Selective Anergy of Vβ8+ T Cells in Human Immunodeficiency Virus–infected Individuals

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Summary

We have analyzed the Vβ usage by CD4+ and CD8+ T cells from human immunodeficiency virus (HIV)–infected individuals in response to an in vitro stimulation with the superantigenic erythrogenic toxin A (ETA) of *Streptococcus pyogenes*. ETA amplifies specifically CD4+ and CD8+ T cells from control donors expressing the Vβ8 and the Vβ12 elements. When peripheral T cells from asymptomatic HIV-infected individuals were stimulated with ETA, there was a complete lack of activation of the Vβ8+ T cell subset, whereas the Vβ12+ T cell subset responded normally to the superantigen. This Vβ-specific anergy, which was also observed in response to staphylococcal enterotoxin E (SEE), affected both CD4+ and CD8+ T cells and represented an intrinsic functional defect rather than a specific lack of response to bacterial superantigens since it was also observed after a stimulation with Vβ8 monoclonal antibodies. The Vβ8 anergic T cells did not express interleukin 2 receptors (IL-2Rs) and failed to proliferate in response to exogenous IL-2 or IL-4, suggesting that this anergy was not a reversible process, at least by the use of these cytokines. The unresponsiveness of the Vβ8 T cell subset is frequent since it was found in 56% of the patients studied, and comparison of the clinical status of responder vs. anergic patients indicated that the only known common factor between them was HIV infection. In addition, it is noteworthy that the anergy of the Vβ8 subset may be a very early phenomenon since it was found in a patient at Centers for Disease Control stage I of the disease. These data provide evidence that a dominant superantigen may be involved in the course of HIV infection and that the contribution of HIV has to be considered.

Some infectious agents such as pathogenic bacteria and retroviruses seem to have evolved towards a similar strategy to influence the immune system of the host and to facilitate infection, by expressing molecules that have features of superantigens. Exogenous bacterial superantigens comprise a set of protein toxins produced by *Staphylococcus*, *Streptococcus*, or *Mycoplasma* that are recognized, in the context of MHC class II molecules, by T cells expressing particular TCR Vβ gene families, causing strong T cell activation associated with toxic shock and autoimmune diseases (1–5). The immunological properties of bacterial superantigens are reminiscent of the Mls antigens that were shown to be encoded by the 3′ open reading frame of mouse mammary tumor virus and were responsible for the activation and subsequent deletion of CD4+ T cell subsets expressing Vβ elements that react with this retroviral superantigen (6–10). Involvement of superantigens in the pathogenesis of murine infections was also reported with a variant of MuLV encoding for a truncated gag fusion protein which possessed superantigenic properties and was shown to be responsible for the murine acquired immunodeficiency syndrome (11–13).

Since HIV-1 is a retrovirus, and since the pathology of HIV infection and AIDS involves predominantly the same CD4+ T cells that are commonly involved in superantigen-associated phenomenons, it was suggested that HIV might cause, in conjunction with class II genes, cell anergy and depletion of noninfected CD4+ T cells bearing TCR Vβ determinants, by encoding a superantigen expressed by activated infected cells (14). Recent studies (15–17) reported significant perturbations of the TCR Vβ repertoire in HIV-infected subjects, suggesting the contribution of superantigens in AIDS pathogenesis.

In vivo studies of peripheral tolerance using either Mls antigens or bacterial enterotoxins have shown that after expansion of T cells bearing the cognate Vβ determinants, the remaining T cells are unresponsive to restimulation by the superantigen in vitro (T cell anergy) (18–21). Therefore, anergy of a given Vβ T cell subset gives evidence of a previous acti-

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vation of this subset by a superantigen. We have used the streptococcal erythrogenic toxin A (ETA)[1] (22) to analyze the Vβ usage of peripheral T cells from asymptomatic HIV-infected subjects in response to this superantigenic activation. Our data indicate the existence, in a large fraction of infected individuals, of a Vβ-specific anergy affecting both CD4+ and CD8+ T cells that express the Vβ8 TCR element. We have characterized this Vβ-specific anergy and have shown that it was not restricted to an ETA stimulation but represented an intrinsic functional defect. The precocity of this phenomenon and the absence of correlation with previous viral or bacterial opportunistic infections suggest the involvement of an HIV-associated superantigen.

Materials and Methods

Blood Samples. Peripheral blood was obtained from 43 HIV-infected individuals in the Service des Maladies infectieuses (Professor R. Roué, Hôpital Militaire Bégin, Saint Mandé, France). Of 38 men and 5 women, 30 were clinically asymptomatic (stage II classification of the Centers for Disease Control [CDC], Atlanta, GA), 13 were CDC stage IIA (CD4 >500/mm^3), 17 were CDC stage IIB (CD4 <500/mm^3), 1 patient was CDC stage I (3 wk after primo infection), 2 patients were CDC stage III, and 9 patients CDC were stage IV. Controls (n = 15) were HIV-seronegative healthy donors and one HIV-seronegative donor with infectious mononucleosis.

Monoclonal Antibodies. The mAbs used in this study were CD4 (IgG1, κ), CD8 (IgG1, κ), CD3 (IgG1, κ), and CD25 (IgG1, κ), purchased from Becton Dickinson (Pont de Claix, France). mAbs specific for human TCR V region epitopes (Diversi-T αβ TCR) were purchased from T Cell Sciences (Bioadvance, Le Perreux, France): mAbs 1C1 and W112 recognize either both Vβ5.2 and Vβ5.3 or only the Vβ5.3 subfamily; mAb LC4 is specific to the Vβ5.1 subfamily; mAbs 16G8 and S511 mAb are specific to the Vβ8 and Vβ12 subfamilies, respectively; and αV2a mAb (clone F_1) is specific to the Vα2 subfamily.

Cell Preparation and Stimulation. PBMC were isolated from heparinized blood by centrifugation on Ficoll-Hypaque (Pharmacia, Sweden) and cultured at 10^6 cells/ml of complete medium composed of RPMI-1640 supplemented with 10% vol/vol heat-inactivated FCS, 2 mM L-glutamine, 20 mM Hepes, 10 U/ml penicillin–streptomycin. Cultures were stimulated with optimal concentrations of bacterial superantigens: 1 μg/ml ETA of *Streptococcus pyogenes* (a gift from Dr. H. Müller-Alouf, Institut Pasteur) or 1 μg/ml staphylococcal enterotoxin E (SEE) (Sigma Chemical Co., St. Louis, MO). Stimulation lasted 4 d for ETA or 11 d for SEE, and in this latter case, 20 U/ml rIL-2 (Boehringer Mannheim, Mannheim, Germany) was added at day 3.

