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22 April 1991; accepted 12 August 1991

Selective Depletion in HIV Infection of T Cells That Bear Specific T Cell Receptor V_β Sequences

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The mechanism of T cell depletion during infection with the human immunodeficiency virus (HIV) is unclear. Examination of the repertoire of T cell receptor V (variable) regions in persons infected with HIV revealed the absence of a common set of V_β regions, whereas V_α usage was normal. The lack of these V_β segments did not appear to correlate with opportunistic infections. The selective elimination of T cells that express a defined set of V_β sequences may indicate the presence of an HIV-encoded superantigen, similar to those encoded by the long terminal repeat of the mouse mammary tumor virus.

MOST ANTIGENS ARE RECOGNIZED through their interaction with the variable V portions of the T cell receptor (TCR) α and β chains (*1*). However, T cells recognize another category of ligands, the superantigens, on the basis of the expressed V_β region alone, independently from the other variable TCR segments (*2–4*). Because the murine mammary tumor virus C-type retrovirus has superantigen properties (*5, 6*), HIV may also encode a superantigen that could participate in T helper cell impairment and destruction. This hypothesis suggests that cell depletion must preferentially affect those T cells expressing the V_β elements that can interact with the retrovirus-encoded superantigen (*7*).

We analyzed the expression of most of the known TCR V_α and V_β genes by polymerase chain reaction (PCR) in peripheral blood cells obtained from six patients affected by the acquired immunodeficiency syndrome (AIDS) (CDC stage IVC1; symptomatic HIV^+ patients with a history of major opportunistic infections and $CD4^+$ lymphocyte counts $<200/mm^3$) and from six healthy HIV^- individuals. Total RNA was prepared from each sample immediately after collection and, at the time of analysis, was reverse-transcribed into cDNA. Aliquots of cDNA were amplified with each of the 22 5′ V_β-specific sense primers and a 3′ C_β-specific antisense primer (*8*) or with each of the 19 5′ V_α-specific sense primers and a 3′ C_α-specific antisense primer (*9*). The expression of each V gene transcript was operationally defined by an optical density value from an enzymatic immunoassay (DEIA), done with a C_β- or a C_α-specific capture probe that mapped to a region internal to the amplified cDNA. The sensitivity and the specificity of the assay are similar to conventional Southern blot (*10*).

There were no major differences between the V_α repertoires of normal individuals and those of AIDS patients (Fig. 1A). Most of the V_α genes were expressed in all samples with no evidence of selective expression. In contrast, comparison of the V_β repertoire of AIDS patients and normal controls revealed differences in the V_β genes expressed by the two groups (Fig. 1B). The V_β14, V_β15,

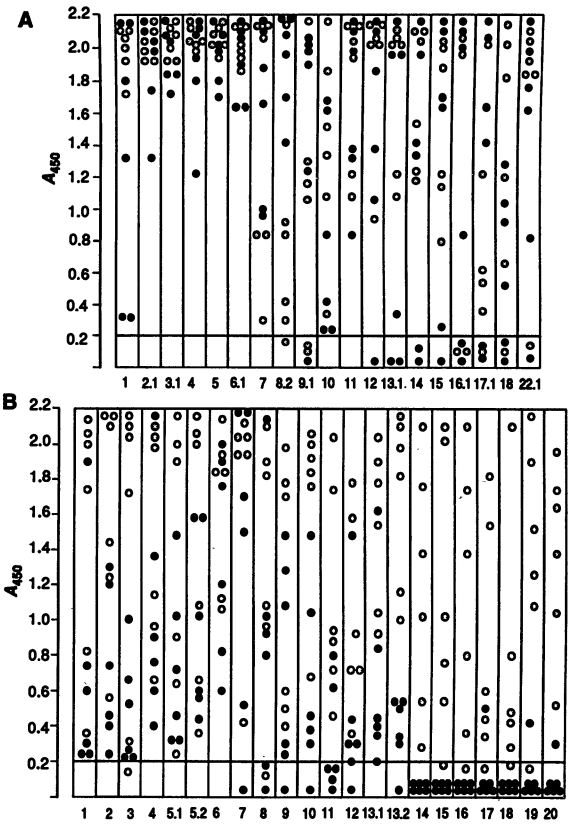

Fig. 1. Expression of TCR (**A**) V_α and (**B**) V_β genes in T cells from AIDS patients (●) and from normal controls (○). The results are expressed as absorbance at 450 nm (A_{450}) from a DEIA test. The cutoff value of 0.20 represents the mean value of ten negative controls ±3 SD. Total RNA was prepared from peripheral lymphocytes by the guanidinium thiocyanate-phenol-chloroform method (*14*). The total RNA (2 μg) preparations were used to synthesize the first strand of cDNA with the Riboclone cDNA Synthesis System (Promega Biotec). Amplification by PCR of the TCR V_α and V_β rearranged genes was as described (*15*), with 19 V_α family–specific (*9*) and 22 V_β family–specific primers (*8*). The specificity of each PCR product was verified by DEIA (*10*) with $C_\beta NH_2$- (ACCCAAAAGGCCACACTGGTGTGCCTGGCC) and $C_\alpha NH_2$- (CAGTGACAAGTCTGTCTGCCTATTCACCGA) specific capture probes, mapping to regions internal to the amplified cDNAs.

