Natural Killer–like T-Cell Lymphoma of the Small Intestine With a Distinct Immunophenotype and Lack of Association With Gluten-Sensitive Enteropathy

Constance M. Yuan, MD, PhD; Steven Stein, MD; John H. Glick, MD; Mariusz A. Wasik, MD

- We report the case of a large natural killer (NK)–like T-cell lymphoma that involved the ileum and displayed a distinct immunophenotype and complex karyotype. The patient exhibited no evidence of gluten-sensitive enteropathy (celiac disease) or any other type of enteropathy as determined by clinical history, endoscopy, and serology for immunoglobulin A (IgA) antiendomysial and IgG antigliadin antibodies. Molecular studies demonstrated a clonal T-cell receptor γ chain gene rearrangement. Immunophenotype analysis showed expression of intestinal epithelium-homing receptor CD103, CD7, cytoplasmic CD3ε, CD56, and CD16 but no other T- or NK-cell markers. Cytogenetic analysis of the malignant cells revealed multiple chromosomal abnormalities indicative of a biologically advanced, high-grade lymphoma. A novel subset of normal intestinal intraepithelial lymphocytes, bearing a similar phenotype, has been described; moreover, this subset diminishes, rather than expands, in gluten-sensitive enteropathy. This case supports the notion that lymphomas involving the small intestine represent a heterogeneous group of lymphomas with diverse pathogenic mechanisms.

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Intestinal T-cell lymphomas are believed to arise from gut-associated lymphoid tissue and can involve all areas of the small bowel, with a particular predilection for the jejunum. The expression of CD103 and other phenotypic similarities between these lymphomas and normal intestinal intraepithelial lymphocytes (IELs) suggests that intestinal T-cell lymphomas represent the malignant counterpart of the immune cells residing in the bowel mucosa. It appears that these lymphomas usually arise from either the major IEL T-cell subset expressing T-cell receptor (TCR) α/β or the minor subset expressing TCR γ/δ. The lymphomas are a rare but well-documented complication of gluten-sensitive enteropathy. Because of the association between intestinal T-cell lymphoma, villous atrophy of jejunal mucosa, and celiac disease, the term ‘enteropathy-type T-cell lymphoma’ is now a widely accepted, distinct clinical-pathological entity incorporated into the World Health Organization classification.

We report the case of an intestinal natural killer (NK)–like T-cell lymphoma occurring in a patient without evidence of gluten-sensitive or non-gluten-related enteropathy. The lymphoma cells bear a distinct immunophenotype corresponding to a novel subset of normal intestinal IELs that are down-regulated, rather than up-regulated, in a gluten-sensitive enteropathy. This case supports the consideration that lymphomas involving the small intestine are derived from various subsets of normal intestinal IELs that are differentially activated by diverse antigenic stimuli. Biologic, diagnostic, and clinical implications of these findings are discussed.

REPORT OF A CASE

A 36-year-old Egyptian-born male, residing in the United States since age 4, presented with recent symptoms of abdominal pain and diarrhea. He had no other associated gastrointestinal symptoms. He had no history of celiac disease, inflammatory bowel disease, or other medical conditions. He had no family history of gastrointestinal illness. He had no symptoms of a lymphoproliferative disorder; specifically, he had no night sweats, fever, or weight loss. Routine hematologic and metabolic laboratory studies were normal. His serum lactate dehydrogenase concentration was 600 U/L (normal range, 313–618 U/L). His serum β2 microglobulin concentration was 1.8 μg/mL (normal range, 1.1–2.4 μg/mL). Serum protein electrophoresis was negative for the presence of a paraprotein. Results of human immunodeficiency virus testing was negative. A physical and computed tomographic abdominal examination revealed a 4 × 6-cm right lower quadrant mass. Upper gastrointestinal and colonic fiberoptic examinations were negative. A small bowel enema showed a large cavitary lesion in the midileum. The lesion involved the entire intestinal wall and extended into the mesentery. A diagnostic biopsy was performed.

An evaluation of the staging bone marrow biopsy showed normal trilineage hematopoiesis without evidence of involvement by lymphoma. The patient was treated with CHOP (cytoxan, Adriamycin, vincristine, and prednisone) chemotherapy. He tolerated the therapy well, and periodic computed tomographic imaging studies showed progressive resolution of his mass. He required 8 full cycles of chemotherapy to achieve a complete remission. A recent small bowel enema study and abdominal computed tomographic scan studies were normal. To date (more than 2 years after the diagnosis), the patient remains asymptomatic and in remission, without gastrointestinal symptoms or complaints.

