Molecular resemblance of an AIDS-associated lymphoma and endemic Burkitt lymphomas: Implications for their pathogenesis

(CHromosome translocations)

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ABSTRACT Non-Hodgkin lymphoma is a common feature of AIDS. Approximately 30–40% of these tumors exhibit clinical features suggestive of endemic Burkitt lymphoma: they are aggressive malignancies that occur in association with Epstein–Barr virus infection, they arise in the setting of immunosuppression, and they carry t(8;14) translocations without detectable rearrangement of the MYC oncogene. To understand the molecular basis of these parallels, we analyzed a case of Epstein–Barr-positive AIDS-associated undifferentiated lymphoma. Southern blots show that the tumor exhibits immunoglobulin joining segment rearrangement but no rearrangement of the MYC oncogene. Cloning of the rearranged joining segment allowed the isolation of recombinant clones encompassing the translocation breakpoint, and sequencing of the translocation junction disclosed that the breakpoint is situated 7 base pairs from the chromosome 14 site involved in a previously described endemic Burkitt lymphoma translocation. Furthermore, the breakpoint is situated far from MYC on chromosome 8, a constant finding in endemic Burkitt lymphomas. That the molecular architecture of the translocation in this case is strikingly similar to previously analyzed translocations from endemic Burkitt lymphomas strongly suggests that common molecular mechanisms must be operative in the pathogenesis of these tumors.

Soon after the first clinical descriptions of AIDS, several reports appeared documenting an increased incidence of Burkitt lymphomas (1, 2) and other non-Hodgkin lymphomas (NHLs) (3) in AIDS patients. Cytogenetic studies of these Burkitt lymphomas demonstrated that they exhibit the same spectrum of chromosome translocations as seen in previously studied Burkitt lymphomas, including the t(8;14) and variant translocations (4–6). Approximately 30–40% of these tumors contain the Epstein–Barr virus (EBV) genome (7, 8). Recently, it has been suggested that the EBV-positive AIDS-associated lymphomas of pleomorphic histopathology, which carry immunoglobulin gene rearrangements but lack detectable MYC oncogene rearrangements, constitute neoplasms of uncertain lineage and distinctly uncommon characteristics that set them apart from most NHLs (9).

However, several features of the EBV-positive subset of AIDS-associated NHLs led us to speculate that these lymphomas might arise via pathogenic mechanisms similar to those seen in endemic Burkitt lymphomas. Endemic EBV-positive Burkitt lymphomas carry t(8;14) translocations, which exhibit a molecular architecture distinct from the t(8;14)-bearing but EBV-negative sporadic Burkitt lymphomas (10, 11). The former are thought to arise through the interplay of several environmental and genetic events (12), including EBV infection and immunosuppression, which together function to expand B-cell populations and enhance the possibility of chromosome translocations and consequent lymphoma (13). The similarities between this scenario for the genesis of endemic Burkitt lymphoma and the clinical features of AIDS-related NHLs are striking. Not only do the AIDS-associated tumors carry both EBV genomes and t(8;14) translocations, they are often preceded by expansion of clonal B-cell populations (14), T-cell regulation of which is abrogated by the immunosuppression of AIDS (15). Thus the clinical parallels between AIDS-associated EBV-negative NHLs and endemic Burkitt lymphomas suggest that molecular similarities between these tumor types might exist as well.

We report here the molecular analysis of an AIDS-associated undifferentiated lymphoma. The lymphoma carried a t(8;14) translocation characteristic of Burkitt lymphoma. Despite the lack of detectable MYC rearrangement by Southern blot, molecular cloning of the chromosome junction demonstrates that the architecture of the translocation typifies that of endemic Burkitt lymphomas and, in fact, is nearly identical to that of a previously analyzed endemic Burkitt lymphoma (10). We thus propose that EBV-positive AIDS-associated NHLs probably arise by mechanisms similar to those operative in endemic Burkitt lymphomas.

CASE REPORT

The patient, a 26-year-old male homosexual, first came to medical attention 6 years before this presentation when he experienced generalized lymphadenopathy and was diagnosed as having mononucleosis. Two years later he suffered an enlarged right axillary lymph node and experienced night sweats; biopsy of the node demonstrated reactive hyperplasia, and the patient was informed that he had chronic mononucleosis. He then remained healthy until 4 weeks before his presentation, when he noticed a painful mass in his right axilla, which subsequently enlarged rapidly. Biopsy of the mass disclosed a NHL, and cytogenetic analysis revealed a t(8;14) translocation. The patient was also shown at that time to harbor the human immunodeficiency virus by ELISA and Western (immunologic) blot and thus was diagnosed as having contracted AIDS.

