HIV Antigens and T-Cell Receptor Variable Beta Chain Families

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The authors investigated whether the human immunodeficiency virus (HIV) has restrictive effects on the variable region of the β chain (Vβ) of the T-cell antigen receptor (TCR), by *in vitro* cultivation of non-HIV-infected peripheral blood lymphocytes with one of six HIV antigens or heat-inactivated whole virus (HIV-HI). Resting and blastic $CD4^+$ and $CD8^+$ cells were assessed with 3-colour cytofluorometry and monoclonal antibodies to various Vβ families/subfamilies. The Vβ families affected include V_β's 13.1/.3, 8, and 21 with gp120; V_β21 with gp160 and RT; V_β8 with p25; V_β's 8 and 21 with Rev; and V_β's 3 and 21 with HIV-2 Vpx. Vβ family-specific effects with HIV-HI did not differ significantly from those found with IL-2 stimulation. Findings differed between $CD4^+$ and $CD8^+$ cells. For $CD4^+$ lymphocytes, significant Vβ-specific decreases were found, not the expansions found with superantigens or mitogens. $CD8^+$ lymphocytes showed slight but significant expansions. The effects on V_β's 8, 13, and 21 are consistent with previous studies of HIV-infected persons. However, it is difficult to accept that antigens encoded by different HIV genetic regions cause proportionate diminutions of similar Vβ families. The authors suggest that these effects may be secondary to changes in cytokine profiles rather than direct interactions with TCR Vβ's.

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INTRODUCTION

Human immunodeficiency virus (HIV) is known to interact with CD4 and subsequently infect $CD4^+$ lymphocytes. Although the proportion of actively infected $CD4^+$ cells is quite small, $CD4^+$ lymphocyte destruction is massive [1]. Furthermore, over the course of HIV infection, the effects of the virus extend well beyond its $CD4^+$ cell target. Most infected individuals exhibit signs of B lymphocyte dysfunction, including poor antibody responses, hypergammaglobulinaemia, and autoimmune phenomena. In patients with endstage disease, $CD8^+$ cells, as well as $CD4^+$ cells, are decimated, although these cells do not appear to be a direct target for the virus [1].

Because of this global destruction, it has been suggested that HIV-1 might have superantigen (SAG)-like effects on subfamilies of the variable region of the beta chain (Vβ) of the TCR, since these can have broadly destructive effects [2–5]. This possibility is intuitively plausible for a number of reasons. First, Mls, the best studied endogenous SAG, is a retroviral gene product affecting specific murine BV-encoded families [6, 7]. Second, in some researchers' hands, the murine AIDS virus, somewhat related to HIV, appears to affect specific murine Vβ families [8, 9]. Third, some homologies exist between HIV proteins and staphylococcal SAGs [10]. Fourth, at least some exogenous SAGs affect $CD8^+$ lymphocytes as well as $CD4^+$ lymphocytes.

Studies to confirm or refute the possibility of direct HIV SAG activity have given conflicting results (Tables 1 & 2). Some studies suggested that Vβ effects occur in early, asymptomatic HIV infection, with no apparent relationship to previous bacterial or viral infections that might be independently associated with SAG activities [11–14]. Others found deletions of entire Vβ families occurring late in disease [3, 4]. One study suggested that SAG activity may be secondary to coinfection with cytomegalovirus (CMV) [15]. Still other studies found no Vβ-specific effects of HIV infection [16–19]. One recent study, described in two strongly overlapping publications, suggested differing Vβ-specific effects of HIV gp160 and gp120 antigens [20, 21]. Oddly, however, the effects of gp160 could be blocked by monoclonal antibody to gp120 [21].

Table 1. Effects found in studies of HIV on TCR Vβ families, by technique used

Technique*	Vβ's examined	Effects found	Reference
IF	5.1–.3, 6.7, 8, 12.1, (α2a)	↑5.2/.3	11
IF	2, 12	None	17
IF	5.1–.3, 6.7, 8, 12.1, (α2a)	↓5.1, ↓12, ↓α2a	12
IF			
IF	2, 3, 5.1–.3, 6.7, 8, 12.1, 13.3, 17, 19**	In $CD8^+$ only: ↑3, ↑5.2/.3 or ↑19**	16
IF	5.1, 6.7, 8, 12.1, (α2a)	↑$gp120^+$: 12, (5.1)†	10
IF	1, 2, 3/14, 5.2/.3‡, 8‡, 8.1/.2/.3, 9, 12.1‡, 13, 13.1, 13.2, 13.3, 17, 19**, 21, 22	$CD4^+$ perturbations: 2, 12, 13, 13.1, 13.2, 13.3, 21	26
IF	2, 3, 8, 13, 19**,§	$CD4^+$: ↓19**	33
IF	2, 3, 5.1/.3, 6.7, 8, 12.1, 13.3, 17, 19, 21, 22	$CD4^+$: 5.3, 21 $CD8^+$: 5.2/.3,12, 21	31
IF	''2, 3, 5, 6, 8, 12''	$CD4^+$ blasts: ↑''3.0, 12.0''	20, 21
2° SAG	ETA: V$_\beta$8,12	$CD4^+$ & $CD8^+$: V$_\beta$8 anergy	14
	SEE: V$_\beta$5.1, 6.1–.3, 8, 18 (Reagents: 5.1-.3, 8, 12, α2	$CD4^+$ & $CD8^+$: V$_\beta$8 anergy	
2° SAG	SED: V$_\beta$5, 12	↓5.2/.3¶, ↓5.3	13
	SEE: V$_\beta$5.1, 6.1–.3, 8, 18 (Reagents: 5.1–.3, 6.7, 8A, 12.1)	↓5.1, ↓8A, ↑12.1	
2° SAG	Rabies nucleocapsid: V$_\beta$8 (Reagents not defined)	General hyporesponsivity	19
HIV Inf	6.7, 8, 12, 17	12 high, 6.7 low, 8 & 17 ±	10
HIV/CMV Inf	6.7a, 12, 17	↑ HIV replication in 12 but only with CMV-infected monocytes	15
Stim	Stimulation index only	None	18

* Immunofluorescence, IF; stimulation with a known superantigen 2° SAG; cell line-directed HIV infectivity, HIV Inf; stimulation by HIV-infected cells in a mixed lymphocyte culture with an HIV-discordant identical twin or of SE-reactive tetanus toxoid-specific clones, Stim.