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MATERIALS AND METHODS

Morphologic Studies

Microscopic evaluation of the tissue specimens were performed on 4-μm hematoxylin-eosin–stained paraffin sections of formalin-fixed or B5-fixed tissue. Wright-stained peripheral blood and bone marrow aspirate smears were also reviewed.

Immunohistochemical Analysis

Paraffin immunohistochemistry was performed using a streptavidin-biotin complex technique (Research Genetics, Huntsville, Ala) with polyclonal antibody against CD3 (Dako Corporation, Carpinteria, Calif) and monoclonal antibodies against CD30 (Dako), ALK (Dako), HLA-DR (Becton Dickinson, San Jose, Calif), CD34 (Becton Dickinson), CD43 (Dako), TIA-1 (Beckman Coulter, Miami, Fla), CD1a (Vector, Burlingame, Calif), and Ki-67 (Beckman Coulter). Detection of Epstein-Barr virus–related messenger RNA expression was performed by in situ hybridization.

Flow Cytometry Analysis

Flow cytometric analysis was performed on a FACSCalibur 4-color flow cytometer (Becton Dickinson) using the following monoclonal antibodies: CD2, CD3, cytoplasmic CD3e, CD4 (Becton Dickinson), CD7 (Caltag, San Francisco, Calif), CD10, CD11d, CD19, CD20, CD34, CD45, CD56, CD57 (Becton Dickinson), CD79a (Pharmingen, San Diego, Calif), CD11a, CD103, TCR α/β, TCR γ/δ, and TdT (Beckman Coulter). Detection of Epstein-Barr virus–related messenger RNA expression was performed by in situ hybridization.

RESULTS

Morphologic and Immunophenotypic Findings

The tumor was composed of a diffuse, pleomorphic, lymphocytic cell infiltrate with clusters of large transformed lymphocytes in a background of mixed small and large lymphocytes (Figure 1, A and B). No definitive angiocentricity or angioinvasion was noted. The mitotic rate was moderate (1–3 per high-power field). Occasional apoptotic cells were seen. No intestinal mucosa could be identified in the multiple-level sections. A Wright-stained touch preparation showed that many of the tumor cells contained abundant cytoplasm with granules typical for large granular lymphocytes. Results of the detailed immunophenotyping performed by both immunohistochemical and flow cytometric analyses are summarized in the Table and are partially presented in Figure 1, C and D, and Figure 2. Briefly, when examined by flow cytometry,
Lymphoma Immunophenotype (Flow Cytometry and Immunohistochemistry)*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Expression</th>
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<tbody>
<tr>
<td>CD1a</td>
<td>–</td>
</tr>
<tr>
<td>CD2</td>
<td>–</td>
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<tr>
<td>Surface CD3</td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic CD3</td>
<td>+</td>
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<tr>
<td>CD4</td>
<td>–</td>
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<td>CD5</td>
<td>–</td>
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<tr>
<td>CD7</td>
<td>+</td>
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<tr>
<td>CD8</td>
<td>–</td>
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<tr>
<td>CD10</td>
<td>–</td>
</tr>
<tr>
<td>CD16</td>
<td>+ (subset)</td>
</tr>
<tr>
<td>CD43</td>
<td>+</td>
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<tr>
<td>CD56</td>
<td>+</td>
</tr>
<tr>
<td>CD57</td>
<td>–</td>
</tr>
<tr>
<td>CD103</td>
<td>+</td>
</tr>
<tr>
<td>TCR  (\alpha/\beta)</td>
<td>–</td>
</tr>
<tr>
<td>TCR  (\gamma/\delta)</td>
<td>–</td>
</tr>
<tr>
<td>TdT</td>
<td>–</td>
</tr>
<tr>
<td>EBER-1</td>
<td>–</td>
</tr>
<tr>
<td>Ki-67</td>
<td>+ (subset)</td>
</tr>
</tbody>
</table>

* TCR indicates T-cell receptor; EBER-1, Epstein-Barr virus–related messenger RNA-1.

