Chromosome Abnormalities in AIDS-Associated Lymphadenopathy

By M. Lita Alonso, Mark E. Richardson, Craig E. Metroka, Janet A. Mouradian, Prasad R.K. Koduru, Daniel A. Filippa, and R.S.K. Chaganti

Cytogenetic studies were performed on direct and 24-hour culture preparations of eight lymph node biopsies from seven patients with acquired immuno deficiency syndrome (AIDS) or AIDS-related complex (ARC)-associated lymphadenopathy in whom histological evidence of lymphoma was not detected. Three of these seven had chromosomal abnormalities, including chromosome instability in one and clonal chromosomal abnormalities in two; one of the latter was a t(8;14)(q24;q32). The remaining five showed normal karyotypes. Epstein-Barr virus (EBV) titers were elevated in all seven patients that exhibited chromosome abnormalities, two of whom later developed malignant lymphoma. A control group of five patients with reactive lymphadenopathy not associated with AIDS failed to reveal chromosomal aberrations, but elevated EBV titers were present in two. These data are consistent with current views on the role of EBV and chromosome change in the development of lymphoma in immunodeficient states and suggest that karyotypically abnormal AIDS-related lymphadenopathy represents a prelymphomatous proliferation.

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THE ACQUIRED immunodeficiency syndrome (AIDS) comprises a group of clinical presentations that have recently emerged as an epidemic among subgroups of homosexual males, intravenous (IV) drug users, blood transfusion recipients, and others. Pneumocystis carinii, cytomegalovirus (CMV), and other opportunistic infections, autoimmune phenomena, generalized lymphadenopathy, lymphoma, and Kaposi's sarcoma (KS) have characterized this disorder. A prodromal phase consisting of fever, weight loss, and lymphadenopathy frequently precedes opportunistic infections. Lymphopenia, cutaneous anergy, decreased in vitro responsiveness to mitogens, decreased T helper cells, and inversion of the T helper/T suppressor ratio are common immunological abnormalities in AIDS. The human T cell lymphotropic virus (HTLV-III) has recently been shown to play an etiologic role in AIDS. Currently, patients in high-risk groups for AIDS with generalized lymphadenopathy but lacking evidence of KS or opportunistic infection are classified as having the AIDS-related complex (ARC). The types of neoplasms seen in this disorder are similar to those seen in posttransplantation immunosuppressed patients and include a Burkitt's-like undifferentiated lymphoma with characteristic rearrangements involving chromosomes 8, 14, and 22 and a probable Epstein-Barr virus (EBV)-related etiology. Although current evidence suggests that the lymphomas in immunosuppressed states arise among polyclonal lymphoid proliferations by selective expansion of monoclonal cells characterized by specific chromosome rearrangements, the cytogenetic nature of the prelymphomatous proliferations is essentially unknown. To address this question, we performed cytogenetic analysis of lymph node biopsies from seven patients with generalized lymphadenopathy arising in a clinical spectrum of AIDS (two cases) or ARC (five cases). For comparison, we also studied a control group of five patients with reactive lymphadenopathy not associated with AIDS or ARC. Our results show that chromosome instability and clonal changes characterize only AIDS or ARC-associated proliferations.

MATERIALS AND METHODS

All patients had lymphadenopathy, which was generalized in the seven patients with AIDS or ARC (group I, Table 1) and localized in five who did have AIDS or ARC (group II). Lymph node histology and laboratory data of all patients studied are summarized in Table 1. All seven group I patients had evidence of an immune dysfunction based on markedly depressed lymphoproliferative responses to a variety of mitogens and antigens. All group I cases were in high-risk groups for AIDS. Those classified as having AIDS had KS or an opportunistic infection, and those classified as having ARC did not. Chromosome preparations were made from single-cell suspensions of freshly obtained lymph node biopsies. The cells were incubated 1 to 3 hours in colcemid (final concentration of 0.1 μg/mL) or were cultured for 24 hours in RPMI 1640 medium supplemented with 20% fetal bovine serum (FBS) and the antibiotic penicillin-streptomycin. Colcemid was added to the cultures during the final hour. Cell harvest and chromosome preparation were made following conventional methods using 0.075 mol/L of potassium chloride as the hypotonic solution and methanol/acetic acid 3:1 as the fixative. Karyotyping was performed from trypsin-Giemsa or quinacrine-banded preparations. All histologic specimens were reviewed, and lymphomas were classified using the Working Formulation.

