Neural Cell Adhesion Molecule-Positive Peripheral T-Cell Lymphoma: A Rare Variant With a Propensity for Unusual Sites of Involvement


A distinct subset of patients with peripheral T-cell lymphoma (PTCL) is described which reacts with Leu-19 (CD56), an antibody that has been shown to identify the neural cell adhesion molecule (NCAM). These NCAM-positive PTCL patients (11 of a series of 46 PTCL; 24%) exhibited a striking predilection for unusual anatomic sites of involvement: central nervous system (36%), muscle (18%), gastrointestinal tract, and nasopharynx (27% each). Additional extranodal sites of involvement included the pituitary, thyroid, parathyroid, adrenals, and pancreas. The NCAM-positive subset also exhibited a characteristic phenotypic profile, with significantly lower expression of CD3 and CD5 compared with the NCAM-negative group. RNA transcripts consistent with the NCAM gene were detected in tissue samples from five Leu-19-positive cases using a reverse transcriptase-polymerase chain reaction assay, supporting the idea that Leu-19 recognizes NCAM in these patient samples. This suggests that the expression of the NCAM plays a role in the behavior and localization of lymphomas. Because of the unique clinical and phenotypic characteristics of this group it may be designated as "NCAM-positive peripheral T-cell lymphoma."

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MATERIALS AND METHODS

Patient selection. All cases of PTCL (46 cases) in the combined Arizona Health Sciences Center (AHSC) lymphoma collection (43 PTCL cases, accumulated over 12 years) plus the recently established Southwest Oncology Group (SWOG) Central Lymphoma Repository (three additional PTCL cases) were studied for Leu-19 expression. PTCL was defined by immunohistochemical reaction for one or more T-cell antigens in the absence of B-cell antigen and precursor T-cell antigen (CD1) expression. Mycosis fungoides was excluded. CD56 detection on B-lineage lymphomas is rare in our experience (weak Leu-19 positivity was found on only 1 of 83 cases of B-lineage diffuse large cell lymphoma), therefore B-cell lymphomas were also excluded.

Histologic classification of cases was made according to the Working Formulation for the Classification of Lymphomas.

Clinical and demographic data. Patients were staged by standard methods, as previously described. Treatment in most cases consisted of curative-intent doxorubicin-containing combination chemotherapy. Patients with localized disease were treated with radiation therapy alone or in combination with chemotherapy.

Complete remission (CR) was defined as the disappearance of all disease that was present at the initiation of therapy. Survival was calculated from the date of diagnosis to the date of death (any cause) or last contact. There was an insufficient number of complete responses in the NCAM-positive group for statistical analysis of disease-free survival.

Tissue immunohistochemistry. A previously described, three-stage immunoperoxidase technique was used on snap-frozen tissue sections to detect B- and T-cell antigens, immunoglobulins, myelo-monocytic antigens, cell adhesion molecules, and a variety of other antigens. This included mouse monoclonal antibodies as the first-stage, a biotin-conjugated second stage, avidin-horseradish peroxidase, and diaminobenzidine tetrahydrochloride chromogen. The following antibodies were used to detect B-cell antigens: CD19 (B4, Coulter Immunology, Hialeah, FL, Leu-12, Becton-Dickinson, Mountain View, CA), CD20 (B1, Leu-16), and CD22 (Leu-14). Antibodies used to detect T-cell antigens included CD1 (Leu-6), CD2 (Leu-5), CD3 (Leu-4), CD4 (Leu-3ab), CD5 (Leu-1), CD7 (Leu-9), and CD8 (Leu-2a); NK cell-associated antigens included CD16 (Leu-11b), CD56 (Leu-19), and CD57 (Leu-7).

Detection of RNA transcripts. RNA was isolated by a modification of the method of Cathala et al. Tissue blocks were embedded in Optimal Cutting Temperature (Miles Diagnostics, Elkhart, IN), snap frozen in isopentane, quenched in liquid nitrogen, and kept frozen until assayed. Tissues were lysed using a polytron (Brinkman, Westbury, NY) in 5 mol/L guanidinium isothiocyanate, 10 mmol/mL EDTA, 50 mmol/mL Tris pH 7.5, 8% β-mercaptoethanol, and precipitated with 0.3 mol/L Na-acetate, and 70% ethanol. To gauge the degree of RNA degradation, RNA was denatured with glyoxal and electrophoresed on 1.2% agarose gels in 10 mmol/L phosphate buffer (pH 7.4) and visualized with ethidium bromide according to standard techniques.

