Immunoglobulin and T-Cell Receptor Gene Rearrangements in Hodgkin’s Disease

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ABSTRACT

We have examined tumor tissue DNA obtained from 32 cases of Hodgkin’s disease of the following subtypes: lymphocyte predominance, six; nodular sclerosing, eight; mixed cellularity, 14; lymphocyte depleted, 4; using immunoglobulin and T-cell receptor β and γ gene probes. Immunoglobulin heavy chain rearrangements were detected in five patients; in three of them only a minor clonal cell population was visible. T-cell receptor gene rearrangement was not observed in any patient examined. Three patients exhibiting minor clonal immunoglobulin gene rearrangements showed polyclonal T-cells in the same sample. There was no correlation between the presence and intensity of the rearranged bands and the number of Reed-Sternberg cells. Our data do not confirm recent reports of a frequent occurrence of immunoglobulin or T-cell receptor gene rearrangements in Hodgkin’s disease and suggest no possible relation between Reed-Sternberg cells and B- or T-lymphocytes, respectively.

INTRODUCTION

Unlike in the majority of acute leukemias and non-Hodgkin lymphomas, where the normal counterparts of each of the different types of malignant cells have been identified, the origin of RS cells remains an enigma. In the past, based on morphological, enzymatic, and antigenic properties, several hypotheses were proposed to explain the nature of RS cells. However, suggestions that RS cells may derive from B- or T-lymphocytes, monocytes, dendritic cells, or an as yet undefined cell population (1-4), have been left unresolved. Recently, developments in molecular biology have provided additional powerful tools for identifying clonal B- and T-cell populations by examination of immunoglobulin and TCR gene rearrangements, respectively (5, 6).

As a result of respective studies in HD, conflicting data emerged claiming monoclonal immunoglobulin (7, 8) and TCR (9) gene rearrangements at various frequencies. We report here our results of Southern blot analyses of tumor tissue DNA obtained from 32 cases of HD using immunoglobulin and TCR gene probes.

MATERIALS AND METHODS

All cases were classified histologically by one of us (T. B.) and by central examination (Institute of Pathology, University of Kiel, Kiel, Federal Republic of Germany) of conventionally processed paraffin-embedded tissue. The number of RS cells was estimated by counting these cells in frozen sections of the same tissue samples subjected to DNA analysis.

High molecular DNA was prepared from frozen lymph node tissue by standard techniques. Fifteen μg DNA were digested with appropriate restriction enzymes (Boehringer Mannheim), electrophoresed on a 0.7% agarose gel, blotted, and hybridized as described (10). To demonstrate immunoglobulin gene rearrangements, BamHI and HindIII digests were hybridized to a 1.3-kb EcoRI Cα probe which detects a 17-kb germline band in BamHI and a 10-kb germline band in HindIII digests (11) (Fig. 1A) and a C-k probes which detects a 12-kb germline band in BamHI digests (11) (Fig. 1B). In addition, EcoRI digests were hybridized to a combined Cα probe which consisted of a 8.0-kb BamHI-EcoRI fragment containing the Ca gene and a 1.2-kb BamHI-EcoRI fragment containing the Clα gene (11) (Fig. 1C). These probes were kindly provided by Dr. P. Leder. EcoRI and BamHI digests were hybridized to a TCR-β probe detecting 12- and 4.2-kb germline bands or a single 23-kb germline band, respectively (12) (Fig. 1D), and to a TCR-γ probe (1.0-kb PstI-EcoRI fragment) hybridizing to both Jα1 and Jα2 segments (12) (Fig. 1E). After hybridization the filters were washed under stringent conditions and exposed to XAR-5 film (Kodak, Rochester, NY) using Dupont Lightning-Plus intensifying screens for 12-48 h at −70°C.

RESULTS AND DISCUSSION

The implications of immunoglobulin and TCR gene configuration in tumor tissue DNA of HD depends on the accuracy of the histological diagnosis. Thus, careful pathological review was performed in all 32 HD patients of this study. Reexaminations were consistent with clinically and morphologically typical HD in every case. The content of RS cells was <5% in seven cases, between 5 and 15% in 16 cases, and between 15 and 30% in nine cases. The cases were subclassified as lymphocyte predominance, nodular sclerosing, mixed cellularity, and lymphocyte depleted in 6, 8, 14, and 4 instances, respectively. We investigated the configuration of immunoglobulin heavy and light chain as well as TCR-β and TCR-γ chain gene loci in each of these 32 cases of HD by Southern blot analyses of cryopreserved tumor tissue DNA. Immunoglobulin light chain genes were in germline configuration in all patients (not shown). Immunoglobulin heavy chain gene rearrangements were detected in five patients (Fig. 2) with varying intensity of the rearranged fragments. In all cases, only a single allele showed rearrangement and the rearranged bands in patients 3, 4, and 5 were considerably less intense than the germline bands. The very faint autoradiographic signal of the rearranged fragment in patient 5 is at the detection limit for rearranged bands in our laboratory but is visible in long-term exposures (not shown). Hybridization to HindIII digests did not reveal further patients exhibiting clonal immunoglobulin heavy chain rearrangements. Mixing experiments of germline and rearranged DNA have shown that the detection limit in our laboratory is at the level of 4% clonal cells in a sample (not shown). As can be seen in Table 1, there was no correlation between the proportion of RS cells in the biopsy specimen and intensity of rearranged fragments in the autoradiograms. In addition, our cases with barely detectable immunoglobulin heavy chain gene rearrangements displayed a variable decrease in the intensity of the 12-kb TCR-β germline band in EcoRI digests (Fig. 3A). In these patients, there was no evidence for Cα2 rearrangement as can be seen in HindIII digests (Fig. 3B). Thus, this pattern represents a marker of polyclonal T-cells as has been demonstrated previously by Flug et al. (13). Rearrangement of TCR-β or TCR-γ sequences was not observed in any patient examined. An example of germline TCR-γ sequences is given for the patients with HD of lymphocyte predominance (Fig. 4A) and lymphocyte depleted subtype (Fig. 4B). Similar results, namely minor clonal

