TCR expression and clonality analysis in peripheral blood and lymph nodes of HIV-infected patients.

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# TCR Expression and Clonality Analysis in Peripheral Blood and Lymph Nodes of HIV-Infected Patients

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**ABSTRACT:** We compared the T cell receptor (TCR) Vβ gene family repertoire in peripheral blood mononuclear cells (PBMC) and lymph node (LN) cells from 7 human immunodeficiency virus (HIV)-infected patients and 3 seronegative healthy controls. Virtually all the Vβ family specificities were represented in patient PBMC and LN cells, and mean values for each specificity were comparable to figures in seronegative controls. In 4 patients, however, some Vβ gene segment transcripts were overrepresented in the LN compartment, compared to the peripheral blood counterpart. To ascertain whether this phenomenon was due to polyclonal or oligoclonal expansion of T cells bearing the relevant Vβ gene product, we sequenced the entire CDR3 region of a panel of 238 PCR clones corresponding to the Vβ transcripts expanded in LN; as control, the same regions were cloned and sequenced in patient's PBMC, and in PBMC and LN cells from seronegative individuals. This analysis disclosed preferential usage of Jβ2 genes in PBMC and LN cells from both seropositive patients and controls, regardless of the Vβ gene segment considered, thus indicating that this skewness in the Vβ-Jβ repertoire could be a consistent feature of at least a part of the Vβ repertoire in different lymphoid compartments, regardless of the pathologic conditions. In addition, in LN from HIV seropositive patients we found the presence of recurrent TCR rearrangements, accounting for 8–23% of the generated clones, in each of the 4 Vβ specificities analyzed; recurrent sequences were not found in PBMC from patients nor in PBMC and LN cells from seronegative controls. These findings suggest that antigen-driven oligoclonal T cell expansions may occur *in vivo* in lymphoid organs of HIV seropositive patients. *Human Immunology 57, 93–103 (1997).* © American Society for Histocompatibility and Immunogenetics, 1997. Published by Elsevier Science Inc.

**ABBREVIATIONS**

| | |
|---|---|
| AIDS | acquired immune deficiency syndrome |
| HIV | human immunodeficiency virus |
| LN | lymph node |
| PBMC | peripheral blood mononuclear cells |
| PCR | polymerase chain reaction |
| SAg | superantigen |
| TCR | T cell receptor |
| WR | Walter Reed |

## INTRODUCTION

Both direct and indirect pathogenetic mechanisms [1–5] have been advanced to explain the progressive depletion of $CD4^+$ T lymphocytes that preceeds the onset of full-blown acquired immunodeficiency syndrome (AIDS), but their relative relevance to the natural history of the disease is unclear. The finding that some microbial antigens, so-called superantigens (SAg), are capable of stimulating and eventually deleting entire subsets of T lymphocytes bearing distinct Vβ specificities [6–9] led to the hypothesis that human immunodeficiency virus (HIV) also might encode for an SAg, that could determine the vast $CD4^+$ T cell depletion seen in seropositive patients. The available experimental evidence, however, is contradictory. Following the initial report by Imberti

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*Received August 28, 1997; accepted October 7, 1997.*

*The first two authors contributed equally to this work.*

Human Immunology 57, 93–103 (1997)

Published by Elsevier Science Inc.

0198-8859/97/$17.00
PII S0198-8859(97)00205-X

**TABLE 1** Clinical, Immunologic and Virologic Features of the HIV-Infected Patients Studied

| Patient | Stage | Therapy | $CD4^+$ cells/$mm^3$ | Serum p24 (pg/ml) | Plasma viral copies/ml |
|---|---|---|---|---|---|
| A | WR4 | Zidovudine | 291 | 16.2 | 124,100 |
| B | WR3 | — | 382 | <10 | 2,020 |
| C | WR2 | — | 373 | <10 | 3,250 |
| D | WR6 | Zidovudine | 114 | 33.8 | 114,180 |
| E | WR4 | Zidovudine | 277 | <10 | 58,060 |
| F | WR4 | Zidovudine | 252 | <10 | 55,870 |
| G | WR4 | Zidovudine | 196 | <10 | 208.910 |

et al. [10] that several Vβ gene transcripts in peripheral blood mononuclear cells (PBMC) of AIDS patients were deleted, other workers did not confirm preferential deletion of Vβ segments [11, 12]; moreover, comparison of the T cell receptor (TCR) repertoire in monozygotic twins discordant for HIV infection [13], as well as in seropositive mothers and their HIV-infected offspring [14], did not reveal any recurrent Vβ gene family deletion. In another setting, analysis of the TCR repertoire in simian immunodeficiency virus-infected macaques also did not show any recurrent Vβ alterations [15].

On the other hand, the body of contradictory findings on the SAg issue [10–15] could fit in with the possibility that the immune response to HIV antigens might shape the immune system in an individual-specific manner, and lead to oligoclonal expansions of some T cell subsets over a polyclonal background. This issue, however, has not been extensively addressed in peripheral lymphoid organs, which constitute the major HIV reservoir and primary site of virus replication [16]. We therefore compared the TCR repertoire in the PBMC and lymph nodes (LN) of 7 HIV-infected patients with different disease progression. We found an increased expression of certain Vβ families in the LN, compared to the corresponding PBMC; in the former, molecular cloning disclosed the presence of identically rearranged CDR3 sequences, thus suggesting that multiple expansions of unique T cell clones may derive from in vivo antigen-driven stimulation.

