CXCR4/CD184 Immunoreactivity in T-Cell Non-Hodgkin Lymphomas With an Overall Th1–Th2+ Immunophenotype

Andrew P. Weng, MD, PhD, Aliakbar Shaha-saferi, MS, and David M. Dorfman, MD, PhD

Key Words: SDF-1/CXCL12; Stromal cell–derived factor 1; Chemokine receptors; CXCR3; OX40/CD134; CCR4; CD30; CD69; Anaplastic large cell lymphoma

DOI: 10.1309/RF4PVCEGGN2XAF86

Abstract

We performed an immunohistochemical analysis of CXCR4/CD184 expression in frozen and paraffin-embedded sections of human peripheral T-cell lymphomas that exhibit a composite Th1 T-cell–like or Th2 T-cell–like immunophenotype, based on expression of Th1-associated markers (CXCR3, CD134/OX40, and CD69) and Th2-associated markers (CD30 and CCR4). In 66 cases examined, CXCR4/CD184 expression correlated significantly with immunoreactivity for other markers of Th2 differentiation (P < .0001). Anaplastic large cell lymphoma, which typically expresses markers of Th2 differentiation, was immunoreactive for CXCR4/CD184 in 22 (88%) of 25 cases. Tumors previously identified as exhibiting a composite Th1-like immunophenotype, which include angioimmunoblastic lymphoma, lymphoepithelioid lymphoma, and other peripheral T-cell lymphomas now termed unspecified, were positive for CXCR4/CD184 in 7 (17%) of 41 cases. These results are consistent with previous findings that a subset of peripheral T-cell lymphomas can be divided into Th1-like and Th2-like categories based on immunoreactivity with a limited set of markers. Our findings also suggest that CXCR4/CD184, which is expressed by a number of malignant neoplasms and may have a role in tumor metastasis, may have a similar function in CXCR4+ T-cell non-Hodgkin lymphomas.

CXCR4/CD184 is a G protein–coupled transmembrane chemokine receptor that is expressed in a broad range of tissues, including the immune and central nervous systems. Interaction between CXCR4/CD184 and its ligand, stromal cell–derived factor-1 (SDF-1)/CXCL12, has been shown to mediate a variety of processes, including cell migration, proliferation, survival, and activation.1-6 The biologic importance of this receptor-ligand interaction is underscored by the finding that homozygous null mice for either CXCR4/CD184 or SDF-1 are nonviable, with mutant embryos showing defects in hematopoiesis, cardiac septum formation, and cerebellar neuronal layer formation.7-10

The hematopoietic defects in CXCR4/CD184/SDF-1 knockout mice include reduced numbers of myeloid progenitors in fetal liver and bone marrow and a severe defect in the generation of B lymphocytes.7-10 Additional studies also have revealed a role for CXCR4/SDF-1 interaction in T-cell development. In early T-cell development, SDF-1 produced by thymic epithelial cells is needed for maturation of CD34+ thymocyte progenitors to immature CD4+ T cells.11 SDF-1 also is a costimulator for CD4+ T-cell activation.6 In later stages of T-cell development, CXCR4/CD184 is expressed preferentially in the Th2 vs the Th1 subset of mature T cells12-15 and is expressed by mature T cells in lymphoid tissues.16

CXCR4/CD184 also is involved in various aspects of human disease. CXCR4/CD184 is a necessary coreceptor for infection of CD4+ T cells by T-cell–tropic HIV,17 and its expression level may be modulated in response to various immunologic stimuli.18-20 Also, signaling through CXCR4 and another chemokine receptor, CCR7, mediates cellular changes in primary tumor cells that facilitate migration and invasion of other tissue sites.21 In fact, CXCR4/CD184 is
expressed on a wide variety of cancer cells, including those in acute lymphoblastic leukemia, ductal and lobular breast carcinoma, melanoma, prostatic carcinoma, ovarian carcinoma, and neuroblastoma. These CXCR4/CD184+ tumor cells have been shown to metastasize preferentially to SDF-1 secreting tissues, including lymph node, lung, liver, and bone marrow. Furthermore, in the case of breast carcinoma cells, this pattern of spread can be abrogated by treatment with a CXCR4/CD184 neutralizing antibody.

