The Journal of Immunology

Clonal Dominance Patterns of CD8 T Cells in Relation to Disease Progression in HIV-Infected Children

Soe Than, Monica Kharbanda, Vivek Chitnis, Saroj Bakshi, Peter K. Gregersen and Savita Pahwa

J Immunol 1999; 162:3680-3686; ;
http://www.jimmunol.org/content/162/6/3680

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Clonal Dominance Patterns of CD8 T Cells in Relation to Disease Progression in HIV-Infected Children[1]

Soe Than,[2]* Monica Kharbanda,* Vivek Chitnis,* Saroj Bakshi,* Peter K. Gregersen,† and Savita Pahwa[3]*

CD8 T cells are important mediators of cellular immune responses as evidenced by clonal expansions in the CD8 TCR Vβ repertoire during primary HIV infection in adults. This study investigated the CD8 TCR Vβ repertoire by complementarity-determining region 3 length analysis using multiplex PCR in purified peripheral blood CD8 T cells of 22 HIV-infected children (age range was 0.75–15 yr, mean was 8.2 ± 4.1 yr). Evidence of clonal dominance in one or more Vβ families was obtained in 15 of 22 children. The patterns of clonal dominance were designated as major, minor, single, and none to indicate the involvement of three or more, two, one, or no Vβ families, respectively. A pattern of major or minor clonal dominance was observed in 12 children (group 1), whereas 10 children had single or no clonal dominance (group 2). In comparison with group 2, the children in group 1 had a higher percentage of CD4 cells (28.3 ± 11.6 vs 8.6 ± 4.8, $p < 0.001$); a higher stimulation index in lymphoproliferative responses to *Candida* (92.0 ± 59.5 vs 12.3 ± 14.4, $p = 0.002$), tetanus (76.3 ± 51.2 vs 11.2 ± 12.7, $p = 0.002$), and alloantigens (178.3 ± 298.9 vs 32.9 ± 35.2, $p < 0.001$); and a lower percentage of $CD8^+HLA\text{-}DR^+CD38^+$ cells (37.4 ± 13.1 vs 54.6 ± 14.2, $p < 0.01$). The number of dominant CD8 T cell clones was significantly correlated with the percentage of CD4 T cells ($r = 0.669$, $p < 0.001$) but not with plasma HIV RNA. Compared with group 1, patients in group 2 had a 4.8 times greater probability of having <15% CD4 cells. These findings indicate that CD8 clonal dominance in HIV-infected children reflects robustness of immune responses, regardless of time since infection and virus load. ***The Journal of Immunology,*** **1999, 162: 3680–3686.**

Cellular immune responses to Ags are initiated by the specific interaction between TCRs and the processed protein Ags bound to the MHC of APCs and result in Ag-specific expansions of T cells. Of the three complementarity-determining regions (CDR1,[4] CDR2, and CDR3) on the α- and β-chains of the TCR that interact with the Ag/MHC complex, the CDR3 loop comes into direct contact with peptide determinants in the Ag/MHC complex. The CDR3 regions of each TCR Vβ family are generated at the time of VJ and C region gene rearrangement and represent the diversity of the TCR repertoire. Nucleotide sequences of the CDR3 region determine the different CDR3 lengths, differing from each other by one nucleotide (1, 2). Consequently, CDR3 size analysis can identify patterns of dominance (3, 4) or depletion among the 24 TCR Vβ families.

A disruption of TCR repertoire diversity can occur in health and disease states in adults and children (5–11), resulting either from depletion/deletion or expansion/overexpression of one or more Vβ families. Panteleo et al. demonstrated that primary infection with HIV in adults frequently results in expansions in TCR Vβ families that are detectable only in CD8 T cells (12, 13). These expansions were oligoclonal and were functionally confirmed to be HIV-specific in nature. More recently, Gorochov et al. also reported CD8 T cell clonal expansions in HIV^+ adults; these expansions persisted for prolonged periods even after effective antiretroviral therapy (ART) (14). In HIV-infected children, although frequent and persistent multiple oligoclonal TCR Vα and Vβ expansions in CD8 T cells have been described, the studies have been limited by the small number of Vβ families examined (15) and/or the failure to investigate the CD4/CD8 repertoire separately (16). Interestingly, no CDR3 length restrictions have been identified in either CD4 or CD8 cells in cord blood (7, 17).

The present study was aimed at determining whether CDR3 length analysis of the CD8 TCR repertoire in HIV-infected children provides insight into the status of the immune system, namely CD4 cell numbers and Th cell function. The underlying assumption of the study was that clonally expanded CD8 T cells comprise a cell population that is important for HIV-specific immunity. Our results indicate that HIV-infected children demonstrate distinct patterns of CD8 T cell clonal dominance, with direct bearing upon the severity of immune compromise. A pattern of major dominance was associated with a more preserved immune system, regardless of age and virus load at time of study.

