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, four; 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.

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

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