Jones et al previously reported that the Th1-associated molecule OX40/CD134 and the Th2-associated molecule CD30 are expressed in nonoverlapping subsets of peripheral T-cell non-Hodgkin lymphomas (NHLs). Further studies with CXCR3, CD69, and CCR4 supported the subdivision of T-cell NHLs into Th1 T-cell–like and Th2 T-cell–like categories. Given the evidence that CXCR4/CD184 has a role in normal T-cell differentiation and in various malignant neoplasms, we examined CXCR4/CD184 expression in cases of T-cell NHL to determine whether CXCR4/CD184 is expressed in these neoplasms, and, if so, whether CXCR4/CD184 immunoreactivity correlates with that of other Th2 T-cell–associated markers.

Materials and Methods

Case material was obtained from the Brigham and Women’s Hospital, Boston, MA, in accordance with institutional policies. All diagnoses were based on the features described in the World Health Organization lymphoma classification system. A total of 32 cases of anaplastic large cell lymphoma (ALCL), including 4 cases that were primary cutaneous, and 28 cases of systemic ALCL were studied, as well as 12 cases of angioimmunoblastic T-cell lymphoma and 29 cases of peripheral T-cell lymphoma, unspecified, which included 4 cases of lymphoepithelioid (Lennert) lymphoma.

Cases were characterized immunophenotypically with antibodies directed against the B-cell marker CD20 (L26) and the T-cell markers CD3, CD45RO, CD43 (Leu22), CD8, CD30, anaplastic lymphoma kinase (ALK)-1, CXCR3, CD134/CD184, CD69, and CXCR4, with CD69 and CCR4 with absence of staining for CXCR3 and CD134/CD184 expression in frozen tissue sections, as previously described.

In cases in which frozen tissue samples were available, immunophenotypic analysis also was performed using antibodies directed against CD4 and CCR4, as previously described. Cases of T-cell NHL were included if they demonstrated CD4 immunoreactivity by frozen section immunohistochemical staining and/or absence of CD8 immunoreactivity by paraffin section immunohistochemical staining and if they exhibited a composite Th1 T-cell–like staining pattern (staining for CXCR3, CD134/CD184, and/or CD69 with absence of staining for CD30 and CCR4) or Th2 T-cell–like staining pattern (staining for CD30 and/or CCR4 with absence of staining for CXCR3, CD134/CD184, and CD69).

Immunostaining for CXCR4/CD184 was performed on formalin-fixed, paraffin-embedded tissue sections at 1:1,000 or on frozen tissue sections at 1:250 with an antihuman CXCR4/CD184 monoclonal antibody (clone 12G5 [IgG2a], R&D Systems, Minneapolis, MN) using a standard indirect avidin-biotin horseradish peroxidase method and diaminobenzidine color development, as previously described. CXCR4/CD184 staining was compared with that of isotype control diluted to identical protein concentration (IgG2a, R&D Systems) for all cases studied, to confirm staining specificity.

Statistical analysis of CXCR4/CD184 staining between various peripheral T-cell lymphoma subtypes was performed using the Student t test.

Results

CXCR4/CD184 Immunostaining in Reactive Lymphoid Tissue

In reactive lymphoid tissue, small T cells in the interfollicular T-cell zone exhibited moderate to strong immunostaining for CXCR4/CD184 Image 1A, along with vascular endothelial cells in small vessels, as previously reported. The majority (>50%) of T cells present in perivascular infiltrates in skin biopsy specimens from 3 patients with acute hypersensitivity reaction, a process characterized by a predominantly Th2 T-cell–type infiltrate, were immunoreactive for CXCR4/CD184, with moderate to strong staining (data not shown). In contrast, fewer than 25% of T cells in synovial tissue from 3 patients with rheumatoid arthritis, a process characterized by a predominantly Th1 T-cell–type infiltrate, were immunoreactive for CXCR4/CD184, with weak to moderate staining (data not shown). These findings are consistent with previous reports that CXCR4/CD184 is expressed preferentially in the Th2 vs the Th1 subset of mature T cells.