T Cell Proliferation Assays. Proliferation assays were performed in 96-well culture plates (Costar, Brumath, France) in a final volume of 200 μl. Polyclonal T cell proliferation was induced by 600 ng/ml anti-CD3 mAbs (Dakopatts, Trappes, France). Specific anti-Vβ–induced proliferation was performed in microwells coated with purified mAbs specific for human TCR V region epitopes (Diversi-T αβ TCR, T Cell Sciences). Coating of microwells was performed as follows. mAbs were diluted at 10 μg/ml in PBS and incubated overnight at 4°C (30 μl/microtiter flat-bottom well). Plates were then washed three times with PBS before adding 4 × 10^5 PBMC/well. rIL-2 was added at 20 U/ml after 3 d, and cultures were pulsed 3 d later with 1 μCi of [^{3}H]thymidine (5 Ci/mmol) during the final 16 h of incubation. Each culture was done in triplicate wells. Results are indicated as stimulating index (SI) which corresponds to mean cpm obtained in cultures coated with anti-Vβ mAbs divided by mean cpm obtained in medium alone.

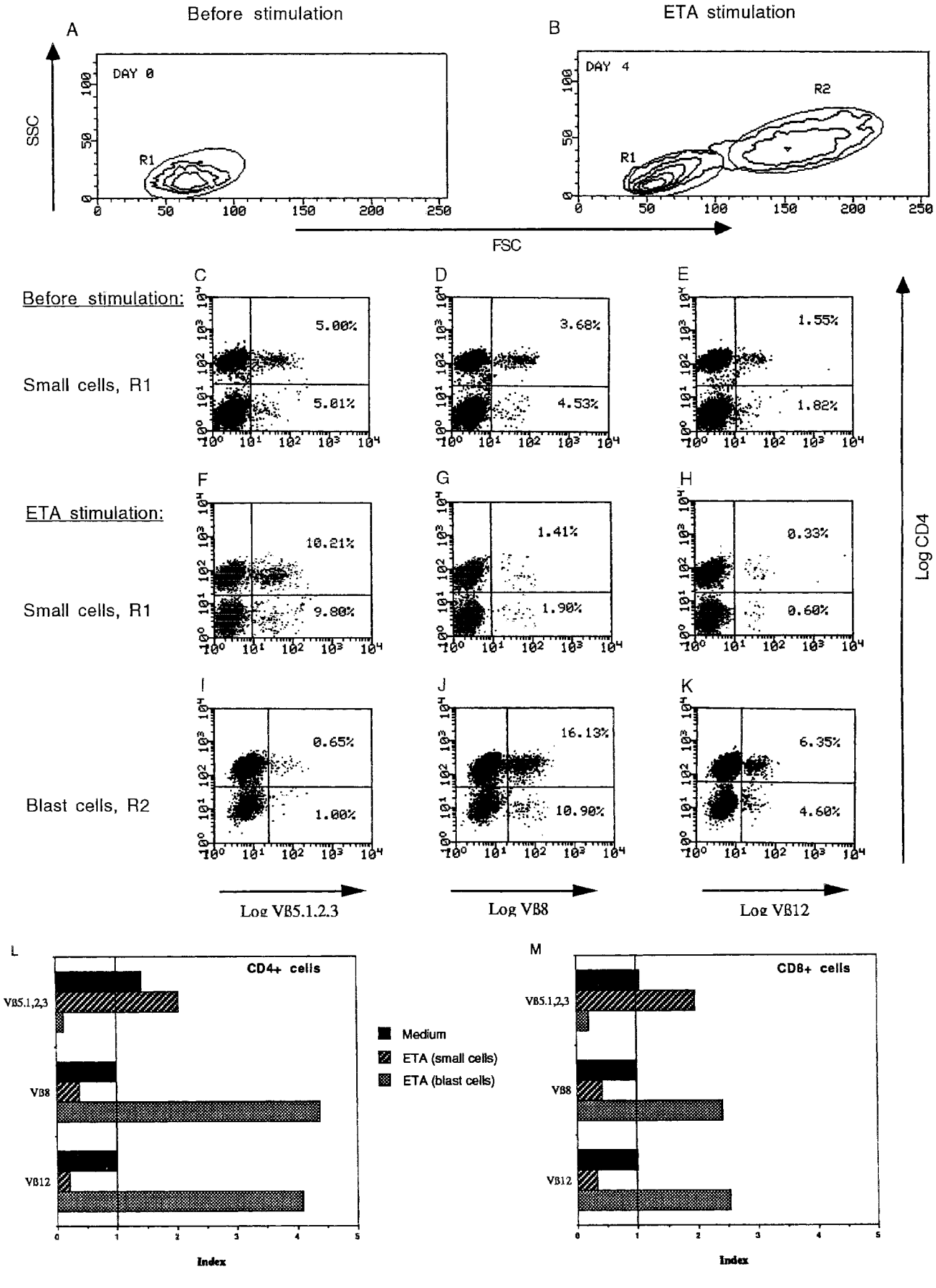
Immunofluorescent Analysis. Comparison of the expression of TCR V gene products by CD3+, CD4+, or CD8+ T cells isolated from HIV-infected patients or controls was done as follows: 5 × 10^5 PBMC were washed three times in PBS containing 1% BSA and 0.1% sodium azide and incubated for 10 min at 4°C with anti-CD3, -CD4, or -CD8 mAbs (Becton Dickinson) conjugated with PE and with a panel of mAbs specific for human TCR V region epitopes (T Cell Sciences), conjugated with FITC. Isotypically matched negative controls were used (FITC or PE-conjugated mouse mAbs from Becton Dickinson). All samples were fixed with 1% paraformaldehyde and immediately acquired in a FACScan® flow cytometer (Becton Dickinson). For each sample, 20,000 double-stained viable lymphocytes were gated following size (FSC) and granulosity (SSC) criteria and analyzed with the Lysis program (Becton Dickinson). The number of positive cells for both CD3 (CD4 or CD8) and the TCR V region for each sample was compared with the corresponding control samples.

Analysis of the Vβ usage by CD4+ or CD8+ T cells from HIV-infected or control individuals in response to a superantigenic activation was performed with the same staining procedure as described above. FACS® analysis of double-stained activated cells was performed simultaneously on two gated populations, e.g., the R1 population corresponding to small cells and the R2 population corresponding to blastic cells (see Fig. 1). To accurately compare, between different donors, T cell subpopulations bearing a given Vβ after in vitro activation, results were calculated as the percentage of T cell blasts bearing a particular Vβ after stimulation divided by the percentage of T cells bearing that Vβ before stimulation. Analysis of IL-2R (CD25) expression on Vβ+ T cell subpopulations consecutively to ETA stimulation was performed as described above, but was restricted to the R1 population (small cells) to allow comparison between the group of anergic HIV-infected donors and groups of responder HIV-infected donors and controls. The Mann-Whitney U test was used to compare the different groups of individuals.

[1] *Abbreviations used in this paper:* ETA, erythrogenic toxin A; SEB, staphylococcal enterotoxin B; SEE, staphylococcal enterotoxin E; SI, stimulation index.