METHODS

DNA Extraction and Blotting Procedures. Cellular DNA was extracted from a lymph node biopsy specimen that had been suspended by passage through a stainless-steel mesh, using established procedures (16). After organic extraction, ethanol precipitation, and solubilization, DNA was digested

Abbreviations: NHL, non-Hodgkin lymphoma; EBV, Epstein–Barr virus; JH, heavy-chain immunoglobulin joining.

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with appropriate restriction endonucleases (Bethesda Research Laboratories) according to manufacturers' specifications. Agarose gel electrophoresis, transfer to nitrocellulose, and hybridization were done essentially according to Southern with modifications (17).

**Molecular Probes.** The probe pHj detects immunoglobulin heavy-chain joining (JH) segments on chromosome 14 (18). The MYC probe (19) and the BCL2 probe (20) have also been described elsewhere. Probes were labeled using 32P by standard nick-translation techniques (21).

**Cloning Procedures.** Genomic cloning in the phage vector EMBL3 was accomplished by using high-molecular-weight DNA essentially as described (10). Recombinant clones were mapped by restriction analysis, and selected DNA fragments were then subcloned after electroelution (International Biotechnologies) of restriction-digested DNA from gels. The purified DNA was then ligated into the plasmid pUC 19 or the bacteriophage M13, and transformation of Escherichia coli competent cells was performed as described (11).

**Nucleotide Sequence Analysis.** Sequencing was accomplished using the Sanger dideoxynucleotide chain-termination method (22) on cloned single-strand M13 DNA and Bethesda Research Laboratory reagents.

**RESULTS**

**Histopathology.** Histopathological examination of stained specimens of the right axillary biopsy demonstrated a diffuse infiltrate that involved the entirety of the node and fat and connective tissue surrounding it. The infiltrate consisted of medium-sized cells with a high nuclear-to-cytoplasmic ratio and intermediate slightly irregular nuclei having somewhat clumped nuclear chromatin without conspicuous nucleoli. The cells were approximately the size of intermixed histiocytes and were relatively uniform throughout the section. These features were interpreted as most consistent with non-Hodgkin lymphoma of undifferentiated (small noncleaved cell) type.

**Molecular Analysis of the t(8;14) Translocation.** Genomic DNA was extracted from the biopsy tissue (designated FI) obtained from the right axillary lymph node. The DNA was digested with various restriction enzymes for Southern analysis. Rearrangement of the immunoglobulin JH segment was observed using the pHj probe, whereas the MYC and BCL2 genes were both in their germ-line configurations (Fig. 1), as were the immunoglobulin λ and κ loci (data not shown). In addition, a dot-blot experiment probed with an EBV probe confirmed the presence of the EBV genome in the patient's tumor sample (data not shown).

**Cloning and Identification of the t(8;14) Breakpoint.** Because no MYC rearrangement was demonstrable, by analogy with the observations made in endemic Burkitt lymphomas, it was hypothesized that the t(8;14) translocation breakpoint would lie far 5' of MYC and, most importantly, within the JH region. Thus we sought to clone and isolate the breakpoint using a strategy that hinged on the previously demonstrated role of immunoglobulin JH region in endemic Burkitt lymphomas (10) and its hypothesized role in the patient's translocation. That is, lacking a rearrangement of a chromosome 8 probe, the chromosome 14 pHj probe was used for screening.

A recombinant genomic library was constructed in the phage EMBL3 and screened with the pHj probe. Clones were obtained which, when physically mapped, correspond to both JH alleles. Representative clones are illustrated in Fig. 2. After determining which fragments lack repetitive sequences, a unique 1.9-kilobase (kb) HindIII fragment from

![FIG. 1. Southern blots of human placental (control) and patient DNAs were probed with oncogene and JH probes. Lanes: 1 and 2, Placental and patient DNAs, respectively, digested with BamHI and probed with the pB3 BCL2 probe; 3 and 4, placental and patient DNAs digested with HindIII and probed with the pMYC7.4 MYC probe; 5 and 6, placental and patient DNAs digested with HindIII and probed with the immunoglobulin JH probe pHj, demonstrating rearrangement of the patient's JH region.](image1)

![FIG. 2. Restriction map of the FI biopsy material recombinant clones. Two rearranged clones encompassing the t(8;14) translocation breakpoint are shown, as are two germ-line clones covering the Cμ, Sμ, and JH regions. The pH1.9 probe is indicated. (Bar = 1 kb.)](image2)
the region 5' of the JH homology was purified and subcloned for use as a probe and was designated H1.9. This probe is identified in Fig. 2.

