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Comparison of activation marker and TCR Vβ gene product expression by CD4+ and CD8+ T cells in peripheral blood and lymph nodes from HIV-infected patients

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SUMMARY

Since lymphoid organs constitute the site of active and progressive HIV disease, analysis of their lymphocytes may provide more accurate information on T cell abnormalities than that obtained from studying peripheral blood lymphocytes. The objective of this study was to compare the expressions of activation markers and T cell receptor (TCR) Vβ gene products by CD4+ and CD8+ T cells in lymph nodes (LN) and peripheral blood (PB) from healthy individuals and asymptomatic HIV-infected patients to determine whether anomalies that could be identified at the HIV replication site could support the hypothesis of T cell activation by HIV-encoded antigens or superantigens. CD4+ and CD8+ T cells in paired LN and PB obtained from six healthy controls and five asymptomatic HIV-infected individuals were analysed by flow cytometry, using anti-CD38, anti-HLA-DR and 13 anti-Vβ MoAbs that cover, approximately, 45% of the T cell repertoire. Analysis of T cell activation marker expression indicated that the percentages of CD4+ and CD8+ T cells bearing CD38 or CD38 and HLA-DR molecules were higher in patients than in controls and, in patients, higher in LN than in PB. Comparison between the Vβ repertoires of CD4+ and CD8+ T cells in LN and PB showed that, in each healthy individual, a limited number of Vβ families expressed by CD4+ or CD8+ T cells had different repartition in LN and PB, whereas in each HIV+ patient, more Vβ families exhibited different distributions and these differences recurred among certain Vβ segments, such as Vβ5.3 and Vβ21 in the CD4+ T cell population and Vβ5.2/5.3, Vβ12 and Vβ21 in the CD8+ T cell population. Taken together, these data argue for a skewed TCR repertoire in HIV infection and sustained activation of T cells by HIV-encoded antigens at the site of HIV replication, and further demonstrate that a high proportion of CD4+ T cells are in an activation state that may, indirectly, participate in their functional abnormalities.

Keywords T cell repertoire HIV infection

INTRODUCTION

HIV infection induces immune system dysfunctions that lead to AIDS and ultimately to death. HIV primarily infects macrophages and CD4+ T helper lymphocytes that progressively and gradually become dysfunctional and are depleted [1]. Although recent data showed that the proportion of HIV-infected peripheral blood (PB) CD4+ T cells was higher than formerly envisioned [2], direct killing of T helper cells does not seem to account for T cell depletion. Thus, indirect mechanisms have been proposed to explain CD4+ T cell abnormalities [1]. Among them, sustained activation of CD4+ T cells by HIV-encoded antigens [3] or superantigens [4] leading to T cell exhaustion or T cell deletion constitutes an attractive hypothesis, supported by the demonstration that CD4+ T cells express activation markers [5,6], and that HIV-infected individuals may have deletions of a large proportion of their Vβ families [7,8].

It is now widely accepted that lymphoid organs constitute the sites where HIV disease is active and progressive and the anti-HIV immune response takes place [9]. Thus, analysis of lymphocytes present in lymphoid organs may provide more accurate information of CD4+ T cell abnormalities than that of PB lymphocytes on which most studies have focused, to date.

The objective of this study was to compare the expressions of activation markers and T cell receptor (TCR) Vβ gene products in lymph nodes (LN) and peripheral blood (PB) from asymptomatic HIV-infected patients to determine whether abnormalities identified at the site of HIV replication

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Table 1. Demographic, clinical and immunological characteristics of HIV-infected patients

Patient number	Age	Sex	Stage (CDC)	Anti-viral therapy	CD4		CD8	
					%	n/mm^3	%	n/mm^3
1	42	M	II	+	6	266	74	3348
2	25	M	II	−	35	955	45	1228
3	34	M	II	+	26	375	46	673
4	26	M	II	+	19	349	59	1057
5	30	M	II	+	14	243	57	972

could support the hypothesis of $CD4^+$ T cell activation by HIV-encoded products.