Materials and Methods

Study population

This study included 22 HIV-infected children ranging in age from 0.75 to 15 yr (mean age was 8.2 ± 4.1 yr). HIV infection was confirmed on the basis of two positive HIV DNA PCR tests and one positive HIV culture. The immunological and clinical status of the study population based on the revised Centers for Disease Control and Prevention (CDC) classification

*Division of Allergy/Immunology, Department of Pediatrics and †Division of Biology and Human Genetics, Department of Internal Medicine, North Shore University Hospital, New York University School of Medicine, Manhasset, NY 11030

Received for publication July 23, 1998. Accepted for publication December 9, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by Grant DA05161 from the National Institutes of Health.

[2] Current address: Advanced BioScience Laboratories, Inc., 5510 Nicholson Lane, Kensington, MD 20895-1078.

[3] Address correspondence and reprint requests to Dr. Savita Pahwa, Division of Allergy/Immunology, Department of Pediatrics, Room 303, Biomedical Research Building, NSUH-NYUSM, 350 Community Drive, Manhasset, NY 11030. E-mail address: spahwa@nsus.edu

[4] Abbreviations used in this paper: CDR, complementarity-determining region; ART, antiretroviral therapy; LTNP, long-term nonprogressor; LPA, lymphoproliferative assay.

0022-1767/99/$02.00

Table I. *Age, immunological, and virological characteristics and treatment of patients*

Subject ID No.	Pattern of Dominance[c]	Dominant Vβ Family(s)	HIV RNA Copies/ml (log_{10})	Age (yr)	CDC Class	CD4 %	CD8 %	ART[a]
Group 1								
964	Major	4, 1, 12, 22	<200 (<2.3)	1.1	N1	31	32	Yes
860	Major	1, 2, 3, 5.2, 7, 22, 11, 15	43,575 (4.6)	1.6	C1	34	45	Yes
677	Major	5.1, 15, 9	9,408 (3.9)	4	B1	40	35	Yes
121	Major	19, 5.1, 5.2, 11, 12, 8	9,068 (3.9)	5	C1	25	42	Yes
117[b]	Major	23, 2, 14, 24, 16, 9	58,812 (4.9)	7	N1	43	33	No
569	Major	2, 14, 21, 15	648,678 (5.8)	8.4	B3	3	58	Yes
804[b]	Major	2, 5.1, 7, 17, 14	20,160 (4.3)	9	N1	30	37	No
113	Major	24, 3, 13.1, 16, 20	1,486 (3.1)	11	B1	26	44	Yes
129	Major	6, 7, 17	4,574 (3.6)	13	A1	37	46	Yes
988	Major	18, 3, 19, 14	394,156 (5.6)	13.5	A3	10	23	Yes
1075	Minor	2, 5.2	1,039 (3.0)	0.75	N1	30	22	Yes
114	Minor	6, 22	76,509 (4.8)	5.4	C1	30	42	No
Group 2								
992	Single	1	89,639 (4.9)	7.5	B3	9	67	Yes
658	Single	18	50,099 (4.6)	8.3	B3	2	47	Yes
109	Single	7	67,037 (4.8)	9.4	B3	7	34	Yes
1028	None	—	25,571 (4.4)	5.4	B3	7	28	Yes
124	None	—	131,924 (5.1)	6.0	B3	17	56	Yes
108	None	—	53,772 (4.7)	9.6	B3	9	65	Yes
029	None	—	21,888 (4.3)	10.4	B3	12	21	Yes
675	None	—	2,022 (3.4)	12.6	B3	14	48	Yes
780	None	—	531,429 (5.7)	14	A3	7	50	Yes
962	None	—	94,356 (4.9)	15	C3	2	59	Yes

[a] ART with dual or triple drug combinations including AZT, 3TC, ddI, d4T, Nevirapine, and the protease inhibitors Nelfinavir, Ritonavir, and Saquinavir.

[b] Patients identified as LTNPs.

[c] Major = three or more Vβ families involved, minor = two Vβ families, single = one Vβ family, none = no clonal dominance.

(18) is listed in Table I. A total of 19 children were on antiretroviral treatment at the time of study, with two or three drug combinations consisting of reverse transcriptase inhibitors and protease inhibitors. The duration of their current therapy ranged from 1 to 18 mo. Two children aged >7 yr who were in immune category 1 had never received any ART and were classified as long-term nonprogressors (LTNPs). In addition, cord blood samples from 10 normal deliveries were studied as well.