** Currently considered V$_\beta$17 [34].

† For 2 of 4 infected patients, a higher proportion of V$_\beta$12.1$^+$ peripheral blood cells stained with anti-gp120 antibody than did V$_\beta$5.1$^+$, V$_\beta$6.7$^+$, or V$_\beta$8$^+$ cells [10]. However, the graphic associated with those data suggested that for 2 of 4 patients, values for V$_\beta$5.1$^+$ cells were higher than for V$_\beta$12.1.

‡ Specificities noted by the supplier differed from those reported in publication as follows: V$_\beta$5.2/.3 by supplier, 5.1 in publication; V$_\beta$8, 8.1/8.2 in publication; V$_\beta$12.1, 12.2 in publication.

§ Specificities noted by the supplier differed from those reported in publication as follows: V$_\beta$8.1/.2 by supplier, 8 in publication; V$_\beta$13.6, 13 in publication; V$_\beta$17.1, 19(17) in publication.

¶ Specificities noted by the supplier as V$_\beta$5.2/.3; 5.3 in publication.

To investigate this issue further, we examined the *in vitro* effects of various HIV antigens on the TCR Vβ family distributions of resting and blastic $CD4^+$ and $CD8^+$ human peripheral blood lymphocytes of volunteer, HIV-uninfected donors. Stimulants included heat-inactivated HIV-1, *env*-encoded HIV-1 gp120 and gp160, *gag*-encoded HIV-1 p25/24, HIV-1 *rev*-encoded protein, *pol*-encoded reverse transcriptase, and HIV-2 *vpx* gene-encoded Vpx.

MATERIALS AND METHODS

Cells and HIV reagents. Peripheral mononuclear cells were obtained and isolated from the blood of 48 normal blood donors and analysed before and after culture with ≥1 (Table 3) of the following: HIV-1 (Hillcrest Biologicals, Cyprus, CA, USA)*, heat-inactivated at 56°C for 45 min, (HIV-HI) 0.001–0.1 μg/ml for 3–7 days; recombinant HIV-1 gp120 (Microgenesis Corp., West Haven, CT, USA) 0.001–0.1 μg/ml for 2–5 days; HIV-1_{IIIB} gp160 (Advanced Biotechnologies, Inc.) 0.001–0.1 μg/ml for 4–7 days, HIV-1_{SF2} p25/24 (p25) (NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA) [22, 23] 0.1 μg/ml for 2–7 days; HIV-1 Rev (Wild Type, *rev* cDNA from pCV-1) (NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA) 0.1 μg/ml for 2–7 days; recombinant reverse transcriptase (RT) (from

* Use of any and all trade names and commercial sources is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

Table 2. Effects found in PCR-based studies of HIV on TCR BV gene families

# Probes	Found	Population	Reference
20	↑2, ↓3, ↓9–15, ↓17, ↓19, ↓20	Adult	4
24	None	Infants	17
22 (AV:19)	↓14–20	Mixed	3
24	Differing ''△'s'':		5
	1, 16, 17, 19, 21	6 infected/ 7 uninfected twin pairs	
	6, (3?, 5S2?)	10 infected/3 uninfected infants compared to their HIV^+ mothers	
	6, 21	1 pair of lymph nodes/PBMC	
22	$CD4^+$: ↑3, 12, 14, 15 ↑17, 20 $CD8^+$: ↑3, 12, 14, 15	cells from 5 non-HIV-infected donors, stimulated *in vitro* with gp160	20, 21

clone Sϕ732 [pMFAT-7], NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA) [24] 0.1 μg/ml for 3–7 days; HIV-2_{NIHZ} Vpx (NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA) 0.1 μg/ml for 4–6 days. All cells were cultured in RPMI 1640 medium containing 10% fetal calf serum and 0.29 mg/ml L-glutamine at a density of 2×10^6 cells/mm^3 at 37°C in humidified 5% CO_2. IL-2 was not added.

Other reagents. Fluorescein isothiocyanate-conjugated (FITC) monoclonal antibodies against the human TCR were purchased from three sources, with reactivities specified to date as follows: Immunotech (Westbrook, ME, USA; BV2S1, clone E22E7.2; BV3S1, clone LE.89; BV5S2, clone 36213; BV8S1/8S2, clone 56C5.2; BV13S6, clone JU74.3; BV17S1, clone E17.5F3.15.13; BV21S3, clone IG.125; BV22S1, clone IMMU546), T Cell Diagnostics (TCD) (Woburn, MA, USA; AV2, clone F1; BV3S1, clone 8F10 [referred to herein as ''3A'']; BV5S1, clone LC4; BV5S2/5S3, clone 1C1; BV5S3, clone W112; BV6S7, clone OT145; BV8, clone 16G8 [referred to herein as ''8A'']; BV8, clone MX6 [referred to herein as ''8B'']; BV12S1, clone S511; BV13S1/13S3, clone BAM13), and BD (A,B TCR). Phycoerythrin-conjugated (PE) monoclonal anti-CD8 and PerCP-conjugated (PerCP) monoclonal anti-CD4 and anti-CD8 were obtained from BD.

Flow cytofluorometry. Three-colour cytofluorometry was undertaken using a FACScan or FACSort (BD) and Lysis II software. Negative control aliquots were stained with FITC mouse IgG1, PE-mouse IgG2a, and PerCP-mouse IgG1. Generally, 30,000 ungated events were collected from each sample. In addition, 3000 events were collected from each sample using the following gating: (a) forward- and side-scattering of $TCR_{\alpha,\beta}+$, cells indicative of large, less dense (blastic) cells (as opposed to small, dense (resting) lymphocytes) and (b) positivity for CD4 or CD8, but not both. This permitted the evaluation of 3000 blastic T lymphocytes for each analysis. Analyses are reported for the following populations: resting $CD4^+$ cells, resting $CD8^+$ cells, blastic $CD4^+$ cells, and blastic $CD8^+$ cells.