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The abbreviations used are: RS, Reed-Sternberg; TCR, T-cell receptor; HD, Hodgkin’s disease; kb, kilobase.

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immunoglobulin gene rearrangements and germline configuration of TCR-β sequences, were reported in recent papers by others (7, 8, 14). However, differences do exist between the findings of these investigations and our own. Patients studied by Weiss et al. (7) were selected for high numbers of RS cells, and minor clonal B-cell populations were detectable only in the nodular sclerosing subtype. In contrast, our data do not indicate that immunoglobulin gene rearrangements in HD are restricted to cases of a particular histological subtype or to high RS cell content in the specimen. On the other hand, the data reported by Knowles et al. (14) differ from our results by the occurrence of polyclonal T-cells only in samples of RS-cell-rich HD, suggesting a polyclonal T-cell origin for RS cells. In this context, our results provide evidence that patients with less than 15% RS cells may entirely lack the 12-kb EcoRI TCR-β band (Fig. 3, lane 6), indicating that at least 80% of the cells in this specimen are polyclonal T-cells (14). However, in the absence of cell separation analyses, we cannot sufficiently exclude the possibility that RS cells in respective cases are a part of the polyclonal T-cell fraction.

Another issue requiring further investigation is the finding of minor clonal immunoglobulin rearrangements and polyclonal T-cells in the same sample of three of our patients. A possible explanation of our data is that these findings may reflect rare instances of composite HD/non-Hodgkin’s lymphoma in patients with HD, and polyclonal T-cells in these cases represent a cell population reactive to the B-cell neoplasm (15). This may explain why rare cases of HD can progress to a B-cell neoplasm (16). However, this interpretation was not supported by the

### Table 1 Summary of gene rearrangement studies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histological subtype</th>
<th>% RS cells</th>
<th>Immunoglobulin heavy chain genes</th>
<th>Immunoglobulin light chain genes</th>
<th>TCR-β gene</th>
<th>TCR-γ gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nodular sclerosing</td>
<td>&lt;5</td>
<td>R/G</td>
<td>G*</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>2</td>
<td>Lymphocyte predominance</td>
<td>5–15</td>
<td>R/G</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>3</td>
<td>Mixed cellularity</td>
<td>&lt;5</td>
<td>R/G</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>4</td>
<td>Nodular sclerosing</td>
<td>15–30</td>
<td>R/G</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>5</td>
<td>Lymphocyte depleted</td>
<td>15–30</td>
<td>R/G</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>6</td>
<td>Mixed cellularity</td>
<td>5–15</td>
<td>G</td>
<td>G</td>
<td>D/G</td>
<td>G</td>
</tr>
</tbody>
</table>

*G, germline band; R, rearranged band; D, deletion.
Fig. 3A. Configuration of TCR-β sequences in four HD patients. EcoR1 digests were electrophoresed, blotted, and hybridized to Cβ sequences that detect 12- and 4.2-kb germline bands. The diminished autoradiographic signal of the 4.2-kb band in patient 6 is due to the reduced amount of DNA (~7 μg) available for analysis. B, rearrangement pattern of TCR-β sequences in four HD patients with diminution of the 12.0 EcoR1 band when hybridized to HindIII digests. Only germline bands of 8, 6.5, and 3.5 kb were detected. N, placental control DNA.

histological features of the cases in our study, particularly in patients 1 and 2, exhibiting strong intensity of the rearranged fragments. Moreover, in previous studies in which the structure of immunoglobulin genes was determined, it was demonstrated that a small clonal population of B-cells could be present in lymph nodes containing mainly T-cells (17). Thus another explanation, although provocative, would be that our results reflect clonal but nonneoplastic subpopulations of cells within these tissues, and it will be of interest to see whether further work confirms or refutes this suggestion. Furthermore, our findings conflict with another study in which TCR-β gene rearrangements were detected in four of eight cases (9) since hybridization of BamHI, EcoRI, and HindIII digests in our series to TCR-β as well as TCR-γ gene sequences showed germline configurations in all patients. At present, we have no explanation for these conflicting results; however, careful pathological review should be performed in all cases in which monoclonal gene rearrangement is detected.

In summary, our work demonstrates that examination of immunoglobulin and TCR gene rearrangements in HD is insufficient to clarify the cellular origin and clonality of RS cells

Fig. 3B. Configuration of TCR-γ sequences in (A) six patients (a-f) with lymphocyte predominant and in (B) four patients (g-j) with lymphocyte depleted HD. EcoR1 digests were electrophoresed, blotted, and hybridized to a TCR-γ probe that detects 3.3- and 1.8-kb germline bands.

but suggests no possible relationship between RS cells and B- or T-lymphocytes, respectively.

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REFERENCES

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