CDR3 length analyses using RT-PCR

PBMCs were isolated from heparinized venous blood by Ficoll-metrizoate (Lymphoprep; Nyegard, Oslo, Norway) density gradient centrifugation. CD8 cells were positively purified using magnetic beads coated with anti-CD8 mAb (Dynal, Lake Success, NY); the purity of these cells by anti-CD8 mAb staining was >98%. The CD8 cells that bound to magnetic beads were lysed in Ultraspec RNA solution (Biotecx, Houston, TX), and RNA was extracted according to the manufacturer's protocol. cDNA was synthesized from 1 to 5 μg of RNA using a Moloney murine leukemia virus reverse transcriptase enzyme (Life Technologies, Grand Island, NY) and TCRβ-chain C region primer (Cβ14). Multiplex PCR was performed as described elsewhere with reaction sets as shown in Table II (4, 19). Briefly, 20 pM of two or three forward Vβ primers and 20 pM of a reverse primer specific for the C region (CβR) were used for each multiplex PCR reaction in the presence of gold *Taq* DNA polymerase enzyme (Perkin-Elmer, Branchburg, NJ). Before the PCR, 3 pM of 20 pM of CβR was labeled with [^{32}P]ATP. The primer sequences are shown in Table II. PCR conditions were as follows: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min for a total of 35 cycles followed by a final extension at 72°C preceded by 10 min of incubation at 95°C to activate *Taq* enzyme (*Taq* gold, Perkin-Elmer). Electrophoresis of the product of the multiplex PCR was performed in sequencing acrylamide gel to generate the spectratype and was followed by analysis in a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) using ImageQuant software (Molecular Dynamics). The intensities of the signals of CDR3 lengths in each TCR Vβ family were quantified, and a signal was defined as "dominant" if its intensity was ≥50% of the combined intensity of the signals of the remaining CDR3 signals within that Vβ family. Patterns of clonal dominance were designated as: "major" if at least three Vβ families were involved, "minor" for two Vβ families, "single" for one Vβ family, and "none" if no clonal dominance was identified. The graphic distribution pattern (immunoscope) of the various CDR3 lengths was subsequently created using ImageQuant software.

Lymphoproliferative responses

PBMCs were cultured in RPMI 1640 medium supplemented with 10% human AB serum (Life Technologies), 2 mM glutamine, 10 mM HEPES buffer, 10 U/ml penicillin, and 10 μg/ml streptomycin at a cell concentration of 0.33×10^6 cells/ml in the presence or absence of 5 μg/ml PHA (Difco Labs, Detroit, MI), the recall Ags tetanus (Connaught Labs, Swiftwater, PA) and *Candida* (Casta, Greer Labs, Lenoir, NC), or irradiated allogeneic lymphocytes in MLCs. After a period of 3 days for PHA and 5 days for MLCs and Ag stimulation, cultures were pulsed with 1 μCi of [^{14}C]thymidine (Amersham, Arlington Heights, IL) for 16 h, harvested on a Skatron Cell Harvester (Skatron, Lier, Norway) and processed in a beta scintillation counter (LKB-Wallac Betaplate 1205, Turku, Finland) for [^{14}C]thymidine incorporation.

Estimation of plasma viral load

HIV RNA in plasma was estimated using an Amplicor HIV monitor kit (Roche Diagnostics, Somerville, NJ) according to the manufacturer's protocol. The sensitivity of the assay was 200 HIV RNA copies per milliliter of plasma.

Statistical analyses

A Mann-Whitney rank-sum analysis was performed to compare differences in age, CD4 and CD8 counts, plasma HIV RNA levels, and lymphoproliferative responses between the selected patient groups. Fisher's exact test was performed to determine the risk of disease progression based on the pattern of CD8 clonal dominance.

Results

TCR repertoire in CD8 T cells from cord blood (Fig. 1)

An examination of the TCR Vβ repertoire by CDR3 length analyses of CD8 T cells was performed in 10 cord blood samples of infants born to healthy HIV-seronegative mothers. Based on the criteria established, no clonal dominance was evident in any of the samples and the repertoire was complete, as reported previously

Table II. *Primer sequences for multiplex PCR*

Primer	Sequence	Position	Reaction Set[a]
Vβ1	5′-CAA CAG TTC CCT GAC TTG CAC-3′	84	A
Vβ2	5′-TCA ACC ATG CAA GCC TGA CCT-3′	86	B
Vβ3	5′-TCT AGA GAG AAG AAG GAG CGC-3′	86	C
Vβ4	5′-CAT ATG AGA GTG GAT TTG TCA TT-3′	122	B
Vβ5S1	5′-TTC AGT GAG ACA CAG AGA AAC-3′	135	D
Vβ5S2	5′-CCT AAC TAT AGC TCT GAG CTG-3′	75	D
Vβ6	5′-AGG CCT GAG GGA TCC GTC TC-3′	81	E
Vβ7	5′-CTG AAT GCC CCA ACA GCT CTC-3′	86	F
Vβ8	5′-TAC TTT AAC AAC AAC GTT CCG-3′	144	B
Vβ9	5′-AAA TCT CCA GAC AAA GCT CAC-3′	84	G
Vβ10	5′-CAA AAA CTC ATC CTG TAC CTT-3′	76	L
Vβ11	5′-ACA GTC TCC AGA ATA AGG ACG-3′	90	H
Vβ12	5′-GAC AAA GGA GAA GTC TCA GAT-3′	117	H
Vβ13S1	5′-GAC CAA GGA GAA GTC CCC AAT-3′	117	C
Vβ13S2	5′-GTT GGT GAG GGT ACA ACT GCC-3′	135	I
Vβ14	5′-TCT CGA AAA GAG AAG AGG AAT-3′	84	J
Vβ15	5′-GTC TCT CGA CAG GCA CAG GCT-3′	87	I
Vβ16	5′-GAG TCT AAA CAG GAT GAG TCC-3′	132	G
Vβ17	5′-CAC AGA TAG TAA ATG ACT TTC AG-3′	137	J
Vβ18	5′-GAG TCA GGA ATG CCA AAG GAA-3′	117	A
Vβ19	5′-CCC CAA GAA CGC ACC CTG C-3′	79	K
Vβ20	5′-TCT GAG GTG CCC CAG AAT CTC-3′	111	E
Vβ21	5′-GAT ATG AGA ATG AGG AAG CAG-3′	143	L
Vβ22	5′-CAG AGA AGT CTG AAA TAT TCG A-3′	122	F
Vβ23	5′-TCA TTT CGT TTT ATG AAA AGA TGC-3′	146	A
Vβ24	5′-AAA GAT TTT AAC AAT GAA GCA GAC-3′	129	K
Cβ14	5′-CTC AGC TCC ACG TG-3′		
CβR	5′-CTT CTG ATG GCT CAA ACA C-3′		

