Involvement of the bcl-6 gene in AIDS-related lymphomas

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Summary

Background: Non-Hodgkin's lymphoma (NHL) represents a major complication of AIDS. Systemic AIDS-related NHLs (AIDS-NHLs) derive from B cells and are classified into four distinct groups, including small noncleaved-cell lymphoma (SNCL), diffuse large-cell lymphoma (DLCL), anaplastic large-cell lymphoma (ALCL), and body-cavity-based lymphoma (BCBL). The molecular pathogenesis of AIDS-NHL is characterized by the association of specific genetic lesions with distinct AIDS-NHL categories. Genetic lesions of AIDS-NHL involve proto-oncogenes (c-myc, Ras), tumor suppressor loci (p53, 6q), and viral infection (Epstein-Barr virus, human herpesvirus type 8).

Design: The aim of this work was to define the involvement of the bcl-6 gene in AIDS-related lymphomagenesis by investigating the distribution of bcl-6 structural alterations throughout the pathologic spectrum of AIDS-NHL. Both gross rearrangements and mutations in the 5' noncoding regions of the gene were investigated.

Results: Gross rearrangements of bcl-6 are confined to a fraction of AIDS-DLCL cases among AIDS-NHLs. Conversely, mutations of the 5' noncoding regions of bcl-6 are detected in a large proportion of AIDS-SNCLs, AIDS-DLCLs and AIDS-ALCLs independent of the concomitant presence of bcl-6 rearrangements.

Conclusions: Mutations of the 5' noncoding regions of bcl-6 represent the most frequent genetic lesion presently detectable among systemic AIDS-NHLs. The frequency of these mutations and their location in the proximity of bcl-6 regulatory regions suggest that they may play a role in AIDS-related lymphomagenesis.

Key words: AIDS, bcl-6, genetic lesion, lymphoma, oncogene

Introduction

Non-Hodgkin's lymphoma (NHL) is the second most frequent cancer associated with AIDS after Kaposi's sarcoma [1]. In some risk groups, namely, the hemophiliacs, NHL overrates Kaposi's sarcoma, representing the most common AIDS-related neoplasm [2]. The incidence of AIDS-related NHL (AIDS-NHL) has continued to rise since the outbreak of the AIDS epidemic and since 1985, the Centers for Disease Control (CDC) have recognized NHL as an AIDS-defining condition [3].

As a group, AIDS-NHLs share a number of distinguishing features [4, 5]. First, AIDS-NHLs are consistently of B-cell origin. Second, AIDS-NHLs are high-grade NHLs that demonstrate a far more aggressive clinical course than that associated with NHLs of similar histology arising in the immunocompetent host. Finally, AIDS-NHLs show a marked predilection for extranodal sites, including the gastrointestinal tract, anus and rectum, central nervous system, bone marrow, kidney, oral cavity, and the body cavities.

The detailed pathological classification of AIDS-NHLs has been a matter of controversy and is being continuously remodeled. At present, systemic AIDS-NHLs are generally distinguished into two major pathologic categories, which include small noncleaved-cell lymphoma (SNCL) and diffuse large-cell lymphoma (DLCL) [4, 5]. AIDS-related SNCL (AIDS-SNCL) and AIDS-related DLCL (AIDS-DLCL) account for approximately 90% of systemic AIDS-NHLs. The remaining fraction of systemic AIDS-NHL is represented by CD30+ anaplastic large-cell lymphoma (ALCL) and by a rare AIDS-NHL variant growing in liquid phase in the body cavities and termed as body-cavity-based lymphoma (BCBL) [6, 7].

