Comparison of Genetic Probe With Immunophenotype Analysis in Lymphoproliferative Disorders: A Study of 87 Cases

By Tawfiq Henni, Philippe Gaulard, Marine Divine, Jean-Pierre Le Couedic, Dario Rocha, Corinne Haioun, Zoubida Henni, Jean-Pierre Marolleau, Yvon Pinaudeau, Michel Goossens, Jean-Pierre Facet, and Félix Reyes

We examined 91 specimens (from 87 patients) for the expression of B-cell– and T-cell–associated differentiation antigens and rearrangements of the Ig and β-chain of the T-cell (β-TCR) genes. Of these, 74 were representative of various histologic subtypes of non-Hodgkin’s lymphoma and related disorders, 11 of Hodgkin’s disease, and 6 of reactive lymphoid hyperplasia. An Ig gene clonal rearrangement correlated to a monotypic (κ/λ) phenotype in 32 of 33 histologically defined lymphoma samples. The genotypic analysis also confirmed clonality in six of seven malignant histologically defined lymphoma samples. Approval was obtained from the Institutional Review Board.

MATERIAL AND METHODS

In the past 10 years, the development of immunohistologic techniques has provided a new tool to recognize the B- or T-cell lineage of proliferating cells and in a significant number of cases to detect a uniform expression of antigenic markers such as the Ig light chain restriction, supporting the identification of a monoclonal process. However, the immunophenotyping technique suffers from some limitations. First, malignant B cells may be intermixed with residual polytypic (κ and λ) cells. Second, T-cell proliferations that often are morphologically polymorphous have posed a major problem, in spite of the absence of a marker of clonality in lymph node specimens comparable to the κ/λ monotypy of B-cell lymphomas, it has been impossible to decide whether these cells are monoclonal. Therefore, an usual criteria for malignancy has been lacking.

To overcome these limitations, a new approach has been recently proposed that is based on the study of the DNA rearrangements that assemble the genes for antigen-receptor molecules in B or T cells. Such rearrangements create at the DNA level markers unique to each individual cell. The detection by the Southern blot assay of uniform rearrangements allows the recognition of a clonal expansion and theoretically of its B- or T-cell derivation.

In the present report, we have focused on the utility of the genotypic approach for the diagnosis in patients with histologically defined lymphomas and related disorders.

THE DIAGNOSIS of malignant non-Hodgkin’s lymphoma is based on the histologic evaluation of tissue biopsies. However, in some cases, the distinction between benign and malignant disorders remains difficult. Furthermore, some metastatic carcinomas may be mistaken for lymphoma.

In the presence of a monoclonal process as revealed by immunophenotypic analysis. DNA analysis was the only way to demonstrate clonality in other samples with either a polymorphous (partial involvement, pseudolymphoma, angioimmunoblastic lymphadenopathy [AILD]) or an undifferentiated (large cell anaplastic) phenotype. It is concluded that although in the majority of cases immunophenotypic alone provides criteria adequate for the diagnosis of lymphoid malignancy, in some, particularly polymorphous or large cell anaplastic processes, genetic probe analysis was additionally discriminative.

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Submitted April 22, 1988; accepted July 26, 1988.

Supported in part by Grant No. 6533 from the Association pour la Recherche Contre le Cancer and Grant No. 949 from Coiffe Nationale de l’Assurance Maladie des Travailleurs Sociaux.

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Table 1. Monoclonal Antibodies Used in This Study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan-B-Cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1*</td>
<td>CD20</td>
<td></td>
</tr>
<tr>
<td>B4*</td>
<td>CD19</td>
<td></td>
</tr>
<tr>
<td>Dako-CD22*</td>
<td>CD22</td>
<td></td>
</tr>
<tr>
<td>Anti-( \lambda, \mu, \delta, \gamma )*</td>
<td>Ig, light, and heavy chains</td>
<td></td>
</tr>
<tr>
<td>Pan-T-cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu-1( \ddagger )</td>
<td>CD5</td>
<td></td>
</tr>
<tr>
<td>Leu-4( \ddagger )</td>
<td>CD3</td>
<td></td>
</tr>
<tr>
<td>Leu-5( \ddagger )</td>
<td>CD2</td>
<td></td>
</tr>
<tr>
<td>Leu-9( \ddagger )</td>
<td>CD7</td>
<td></td>
</tr>
<tr>
<td>T-cell subset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu-2( \ddagger )</td>
<td>CD8</td>
<td>Cytotoxic/suppressor</td>
</tr>
<tr>
<td>Leu-3( \ddagger )</td>
<td>CD4</td>
<td>Helper/inducer</td>
</tr>
<tr>
<td>Leu-6( \ddagger )</td>
<td>CD1</td>
<td>Thymocytes</td>
</tr>
<tr>
<td>Other</td>
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<td></td>
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<tr>
<td>Ki-1( $ )</td>
<td>CD30</td>
<td>Activated cells</td>
</tr>
<tr>
<td>Leu-M1( \ddagger )</td>
<td>CD15</td>
<td>Myelomonocytic series</td>
</tr>
<tr>
<td>Leu-M3( \ddagger )</td>
<td>CD14</td>
<td>Myelomonocytic series</td>
</tr>
<tr>
<td>OK-M1( | )</td>
<td>CD11b</td>
<td>Myelomonocytic series</td>
</tr>
</tbody>
</table>