Figure 1. Vβ usage of CD4+ and CD8+ human T cells in response to ETA. PBMC from normal donors were analyzed before (*A*) and after (*B*) 4-d ETA stimulation (1 μg/ml) and two gates were defined following size (FSC) and granulosity (SSC) criteria. Gate R1 corresponded to small cells (*A*) and gate R2 to blast cells (*B*). Analysis of the Vβ usage by CD4+ T cells in response to ETA was realized by dual staining with CD4-PE mAb and anti-Vβ-FITC mAbs before (*C–E*) and after (*F–K*) stimulation. Numbers in quadrants indicate the percentage of CD4+Vβ+ or CD4−Vβ+ T cells subsets in gate R1 or gate R2. To visualize Vβ-specific stimulation by ETA, results are expressed as index calculated as the percentage of CD4+ T cell blasts bearing a particular Vβ after stimulation divided by the percentage of CD4+ T cells bearing that Vβ before stimulation (*L*). Thus an index superior to 1 corresponds to the amplification of a given Vβ subset. This calculation was also used for the CD8+ T cell subset (*M*). (■) Medium; (■) ETA small cells; (▨) ETA blast cells.

SERONEGATIVE DONOR C5



Results

Vβ8- and Vβ12-specific Stimulation of Peripheral Human T Cells by ETA. Streptococcal ETA is a potent superantigen (22, 24). In vitro activation of PBMC from control donors by ETA leads to blastogenesis easily identified by FACS® analysis. Two populations (R1 and R2) can be distinguished considering both the size (FSC) and the granulosity (SSC) of activated cells (Fig. 1 *B*): blastic cells (gate *R2*) appear larger and more granular than nonblastic cells (gate *R1*), the latter exhibiting size and granulosity of ex vivo, nonstimulated lymphocytes (Fig. 1 *A*). Analysis of the Vβ usage by CD4+ and CD8+ T cells responding to ETA was performed with Abs against members of the Vβ5, Vβ8, and Vβ12 families. Fig. 1, *F–K* show the percentages of CD4+ T cells stained with each anti-Vβ among the two distinct populations, small cells in R1 and blastic cells in R2. It appeared that ETA stimulates preferentially Vβ8+ T cells as well as Vβ12+ T cells (Fig. 1, *J* and *K*). Indeed T cells bearing Vβ8 or Vβ12 were almost all found in the R2 population (compare Fig. 1, *J* to *G* and *K* to *H*). By contrast, T cells expressing Vβ5 were especially found in the nonblastic R1 population (Fig. 1 *F*). These same results were found for the CD8+ T cell population (Fig. 1 *M*), confirming previous studies indicating that, in vitro, both CD4+ and CD8+ T cells are stimulated by ETA (22, 25).

To adequately compare among different donors, T cell subpopulations bearing a given Vβ after ETA stimulation, results were calculated as the percentage of T cell blasts bearing a particular Vβ after stimulation divided by the percentage of T cells bearing that Vβ before stimulation. This calculation, proposed initially by Kappler et al. (1) was designed to correct for variations in Vβ expression from one person to another. In addition, it allowed one to visualize an amplification of a given Vβ T cell subset after stimulation, namely when the index was superior to 1. This calculation was always used throughout this study.

The ratio of T cells bearing a particular Vβ before (Fig.

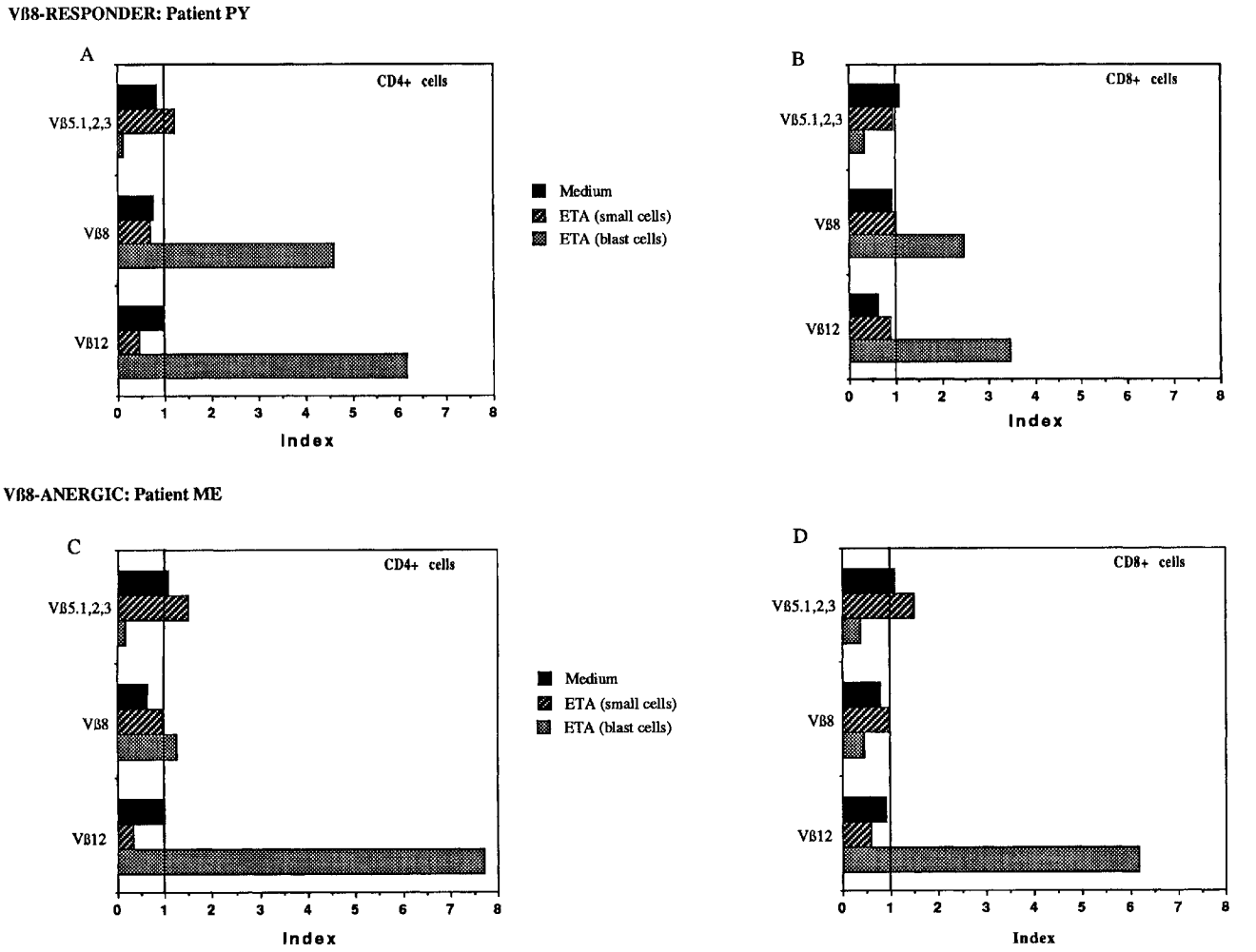


Figure 2. Vβ-specific stimulation of CD4+ and CD8+ T cells from HIV-infected donors. PBMC from HIV-infected donors were stimulated by ETA as described in Fig. 1. Analysis of Vβ usage of CD4+ and CD8+ T cells in response to ETA and index calculation were performed as described in Fig. 1. Results of a representative experiment are shown.

