. Hybridization was carried out for 24 hours at 65°C in a solution of 5X saline sodium citrate buffer (SSC), 5X Denhardt’s, 100 μg/mL denatured salmon sperm DNA, and 10% dextran sulfate containing 32P-labeled cDNA probe. Filters were washed at 65°C with 3x SSC containing 0.1% SDS. Rearrangement was scored by loss of the germline band or the appearance of new bands.

DNA probes. Rearrangements of the T-cell receptor (TCR) δ chain gene were examined with a 1.0-kb germline Pst-EcoRI frag.
ment containing the first J region (\(J_\delta1\)). The probe for the TCR\(\beta\) chain gene was a C\(\beta\) fragment, 720 bp in length, isolated from the HBVT 96 clone. To detect TCR\(\gamma\) and immunoglobulin gene rearrangements, we used a J\(\gamma\) probe and a JH probe. All probes were labeled by the DNA random-primer oligolabeling method (Amersham Kit; Amersham, UK), following the manufacturer’s recommendation.

### RESULTS

Clinical characteristics of the patients with sinonasal NHL are listed in Table 1. There were three men and six women; their ages ranged from 38 to 72 years, with a median of 43 years. Most of the patients presented with nasal obstruction with or without discharge. On admission, all the patients were diagnosed as having NHL, stage I or II. Three patients remain alive without disease, three patients suffered disease relapse, and three patients died within 1 year after diagnosis. One patient died of sepsis during chemotherapy-induced neutropenia. In eight patients (all but case no. 8), the serum lactate dehydrogenase (LDH) level was within normal limits. The serum lactate dehydrogenase (LDH) level in patient no. 8 increased only slightly. The serum LDH level in patient no. 9 was within normal limits. The control group consisted of two men and four women, ranging in age from 46 to 78 years, with a median of 54.5 years.

**Histologic findings.** All patients with sinonasal NHL were tentatively diagnosed as rare type, angiocentric lymphoma according to the updated Kiel classification. Histologically, their characteristics were diffuse proliferation of tumor cells with various sized nuclei and rather clear cytoplasm. The nuclei were also irregularly shaped. Angioinvasion and necrosis of the tumor tissue was found in all patients (Fig 1). Of the six control patients, four had NHL of a diffuse, pleomorphic type with medium and large cells, and two had immunoblastic-type NHL. In control cases, necrosis was absent, except for one case accompanied by necrosis, which showed angiodestructive features.

**Cytology.** Specimens from six of nine patients with sinonasal NHL and six of six patients with tonsil/pharynx-involved non–B-cell lymphoma were available for cytologic examination. Five of six available imprint smear specimens of sinonasal NHL showed that some tumor cells had varying degrees of azurophilic granules in the cytoplasm (Fig 2). In contrast, azurophilic granules were absent in the cytoplasm of tumor cells of control cases.

**Immunohistochemical study.** The immunophenotypes of the sinonasal NHL and control groups are listed in Table 2. In sinonasal NHL, CD2 was positive in all cases. CD56 was also expressed in all cases of sinonasal NHL. CD16 was positive in five of nine patients with CD56 positivity. Among nine cases of sinonasal NHL, three patients had CD56\(\text{(Leu-19)}^+\)CD3\(\text{(Leu-4)}^+\) NHL, and six patients had CD56\(\text{(Leu-19)}^+\)CD3\(\text{(Leu-4)}^+\) NHL. Typical CD56\(\text{(Leu-19)}^+\)CD3\(\text{(Leu-4)}^+\) and CD56\(\text{(Leu-19)}^+\)CD3\(\text{(Leu-4)}^+\) specimens are shown in Figs 3 and 4, respectively. CD3\(\epsilon\) (DAKO) was expressed in eight of all patients with sinonasal NHL. Among these patients, two did not express CD3\(\text{(Leu-4)}\) antigen. In the control group, none of the patients had CD16\(^+\) or CD56\(^+\) cells, although they strongly expressed CD3\(\text{(Leu-4)}\). Seven of nine patients with sinonasal NHL expressed CD7 in lymphoma cells. CD34 and TdT were not expressed in all sinonasal NHLs and control’s NHLs (data not included in Table 2). Similarly, B-cell markers (CD19 and CD20) were

![Fig 1. Biopsy specimen of sinonasal NHL. There is diffuse proliferation of medium-sized lymphoma cells with nuclear convolution. Angiocentric pattern is also noted. (H&E stain; original magnification \(\times 350\).)](image-url)
not detected in all nine patients with sinonasal NHL and the six control patients.

**Southern blot analysis of TCR and immunoglobulin genes.**
Eight of nine patients with sinonasal NHLs showed a germ-line configuration of TCRs, including β, γ, and δ genes and JH. One patient (no. 2) had deletion of Jδ1, and rearranged bands of one allele of the γ gene, and the Cβ1 gene (Fig 5). In contrast, all five control patients examined showed the rearrangement of the TCRβ and TCRγ genes (Fig 5).