## MATERIALS AND METHODS

### Study Population

Seven HIV-infected patients underwent routine clinical and laboratory evaluation, and were staged according to the Walter Reed (WR) staging classification [17]; their clinical, virologic and immunologic features are summarized in Table 1. These patients underwent LN biopsy for diagnostic purposes; in every case, the histopathologic picture was compatible with HIV-related alterations. A blood sample was taken just prior to biopsy. Ten healthy laboratory staff members served as seronegative blood donors; control LN and PBMC samples were obtained from 3 seronegative patients who underwent minor surgery. All the participants gave their informed consent to this study.

### Preparation of RNA and cDNA Synthesis

PBMC were isolated from heparin-anticoagulated venous samples by Ficoll-Paque (Pharmacia-LKB, Uppsala, Sweden) gradient centrifugation, as described [18]. Total RNA was extracted from liquid nitrogen-frozen LN and freshly isolated PBMC using the RNAZolB method (Biotex Laboratories, Inc., Houston, TX), as previously reported [19, 20]. One μg of total RNA was used for the synthesis of first strand cDNA using reverse transcriptase (Gibco Brl, Gaithersburg, MD) and random hexamer primers, according to the manufacturer's instructions.

### Polymerase Chain Reaction (PCR) Analysis of Vβ Gene Family Expression

We utilized a quantitative PCR method to estimate the proportion of T cells expressing particular Vβ gene segments. For each PCR, an aliquot of each cDNA sample was co-amplified using Vβ-specific primer and an oligomer from the downstream β chain region (Cβ primer) as one pair, and two Cα primers as the other. The sequences of the specific primers used, and details of the PCR technique were described elsewhere [21]. Briefly, amplification was performed in a 50 μl volume containing 0.2 μM of each primer and 0.7 U of Taq polymerase (Perkin-Elmer, Emeryville, CA) under the following conditions: 95°C denaturation, 52°C annealing, and 72°C extension, 1 min each, for 30 cycles. To quantify the amplified products, co-amplification was performed with reverse primers end-labeled with $^{33}P$. The amplified products were separated on 5% acrylamide gels, dried, exposed to X-ray film at −80°C, and analyzed by an ultrascan XL enhanced laser densitometer (Pharmacia-LKB). The data were expressed as the amount of Vβ product over the amount of Cα product; the percent expression of each Vβ region was calculated in relation to the sum of all 22 ratios according to the following formula:

$$\%V\beta_s = \frac{V\beta_s/C\alpha_s}{\sum(v\beta/C\alpha)_{1-22}}$$

where $s$ is sample.

To evaluate the variability of our PCR assay, we conducted independent amplifications of the same cDNA sample: the findings (data not shown) showed that inter-assay variability did not exceed 20%.

