Immunoglobulin Gene Rearrangements in Hairy Cell Leukemia

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Studies of hairy cell leukemia have yielded conflicting data about the cell of origin in this disease. To investigate this issue, we have examined the state of immunoglobulin genes in the cells of 11 randomly selected splenectomies showing histologic involvement with hairy cell leukemia. DNA was extracted from splenic tissue samples and digested with restriction endonucleases. Following agarose gel electrophoresis and transfer to nitrocellulose filters or activated nylon membranes, splenic DNA was hybridized with radio-labeled DNA fragment probes specific for the constant regions of the immunoglobulin heavy chain and kappa and lambda light chain genes. Autoradiograms of the hybridized DNA in each case revealed rearrangements of a heavy chain gene and at least one light chain gene. In addition, immunophenotyping of cellular immunoglobulin polypeptides was carried out on frozen tissue sections from all but one case. In each case in which an immunoglobulin polypeptide could be detected, a rearrangement was present in the DNA of the corresponding immunoglobulin gene. These studies offer strong evidence for endogenous immunoglobulin synthesis in hairy cells and for the B lymphocytic character of this leukemia.

A SIGNIFICANT AMOUNT of research in recent years has been directed at determining the cell of origin in hairy cell leukemia.1-3 Some of the evidence collected favors derivation from a monocytic or histiocytic progenitor. For example, in vitro hairy cells often show weak phagocytic activity, as assayed by ingestion of small particles or microorganisms.4,5 These cells also frequently possess tartrate-resistant acid phosphatase and receptors for the Fc portion of IgG.5,7 In addition, scanning electron microscopy reveals extensive ruffling of the cell surface,5 a feature occasionally seen with normal monocytes. Like monocytes, many hairy cells display surface antigens reactive with OKM1 antibody,8 although this antibody binds to certain myeloid cells as well as to monocytes.

In contrast, other evidence strongly suggests that hairy cell leukemia most often arises from B lymphocytes rather than from monocytes.9,10 This evidence comes primarily from studies that show surface immunoglobulin on cells obtained from hairy cell patients.8,10,11 However, these studies are frequently complicated by the propensity of hairy cells to bind, nonspecifically, antibody reagents used to characterize antigenic markers and/or to bind exogenous antibody in vivo. Several approaches have been adopted to circumvent these problems, including prewashing cells prior to incubation with antibody,5 attempts to detect antibody secretion from hairy cells in culture,4,9 and demonstration of surface immunoglobulin restricted to a single light chain type.4,5

In this article, we offer further evidence supporting a B cell derivation for hairy cell leukemia. We have studied the configuration of immunoglobulin genes in splenic hairy cells of 11 randomly selected cases of hairy cell leukemia. Each case shows rearrangements of the DNA for these genes, indicating the potential for synthesizing immunoglobulin polypeptides. Studies of cellular immunoglobulin were carried out on most cases, and a rearrangement was found for each immunoglobulin gene for which an immunoglobulin antigen could be detected by immunophenotyping of frozen tissue sections.

MATERIALS AND METHODS

The diagnosis of hairy cell leukemia was originally made in each case on the basis of microscopic examination of bone marrow biopsy specimens. Each patient subsequently underwent splenectomy for pancytopenia and/or splenomegaly, at which time the diagnosis of hairy cell leukemia was confirmed by microscopic examination of the resected spleen. Stains of splenic tissue for tartrate-resistant acid phosphatase were performed in each patient, except patient no. 11, and were uniformly positive.

Splenitic tissue samples were obtained at the time of splenectomy, frozen in air-tight plastic capsules by immersion in a dry ice-isopentane bath, and stored at -70°C for up to 5 years prior to use. DNA was purified from about 25 mg of wet tissue by methods described elsewhere.9

High molecular weight DNA from each case was digested with appropriate restriction enzymes and the products electrophoresed in a 0.8% agarose gel. DNA fragments separated by electrophoresis were transferred out of the gels onto nitrocellulose filters or activated nylon membranes (Plasco, Inc., Woburn, Mass), as described by Southern.13 Filters were hybridized with nick- or radio-labeled pBR322 plasmid DNA carrying human immunoglobulin gene DNA fragments. The precise locations of these fragments within chromosomal DNA in the region of the immunoglobulin genes are shown in Fig 1. The DNA fragment used as a hybridization probe for heavy chain gene rearrangements is specific for the joining, or J, region of the heavy chain gene and detects heavy chain gene rearrangements, regardless of the expressed heavy chain class. The Cµ and mixed Cλ light chain hybridization probes detect kappa and lambda light chain gene rearrangements, respectively. All plasmids...
containing human DNA were handled according to National Institutes of Health (NIH) guidelines for research involving recombinant DNA. Hybridization reactions were carried out under conditions described elsewhere, using 50% formamide and 4.8% dextran sulfate at 42°C. After extensive washing and drying of filters, autoradiography was performed against a sheet of x-ray film, using a single intensifying screen, for 12–72 hours at -70°C.