SUBJECTS AND METHODS

Subjects

PB samples and LN were obtained from five HIV^+ asymptomatic individuals. Two patients underwent surgery for suspected lymphoma that was not confirmed by histopathological analysis, and three patients for reasons unrelated to HIV infection. All patients belonged to the CDC group II (Centers for Disease Control, Atlanta, GA) and were males. Four of them had been taking zidovudine (Burroughs Wellcome, Paris, France). Their clinical and immunological status is presented in Table 1. PB samples and LN were also obtained from six healthy volunteers (three males and three females) who underwent surgery for varicose vein stripping or an inguinal hernia. All patients gave written informed consent. The protocol was approved by the Charles Nicolle Hospital Ethic Committee (Rouen, France).

Separation and purification of cells

PB and LN mononuclear cells (MNC) were prepared by density gradient centrifugation on Ficoll–Hypaque (Nycomed Pharma, A.S., Oslo, Norway). Cells at the interface were collected, washed twice with PBS and adjusted to 5×10^6 cells/ml in PBS containing 5% fetal bovine serum (FBS) and 0·1% sodium azide and used for the following experiments.

Lymphocyte subpopulation analysis

PB and LN MNC from the HIV-infected individuals and the seronegative healthy volunteers were analysed by three-colour automated flow cytometry using PE-conjugated anti-CD38 (Becton Dickinson, Mountain View, CA), FITC conjugated anti-HLA-DR (Immunotech, Marseille, France), tri-colour conjugated anti-CD4 or anti-CD8 (Caltag Laboratories Inc., San Francisco, CA) MoAbs. MNC (100 μl; 5×10^5 cells) were incubated simultaneously with three fluorochrome-labelled antibodies, each diluted at its optimal concentration, for 20 min at room temperature. After three washes, the cells were fixed with paraformaldehyde.

PB and LN MNC were also analysed by two-colour automated flow cytometry to assess the Vβ segment frequencies in the $CD4^+$ and $CD8^+$ T cell subsets. MNC (5×10^5) were incubated simultaneously with PE anti-CD4 or anti-CD8 and FITC-anti-Vβ gene-specific MoAb (Immunotech) for 20 min at room temperature, washed and fixed with paraformaldehyde. The different anti-Vβ MoAbs used in the study were the same as those reported previously [6]. For unconjugated anti-Vβ MoAb (anti-Vβ17, anti-Vβ21 and anti-Vβ22), a two-step procedure was used. First, 5×10^5 MNC were incubated with anti-Vβ MoAb for 20 min at room temperature, washed twice and then labelled with FITC-conjugated goat $F(ab')_2$ anti-mouse immunoglobulins (Immunotech). After washing, the cells were saturated with PBS–0·1% sodium azide–5% FBS, containing 1% mouse serum, for 5 min at room temperature. Second, the cells were incubated with PE anti-CD4 or anti-CD8 and processed as described above.

Cell surface markers were analysed on 100 000 lymphocytes using an EPICS ELITE flow cytometer (Coulter Electronics Inc., Hialeah, FL) equipped with a 15-mW argon laser. For Vβ analysis, the cells were counted until at least 200 Vβ-positive cells had been evaluated. Cell labelling was analysed on a log fluorescence scale. FITC was measured using a 525-nm bandpass filter, PE with a 575-nm bandpass filter, and tri-colour with a 675-nm bandpass filter. Positive cells were determined by setting the threshold with reference to non-specific labelled MoAb.

Statistical analysis

The non-parametric, two-sided Wilcoxon test and χ^2 test were used for statistical comparison of experimental groups.

RESULTS

Comparison of T cell activation markers in peripheral blood and lymph nodes from HIV-infected individuals and healthy controls

We previously showed, by three-colour automated flow cytometry using tri-colour anti-CD4, FITC anti-HLA-DR and PE anti-CD38, that the percentages of circulating $CD4^+$ $CD38^-DR^+$, $CD4^+CD38^+DR^-$ and $CD4^+CD38^+DR^+$ T cells were significantly higher in HIV-infected patients than in healthy controls [6]. A similar study was performed on paired PB and LN T cells in six healthy controls and five HIV-infected individuals. Tables 2 and 3 show that, in healthy controls, the $CD4^+CD38^-DR^-$ and $CD8^+CD38^-DR^-$ T cells were major subsets present and similar in both PB and LN. $CD38^+DR^-$ T cells represented approximately 30% of $CD4^+$ or $CD8^+$ T cells, and the levels of DR^+ T cells ($CD38^-DR^+$ and $CD38^+DR^+$) were low and comparable in PB and LN. In HIV-infected individuals, the percentages of circulating $CD4^+$ and $CD8^+$ T cells expressing the CD38 molecule were not different in PB and LN, and higher than those observed in healthy control PB and LN. Analysis of the data presented in