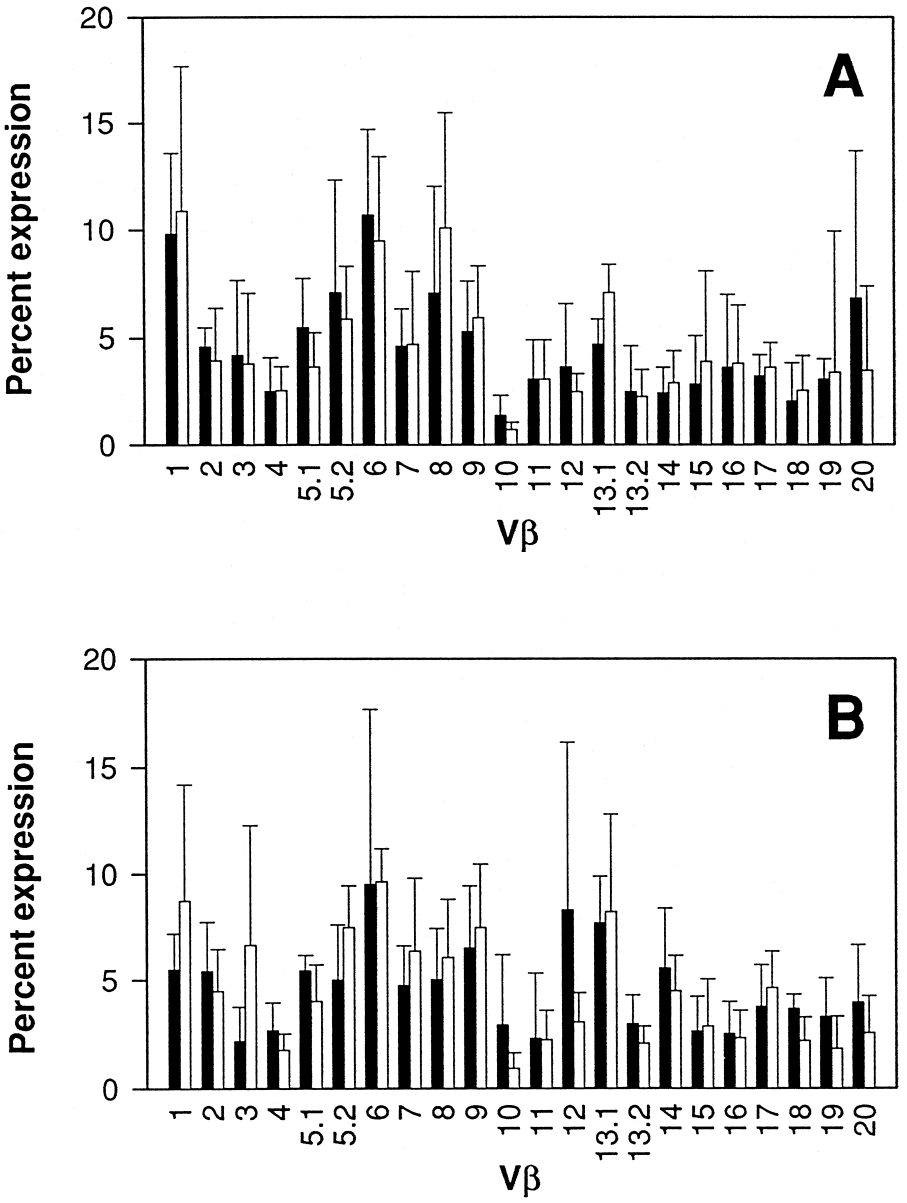


**FIGURE 1** TCR Vβ gene-segment expression in PBMC (panel A) and LN cells (panel B) from 10 healthy seronegative individuals (closed bars) and 7 $HIV^+$ patients (open bars). The columns represent mean values (±1 SD) of Vβ densitometric determination.

### Cloning and Sequencing

For cloning purposes, the PCR product of the selected Vβ was migrated on a 2% agarose gel. Bands with the expected size were isolated, and the PCR product purified by the Wizard PCR purification kit (Promega, Madison, WS).

The material was cloned into a TA cloning vector using the TA Cloning System kit (Invitrogen, San Diego, CA), according to the manufacturer's instructions, and used to transform INVαF′ *Escherichia coli* strains. Plasmid DNA was extracted from positive colonies, and sequenced by the dideoxy chain-termination procedure, using an appropriate sequencing kit (Promega). To prevent artifacts introduced by the cloning procedure, and minimize the inter-test variability, PBMC and LN samples from a same individual were always cloned and sequenced during the same experimental session.

### Viral RNA Quantitation

Genomic HIV-RNA was quantified in plasma using a competitive reverse transcriptase PCR, as previously described [22].

### Statistical Analysis

Data were managed by the Mann-Whitney and Wilcoxon test, as appropriate.