the lymphoma cells displayed T- and NK-cell–associated antigens CD7, cytoplasmic CD3\(^e\), CD56, and CD16 (dim expression). In addition, the lymphoma cells expressed the CD103 antigen (a member of the integrin \(\alpha\) chain family involved in epithelial homing) that is typically seen in intestinal T lymphocytes, both normal and malignant. Absent T-cell–associated antigens included surface CD3, TCR \(\alpha/\beta\), TCR \(\gamma/\delta\), CD2, CD5, CD4, and CD8. The malignant lymphocytes also did not express the B-cell–associated antigens and immature cell markers TdT, CD1a, and CD34. The density of CD45 expression by the lymphoma matched the expression of CD45 seen in mature lymphocytes. Results of paraffin immunohistochemistry were consistent with a lymphoma of T-cell origin, showing CD3- and CD43-positive staining in the malignant-appearing cells. The lymphoma cells were negative for CD30, TIA-1, ALK, and Epstein-Barr virus–related messenger RNA expression. Cell cycle–related MIB-1 (Ki-67) antigen was strongly expressed in approximately 60% of the cells.

**Molecular Studies**

Analysis of the TCR \(\gamma\) gene locus for rearrangement (Figure 3) showed a single large peak when using the Vy 1 to 8 primers (238-bp product). The Vy 9 to 11 primers showed a major, dominant peak at 180 bp and a smaller peak at 156 bp. These results indicate the presence of a major clonal cell population. The presence of the large peaks using both primer sets is likely due to cross-reactivity rather than detection of 2 separate clones.

**Cytogenetic Analysis**

Cytogenetic analysis was performed on 34 dividing cells, with only one normal metaphase noted. The other 33 metaphases exhibited multiple structural and numeric chromosomal abnormalities. Five of the cells exhibited aneuploidy, with an average of 40 chromosomes per cell. Additional chromosomal material was noted in the following: add(1p), add(2q), add(4q), add(6p), add(6q), add(7q), add(9q), add(18p), and add(19q). Multiple marker chromosomes and rings were also noted. A marker chromo-

![Figure 2](image.png)

**Figure 2.** Flow cytometric analysis of the intestinal T-cell lymphoma. Flow cytometry analysis of cells falling into the large lymphocyte gate by forward- and side-scatter characteristics exhibited the following immunophenotypes: A, 93% of the cells were CD56\(^e\), surface CD3\(^+\). B, 95% of the cells were cytoplasmic CD3\(^e\), TdT\(^+\). C, 70% of the cells were CD103\(^+\) surface CD3\(^+\).

some appeared to exhibit material resulting from a possible duplication of 11q. Deletion of 3q was noted. Unisomy X and unisomy 17 were present. A potential t(21:22) translocation was also seen. Among all of these abnormalities, loss of chromosome 17, where the p53 tumor suppressor gene is coded, was the most consistent. Nevertheless, none of the chromosomal alterations was characteristic for a particular lymphoid cell lineage.

**Serologic Studies**

Antiendomysial antibodies (IgA) and antigliadin antibodies (IgG and IgA) were analyzed in this patient's se-
IELs is still not absolutely clear, the evidence shows a pre-
derivation of the lymphoma.

teropathy raise questions regarding pathogenesis and cell

advanced, high-grade tumor. Molecular studies revealed a
mosomal abnormalities indicative of a biologically ad-
alysis of the lymphoma cells revealed multiple chro-

Figure 3. T-cell receptor (TCR) γ chain gene rearrangement. TCR γ gene rearrangement was determined by polymerase chain reaction, followed by product analysis by capillary electrophoresis. A, Primers directed against the Vγ1 to 8 subfamily detected a 238-bp (base pair) polymerase chain reaction product. B, Primers directed against the Vγ9 to 11 subfamily detected 156- and 180-bp products.

rum, as increased concentrations of these antibodies are present in gluten-sensitive enteropathy/celiac disease. Antiendomysial IgA antibodies were not detected in this case. Antigliadin IgG antibodies were also not detected. Antigliadin IgA antibodies were interpreted as indeterminate according to reference ranges. The antigliadin antibody assays are less sensitive (76% for IgG and 91% for IgA) and less specific (88% for IgG and 85% for IgA) for enteropathic disease than IgA antiendomysial antibodies, which have a sensitivity and specificity greater than 99%. Additionally, the antigliadin IgA antibodies have a positive predictive value of only 45%.

