CONCISE REPORT

The Structure of the T Cell Gamma Chain Gene in Lymphoproliferative Disorders and Lymphoma Cell Lines

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During the development of B and T cells, a number of genes undergo rearrangement. In this paper we have studied the structure of the T cell γ-chain gene in primary lymphomas and cell lines derived from patients with lymphoma. Samples derived from patients with angioimmunoblastic lymphadenopathy (AIL), Lennert’s lymphoma, and Hodgkin’s disease were also examined. TcR γ was rearranged in all T cell lymphomas and B cell lymphoma cell lines. Rearrangement of the γ-chain gene was also found in AIL, Lennert’s lymphoma, and four of eight cases of Hodgkin’s disease. These studies indicate that rearrangement of TcR γ is a useful clonal marker but does not aid in the identification of cell lineage.

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MATERIALS AND METHODS

Patient material. Lymph nodes of individuals undergoing investigation of lymphoma were snap frozen and stored at −70°C. The lymphomas were characterized by routine histology and histochemistry and as previously described. There were 14 cases of T cell lymphoma (two lymphoblastic lymphomas and 12 peripheral T cell lymphomas); seven cases of B cell lymphoma (one B cell chronic lymphocytic leukemia, one centrocytic lymphoma, one centroblastic/centrocytic lymphoma, two immunoblastic lymphomas and two cases with monotypic B cells and a large proportion of pleomorphic T cells); five cases of Lennert’s lymphoma; eight cases of Hodgkin’s disease; ten cases of non-Hodgkin’s lymphoma containing large anaplastic Ki 1+ cells (Ki 1+ lymphoma) (ten); nine cases of AIL; and two cases of AIL-like hyperimmunoreaction.

Cell lines. Recently, eight cell lines derived from patients with non-Hodgkin’s lymphoma have been established at the Ontario Cancer Institute. All of the cell lines are Epstein-Barr virus negative and express surface or cytoplasmic immunoglobulin.

Analysis of DNA. DNA was extracted from the lymph nodes or cell lines as previously described and digested with one of the restriction enzymes BamHI, EcoRI, or HindIII, according to the supplier’s recommendations. The cut DNA was separated by electrophoresis on a 0.8% agarose gel, transferred to nitrocellulose according to the method described by Southern, and hybridized to a 32P-labeled cDNA containing the γ-gene (a gift from Dr D. Littman, University of California, San Francisco). Rearrangement was scored by the loss of the germine band or the appearance of new bands.

RESULTS

The results of the Southern blot analysis using a γ-cDNA probe are summarized in Table 1, and representative autoradiographs shown in Fig 1. Rearrangement of TcR γ chain gene resulted in the appearance of new bands and variable loss of the germine band. Rearrangement of TcR γ gene was found in 14 of 14 cases of T cell lymphoma (Fig 1A); six of seven B cell lymphomas; five of five cases of Lennert’s lymphoma; eight of eight Ki 1+ lymphomas; nine of nine cases of AIL; and four of eight cases of Hodgkin’s disease.
T CELL γ-CHAIN REARRANGEMENT IN LYMPHOMA

Table 1. Structure of Rearranging Genes in Lymphohematopoietic Malignancies

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients</th>
<th>Germ-line</th>
<th>Ig HC + LC</th>
<th>Ig HC + LC + TcRγ</th>
<th>Ig HC + LC + TcRγ + TcRβ</th>
<th>TcRβ</th>
<th>TcRβ/γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cell lymphoma</td>
<td>14</td>
<td></td>
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<td></td>
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<tr>
<td>B cell lymphoma</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B cell lymphoma cell lines</td>
<td>8</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lennert’s lymphoma</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki 1 + lymphoma</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angioimmunoblastic lymphadenopathy</td>
<td>11</td>
<td>2*</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*These two cases were diagnosed as ALL-like hyperimmune reaction.

(Fig 1b). Rearrangement was not seen in two cases of ALL-like HR.

In the B cell lymphoma cell lines, rearrangement of TcRγ chain was found in three of the eight cell lines.

A number of patterns of rearrangement were seen; however, within and between different classes of tumors, similar rearranged bands could be seen. This is illustrated in Fig 1A, in which lanes 2, 3, 6, 7, and 11 all contain an apparently identical rearranged band.

