Frequent c-myc Oncogene Activation and Infrquent Presence of Epstein-Barr Virus Genome in AIDS-Associated Lymphoma

By Milayna Subar, Antonino Neri, Giorgio Inghirami, Daniel M. Knowles, and Riccardo Dalla-Favera

Sixteen cases of histologic intermediate-grade and high-grade AIDS-associated non-Hodgkin's lymphoma (NHL) were studied for the presence and patterns of c-myc gene and bcl-2 locus rearrangements. The presence of Epstein-Barr virus (EBV) sequences and proteins and HTLV-I sequences were also investigated. c-myc gene rearrangements analogous to those observed in sporadic Burkitt lymphomas were detected in 12 of 16 cases. Six of 16 cases had detectable EBV sequences and proteins. None of the cases displayed bcl-2 rearrangements or contained HTLV-I sequences. These data suggest a frequent role for c-myc activation in the pathogenesis of AIDS-associated NHL, independent of histologic type. Conversely, EBV does not appear to be directly involved in lymphomagenesis in the majority of AIDS-associated NHLs.

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fragment FITC-conjugated goat immunoglobulin anti-human C3(1:20) (Organon Teknike) at room temperature for 30 minutes. After counterstaining in Evans-blue, the slides were read with an immunofluorescence microscope.

RESULTS

Frequency of c-myc gene rearrangements. Chromosomal translocations involving the c-myc locus in Burkitt lymphoma (BL) have been shown to lead to (a) truncations of the c-myc gene within its first intron, first exon, or flanking sequences in (8:14) translocations in sporadic BL (sBL); or (b) mutations of sequences within the first c-myc exon in (8:14) translocations in endemic BL (eBL) as well as in (8:22) and (2:8) translocations in both eBL and sBL. The truncations can be detected by Southern blot hybridization using restriction enzymes cutting outside c-myc sequences (eg, EcoRI and HindIII), whereas most (60%) of the mutations are detectable as polymorphisms of a PvuII restriction site located at the 3' side of exon 1.

With these assays, rearrangements of the c-myc locus were detectable by Southern blot hybridization of EcoRI-digested DNA in 7 of 10 SNCC, 3 of 4 LNCC, and 1 of 2 LC-IBP NHLs (Fig 1 shows representative results). The cases lacking detectable rearrangement were analyzed for PvuII site mutations, and a single SNCC case (not shown; Table 1) scored positive. Thus, 12 of 16 AIDS NHLs displayed patterns of c-myc oncogene activation generally consistent with those observed in sBL. This may represent a minimal estimate, since additional cases carrying mutations in the first exon may exist and not be detected by the PvuII analysis.

Characterization of chromosomal recombinations. The demonstration of c-myc gene rearrangements in most AIDS-NHLs led us to examine whether these rearrangements corresponded to the juxtapositions of c-myc on chromosome 8 with immunoglobulin heavy-chain (IgH) loci on chromosome 14 characteristic of (8:14) translocations in BL. In particular, two distinct patterns of t(8:14) translocations have been described in BL. The first involves recombination between the JH region of IgH with sequences >100 kilobases (kb) 5' to c-myc and is typical of eBL. The second involves recombination between the Switch (S\textsubscript{\text{\textasciitilde}}) region of IgH and sequences within the c-myc locus and is typical of sBL. We attempted to identify and map chromosomal breakpoints in our panel of AIDS-NHLs by studying the linkage between c-myc and various IgH regions, namely J\textsubscript{\text{\textasciitilde}}, S\textsubscript{\text{\textasciitilde}}, and C\textsubscript{\text{\textasciitilde}} (Fig 2C) in Southern blot hybridization experiments. In the AIDS-NHL cases that carry rearranged c-myc alleles, breakpoints were found within or in close proximity to the S\textsubscript{\text{\textasciitilde}} region, as shown by the linkage between c-myc and C\textsubscript{\text{\textasciitilde}} (but not J\textsubscript{\text{\textasciitilde}}) sequences in BamHI and HindIII digests and, indirectly, by the lack of linkage between J\textsubscript{\text{\textasciitilde}} and c-myc sequences in EcoRI digests (Fig 2). We conclude that 11 of 16 AIDS-NHLs carry IgH/c-myc recombinations analogous to sBL.