[a] A total of 12 reaction sets (A—L) with two or more primers were used for multiplex PCR, as described in *Materials and Methods*.

(17). Fig. 1 shows a representative immunoscope pattern of CD8 cells from normal cord blood.

TCR repertoire pattern in CD8 T cells of HIV-infected children

The results of the TCR Vβ families in the CD8 T cells of the 22 HIV-infected children are summarized in Table I. Clonal dominance was observed in 15 children, and the patterns were considered to be major in 10 children (45.5%), minor in 2 children (9.1%), single in 3 children (13.6%), and none in 7 children (31.8%). Based on the patterns of clonal dominance, children were arbitrarily divided into group 1 (major/minor clonal dominance) and group 2 (single/no clonal dominance). In group 1, 10 of 12 children were in CDC immune category 1; the two children classified as LTNPs also fell into this group. All children in group 2 were in immune category 3. No particular Vβ usage was noted in the dominant clones. A representative immunoscope pattern of major clonal dominance is shown in Fig. 2. Based on Fisher's exact test, children in group 2 had a 4.8 times greater probability of being in immunological category 3 (CD4 cells $<$15% (95% confidence interval = 1.3–17.6, $p = 0.008$)) than those in group 1.

Lymphoproliferative responses in HIV-infected children with different patterns of clonal dominance

We evaluated the functional lymphocyte responses of the study patients by lymphoproliferative assays (LPAs) in response to the mitogen PHA, the recall Ags tetanus and *Candida*, and the alloantigens in MLCs (Fig. 3). The children in group 1 had significantly higher responses to recall Ags and alloantigens than the children in group 2, but responses to PHA were equivalent. A stimulation index of $>$50 for recall Ags was noted in 80% of children with major/minor clonal dominance but was not seen in any children with single/no clonal dominance.

Comparison of immunological responses and other variables between children exhibiting major/minor dominance and those with single/no clonal dominance (Table III)

Based on the parameters evaluated, the immune system appeared to be preserved better in children in group 1 compared with those in group 2. CD4 counts were significantly higher in children in group 1 vs children in group 2. A strong correlation was noted between the percentage of CD4 cells and the number of dominant CD8 clones ($r = 0.669$, $p < 0.001$, Fig. 4). Although CD8 T cell percentages were equivalent between the two patient groups, the percentage of HLA-DR$^+$CD38$^+$-expressing CD8 cells was significantly higher in the children from group 2. As described earlier, mean lymphoproliferative responses for recall Ags and alloantigens were significantly higher in the children from group 1. The mean age of the children in group 1 was lower (6.8 $\pm$ 4.4 yr) than that of the children in group 2 (9.8 $\pm$ 3.2 yr), but the difference in age was not statistically significant. Moreover, even if children of $<$5 yr of age were excluded, there was a significant association between the presence of major clonal dominance and a higher percentage of CD4 cells (data not shown). The mean virus load in the children from group 1 was slightly lower than that seen in the children from group 2; the difference was not significant. Further investigation of the relation of plasma viral load to the pattern of clonal dominance showed a trend for major clonal dominance to be associated with low viral load; however, this relationship was not statistically significant ($r = -0.236$, $p = 0.285$, Fig. 5).

Discussion

CD8 T cells are important components of HIV-specific immunity, as they mediate cytolytic and noncytolytic antiviral activities (20,