For reverse transcriptase-PCR, the RT-Gene Amplification Kit (Perkin-Elmer, Norwalk, CT) was used as directed. Total RNA (1 μg) was reverse transcribed using random primers, then subjected to 35 cycles of PCR alternating 1-minute incubations at 65°C and 94°C, and consisted of the sequences 5'-GGC TGT GGG CTG GGC and 5'-GAGGCCACAGGTGGGOTGCC-5'. The samples were extracted with buffered phenol/chloroform, chloroform, and precipitated with 0.3 mol/L Na-acetate, and 70% ethanol. To gauge the degree of RNA degradation, RNA was denatured with glyoxal and electrophoresed on 1.2% agarose gels in 10 mmol/L phosphate buffer (pH 7.4) and visualized with ethidium bromide according to standard techniques.

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Statistical analysis. Differences in sites of involvement, sex, stage at presentation, histology, and CR rate were analyzed using Fisher’s two-tailed Exact Test. Differences in antigen expression were compared using the hypothesis test for proportions between two populations (Z test). Survival curves were estimated by the Kaplan-Meier method and comparisons were based on the log-rank test.

RESULTS

Patient population. The study population consisted of 46 cases of PTCL representing 6.4% of the total cases (708) in the combined AHSC and SWOG repository collections. Of these, 11 (24%) were NCAM-positive; 35 (76%) were NCAM-negative.

Clinical characteristics. Clinical characteristics of the NCAM-positive and NCAM-negative patients are summarized and compared in Table 1. These immunophenotypic groups appear to be similar with regard to most of the usual clinical parameters associated with outcome, including age (median years v 61 years for the NCAM-positive and NCAM-negative groups, respectively), stage at presentation (stage III-IV; 64% v 69%), histology (diffuse large cell, 64% v 63%), and complete remission rate (50% v 56%). Interestingly, as measured by median survival, outcome varies with the NCAM-positive group having a median survival less than half that of the NCAM-negative patients (21 months v 47 months). However, overall survival is not significantly different as measured by the log-rank test.

Selected anatomic sites of involvement for the two groups are compared in Table 2; the NCAM-positive group had a lower incidence of lymph node involvement, but a higher incidence of involvement at every extranodal site studied. These differences are statistically significant for central nervous system (CNS) and nasopharynx (P = .01 and .05, respectively). The NCAM-positive group exhibited a remarkable pattern of disease involvement, including: 36% involvement of the CNS (brain, meninges, and cerebrospinal fluid); 36% of the lung parenchyma; 24% of the liver; 24% of the gastrointestinal tract; and 24% of the lymph nodes. The NCAM-negative group had a lower incidence of involvement at every extranodal site studied.
nal fluid), 27% involvement in the nasopharynx and in the gastrointestinal tract (including gallbladder), and 18% in skeletal muscle and in kidney. In addition, there was involvement of such unusual sites as pituitary, thyroid, parathyroids, adrenals, and pancreas.

**Phenotypes.** Selected immunohistochemistry results are listed in Table 3. CD2 was the most common T-cell antigen expressed on the NCAM-positive group; this was also expressed on the majority of the NCAM-negative group (82% and 68%, respectively). The NCAM-positive group exhibited a striking and statistically significant decrease in the expression of CD3 and CD5, two important T-cell antigens, compared with the NCAM-negative group. There was a complete absence of expression of CD16 and CD57, considered NK cell-associated antigens (as is CD56), on the CD56-positive PTCL cells.

Figure 2 shows photomicrographs of histology (hematoxylin and eosin) and immunohistochemical staining for Leu-19 of a representative case of a diffuse large cell lymphoma (corresponding to patient number 3 in Fig 3).