1, *C–E*) and after culture in the absence of stimulation (in medium alone) was close to 1 (Fig. 1, *L* and *M*). In contrast, it was clear that T cells bearing Vβ8 and Vβ12 were enriched in blasts produced by activation with ETA (ratio 4, 4 and 4, 1, respectively for $CD4^+$ T cells and 2,3 and 2,5 for $CD8^+$ T cells), whereas T cells bearing Vβ5 were specifically excluded from the ETA blasts (ratio ~0.22). Indeed $V\beta5^+$ T cells remained in the R1 nonblastic population. The enhanced ratio observed for this latter population does not correspond to a specific proliferation, but rather is a consequence of an apparent enrichment of the R1 population with $V\beta5^+$ cells due to the disappearance from this population of the other subsets ($V\beta8^+$ and $V\beta12^+$) (Fig. 1, *L* and *M*).

Analysis of the Vβ Usage by $CD4^+$ and $CD8^+$ T Cells from HIV-infected Patients in Response to ETA Stimulation. An extensive study of the Vβ usage of patients' T cells responding to ETA (summarized in Table 1) was performed in HIV-infected patients, most of which were asymptomatic at the beginning of this study. Experiments performed as described in Fig. 1 and index of Vβ stimulation calculated as mentioned above revealed that HIV-infected patients could be divided into two groups. The first, named "responder," is represented by the patient PY in Fig. 2. The $V\beta^+$ T cell subsets of these patients behaved as did those of the controls (Fig. 1) to a stimulation by ETA with a specific activation of the $V\beta8^+$ and $V\beta12^+$ subsets among $CD4^+$ (Fig. 2 *A*) and $CD8^+$ (Fig. 2 *B*) T cells. The second, named "anergic," represented

Table 1. *Vβ usage by $CD4^+$ T Cells from HIV-infected Donors in Response to ETA Stimulation**

	HIV^- controls			HIV^+ Vβ8 responders				HIV^+ Vβ8 anergic			
Donors	Vβ5.1,2,3	Vβ8	Vβ12	Patients	Vβ5.1,2,3	Vβ8	Vβ12	Patients	Vβ5.1,2,3	Vβ8	Vβ12
C1	0.44	2.80	3.40	AT	0.05	2.41	4.86	AI	0.58	1.10	8.00
C2	0.44	3.55	4.63	CE	0.27	2.93	7.17	BT	0.66	0.96	3.16
C3	0.42	4.51	5.62	CT	0.10	3.58	4.63	BU	0.20	1.11	5.85
C4	0.30	3.30	5.48	DO	0.02	2.57	4.49	CR	0.06	0.26	9.60
BR‡	0.05	2.50	3.60	DS	0.25	3.78	8.70	CS§	0.30	0.66	2.20
CY	0.40	3.53	5.13	DY	0.29	2.67	5.50	DA	0.20	0.80	10.93
C5	0.13	4.38	4.10	EA	0.37	4.26	6.61	DD	0.15	1.52	4.63
C6	0.16	3.81	6.97	GO	0.18	4.50	2.24	DI	0.46	1.03	8.14
MA	0.19	4.05	4.34	GR	0.37	4.02	12.54	DR	0.32	0.42	5.26
C7	0.24	2.55	3.85	HY	0.16	2.40	4.90	DT	0.26	0.79	3.26
C8	0.27	3.38	4.32	MA	0.15	4.29	12.24	GE	0.09	1.29	2.92
C9	0.10	2.76	2.61	MT	0.06	4.07	4.23	GG	0.25	0.31	3.45
GN	0.38	2.60	3.22	PT	0.09	5.09	5.10	GT	0.35	1.56	8.62
C10	0.15	2.37	5.11	PY	0.12	5.60	7.16	HE	0.06	0.83	11.42
C11	0.13	4.81	4.40	RE	0.30	2.40	4.58	HT	0.60	0.64	4.30
				RL	0.41	6.06	6.81	HU	0.90	0.59	5.88
				RR	0.49	3.56	10.38	HZ	0.16	0.94	8.70
				TD	0.07	2.96	6.37	LE	0.38	1.05	3.54
				ZI	0.11	3.10	6.09	LI	0.56	1.52	5.97
								ME	0.17	1.26	7.72
								MN	0.46	0.98	7.21
								MY	0.10	1.20	9.79
								SN	0.17	1.05	4.70
								VT	0.36	0.33	6.44
Mean	0.25	3.39	4.45		0.20	3.70	6.56		0.33	0.93	6.32
(± SD)	(0.14)	(0.79)	(1.10)		(0.14)	(1.10)	(2.72)		(0.22)	(0.38)	(2.68)

* Results are expressed as stimulation index corresponding to the percentage of $CD4^+$ T cell blasts bearing a given Vβ after 4-d stimulation by ETA divided by the percentage of $CD4^+$ T cells bearing that Vβ before stimulation.
‡ Infectious mononucleosis (MNI).
§ Patient in CDC stage I.

by the patient ME in Fig. 2, showed a Vβ8-specific lack of response to ETA stimulation: the majority of Vβ8+ T cells were found in the small cell population (population R1). Indeed, after ETA stimulation, the indexes of Vβ8+ T cell in the blasts were around or below 1 in contrast to those of Vβ12+ T cells which could reach 6–8 in the blasts of the same patient (Fig. 2 and Table 1). The fact that the Vβ8+ T cells did not disappear after ETA stimulation but remained in the small cell population (R1) indicated the existence of an anergic state in this subset rather than a specific deletion. Fig. 2 shows that this Vβ-specific anergy was found in both CD4+ and CD8+ T cells.

Table 1 summarizes the results from a series of experiments performed with lymphocytes from 43 HIV-infected patients and 15 seronegative donors. Interestingly, a large fraction of HIV-infected patients showed a Vβ8-specific anergy in response to ETA since it concerned 56% of the patients studied (24 over 43). This phenomenon was never observed in lymphocytes from seronegative controls, including a patient infected by EBV infectious mononucleosis (MNI) (Table 1). This anergy was not associated with a general unresponsiveness to ETA, since all the patients from the nonresponder group showed a normal expansion of the Vβ12 T cell subset in response to ETA, and the corresponding mean index in T cell blasts was comparable to that of the responder group. By contrast, the absence of amplification of the Vβ8+ T cell subset in ETA-stimulated cultures from anergic patients was underlined by a mean index below 1 in T cell blasts as compared to 3.5 for the two other groups, responders and controls (Table 1).