Table 2. Percentages* of the different $CD4^+$ T cell subsets in the peripheral blood (PB) and lymph nodes (LN) of five HIV-infected individuals and six healthy controls

	$CD4^+CD38^-DR^-$ (%)			$CD4^+CD38^-DR^+$ (%)			$CD4^+CD38^+DR^-$ (%)			$CD4^+CD38^+DR^+$ (%)		
	PB	LN		PB	LN		PB	LN		PB	LN	
HIV patients												
1	20·7	13·9		8·1	1·1		45·2	55·3		26·1	29·7	
2	32·1	12·4		3·0	1·8		62·3	58·2		2·7	27·6	
3	30·6	7·3		9·7	1·1		46·3	51·3		13·4	40·2	
4	23·5	7·8		4·0	0·9		60·9	69·8		11·5	21·5	
5	22·9	4·3		3·9	0·2		47·4	62·8		25·8	32·8	
Mean	26·0	9·1	($P < 0·05$)	5·7	1·0	($P < 0·05$)	52·4	59·5	(NS)	15·9	30·4	($P < 0·05$)
s.d.	5·1	3·9		3·0	0·6		8·4	7·1		10·0	6·9	
Controls												
A	74·3	66·2		0·4	2·6		25·0	30·5		0·3	0·7	
B	64·0	60·5		1·6	2·0		31·7	33·7		2·7	3·8	
C	62·5	64·5		3·4	3·9		33·0	30·3		1·0	1·4	
D	67·9	62·4		5·4	4·5		20·1	26·8		6·6	6·3	
E	60·9	49·3		2·1	3·6		36·1	41·8		0·8	5·2	
F	64·6	50·1		8·8	10·9		23·4	34·2		3·3		4·9
Mean	65·7	58·8	(NS)	3·6	4.6	(NS)	28·2	32·9	(NS)	2·5	3·7	(NS)
s.d.	4·8	7·3		3·1	3·2		6·3	5·1		2·3	2·2	

* Expressed as the percentages of $CD4^+$ T cells. Values between parentheses are the *P* values obtained using the Wilcoxon test. NS, Not significant.

Table 3. Percentages* of the different $CD8^+$ T cell subsets in the peripheral blood (PB) and lymph nodes (LN) of five HIV-infected individuals and six healthy controls

	$CD8^+CD38^-DR^-$ (%)			$CD8^+CD38^-DR^+$ (%)			$CD8^+CD38^+DR^-$ (%)			$CD8^+CD38^+DR^+$ (%)		
	PB	LN		PB	LN		PB	LN		PB	LN	
HIV patients												
1	20·9	9·6		8·1	0·6		38·4	43·8		32·6	46·0	
2	44·4	14·8		11·6	1·2		41·2	55·3		2·8	23·7	
3	29·3	2·0		13·4	0·8		29·3	28·3		28·0	68·7	
4	8·0	5·7		6·4	0·9		30·5	33·4		55·1	59·9	
5	0·8	0·1		5·2	0·5		63·1	64·2		30·8	35·1	
Mean	20·7	6·4	($P < 0·05$)	8·9	0·8	($P < 0·05$)	40·5	45·0	(NS)	29·9	46·7	($P < 0·05$)
s.d.	17·3	5·9		3·5	0·3		13·6	14·9		18·6	18·2	
Controls												
A	61·7	71·9		0·4	2·3		31·1	23·4		6·8	2·4	
B	63·5	67·1		2·6	3·7		30·7	27·1		3·2	2·1	
C	56·8	58·1		9·5	11·0		27·9	26·1		5·7	4·8	
D	56·6	54·2		9·1	3·5		26·2	35·5		8·1	6·8	
E	77·3	51·7		1·7	3·4		19·2	35·6		1·7	9·4	
F	51·1	50·3		6·9	9·8		38·4	36·8		3·6	3·1	
Mean	61·2	58·9	(NS)	5·0	5·6	(NS)	28·9	30·8	(NS)	4·9	4·8	(NS)
s.d.	9·0	8·8		4·0	3·8		6·3	5·9		2·4	2·9	

* Expressed as the percentages of $CD8^+$ T cells. Values between parentheses are the *P* values obtained using the Wilcoxon test. NS, Not significant.