*Coulter Immunology, Hialeah, FL.
†Dakopatts, Denmark.
‡Becton-Dickinson, Mountain View, CA.
§Immunotech, Luminy, Marseille, France.
∥Ortho Pharmaceutical, Raritan, NJ.

Ig and APAAP complexes were obtained from Dako (Dakopatts A/S, Denmark). For surface Ig evaluation, slides were first covered with normal fetal calf serum for 20 minutes. Ig light chains were detected by using at least two dilutions of the relevant antibody. Alkaline phosphatase was revealed after incubation in a solution of Fast-Red TR, 1 mg/mL, and naphtol AS-TR, 0.2 mg/mL (Sigma Chemical Co, St Louis) containing levamisole, which blocks endogenous alkaline phosphatase activities.

Immunophenotypic criteria. Lymphomas were considered to be of B-cell derivation if the tumor cells were positive for the presence of surface Ig and/or at least two pan-B antigens (CD19, CD20, CD22) and negative for the CD3, CD4, CD8 antigens. They were considered as monotypic if there was a predominance (over 90%) of the expression of one \( \kappa \)- or \( \lambda \)-light chain. Lymphomas were considered to be of peripheral T-cell derivation if the tumor cells were negative for the presence of the CD1 antigen, positive for the presence of the CD3 antigen as well as of other pan-T and T-cell subset antigens, and negative for surface Ig and the CD19, CD20 antigens. The recognition of a pan-T antigenic loss required that >50% of the tumor cells lacked expression of one antigen that could be expressed on small apparently reactive T lymphocytes.

Immunogenotypic study. DNA was extracted from tissue samples and analyzed according to standard methods detailed elsewhere. Ten micrograms of DNA from each sample were digested with the appropriate restriction endonucleases, subjected to electrophoresis in 0.6% to 1% agarose gel, transferred onto a nitrocellulose filter by the method of Southern, and hybridized to \( ^{32} \)p-labeled DNA probes.

Rearrangements of the T cell receptor \( \beta \)-chain gene (\( \beta \)-TCR) were analyzed using a probe specific for the constant regions (C\( \beta 1 \) and C\( \beta 2 \)) after DNA digestion with EcoRI, BamHI, and HindIII endonucleases. Analysis of immunoglobulin gene rearrangements

<table>
<thead>
<tr>
<th>Histology (WF*)</th>
<th>No. of Cases</th>
<th>JH R</th>
<th>JH R</th>
<th>JH R</th>
<th>JH R</th>
<th>JH R</th>
<th>JH R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>JH R</td>
<td>JH R</td>
<td>JH R</td>
<td>JH R</td>
<td>JH R</td>
<td>JH R</td>
</tr>
<tr>
<td>Small lymphocytic</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular small cleaved</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular mixed</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse small cleaved</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse mixed</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse large</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse large immunoblastic</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small noncleaved</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>31</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Samples expressed at least two pan-B antigens (CD19, CD20, CD22) and were negative for CD3, CD4, and CD8 antigens.

Abbreviations: R, rearranged; GL, germ-line.
was performed by using a JH probe specific for the heavy chain joining region2 after BamHI-HindIII double digestion (all probes were gifts from T. Rabbits).

**RESULTS**

The histopathologic features of the samples under study are listed in Tables 2 through 5.