Because these clones might represent either physiologic immunoglobulin gene rearrangements or translocation breakpoints, it was necessary to show that the H1.9 probe originated from chromosome 8. This probe was hybridized to Southern-blotted DNAs from a panel of somatic cell hybrids containing segregated human chromosomes. Fig. 3 demonstrates that H1.9 hydridizes only to DNA containing the entire chromosome 8. [The filter from Fig. 3 was subsequently stripped and hybridized to the pRYC7.4 MYC probe; the hybridization pattern obtained (data not shown) is identical to that obtained using H1.9, further demonstrating synten of pH1.9 and MYC.] We next sought to determine whether the region of the genome including the H1.9 probe is amplified in the COLO 320 cell line, which carries an amplification unit that encompasses regions surrounding MYC.

FIG. 3. Southern blot of HindIII-digested somatic cell hybrid and human DNAs probed with pH1.9. Lanes: 1, Placenta; 2, patient FID; 3, NP3 mouse myeloma control; 4, hybrid GM637 × C57 VIII 3a, which contains human chromosome 14 but not 8; 5, clone 77-5, which contains human chromosome 8 but not 14; 6, clone 77-30, which contains a human chromosome 8 from which the MYC gene has been deleted. The pH1.9 probe hybridizes to a 1.9-kb band only in human and hybrid DNAs carrying the region around MYC.

(23). Previous work had shown that the p38b9 probe, which is localized near the chromosome 8 translocation breakpoints of several endemic Burkitt lymphomas (10, 11), is included in the COLO 320 amplification unit (F.H., unpublished results). Fig. 4 illustrates that H1.9 is, indeed, amplified in COLO 320. These lines of evidence show that H1.9 contains chromosome 8 sequences that lie some undetermined distance from MYC (as in endemic Burkitt lymphomas) and that, therefore, the phage clones, indeed, include the t(8;14) junction.

Nucleotide Sequence Analysis. To examine the structure of the translocated breakpoint, the nucleotide sequence across the chromosome junction was obtained. A portion of the sequence is shown in Fig. 5. It was found that the breakpoint lies 7 nucleotides from the previously described cell line P3HR-1 endemic Burkitt lymphoma breakpoint, upstream of the J5 segment (10). Thus, at the molecular level this translocation is nearly identical to a previously analyzed translocation carried by an endemic, EBV-positive Burkitt lymphoma.

DISCUSSION

It is well recognized that aggressive NHLs occur in AIDS patients, accounting for from between 2.5% and 5% of primary AIDS diagnoses in a recent series (8). Cytogenetic

FIG. 4. HindIII-digested DNAs probed with pH1.9. Lanes: 1, Placenta (HP); 2, 697 lymphoblastic leukemia cell line (control); 3, HL-60 promyelocytic leukemia cell line; 4, COLO 320 colon carcinoma line. HL-60 and COLO 320 carry amplification units surrounding MYC, and pH1.9 is included in the COLO 320 amplification unit.

Fig. 5. Nucleotide sequence of the t(8;14) breakpoint (Top) and the normal chromosome 14 region (Bottom). Vertical lines denote sequence identity. The Fl (patient) and previously cloned P3HR-1 endemic Burkitt lymphoma breakpoints are indicated. A heptamer recombinase signal is bracketed; the upstream nonamer is not illustrated.

\[
\text{t(8;14)} \quad \text{T T T T A C A G G A A T AT C T A T G C A A C C C G G G C C T C T C G G A C T C A G T C T} \\
\text{I g J H} \quad \text{G C C T C T G G G T C C A A T G C C A A C} \\
\Delta \quad \text{F l} \quad \text{P 3 H R - 1}
\]
of Burkitt lymphoma gene rearrangements of cases analysis J5.
P3HR-1 EBV-positive patients found respect histopathology cations analyzed from NHL that 23 base consequences that interrupt the region immediately surrounding MYC is not rearranged by Southern blot. Yet regions far 5' of MYC on chromosome 8 are involved. This is the general case for endemic Burkitt lymphomas, as has been shown by our laboratory (10, 11) and others (24). Thus, with regard to the chromosome 8 breakpoint localization, this case fits the pattern established by analysis of endemic Burkitt lymphoma translocation breakpoints.

