A VH Clonal Deficit in Human Immunodeficiency Virus-Positive Individuals Reflects a B-Cell Maturational Arrest

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A major feature of human immunodeficiency virus (HIV) infection is disordered B-cell function, which paradoxically includes both pathologic overactivity (elevated serum antibodies, lymphadenopathy, and increased risk for lymphoma) and underactivity (impaired antibody immunity, particularly to bacterial polysaccharide antigens). B-cell immune dysfunction contributes significantly to HIV-related morbidity and also represents an obstacle to eventual definitive treatment by anti-HIV immunization. Our laboratory has recently identified in normal B-cell populations certain VH gene subfamilies with a developmentally regulated pattern of utilization. In particular, B cells bearing rearranged VH3L were rare in the germinal center but uniformly abundant in the blood and lymphoid mantle zone. We used this index gene subfamily as a clonal criterion for the pattern of B-cell development in lymphocytes of HIV-positive individuals. In a series of 19 HIV-positive subjects, a striking deficit of VH3L B cells was observed; in contrast, none of the 16 normal subjects showed this abnormality. Other VH subfamilies (VH1N, VH4/6, and VH3N) were unaffected in the HIV-positive patients. This VH3L clonal deficit and other recent phenotype and histopathologic findings suggest that the general B-cell dysfunction in HIV is due to a discreet maturational arrest at the germinal center stage.

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IN NORMAL individuals a critical step in memory B-cell differentiation occurs in the germinal center, a specialized lymphoid microenvironment transiently inhabited by activated B cells. Cells successfully completing this developmental stage become part of a recirculating pool of mature memory cells that form the predominant B-cell population in blood and the lymph node mantle zone. The orderly transition from germinal center stage to the quiescent recirculating pool is a key event in B-cell differentiation.

One striking manifestation of human immunodeficiency virus (HIV) infection is the substantial disturbance in differentiation and function of B lymphocytes. On one hand, this involves hypergammaglobulinemia, generalized lymphadenopathy due to B cell hyperplasia, and frequent development of high-grade B-cell lymphomas including Burkitt’s lymphoma. On the other hand, this paradoxically involves a markedly impaired ability to generate a primary response to neoantigens, and a poor response to polysaccharides in vivo and pokeweed mitogen in vitro. A peculiar “bare follicle” lymph node histology and a decline in B cells expressing certain differentiation antigens (Leu-8 and CD21) are also frequent findings in HIV infection. Many of these B-cell abnormalities occur early in the infection, before substantial depletion of CD4+ T cells or clinically evident disease. Moreover, they are manifested even when purified B cells from HIV-positive individuals are tested with normal T cells, suggesting that the functional deficits are intrinsic to the B cell.

Previously, we have observed that B cells bearing certain rearranged VH3 genes (VH3L) were developmentally restricted. That is, VH3L B cells were uniquely deficient in germinal center cells but abundant in blood and the lymph node mantle zone B-cell population. This restriction appears to be due to a selective expansion of VH3L B cells in the postgerminal center B-cell population. Using a polymerase chain reaction (PCR) based strategy, we have now tested VH3L as an index gene to evaluate this transitional event of B-cell development in HIV-positive individuals.

MATERIALS AND METHODS

Subjects and specimen preparation. Peripheral blood samples were obtained from patients diagnosed with acquired immunodeficiency syndrome (AIDS) according to CDC criteria and normal subjects. Blood mononuclear cells were fractionated by Ficoll-Hypaque gradient. To examine DNA in fixed paraffin-embedded tissues, 10-μm sections were used for each PCR analysis as described by Shibata et al. Specimen procurement from patients and normal subjects was in accordance with protocols approved by the UCLA Human Subject Protection Committee.