Infrequent presence of EBV sequences. We investigated the presence of EBV sequences by Southern blot hybridization using an EBV probe for the EBNA-1 gene and the EBV OriP, since these regions are consistently present in EBV-infected cells. EBV sequences were not detectable in 10 of 16 AIDS-NHLs, whereas hybridization bands were detected in six cases, either in the EBV genomic configuration or as rearranged or partially deleted fragments (Fig 3). Although examination of the intensity of the hybridization bands suggests that EBV sequences are present within the malignant cells representing >90% of the biopsy, the same result could theoretically be obtained if EBV sequences are present at high copy numbers in minor B-cell populations. We

<table>
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<th>Case</th>
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<th>Breakpoint*</th>
<th>EBV†</th>
<th>SB</th>
<th>IF</th>
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SNCC, small noncleaved cell lymphoma; LNCC, large noncleaved cell lymphoma; LC-IBP, large cell, immunoblastic-plasmacytoid lymphoma; R, rearranged; G, germline.

*Region of the IgH gene involved in the c-myc-Ig rec combination event (described in text). S\textsubscript{\text{\textasciitilde}}, switch region. NT, not tested.
†Presence (+) or absence (−) of EBV sequences detected by Southern blot hybridization (SB) or of EBNA proteins detected by indirect immunofluorescence (IF).

IgH-c-myc is rearranged by PvuII digestion analysis (described in Results Section).

Fig 1. Analysis of c-myc gene rearrangements. Representative cases display c-myc oncogene rearrangements. Lane numbers correspond to cases in Table 1. The 13-kb germline band is evident in control lane (C) obtained from normal lymphoid cells.
C-MYC ACTIVATION AND EBV SEQUENCES IN NHL

Fig 2. Southern blot analysis of Ig and c-myc gene organization (A and B). DNA from two representative cases (4.15) in Table 1 was digested with the indicated restriction endonucleases, size-separated on 1% agarose gels and transferred to nitrocellulose filters. Comigrating fragments are indicated by dashes between lanes. Arrows indicate positions of germline fragments from control DNAs run on the same gel; their sizes are indicated in kilobases (kb). The two germline HindIII fragments containing Jb and Cb display similar sizes under our experimental conditions. (C) Schematic representations of the human Ig, and c-myc loci together with the Ig, and c-myc probes used. Restriction sites in the map: (R) EcoRl; (B) BamHl; (H) HindIII.

Therefore directly investigated the presence of EBV proteins within the malignant cells by indirect immunofluorescence using a human α-EBNA positive sera. Typical patchy nuclear fluorescence was detectable in the majority of the malignant cells (80%) in the cases that contained EBV sequences, but not in cases negative by Southern blot hybridization (Table 1).

Lack of bcl-2 rearrangements and HTLV-I sequences. Most low-grade follicular B-NHLs, 40% of diffuse large cell B-NHLs, and 30% of undifferentiated BL have been reported to carry rearrangements involving the bcl-2 locus on chromosome 18. To determine if bcl-2 rearrangements were present in our AIDS-NHL cases, Southern blot hybridization analysis of the tumor DNAs was performed with probes from both the major and minor bcl-2 gene breakpoint regions. No rearrangement was detected by any of these probes in the 16 AIDS-NHLs (data not shown). All of the cases were also negative for HTLV-I and HIV sequences (data not shown).

Fig 3. EBV DNA hybridization. The presence of EBV sequences was detected by BamHl digestion and hybridization to a probe containing the genes EBNA-1 and OriP corresponding to the 9.0-kb BamHl C and the 4.8-kb BamHl K fragments (described in text). Control lane (C) represents hybridization to DNA from the EBV + eBL cell line Daudi. Lane numbers correspond to cases in Table 1.