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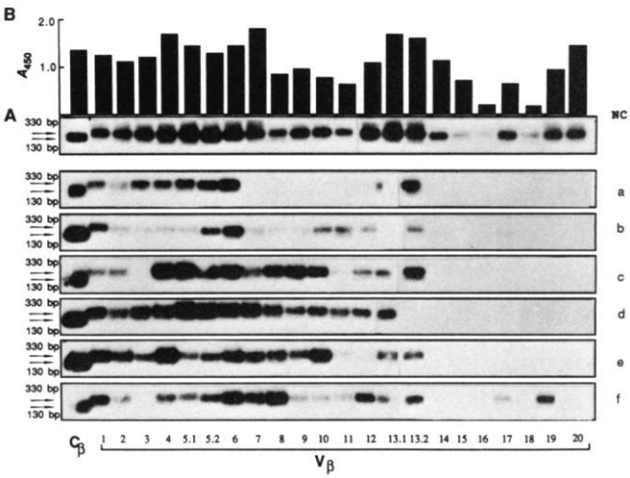

Fig. 2. (**A**) Southern (DNA) blot analysis of amplified TCR V_β and C_β transcripts, obtained with T cells of AIDS patients (a to f) and T cells of a normal control (NC). C_β transcripts were amplified with the $C_\beta 3'$ and the $C_\beta 5'$ oligonucleotides (*8*). DNA was transferred to Hybond N nylon-blotting membrane (Amersham International) as indicated by the manufacturer. The filters were prehybridized for 2 hours at 65°C in 5× saline sodium citrate (SSC), 5× Denhardt's solution (1× Denhardt's: 0.02% polyvinyl pyrrolidone, 0.02% Ficoll, and 0.02% BSA), 0.5% SDS, and DNA salmon sperm (100 μg/ml). Hybridization was carried out overnight at 65°C with a $C_\beta 5'$-specific labeled oligonucleotide (*8*) that was specific for a region internal to the amplified product. The oligonucleotide was labeled with γ [^{32}Pd]ATP (specific activity 3000 Ci/mmol; Amersham International) with the polynucleotide kinase (New England Biolabs). After hybridization, the filters were washed twice for 10 min at room temperature in 2× SSC and 0.2% SDS and autoradiographed at −70°C. (**B**) Results of the DEIA of the amplified TCR V_β transcripts obtained with T cells of the NC. The test was carried out as described (*10*) with the $C_\beta NH_2$ (*8*) as capture probe.

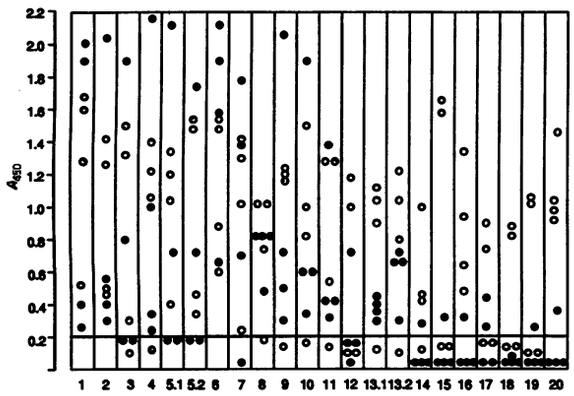

Fig. 3. Expression of TCR V_β genes in T cells of group A (●) and group B (○) HIV$^+$ patients. The results are expressed as optical density values, obtained by DEIA as described in Fig. 1.

$V_\beta 16$, and $V_\beta 18$ sequences could not be detected in any lymphocyte sample from AIDS patients. Similarly, $V_\beta 17$, $V_\beta 19$, and $V_\beta 20$ were expressed in only one sample. All other V_β genes were randomly represented in the T cells from AIDS patients, suggesting that the observed impairment in V_β gene expression is V_β family–specific.