**DISCUSSION**

NK cells have been defined as CD3-CD56+ lymphocytes that mediate MHC-unrestricted cytotoxicity. Unlike T lymphocytes, TCR genes of NK cells are not rearranged or expressed productively. Recently, some patients were reported to have lymphoma cells expressing CD3δ and CD56δ, consistent with the NK-cell phenotype.11,17,18 Unfortunately, however, immunogenetic characteristics of these cells remained unclear, because only few cases were analyzed by Southern blotting. In the present study, all nine cases of sinonasal NHL were CD56-CD2+, whereas controls were CD56 CD2+. Furthermore, according to the rearrangement of TCR and expression of CD3, the sinonasal NHL cases were classified into three groups: TCR CD56-CD2+ (three of nine patients), TCR-CD56CD2+ (five of nine patients), and TCR+CD56-CD2+ (one of nine patients). TCR+CD56-CD2+ (three of nine patients) with sinonasal NHL had the same phenotype as the previously reported lymphoma cases, which originated from NK cells. Interestingly, the CD56+ tumors in five of nine patients with sinonasal lymphoma expressed CD3(Leu-4) and lacked rearrangement of TCR. Although a few cases of lymphomas expressing both CD3 and CD56 were previously reported,11,17,18 they were classified as T-cell lymphomas. This is because expression of CD3 protein had been believed to be a definitive marker of the T-cell lineage.

**Table 1. Immunohistology and Southern Blot Analysis**

<table>
<thead>
<tr>
<th>Sinonasal lymphoma group</th>
<th>Immunohistology (CD)</th>
<th>Southern Blot</th>
<th>HTLV-I (A1/Genome)</th>
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<tr>
<td>1</td>
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<td>G G G G</td>
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<th>Southern Blot</th>
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<td>NE + + +</td>
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Abbreviations: CD3+, Leu-4; CD3-, CD56δ (DAKO); G, germline configuration; R, rearrangement; D, deletion; Azur., azurophilic granules in the cytoplasm of tumor cells; HTLV-I, human T-lymphotrophic virus type I; Ab: antibody; NE, not examined.
Immunohistochemistry demonstrates (A) CD56(Leu-19)^+ and (B) CD3(Leu-4)^+ from a representative case of sinonasal NHL. Immunohistochemistry demonstrates strong reactivity for CD56 by the tumor cells. In contrast, reactivity for CD3 is not shown in the tumor cells. (Original magnification × 700.)

The monoclonal antibody CD56 is not specific for NK cells, but may also be found in T lymphocytes, which have NK activity. Analysis of the rearrangement and expression of TCR and immunoglobulin genes in human lymphomas is a useful method to classify tumors according to the lineage and to model normal receptor ontogeny. While T lymphocytes and NK cells have remarkable similarities with respect to expression of membrane receptors and immune effector cell functions as mentioned earlier, they can be clearly discriminated from each other by TCR gene rearrangement. Furthermore, the TCRδ chain gene rearranges at a very early stage of T-cell ontogeny, before the other TCR genes. From these findings, TCR^+CD3^+CD56^- sinonasal NHL is not of T-cell lineage. After in vitro activation, adult NK cells, which have essentially undetectable levels of CD3e protein, transcribe CD3e and express cytoplasmic CD3e proteins. These activated adult NK cells express CD3e alone, whereas fetal NK cells express CD3y, CD3δ, and CD3e. Salmerón et al have shown that Leu-4, the monoclonal antibody specific for CD3, recognizes CD3yε and CD3δε complexes, but not CD3ε alone. These findings indicate that lymphoma cells having the phenotype TCR^+CD3^+CD56^-CD3ε^+ in the five cases we identified are consistent with fetal NK cells. We propose that CD3γεδε and CD56^- lymphoma cells lacking TCR rearrangement are not of T-cell origin, but rather similar to the phenotype of fetal NK cells.

Rabbit anti-CD3ε (DAKO) was generated against amino acids 156 to 168 in the cytoplasmic domain. It has been reported that CD3ε (DAKO) specifically detected cytoplasmic CD3ε antigen, while Leu-4 recognized CD3γε and CD3δε complexes. As mentioned earlier, activated adult NK cells express CD3ε alone, whereas fetal NK cells express CD3γ, CD3δ, and CD3ε. Therefore, in the current study, two cases of sinonasal NHL with TCR^+CD3^+CD56^-CD3ε^+ are considered to be activated adult NK cells. In one patient (no. 2), tumor cells expressed CD3(Leu-4) and CD56, in association with the rearrangement of TCRβ and TCRγ chain genes. This tumor may be considered to be of T-cell lineage.

The cytologic appearance and histologic profile of the tumor cells varied from case to case. Sinonasal NHL could also be characterized by the presence of two morphologic features: the presence of azurophilic granules in the cytoplasm of the neoplastic cells, and the frequent occurrence of...
markers are defined, the results reported here show that the majority of sinonasal NHLs seem to originate from T-cell/NK-cell progenitor cells, as proposed by Lanier et al. Variable expression patterns of CD3 and CD56, and the rearrangement of TCR on sinonasal NHL, may reflect varying degrees of differentiation of these cells. At least, we can conclude that sinonasal NHL is distinctly different from conventional nodal and extranodal lymphoma.

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REFERENCES

Expression of adult and fetal natural killer cell markers in sinonasal lymphomas [see comments]

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