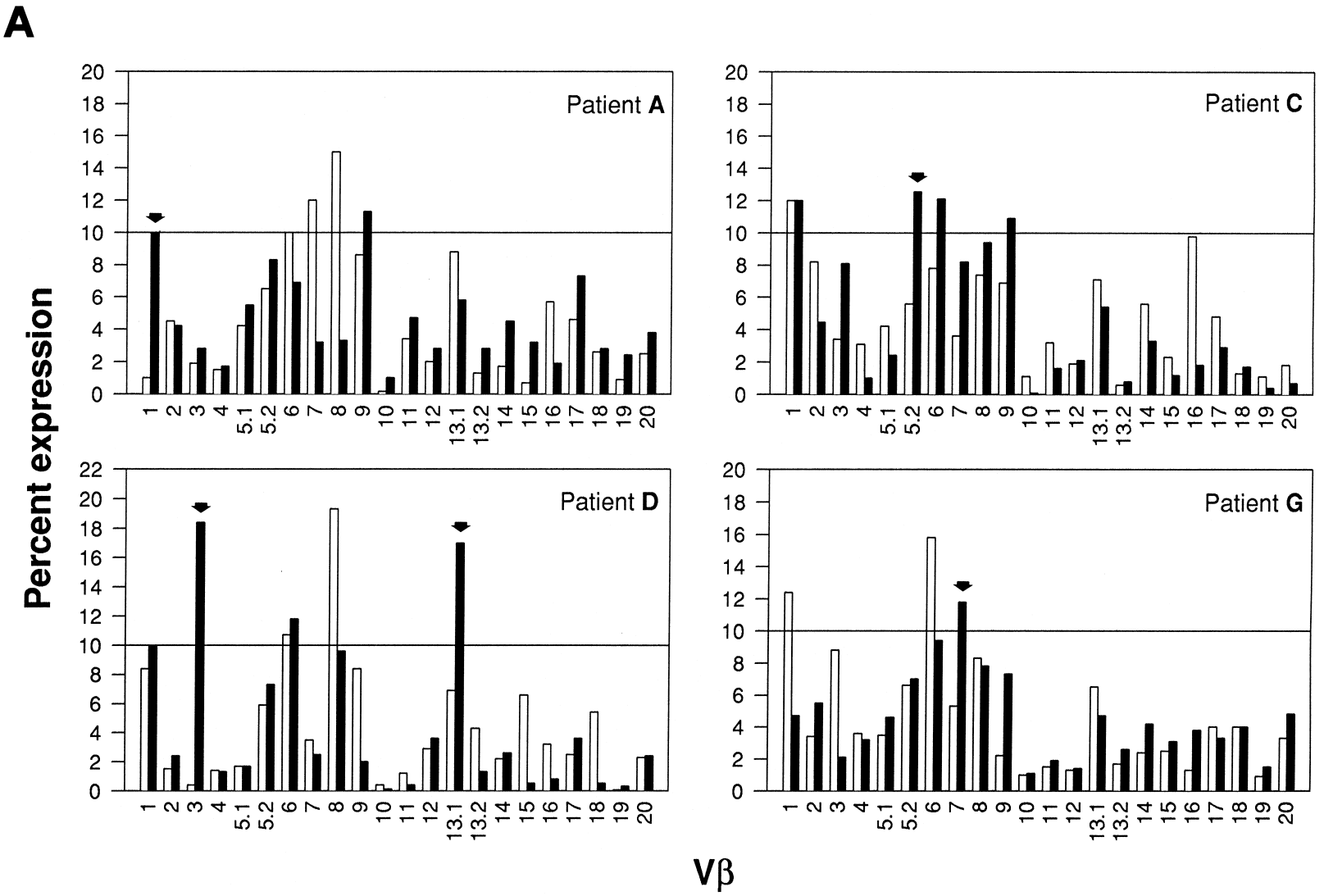


**FIGURE 2** Comparative analysis of the TCR Vβ repertoire in peripheral blood (open columns) and lymph nodes (closed columns) from HIV-infected patients (panel A) and seronegative controls (panel B). The columns represent the Vβ expression percentages, as determined in *Methods*; the arrows identify Vβ segments showing significant overexpression, compared to the PBMC counterpart (according to the criteria described in text), and the horizontal lines denote the arbitrary 10% cut-off value.