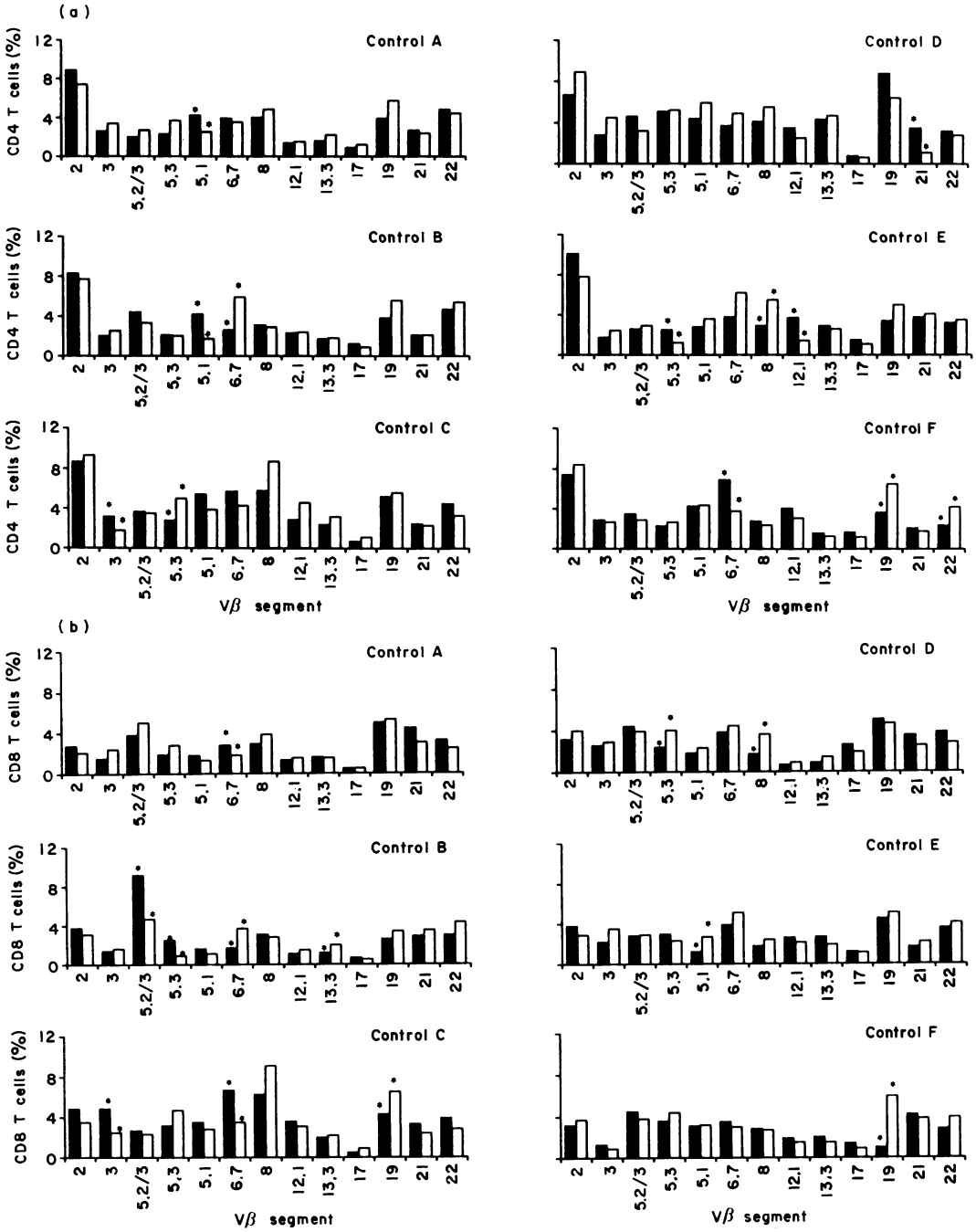

Fig. 1. Comparative analysis of the T cell receptor (TCR) repertoire expressed by $CD4^+$ (a) and $CD8^+$ (b) T cells in peripheral blood (■) and lymph nodes (□) from six healthy individuals (A, B, C, D, E and F). TCR Vβ expression percentages are shown as obtained by two-colour cytofluorometry analysis using anti-CD4 or anti-CD8 MoAbs and 13 anti-Vβ MoAbs covering approximately 45% of the repertoire. * Significant differences in Vβ expression (> 40%).