Analytical and statistical techniques. The proportions of resting and blastic $CD4^+$ and $CD8^+$ lymphocytes expressing a given BV gene product at the end of culture were compared to the proportions of resting $CD4^+$ or $CD8^+$ lymphocytes expressing that BV product at the beginning of culture. The same reagent panel was used at the beginning and end of each culture but the monoclonal antibody panel used was enlarged as additional reagents became available (Table 3). Comparisons were carried out using, as the denominator, the sum of values for all monoclonal antibodies to non-overlapping Vβ examined for that particular experiment. For example, the proportion of $CD4^+$ blastic cells expressing V_β''X'' = $\%V_\beta$''X+''/($\%V_\beta$''X+'' + $\%V_\beta$''Y+'' + $\%V_\beta$''Z+''), when monoclonal antibodies to V_β''X'', V_β''Y'', and V_β''Z'' were used in that particular experiment.

A Wilcoxon signed rank test was used to compare the proportions of cells expressing gene product V_β''X'' at the end of culture to the proportion of cells expressing V_β''X'' at the beginning of culture (day 0). This test provides a nonparametric matched (pre- to post-) analysis of all samples stimulated with a given reagent. Data herein will be expressed as the median subtractive differences between the proportion of cells expressing a given Vβ at the end of culture and the proportion of resting $CD4^+$ or $CD8^+$ cells expressing that Vβ product on day 0, for all cultures done with that antigen.

Results could not be obtained for the effects of media alone, without IL-2, because without IL-2 there were insufficient numbers of blastic cells for analyses to be performed. We therefore used IL-2 cultures as our negative control. (We did not measure cytokine levels in the current study; it is possible that IL-2 was generated in the stimulated cultures.) Specifically, in addition to comparing results at the end of culture to those on day 0, we also compared the differences associated with each antigen to those seen with IL-2 ($n = 6$–14), using a Wilcoxon rank sum test. For further comparison, data are also provided concerning the effects of a known superantigen, staphylococcal enterotoxin D (SED). Results concerning IL-2 and SED are presented in [25].

Results for all BV gene families listed above are available from the authors. Data presented in Figs 1–7 include only those Vβ families for which a significant increase or decrease was found in blastic cells to at least one antigen ($P < 0.01$).

Table 3. Number of evaluations, by antigen, Vβ reagent, and lymphocyte population being compared to day 0 resting lymphocytes

	$CD4^+$ Lymphocytes													
	HIV-HI	GP 120	GP 160	P25	REV	RT	Vpx	HIV-HI	GP 120	GP 160	P25	REV	RT	Vpx
	Resting							Blastic						
V_β														
(α2)	9	13	7	5	5	5	0	9	13	7	5	5	5	0
2	16	16	11	14	17	21	17	16	16	11	13	17	21	16
3	6	8	6	14	17	21	17	6	8	6	14	16	21	17
3.1	14	16	12	14	17	21	17	14	16	12	14	17	21	17
5.1	10	12	10	14	17	21	17	10	12	10	14	17	21	17
5.2	5	5	3	14	17	21	16	5	5	3	14	17	21	16
5.2/.3	18	17	12	14	17	21	17	18	17	12	14	17	21	17
5.3	8	10	8	14	17	21	17	8	10	8	14	17	21	17
6.7	11	13	10	14	17	21	17	11	13	10	14	17	21	17
8.1/.2	15	14	10	14	17	21	17	15	14	10	14	17	21	17
8A	15	17	12	14	17	21	17	15	17	12	14	17	21	17
8B	5	7	6	14	17	21	17	5	7	6	14	17	21	17
12.1	18	17	12	14	17	21	17	18	17	12	14	17	21	17
13.1/.3	9	11	10	13	17	21	17	9	11	10	14	17	21	17
13.6	7	7	3	14	17	20	17	7	7	3	14	17	21	17
17.1	18	17	12	14	17	21	17	18	17	12	14	17	21	17
21.3	13	15	11	14	17	21	17	13	15	11	14	17	21	17
22.1	13	15	12	14	17	21	17	13	15	12	14	17	21	17
	$CD8^+$ Lymphocytes													
	HIV-HI	GP 120	GP 160	P25	REV	RT	Vpx	HIV-HI	GP 120	GP 160	P25	REV	RT	Vpx
	Resting							Blastic						
(α2)	6	10	4	4	4	3	0	6	10	4	4	4	3	0
2	9	11	9	12	16	18	15	9	11	9	12	15	18	15
3	4	6	5	13	16	18	15	4	6	5	13	16	18	15
3.1	9	12	9	13	16	18	15	10	12	9	13	16	18	15
5.1	7	9	7	13	16	18	15	7	9	7	13	16	18	15
5.2	4	4	3	13	16	18	14	4	4	3	13	16	18	14
5.2/.3	10	12	9	13	16	18	15	10	12	9	13	16	18	15
5.3	6	8	7	13	16	18	15	6	8	7	13	16	18	15
6.7	8	10	7	13	16	18	15	8	10	7	13	16	18	15
8.1/.2	8	10	9	13	16	18	15	8	10	9	13	16	18	15
8A	10	12	9	13	16	18	15	10	12	9	13	16	18	15
8B	4	6	5	13	16	18	15	4	6	5	13	16	18	15
12.1	10	12	9	13	16	18	15	9	12	9	13	16	18	15
13.1/.3	7	9	7	13	16	18	15	7	9	7	13	16	18	15
13.6	5	5	3	13	16	17	15	5	5	3	13	16	18	15
17.1	10	12	9	13	16	18	15	10	12	9	13	16	18	15
21.3	9	11	9	13	16	18	15	9	11	9	13	16	18	15
22.1	9	11	9	13	16	18	15	9	10	9	13	16	18	15

RESULTS

Stimulation with SED, a known SAG, produced extensive blastogenesis (data not shown) and an increased proportion of cells expressing V_β5.2/5.3 in both $CD4^+$ and $CD8^+$ blastic (''Bl'') cells at the end of culture, compared with resting cells placed into culture (''D0'') ($CD4^+$ V_β5.2/5.3: median D0 6.0%, Bl 26.5%; $CD8^+$: 8.0%, Bl 34.0%) ($CD4^+$ V_β5.3: D0 2.6%, Bl