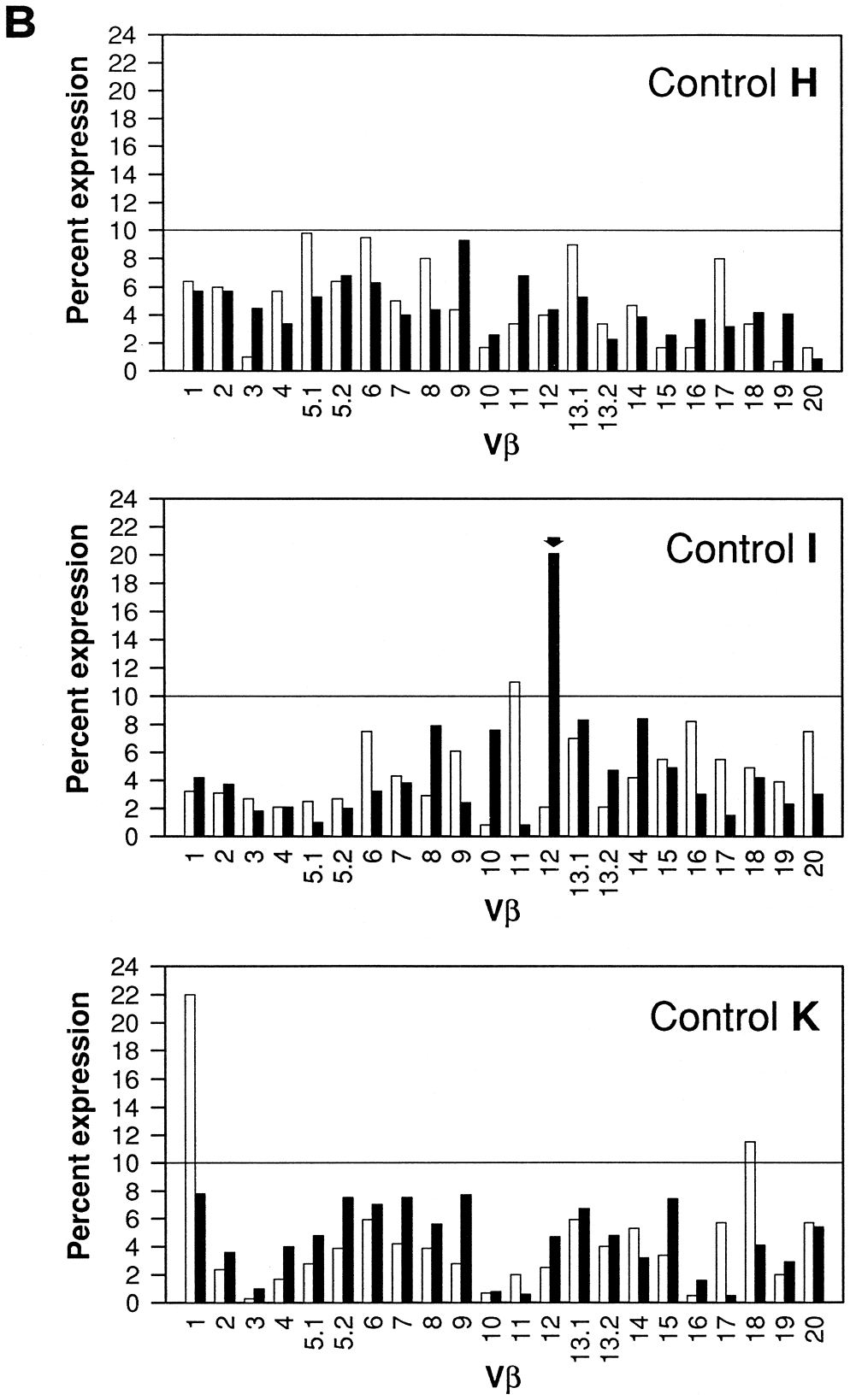


FIGURE 2 continued

## RESULTS

### Vβ Gene Expression in PBMC and LN Cells from HIV-Infected Patients and Controls

We first compared the TCR Vβ gene family profile in peripheral blood of HIV-infected patients and seronegative controls (Fig. 1A). As also reported by others [23], the mean percent expression of each Vβ gene family in healthy donors (closed columns) ranged from 0.5 to 10%, with marked individual differences in the usage of some specificities (see Fig. 2, panel B). All the Vβ family specificities were also detectable in PBMC from the HIV-infected individuals (Fig. 1A, open columns); no statistically significant differences in Vβ gene usage were seen in these patients, compared to control figures. Similar results were obtained when Vβ family usage in LN cells from seropositive and seronegative individuals was compared (Fig. 1B).

### Comparison of Vβ Gene Family Repertoire in PBMC and LN from HIV-Infected Patients

Comparative analysis of the LN and PBMC Vβ profile in individual patients revealed profound differences between the two lymphoid compartments; in three patients (B, E, and F), the LN Vβ gene usage pattern was comparable to that seen in the corresponding PBMC (not shown), whereas in the other 4 patients (A, C, D, and G) some gene transcripts were differently represented in the two compartments (Fig. 2, panel A). To compare PBMC and LN data, we arbitrarily considered as significant only Vβ family expression values that at least doubled those recorded in PBMC, and exceeded 10% of the total repertoire; this threshold should exclude minor Vβ expression changes due to technical variability or lymphocyte trafficking between the two compartments. According to these criteria, Vβ3 and Vβ13.1 were over-represented in the LN from patient D, while the Vβ1, Vβ5.2, and Vβ7 families were expanded in LN from patients A, C, and G, respectively (Fig. 2, panel A, arrows). These figures were confirmed in at least 2 independent experiments; as outlined above, repeated PCR amplifications on a same PBMC sample showed that experimental PCR variability did not exceed 20%. Indeed, according to the above criteria, we also occasionally observed individual-specific reductions in the expression of certain Vβ specificities in LN, compared to the corresponding PBMC; in particular, Vβ7 in patient A, Vβ8 in patient D, and Vβ1 in patient G were under-represented in the LN compartment (Fig. 2, panel A).

As the physiological distribution of the various Vβ specificities in the two lymphoid compartments has only been partially characterized in humans [24, 25], and unequal distribution of certain specificities might occur due to lymphocyte trafficking, we compared Vβ usage in PBMC and LN from 3 seronegative subjects (Fig. 2, panel B). In two of these individuals (Controls H and K), no Vβ gene product showed a significant prevalence in LN, compared to PBMC; in Control I, instead, the Vβ12 gene segment was significantly overexpressed, compared to PBMC figures. In addition, as stated above, occasional overexpression of some Vβ families was observed in PBMC (Vβ11 in Control I, and Vβ1 in Control K; Fig. 2B), compared to LN figures.

### Analysis of the CDR3 Region of Vβ Transcripts: Jβ Family Frequency Distribution

To discern whether the observed increase in the expression of certain Vβ segments in LN from some seropositive patients was due to clustering of T cells bearing identical TCR rearrangements or polyclonal expansion of T cells expressing these Vβ products, we cloned and sequenced cDNAs corresponding to the Vβ specificities expanded in LN of seropositive patients. In patient A we chose Vβ1, in patient C Vβ 5.2, in patient D Vβ3, and in patient G Vβ7; the same Vβ segments were also cloned from the corresponding PBMC. As control, we cloned and sequenced the above segments from PBMC and LN cells of healthy donors; in Control I, we also analysed the Vβ12 specificity, as its LN expression was relatively increased compared to the corresponding PBMC (see Fig. 2, panel B). Experimental data were obtained in a total of 238 molecular clones (Fig. 3 and Table 2).

Analysis of the Jβ repertoire in PBMC from control individuals disclosed a prevalence of Jβ2 cluster usage, regardless of the Vβ segment studied (Table 2); as shown in Fig. 3 (panel A, open columns), Jβ2-using clones accounted for 83% of the overall repertoire, and Jβ2.1 and Jβ2.7 were mostly represented. This finding was not surprising, as other workers reported similar figures in PBMC [24, 25]. Data regarding Jβ usage in LN are not available in the literature, so we sequenced about 50 clones from 4 different Vβ specificities in the LN from seronegative individuals. As shown in Table 2 and Fig. 3 (panel A, closed columns), a preferential usage of Jβ2 region was also found in this lymphoid compartment (73%); in addition, the Jβ2.4 and Jβ2.6 segments were not expressed in association with the Vβ segments considered in PBMC or LN cells of these subjects (Fig. 3A and Table 2).