**B-cell proliferations.** Monotypic surface Ig was expressed in 33 lymphoma samples. In all but one, the presence of a monoclonal population of B cells was demonstrated by the finding of a JH rearrangement (Table 2). Ten other cases were recognized as B-cell proliferations because of the expression of at least two of the CD19, CD20, and CD22 pan-B antigens. However, a monotypic surface Ig could not be demonstrated, due to either the absence of a detectable light chain or, conversely, to the presence of both k and l isotypes in the sections (Table 3). In nine of these ten samples, the JH probe also revealed clonal rearrangements of Ig genes.

In the majority of these B-cell samples, the JH probe revealed one (11 cases) or two (26 cases) bands in addition to the germ-line one, corresponding to the monoclonal rearrangement of one or two alleles (Fig 1A). However, in three cases (nos. 37, 39, and 40), more than two bands were detected, indicating the presence of at least two separate clones (Fig 1B). Case no. 38 corresponded to the transforma-

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**Table 3. B-cell Lymphomas Without Ig Light Chain Restriction**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tissue</th>
<th>Histology (WF)</th>
<th>Phenotype</th>
<th>Genotype</th>
<th>JH β-TCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Mediastinal</td>
<td>Diffuse large</td>
<td>-</td>
<td>-</td>
<td>R GL</td>
</tr>
<tr>
<td>35</td>
<td>Mediastinal</td>
<td>Diffuse large</td>
<td>-</td>
<td>-</td>
<td>R GL</td>
</tr>
<tr>
<td>36</td>
<td>Mediastinal</td>
<td>Diffuse large</td>
<td>-</td>
<td>-</td>
<td>GL GL</td>
</tr>
<tr>
<td>37</td>
<td>Lymph node</td>
<td>Diffuse large, transformation of follicular small cleaved</td>
<td>(+) (+) + + - 3R GL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Lymph node</td>
<td>Diffuse large, transformation of small lymphocytic</td>
<td>(+) (+) + + - 2R GL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Lymph node</td>
<td>Diffuse large</td>
<td>(+) (+) + + - 3R GL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Soft tissue</td>
<td>Diffuse small noncleaved*</td>
<td>(+) (+) + + - 3R GL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>Lymph node</td>
<td>Suspended partial involvement, small lymphocytic</td>
<td>(+) (+) + + - R GL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Lymph node</td>
<td>Suspended partial involvement, large cell</td>
<td>(+) (+) + + - R GL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Skin</td>
<td>Pseudolymphoma</td>
<td>(+) (+) + + - R GL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Samples were negative for the CD3, CD4, and CD8 antigens.

Abbreviations: R, rearranged; GL, germ-line; 3R, three rearranged fragments; 2R, a second rearranged fragment is detected in addition to the single one already present at the time of small lymphocytic lymphoma.

*Human Immunodeficiency Virus (HIV)-related.

†k and l refer to the respective proportions of k- and l-bearing cells.
phenotype of infiltrating cells (Table 4). Most of them
conventional histology and the CD1-negative, CD3-positive
a rearrangement of the addition of a new rearranged
sive specimens showed
surface Ig into a nonmonotypic large-cell lymphoma; succes-
sion of a small lymphocytic lymphoma with a monotypic
peripheral T-cell malignant lymphomas on the basis of
rearrangement in two cases (one follicular mixed, one diffuse
hand,
ance of an additional rearranged fragment.
for myelomonocytic antigens, and their DNA con-
tained a clonal Cβ rearrangement. By contrast, DNA from
case no. 22 displayed a germ-line configuration of β-TCR
and Ig genes and tumor cells expressed CD14, CD15, and
CD11b myelomonocytic antigens.

Related disorders. Five specimens were studied (Table
5). One case of lymphomatoid papulosis and one case of
lymphomatoid granulomatosis were shown to have a CD3,
CD4-positive phenotype and had clonally rearranged the β-TCR gene. In three cases morphologically classified as
angioimmunoblastic lymphadenopathy (AILD), immuno-
histology revealed a mixture of T and B cells. DNA analysis
demonstrated a B-cell clone in case no. 3, a T-cell clone in
case no. 4, and the absence of rearranged fragments in case
no. 5.