The pathogenesis of AIDS-NHL is thought to involve several elements, including factors contributed by the immunocompromised host as well as genetic alterations of the tumor clone [4, 5]. Host factors contributing to AIDS-NHL development are mainly represented by disrupted immunosurveillance per se and chronic antigen stimulation. The role of disrupted immunosurveillance is exemplified by the close association between low CD4+ T cells in the host's peripheral blood and increased AIDS-NHL risk [8]. Such association is particularly marked in the case of AIDS-DLCL and AIDS-ALCL, whereas AIDS-SNCL may develop also in the context of a relatively preserved immunity. On the other hand, the con-
tribution of chronic antigen stimulation and selection to AIDS-related lymphomagenesis is documented by the high rate of somatic mutations accumulating in the hypervariable regions of immunoglobulin genes expressed by AIDS-NHL cells [9].

In addition to host factors, the pathogenesis of AIDS-NHL is characterized by the clonal accumulation of a number of genetic lesions within the tumor cells. Such genetic lesions are represented by activation of proto-oncogenes (c-myc, Ras), disruption of tumor-suppressor loci (p53, 6q), as well as infection by viruses, including Epstein–Barr virus (EBV) and human herpesvirus type 8 (HHV-8) [4, 5, 10–14]. The repertoire of genetic lesions in AIDS-NHL differs substantially according to histology. The molecular pathogenesis of AIDS-SNCCl involves c-myc activation and EBV infection in 100% and 30% of the cases, respectively, whereas mutations of p53 are present in 60% of patients [10]. Activation of the Ras genes also occurs in 15% of AIDS-SNCCl cases [10]. AIDS-related DLCL displays EBV infection in approximately 70% of cases and c-myc translocations in 20% of patients [10]. Deletions of 6q have also been reported in a substantial fraction of AIDS-DLCLs [11]. AIDS-ALCLs carry EBV infection in virtually all cases, whereas the occurrence of other genetic lesions has not been investigated extensively in this AIDS-NHL group [15]. Finally, AIDS-BCBL consistently associates with HHV-8 and EBV infection of the tumor clone in the absence of known alterations of proto-oncogenes or tumor-suppressor genes [12–14].

In an effort to deepen our understanding of AIDS-related lymphomagenesis, we have undertaken several investigations aimed at defining the status of the bcl-6 gene in AIDS-NHLs belonging to different histologic categories. Here we describe the evidence supporting the involvement of genetic lesions of bcl-6 in the pathogenesis of AIDS-NHL.

Genetic lesions of bcl-6 in AIDS-related lymphomas

The bcl-6 gene has been originally identified by virtue of its involvement in chromosomal translocations affecting the chromosomal band 3q27 in DLCL of the immunocompetent host [16–20]. Among NHLs of the immunocompetent host, the same gene was subsequently found to be rearranged in approximately 40% of B-cell DLCLs, including cases with cytogenetically normal 3q27 chromosomes [21]. The rearrangement breakpoints among NHLs of the immunocompetent host cluster within a 4 kb region spanning the bcl-6 promoter sequences and the first noncoding exon, and result in the fusion of bcl-6 coding sequences (exons 2 through 10) to heterologous promoters from other chromosomes, presumably leading to the deregulated expression of the bcl-6 protein [16–22].

The bcl-6 protein belongs to the family of transcription factors containing zinc-finger motifs [17, 18, 23, 24]. Functional studies have indicated that bcl-6 can function as a transcription factor that can bind a specific DNA sequence and repress transcription from linked promoters [23]. Thus, the physiologic function of bcl-6 may be to repress the expression of genes carrying its specific DNA binding motif. Since the bcl-6 gene is expressed in germinal-center (GC) B cells, but not in their differentiated cell progenies (plasma cells and memory B cells), it is conceivable that it may be involved in the induction of GC-associated functions and that its down-regulation may be necessary for B cells to progress toward further differentiation into memory B cells or plasma cells [24]. In NHLs carrying a rearranged bcl-6, the down-regulation of the bcl-6 protein may be prevented by the juxtaposition of the gene to heterologous promoters [22].