Analysis of surface and/or cytoplasmic immunoglobulin was carried out on frozen sections using methods and antibody reagents discussed previously. All cases were stained with monoclonal antibodies reactive with kappa, lambda, mu, delta, gamma, and alpha chains (Becton Dickinson, Palo Alto, Calif). In addition, cases lacking an immunoglobulin light chain and/or heavy chain were stained with goat antisera to human antibody, and in some cases, F(ab')2 antibody fragments, reactive with kappa, lambda, or mu chains (Tago, Inc, Burlingame, Calif).

RESULTS

To determine the configuration of immunoglobulin genes within leukemic hairy cells, DNA was extracted from each of 11 frozen leukemic spleen specimens, cut with the appropriate restriction enzyme, and subjected to the Southern blot hybridization protocol. Figure 2 shows the results obtained when splenic DNA from the 11 hairy cell leukemia patients was analyzed for heavy chain immunoglobulin gene rearrangements using a DNA hybridization probe specific for the joining region \( J_H \) of the heavy chain locus. In each lane, the band at 17 kilobases (kb) represents the so-called "germline," or unrearranged immunoglobulin locus. Any bands located in a position other than that of the 17-kb band represent clonally rearranged heavy chain immunoglobulin alleles (denoted by arrows in Fig 2). As summarized in Table 1, at least one rearranged allele was detected in all cases. In several cases, the presence of two rearrangements is consistent with the interpretation that both heavy chain alleles have undergone rearrangement. The presence of germline bands of variable intensity in all of the cases indicates that the tissue specimens contained unrearranged immunoglobulin alleles in addition to the rearranged allele(s). The germline alleles are contributed either by a single unrearranged allele in the tumor cells and/or by allelic loci in contaminating nonleukemic splenic...
Ig DNA rearrangements in hairy cells

Table 1. Summary of Immunoglobulin Gene Rearrangements and Immunoglobulin Antigens in Cases of Hairy Cell Leukemia

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Ig Genes (Heavy Chains, Light Chains)</th>
<th>Heavy Chain Locus</th>
<th>x Locus</th>
<th>λ Locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>μ, δ, λ</td>
<td>R (G)</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>μ, δ, λ</td>
<td>G</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>μ, δ, λ</td>
<td>R (G)</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>μ, δ, λ</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>—, x</td>
<td>R</td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>6</td>
<td>μ, δ, λ</td>
<td>R (G)</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>—, x</td>
<td>R</td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>8</td>
<td>μ, δ, λ</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>9</td>
<td>μ, δ, λ</td>
<td>R</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>—, —</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>11</td>
<td>NA</td>
<td>R</td>
<td>R</td>
<td>G</td>
</tr>
</tbody>
</table>

R, rearranged Ig gene for at least one allele; G, only germline configuration detected; (G), germline configuration but band intensity was light.

NA, not available.

*Analysis of immunoglobulin antigens in this case was somewhat ambiguous. Although a clear majority of cells stained unequivocally for μ and δ heavy chains, a much smaller portion showed lambda (about 14%) and kappa (about 4%) light chains.

Each specimen contained a significant clonal population of B lymphocytes, as indicated by the presence of rearranged immunoglobulin alleles. A possible, but unlikely, alternative interpretation is that presumed rearranged bands actually resulted from inherited mutations in the immunoglobulin gene DNA. Nonlymphoid control tissues uninvolved by hairy cell leukemia, which could have been used to test this possibility, were not available for these patients due to the retrospective nature of this study. However, as described in earlier studies, inherited polymorphisms of the germline heavy chain gene analyzed with the EcoRI restriction enzyme appear to be very rare in the general population, and none have been observed by us or others in a large series of patients. Normal splenic tissue from patients other than those in the present study have shown no detectable heavy chain bands in Southern blot autoradiograms except for the unarranged germline bands (M.L.C. and J.S., unpublished observations).