Quantitation of genomic VH subfamily utilization. Quantitative PCR analysis of VH subfamily utilization was essentially as described by Valles-Ayoub et al. Genomic DNA was prepared from ~2 x 10^7 cells by standard methods. Quantitative PCR reaction conditions were established for a template range of 50 to 250 ng. Because human B cells usually bear only one fully rearranged allele, the abundance of VDJ segments for a particular VH subfamily directly corresponds to the abundance of B cells in the population expressing that VH subfamily. Oligonucleotides were prepared by Dr Thomas Sutherland (UCLA Molecular Biology Institute Oligonucleotide Synthesis Laboratory, Los Angeles, CA). Oligonucleotide primers used: VH1N, 5’dTCTGGGCT-GAGGTTGAGAAC (coding strand sequence codons 7 through 13, FR1, VH1); VH3L, 5’dCTGGTGGAGTCTGGGGGAGGCC (coding strand sequence, FR1, codons 6 through 12, VH3); VH4/6, 5’dCTGGAGCTGGTGAGAAC (coding strand sequence, FR1, codons 9 through 14, VH4 and VH6); VH3N, 5’dCTCCTCA-CAGGGTGAGTATCC (coding strand sequence, 5’ flank, –68 to –50 upstream of translational start site, VHS); JHKOR, 5’dAGT-GCCAACCGCCTCACAGG (noncoding strand sequence, codons 176 to 186) of Pathology CHS 13-186, UCLA School of Medicine, Los Angeles, CA 90024-1732.

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Fig 1. Selective deficit of VH3L B cells in HIV infection. PCR amplification of rearranged Ig subfamilies (VH1N, VH3L, VH4/6) and N-ras in PBLs from patients with HIV infection and normal individuals. Photographs of agarose gels stained with ethidium bromide.

108 to 2 nt 3’ of codon 113A, consensus human JH); ras83, 5’ dGGGATTCTTACCGAAAGCAAGTG (coding strand sequence of exon I1 of the human N-ras proto-oncogene; ras84, 5’ dATAATAAACTACCGTTTATGTG (noncoding strand sequence within exon I1 of the human N-ras proto-oncogene.

Amplification reactions were performed based on standard conditions using 200 ng of predenatured (94°C, 2 minutes) genomic DNA templates, 0.5 U Thermus aquaticus DNA polymerase (Taq) (Perkin-Elmer/Cetus, Emeryville, CA), 100 pmol oligonucleotide (each), 200 μmol dNTP, and 0.1 μCi 32P-[α]-dCTP (Amersham, Chicago, IL) with a final reaction volume of 100 μL. Reaction mixtures for VH3L were subjected to 40 successive cycles consisting of: heat denaturation (94°C, 1 minute), annealing, and primer extension (72°C, 2 minutes). Reaction mixtures for N-ras and the remaining VH subfamilies were subjected to 40 successive cycles consisting of: heat denaturation (94°C, 1 minute), annealing (55°C, 1 minute), and primer extension (72°C, 2 minutes) using the Bellco DNA Pacer (Vineland, NJ). PCR products were fractionated using 1%/1% Nu-Sieve/LE agarose (FMC, Rockland, ME) in 1x TPE (0.08 mol/L TRIS-phosphate, 0.008 mol/L EDTA) buffer. Gels were briefly stained with ethidium bromide to visualize bands; the apparent sizes of the amplified products were: VH1N, 440 bp; VH3L, 380 bp; VH4/6, 434 bp; VH5N, 583 bp and N-ras, 147 bp. N-ras, a single copy gene, was included as a control for the quality of DNA harvested and a normalizing denominator for PCR reactions. For quantitative data, bands from agarose gels were excised and Cerenkov counts measured using Beckman LS-7000 scintillation counter (Palo Alto, CA).

RESULTS

The abundance of B cells bearing rearranged VH subfamilies was determined using VH-specific oligonucleotides in a quantitative PCR assay (see Materials and Methods). An initial examination of blood samples from five HIV-positive and three normal individuals is shown in Fig 1. B cells bearing rearranged VH subfamilies (VH1N, VH4/6, VH5N) were abundant in both populations. VH3L B cells were also abundant in the normal individuals, but unexpectedly were deficient in this initial study of HIV-positive individuals. To confirm whether these distinct levels of VH3L B cells were characteristic of each population, an expanded panel of additional subjects was tested (Fig 2). This panel included 13 HIV-positive subjects and 10 normal subjects. Two samples from HIV-positive individuals (lanes 5 and 13) were excluded because of insufficient DNA. Again, a uniform deficit of VH3L B cells was observed exclusively in HIV-positive subjects. It is interesting to note an apparent overabundance of VH1 and VH4/6 in the HIV-positive patients in Fig 1. If so, this could suggest a compensatory mechanism or an additional aspect of the clonal selection process affecting HIV B-cell populations that might merit further study.