Recent evidence has suggested that the bcl-6 gene may be altered by mechanisms other than chromosomal rearrangements in NHLs [25]. In approximately 70% of DLCLs and 50% of follicular lymphomas of the immunocompetent host, the bcl-6 gene is altered by multiple mutations clustering within its 5' noncoding region [25]. These mutations frequently occur in the absence of any recognizable chromosomal abnormality affecting band 3q27 or molecular rearrangement of the bcl-6 locus. The genomic sequences most frequently involved by the mutations are adjacent to the bcl-6 promoter region and overlap with the major cluster of chromosomal breakpoints, suggesting that mutations and rearrangements may be selected for their ability to alter the same region, which may be important for the normal regulation of bcl-6. The combined frequency of mutations and rearrangements approaches 100% of DLCL cases arising in the immunocompetent host, suggesting that structural alterations of the 5' noncoding region of the bcl-6 gene are necessary for the development of these tumors [25].

Based on the evidence that cytogenetic breakpoints at 3q27, the chromosomal site of bcl-6, had been detected in AIDS-NHL karyotypes [26], we decided to investigate the distribution of bcl-6 genetic lesions, including both gross rearrangements and mutations of the 5' noncoding regions, throughout the pathologic spectrum of AIDS-NHLs. As a first step, the presence of bcl-6 rearrangements was tested in a panel of 40 AIDS-NHL cases. This study demonstrated that, among AIDS-NHLs, gross rearrangements of bcl-6 are restricted to approximately

Table 1. Frequency of genetic lesions in AIDS-NHL.

<table>
<thead>
<tr>
<th>Histology*</th>
<th>bcl-6</th>
<th>c-myc</th>
<th>Ras</th>
<th>p53</th>
<th>EBV</th>
<th>HHV-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS-SNCCl</td>
<td>-</td>
<td>70%</td>
<td>100%</td>
<td>15%</td>
<td>60%</td>
<td>30%</td>
</tr>
<tr>
<td>AIDS-DLCL</td>
<td>20%</td>
<td>65%</td>
<td>20%</td>
<td>15%</td>
<td>-</td>
<td>70%</td>
</tr>
<tr>
<td>AIDS-ALCL</td>
<td>-</td>
<td>65%</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>90%</td>
</tr>
<tr>
<td>AIDS-BCBL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100%</td>
</tr>
</tbody>
</table>


* Mutations of the bcl-6 5' noncoding regions.

* Genetic lesion not involved.
20% of AIDS-DLCL cases [27]. Rearrangements of bcl-6 in AIDS-DLCL occurred in cases both with and without EBV infection [27]. Although 20% of the AIDS-DLCLs included in the panel also carried a rearranged c-myc, no AIDS-DLCL sample harbored simultaneously a rearrangement of c-myc and bcl-6 [27]. Notably, the frequency of bcl-6 gross rearrangements in AIDS-DLCL was significantly lower than that detected among DCLCs of the immunocompetent host [17, 21, 27], confirming the notion that the pathogenesis of these two groups of neoplasms is different. In this respect, it is notable that AIDS-DLCL is consistently devoid of bcl-2 rearrangements, which instead are detected in 20% of DCLCs of the immunocompetent host [4, 5, 28]. Conversely, AIDS-DLCL frequently harbors EBV infection, which is generally absent in DCLCs of the immunocompetent host [10, 28].

In addition to rearrangements, the presence of mutations of the 5' noncoding regions of bcl-6 was also investigated in AIDS-NHL. Mutational analysis of bcl-6 5' noncoding regions was performed by a combination of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing, as previously reported [25]. Five distinct fragments of the bcl-6 gene were analyzed by PCR-SSCP. These fragments, spanning the first exon and 740 bp of the first intron of bcl-6, were selected for mutational analysis since they are the site of the overwhelming majority of mutations affecting the bcl-6 gene in NHLs of the immunocompetent host [25]. Overall, mutations of the 5' noncoding regions of bcl-6 were detected in approximately 60% of AIDS-NHLs. According to AIDS-NHL histology, mutations clustered with AIDS-SNCCCL (70% of cases), AIDS-DLCL (65% of cases), and AIDS-ALCL (65% of cases). No bcl-6 5' mutations were detected in AIDS-BCBL cases. Within the AIDS-DLCL group, mutations of the bcl-6 5' noncoding regions occurred both in the presence and in the absence of bcl-6 rearrangements. With respect to other genetic lesions frequently encountered in AIDS-NHL, the presence of bcl-6 mutations in a given AIDS-NHL sample was independent of the concomitant presence of c-myc rearrangements, p53 mutations, or EBV infection.