DNAs from all hairy cell leukemic spleens were also analyzed for light chain immunoglobulin gene rearrangements. All specimens showed one or more light chain gene rearrangements. The finding of rearranged light chain bands in all cases in addition to rearranged heavy chain bands further argues against the possible misinterpretation of DNA polymorphisms as rearranged immunoglobulin genes. DNA hybridized with a kappa light chain-specific probe after BamHI digestion revealed clonal rearrangement of kappa alleles in six of the 11 patients, as in each of these patients, bands were found in positions other than that of the germline band at 12 kb (Fig 3A). The presence of two rearranged bands in several specimens is again consistent with rearrangement of both light chain gene alleles. Eight cases showed rearranged lambda light chain bands when analyzed with the combined C, constant region probe (Fig 3B). Three of these patients (patients no. 4, 8, and 10) contained rearrangements of at least one allele for both the kappa and lambda light chain loci. However, lambda gene rearrangements were found in five patients (patients no. 1, 2, 3, 6, and 9) in the absence of evidence for kappa gene rearrangements. These results conflict with the proposed hierarchy of immunoglobulin gene rearrangement, whereby lambda gene rearrangements in developing B lymphocytes occur only after defective, nonproductive rearrangements of both kappa alleles. For three of these patients (nos. 1, 3, and 6), the data support a possible explanation for this apparent conflict. In these three cases, the kappa bands in the autoradiograms were in the germline position, but were considerably less intense than the kappa germline bands detected from blots of comparable amounts of DNA prepared from normal nonlymphoid human tissue. This finding suggests that both kappa genes in the leukemic cells may have been deleted, a frequent result of defective kappa light chain gene rearrangement. Therefore, the less intense kappa germline bands found in splenic tissues from these patients are probably derived from contaminating nonleukemic splenic tissues (ie, blood vessels, fibrous tissue, and nonleukemic blood cells), although retention of a single germline kappa allele in the leukemia cells cannot be ruled out.

Analyses for cellular immunoglobulins (summarized in Table 1) demonstrated heavy chain polypeptides associated with the cells of only seven of the ten patients examined, despite rearrangements of the heavy chain locus in all ten patients. Each of the seven patients with heavy chain markers concurrently expressed both μ and δ polypeptides. No other heavy chain classes were detected with either monoclonal antibodies or polyvalent antisera.

Analyses for light chains (Table 1) showed that each of seven patients whose samples stained for lambda light chains also contained a rearranged lambda immunoglobulin gene. In addition, two samples that stained for kappa contained a kappa gene rearrangement in the absence of lambda gene rearrangements. Patient no. 10 was the sole example of a leukemia containing no detectable immunoglobulin polypeptides, despite the presence of rearrangements in the heavy chain and both light chain loci.
Fig 3. Autoradiograms obtained from DNA of hairy cell leukemia spleens hybridized with light chain immunoglobulin-specific DNA probes. (A) Specimens were hybridized with radiolabeled kappa-specific probes and (B) with lambda-specific probes. Analyses for light chain gene rearrangements were performed on DNAs digested with either the BamHI (A) or EcoRI (B) restriction enzyme. The patient numbers are the same as those in Table 1. Dashes indicate germ-line bands of 12 kb for the kappa-specific probe (A) and 8, 16, and 18 kb for the lambda-specific probe (B). Cases 1, 2, and 4 also show a 23-kb polymorphic germ-line band, as has been described previously. Arrows indicate bands corresponding to clonal rearrangements of light chain immunoglobulin genes.

DISCUSSION

In germline cells, the variable and constant domains of each type of immunoglobulin polypeptide chain are encoded as separate gene segments in DNA distantly linked within the respective chromosomal loci of these polypeptides. Prior to immunoglobulin synthesis, these gene segments within individual B cells must undergo a series of somatic recombination events. These events bring a variable region gene segment into close proximity with a constant region segment, resulting in the formation of a transcriptionally active immunoglobulin gene. The altered configuration of immunoglobulin gene segments with respect to that of germline cells provides a molecular marker for B cell development, as rearrangement of immunoglobulin gene segments appears to be virtually restricted to cells of B lymphocytic lineage. Therefore, our detection of immunoglobulin gene rearrangements in 11 spleens of patients with hairy cell leukemia is consistent with the B lymphocytic character of the leukemia in these cases. Recently and independently, Korsmeyer et al have reached a similar conclusion in studies comparable to our own. The potential for monocytes and histiocytes to undergo immunoglobulin gene rearrangement has not been extensively examined, and is of special relevance to the controversy over the cell of origin in hairy cell leukemia. However, no rearrangements have been found in single cases of either monocytic leukemia or malignant histiocytosis, nor have rearrangements been detected in HL-60, a promyelocytic leukemia cultured cell line inducible for monocytic differentiation (authors' unpublished observations).

Rare T cell–derived hairy cell leukemia has been reported. In previous studies, we have been unable to detect immunoglobulin gene rearrangements in biopsy specimens of T cell lymphomas. This result contrasts with studies on a small number of mouse T cell tissue culture lines in which occasional heavy chain rearrangements were found in the absence of light chain rearrangements. Korsmeyer et al have described a single TALL-derived cell line that contained a non-productive heavy chain gene rearrangement but retained the germline configuration of kappa and lambda genes. These reports suggest that rare heavy chain gene alterations may occur in T cell tumors,
although light chain gene rearrangements have never been found either in T cell tumors or in cultured T cell lines. In any event, besides demonstrating immunoglobulin gene rearrangements, the patients in the present study fail to show T cell surface markers (Leu-1-7 and 9 antigens) by frozen section immunophenotyping (authors' unpublished observations). We conclude that none of our cases falls into the T cell subset of hairy cell leukemia.