16.6%; $CD8^+$: 1.3%, Bl 18.2%). In 14 of the 17 donors, SED also produced an increase in the proportion of blastic $CD4^+$ cells expressing $V_\beta8$ ($CD4^+$ $V_\beta8.1/.2$: D0 13.2%, Bl 29.0%) ($CD4^+$ $V_\beta8A$: D0 12.5%, Bl 30.2%) ($CD4^+$ $V_\beta8B$: D0 8.8%, Bl 31.8%, $n = 4$, $P = 0.0015$)*. These alterations are consistent with activation and expansion of $CD4^+$ and $CD8^+$ $V_\beta5.2/5.3^+$ cells and $CD4^+$ $V_\beta8^+$ cells. Stimulation with IL-2 produced minimal blastogenesis and increased proportions of blastic $CD4^+$ cells expressing $V_\beta5.1$ (D0 8.4%, Bl 12.6%) and blastic $CD8^+$ cells expressing $V_\beta5.3$ (D0 2.9%, Bl 4.6%).

The presence of HIV-HI in a culture was associated with a higher proportion of blastic $CD4^+$ cells expressing $V_\beta5.1$ at the end of culture, compared to the resting lymphocytes when they were put into culture (median D0 9.3%, Bl 13.3%) (10 of 10 cultures showing increases) (Fig. 1). The proportions of blastic $CD4^+$ cells expressing $V_\beta13.1/.3$ (median D0 11.5%, Bl 8.7%) and of resting $CD4^+$ cells (referred to as ''R'') expressing $V_\beta8.1/.2$ were lower than at culture initiation (median D0 12.1%, R 10.3%) (9 of 9 and 12 of 15 cultures showing declines, respectively) (Fig. 1). However, none of these changes were significantly different from those seen in cultures stimulated with 10% IL-2 alone ($V_\beta5.1$: median D0 8.4%, Bl 12.6%, $n = 13$; $V_\beta13.1/.3$: median D0 12.0%, Bl 10.2%, $n = 12$; $V_\beta8.1/.2$: median D0 8.9%, R 7.6%, $n = 13$).

With gp120, there was an increased proportion of blastic $CD4^+$ cells expressing $V_\beta5.1$ (D0 9.5%, Bl 13.1%) (10 of 12 cultures showing increases) and blastic $CD8^+$ cells expressing $V_\beta21.3$ (D0 1.0%, Bl 4.3%) (10 of 11 cultures with increases) (Fig. 2). GP120 was also associated with the following changes: a decreased proportion of resting $CD4^+$ cells expressing $V_\beta8.1/.2$ (D0 12.0%, R 9.4%) (11 of 14 cultures) and $V_\beta13.1/.3$ (D0 12.1%, R 11.0%) (10 of 11 cultures), a decreased proportion of blastic $CD4^+$ cells expressing $V_\beta8$ by reagent to 8.1/.2 (D0 12.0%, Bl 6.7%) (13 of 14 cultures) and reagent 8A (D0 10.9%, Bl 10.8%) (13 of 17 cultures) (11 of 14 cultures with declines by both reagent 8 and 8A), a decreased proportion of blastic $CD4^+$ cells expressing $V_\beta13.1/.3$ (D0 12.1%, Bl 8.3%) (11 of 11 cultures), and a decreased proportion of resting $CD8^+$ cells expressing $V_\beta8.1/.2$ (D0 10.6%, R 9.0%) (9 of 10 cultures) and $V_\beta22.1$ (D0 9.0%, R 4.7%) (10 of 11 cultures) (Fig. 2). Changes that were significantly different from changes seen with IL-2 cultures included those for $V_\beta21.3^+$ blastic $CD8^+$ cells (IL-2: D0 0.4%, Bl 0.4%, $n = 9$, $P = 0.01$ for gp120 vs IL-2), $V_\beta13.1/.3^+$ resting $CD4^+$ cells (D0 12.0%, R 11.6%, $n = 12$, $P = 0.04$ for gp120 vs IL-2), and $V_\beta8.1/.2^+$ blastic $CD4^+$ cells (D0 8.5%, Bl 8.3%, $n = 13$, $P = 0.02$ for gp120 vs IL-2).

No significant expansions were seen with gp160 (Fig. 3). However, it was associated with a decreased proportion of resting $CD4^+$ cells expressing $V_\beta8.1/.2$ (D0 9.4%, R 6.9%) (10 of 10 cultures) and $V_\beta21.3$ (D0 3.9%, R 3.2%) (11 of 11 cultures)

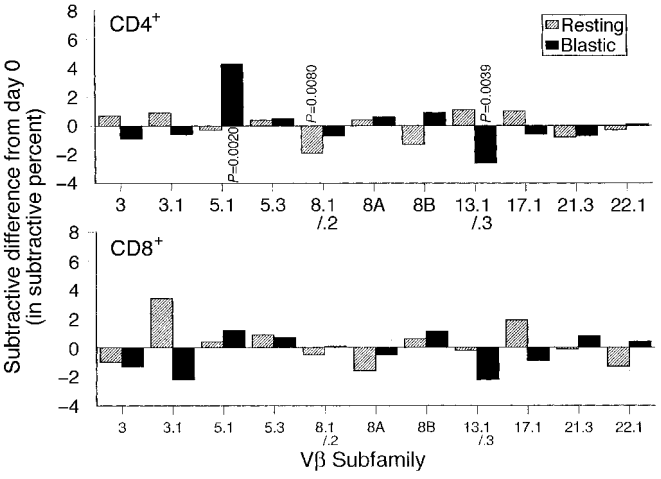

Fig. 1. Change in median percentage of $CD4^+$ and $CD8^+$ lymphocytes expressing TCR Vβ subfamily post HIV-HI stimulation by cell state. Medians of results for all samples tested. No finding differs from that of cultures with 10% IL-2 alone ($P < 0.05$).

(Fig. 3). The latter differed significantly from the effects of IL-2 on cultures (IL-2: D0 1.0%, R 1.1%, $n = 11$, $P = 0.02$ for gp160 vs IL-2).