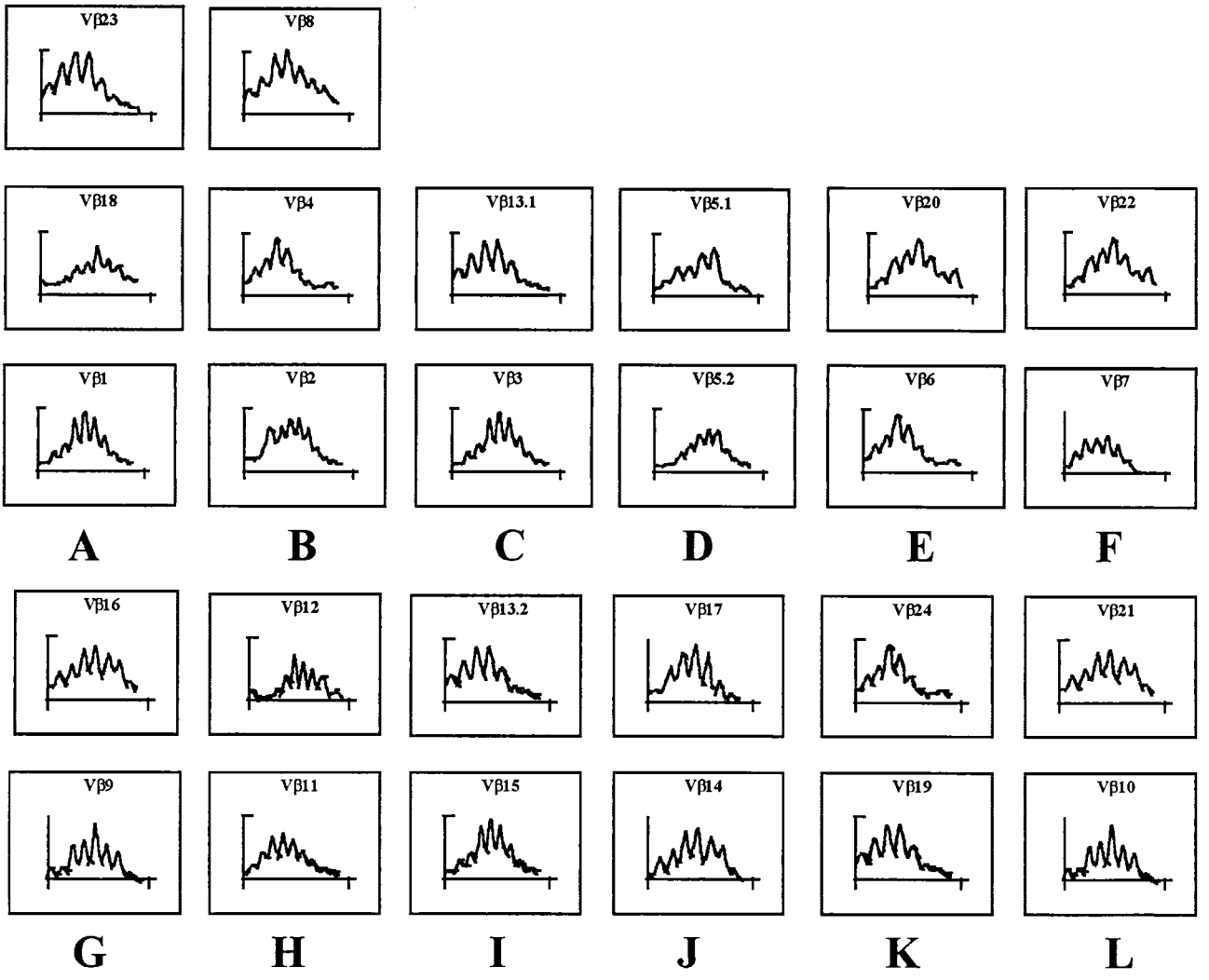

FIGURE 1. TCR Vβ repertoire in CD8 cells from normal cord blood: Immunoscope pattern shows no clonal dominance. *A–L* indicate reaction sets of multiplex PCRs (see Table II).

21). Recent data from several laboratories have shown that analyses of the CD8 T cell TCR repertoire provide an incisive glimpse into CD8 T cell immune responses in primary (12, 13) and chronic HIV infection (14). We have studied the TCR Vβ repertoire by CDR3 size analyses in CD8 cells from 22 children with perinatal HIV infection across a wide age range. Distinct patterns of clonal dominance (identified as major, minor, or single) were noted in 15 of 22 children. Children manifesting patterns of major/minor clonal dominance (designated as group 1) had higher CD4 counts and better lymphoproliferative responses to recall Ags and alloantigens than children who exhibited patterns of single or no clonal dominance (group 2). The expression of activation markers HLA-DR and CD38 on CD8 cells was higher in children from group 2. Plasma HIV RNA, although lower in children in group 1, was not significantly different between the two groups. These findings implicate an important role for clonal dominance in CD8 cells for host-immune responses in HIV-infected children.

CDR3 length analysis of TCR Vβ families is a sensitive indicator of Ag-specific clonal dominance, because it is the CDR3 region that comes in direct contact with Ag. T cell changes manifesting as clonal expansions of the TCR repertoire in CD4 or CD8 T cell populations have been reported in various disease states (e.g., rheumatoid arthritis, acute infectious mononucleosis, systemic lupus erythematosus, and Kawasaki disease) (8–11) and are believed to represent the responses of T cells to inciting Ags. Previous studies in patients with HIV infection have demonstrated that dominance/expansions in the CD8 T cell Vβ repertoire are oligoclonal as determined by an analysis of the TCR J region sequence (4, 12, 13). We analyzed the J region sequence of four dominant clones in CD8 cells from two patients in this cohort and confirmed that the observed Vβ family dominance was oligoclonal (data not shown).