Additional evidence for B cell derivation of hairy cell leukemia is provided in the present study by the correlation of immunoglobulin gene rearrangements and expression of immunoglobulin antigens within tissues of individual patients. A rearrangement of the corresponding gene could be found for each immunoglobulin antigen in all patients. In patients showing immunoglobulin light chains, these polypeptides were restricted to a single light chain type. Spleenic tissue from each of these cases contained rearrangements of DNA for the same light chain type, strongly suggesting that the rearranged light chain genes in these patients were synthetically active and that the detected immunoglobulin was endogenously derived.

In contrast, rearrangements were noted in several instances where no corresponding cellular immunoglobulin was found (Table 1, patients no. 5, 7, and 10). The findings in patient no. 10 can be explained by some type of postrearrangement block in immunoglobulin gene expression, as has been seen in numerous examples of acute lymphocytic leukemia and so-called null cell lymphoma. Another possibility is that immunoglobulin polypeptides were expressed in amounts insufficient to be detected by our reagents and methods. The presence of light chains, but absence of heavy chain antigens, in patients no. 5 and 7 is unexpected, although failure to detect heavy chain antigens could conceivably be due to synthesis of an abnormal heavy chain polypeptide or to the idiosyncratic failure of some antibody reagents against heavy chains to detect the immunoglobulin heavy chains of certain tumors.

In general, the cases analyzed appear to conform to the proposed hierarchy of immunoglobulin gene rearrangements, such that heavy-chain rearrangement precedes light-chain rearrangement, and lambda gene rearrangements only occur after both kappa alleles have undergone aberrant nonproductive rearrangements or deletions. Superficially, patients no. 2 and 9 appear to be exceptions to this rule. Repeated Southern blots of DNA from these two patients provided no evidence of rearranged kappa bands; however, the possibility that both kappa alleles in these patients have been deleted was excluded only by subjective assessment of the relative intensity of the kappa germ line band, and this cannot be taken as a strong argument against deletions of the kappa loci. For both patients no. 2 and 9, the percentage of leukemic cells in the specimens, as judged both histologically and by the intensity of the rearranged lambda bands, was low. Therefore, if both leukemic kappa alleles were deleted, the decrease in intensity of the kappa germline band might be difficult to discern because of the presence in the samples of a relatively large proportion of nonleukemic contaminating cells.

In addition to indicating the B cell derivation of hairy cell leukemia, the detection of immunoglobulin gene rearrangements by the Southern blot hybridization technique also supports the clonal nature of this neoplasm. This determination is based on the fact that the Southern blot hybridization technique detects rearrangements only of clonal lymphocyte populations. Normal, reactive, or hyperplastic lymphoid tissues contain innumerable, but nonidentical, immunoglobulin gene rearrangements. As shown previously, none of these rearrangements is sufficiently abundant to be detected by the Southern blot hybridization technique.

Several authors have attempted to place the hairy cell at various points on the developmental pathway of B lymphocytes and thereby possibly account for the unusual phenotype of these cells. B lymphocyte development in those studies was defined in terms of acquisition of B cell surface antigens. The hierarchy of immunoglobulin gene rearrangements also constitutes a developmental pathway in which steps are denoted by successive rearrangements in heavy and light chain loci. Based on the patterns of immunoglobulin gene rearrangement observed in the present study, hairy cells represent a mature stage of B cell development (ie, cells containing rearrangements of both heavy and light chain genes). Earlier stages of B cell development, signified by total absence of immunoglobulin gene rearrangements and by rearrangements only of heavy chain loci without light chain rearrangements, were not observed for the 11 patients in this study.

The results described in this study agree with the impression of other authors that hairy cells frequently show features of B lymphocytes. We have demonstrated that immunoglobulin gene rearrangements occur commonly in hairy cells, indicating the genetic capacity for immunoglobulin synthesis, and that immunoglobulin polypeptides consistent with the expression of these rearranged genes can be found on or in hairy cells when careful immunophenotyping of frozen tissue sections is performed. None of our observations address the question of why hairy cells may concurrently show other features suggesting monocytic differentiation. Based on our results, it is clear, at least in the patients analyzed by us, that hairy cells are not
simply monocytes. We can only propose, as have others, that although they closely resemble B lymphocytes, hairy cells may be phenotypic hybrids with some monocytic characteristics and, as such, have no known normal counterpart among cells of the hematopoietic system.

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REFERENCES

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