We also analyzed TCR V_β expression in T cells from each AIDS patient and from one normal control by transferring the amplified cDNA to nitrocellulose filters and hybridizing with a ^{32}P-labeled C_β-specific probe (Fig. 2A). For comparison, the results obtained with T cells from the normal healthy control are also expressed as DEIA values (Fig. 2B). The control expressed all of the V_β genes, but the amount of the individual transcripts differed considerably. In this sample, $V_\beta 15$, $V_\beta 16$, and $V_\beta 18$ were the least expressed; $V_\beta 5.1$ and $V_\beta 13.1$ were the most abundant.

The V_β repertoires of the AIDS patients were, in general, more restricted as compared to that of the normal control, but the degree of this restriction varied considerably among the different samples. Patient b expressed only $V_\beta 1$, $V_\beta 5.2$, and $V_\beta 6$; all other transcripts were either absent or barely detectable. The next most compromised repertoire was that of patient a, in which we detected only eight of the 22 transcripts tested. Patient d expressed the most V_β genes, but the transcription of eight V_β segments was not detected. Although there was individual variation, all samples lacked the expression of a common set of V_β genes: $V_\beta 14$, $V_\beta 15$, $V_\beta 16$, $V_\beta 17$, $V_\beta 18$, $V_\beta 19$, and $V_\beta 20$. The only exception was patient f, who expressed $V_\beta 19$ and barely detectable $V_\beta 17$. Thus, HIV infection appears to result in a severely compromised V_β repertoire in which members of particular V_β families are preferentially affected.

We investigated whether V_β depletion was caused directly by HIV or by some of the opportunistic infections that arise during disease progression by analyzing the expression of V_β genes in two other groups of HIV$^+$ patients. One group consisted of asymptomatic patients with severe lymphocytes depletion (CD4$^+$ lymphocyte count $<200/mm^3$), but without malignancy or opportunistic infection (group A, CDC stage III). The other group consisted of asymptomatic patients who were HIV positive for more than 5 years and had normal lymphocyte numbers (group B, CDC stage II). The two groups displayed a distinct pattern of V_β expression (Fig. 3). The pattern of group A resembled that of the AIDS patients from Fig. 1B, in which $V_\beta 14$, $V_\beta 15$, $V_\beta 16$, $V_\beta 17$, $V_\beta 18$, $V_\beta 19$, and $V_\beta 20$ were either not expressed or barely detectable. Conversely, group B V_β expression was similar to that of the normal controls, in which most of the V_β genes were randomly represented.

These results are compatible with a hypothesis proposed after the discovery that murine endogenous superantigens are encoded by the mammary tumor virus in the open reading frame of the 3′ long terminal repeat (*6*). Because superantigens cause the elimination of responsive T cells, it was suggested that HIV might cause cell anergy and depletion of noninfected CD4$^+$ T cells by encoding a superantigen expressed on activated infected cells, in conjunction with the major histocompatibility complex (MHC) class II proteins (*7, 11*). The hypothesis also states that progression of CD4$^+$ T cell depletion requires cycles of mutation in the retroviral superantigen gene, resulting over time in the elimination of CD4$^+$ T cells bearing different V_βs. Deletion of some V_β segments in our study was almost complete; thus, both CD4$^+$ and CD8$^+$ cells that express the relevant V_β genes are probably targets of inactivation. Therefore, HIV superantigens may eliminate CD4$^+$ cells expressing several V_βs, whereas only those CD8$^+$ cells that express V_β elements with high affinity for the retrovirus-encoded superantigens are deleted.

The V_β-specific deletions correlated with the pathogenesis of the disease and not with the secondary effects of opportunistic infection. If HIV encodes a superantigen responsible for T cell depletion, the individual differences in development of clinical symptoms of AIDS may be explained by the documented variability of the affinity of the various MHC class II alleles for some superantigens (*12*). In addition, TCR V gene polymorphism (*13*) may also affect the differences of the affinity of interaction between the different alleles of a given V_β gene and the retrovirus-encoded superantigen.

Our data confirm that no major V_α or V_β

deletions exist in the repertoire of the normal subjects (*13*) and show a pattern of specific V_β deletion in retrovirally infected humans. In mice, some V_β clonotypes susceptible to clonal deletions mediated by endogenous superantigens cross-react with exogenous superantigens (*4*). Thus, elimination of these cells early in ontogenesis may prevent the adverse effects that might occur later in life if such antigens are encountered (*4*). However, the absence of somatically imposed V_β deletions suggests that this concept cannot be applied to humans. Hence, the evolutionary pressures that have favored the advantages of maintaining a large T cell repertoire in humans may be ultimately responsible for the devastating pathogenic effect of HIV infection.

In conclusion, our results lend support to the hypothesis that HIV-encoded proteins may deliver anergic signals to noninfected T cells through interactions with specific V_β sequences. The final proof of this model will require the identification and the molecular characterization of the HIV-encoded superantigens. Such a characterization may also aid in avoiding deleterious effects of candidate vaccines.

