We describe a patient in whom two lymph node biopsies removed 18 months apart disclosed histologic and immunophenotypic evidence of a non-Hodgkin's lymphoma containing neoplastic lymphocytes of both B and T type. Analyses of immunoglobulin and T cell receptor genes confirmed the presence of separate B and T cell clones. In addition, immunogenotyping revealed the possibility of a second B cell clone within the patient's tumor. Development of a multiclonal lymphoma in this patient may relate to the carcinogenic effects of chemotherapy or to a predisposition for neoplastic transformation of lymphocytes due to a previously diagnosed autoimmune condition. Another possible explanation is that the lymphoma implies the existence in this patient of a transformed lymphocyte-committed stem cell that is capable of generating both B and T lineage clones.

M ost neoplasms are believed to consist of a single clone of neoplastic cells. A possible exception to this rule has recently been described among non-Hodgkin's lymphomas. Within a subset of these neoplasms, two proliferating clones of neoplastic B lymphocytes have been found in individual patients. Distinction between the clones in these cases was based on differences detected in the configurations of DNA in rearranged immunoglobulin genes. The results of immunoglobulin DNA analysis did not preclude a common origin of both clones from a single progenitor cell in which the immunoglobulin genes had not yet undergone DNA rearrangement, however. Furthermore, the interpretation of biclonality among B cell lymphomas has been complicated by the discovery that rearranged immunoglobulin genes in some B cell tumors can undergo somatic mutation. Occasional mutations may alter the position of bands in Southern blot autoradiograms, thereby simulating differences in gene rearrangements among individual cells from a monoclonal tumor.

The lymphomas so far presented as candidates for biclonality have included only neoplasms composed exclusively of B cells. We report here a case of non-Hodgkin's lymphoma in which separate clones of B and T lymphocytes were detected using a combination of immunogenotyping and immunophenotyping techniques. Because clones of both B and T lineage were present in this tumor, deductions about multiclonality in this case are not affected by somatic mutation of rearranged genes, a process limited to immunoglobulin genes of B cells. In addition, in different biopsy specimens from this case, two different B cell populations were identified in which no shared rearrangements of immunoglobulin genes could be found. Therefore, this tumor may contain at a minimum two, and possibly as many as three, apparently distinct clonal lines of lymphocytes, which may or may not be related through a common precursor.

MATERIALS AND METHODS

Case report. The patient is a 63-year-old white woman who presented in 1981 with symptoms of diffuse myalgias, especially in her arms and legs, associated with peripheral eosinophilia. Subsequently, she developed a diffuse erythematous rash with induration and thickening of the skin, predominantly on the trunk and extremities. A biopsy of the skin and soft tissues of the left thigh was performed, and the histologic diagnosis of eosinophilic fasciitis was made. The patient was treated with systemic steroids, resulting in resolution of adenopathy and pulmonary nodules.

In June 1983, the patient developed diffuse peripheral lymphadenopathy and was first seen at the Stanford University Medical Center. Biopsy tissue from an enlarged left axillary lymph node was diagnosed as containing diffuse large noncleaved cell lymphoma (DLCL). Staging workup revealed a normal bone marrow biopsy, but the bipedal lympangiogram demonstrated extensive involvement of retroperitoneal lymph nodes, and a chest x-ray film showed bilateral nodular lesions consistent with lymphoma. The patient was classified as a stage IVA DLCL and treated from June to November 1983 with six cycles of CHOP (cyclophosphamide, Adriamycin, vincristine, prednisone) chemotherapy, followed by the complete resolution of adenopathy and pulmonary nodules.

Soon after cessation of chemotherapy, the patient's initial skin lesions reappeared but responded to treatment with steroids combined briefly with small doses of cyclophosphamide. Late in 1984, she developed recurrent peripheral adenopathy. Biopsy of an enlarged left axillary lymph node in January 1985 revealed diffuse, mixed small and large cell lymphoma. Repeat staging indicated that her disease was confined to lymph nodes. Treatment was begun with BACOP (bleomycin, Adriamycin, cyclophosphamide, oncovin, prednisone), producing a continued gradual regression of the patient's disease up to the present time.

Immunophenotype and immunogenotype studies. Biopsy tissues collected from the operating room were snap frozen in air-tight plastic capsules by immersion in a dry ice/isopentane bath and stored at −70°C. All tissues studied were obtained with the informed consent of the patient, in accord with institutional guidelines. Techniques for frozen-section immunoperoxidase studies have been described in detail elsewhere. Staining of frozen sections was performed using monoclonal antibodies directed against μ-, κ, and λ immunoglobulin light chains (Becton Dickinson, Mountain View, CA); Leu 1 through 5 (CD 20) (Coulter Clone, Hialeah, FL); 4G7 (CD 19) (Dr Ronald Levy, Stanford University); and TO15 (Dr David Mason, Oxford University).