Longitudinal study of 14 patients for over 1 yr indicated that anergy represented a constant and long-lasting phenomenon. Comparison of the clinical status of responder vs. anergic patients indicate no correlation with the stage of the disease and previous viral or bacterial infections. In addition it should be noted that the unresponsiveness of the Vβ8 subset may

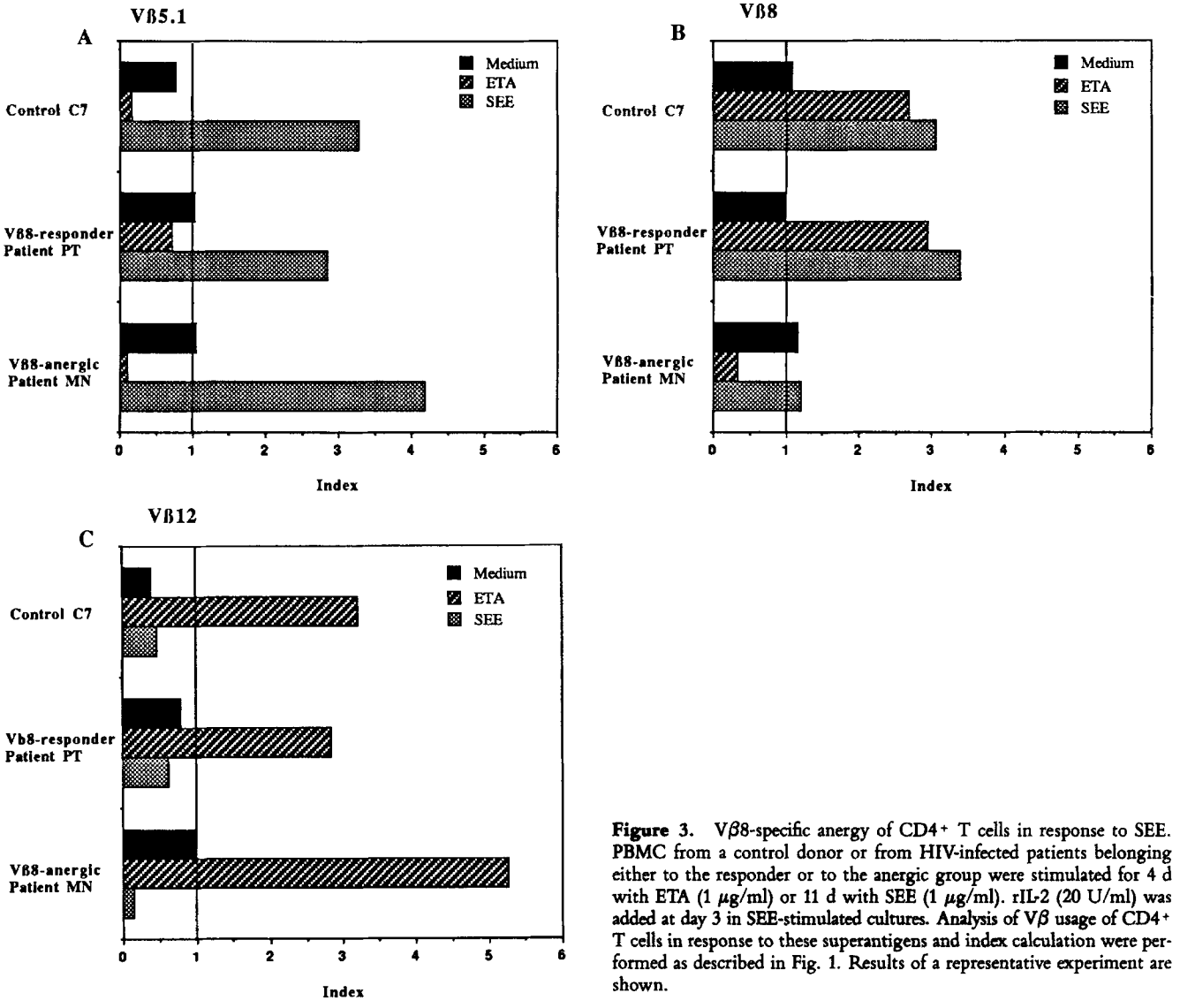


Figure 3. Vβ8-specific anergy of CD4+ T cells in response to SEE. PBMC from a control donor or from HIV-infected patients belonging either to the responder or to the anergic group were stimulated for 4 d with ETA (1 μg/ml) or 11 d with SEE (1 μg/ml). rIL-2 (20 U/ml) was added at day 3 in SEE-stimulated cultures. Analysis of Vβ usage of CD4+ T cells in response to these superantigens and index calculation were performed as described in Fig. 1. Results of a representative experiment are shown.

be a very early phenomenon since it was found in a patient at CDC stage I of the disease (see Table 1). Thus it appeared that the only known common factor between patients in all groups was HIV infection.

Vβ8-specific Unresponsiveness Is Not Restricted to an ETA Stimulation but Represents an Intrinsic Functional Defect. Since unresponsiveness of the Vβ8 T cell subset could be the consequence of a specific defect in the interaction between this subset and streptococcal ETA, we analyzed the response to another bacterial superantigen, SEE. In addition to stimulating T cell subsets expressing the Vβ5.1, Vβ6, and Vβ18 elements, SEE activates also the Vβ8$^+$ T cell subset (1, 26). Comparison of the in vitro SEE and ETA stimulation on the expansion of the Vβ8 CD4$^+$ T cell subset from the two groups of patients is shown in Fig. 3. No amplification of the Vβ8 T cell subset was observed after SEE stimulation of lymphocytes from a nonresponder patient, whereas in the same culture, the expected amplification of the Vβ5.1 subset occurred. The same results were observed in the CD8$^+$ T cell subpopulation (data not shown), although the SEE-activated population which was predominantly CD4$^+$ blast cells in response to SEE, is composed of 90% CD4$^+$ and 10% CD8$^+$ T cells as compared to 50% CD4$^+$ and 50% CD8$^+$ T cells in response to ETA. A comparison of SEE and ETA stimulations on lymphocytes from eight patients from the anergic group confirmed that the anergy was never found in the Vβ5.1 and Vβ12 subsets (data not shown).

To confirm that the Vβ-specific anergy was not only associated to a superantigenic activation but rather represented an intrinsic functional defect, stimulations were performed with anti-Vβ mAbs and compared to those induced by ETA and SEE. Table 2 shows a representative experiment in which T cells from three responder patients were significantly stimulated by coated anti-Vβ8 mAbs, as well as by anti-Vβ5.1 and anti-Vβ12 mAbs. On the contrary, T cells from three anergic patients were not sensitive to anti-Vβ8 stimulation whereas they were normally stimulated by anti-Vβ12 and anti-Vβ5.1 mAbs. As expected, the Vβ8-specific anergy was also observed in response to ETA and SEE, each superantigen representing a control of the mAb stimulation since SEE activated Vβ5.1 and Vβ8 subsets whereas ETA activated Vβ8 and Vβ12 subsets. Thus, the anergic state observed in response to superantigens was specific and intrinsic to the Vβ8 T cell subset.