Table 4 shows that with the exception of three cases (case
nos. 1, 10, and 18) all CD3-positive and Cβ-rearranged
lymphoma samples were found by immunohistology to have
an abnormal antigenic profile. Tumor cells lacked one or
several pan-T antigens (CD2, CD5, CD7) or both CD4 and
CD8 subset antigens. A loss of pan-T antigens was also
evidenced in the two cases of lymphomatoid papulosis and

\[ \text{Table 5. Related Lymphoproliferative Disorders} \]

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tissue</th>
<th>Histology</th>
<th>Phenotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Skin</td>
<td>Lymphomatoid papulosis</td>
<td>Ig CD19, CD20 CD3</td>
<td>GL R</td>
</tr>
<tr>
<td>2</td>
<td>Nasal</td>
<td>Lymphomatoid granulomatosis</td>
<td>Ig CD3</td>
<td>GL R</td>
</tr>
<tr>
<td>3</td>
<td>Node</td>
<td>AILD</td>
<td>Ig CD5</td>
<td>R GL</td>
</tr>
<tr>
<td>4</td>
<td>Node</td>
<td>AILD</td>
<td>CD2</td>
<td>R GL</td>
</tr>
<tr>
<td>5</td>
<td>Node</td>
<td>AILD</td>
<td>CD7</td>
<td>GL R</td>
</tr>
</tbody>
</table>

Abbreviations: GL, germ-line; R, rearranged.

The results listed in Tables 2 and 3 show that one case of
B-cell lymphoma (small lymphocytic, monotypic) displayed
a rearrangement of both Ig and β-TCR genes. On the other
hand, the JH and Cβ probes failed to demonstrate any
rearrangement in two cases (one follicular mixed, one diffuse
large-cell).

T-cell proliferations. Eighteen cases were diagnosed as
peripheral T-cell malignant lymphomas on the basis of
conventional histology and the CD1-negative, CD3-positive
phenotype of infiltrating cells (Table 4). Most of them
belonged to the diffuse mixed and diffuse large-cell histo-
logic subgroups of the WF. In all cases, including recurrent
disease material in case nos. 12 and 13, DNA analysis with
the Cβ probe demonstrated the presence of a T-cell mono-
clonal process by showing the rearrangement of one (17
cases) or two (one case) alleles (Fig 2).

Four additional cases of diffuse large-cell lymphoma (case
nos. 19 through 22) were considered as large-cell anaplastic
and were found to be CD30 (Ki-1)-positive. Case no. 19
had a CD3, CD4-positive T-cell phenotype and had clonally
rearranged the β-TCR gene. The three remaining cases were
negative for pan-B and pan-T antigens and expressed only
the CD4 subset antigen. This finding prompted us to look for
additional differentiation markers. Case nos. 20 and 21 were
negative for myelomonocytic antigens, and their DNA con-
tained a clonal Cβ rearrangement. By contrast, DNA from
case no. 22 displayed a germ-line configuration of β-TCR
and Ig genes and tumor cells expressed CD14, CD15, and
CD11b myelomonocytic antigens.

Fig 1. DNA rearrangements of the Ig heavy chain gene using
the JH probe after BamH1-HindIII double digestion. The size of
the germ-line band is shown at the top, and arrows indicate the
rearranged fragments. (A) Lane 1, germ-line control; lanes 2 and
3, B-cell lymphoma samples showing the rearrangement of one or
two alleles, respectively. (B) Lane 1, germ-line control; lane 2,
case no. 37 (Table 3) showing three rearranged fragments that
correspond to at least two separate clones; lanes 3 and 4,
successive samples of case no. 38 (Table 3) showing the appear-
ance of an additional rearranged fragment.

Fig 2. DNA rearrangements of the β-TCR gene using the Cβ probe after digestion with (A) BamH1, (B) EcoRI, and (C) HindIII. Sizes of the
germ-line bands are shown at the top, and arrows indicate the rearranged fragments. Lane 1, germ-
line control; lanes 2 and 3, two T-cell lymphoma samples showing the rearrangement of one allele
of the Cβ1 segment; lane 4, another sample showing the rearrangement of one allele of the Cβ2
segment (see text).
lymphomatoid granulomatosis. Finally, a complete loss of pan-T antigens (including CD3) was associated with the presence of a clonal rearrangement of the β-TCR gene in two cases of Ki-1-positive large-cell anaplastic lymphoma.

The majority of β-TCR rearrangements observed in this study involved the Cβ1 segment of the β-chain gene, since they were detected in both EcoRI- and BamHI-digested DNA, with a germ-line configuration of HindIII-digested DNA (Fig 2, lanes 2 and 3). In two cases, rearranged Cβ2 fragments were revealed in BamHI- and HindIII-digested DNA, with a germ-line configuration after EcoRI digestion14 (Fig 2, lanes 4). Thus, digestion with three endonucleases excluded that observed fragments could result from polymorphism or partial digestion. Two cases (case nos. 1 and 9) were found to have simultaneously rearranged both the β-TCR and Ig genes.