As with gp160, no significant blastic expansions were found in cells cultured with p25. There was a proportionate increase in resting $CD4^+$ cells expressing $V_\beta3.1$ (D0 11.0%, R 12.7%) (13 of 14 cultures) and resting $CD8^+$ cells expressing $V_\beta6.7$ (D0 3.4%, R 5.8%) (11 of 13 cultures) (Fig. 4). P25 was also associated with a decreased proportion of resting $CD4^+$ cells expressing $V_\beta8$, using reagent 8B (D0 9.5%, R 6.7%) and reagent to $V_\beta8.1/.2$ (D0 9.6%, R 7.1%) (13 of 14 cultures with declines with both reagents), and of blastic $CD4^+$ cells expressing $V_\beta8.1/.2$ (D0

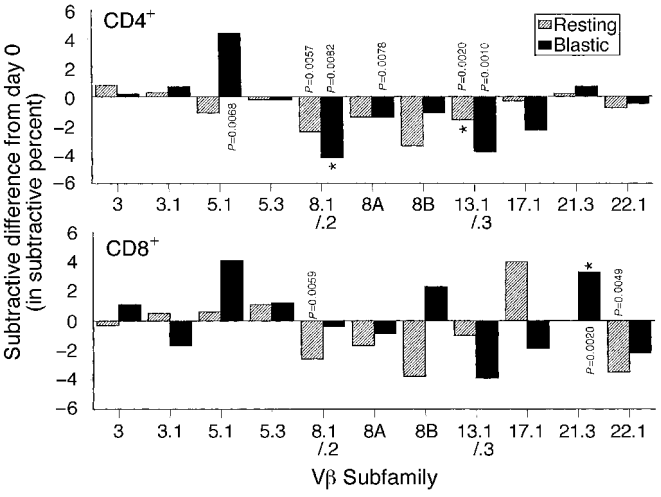

Fig. 2. Change in median percentage of $CD4^+$ and $CD8^+$ lymphocytes expressing TCR Vβ subfamily post GP120 stimulation by cell state. Medians of results for all samples tested. *Finding differs from that of cultures with 10% IL-2 alone ($P < 0.05$).

*These results are consistent with findings presented at the Human TCR Monoclonal Antibody Workshop, San Francisco, CA, USA, on July 26, 1995, in that: (1) There is evidence that the clone 8F10 reagent may be contaminated; the clone LE89 reagent is to a subset of $V_\beta3$; and (2) The three anti-$V_\beta8$ reagents discussed herein likely do not react with identical subsets of the $V_\beta8$ family.

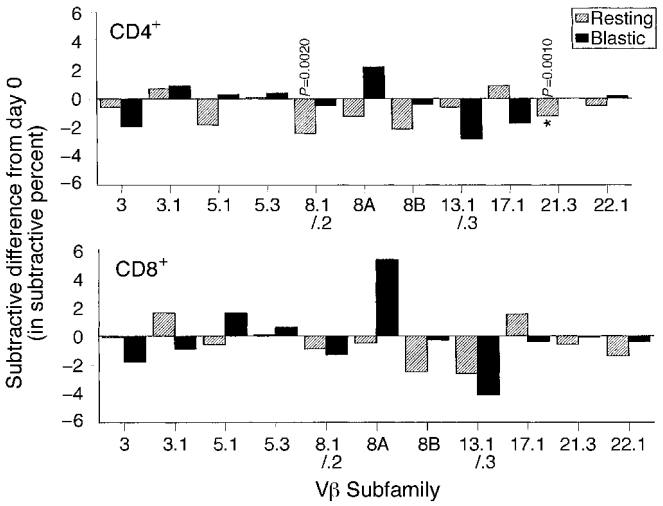

Fig. 3. Change in median percentage of $CD4^+$ and $CD8^+$ lymphocytes expressing TCR Vβ subfamily post GP160 stimulation by cell state. Medians of results for all samples tested. *Finding differs from that of cultures with 10% IL-2 alone ($P < 0.05$).

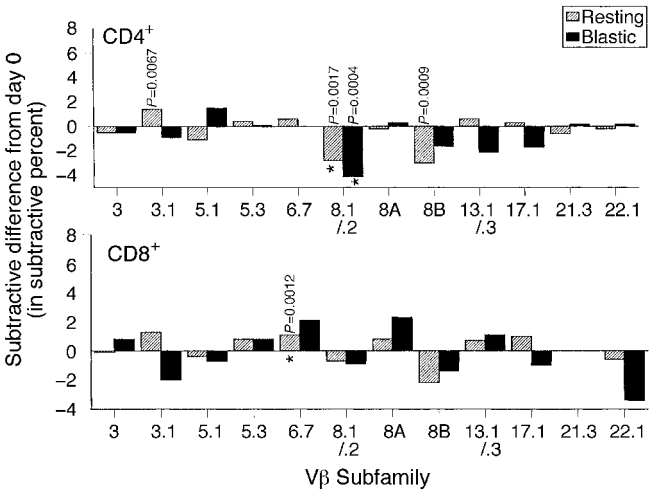

Fig. 4. Change in median percentage of $CD4^+$ and $CD8^+$ lymphocytes expressing TCR Vβ subfamily post P25/24 stimulation by cell state. Medians of results for all samples tested. *Finding differs from that of cultures with 10% IL-2 alone ($P < 0.05$).

9.6%, Bl 5.4%) (13 of 14 cultures) (Fig. 4). The latter two findings differ significantly from those of IL-2 cultures (IL-2, resting $CD4^+$ cells: D0 8.9%, R 7.6%, $n = 13$, $P = 0.02$ for p25 vs IL-2) (IL-2, blastic $CD4^+$ cells: D0 8.5%, R 8.3%, $n = 13$, $P = 0.01$ for p25 vs IL-2). The finding for $V_\beta 6.7^+$ resting $CD8^+$ cells also differed from those of IL-2 cultures (IL-2: D0 4.3%, R 3.7%, $n = 10$, $P < 0.01$ for p25 vs IL-2).