Tables 2 and 3 revealed two significant differences between PB and LN: (i) higher percentages of T cells expressing CD38 and DR molecules were found in LN than in PB; (ii) lower percentages of $CD4^+$ or $CD8^+CD38^-DR^+$ T cells were found in LN than in PB. Interestingly, in patient 2, whose PB $CD4^+$ T cell count was normal with a low percentage of these cells expressing activation markers, almost one-quarter of the LN $CD4^+$ T cells bore the CD38 and DR molecules. One should also note that, in both PB and LN, $CD8^+$ T cells more frequently expressed the CD38 or CD38 and DR molecules than $CD4^+$ T cells.

Taken together, these data indicate that T cell activation markers are strongly expressed in lymphoid organs where HIV is active.

Comparison of the TCR Vβ gene repertoire of $CD4^+$ and $CD8^+$ T cells in peripheral blood and lymph nodes

The TCR Vβ gene products expressed by paired LN and PB, $CD4^+$ and $CD8^+$ T cells were determined in six healthy and five HIV-infected individuals. Preliminary experiments showed that the variability in each independent flow cytometry analysis did not exceed 18%. Therefore, differences which exceeded 2 s.d.

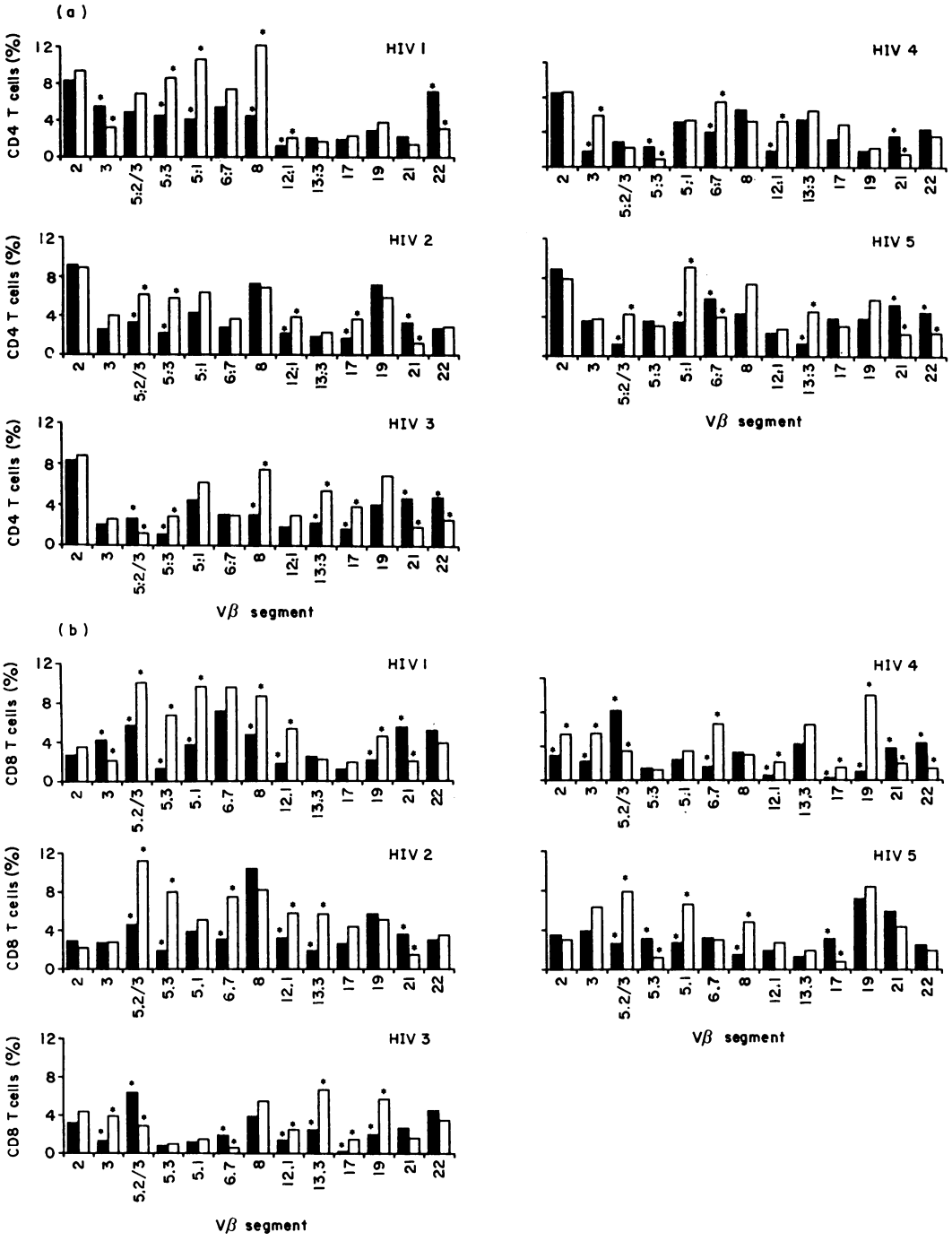

Fig. 2. Comparative analysis of the T cell receptor (TCR) repertoire expressed by $CD4^+$ (a) and $CD8^+$ (b) T cells in peripheral blood (■) and lymph nodes (□) from five HIV-infected individuals (1, 2, 3, 4 and 5). TCR Vβ expression percentages are shown. * Significant differences in Vβ expression (> 40%).

(≈40%) in the relative proportion of each Vβ gene family between LN and PB were considered significant.

The Vβ family distributions in $CD4^+$ and $CD8^+$ T cells, isolated from paired LN and PB obtained from six healthy individuals, are shown in Fig. 1. In each individual, some differences in Vβ family frequencies were found between LN and PB $CD4^+$ and $CD8^+$ T cells. However, in both T cell subsets, these differences never involved more than three Vβ families and most often were limited to one or two Vβ gene families. In addition, differences were not restricted to one or two Vβ families, but rather were scattered throughout the different Vβ segments studied.