In control specimens, T cells had an homogeneous phenotype and DNA analysis revealed no clonal rearrangements of either β-TCR or Ig genes. Occasionally, the Cβ probe gave a faint band of 8 kb (EcoRI digestion) or 11 kb (HindIII digestion) that was not ascribed to a clonal process in view of the germ-line configuration of BamHI-digested DNA.

DISCUSSION

Immunophenotypic and, more recently, immunogenotypic methods have become a critical contribution in determining lineage and clonality of lymphoid tumors.15-21 We have addressed the issue of the respective value of these methods by analyzing the patterns of rearrangement of Ig and β-TCR genes and the expression of B and T cell-associated differentiation antigens in a collection of lymphoproliferative specimens.

Sixty-two cases were histologically considered as non-Hodgkin’s lymphoma (Tables 2 and 3, case nos. 34 through 40; Table 4). As expected, DNA analysis by the Southern blot assay for Ig and TCR genes provided a reliable marker of clonality, since it was found positive in 59 of the 62 cases. This observation is in keeping with the concept that the vast majority of human lymphoid neoplasms are clonal in origin.3,22 DNA analysis demonstrated the presence of a clonal process in seven additional cases that included partial node involvement by lymphoma cells and pseudolymphoma (Table 3), lymphomatoid granulomatosis, lymphomatoid papulosis, and AILD (Table 5). By contrast, we found no rearrangements of either Ig or β-TCR genes in a control group that included reactive lymphoid hyperplasia and Hodgkin’s disease. This latter finding is in variance with reports of occasional B-cell or T-cell clones in specimens from patients with Hodgkin’s disease.9,23,24

By using combined phenotypic and genotypic criteria, 43 lymphoid tumors were assigned to the B-cell lineage. In 33 cases (74%) of histologically malignant lymphoma, a phenotypic pattern of light chain restriction suggested a B-cell monoclonal process (Table 2). This was confirmed by DNA analysis in all cases but one (discussed below). These results confirm the value of Ig light chain restriction as a marker of clonality.1,5,10,20,21 Seven cases also considered histologically as diffuse malignant lymphoma were assigned to the B-cell lineage, since tumor cells expressed at least two pan-B antigens (case nos. 34 through 40, Table 3). In these cases, monoclonality could not be ascertained due to the absence of surface light chain monotype. In four of these cases, κ or λ chains were undetectable due to their low density, as already reported in large-cell mediastinal lymphoma.23,24 Conversely, both κ- and λ-positive cells were detected in sections of three cases. With the exception of case no. 36 (discussed below), DNA analysis confirmed the presence of a clonal process in these nonmonotypic B-cell lymphomas.

The genotypic study of these nonmonotypic lymphomas also established that an oligoclonal process occurred within individual tumors. Thus, the configuration observed in case no. 38 resulted from the addition of a second rearranged fragment to the unique JH band present in the initial biopsy sample. In case nos. 37, 39, and 40, the finding of three distinct nongerm-line JH bands indicated that more than one clone coexisted in the same tumor site. These findings are in agreement with the observation that histologic conversion (as in case nos. 37 and 38) correlates with the presence of different clones of B cells27 and that acquired immunodeficiency syndrome (AIDS)-related lymphomas (as in case no. 40) are frequently oligoclonal.28

To note, we found a nonexpression of one of the three pan-B antigens in only three samples, and this is in contrast to what we observed in T-cell lymphoma, as discussed below. Others have already reported that in malignant lymphoma, the loss of pan-B antigens occurs less often than that of pan-T antigens and therefore cannot be considered as a sensitive marker of B-cell malignancy.8

Thus, the results of the genotypic study show that a valid diagnosis of malignant B-cell lymphoma had been proposed in 40 cases on the basis of histologic and immunophenotypic methods. In three other cases (case nos. 41 through 43, Table 3) in which these methods were inconclusive, DNA analysis demonstrated the presence of a clonal expansion of B cells. These findings are in accordance with previous reports on the sensitivity of the Southern blot assay in detecting minor clonal populations that compose as little as 1%13 to 10%20 of the total cells in a tissue sample. However, among the 40 biopsy specimens that were recognized as B-cell lymphoma by morphologic and immunohistologic methods, two failed to show clonal JH rearrangements: one was a lymph node specimen with a monoclonic light chain expression (Table 2), the other was a nonmonotypic mediastinal sample (Table 3). The failure of genotypic analysis may relate to the low number of lymphoma cells in the tissue fragment subjected to DNA extraction,29 since in one case the architecture was follicular and in the other case malignant cells were intermixed with abundant fibrosis.