Culturing with Rev produced increased proportions of blastic $CD4^+$ cells expressing $V_\beta 5.1$ (D0 8.7%, Bl 14.6%) (16 of 17 cultures), blastic $CD8^+$ cells expressing $V_\beta 5.3$ (D0 2.8%, Bl 4.9%) (14 of 16 cultures), and resting $CD8^+$ cells expressing $V_\beta 8$ (reagent 8A:D0 10.2%, R 11.2%) (13 of 16 cultures) (Fig. 5). There was a proportionate decrease in resting $CD4^+$ cells expressing $V_\beta 8.1/.2$ (D0 9.5%, R 7.4%) (16 of 17 cultures) and $V_\beta 21.3$ (D0 3.7%, R 1.8%) (11 of 17 cultures) and in blastic $CD4^+$ cells expressing V_β's 8.1/.2 (D0 9.5%, Bl 6.3%, $P = 0.007$) (15 of 17 cultures) and 17.1 (D0 10.8%, Bl 7.7%) (15 of 17 cultures) (Fig. 5). Of these findings, those concerning $V_\beta 21.3$ (IL-2: D0 1.0%, R 1.1%, $n = 11$, $P = 0.03$ for Rev vs IL-2) and $V_\beta 8.1/.2$ differ significantly from those for IL-2 cultures (IL-2 resting $CD4^+$ cells: D0 8.5%, R 7.6%, $n = 13$, $P = 0.03$ for Rev vs IL-2) (IL-2 blastic $CD4^+$ cells: D0 8.5%, Bl 8.3%, $n = 13$, $P = 0.01$ for Rev vs IL-2).

Post-culturing with RT, in the $CD4^+$ blastic population, there were proportionate increases in V_β's 5.1 (D0 9.4%, Bl 13.8%) (18 of 21 cultures), 5.3 (D0 2.0%, Bl 2.7%) (17 of 21 cultures), and 8 (reagent 8A:D0 9.7%, Bl 11.2%) (18 of 21 cultures) and a proportionate decrease in $V_\beta 21.3$ (D0 3.4%, Bl 0.9%) (15 of 21 cultures) (Fig. 6). There were also proportionate increases in resting $V_\beta 3.1^+$ $CD4^+$ (D0 8.8%, R 9.7%) (18 of 21 cultures) and $V_\beta 5.3^+$ $CD4^+$ lymphocytes (D0 2.0%, R 2.5%) (17 of 21 cultures) (Fig. 6). Of these findings, only that for $V_\beta 21.3^+$ $CD4^+$ blastic cells differed from those for IL-2 cultures (D0 1.0%, Bl 0.9%, $n = 11$, $P = 0.04$ for RT vs IL-2).

With HIV-2 Vpx, there were proportionate increases in blastic $CD4^+$ cells expressing $V_\beta 5.1$ (D0 9.7%, Bl 16.9%) (17 of 17 cultures), $V_\beta 5.3$ (D0 1.9%, Bl 3.8%) (12 of 17 cultures), and $V_\beta 8$ with reagent 8A (D0 9.7%, Bl 12.0%) (16 of 17 cultures) and reagent 8B (D0 7.9%, Bl 10.0%) (12 of 17 cultures, 12 of 17 cultures with increases with both reagents). (In the case of HIV-2 Vpx, results obviously differ greatly for reagents 8A/8B and 8. This was not found with other antigens but could possibly be related to differences in the $V_\beta 8$ subset reactivity of these reagents.) There were proportionate increases in blastic $CD8^+$ cells expressing $V_\beta 3$ (D0 0.6%, Bl 4.6%) (12 of 15 cultures) and

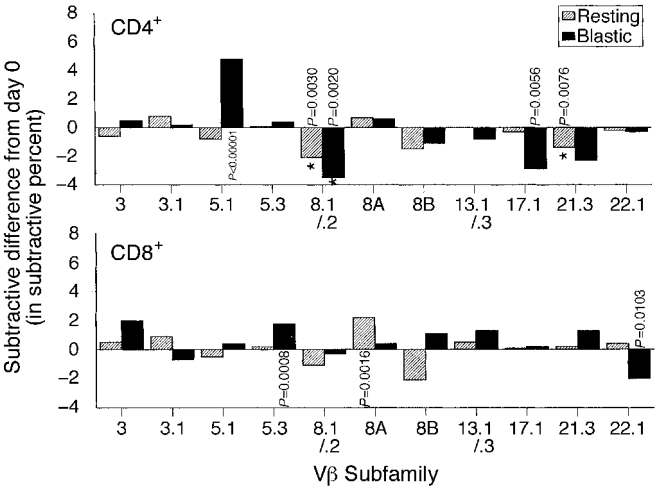

Fig. 5. Change in median percentage of $CD4^+$ and $CD8^+$ lymphocytes expressing TCR Vβ subfamily post Rev stimulation by cell state. Medians of results for all samples tested. *Finding differs from that of cultures with 10% IL-2 alone ($P < 0.05$).

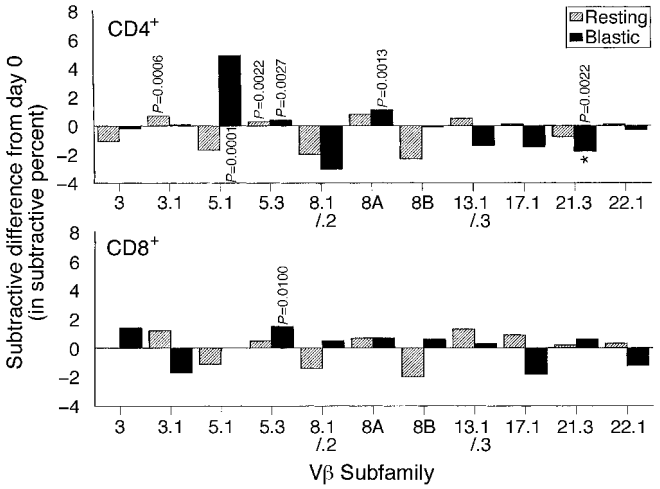

Fig. 6. Change in median percentage of $CD4^+$ and $CD8^+$ lymphocytes expressing TCR Vβ subfamily post RT stimulation by cell state. Medians of results for all samples tested. *Finding differs from that of cultures with 10% IL-2 alone ($P < 0.05$).