**Detection of NCAM RNA.** RNA was isolated as described from five patients with abundant archival material. Samples from patients 1, 2, 3, and 5 were each generated from 1 μg of total RNA, while patient 4 had only 0.5 μg of total RNA retrieved. Ten micrograms of total RNA from patients 1, 2, 3, and 5 were electrophoresed to examine whether the RNA preparations were intact. Patient 1 had negligible ribosomal RNA, suggesting extensive degradation; RNA from the other patients appeared largely intact (data not shown). Agarose gel electrophoresis of the reverse transcriptase-PCR products demonstrated bands of approximately 525 bp (as predicted) in all five patients tested (Fig 3). Similar 525-bp bands from a strongly Leu-19-positive multiple myeloma cell line (8226 Dox 40) have been sequenced, confirming their identity with NCAM mRNA (E.H. Hanneman, manuscript in preparation). A high level of expression was found in one case (patient 3); this patient appeared to have the most intact RNA by ethidium bromide staining. The 525-bp signal was present but less prominent in patients 2 and 4, and faint signals were detected in patients 1 and 5. The low level of PCR product in patient 5 may reflect weak expression of CD56 by immunoperoxidase in this patient; the low level in patient 1 reflects extensive RNA degradation in that case. Shown on the gel are RT-PCR products from cell lines with low NCAM expression (8226) and high NCAM expression (8226 Dox 40), showing differential levels of signal detection. Controls performed on other gels included no mRNA, and RNA from an NCAM-negative lymphoid cell line (NC37) that showed no detectable bands (data not shown).

**DISCUSSION**

These results suggest that expression of the cell adhesion molecule NCAM on peripheral T-cell lymphomas has relevance for the biologic behavior of PTCL. NCAM expression identifies a distinct subset of PTCL with a characteristic phenotype (CD2+, CD56+; CD3-, CD5-) and behavior that differs from NCAM-negative PTCL. The extent of these differences justifies the delineation of NCAM-positive PTCL as a unique subgroup within the larger category of PTCL. Overall, NCAM-positive patients have a predilection for widespread extranodal involvement; all sites examined showed a higher incidence of involvement in this subgroup, with the exception of lymph nodes. The high incidence of CNS involvement is particularly striking because CNS involvement in PTCL is rare; most lymphomas that involve the CNS are of B-cell lineage. This predilection for CNS involvement correlates with the high expression of NCAM in the CNS and may relate to the homophilic binding characteristics of NCAM. Similarly, gastrointestinal involvement by PTCL is equally unusual; in one large series, only 1.1% of cases had a T-cell phenotype. This may also relate to the homophilic binding of NCAM, as NCAM has been reported in the mouse GI tract and we have observed strong Leu-19 staining in the muscularis of the human GI tract. The predilection for nasopharyngeal involvement in our series confirms previous studies (in a predominantly Chinese population) that found that a high proportion of nasopharyngeal lymphomas have a T-cell phenotype and express "natural killer cell" markers. Of 708 lymphoma patients phenotyped at the AHSC, only eight primarily involved the nasopharynx; three of these eight patients are among the 11 NCAM-positive PTCL (P < .0001; Chi-squared test).

The phenotypic differences between the NCAM-positive and NCAM-negative groups are also worthy of note. The significantly lower expression of CD3 and CD5 confirm the uniqueness of the NCAM-positive group. The absence of expression of the NK cell-associated antigens CD16 and CD57 is striking; CD16 in particular is described as the most constant marker of NK cells. This suggests that, in the context of PTCL, CD56 is not a marker of NK cells.

The identification of NCAM expression in PTCL appears to have clinical implications. The predilection for unusual

### Table 2. Sites of Involvement Compared

<table>
<thead>
<tr>
<th>Site</th>
<th>NCAM-Positive (%)</th>
<th>NCAM-Negative (%)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node</td>
<td>55</td>
<td>70</td>
<td>.46</td>
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<tr>
<td>Skin</td>
<td>64</td>
<td>40</td>
<td>.29</td>
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<tr>
<td>CNS</td>
<td>36</td>
<td>3</td>
<td>.01</td>
</tr>
<tr>
<td>Nerve</td>
<td>9</td>
<td>3</td>
<td>.47</td>
</tr>
<tr>
<td>Muscle</td>
<td>18</td>
<td>3</td>
<td>.17</td>
</tr>
<tr>
<td>Marrow</td>
<td>45</td>
<td>37</td>
<td>.72</td>
</tr>
<tr>
<td>Liver</td>
<td>45</td>
<td>27</td>
<td>.28</td>
</tr>
<tr>
<td>Spleen</td>
<td>45</td>
<td>39</td>
<td>.73</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>27</td>
<td>3</td>
<td>.05</td>
</tr>
<tr>
<td>GI tract</td>
<td>27</td>
<td>7</td>
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</tr>
<tr>
<td>Heart</td>
<td>9</td>
<td>3</td>
<td>.47</td>
</tr>
<tr>
<td>Kidney</td>
<td>18</td>
<td>3</td>
<td>.17</td>
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</table>

*Fisher's two-tailed exact test.