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9. Primers for V_α used in this study: V_α1, CTGAGGTGCAACTACTCA; V_α2, AGAGGGAGCCTTAGCCTCTCTCAA; V_α3, AATGCCACCATGAACTGCAGTTAC; V_α4, ACAAGCATTACTGTACTCCTA; V_α5, GGCCCTGAACATTCAGGA; V_α6, TGACCAGCAAAATGCAACAGAAGG; V_α7, AGGAGCCATTGTCCAGATAAA; V_α8, GCTTATTCAAACAGCGCCTCAGAC; V_α9, CAGAGAGTGACTCAGCCCGAGAAG; V_α10, ACCCAGCTGGTGGAGCAGAGCCCT; V_α11, AGAAAGCAAGGACCAAGTGTT; V_α12, CACAACCTAACTCAAGCGCAGACT; V_α13, CTCATCAACCTGTTTTACATTCCC; V_α14, GCAGCTTCCCTTCCAGCAAT; V_α15, AGAACCTGACTGCCCAGGAA; V_α16, CCTCCAGTTCCTTCTGAA; V_α17, CAGCAGGTGAAACAAAGTCCTCAA; V_α18, TGTCAGGCAATGACAAGG; V_α22, TACACAGCCACAGGATACCCTTCC; C_α3′, ATGTCTAGCACAGTTTTGTCTGTG; and C_α5′, ATATCCAGAACCCTGACCCTGCCG.
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16. Supported by Sorin Biomedical (Saluggia, Italy) and IV Progetto AIDS, Istituto Superiore di Sanità.

26 June 1991; accepted 10 September 1991

Structure of a Legume Lectin with an Ordered N-linked Carbohydrate in Complex with Lactose

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The three-dimensional structure of the lactose complex of the *Erythrina corallodendron* lectin (EcorL), a dimer of N-glycosylated subunits, was determined crystallographically and refined at 2.0 angstrom resolution to an *R* value of 0.19. The tertiary structure of the subunit is similar to that of other legume lectins, but interference by the bulky N-linked heptasaccharide, which is exceptionally well ordered in the crystal, forces the EcorL dimer into a drastically different quaternary structure. Only the galactose moiety of the lactose ligand resides within the combining site. The galactose moiety is oriented differently from ligands in the mannose-glucose specific legume lectins and is held by hydrophobic interactions with Ala^{88}, Tyr^{106}, Phe^{131}, and Ala^{218} and by seven hydrogen bonds, four of which are to the conserved Asp^{89}, Asn^{133}, and NH of Gly^{107}. The specificity of legume lectins toward the different C-4 epimers appears to be associated with extensive variations in the outline of the variable parts of the binding sites.

RECOGNITION OF COMPLEX CARBOhydrates by lectins (*1*) has been implicated in important biological processes such as protein targeting to cellular compartments (*2*), homing of leukocytes (*3*), and host-pathogen interactions (*1*). The readily available plant lectins (*4, 5*) have become a paradigm for protein-carbohydrate recognition at the cell surface because of their ability to detect subtle variations in carbohydrate structures found on proteins and lipids from various sources. Structures of several plant lectins specific for mannose-glucose (Man-Glc), *N*-acetylglucosamine (GlcNAc)–sialic acid, and complexes of these lectins with sugars have been determined by crystallographic methods (*6–10*).

We report the high-resolution crystal structure of the *Erythrina corallodendron* lectin (EcorL) (*11*), which is specific for galactose and its derivatives, in complex with lactose (*12*). This legume lectin is a dimer of a 30-kD glycosylated subunit, homologous to other lectins of the same family (*5*), with a plant-specific heptasaccharide Manα6(Manα3)(Xylβ2)Manβ4GlcNAcβ4(L-Fucα3)GlcNAcβ N-linked to Asn^{17} of each subunit (*13*) (Xyl, xylose, and Fuc, fucose). Each subunit contains Mn^{2+} and Ca^{2+}, which are essential for the lectin activity (*11*). Beyond aspects related to the basis of sugar-binding specificity of the legume lectins, this report deals with the effect of glycosylation on protein assembly as well as with the interactions that stabilize branched surface carbohydrates in a single conformation.

The crystals of the EcorL-lactose complex are monoclinic *C*2, with cell dimensions a = 84.40 Å, b = 73.05 Å, c = 71.40 Å, and β = 113.42°, and contain one monomer in the asymmetric unit (*14*). The structure was determined by molecular replacement (*15*) by using the known structures of concanavalin A [Con A; (*16, 17*)] and pea lectin [PL (*9*)] as models. The *R* value of the current model is 0.190 at 2.0 Å resolution (*15*).

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