Anergic Vβ8 T Cells Do Not Express IL-2Rs and Are Unresponsive to Exogenous Cytokines. In vivo administration of the superantigen staphylococcal enterotoxin B (SEB) (19–21) or immunization of Mls-1^b mice by Mls-1^a spleen cells (18) have been reported to induce in mice a peripheral T cell tolerance characterized by the anergy of T cell subsets expressing specific Vβ determinants. In these models, the anergic subset expressed the IL-2Rs partially (19) or normally (18), but failed to respond to exogenous IL-2 (18, 19, 27). Since our results suggest the induction, in the course of HIV infection, of a Vβ-specific peripheral tolerance, we analyzed whether the IL-2/IL-2Rs pathway was affected in the Vβ8 anergic subset.

IL-2Rs (CD25) expression was studied on the Vβ5, Vβ8, and Vβ12 subsets after 4 d of stimulation with ETA. To compare the Vβ8 anergic subset to the others, CD25 expression was analyzed on the R1 (small cells) population. Results in Fig. 4 show that ETA stimulation induced the expression

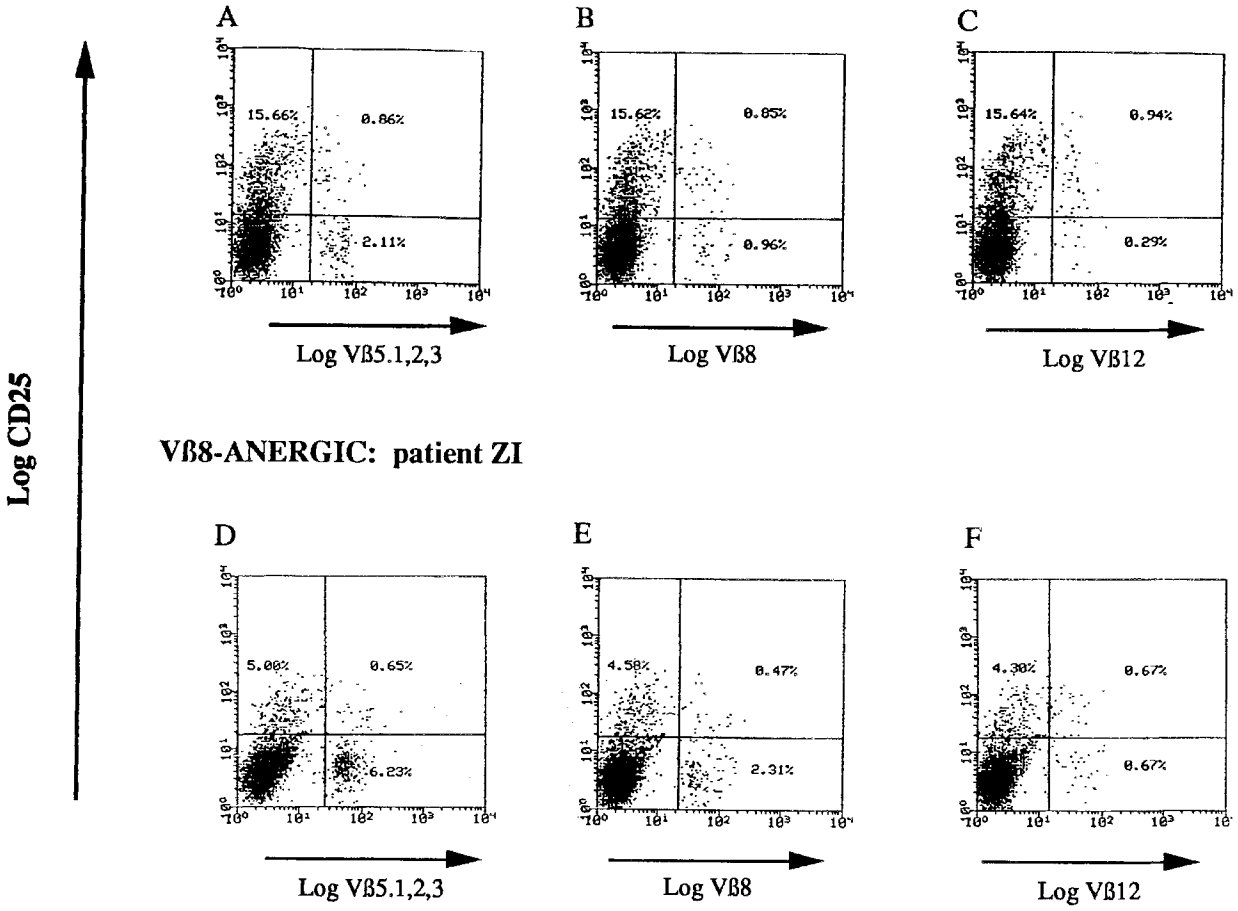
Table 2. *Vβ8-specific Anergy Is Maintained after Activation by Anti-Vβ8 mAbs*

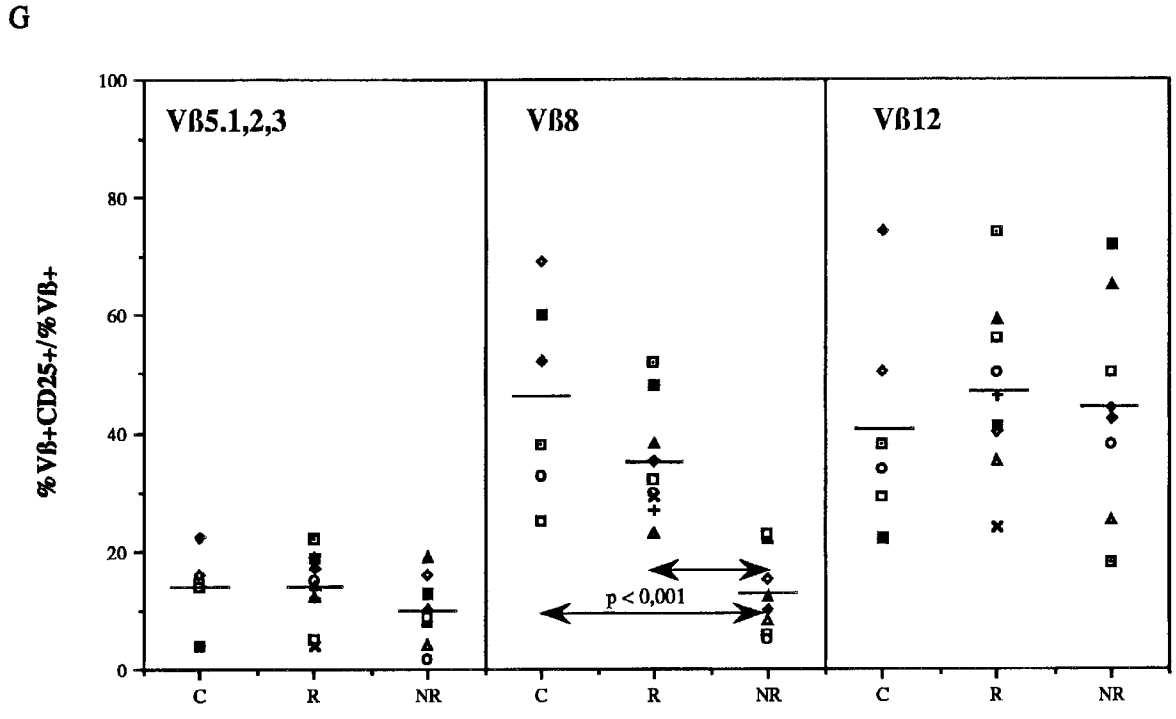
Vβ8 responder patients				Vβ8 anergic patients				
		Vβ stimulation of PBMC with*				Vβ stimulation of PBMC with*		
Patients	Vβ expression studied	ETA (index)‡	mAbs (SI)§	Patients	Vβ expression studied	ETA (index)‡	SEE (index)‡	mAbs (SI)§
CT	Vβ5.1	0.13	7.87	ME	Vβ5.1	0.17	5.03	3.54
	Vβ8	3.58	11.33		Vβ8	0.90	1.24	1.19
	Vβ12	5.83	8.80		Vβ12	12.9	0.98	6.23
GO	Vβ8	3.86	2.40	AI	Vβ8	0.49	ND	0.32
	Vβ12	2.36	2.20		Vβ12	8.01	ND	2.55
CE	Vβ8	3.25	2.47	HT	Vβ8	0.18	ND	0.99
	Vβ12	7.42	2.41		Vβ12	6.26	ND	2.69