DNA was extracted from tissue specimens and purified by standard procedures. Ten micrograms of DNA was digested with an appropriate restriction enzyme (New England BioLabs, Beverly, Mass.).

From the Departments of Pathology and Medicine, Stanford University, CA.

Submitted January 12, 1987; accepted March 14, 1987.

Supported in part by Grants No. CA34233 and CA38621 from the National Institutes of Health, Bethesda, MD.

Address reprint requests to Dr Jeffrey Sklar, Department of Pathology, Stanford University Medical Center, Stanford, CA 94305.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1987 by Grune & Stratton, Inc.

@0006-4971/87/7001-0046$3.00/0

Fig 1. Analysis of immunoglobulin and T cell receptor gene DNA. Autoradiograms resulting from analyses for rearrangements of immunoglobulin heavy chain genes (A) and T cell receptor genes (B). The hybridization probes used are indicated above each panel. Lane 1 shows analyses of DNA from the initial (1983) biopsy specimen; lanes 2a and 2b show analyses of DNA from the two parts of the second (1985) biopsy specimen. Dashes indicate unrearranged germline bands; size of the corresponding fragments is given in kilobases. Arrows indicate clonal, rearranged bands. The lanes in each panel were assembled from different analyses. Within a panel, the analyses were made under similar conditions (including length of electrophoresis) and were aligned using marker bands run in parallel for each analysis, but not shown. Letters below each panel represent the restriction enzymes used in the analyses: H, HindIII; B, BamHI, S, SacI, and E, EcoRI. The analysis in the middle panel of B is identical to that in the panel to the left, except that electrophoresis was allowed to proceed for about four times as long.
MA) and the resulting fragments were analyzed by the Southern blot hybridization procedure. DNA probe fragments were radiolabeled with α-[32P]-dCTP by the random hexamer priming method. Genomic DNA fragments used for analysis were specific for the heavy chain joining region, the constant and joining regions of the κ light chain gene, the constant region of the λ immunoglobulin light chain gene, the constant region for the β T cell receptor gene, and the joining region of γ T cell receptor gene. The structures of each of these probes has been described in detail previously, as have been methods for hybridization and autoradiography.

RESULTS

Analysis of this patient’s neoplasm was based on tissue obtained from two lymph node biopsies—initially the diagnostic biopsy performed in June 1983 and the biopsy performed at relapse in January 1985. Histologic review of the lymph node biopsy specimen from 1983 showed complete effacement of the normal lymph node architecture by a polymorphous population of atypical small and large lymphoid cells. Large cells with vesicular noncleaved nuclei predominated in most areas examined, leading to the classification of the tumor as an intermediate grade, diffuse large nonecleaved cell lymphoma according to the International Working Formulation for classification of non-Hodgkin’s lymphoma.

Immunoperoxidase studies on fresh-frozen tissues from this lymph node demonstrated that the large atypical cells expressed the B lineage antigens TO15, B1 (CD 20), and 4G7 (CD 19); however, staining for immunoglobulin light chains or μ heavy chain was absent. Similarly, the T cell antigens Leu 1 through 5 (CD 2-5, 8) and 9 (CD 7) could not be detected on these cells. In contrast, many of the smaller lymphocytes stained for normal patterns of T cell antigens. The immunologic findings were interpreted as consistent with a B lineage lymphoma lacking immunoglobulin expression, surrounded by a polyclonal T cell host response.

The B cell lineage of the neoplasm in this biopsy specimen was confirmed by analysis of immunoglobulin gene rearrangements (Fig 1, lane 1). Two clonal rearranged bands were detected in the analysis of the heavy chain gene DNA with one enzyme (HindIII), and a single rearranged band with a second enzyme (BanHI). One clonal rearrangement was found in an analysis of the λ light chain gene DNA. Analysis of DNA for the β and γ T cell receptor demonstrated bands only in the position of unrearranged germline DNA. The results obtained with this tissue specimen are consistent with a monoclonal B lineage lymphoma, in which both heavy chain alleles were probably rearranged and one of the two λ light chain alleles was rearranged.

The 1985 lymph node biopsy specimen showed different histologic features from those noted in the previous biopsy specimen. The tumor population again consisted of a polymorphous population of atypical small and large lymphoid cells, with a tendency for the larger cells to be found clustered around vessels (Fig 2). Because the number of small and large cells in this specimen appeared to be more even, the tumor was classified as a diffuse, mixed small and large cell lymphoma.