The functional significance of the clonally dominant CD8 cells in relation to anti-HIV responses in HIV-infected children is unknown. It is possible that in addition to HIV, other Ags such as those used for recent immunization or coinfections contribute to the appearance of clonal dominance. This possibility seems unlikely to explain the differences among patients in our study, as neither group had associated coinfections and all of the children were under similar childhood immunization protocols. If the clonally dominant CD8 T cells are HIV-specific, it is important to point out that HIV-infected infants in the first year of life are reportedly deficient in CTL precursors (22). Thus, it is possible that the clonally expanded cells in infancy represent predominantly noncytolytic CD8 T cells producing cell-associated antiviral factor or β-chemokines. The HIV-directed suppressor activity mediated

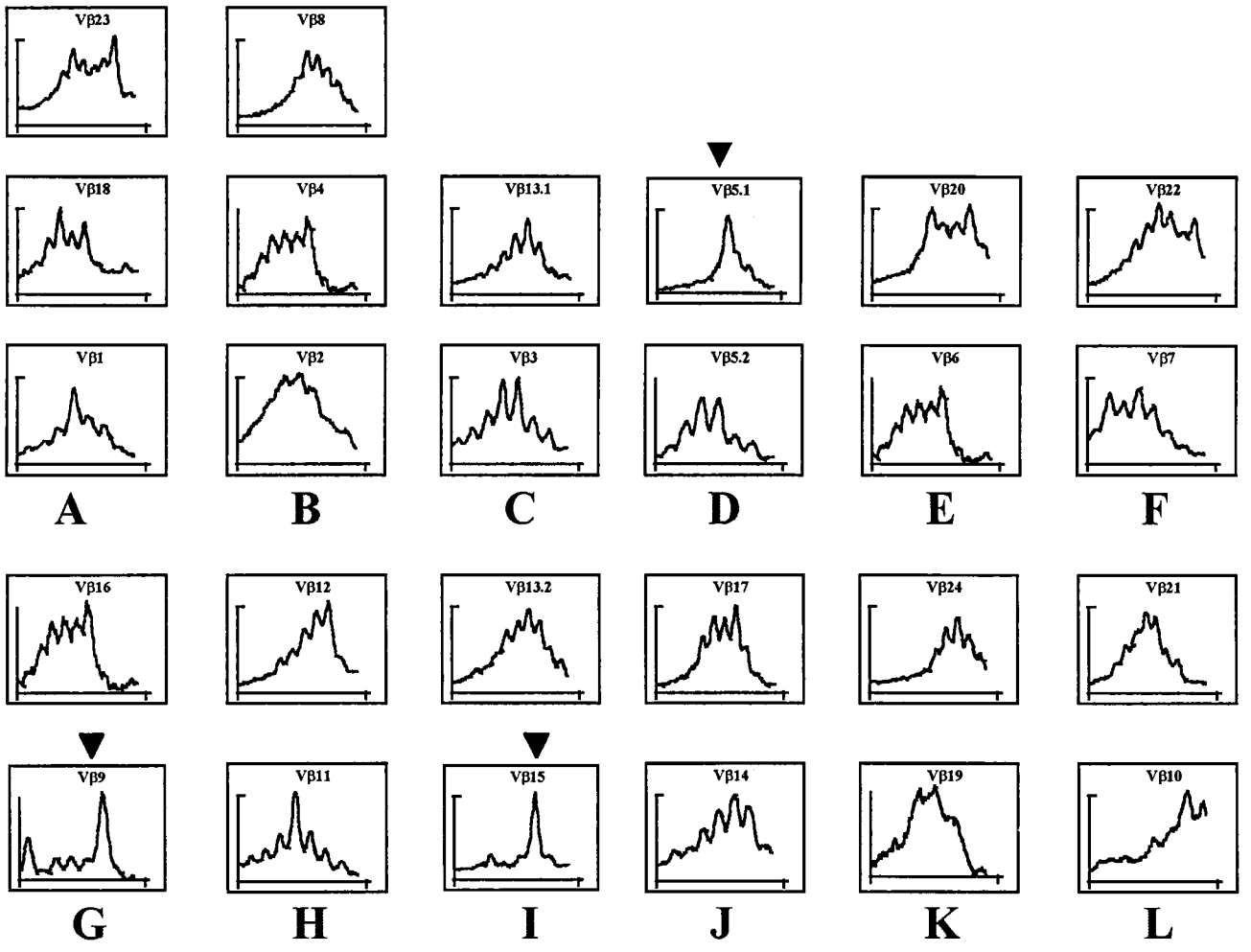

FIGURE 2. Representative immunoscope pattern of CD8 TCR Vβ repertoire showing major clonal dominance in CD8 cells from an HIV-infected child. Arrowheads indicate dominant clones that appear as single peaks to reflect clonal dominance in affected Vβ families. *A–L* indicate reaction sets of multiplex PCRs (see Table II).

by CD8 T cells in HIV-infected infants has been shown to strongly influence the course of the disease (23). In older children, the CD8 T cell clones might preferentially represent CTLs, as reported previously (12, 13). Thus, further studies to evaluate the functional attributes of oligoclonal CD8 T cell expansions need to be performed in an age-specific manner.