DISCUSSION

The major findings of this report are the frequency of c-myc gene rearrangements in AIDS-NHLs of different histologies and, concomitantly, the absence of EBV sequences in most of these tumors. Although c-myc oncogene activation and EBV infection in the context of immunosuppression has been repeatedly suggested as the main steps of lymphomagenesis in AIDS, no direct evidence for this has been reported in sizable panels of cases. Our survey of 16 cases clearly points to a role for c-myc activation in most AIDS-NHLs, whereas the surprising finding of the absence of EBV sequences in most of these cases suggests a general reevaluation of the problem of AIDS-NHL pathogenesis.

Specific chromosomal recombinations [(8:14), (8:22), and (2:8)] involving the c-myc and Ig loci represent features of both the endemic and sporadic form of BL and of the L,-type of acute lymphocytic leukemia. A role for these alterations in malignant B-cell transformation is strongly suggested by (a) the evidence of c-myc/Ig juxtapositions in B-cell malignancies in mice and rats; (b) the increased incidence of B-cell tumors in transgenic mice carrying Ig-myc chimeric constructs, and (c) the in vitro transforming activity of activated c-myc genes on human B-lymphoblastoid cells. It is therefore likely that, by analogy with non-AIDS-associated BL, the c-myc rearrangements shown in 12 of 16 AIDS-NHLs contribute to the pathogenesis of these tumors by disrupting the normal control of c-myc gene expression. Our data indicate an association between c-myc rearrangements and a variety of NHL types, including high-grade (Burkitt and non-Burkitt-type) and intermediate-grade lymphomas, and indicate a specific association between immunosuppres-
sion and B-cell tumors involving c-myc oncogene activation. In this context, one or more of the biologic alterations present in AIDS theoretically may favor the occurrence of chromosomal translocations involving c-myc. Alternatively, and perhaps more likely, cells in which these translocations have occurred may acquire specific biologic modifications that make them particularly suited to expand and progress toward malignancy in the context of immunodeficiency states. BL cells were recently reported to lack specific cell-surface molecules involved in immunorecognition by T cells and are unable to elicit either autologous or allogeneic T-cell responses in vitro. The effects of these alterations would obviously be amplified in AIDS.

A suggested role for EBV in AIDS lymphomagenesis was prompted by the association of this virus with B-cell lymphomas occurring in a variety of inherited and acquired immunodeficiencies and by the observation that the immunosurveillance of EBV-infected B cells is defective in AIDS and ARC. In addition, multiple B-cell clonal expansions, presumably carrying EBV, have been detected in the lymphadenopathy syndrome (LAS) or AIDS-NHL nodal biopsies, and EBV-positive B-lymphoblastoid cell lines can be readily established in vitro from the peripheral blood of AIDS patients. These observations had previously led us and other researchers to propose that AIDS-associated immunosuppression and EBV infection may favor the clonal expansion of EBV-infected B cell populations, increasing the probability of the occurrence of genetic alterations, namely translocations of the c-myc gene, which can lead or contribute to NHL development. This model was supported by intra vitro data showing that the expression of an activated c-myc oncogene can cause the tumorigenic conversion of EBV-infected lymphoblasts from an AIDS patient. Despite these observations, it is now clear that most AIDS-NHLs do not carry EBV sequences in their genome; thus, EBV is not directly involved in cell transformation in these cases. Models involving EBV remain valid for the approximate one-third of cases that do contain viral sequences. Further studies are required, however, to establish whether in these cases EBV has a primary role in lymphomagenesis or secondarily infects the malignant cells.

In general, the concurrence of c-myc gene rearrangements with the relatively low frequency of EBV sequences suggest a similarity between AIDS-NHL and the sporadic rather than the endemic form of BL, as previously suggested. This similarity is enhanced by the observation that AIDS-NHLs carry truncations of the c-myc gene and recombinations with the Switch region of the immunoglobulin heavy chain in the cases in which no alteration of c-myc was detected in this study.

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