In HIV-infected subjects as well, Jβ segment usage showed a considerable similarity in the two compartments. As shown in Fig. 3 (panel B), PBMC and LN Jβ2 rearrangements accounted for 67% and 74% of the total repertoire, respectively, and Jβ2.1 and Jβ2.7 were the most frequently used segments in both compartments. In addition, the Jβ1.3, Jβ2.4, and Jβ2.6 segments were undetectable in both PBMC and LN of the HIV-infected

**TABLE 2** Jβ Gene Segment Usage in PBMC Versus LN Cells From HIV$^+$ Patients and Seronegative Controls

| Subject | Sample | Vβ | Jβ segment | | | | | | | | | | | | No. of clones |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1.1 | 1.2 | 1.3 | 1.4 | 1.5 | 1.6 | 2.1 | 2.2 | 2.3 | 2.4 | 2.5 | 2.6 | 2.7 | |
| Patient A | LN | 1 | — | 1 | — | — | — | 1 | 5 | — | 4 | — | 4 | — | 10 | 25 |
| | PBMC | 1 | 2 | 1 | — | 1 | 2 | — | 1 | — | 2 | — | 1 | — | 3 | 13 |
| Patient D | LN | 3 | — | 1 | — | — | 3 | 1 | 1 | — | 2 | — | — | — | 5 | 13 |
| | PBMC | 3 | | — | — | 1 | — | — | 1 | — | 1 | — | 2 | — | 3 | 8 |
| Patient C | LN | 5.2 | 5 | 2 | — | — | — | — | 7 | — | 2 | — | — | — | 1 | 17 |
| | PBMC | 5.2 | 2 | 1 | — | — | 1 | — | 3 | — | 3 | — | — | — | 1 | 11 |
| Patient G | LN | 7 | 4 | — | — | — | — | — | 1 | — | 2 | — | — | — | 8 | 15 |
| | PBMC | 7 | 2 | — | — | — | — | 3 | 3 | — | 1 | — | 1 | — | 7 | 17 |
| Control H | LN | 1 | 2 | — | — | — | 2 | — | 3 | 1 | 2 | — | 2 | — | 3 | 15 |
| | PBMC | 1 | 2 | — | — | — | 1 | 1 | 3 | 1 | 2 | — | — | — | 2 | 12 |
| | LN | 3 | 1 | — | — | 1 | — | 1 | 1 | 1 | — | — | — | — | 7 | 12 |
| | PBMC | 3 | 2 | — | — | — | — | — | 2 | — | — | — | — | — | 4 | 8 |
| | LN | 5.2 | 2 | — | 1 | — | — | — | 2 | 1 | — | — | 2 | — | 3 | 11 |
| | PBMC | 5.2 | — | 1 | — | — | — | — | 5 | 1 | 3 | — | — | — | 2 | 12 |
| | PBMC | 7 | 1 | — | — | — | — | — | 6 | — | — | — | — | — | 7 | 14 |
| | LN | 7 | 1 | 1 | — | — | — | — | 5 | — | 1 | — | 1 | — | 3 | 12 |
| Control I | LN | 12 | 1 | — | 1 | — | 1 | — | 2 | — | 2 | — | 2 | — | 2 | 11 |
| | PBMC | 12 | 1 | — | 1 | — | 1 | — | 5 | — | — | — | 1 | — | 3 | 12 |
| Total | | | | | | | | | | | | | | | | 238 |

subjects studied (Fig. 3B and Table 2). These data seemed to indicate that, at least for the Vβ segments considered here, a skewness towards the usage of Jβ2 segments is a consistent feature of the Vβ repertoire in different lymphoid compartments, regardless of the pathologic conditions.

### Analysis of the CDR3 Region of Vβ Transcripts: Identification of Recurrent Rearrangements in LN Cells

We next examined the sequences of 70 clones obtained from 4 different Vβ families expanded in LN from HIV-infected patients. As shown in Table 3, we found recurrent identical CDR3 transcripts in all the Vβ families considered. Of a total of 25 clones of the Vβ1 transcript (patient A), 4 containing a Vβ1–Jβ2.5 rearrangement (16%) and 2 a Vβ1–Jβ2.7 rearrangement (8%) shared the same CDR3 region. In patient C, 3 clones containing a Jβ2.7 segment (18%) shared an identical CDR3 region. In patient D, 3 Vβ3–Jβ2.7 (23%) and 2 Vβ3–Jβ2.3 (15%) identical rearrangements were detected; finally, in patient G, 2 Vβ7–Jβ1.1 (13%) and 2 Vβ7–Jβ2.7 (13%) clones had the same CDR3 region (Table 3). No identical CDR3 transcripts were found in over 160 clones of the same Vβ from the corresponding PBMC (not shown), nor from PBMC (not shown) and LN cells from seronegative subjects (Table 3), even considering the Vβ12 segment, which was relatively expanded in LN cells of Control I, compared to the autologous PBMC sample (see Fig. 2, panel B). Altogether, this sequence analysis suggested an oligoclonal expansion of T lymphocytes bearing Vβ1 (Patient A), Vβ3 (Patient D), Vβ5.2 (Patient C) and Vβ7 (Patient G) products.