COMMENT

We describe an unusual case of a patient with an intestinal large NK-like T-cell lymphoma exhibiting a distinct immunophenotype, complex cytogenetic abnormalities, and lack of evidence of celiac disease or any other type of enteropathy. Although the patient presented with an ulcerated large mass in the ileum, no clinical, endoscopic, or serologic evidence of preceding and/or concomitant enteropathy was noted. The lymphoma cells expressed a combination of the T- and NK-cell–associated antigens CD7, cytoplasmic CD3ε, CD56, and CD16 and in the absence of the following T-cell markers: surface CD3, TCR α/β and γ/δ, CD2, CD5, CD4, and CD8. The lymphoma cells expressed the CD103 antigen typically seen in normal and malignant intraepithelial T lymphocytes. Cytogenetic analysis of the lymphoma cells revealed multiple chromosomal abnormalities indicative of a biologically advanced, high-grade tumor. Molecular studies revealed a clonal TCR γ chain gene rearrangement. The unusual immunophenotype and the lack of association with overt enteropathy raise questions regarding pathogenesis and cell derivation of the lymphoma.

Although the origin of the different subsets of normal IELs is still not absolutely clear, the evidence shows a predominant CD3ε, TCR α/β, CD8 cytotoxic T-cell phenotype with a lesser population (usually <10%) expressing TCR γ/δ. Jejunal IELs are especially enriched in CD56+ T cells, constituting in this site up to 15% of TCR α/β IELs. Similarly, the immunophenotypes of tumor cells are typically CD3+, CD5+, CD7+, CD8+, CD4+, and CD103-. Regarding lymphomagenesis, a number of studies have shown that most intestinal T-cell lymphomas involving the jejunum are associated with gluten-sensitive enteropathy and arise from TCR α/β+ T cells; well-documented cases of intestinal γ/δ T-cell lymphomas have also been described.

Several lines of evidence clearly indicate that our case does not represent a typical celiac disease–associated intestinal T-cell lymphoma. First, no evidence of gluten-sensitive or other types of enteropathy was documented by clinical, endoscopic, or serologic evaluation. Furthermore, the lymphoma involved the ileum, rather than the jejunum, the usual site of involvement in celiac disease–associated intestinal T-cell lymphoma. Except for the recent lymphoma-related symptoms, the patient had no personal or family history of celiac disease, gastrointestinal disorders, or other entities associated with celiac disease, such as dermatitis herpetiformis. To date, more than 2 years after the diagnosis, the patient remains asymptomatic, without gastrointestinal complaints. Although intestinal biopsy remains the gold standard for defining enteropathy, serologic studies for antiendomysial IgA antibodies (that were negative in this case) have been shown to be rather specific and sensitive in detecting celiac disease. The high sensitivity and specificity of antiendomysial antibodies is illustrated when tested against untreated celiac disease patients versus patients with various conditions, including inflammatory bowel disease (Crohn disease and ulcerative colitis), systemic lupus, and rheumatoid arthritis. Finally, serologic tests have been reported as positive in some subclinical cases of celiac disease, including asymptomatic relatives of patients with celiac disease.

The occurrence of intestinal T-cell lymphoma without gluten-sensitive or other apparent types of enteropathy has been reported. Although such a clinical setting appears to occur in a minority of intestinal T-cell lymphomas, it suggests a diverse etiology for these lymphomas.