CXCR4/CD184 Immunostaining in Frozen Tissue Sections of Peripheral T-Cell Lymphoma

Frozen tissue samples from 19 cases of peripheral T-cell lymphoma exhibiting a composite Th1 T-cell–like or Th2 T-cell–like immunophenotype were studied for CXCR4/CD184 immunoreactivity. Five of the 19 cases fulfilled diagnostic criteria for ALCL and demonstrated a composite Th2 T-cell–like immunophenotype, based on previous studies (positive staining for CD30 in 5/5 cases;
positive staining for CCR4 in 3/5 cases; absence of staining for CXCR3, OX40/CD134, and CD69 in 5/5 cases). All cases exhibited moderate to strong immunostaining for CXCR4/CD184 in 5% to 25% of neoplastic cells (1 case), 25% to 75% of neoplastic cells (1 case), or more than 75% of neoplastic cells (3 cases) Table II. Two of the 5 cases were immunoreactive for ALK. Fourteen cases demonstrated a composite Th1 T-cell-like immunophenotype, based on previous studies (positive staining for CXCR3 in 10/14 cases, positive staining for OX40/CD134 in 10/14 cases, positive staining for CD69 in 14/14 cases, absence of staining for CD30 and CCR4 in 14/14 cases). Eleven of 14 cases were negative for CXCR4/CD184 staining, defined as immunostaining in fewer than 5% of neoplastic cells. Three
of the 14 Th1 T-cell–like cases exhibited strong immunostaining for CXCR4/CD184 in more than 25% of neoplastic cells (Table 1). Interestingly, these 3 CXCR4/CD184+ Th1 T-cell–like cases of peripheral T-cell lymphoma were all immunoreactive for Th1 T-cell–associated markers CXCR3 and CD69 but were negative for OX40/CD134. One additional OX40/CD134– case with a composite Th1 T-cell–like immunophenotype was negative for CXCR4/CD184.

**CXCR4/CD184 Immunostaining in Paraffin-Embedded Tissue Sections of Peripheral T-Cell Lymphoma**

Forty-seven cases of T-cell NHL exhibiting a composite Th1 T-cell–like or Th2 T-cell–like immunophenotype, as defined in the preceding text, were analyzed for CXCR4/CD184 immunoreactivity using formalin-fixed, paraffin-embedded tissue sections. The cases included 16 cases of systemic ALCL (10 ALK-1+, 6 ALK-1–), of which 13 exhibited moderate to strong staining for CXCR4/CD184 in 5% to 25% of neoplastic cells (1 case), 25% to 50% of neoplastic cells (5 cases), or more than 75% of neoplastic cells (7 cases). Of the 13 CXCR4/CD184+ cases, 9 were ALK-1+. A representative case is shown in [Image 1B](#). One additional OX40/CD134– case with a composite Th1 T-cell–like immunophenotype was negative for CXCR4/CD184.

<table>
<thead>
<tr>
<th>Lymphoma Type</th>
<th>CXCR4+</th>
<th>CXCR4–</th>
<th>Total No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 T-cell–like NHL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen tissue samples T-NOS</td>
<td>3</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Fixed tissue samples T-NOS</td>
<td>3</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>AIL</td>
<td>1</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>7†</td>
<td>34‡</td>
<td>41</td>
</tr>
<tr>
<td>Th2 T-cell–like NHL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen tissue samples ALCL</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Fixed tissue samples ALCL</td>
<td>13</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>cALCL</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>22‡</td>
<td>3†</td>
<td>25</td>
</tr>
</tbody>
</table>

† The association between CXCR4/CD184 immunostaining and a Th2 T-cell–like immunophenotype was significant (*P* < .0001).