### Table 3. Selected Phenotypes

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CD2</th>
<th>CD3</th>
<th>CD4</th>
<th>CD5</th>
<th>CD16</th>
<th>CD57</th>
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<tr>
<td>NCAM+ (%)</td>
<td>82</td>
<td>18</td>
<td>45</td>
<td>9</td>
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<td>0</td>
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<tr>
<td>NCAM− (%)</td>
<td>88</td>
<td>66</td>
<td>63</td>
<td>46</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>P value</td>
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<td>.006</td>
<td>.31</td>
<td>.03</td>
<td>.38</td>
<td>1.0</td>
</tr>
</tbody>
</table>

NCAM (Y0) 68 66 63 46 7 0
NCAM+(Y0) 82 18 45 9 0 0

NCAM-POSITIVE PTCL

Fig 2. Photomicrographs demonstrating the histology (A: hematoxylin and eosin) and immunohistochemistry for Leu-19 (B) from a representative case of NCAM-positive PTCL (corresponding to patient 3 in Fig 3). The histology is that of a diffuse large cell lymphoma; immunohistochemistry demonstrates strong surface reactivity for Leu-19 by the malignant cells.

sites of disease, particularly the CNS, implies a need for staging evaluation and special radiographic procedures (ie, cranial CT scans) not usually included in the evaluation of lymphoma patients. Some of the unusual sites of disease seen might require modification of conventional treatment regimens, such as CNS prophylaxis. The lower median survival in these patients, less than half that in those lacking NCAM expression, suggests that the disease may be prone to a more aggressive course, although overall survival between these two groups was not significantly different.

The results of the reverse transcriptase-PCR assay finding RNA transcripts consistent with NCAM in all five patient samples studied support the likelihood that the staining of these lymphomas by Leu-19 is related to NCAM and not cross-reactivity or Leu-19 false positivity. In particular, the very strong reverse transcriptase (RT)-PCR signal detected in patient 3 correlates well with the intense Leu-19 positivity seen in that case by immunohistochemistry. The weak signal detected in two cases is not inconsistent with the presence of NCAM mRNA; the low level of NCAM RNA detected in patient 1 reflects RNA degradation in that sample, and the low signal in patient 5 may be a reflection of weak immunoperoxidase staining in that case. Nonetheless, we cannot entirely exclude the possibility that the faint signal detected in some of the RT-PCR reactions might be caused by NCAM on tumor-infiltrating NK cells.

The process of cancer metastasis and invasion is complex. The metastatic cascade as proposed by Liotta and Stetler-Stevenson involves the differential expression (upregulation and downregulation) of a number of cell surface and extracellular matrix adhesion molecules and degradative enzymes. Lymphocytes, unlike other adult tissues, normally recirculate through the bloodstream and reenter extravascular tissues; thus, they form a model for the metastatic spread of malignant cells. A series of remarkable discoveries have outlined many of the determinants of lymphocyte recognition and recirculation. Differential expression of a number of cell adhesion molecules and homing receptors results in selective recirculation of lymphocytes to different anatomic sites. These mechanisms governing normal lymphocyte recirculation may also be operative in neoplastic lymphocyte dissemination; thus, the presence or absence of cell adhesion molecules or homing receptors on lymphomas may determine whether or not lymphomas disseminate, the mechanisms of dissemination, and specific sites of involvement. CD56/NCAM has not previously been considered to be an important determinant in the dissemination or behavior of lymphomas; we suggest that our work indicates...
that it has such a role in this select subgroup. Supporting evidence for the significance of NCAM in leukocyte-associated tumors is provided by parallel work in our laboratory, which has detected NCAM on a large proportion of multiple myelomas; the NCAM-positive myeloma cases also appear to have major differences in biologic behavior from myelomas lacking NCAM expression.\textsuperscript{18} We do not suggest that CD56/NCAM is the only determinant of localization and behavior in NCAM-positive PTCL cases; it will require study of a large number of cell adhesion molecules and homing receptors to elucidate the mechanisms that result in the differing patterns of lymphoma localization. In the end, perhaps NCAM will be found to determine, in part, the extranodal dissemination of PTCL.

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REFERENCES

Neural cell adhesion molecule-positive peripheral T-cell lymphoma: a rare variant with a propensity for unusual sites of involvement [see comments]

WF Kern, CM Spier, EH Hanneman, TP Miller, M Matzner and TM Grogan