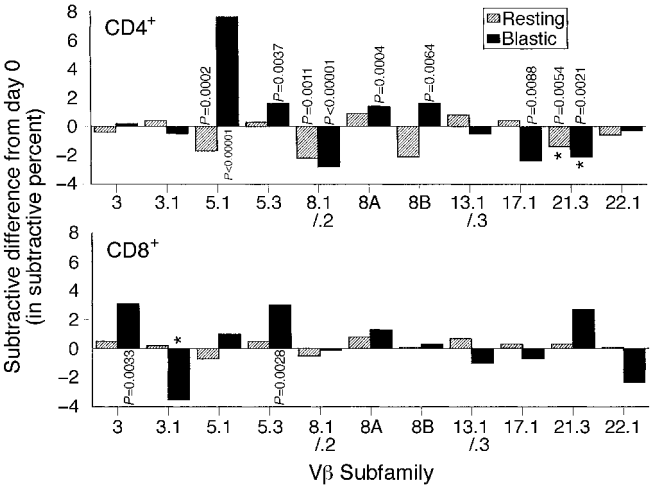

Fig. 7. Change in median Percentage of $CD4^+$ and $CD8^+$ lymphocytes expressing TCR Vβ subfamily post HIV-2 Vpx stimulation by cell state. Medians of results for all samples tested. *Finding differs from that of cultures with 10% IL-2 alone ($P < 0.05$).

V_β5.3 (D0 2.7%, Bl 4.9%) (12 of 15 cultures). Proportionate decreases occurred in resting $CD4^+$ cells expressing V_β's 5.1 (D0 9.7%, R 7.9%) (14 of 17 cultures), 8.1/.2 (D0 9.5%, R 7.6%) (14 of 17 cultures), and 21.3 (D0 3.8%, R 2.6%) (13 of 17 cultures) and blastic $CD4^+$ cells expressing V_β's 8.1/.2 (D0 9.5%, Bl 6.7%) (17 of 17 cultures), 17.1 (D0 11.1%, Bl 8.7%) (15 of 17 cultures), and 21.3 (D0 3.8%, Bl 2.1%) (15 of 17 cultures) (Fig. 7). Changes differed significantly from those found with IL-2 stimulated cultures in regard to $V_\beta 21.3^+$ $CD4^+$ resting (IL-2: D0 1.0%, R 1.1%, $n = 11$, $P = 0.01$ for HIV-2 Vpx vs IL-2) and blastic cells (IL-2: D0 1.0%, Bl 0.9%, $n = 11$, $P < 0.01$ for HIV-2 Vpx vs IL-2) and $V_\beta 3^+$ $CD8^+$ blastic cells (IL-2: D0 2.0%, Bl 4.1%, $n = 10$, $P < 0.05$ for HIV-2 Vpx vs IL-2).

To reiterate, contrary to some published studies summarized herein, following stimulation with HIV antigens, we did not find expansion of the V_β2.1 or 12.1 subfamilies within the blastic $CD4^+$ or $CD8^+$ populations. With HIV-2vpx, we did find expansion of $V_\beta 3^+$ cells in the CD8+ blastic population.

DISCUSSION

We provide evidence for TCR Vβ-specific alterations secondary to *in vitro* cultivation of uninfected cells with one of six HIV antigens or HIV-HI. The Vβ families affected include V_β's 13.1/.3, 8, and 21 with gp120; V_β21 with gp160 and RT; V_β8 with p25; V_β's 8 and 21 with Rev; and V_β's 3 and 21 with HIV-2 Vpx. Results were remarkably consistent among samples stimulated with a given antigen. Findings differed for the $CD4^+$ and $CD8^+$ cells. For $CD4^+$ lymphocytes, significant Vβ-specific decreases were found, not the expansions found with SAGs or mitogens using this system [25]. Vβ family-specific effects were found with HIV-HI but these findings did not differ significantly from those found with IL-2 stimulation.

The question of whether HIV or HIV antigens possess SAG-like activity is highly controversial. Previous studies have examined the relationships between natural HIV infection and the *in vivo* or *in vitro* activation, expansion, or deletion of T cells with particular TCR Vβ families. These studies have employed immunofluorescence, SAG stimulation of HIV-infected cells, HIV infectivity, and/or quantitative polymerase chain reaction (PCR) techniques. Results are summarized in Tables 1 & 2.

Studies using any of the non-PCR techniques have been restricted by small sample sizes, a limited supply of Vβ-typed clones/T-cell lines and, most importantly, the availability of monoclonal antibodies to various Vβ subfamilies (Table 1). Studies examining SAG stimulation of HIV-infected cells are, in addition, limited by the specificities of SAGs used. Reagents used, Vβ families investigated, cell populations examined ($CD3^+$, $CD4^+$, or $CD8^+$), and activation status of the cells examined vary from study to study. Despite these limitations and the discrepancies from study to study, certain Vβ families have been implicated in multiple studies, i.e. V_β5, V_β8, V_β12, and V_β21.

PCR-based studies have benefitted from the availability of probes to many BV families (Table 2) but suffer from concerns about the quantitative accuracy of this technique. The first published study using PCR reported a virtual absence of BV 14-20 gene expression in six patients with AIDS, as well as additional deletions varying from patient to patient [3]. Based on 11 infected patients, Hodara *et al*. reported that in advanced disease, there is an overexpression of BV2 and underexpression of various BV subfamilies in $CD4^+$ cells, including deletions or underexpression of BV's 10, 12, 13.1, 13.2, 14, 15, and 17 in 3 of 4 patients with AIDS [4]. Only BV3 was underexpressed in both $CD4^+$ and $CD8^+$ cells. One assessment of seven HIV-infected infants found no BV perturbation, compared to two healthy neonates [17]. A recently published review summarized three

PCR analyses undertaken by one group of collaborators [5]. It included three study groups, one of which apparently consisted of 6 of 7 twin pairs also described in a separate publication [26]. Results were inconsistent among the three groups. The only overlapping perturbations were in BV's 6 and 21.