‡ Two cases were positive for CD69, 4 for CD134/OX40, and 1 for CXCR3.

**Table 1**

CXCR4/CD184 Immunohistochemical Staining Profile in T-Cell NHL

<table>
<thead>
<tr>
<th>Lymphoma Type</th>
<th>CXCR4+</th>
<th>CXCR4–</th>
<th>Total No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 T-cell–like NHL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen tissue samples T-NOS</td>
<td>3</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Fixed tissue samples T-NOS</td>
<td>3</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>AIL</td>
<td>1</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>7†</td>
<td>34‡</td>
<td>41</td>
</tr>
<tr>
<td>Th2 T-cell–like NHL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen tissue samples ALCL</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Fixed tissue samples ALCL</td>
<td>13</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>cALCL</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>22‡</td>
<td>3†</td>
<td>25</td>
</tr>
</tbody>
</table>

† The association between CXCR4/CD184 immunostaining and a Th2 T-cell–like immunophenotype was significant (*P* < .0001).

‡ Two cases were positive for CD69, 4 for CD134/OX40, and 1 for CXCR3.

**CXCR4/CD184 Immunostaining in Paraffin-Embedded Tissue Sections of Peripheral T-Cell Lymphoma**

In total, 66 cases of peripheral T-cell lymphoma with a composite Th1 T-cell–like or Th2 T-cell–like immunophenotype were examined for CXCR4/CD184 immunoreactivity. Of 25 cases, 22 (88%) with a Th2 T-cell–like immunophenotype were immunoreactive for CXCR4/CD184, whereas only 7 (17%) of 41 cases with a Th1 T-cell–like immunophenotype were immunoreactive for CXCR4/CD184, indicating a significant association between CXCR4/CD184 immunostaining and a Th2 T-cell–like immunophenotype (*P* < .0001). Of 7 CXCR4/CD184+ Th1-like tumors, 6 were immunoreactive for the Th1-associated markers CXCR3 and CD69 but were negative for OX40/CD134, suggesting that they may represent a unique subset of T-cell tumors.

We studied 7 additional cases of systemic ALCL that exhibited an overall Th2 T-cell–like immunophenotype, with
the exception of immunoreactivity for 1 Th1 T-cell–associated marker. These 7 cases included 2 that were immunoreactive for CD69, 4 that were immunoreactive for OX40/CD134, and 1 that was immunoreactive for CXCR3. All 7 cases exhibited moderate to strong immunostaining for CXCR4/CD184 in 5% to 25% of neoplastic cells (1 case), 25% to 75% of neoplastic cells (2 cases), or more than 75% of neoplastic cells (4 cases) (data not shown).

Discussion

In a manner analogous to the categorization of B-cell NHLs according to their similarity to subsets of normal developing B cells, we endeavored to determine whether T-cell NHLs can be categorized based on their similarity to normal functional T-cell subsets. We described the preferential expression of the CXCR4/CD184 receptor on a subset of peripheral T-cell lymphomas that coexpress other markers associated with Th2-like differentiation, including CD30 and CCR4. In combination with previous work with the Th1-associated markers CXCR3, CD134/OX40, and CD69 on specimens of T-cell NHL,27-29 these data provide further support for a model in which many peripheral T-cell lymphomas can be subdivided into distinct subsets that resemble normal T cells of either Th1 or Th2 differentiation. The associated expression of Th2 activation markers CD30 and CXCR4 may have direct functional significance: recently, it was found that CD30 expression in cells may induce CXCR4 expression, possibly as a means to regulate the migration of activated cells.34

CXCR4 has been shown to be expressed in a number of malignant neoplasms and to affect tumor spread and metastasis. In acute lymphoblastic leukemia of precursor B-cell, B-cell, and T-cell types, CXCR4 overexpression is associated with extramedullary organ infiltration.22 CXCR4 also may have a role in the dissemination of B-cell chronic lymphocytic leukemia and follicular NHL.35,36 Our findings showing that CXCR4 is expressed in a subset of T-cell NHLs suggest that this chemokine receptor may have a role in the spread and metastasis of T-cell neoplasms as well.