## DISCUSSION

It was advanced that an as yet unidentified HIV-associated SAg might trigger *in vivo* T lymphocytes expressing specific Vβ gene family products, leading to their selective deletion in the PBMC of HIV-infected individuals [10]. However, the body of contradictory findings on this issue [10–15] could also fit in with an alternative model, which holds that the immune response to antigens might shape the immune repertoire in an individual-specific, non-random manner, leading to the oligoclonal expansion of some T cell subsets. Indeed, this view is strengthened by several examples of unrelated pathological conditions in both animal models and humans, including the response to myelin basic protein in mice [26], cardiac allograft rejection in rats [27], infiltrates associated with melanomas and head-and-neck carcinomas [28, 29], and the histopathological picture of synovial tissues in rheumatoid arthritis patients [30]. In reference to HIV infection, a partial cytofluorographic analysis of the Vβ specificities in LN from HIV-infected patients revealed a skewed TCR repertoire [31], thus supporting T cell activation by HIV-encoded antigens at the site of HIV replication; furthermore, several workers described oligoclonal expansions of HIV-specific CTL during the primary immune response to HIV [32, 33]. Our PCR findings on TCR Vβ expression did not dis-

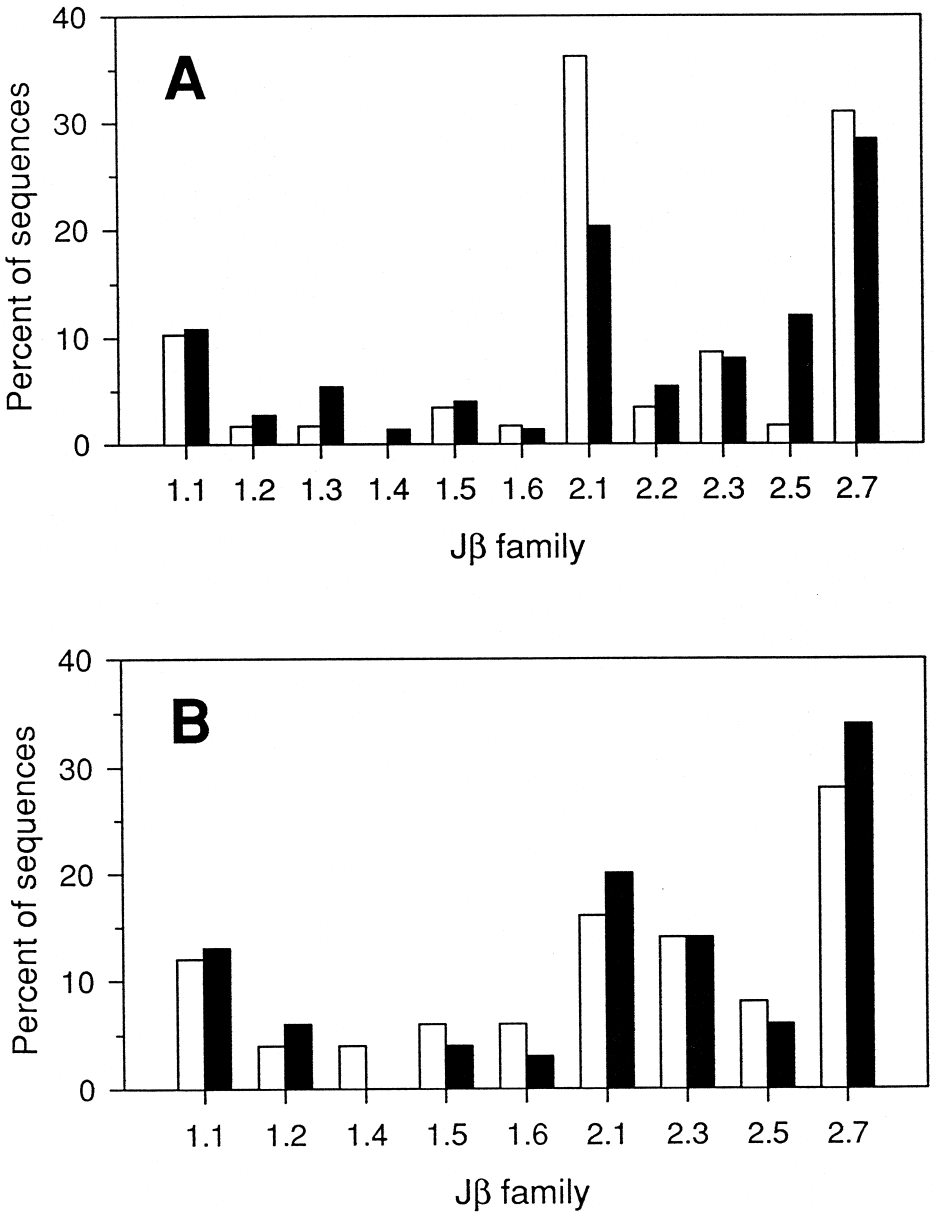


**FIGURE 3** Frequency of Jβ cluster usage among PBMC (open bars) and lymph node cells (closed bars) from 3 seronegative controls (panel A) and 4 HIV-infected patients (panel B). The columns represent mean values of the Jβ region frequencies, calculated from the data presented in detail in Table 2.

close recurrent modulation or expansion of any Vβ gene product in PBMC and LN cells from the patients studied, thus not supporting the idea of an SAg involvement in AIDS immunopathogenesis.