DISCUSSION

In the present study we have examined the structure of the T cell γ-chain gene in 55 primary lymphomas and eight lymphoma cell lines. The structure of the Ig LC and HC genes and the TcRγ chain gene of the lymphoma samples have been reported; those results are summarized in Table 1. Similar studies have been carried out on the lymphoma cell lines, and the results are also presented in Table 1 (manuscript in preparation). As can be seen in Table 1, rearrangement of TcRγ chain gene may occur in a number of settings.

In agreement with our previous studies, in all cases in which there was rearrangement of TcRβ, there was also rearrangement of TcRγ. However, in the present study we also found TcRγ rearrangements in association with rearranged Ig HC and LC genes; this could be with or without TcRβ rearrangement. Furthermore, two cases were identified in which there was TcRγ rearrangement in the absence of Ig or TcRβ rearrangement.

The two cases in which there was only TcRγ rearrangement were cases of Ki 1 + lymphoma. The Ki 1 antigen is detected by a monoclonal antibody raised against a Reed-Sternberg cell line. The antibody reacts with Reed-Sternberg cells, activated normal T lymphocytes, and almost all of the cells in some large anaplastic lymphomas; the latter are designated Ki 1 + lymphomas. Immunohistochemical studies indicate that these lymphomas are mainly of T cell origin, occasionally of B cell origin, and seldom of histiocytic origin. In a previous report we suggested that the two cases in which there was no clonal rearrangement of the Ig and TcRβ genes may be a polyclonal proliferation of lymphocytes. However, the detection of a rearranged γ-chain gene indicates that they are clonal in origin.

The nature of the cell in the Ki 1 + lymphomas that only have TcRγ rearrangement is unknown. Whether it represents an early lymphocyte precursor that became frozen in differentiation as a result of malignant transformation or is the malignant counterpart of a mature functional cell type not yet recognized will be resolved by more intensive study of the cell involved in such lymphomas.

Rearrangement of the γ-chain gene was also found in a large proportion of B cell lymphomas and B cell lymphoma cell lines. These tumor cells have rearranged their immuno-

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Fig 1. Southern blot analysis of DNA cut with BamH1 and probed with 32p-labeled gamma chain cDNA. (A) DNA derived from ten T cell lymphomas. (B) DNA from two cases of Hodgkin’s lymphoma. The lane G in each sample is DNA derived from granulocytes and represents the germline pattern: □, germline bands; △, rearranged bands; ○, deleted germline bands.
globular and heavy chain genes and express either cytoplasmic or surface Ig. In some of these cases, γ-gene rearrangement was present in the absence of TcRβ rearrangement.

These findings, along with our previous studies of T cell leukemia cell lines and functional T cell clones and studies in mouse, suggest that rearrangement of the γ-chain gene is an early event in the development of lymphocytes. The present data are consistent with the following developmental scheme. In the development of a functional T cell, rearrangement of TcRβ occurs only if the γ-chain gene has rearranged. In the development of a functional B cell prior to rearrangement of the γ-gene, neither appears to be essential for the subsequent rearrangement of the HC genes nor does it restrict a cell from differentiating along the B cell pathway. However, the frequent association of γ-chain rearrangement with Ig rearrangement in mature B cells suggests that early events involved in lymphocyte differentiation that affect DNA conformation and recombination activity are relatively indiscriminate and can act on Ig HC, TcRγ, and TcRβ chain genes. Second, the association of Ig and TcRγ rearrangements in the same B cell suggests that these rearrangements are occurring in the bone marrow. This is in contrast with other studies suggesting that TcRγ rearrangement occurs in the thymus.14

The finding of gamma rearrangement in lymph nodes from patients with Hodgkin’s disease confirms our previous observation of a clonal population of cells in some cases of this disease. Studies are now in progress to determine whether this finding can identify a subset of patients with regard to histologic subtype or clinical behavior. One of the samples contained 50% Reed-Sternberg cells; in this case no rearrangements were detected. In Fig 1, new bands of different molecular weights are apparent. However, similar size bands are also present in different samples. This observation persists even when different restriction enzymes are used. A possible reason for this is the limited repertoire of TcRγ chain gene. Analysis of γ cDNA clones reveals that there are, at most, ten variable regions and six joining regions.19 However, enough variability in the size of the rearranged fragment exists so that in a polyclonal population of T cells, such as obtained from peripheral blood, rearrangement is not evident.

As rearrangement of TcRγ chain gene may be associated with TcRβ rearrangement or Ig rearrangement, it is not a useful marker of lineage. However, since there appears to be a range of possible rearrangements of the gamma chain gene, it should prove to be a useful marker of clonality of both T and B cell malignancies. In addition, a new cell type having only gamma gene rearrangement may have been identified.

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