Immunoperoxidase studies on the 1985 lymph node suggested two distinct subpopulations (Fig 3). Cells in some areas within the sections from this node, particularly in those areas adjacent to vessels, showed expression of the B lineage antigens TO15, B1 (CD 20), and 4G7 (CD 19), but did not express T cell antigens. This cell population failed to bind anti-λ, κ, or μ immunoglobulin antibodies. Cells in other areas of the sections expressed the T cell antigens Leu 1, 3, 4, and 5 (CD 2-5). Only scattered cells expressed Leu 2 (CD 8). Staining for Leu 9 (CD 7) on these cells was variable, from absent to moderate intensity on different cells. The immunologic findings in this lymph node biopsy specimen were interpreted as suggesting both a B cell lymphoma, based on the presence of immunoglobulin-negative B lineage cells, and a T cell lymphoma, based on the monotypic helper phenotype with abnormal, inconsistent staining for Leu 9 (CD 7).

Lymph node tissue from the 1985 biopsy had been divided into two parts (a and b) immediately after removal from the patient; tissue fragments representing the two parts of this specimen were separately analyzed for gene rearrangements (Fig 1, lanes 2a and b). Separate immunophenotypic studies were not available on these two portions of the 1985 biopsy. No clonal rearranged bands were detected in analyses of any immunoglobulin genes prepared with DNA extracted from tissue of part a. Analysis of the T cell receptor gene demonstrated two closely migrating bands, however, one in the germline position and a second band slightly below the germline band. Analysis of Ig T cell receptor genes in this tissue showed two rearranged bands, in addition to unarranged, germline bands. Therefore, these data indicate a clonal population of T cells within this part of the specimen.

Examination of DNA from tissue of part b of the 1985 biopsy specimen produced different results. This tissue showed two clonal rearrangements in analyses of the immunoglobulin heavy chain gene with one enzyme (HindIII), and no detectable rearrangements with the other (BanHI). A
single clonal rearrangement was found for the \( \lambda \) light chain gene. Analyses of the \( \beta \) and \( \gamma \) T cell receptor genes produced results similar to those seen for part a. These findings imply that the T cell clone in part b was admixed with a B cell clone in part b.

None of the rearranged immunoglobulin gene bands detected in part b of the 1985 tissue matched those found in the earlier biopsy specimen, suggesting the emergence of a second clonal population of B cells in the patient. As a further test of the non-identity of the B cell populations, we investigated the configurations of DNA in the \( \kappa \) immunoglobulin locus of these two tissues using a hybridization probe for the \( \kappa \) gene joining region.\(^{10}\) Although the \( \kappa \) constant regions in the clonal population of both specimens had apparently been deleted, the \( \kappa \) joining regions were retained and produced bands in different positions between the two tissues, as detected in analyses with \( Sacl, BamHI, \) and \( HindIII \) enzymes (the latter two analyses not shown).

**DISCUSSION**

The histology and immunophenotyping of the two lymph node tissue samples in this case support the diagnosis of a non-Hodgkin's lymphoma containing B and T cell clones. DNA analyses confirmed the presence of separate clonal B and T cell populations in the second biopsy specimen. In addition, a possible distinction between the B cell clone in the first biopsy sample and the B cell clone in the later biopsy sample was demonstrated by analysis of DNA.

Past reports have described coexistent rearrangements of T cell receptor genes and immunoglobulin genes in the same cells within some lymphoid neoplasms.\(^{15-19}\) This cannot be the situation in the present case. Clonal rearrangements of the T cell receptor genes and immunoglobulin genes were both found in part b of the 1985 biopsy. Part a, however, contained only T cell receptor gene rearrangements in the absence of immunoglobulin gene rearrangements. The conclusion that T cell receptor and immunoglobulin gene rearrangements occurred in different cells is consistent with the immunophenotypic data, which showed separate B and T lineage populations among the histologically atypical cells of tissue sections.

On first appraisal, failure to detect any common immunoglobulin gene rearrangements between the two clonal B cell populations in this patient suggests that the two populations are unrelated or at least arose from a common precursor which lacked rearranged genes. As described previously, however, postrearrangement somatic mutations in immunoglobulin DNA may change the position of bands derived from a single recombination of immunoglobulin gene segments. Such changes may be extensive, affecting several different genes within a single monoclonal tumor (M.L. Cleary and J. Sklar, unpublished observations). In this regard, the immunophenotyping and DNA studies demonstrated noteworthy similarities among the two B cell populations in this patient. Both clones failed to express detectable levels of cellular immunoglobulins, a feature associated with \( \sim 15\% \) of all large cell lymphomas. In addition, both populations showed rearrangements of lambda light chain genes, an occurrence in less than one-third of monoclonal B cell tumors.