Thus, the PCR data are in some respects consistent with studies using immunofluorescence or SAG stimulation but suggest broader defects than those found with the other techniques. One group of investigators who published their PCR-based results have subsequently suggested that this technique is insufficiently quantitative (and that an insufficient number of BV subfamily-specific probes are available) for PCR to be used to examine the TCR repertoire [26]. An additional problem with PCR may be the variation in amplification efficiency among BV families. Even allowing for the problems inherent in using PCR to study the TCR, it remains difficult to explain the virtual absences of expression of entire BV families that have been reported for HIV-infected persons when PCR techniques have been used. A study comparing immunofluorescence and quantitative PCR determinations of the TCR repertoire of normal donors found that PCR findings were not related to surface expression in any predictable or even consistent fashion [27]. Until these issues are resolved, PCR-based results might best be viewed with caution.

One recently published study used an *in vitro* stimulation approach similar to our own [20, 21]. With gp160 stimulation, increases were found in $CD4^+$ cell levels of TCRBV 3, 12, 14, and 15 (5 of 5 donors), BV17 (3 of 5 donors), and BV20 (2 of 5 donors) [20, 21]. For $CD8^+$ cells, increases were reported in BV 3, 12, 14, 15, but ''only in about half of the individuals on average'' [21]. Based on figures duplicated in both manuscripts, the largest increases appear to have been in BV 3, 14, and 15. Confirmation with immunofluorescence was done for two cultures, using TCD's reagents to V_β's 2, 3, 5, 6, 8, and 12. Increased percentages of $V_\beta 3^+$ and 12^+ cells were found. With gp120, increases were found for a different cluster of BV's by PCR, i.e. 2, 15, and 20 ($n = 2$) and for $V_\beta 2$ by immunofluorescence ($n = 1$) [20]. No statistical testing was done for any of these results. The effects of gp160 could be blocked with monoclonal antibody to gp120 [21]. We cannot readily explain why this study's Vβ findings for gp160 and gp120 differ from our own. At least at some point in this described study, the source of gp160 was the same as our own (J. Silver, personal communication, 1995) and the monoclonal reagents used appear to be a subset of those used by us. Two notable differences from our own study include smaller numbers ($n = 2$ and $n = 1$) and the addition of IL-2 during the last 24 h of culture. We and others have found an effect of IL-2 on Vβ distributions, albeit not in the pattern described by these authors [25, 28].

In the experiments described herein, we examined *in vitro* Vβ-specific responses to various HIV antigens, as well as to HIV-HI. Our approach has at least two advantages over patient-based studies. First, it obviates concerns about HLA discrepancies between comparison groups [5]. Second, it permits direct comparisons of several HIV antigens using any given donor's cells. All the HIV antigens assessed caused cellular depletion; no obvious expansion phase was seen, as found with staphylococcal SAGs or mitogens [25, 29]. All antigens except p25 were associated with perturbations of $V_\beta 21.3$. These changes included Vβ-specific proportionate decreases in the resting (gp160, Rev, and HIV-2 Vpx) and/or blastic (RT and HIV-2 Vpx) $CD4^+$ lymphocyte populations or Vβ-specific proportionate expansion in the blastic $CD8^+$ population (gp120). Similarly, gp120, p25, and Rev were associated with proportionate decreases of $V_\beta 8$ in the resting and/or blastic $CD4^+$ populations. GP120 was also associated with decreased proportion of $V_\beta 13.1/.3^+$ cells in the resting $CD4^+$ population and HIV-2 Vpx was associated with an increased proportion of $V_\beta 3^+$ cells in the $CD8^+$ blastic population. Additional alterations in the distributions of Vβ families were found; however, these changes did not differ significantly from those found with IL-2 stimulation. We did not measure cytokine levels, but it is possible that the findings are secondary to *in vitro* cytokine production.

We provide data concerning Vβ effects of various HIV antigens, and differentiate effects on $CD4^+$ from those on $CD8^+$ lymphocytes. Certain Vβ families were relatively expanded in $CD8^+$ cells, suggesting a slightly Vβ-biased cytotoxic T-cell response to gp120 and HIV-2 vpx, reminiscent of that reported with HIV-1 gp160 in a murine system [30]. Our other findings are consistent with, and of magnitude comparable to, many of those found in HIV-infected patients, with effects found on V_β's 8, 13, and 21 [2, 5, 13, 14, 19, 26, 31]. (It might be noted that $V_\beta 13$ and $V_\beta 21$ are reported to be highly homologous in complementarity determining region 4, thought to be associated with SAG activity [26].) We did not see any effects on $V_\beta 12$, as reported by others [2, 12, 13, 20, 21, 26, 31]. However, the subfamily specificities of the $V_\beta 12$ monoclonal reagents we used have not yet been confirmed. (The Human TCR Monoclonal Antibody Workshop, San Francisco, CA, USA, on July 26, 1995.) We did find effects on $V_\beta 5$ subfamilies, as reported by some investigators [2, 11, 13, 16] but these findings did not differ significantly from those found with IL-2 stimulation, suggesting that they may not be primary in nature.

It is difficult to imagine that these Vβ-specific effects of HIV antigens are physiologically meaningful. First, they represent only slight perturbations, albeit statistically significant and comparable to those found in most of the published studies. Second, the involved $CD4^+$ cell populations were decreased, not expanded. Vigorous blastogenesis did not occur in these cultures (data not shown). While these findings are again consistent with many reports in HIV-infected persons, they are unlike the effects found with most exogenous SAGs. Third, these effects were not absolute. Cells expressing a given Vβ family may have been proportionately decreased but were not entirely deleted. Thus, there were no obvious ''gaps'' in the Vβ repertoire, granted that the remaining cells may have been anergic [13, 14]. Similarly, the proportionate increase in $CD8^+$ cells expressing a given Vβ family was only slight. However, despite our own reservations, it is admittedly intriguing that the Vβ families found to be perturbed were those most frequently cited in the literature.

We cannot explain why antigens encoded by different HIV genetic regions might cause proportionate diminutions of similar Vβ families. Homologies have been reported between a number of these antigens and normal cellular proteins and/or staphylococcal SAGs [10, 32]. However, to our knowledge, these HIV antigens (other than gp120 and gp160) are not homologous to one another nor would homology be expected. We thus suggest that these Vβ effects may be secondary to *in vitro* changes in the levels of various cytokines. We and others have shown that IL-2 stimulation leads to slight skewing of the Vβ repertoire [25, 28]. Although it has not been reported, other cytokines might also alter the Vβ repertoire. We did not study cytokine profiles in this study but this merits further examination.

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