The factors that determine the pattern of CD8 T cell clonal response or the duration for which such clones persist are important and could not be ascertained in this study. It is possible that the dominant CD8 clones in older children in immune category 1 were

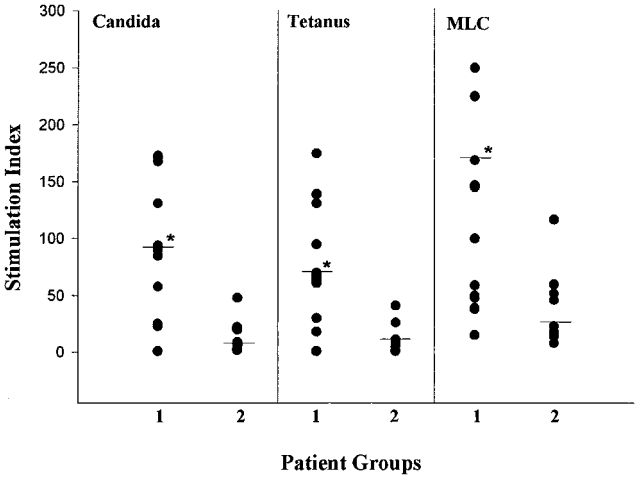

FIGURE 3. Lymphoproliferative responses in HIV-infected children. LPAs were performed with PBMCs as described in *Materials and Methods*. Patient group 1 = major/minor clonal dominance; patient group 2 = single/no clonal dominance. ∗, Mean stimulation index values in group 1 were significantly higher than in group 2 ($p = 0.002$ for *Candida* and tetanus responses and $p < 0.001$ for responses to alloantigens).

Table III. *Immunological and virological characteristics and lymphoproliferative responses of patients with major/minor or single/none pattern of CD8 clonal dominance*

	Major + Minor ($n = 12$)	Single + None ($n = 10$)	p Values
Age (yr)	6.8 ± 4.4	9.8 ± 3.2	NS
V.L. ($\log_{10}$)[a]	4.1 ± 1.1	4.8 ± 0.7	NS
% CD4	28.3 ± 11.6	8.6 ± 4.8	<0.001
% CD8	38.3 ± 10.1	47.5 ± 15.5	NS
DR^+CD38^+[b]	37.4 ± 13.1	54.6 ± 14.2	<0.01
LPA			
PHA	400.2 ± 518.5	258.4 ± 144.7	NS
Candida	92.0 ± 59.5	12.3 ± 14.4	0.002
Tetanus	76.3 ± 51.2	11.2 ± 12.7	0.002
MLC	178.3 ± 298.9	32.9 ± 35.2	<0.001

[a] V.L. = Plasma HIV RNA ($\log_{10}$)
[b] Expressed as % CD8 cells.

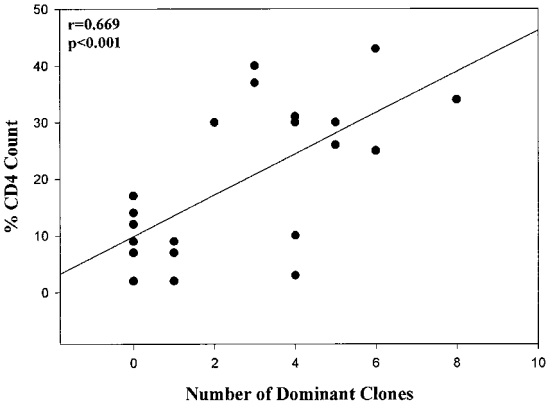

FIGURE 4. Correlation between the number of CD8 TCR Vβ-dominant clones and the percentage of CD4 cells in HIV-infected children (r = 0.669, $p < 0.001$).

representative of clones that had persisted since the onset of infection. Alternatively, they could represent clones that had been selected out or new clones originating from new thymic progeny in response to different viral epitopes. Also unclear are the reasons for the absence or paucity of these clones in some children; it is not known whether the clonal response in these cases never developed or whether it was lost. The failure of children to develop HIV-specific dominant clones could be due to genetic factors or to immune distraction during an early period of their lives in which they were faced with other serious infections. Reasons for the loss of clonally dominant T cells could be multiple (e.g., increased apoptosis (24), absence of persistent Ag stimulation (25), or decreased helper activity for CD8 cells) (26). Viral load as a contributing factor for loss of clonal dominance was ruled out, as plasma HIV RNA was not significantly higher in children from group 2 compared with those in group 1. Long-term prospective studies are required to address these questions.