* Cells were stimulated for 4 d with ETA (1 μg/ml), for 11 d with SEE (1 μg/ml) or for 6 d with anti-Vβ mAbs. rIL-2 was added (20 U/ml) at day 3 in the last two cultures.

‡ Index represents the percentage of CD3$^+$ T cells bearing a given Vβ after ETA or SEE stimulation divided by the percentage of CD3$^+$ T cells bearing that Vβ before simulation.

§ SI corresponds to cpm obtained for cultures in microwells coated with corresponding Vβ mAbs, divided by cpm obtained in medium alone. Values correspond to mean index of triplicate wells.


Vß8-RESPONDER: patient PT
A
B
C
15.66%
0.86%
2.11%
15.62%
0.85%
0.96%
15.64%
0.94%
0.29%
Log Vß5.1,2,3
Log Vß8
Log Vß12
Log CD25
Vß8-ANERGIC: patient ZI
D
E
F
5.00%
0.65%
6.23%
4.58%
0.47%
2.31%
4.30%
0.67%
0.67%
Log Vß5.1,2,3
Log Vß8
Log Vß12


G
Vß5.1,2,3
Vß8
Vß12
% Vß+CD25+/% Vß+
100
80
60
40
20
0
p < 0,001
C
R
NR

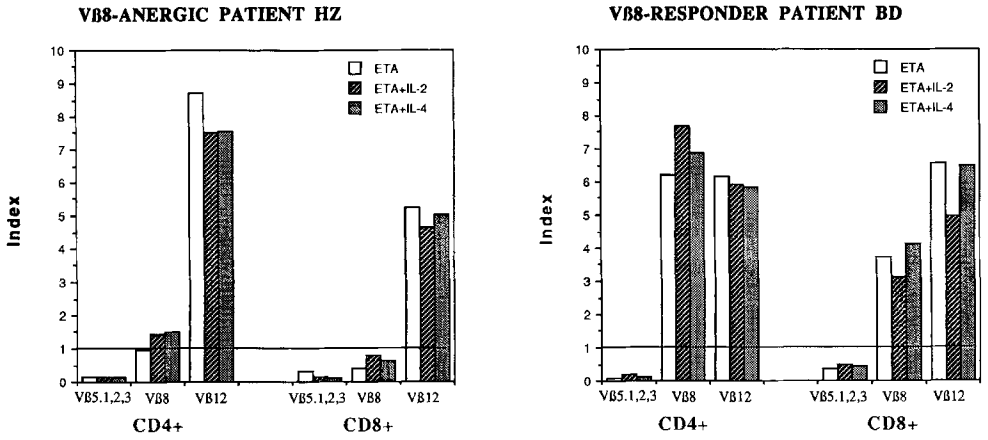


Figure 5. Anergic Vβ8 T cells are unresponsive to exogenous IL-2 and IL-4. PBMC from anergic patient HZ (*left*) or responder patient BD (*right*) were stimulated for 6 d by ETA (1 μg/ml) in the absence or in the presence of rIL-2 (20 U/ml) or rIL-4 (4 ng/ml). Analysis of Vβ usage of CD4+ and CD8+ T cells in response to ETA stimulation and index calculation were performed as described in Fig. 1. Results of this experiment are representative of a number of similar experiments performed on anergic and responder patients.

of IL-2Rs on a high percentage of small T cells expressing Vβ12 element in the three groups of donors, indicating that expression of IL-2Rs precedes blastogenesis. Although a dispersion of CD25 expression among donors was noticed (probably due to an asynchronous activation), no significative difference in the Vβ12 subset was found between controls (*C*), responder (*R*), and nonresponder (*NR*) groups.

By contrast, CD25 expression by the anergic Vβ8 subset was very low for all the nonresponder patients and a significant difference ($p < 0.001$) was found between the anergic group and the two other groups (Fig. 4). It is noteworthy that the anergic Vβ8 subset behaves like the Vβ5 subset from the three groups, this latter subset being insensitive to ETA activation (Fig. 4).

Addition of rIL-2 at the initiation of ETA stimulation could not reverse the anergic state of the Vβ8 subset (Fig. 5). Similar results were obtained with a crude preparation of cytokines (TCGF) (data not shown). The influence of IL-4 was also studied since a recent study indicated that infection of SEB-tolerant mice with *Nippostrongylus brasiliensis* (known to induce a high IL-4 secretion in normal mice) circumvented the anergy of the CD4+Vβ8+ T cells (28). Fig. 5 shows that the Vβ-specific anergy observed in the course of HIV infection could not be reversed by IL-4.

Discussion

Peripheral tolerance, due either to T cell deletion or anergy, plays a major role in the suppression of autoimmunity (29, 30). Whereas deletion has been convincingly demonstrated for immature T cells recognizing self-determinants during thymic development (29, 31–33), functional anergy has been recently demonstrated for peripheral T cells exposed to superantigens in vivo. Thus, murine T cells bearing Vβ6+ TCR, specific for determinants encoded by the Mls-1a locus, are rendered unresponsive to Mls-1a in vitro after prior exposure to Mls-1a–bearing cells in vivo (18, 27, 34). Similarly, Vβ8+ T cells no longer respond in vitro to SEB when taken from SEB-primed mice (19–21, 35). In this report, we show the existence, in an important fraction of HIV-infected individuals, of a Vβ-specific clonal anergy that affects both CD4+ and CD8+ T cells. The Vβ8–specific unresponsiveness we observed in response to streptococcal superantigen ETA was not the consequence of a defective presentation of ETA since in the same cultures, the Vβ12+ population was always normally stimulated by this antigen. In addition, the Vβ8 anergy was also observed in response to another bacterial superantigen, SEE. The failure of anti-Vβ8 mAbs to stimulate this subset indicates that this Vβ-specific anergy represents an intrinsic functional defect rather than a specific lack of response to certain bacterial superantigens.