An important finding of our work was that major individual TCR repertoire perturbations, consisting of increased expression of certain Vβ transcripts, occur in LN from most HIV-infected patients, and occasionally in seronegative subjects. In humans, TCR Vβ usage and molecular structure in the lymphnodal compartment is mostly unexplored; TCR analysis by cloning and CDR3 sequencing of 4 Vβ-Cβ transcripts overexpressed in LN from 4 different HIV-infected subjects disclosed a skewness in Jβ usage in both PBMC and LN compartments. The finding in PBMC was not completely unexpected, as non-random TCR Jβ usage in circulating T cells has already been described [24, 25, 34]; however, our data show for the first time that a similar profile is also observed in secondary lymphoid organs in both HIV-infected and seronegative healthy individuals, thus suggesting that a skewness towards the usage of Jβ2 segments might be a hallmark of at least a part of the Vβ repertoire, regardless of the pathologic conditions.

On the other hand, multiple identical transcripts among different clones were found in LN cells, but not in PBMC, from HIV-infected patients. While these findings suggest oligoclonal T cell expansions in LN of these patients, the absence of recurrent transcripts in PBMC

**TABLE 3** Cloning and Sequencing of Vβ Specificities in LN From HIV-Infected Patients and Seronegative Controls

| Subject | Vβ | No. of cloned sequences | No. of occurrences (%) | *N* | Jβ |
|---|---|---|---|---|---|
| Patient A | Vβ1 | 25 | 4 (16%) | LGLGV | 2.5 |
| | | | 2 (8%) | DLFGVLD | 2.7 |
| Patient C | Vβ5.2 | 17 | 3 (18%) | SQRGST | 2.1 |
| | | | 2 (12%) | SPPVA | 1.1 |
| | | | 2 (12%) | SLFGG | 2.3 |
| Patient D | Vβ3 | 13 | 3 (23%) | FSAW | 2.7 |
| | | | 2 (15%) | FRQGRGG | 2.3 |
| Patient G | Vβ7 | 15 | 2 (13%) | SQDRT | 1.1 |
| | | | 2 (13%) | QESPGQKVA | 2.7 |
| Control H | Vβ1 | 15 | 0 | | |
| | Vβ5.2 | 11 | 0 | | |
| | Vβ3 | 12 | 0 | | |
| | Vβ7 | 12 | 0 | | |
| Control I | Vβ12 | 11 | 0 | | |

and LN cells of seronegative controls, even in Vβ which displayed a gross overexpression, seems to indicate that simple evaluation by PCR or cytofluorographic analysis of Vβ expression does not really reflect clonally restricted responses. This could be a useful take-home lesson in particular settings, and especially in autoimmune disorders, where the importance of the TCR repertoire is well known [35].

Our results constitute preliminary evidence in support of an oligoclonal expansion of T lymphocytes bearing distinct Vβ products in LN of HIV-infected patients, and suggest that these expansions might be antigen-driven. As none of our patients experienced opportunistic infections at the time of this study, it is unlikely that these expansions reflected clonally restricted responses to intercurrent pathogens; it is therefore probable that this oligoclonality might depend on antigenic stimulation by HIV. In this regard, we could not establish any correlation between the patient's immunologic or virologic status and the presence of Vβ expansions in LN (see Table 1), as this phenomenon was also seen in patients with low viral burden, as judged by plasma viral copy number (patient C). In any case, our study cohort is too small to allow definitive conclusions in this regard, nor could we distinguish whether the observed oligoclonal T cell expansions preferentially involved the $CD4^+$ or $CD8^+$ T cell compartment, or both, since total RNA was extracted from frozen LN biopsies. While molecular analysis of HIV-specific T cell clones derived from lymphoid tissues of infected individuals might provide formal proof for the hypothesis that the oligoclonal Vβ expansions observed here are related to an anti-HIV response, our finding of moderate oligoclonal expansions in LN (ranging from 8% to 23%) is consistent with previous data on the frequency of antigen-specific T cells in non-HIV-related pathologic lesions [36, 37]; moreover, over-representation of some Vβ–Jβ combinations was also detected among T cells infiltrating salivary glands in the sicca syndrome associated with HIV infection [38], and in the course of the primary immune response to HIV [32, 33]. Furthermore, it would be intriguing to characterize the Vβ repertoire and CDR3 usage in LN from subjects in different stages of disease, and in particular the so-called long-term non-progressor patients [39–41], in order to verify whether an expansion of some Vβ-bearing T cell clones plays a role in controlling or favoring HIV spread at the site of virus replication, and thus is endowed with prognostic significance.

ACKNOWLEDGMENTS

The help of Ms. Patricia Segato in the preparation of this manuscript is gratefully acknowledged. The authors wish to thank Dr. A. De Rossi for helpful discussion, and P. Gallo for artwork. These studies were supported in part by grants from the Istituto Superiore di Sanità (AIDS Project); the National Research Council (PF FATMA); the Associazione Italiana per la Ricerca sul Cancro (AIRC); and MURST 60%.

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