We questioned whether this was a distinctive pattern in the blood compartment or reflected a systemic lymphoid deficit. To address this issue, we selected archival tissues of spleen and lymph node from three HIV-positive and three HIV-negative autopsies (Fig 3). These six autopsy cases were performed during 1989 through 1990, all were males and matched for age (20 to 35 years). All three HIV-positive cases had documented physical findings of lymphadenopathy. The histologic findings in this group included
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marked follicular dissolution without evidence of lymphoma; case 2 was notable for lymphocyte depletion. The HIV-negative cases included two cases of accidental death (cases 1 and 2) and one case of congenital heart disease (case 3). VH3L, the developmentally restricted subfamily, was compared with VH5N, a nonrestricted B-cell subfamily. As expected, VH5N was abundant in spleen and lymph node tissue from HIV-negative and HIV-positive cases. The one exception was the lymph node from case 2 of the HIV-positive group. This low abundance of VH5N was consistent with the histologic finding of marked lymphopenia in this specimen. In contrast, VH3L B cells, while abundant in spleen and lymph node in the HIV-negative group, were uniformly deficient in the HIV-positive group. This finding confirms that this deficit was reflective of a systemic change in B-cell clonality.

Lymphopenia is a major abnormality of immune function in AIDS. In a previous study we have shown that the abundance of unrestricted clones, such as VH5N, are representative of lymphocytes in a mixed cellular population. Therefore, we adopted this analysis and recalculated the data from Fig 3 to examine the possibility that this recurring VH3L deficit might be part of a general lymphocyte depletion. We compared the relative abundance (measured Cerenkov counts) of VH3L with VH5N in postmortem cases (Fig 4). Generally, VH3L B cells were five times more abundant in normal spleen and three to four times more abundant in normal lymph node compared with corresponding HIV-positive tissues. This examination of peripheral blood lymphocytes (PBLs) and autopsy tissues identifies a VH clonal B-cell deficit in HIV-positive individuals.

DISCUSSION

This study describes a profound deficit of VH3L B cells in HIV-positive individuals. In contrast to this selective deficit, other VH gene subfamilies remained relatively unaffected. This depletion was not only localized to blood, but also present in spleen and lymph node populations. A simple explanation for this clonal deficit would be that VH3L represents a B-cell subset that is a natural target for HIV infection, analogous to the CD4⁺ T-cell subset. However, most evidence indicates that B cells are not susceptible to direct HIV infection. A more promising explanation is that the B-cell deficit is a result of an immunophysiologic disturbance interfering with proper B-cell maturation with associated impairment in B-cell function. We became
intrigued by the similarity between the peculiar pattern of differentiation antigens in most HIV-positive individuals' PBLs and the phenotype of germinal center B cells (CD20<sup>hi</sup>, CD21<sup>high</sup>, Leu<sup>8</sup>).<sup>9</sup> Also, the selective unresponsiveness of B cells from HIV-positive to PWM in vitro is a feature of normal germinal center B cells. Moreover, the lymphoid histology of HIV-positive individuals with lymphadenopathy most commonly involves expanded germinal center but attenuated mantle zone areas.<sup>8,12,18</sup>

We have used VH3L B cells as index clones of B cells at a stage of normal development, the recirculating pool of post germinal center lymphocytes. A disturbance in completion of germinal center differentiation would explain the eventual attrition of memory cells which accounts for the main feature of this humoral immunodeficiency. Numerous immunoregulatory abnormalities in HIV infection may contribute to this maturational arrest. Among them, we are particularly intrigued by the possible early depletion of CD29, CD4 T cells, the predominant memory T-cell population, and probable cell type driving entry of B cells into germinal center differentiation<sup>19-21</sup>; and that the follicular dendritic cell, a unique and critical component of the germinal center microenvironment, is a direct target of HIV infection.<sup>22-24</sup>

We have recently reported that common variable immunodeficiency (CVI), a rare disorder of quantitative and qualitative antibody production, is also characterized in most cases by a germinal center maturational arrest of B cells, including the same functional, phenotypic, and clonal (eg, VH3L) abnormalities noted in HIV infection.<sup>25,26</sup> Causally, CVI and HIV infection also share the feature of systemically elevated interleukin-6 with its immunopathologic and clinical sequelae.<sup>27,28</sup> Taken together, these findings strongly support the idea that HIV-associated defect in B-cell development defines a class of immunodeficiency diseases that involves a maturational arrest at the germinal center stage.

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