Inactivation or deletion of superantigen-reactive T cells by acute confrontation with superantigen is preceded by specific activation of these cells (35–37). Several recent reports have discussed attempts to indirectly reveal the presence of an HIV-associated superantigen by looking for consistent amplifications and/or deletions in the peripheral TCR Vβ repertoire of HIV-infected individuals. A more restricted Vβ repertoire (Vβ14 through Vβ20 appeared to be deleted) was found in HIV-infected patients with advanced disease (15) and a significant increase of peripheral CD4+ T cells of the Vβ5.3 subfamily was reported in asymptomatic subjects (16). Perturbations in the Vβ repertoire were also found in several pairs of monozygotic twins discordant for HIV with identical MHC (17), allowing meaningful comparisons of their Vβ repertoire, since one of the major factors that influence

Figure 4. Anergic Vβ8 T cells do not express IL-2R after ETA-activation. (*A–F*) PBMC from an HIV-infected Vβ8-responder (patient PT) and Vβ8 anergic (patient ZI) were stimulated for 4 d by ETA (1 μg/ml). Expression of CD25 was determined in the R1 population (small cells) by dual staining of T cell subsets expressing Vβ5.1,2,3 (*A* and *D*), Vβ8 (*B* and *E*) and Vβ12 (*C* and *F*) elements. (*G*) This same analysis was performed after ETA activation of T cells from seronegative controls C ($n = 6$), Vβ8 responders R ($n = 9$) or anergic NR ($n = 8$) HIV-infected donors. Results are expressed as the percentage of CD25+ T cells in the R1 population among a given Vβ T cell subset. Each symbol corresponds to a single individual. A significative difference in the expression of CD25 was only observed for the Vβ8+ subset from the anergic patients as compared to that of control or responder donors ($p \leq 0.001$ by Mann-Whitney U test).

the nature of the peripheral TCR Vβ repertoire is the MHC class II haplotype of the individual (38). Our study provides evidence that a dominant superantigen may be involved early in the course of HIV infection.

Since in mice in vivo administration of a superantigen induces, in addition to clonal anergy, varying degrees of clonal deletion (2, 18–21, 36, 39, 40), we compared ex vivo Vβ8 expression by CD4+ and CD8+ T cells from anergic subjects to that of responder and control subjects, but we could not detect any deletion or underrepresentation of this subset in anergic patients (mean values: 4.95% $V\beta8^+CD4^+$ ± 1.90; 4.03% $V\beta8^+CD8^+$ ± 1.88 in nonresponders vs. 4.24% $V\beta8^+CD4^+$ ± 1.26 and 4.04% $V\beta8^+CD8^+$ ± 2.17 in responders). Clonal deletion, however, is not an obligatory consequence of anergy induction and anergized cells may persist for extended periods of time (18, 27, 36). In addition, the outbred nature of human populations is an important obstacle to an accurate comparison of Vβ repertoires.

In vitro models of T cell anergy suggest that a major defect in these cells is their inability to produce IL-2, whereas anergized cells are fully responsive to exogenous IL-2 (41). Models of in vivo–induced anergy (18, 19, 21, 27, 37) and the present data, suggest a more profound defect. Upon in vitro restimulation with ETA, anergic $V\beta8^+$ T cells from HIV-infected individuals do not express IL-2Rs at all. These cells are also unresponsive to exogenous IL-2 or IL-4. It suggests that unresponsiveness of the anergized $V\beta8^+$ T cells appearing in the course of HIV infection is not a reversible process, at least by the use of these cytokines. Indeed, a 1-yr-longitudinal study of HIV-infected subjects belonging to responder or anergic group indicates that the Vβ anergic status is not a transient phenomenon, which may reflect a chronic exposure to the superantigen.

Several observations are in favor of a direct involvement of HIV in the Vβ-specific anergy observed in asymptomatic HIV-infected individuals: (*a*) anergy can be observed very early in the course of HIV infection e.g., in T cells from a primo-infected patient (CDC stage I); (*b*) comparison of the clinical status of responder vs. anergic patients showed no correlation with previous viral or bacterial infections, suggesting that anergy may not be induced by opportunistic pathogens; (*c*) inactivated HIV is able to induce, in vitro, a strong proliferation of normal peripheral lymphocytes and, concomitantly, the selective expansion of the $V\beta8^+$ T cell subset is reproducibly detected (Poccia, F., and M. L. Gougeon, manuscript in preparation).

Except for the acute pathogenic variant of simian immunodeficiency virus PBj14 (42), no report has shown until now the ability of HIV to activate normal peripheral T cells. Since a selective expansion of superantigenic-reactive T cells is known to precede anergy, one can speculate that in vivo infection of $CD4^+$ cells will release viral protein(s) containing Vβ-specific elements and presentation of this superantigen to T cells, in association with MHC class II molecules, will induce activation followed by anergy of subsets bearing the cognate Vβ determinants. It is interesting to note that the putative viral superantigen involved in this process has no selective tropism for $CD4^+$ T cells, since both $CD4^+$ and $CD8^+$ T cells are found to be anergic in patients. A recent report (43) described the dependence of HIV-1 replication on a superantigen and it concerned particularly the $V\beta12^+$ $CD4^+$ cell subset which replicated more efficiently HIV in vitro and which was found enriched for gp120-expressing cells in vivo. Our study is probably concerned with another superantigen, since the Vβ specificity involved is different and its mode of action also may be different since we never found a Vβ12 anergy in patients' peripheral T cells. The fact that Vβ8 anergy is not observed in all HIV-infected individuals but in 56% of them may be related to at least two factors: the dependence of the HLA-DR phenotype of presenting cells for the efficacy of the putative superantigen (38, 44, 45), and the possible requirement, for the acquisition of superantigenic activity, of mutations in the viral protein.

The involvement of superantigens has been recently suggested in autoimmune diseases such as rheumatoid arthritis (46) or multiple sclerosis (5, 47) and the expansion of T cells expressing TCR Vβ2 and Vβ8 has been reported in patients in the acute phase of Kawasaki disease (48). The influence of a superantigen in these pathologies was suggested by enhancement of T cell subsets expressing given Vβ elements or by skewed TCR repertoire. Our study reports for the first time in humans a specific Vβ anergy associated with a retroviral infection. It is unclear what role a viral superantigen may play in rendering target T cells anergic or susceptible to deletion by apoptosis (49–52). Activation of a significant fraction of CD4 cells by a superantigen would greatly increase the number of cells susceptible to virus infection and replication (43), contributing to viral dissemination and progressive immune failure. Encoding for superantigenic protein would also help the virus to escape from the immune system by inducing anergy in $CD8^+$ cytotoxic T cells that kill infected cells. Thus an as yet unidentified HIV-1–encoded superantigen could contribute, in addition to other cytopathogenic mechanisms, to the profound immunodeficiency observed in HIV infection.

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