RESULTS

Of the 13 nodes studied, three showed chromosomal abnormalities [patients 1 (first biopsy), 3, and 6–group I, Table 1]. Biopsies from patients 1 and 3 exhibited mixtures of normal and clonally abnormal cells. In biopsy 1 of patient 1, of the 30 cells analyzed, 27 were normal (46,XY or hypodiploid due to random loss of chromosomes) and three had rearranged chromosomes. The chromosomal complements of the latter were the following respectively: (a) 46,XY,t[8;14](q24;q32), (b) 46,X,Y,t[8;14](q24;q32), +mar1, and (c) 43,X,Y,-8,-14,-16,-19,+8q,+mar1. The mar1 chromosome was comprised of the entire long arm of 18pter→cen.
Table 1. Clinical Features and Results of Laboratory and Cytogenetic Evaluation of Patients Having Lymphadenopathy, Either in Association With (Group I) or Lacking (Group II) AIDS or ARC

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Biopsy Site</th>
<th>Histology</th>
<th>CMV Titers</th>
<th>EBV Titers</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of Cells/Karyotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I, lymphadenopathy associated with AIDS or ARC</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1 (AIDS)</td>
<td>38/M</td>
<td>Left epitrochlear lymph node</td>
<td>Marked follicular hyperplasia with pericapsular small foci of KS</td>
<td>1:64</td>
<td>1:2560</td>
<td>24: 46,XY 3: 45,XY* 1: 46;XY,t(8;14)(q24; q32), + mar 1 1: 46;X, Y,t(8;14)(q24; q32), + mar 1 1: 43;X, -Y, -8, -14, -16, -19, +8q-, +mar 1</td>
</tr>
<tr>
<td>2 (ARC)</td>
<td>26/M</td>
<td>Left cervical lymph node</td>
<td>Marked follicular hyperplasia</td>
<td>ND</td>
<td>1:160</td>
<td>14: 46,XY 2: 45,XY* 1: 44,XY*</td>
</tr>
<tr>
<td>3 (ARC)</td>
<td>33/M</td>
<td>Right cervical lymph node</td>
<td>Marked follicular hyperplasia</td>
<td>ND</td>
<td>1:320</td>
<td>14: 46,XY 3: 44,XY* 3: 47;XY, + mar 1</td>
</tr>
<tr>
<td>4 (ARC)</td>
<td>26/M</td>
<td>Right cervical lymph node</td>
<td>Marked follicular hyperplasia</td>
<td>ND</td>
<td>1:1280</td>
<td>16: 46,XY 2: 45,XY*</td>
</tr>
<tr>
<td>5 (ARC)</td>
<td>30/M</td>
<td>Right cervical lymph node</td>
<td>Marked follicular hyperplasia</td>
<td>1:64</td>
<td>ND</td>
<td>20: 46,XY</td>
</tr>
<tr>
<td>6 (ARC)</td>
<td>32/M</td>
<td>Left axillary lymph node</td>
<td>Marked follicular hyperplasia</td>
<td>1:128</td>
<td>1:5120</td>
<td>9: 46,XY 1: 45,XY* 3: 44,XY* 1: Chromosome breakage</td>
</tr>
<tr>
<td>7 (AIDS)</td>
<td>31/M</td>
<td>Right cervical lymph node</td>
<td>Marked follicular hyperplasia</td>
<td>1:256</td>
<td>1:10,240</td>
<td>25: 46,XY</td>
</tr>
<tr>
<td>Group II, lymphadenopathy not associated with AIDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>17/M</td>
<td>Right cervical lymph node</td>
<td>Follicular hyperplasia with few clusters of epitheloid cells</td>
<td>ND</td>
<td>ND</td>
<td>25: 46,XY</td>
</tr>
<tr>
<td>9</td>
<td>62/F</td>
<td>Left axillary lymph node</td>
<td>Follicular hyperplasia</td>
<td>ND</td>
<td>ND</td>
<td>25: 46,XX</td>
</tr>
<tr>
<td>10</td>
<td>58/M</td>
<td>Right cervical lymph node</td>
<td>Follicular hyperplasia with few clusters of epitheloid cells</td>
<td>ND</td>
<td>1:1280</td>
<td>25: 46,XX</td>
</tr>
<tr>
<td>11</td>
<td>33/F</td>
<td>Left submandibular lymph node</td>
<td>Follicular hyperplasia</td>
<td>ND</td>
<td>ND</td>
<td>25: 46,XY</td>
</tr>
<tr>
<td>12</td>
<td>33/F</td>
<td>Left submandibular lymph node</td>
<td>Follicular hyperplasia</td>
<td>ND</td>
<td>1:1280</td>
<td>25: 46,XX</td>
</tr>
</tbody>
</table>

ARC, AIDS-related complex; CMV, cytomegalovirus; EBV, Epstein-Barr virus; KS, Kaposi's sarcoma; ND, not done.