The distinct immunophenotype of the lymphoma described also argues against its association with celiac disease. During chronic antigen exposure, particularly in active celiac disease, an increase in the population of both CD3+ TCR α/β and γ/δ T lymphocytes has been observed. The lack of surface CD3/TCR complex expression, as well as either the α/β or γ/δ chains, precludes our ability to provide definitive evidence of derivation from either of these 2 T-cell lineages. Furthermore, Eiras et al have recently identified a population of normal IELs with an immunophenotype essentially the same as our lymphoma cells. These IELs express CD7 and CD103 but lack a number of T-cell–associated markers such as CD3, TCR α/β and γ/δ, CD4, CD5, and CD8. Additionally, the cells exhibit NK-cell markers (44% of the analyzed CD3– and CD7+ cells were CD56+, with 12% being CD16+, 98% being CD161+, and 92% being CD94+) and an activated cell phenotype, as indicated by the expression of CD45RO, CD122, CD69, and CD38. The functional significance and cellular derivation of this population are currently uncertain but may be more consistent with NK- than with T-cell lineage. According to the authors, this IEL subset was markedly reduced in patients with celiac disease compared to the normal controls. In 15 patients with celiac disease tested, these cells represented only about 2% of IELs, compared to approximately 40% in the normal subjects. As the authors postulate, the decrease in this IEL
population may be a useful marker for celiac disease, especially when examined as a ratio to the population of CD3+ and TCR+ T lymphocytes. Cellier et al have also described a subset of lymphocytes bearing the same immunophenotype as that of our lymphoma cells: CD7+, cytoplasmic CD3ε+, surface CD3+, TCR+, CD4+, and CD8+, with restricted TCR γ gene rearrangement. Furthermore, they have observed an increase in this lymphocyte subset in patients with refractory sprue but not in normal patients or patients with celiac disease. To our knowledge, our case represents the first description of lymphoma apparently derived from this unique subset of intestinal IEL. These observations indicate that lymphomas involving the small intestine have diverse etiologies and may arise from normal cell counterparts outside the context of celiac disease, especially since lymphocyte subsets bearing that specific immunophenotype decrease in number in celiac disease. This, in turn, suggests that antigenic stimuli unrelated to gladin drive these normal intestinal cells toward malignant transformation.

The cells in this lymphoma, as well as their apparent normal IEL counterparts, cannot be easily categorized as either T lymphocytes or NK cells. Moreover, in many instances, distinguishing T-cell from NK-cell origin may be impossible, because certain normal and malignant T lymphocytes coexpress antigens considered specific for NK cells, such as killer cell inhibitory receptors. Despite the substantial karyotypic complexity of the lymphoma we describe, it lacks the cytogenetic abnormalities characteristic of either a T- or NK-cell lineage. Although the presence of a TCR gene rearrangement strongly suggests a T-cell origin of the lymphoma, the immunophenotype is more ambivalent and consistent with cells of either T- or NK-cell origin. Expressions of T-cell antigens CD7 and cytoplasmic CD3ε are also observed in cells of NK lineage. The T-cell lineage-specific antigens, such as surface CD3, TCR α/β or γ/δ, CD2, CD5, and CD4 or CD8, were not expressed in this case. The lymphoma cells expressed 2 NK-cell–associated markers: CD56 (detected on most of the malignant cells) and CD16 (dimly expressed by a minor subset of the cells [Table]). However, the expression of these 2 NK-cell–associated markers is not specific for the NK lineage. CD56 (a neural-cell adhesion molecule) is also expressed by subsets of normal CD4+ and CD8+ T cells, CD3+ and CD8+ T-cell large granular lymphocyte leukemia, and other non–NK-cell hematopoietic malignancies such as myelogenous leukemia and multiple myeloma. Furthermore, the majority of CD56+ intestinal lymphomas are most likely derived from intraepithelial CD8+ cytotoxic T cells. As opposed to NK-cell leukemia and nasal NK-cell lymphoma, primary intestinal NK-cell lymphomas are exceptionally rare entities, typically expressing CD3+, CD5+, CD2+, CD7+, CD56+, perforin+, TCR α/βγδ, or TCR γ/δ. In our case, lacking a TCR gene rearrangement. It is also possible that the lymphoma is derived from TCR+ γ/δ T cells normally lacking expression of several T-cell markers, such as CD4 and CD8. Although our lymphoma lacks expression of a surface TCR γ/δ/CD3 complex, this expression could have been lost or down-regulated. Accordingly, down-regulation of the TCR γ/δ has been demonstrated in apparently normal, clonally expanded T-cell populations stimulated by various antigens. Considering the uncertainty of the exact cell origin (T cell vs NK cell), we believe that the designation of NK-like T-cell lymphoma is the most appropriate, at present, for our case.

In summary, we describe the case of an NK-like T-cell malignancy of the small intestine with a distinct phenotype, a clonal TCR gene rearrangement, and a complex karyotype. The combined evidence presented in this case supports the consideration that lymphomas involving the small intestine represent a heterogeneous group of malignancies, most likely derived from normal subsets of immunoreactive IELs chronically activated by diverse antigenic stimuli.

References