On chromosome 14, the analogy is also substantiated. The translocation junction is 7 base pairs from the previously characterized P3HR-1 endemic Burkitt lymphoma breakpoint. The breakpoint lies within the JH regions, upstream of J5. A conserved signal sequence homologous to the immunoglobulin recombine heptamer–nonamer (25) is also present. Upstream of the breakpoint on chromosome 14 is a heptamer that matches the functional recombination signal actually used by J5 at 5 of 7 positions: CAATGTG is near the break, whereas CAATGTTG is seen at J5. Farther upstream, 23 base pairs from the heptamer, is a nonamer previously described for P3HR-1 endemic Burkitt lymphoma. In sum, the molecular features of the (t(8;14) translocation in this AIDS-associated NHL are strongly reminiscent of those of the endemic Burkitt lymphoma cases.

The relationship between the epidemiology and the molecular genetics of Burkitt lymphoma is now quite well understood. Burkitt lymphomas occur in two distributions throughout the world. The endemic form occurs primarily in equatorial Africa, where it is associated in over 97% of cases with EBV infection (12). The sporadic form, in contrast, is found largely in Europe and North America and is associated with EBV in ~20% of cases. Both types of tumors exhibit characteristic translocations, most commonly the t(8;14)- (q24;q32), which joins the immunoglobulin heavy chain locus to the MYC oncogene (for review see ref. 13). Now a correlation exists between the architecture of the t(8;14) translocation and the form (endemic versus sporadic) of Burkitt lymphoma. The sporadic tumors have translocations that interrupt the region immediately surrounding MYC and that appear to be catalyzed by immunoglobulin isotype switching enzymes. These translocations are easily detectable by Southern blot. Endemic Burkitt lymphomas carry translocations localized far from MYC and that arise as a consequence of mistakes made by the immunoglobulin V—D-J recombine enzymes (10), which catalyze physiologic recombination at the immunoglobulin and T-cell receptor loci in early lymphocytes (25). MYC rearrangements are not detectable in these latter tumors. Thus a strong correlation exists between the type of Burkitt lymphoma (endemic versus sporadic), the structure of the translocated chromosome, and the point during lymphocyte differentiation (pre-B-cell V—D-J joining versus mature B-cell isotype switching) at which the translocation occurs.

This correlation has several consequences for current thinking regarding the pathogenesis of these tumors. Crucial to this discussion is the role of EBV (12). EBV can immortalize B cells and is routinely used in vitro to establish lymphoblastoid cell lines: In vivo it engenders polyclonal B-cell activation, which is subject to T-cell regulation. Taking these findings into account, the hypothetical scenario for the pathogenesis of endemic Burkitt lymphoma is as follows. In endemic regions, infection of a young individual occurs without clinical disease. However, if the EBV-infected individual contracts malaria, immunosuppression ensues. Polyclonal B lymphocyte proliferation proceeds unchecked in the absence of T-cell suppression, probably enlarging the population of B cells subject to genetic change. This enhances the probability of recombinease error, which may ultimately lead to translocation and lymphomagenesis.

In AIDS-associated, EBV-positive NHL cases, such as the one described here, the parallel with the pathogenesis of endemic cases is evident. EBV infection and profound immunosuppression facilitate abnormal B-lymphocyte proliferation, with the lack of normal regulation of the immune response to EBV has been studied directly in AIDS patients (15); T-cell suppression of EBV-induced B-cell proliferation is totally absent in these patients. The resultant polyclonal B-cell expansion is easily detected in patients with the lymphadenopathy syndrome, as well as with AIDS (14). This pool of B cells is the substrate for recombinease error during immunoglobulin rearrangement, just as in endemic cases.

This case also underscores the fact that several types of malignancies have been shown to carry (t8;14) translocations. These genetic lesions have now been described in Burkitt lymphoma, AIDS-associated NHL, acute lymphoblastic leukemia, and in follicular lymphomas that evolve into acute leukemia (26). The variety of lymphoid neoplasms that may share common molecular mechanisms of lymphomagenesis. In particular, AIDS-associated NHLs such as the case described here may prove to carry remarkably uniform genetic alterations.

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