*Hypodiploid chromosome numbers represent random chromosome loss due to cell rupture during preparation.

Thus, the presence of the 8q- and mar1 chromosomes in the third cell indicates that it also belongs to the same abnormal clone as the other two cells. The absence of the 14q+ chromosome in it probably represents random loss of this chromosome, only a single copy of chromosome 14 being present in this cell. In the biopsy from patient 3 (Table 1), of 20 cells analyzed, 18 were normal (46,XY or hypodiploid due to random loss of chromosomes) and two had a small acrocentric marker chromosome in addition to an otherwise normal chromosome complement. The two cells with the marker chromosome thus identify a clonal proliferation with the karyotype 47,XY,+mar1. The derivation of mar1 in these cells could not be determined due to its nonspecific banding pattern. Among the cells analyzed from the biopsy of patient 6, nonspecific chromosome and chromosomal breakage was seen in 3 of 16 (19%) (Table 1). The remaining ten nodes including five each from groups I and II revealed only normal chromosomal complements (46,XY or hypodiploid due to random loss of chromosomes).

EBV titers, shown in Table 1, were elevated in two group II patients with negative monospot tests. No chromosome abnormalities were present in biopsies from these patients. EBV titers were elevated in six of the seven group I patients. The patient with the highest EBV titer (patient 7, group I, Table 1) lacked cytogenetic aberrations. A subsequent non-Hodgkin's lymphoma developed in patients 1, 4, and 6. The lymphoma in patient 1 was classified as immunoblastic B cell type, and in patient 4 was classified as diffuse large cell type. Histologic slides showing lymphoma in case 6 were unavailable for review for classification.
CHROMOSOME BREAKAGE IN AIDS LYMPHADENOPATHY

DISCUSSION

The pattern of lymphoma development in AIDS is strikingly similar to that in two other immunodeficiency states, namely, posttransplantation immunodeficiency and X-linked lymphoproliferative syndrome. Lymphomagenesis in both disorders is increasingly recognized to be linked to EBV infection. In these cases, a polyclonal proliferation of B cells, probably initiated by viral infection, is suggested to lead to monoclonal expansion of malignant cells characterized by specific chromosome rearrangements that bring about juxtaposition of the c-myc oncogene and the immunoglobulin loci, resulting in c-myc deregulation. Such a model implies that the proliferating polyclonal cells which may be preneoplastic are genetically unstable and exhibit chromosome rearrangements that include the abovementioned lymphoma-specific ones. Cytogenetic data pertaining to this proliferation have not been available until now.

In our study, three of eight reactive lymph nodes associated with AIDS or ARC (group I) revealed chromosome abnormalities. All three patients, two of whom later developed lymphoma also had high EBV titers. Another group I patient with high EBV titers eventually developed a non-Hodgkin’s lymphoma, and two group II patients with high titers remain alive and well 12 to 19 months following diagnosis. We did not detect a chromosome abnormality in the lymph node biopsies from these latter three patients, suggesting that EBV infection may precede the appearance of cytogenetic abnormalities. Unfortunately, tissues from lymphomas were not available to us for cytogenetic analysis. In contrast, five reactive lymphadenopathies unassociated with AIDS or ARC (group II) failed to reveal chromosome aberrations despite elevated EBV titers in two.

The cytogenetic evidence presented here strongly suggest that the lymphadenopathies of AIDS or ARC patients represent prelymphomatous proliferations, which is in accord with histopathological evidence for the development of non-Hodgkin’s lymphoma correlated with AIDS lymphadenopathy presented by other investigators. EBV infection may be related to the chromosome breakage, which precedes lymphoma development. Further cytogenetic and molecular studies of AIDS-related lymphadenopathy should clarify the significance of this proliferation to lymphoma development.

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