Conclusions

The evidence summarized in this report demonstrates that structural alterations of the bcl-6 gene are frequently involved in AIDS-NHL. Whereas gross rearrangements of bcl-6 are confined to a fraction of AIDS-DLCL cases, mutations of bcl-6 5' noncoding regions occur at high frequency in the major histologic categories of AIDS-NHL, namely, AIDS-SNCCCL, AIDS-DLCL, and AIDS-ALCL. In this respect, bcl-6 5' mutations add to the number of genetic lesions detected in AIDS-NHL, which also include gross rearrangements of bcl-6 and c-myc, inactivation of p53, as well as infection by EBV and HHV-8 [4, 5, 10-14].

The molecular characteristics of the mutations of the bcl-6 5' noncoding regions detected in AIDS-NHLs resemble those of NHLs of the immunocompetent host under several aspects [25]. First, in both NHL groups, the mutations cluster within a 740 bp fragment of the bcl-6 intron 1 that is thought to contain the regulatory sequences of the gene. Second, in both AIDS-NHLs and NHLs of the immunocompetent host, most mutations are single nucleotide substitutions. Finally, in both AIDS-related and AIDS-unrelated NHLs, bcl-6 5' mutations occur independently of the concomitant presence of bcl-6 gross rearrangements.

The detection of mutations of bcl-6 5' noncoding regions in AIDS-NHLs may shed some light on the histogenesis of the AIDS-NHL categories associated with these mutations. Among NHLs of the immunocompetent host, it has been proposed that bcl-6 5' mutations represent a marker of GC or post-GC B cells, based on the indirect evidence that mutations cluster with lymphoma types, such as follicular lymphoma, DLCL, and SNCL, that derive from GC or post-GC cells, whereas mutations are rare in lymphomas deriving from virgin B cells [25; A. Migliazza et al., unpublished observation]. Thus, the frequent association of AIDS-SNCCCL, AIDS-DLCL, and AIDS-ALCL with bcl-6 5' mutations may be taken to suggest that these AIDS-NHLs originate from GC or post-GC B cells. Indeed, the rate of bcl-6 5' mutations in AIDS-SNCCCL and AIDS-DLCL is superimposable to that detected in NHLs of similar histology arising in the immunocompetent host [25; A. Migliazza et al., in preparation].

Despite the small number of AIDS-BCBLs included in this study, it is notable that no mutations of the bcl-6 5' noncoding regions were detected in this AIDS-NHL category. The absence of bcl-6 5' mutations in AIDS-BCBL, as compared to the high frequency of mutations detected in AIDS-SNCCCL, AIDS-DLCL, and AIDS-ALCL, may be taken to suggest that this rare AIDS-NHL type originates from a B-cell population distinct from that of other AIDS-NHL groups. Although immunophenotypic and virological data of AIDS-BCBL are compatible with this hypothesis [7, 12-14], large panels of AIDS-BCBL cases need to be tested for bcl-6 5' mutations in order to answer this question conclusively.

Finally, the high rate of mutations of bcl-6 5' regulatory regions in AIDS-NHLs raises several issues concerning their actual contribution to AIDS-NHL pathogenesis. Although the frequency and clustering of bcl-6 5' mutations strongly suggest that mutations may have been selected based on their functional role during tumorigenesis, a direct proof of their pathogenicity is presently lacking. Future in vitro studies aimed at transfecting B cells with mutated bcl-6 alleles may be a suitable model to clarify this issue.

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