In HIV^+ patients, comparative analysis of the Vβ repertoires in $CD4^+$ and $CD8^+$ T cells showed major differences between paired LN and PB. The data presented in Fig. 2 indicate that the number of significant variations in Vβ

family between PB and LN was higher and significant for $CD4^+$ (χ^2 tests, $P < 0{\cdot}001$) and $CD8^+$ (χ^2 tests, $P < 0{\cdot}001$) T cells than that observed in healthy controls. Within each HIV-infected individual, this number ranged from 5 to 9, and tended to be higher in $CD8^+$ than in $CD4^+$ T cells. No deletions of Vβ families were observed in LN or PB. Moreover, analysis of the Vβ segments whose expression was different in LN and PB from HIV^+ patients indicated that certain Vβ segments were frequently or constantly involved, such as the Vβ5.3 and Vβ21 families in the $CD4^+$ T cell population and the Vβ5.2/5.3, Vβ12.1 and Vβ21 families in the $CD8^+$ T cell population. It is interesting to note that the Vβ21 subset was reduced in $CD4^+$ and $CD8^+$ LN T cells and that, conversely, the Vβ12.1 was increased in $CD4^+$ LN T cells from all five HIV-infected patients. The number of Vβ segment discordances in the $CD4^+$ and the $CD8^+$ T cells between PB and LN was not correlated with their absolute numbers or their level of activation in PB.

DISCUSSION

It has been estimated that approximately 2% of total body lymphocytes are found in the PB, and that their phenotypes, functions and alterations may not be representative of those of lymphocytes present in lymphoid organs involved in a pathological process. This might be particularly relevant to HIV infection, during which lymphoid organs, in particular LN, bear the bulk of the HIV burden [9]. These observations prompted us to compare the phenotypes of LN and PB, $CD4^+$ and $CD8^+$ T cells from five HIV-infected individuals, particularly regarding the expression of activation markers and Vβ gene families. All the patients were asymptomatic and did not have opportunistic infections. LN were obtained from three patients during surgical procedures unrelated to their HIV disease, and from two patients during surgery for suspected lymphoma that was not confirmed by histopathological analysis. Thus, one can presume that all changes observed were directly related to HIV replication and exposure to viral products.