On the other hand, extensive somatic mutation of immunoglobulin genes has so far been documented only in follicular B cell lymphomas, not in large cell lymphomas, such as in this patient. Moreover, differences in detectable rearrangements in this case extend even as far as the nonfunctional \( \kappa \)-genes that have undergone partial deletion. At present, it is not known if such nonfunctional genes are subject to somatic mutation, but whether or not these two apparently different B cell clones are actually mutational variants of the same
LympHoma containing B and T cell clones

A clone does not detract from the conclusion that a separate T cell clone was present within this patient's tumor. Although the results of DNA analyses offer strong evidence for at least two different clones of lymphocytes in this patient's neoplasm, they do not specify the neoplastic nature of these clones. Could only one of the clones actually be neoplastic while the others represent immunologic reactions against the neoplastic clone? This seems unlikely for at least two reasons. First, detectable clonal proliferations of lymphocytes seem to be unusual outside of malignancy and have not previously been associated with immunological responses. Second, the immunophenotype of each clone was abnormal: Both B cell clones lacked immunoglobulin expression, and the T cell clone showed an anomalous, monocytic pattern of T cell antigens commonly found in peripheral T cell lymphomas. In addition, the absence of detectable surface or cytoplasmic immunoglobulin from the B cell clones argues against their role in a normal immune response.

Several mechanisms could explain the occurrence of multiple clones of lymphocytes within this patient's tumor. One obvious possibility is that it is the result of a very rare coincidence in which two or three lymphocytes were independently transformed to malignancy more or less simultaneously. An interesting feature of this case, however, is that the patient initially had the rare autoimmune skin disorder, eosinophilic fasciitis. Lymphomas appear with greater than average frequency among patients with certain other autoimmune disorders, such as celiac disease, Sjögren's disease, and chronic lymphocytic thyroiditis. In addition, one patient with diffuse fasciitis has been described in whom a lymphoproliferative disorder was diagnosed at autopsy. Therefore, one or more of the neoplastic lymphocytic clones detected within our patient may have developed as a result of a high rate of lymphocytic transformation due to autoimmunity.

Another possible cause for multiple clones in this patient's lymphoma is the chemotherapy she received after the diagnosis of her first neoplastic B cell clone. Most second neoplasms arising after chemotherapy are acute nonlymphocytic leukemias, although non-Hodgkin's lymphomas are also overrepresented among second tumors. The greatest increase in relative risk appears to occur among patients treated by combined radiation and chemotherapy. The interval from therapy to detection of the second cancer averages between 3 and 10 years. Given these facts, the chance that our patient's therapy for her initial clone could have induced either or both of the other two clones does not seem great since she received neither radiation nor particularly intensive chemotherapy at any time. Moreover, the interval of ~18 months from the beginning of chemotherapy to diagnosis of the second and third clones was shorter than that in most cases of posttherapy second tumors.

Any speculation about chemotherapy-induced tumors in this patient must take into consideration that there are no published studies of second neoplasms in non-Hodgkin's lymphoma patients who have received the same CHOP regimen administered to the patient in this study. Furthermore, the ability to distinguish different clones of cells among neoplastic lymphocytes is a relatively recent development. Consequently, in the past, additional clones of neoplastic lymphocytes induced by therapy for non-Hodgkin's lymphoma would probably have been regarded as a relapse attributable to reemergence of the original clone. Therefore, induction of additional clones of neoplastic lymphocytes by chemotherapy may be more common in non-Hodgkin's lymphoma than is currently recognized.

A final possible mechanism to account for this patient's lymphoma is derivation from a single transformed early lymphocytic precursor. Lymphocyte-committed stem cells capable of generating both B and T cells are presumed to exist, although the evidence accumulated to date for this lymphocyte precursor is indirect. Among the best indications for the existence of such a cell is the deficiency of both B and T cells in patients with severe combined immune deficiency. More recently, the discovery of certain lymphocytic tumors containing a clone of cells that possess both immunoglobulin and T cell receptor gene rearrangements further supports existence of a lymphocytic stem cell. The possibility that the B and T cell clones in the present case of lymphoma arose from a single transformed cell may provide additional indirect evidence for a stem cell with pluripotential capacity for differentiation along both B and T cell lines.

ACKNOWLEDGMENT

We are indebted to Kathleen Shaw for assistance in preparation of the manuscript and to Richard Coffin and Pamílfo Verzola for photography. We thank Dr Saul A. Rosenberg for his advice and comments on the manuscript.

REFERENCES


Non-Hodgkin's lymphoma containing both B and T cell clones

E Hu, LM Weiss, R Warnke and J Sklar