A diagnosis of malignant peripheral T-cell lymphoma was considered in 19 cases (nos. 1 through 19, Table 4) on the basis of morphology and of the CD1-negative, CD3-positive phenotype of tumoral cells. DNA analysis demonstrated monoclonality in each case. This series included four patients in whom successive biopsies were obtained. In case nos. 12 and 13, monoclonality was demonstrated only in recurrent disease material. In case nos. 9 and 14, the size of rearranged bands was identical in successive biopsies, indicating that the same clone was present throughout the course of the disease.
With the exception of three cases (case nos. 1, 10, and 18), a striking finding was the nonexpression by lymphoma cells of one or several of the CD2, CD5, CD7 pan-T antigens, or of both CD4/CD8 subset antigens (Table 4). Since it was first reported as suggestive of malignant T-cell lymphoma, such a feature has been confirmed in several studies. We also observed such an incomplete T-cell phenotype in two patients with lymphomatoid papulosis and granulomatosis that had clonally rearranged one allele of the β-TCR gene. These conditions have been recently recognized as clonal T-cell disorders. Thus, in our study, the expression of an incomplete T-cell phenotype always correlated with the presence of a monoclonal process as revealed by DNA analysis. This was well illustrated by case nos. 12 and 13 (Table 4), in which the initial biopsy samples showed only a germ-line configuration of the β-TCR gene that was associated with an homogeneous antigenic profile. Subsequent biopsies revealed that the CD7 antigen had been lost when DNA contained a clonal rearrangement. It has to be noted that in this study the most commonly "lost" pan-T antigen was CD7, as already reported. Finally, we found no abnormal expression of the T-cell–associated antigens in the control group in which DNA analysis failed to detect clonal rearrangements. Therefore, our data show that an abnormal T-cell phenotype indicates the presence of a clonal process. This substantiates the validity of the combined morphologic and immunophenotypic approach in establishing a definitive diagnosis of malignant peripheral T-cell lymphoma.

However, in some cases, this approach did not allow a firm conclusion. In such cases, which fit into two groups, DNA analysis provided a critical support by identifying a clonal population within tumor cells. The first group consisted of three cases of Ki-1–positive large-cell anaplastic lymphoma that expressed only the CD4 marker without B-cell– or T-cell–associated antigens (Table 4, case nos. 20 through 22). One was identified as a true histiocytic proliferation because of the germ-line configuration of both Ig and β-TCR genes and of the expression of myelomonocytic differentiation markers. It is known that the CD4 marker is normally shared by both the T lymphocyte and the monocyte-macrophage series. The two other cases were negative for myelomonocytic markers and showed a clonal rearrangement of the β-TCR gene, suggesting a T-cell differentiation. In a previous study of ten Ki-1–positive lymphomas, seven were identified as T-cell malignancies on the basis of the genotypic analysis. The second group consisted of three cases considered as AILD (Table 5) showing a predominance of T cells, with both CD4 and CD8 subsets and no evidence of pan-T antigen loss. A JH rearrangement was found in one case, a Cβ one in another. These results are in agreement with other studies showing that a significant proportion of AILD samples contain T-cell and occasionally B-cell clones, an observation that correlates with the fact that malignant lymphoma may subsequently develop in such a setting.

It should be noted that three lymphoma samples (4.5%) contained a rearrangement of both Ig and TCR genes. Such "bigenotypic" tumors have been recognized in up to 10% of malignant lymphomas. In these cases as in the present study, however, bigenotypic tumor cells retain a completely fidelous immunophenotypic pattern: one case had a B-cell phenotype with a monotypic light chain and was CD3-negative. The others were CD3-positive and negative for pan-B antigens. It thus appears that the lineage specificity of clonal DNA rearrangements is not absolute and that a conclusive lineage definition requires an immunophenotypic study.

We conclude that genomic study should be used only when histologic and immunohistologic techniques are inconclusive. This is the case when either the polymorphism of the cellular infiltrate makes it impossible to determine accurately the phenotype of tumoral cells or lymphoma cells do not express any pan-T or pan-B antigens.

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