We first studied the expression of HLA-DR and CD38 molecules by LN and PB from HIV-infected patients and healthy controls. CD38 or CD38 and DR expression by $CD4^+$ and $CD8^+$ T cells was higher in the LN than the PB of all HIV-infected patients, and their PB levels exceeded those of normal individuals. Furthermore, T lymphocytes bearing both activation markers, which is infrequent in healthy controls, were strongly represented in LN from HIV-infected individuals, and constituted close to one-third of $CD4^+$ T cells and half of $CD8^+$ T cells. Thus, the demonstration that T cells expressing activation markers predominate in lymphoid organs where HIV infection is active suggests that they are cells in close proximity to viral antigenic products. One should also note that, in all five CDC stage II HIV-infected individuals, the percentages of $CD4^+$ or $CD8^+$ $CD38^-DR^+$ subsets were lower in LN than in PB. We previously reported that the PB of CDC stage IV patients was characterized by an increased proportion of $CD38^+DR^+$ T cells and a decreased level of $CD38^-DR^+$ T cells [6]. Therefore, the phenotypes of $CD4^+$ and $CD8^+$ T cells present in LN from CDC stage II patients resembled those of PB from CDC stage IV patients. On the basis of this observation, it is likely that circulating $CD38^+DR^+$ T cell populations, namely $CD4^+CD38^+DR^+$ T cells, may constitute a surrogate marker for disease progression.

The second objective of this study was to compare the expressions of TCR Vβ gene products in $CD4^+$ and $CD8^+$ T cells isolated from PB and LN from HIV^+ patients. This approach may circumvent the difficulty encountered in comparing the Vβ family frequencies between HLA-mismatched individuals, since the HLA complex may alter the expression of the $\alpha\beta$ TCR repertoire. We used two-colour automated flow cytometry, which, indeed, gave a limited picture of the TCR Vβ repertoire but enabled us to analyse $CD4^+$ and $CD8^+$ T cell subsets separately, and to eliminate bias introduced by the polymerase chain reaction (PCR) technique. The Vβ segment expression was first studied in healthy individuals. It has been established that several factors, such as the TCR locus, HLA background and environment, influence the peripheral $\alpha\beta$ TCR repertoire expression. Our findings indicate that Vβ gene product expression may also differ according to the lymphoid compartment considered. Although limited in number, differences in the Vβ family frequencies as high as 200% were seen between LN and PB, an observation that must be taken into consideration when such an analysis is performed in patients.

Results obtained from HIV-infected patients were dramatically different from those of normal individuals. Within a single HIV-infected individual, the differences in the TCR Vβ gene product expression between PB and LN T cells involved at least half of Vβ families studied, demonstrating perturbations of the Vβ repertoire at the site of HIV replication. These differences were observed in both $CD4^+$ and $CD8^+$ T cells, and recurred in certain Vβ segments, whereas in healthy individuals they involved only a few distinct Vβ gene families. In this regard, it is interesting to note that the Vβ21 family was frequently perturbed in T cells from HIV-infected patients, in agreement with earlier comparative analyses of the Vβ repertoire expressed by circulating T cells in monozygotic twins discordant for HIV [10] or by PB and LN T cells from one HIV-infected patient [11]. In addition, the absence in LN of deletion of the Vβ families previously reported to be deleted in circulating T cells [7,8] argues against a superantigen effect of HIV-encoded products.

Finally, significant disturbances of the Vβ repertoire expressed by $CD4^+$ and $CD8^+$ T cells were observed in LN compared with the PB during the clinical latency period of HIV infection. Recurrent perturbations of distinct Vβ segments observed in the different HIV-infected patients and in both $CD4^+$ and $CD8^+$ T cell compartments provide direct evidence for a skewed TCR repertoire in HIV infection that could be attributed to sustained T cell activation by various HIV-encoded products. This concept is further supported by the high percentage of activated T cells at the site of HIV replication that may exhibit severe functional abnormalities and thereby contribute to the immune dysregulation characterizing AIDS.

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