A soluble CXCR4 inhibitor, AMD3100, has been used to block HIV entry into CXCR4+ cells and to decrease inflammation and pathologic effects in a mouse model of asthma.14,15 CXCR4 neutralizing antibody also has been used to block the spread of breast carcinoma cells in a mouse model.23 These results raise the possibility of novel treatment approaches based on chemokine receptor blockade or inhibition that may be effective for hematopoietic neoplasms, including CXCR4+ T-cell NHLs.

As data accumulate on the expression of chemokine receptors in T-cell NHLs, it seems that certain composite immunophenotypes are associated with specific lymphoma subtypes. For example, cases of ALCL typically express markers of Th2 T cells, while cases of angioimmunoblastic T-cell lymphoma, lymphoepithelioid T-cell lymphoma, and angiocentric T-cell lymphoma exhibit a Th1 T-cell–like immunophenotype.28,29 The present study of CXCR4 expression in T-cell lymphomas supports these earlier observations. In the present study, we also found that cases of ALCL that expressed 1 marker of Th1-activated T cells were uniformly immunoreactive for CXCR4/CD184. A comprehensive survey of cases of T-cell NHL—including cases that do not obviously correspond to a Th1 T-cell or Th2 T-cell immunophenotype—may reveal whether certain Th1 and Th2 T-cell–associated markers, such as CXCR4, correlate with specific histologic types regardless of a clear Th1 or Th2 T-cell immunophenotype, which would suggest particular biologic significance for such histologic subtype-specific markers.

What are the prognostic implications of CXCR4 expression in T-cell NHL? We have not studied this directly, but have found that CXCR4 expression is correlated closely with the ALCL subtype of T-cell lymphoma, which typically has a Th2 T-cell–like immunophenotype. Previous studies have shown that ALCL generally has a better prognosis than other T-cell lymphomas, which would include those with a Th1 T-cell–like immunophenotype.37-39 Studies to assess the functional significance of CXCR4 expression in neoplastic T cells and the prognostic significance of CXCR4 expression in cases of T-cell lymphoma are needed. Study of additional cases also may answer the question of whether OX40/CD134+, CXCR4/CD184+ T-cell lymphoma with an overall Th1 T-cell–like immunophenotype represents a specific tumor subtype.

The advent of high-density complementary DNA microarray technology has permitted simultaneous quantitative analysis of substantial portions of the transcriptome, which has facilitated the generation of expression profiles from limited tissue sources such as primary human tumors. Several pilot studies have demonstrated further that such data can be used to identify novel tumor markers that can serve as adjuncts in the diagnosis of histologically similar-appearing tumors such as ALCL,40 or panels of markers that can define biologically and/or prognostically relevant subgroups within otherwise heterogeneous entities such as diffuse large B-cell lymphoma41,42 and T-cell acute lymphoblastic leukemia.43 While peripheral T-cell lymphomas as a group are nearly uniform in their poor prognosis, the diagnostic category clearly subsumes multiple histologic subtypes and, presumably, multiple biologic subtypes as well. The subdivision of peripheral T-cell lymphomas into Th1 and Th2 T-cell–like subsets may facilitate the discovery of unifying histogenetic mechanisms and may reveal potential means of therapeutic...
intervention that exploit preserved stereotypical cellular responses to chemokine stimulation, blockade, or both. Future studies are needed to validate the biologic and clinical significance of Th1 vs Th2 T-cell–like distinction among peripheral T-cell lymphomas.

From the Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA.

Address reprint requests to Dr Dorfman: Dept of Pathology, Brigham and Women’s Hospital, 75 Francis St, Boston, MA 02115.

References