Recently, Gorochov et al. reported that the CD8 TCR Vβ repertoire was drastically altered at all disease stages in HIV-infected patients (14). Interestingly, the disruption in the CD8 T cell repertoire persisted for longer periods than that in CD4 cells after highly active ART. These investigators found that the CD8 TCR repertoire perturbations in HIV-infected patients were independent of clinical and immunological status or plasma viral load. In contrast to our study, these investigators evaluated only the deviations from normal and did not, as reported here, examine numbers of dominant clones. It was the latter component of our study that led us to identify the relationship of the different patterns of clonal dominance to CD4 counts and to the functional responses to recall Ags. Another study that evaluated the TCR repertoire using semiquantitative PCR in patients demonstrated that during primary HIV infection, patients who exhibited expansions in at least two Vβ families or no expansions with polyclonal activation had a slow decline of CD4 cells regardless of plasma viral load, whereas the presence of single expansions was associated with faster CD4 cell loss (13). These findings are in agreement with our observations, as the patients with major CD8 clonal dominance in the CDR3 length analysis of the TCR Vβ repertoire used in our study could appear either as multiple expansions or no expansions in a semiquantitative PCR that does not identify clonality.

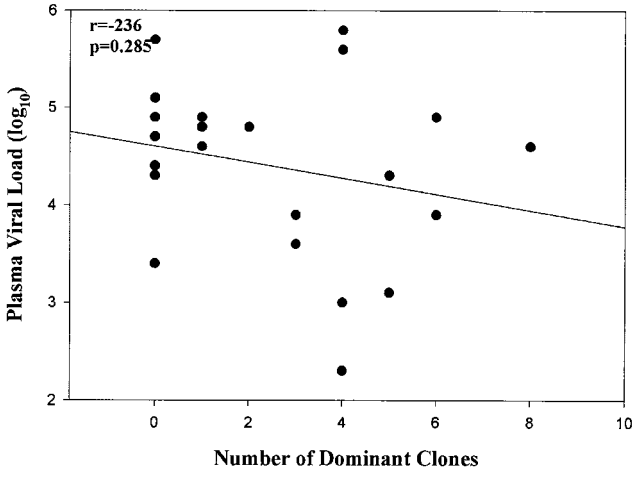

FIGURE 5. Correlation between the number of CD8 TCR Vβ-dominant clones and plasma HIV RNA in HIV-infected children. There is no statistically significant correlation ($r = -0.236$, $p = 0.285$).

It is well known that CD4 T cell help is essential for efficient CD8 T cell function. The importance of CD4 Th function for CD8 T cell CTL effector function was elaborated by Rosenberg et al. (26). These investigators reported that a polyclonal, persistent, and vigorous HIV-1-specific CD4 T cell response is associated with an increased production of IFN-γ and chemokines and occurs in individuals who control viremia without any ART. Thus, efficient CD4 help was considered essential for the production of type 1 cytokines and chemokine responses as well as for CD8 T cell antiviral activity. In our study, efficient lymphoproliferative responses to recall Ag were much higher in the children from group 1; although indirect, these findings are in concurrence with the view that good Th function is associated with CD8 T cell clonal dominance.

This study is the first report to focus on the association of CD8 T cell clonal dominance with disease severity and T lymphoproliferative responses in children with perinatal HIV infection. Studies of the TCR repertoire reported thus far in adults have been performed either during primary infection or have evaluated the influence of ART. Our studies indicate that the CD8 TCR repertoire provides insights into the host-immune response regardless of age and viral load. A central question left unanswered in this study is whether the presence of CD8 T cell clonal dominance is simply the consequence of a relatively intact immune response or whether it represents an anti-HIV response in some way. In either case, the function of clonally expanding CD8 cells in infancy may be significantly different from their counterparts in adults, and it is important to further elucidate the function of these cells. The quantitation of Ag-specific MHC class I-restricted CD8 T cells by Ag-loaded MHC tetramers (27) in a clonally expanded CD8 T cell population could potentially elucidate the biological nature of CD8 T cell clonal dominance in vivo.

References

1. Chothia, C., D. Boswell, and A. Lesk. 1988. The outline structure of T cell receptor $\alpha\beta$. *EMBO J. 7:3745.*
2. Davis, M., and P. Bjorkman. 1988. T cell antigen receptor genes and T cell recognition. *Nature 334:395.*
3. Pannetier, C., J. Even, and P. Kourilsky. 1995. T-cell repertoire diversity and clonal expansions in normal and clinical samples. *Immunol. Today 16:176.*
4. Gregersen, P. K., R. Hingorani, and J. Monteiro. 1995. Oligoclonality in the CD8 T cell population. *Ann. N. Y. Acad. Sci. 19.*
5. Batliwalla, F., J. Monteiro, D. Serrano, and P. Gregersen. 1996. Oligoclonality of $CD8^+$ T cells in health and disease: aging, infection, or immune regulation? *Hum. Immunol. 48:68.*
6. Schwab, R., P. Szabo, J. S. Manavalan, M. E. Weksler, D. N. Posnett, C. Pannetier, P. Kourilsky, and J. Even. 1997. Expanded CD4 and CD8 T cell clones in elderly humans. *J. Immunol. 158:4493.*
7. Hingorani, R., I.-H. Choi, P. Akolkar, B. Gulwani-Akolkar, R. Pergolizzi, J. Silver, and P. K. Gregersen. 1993. Clonal predominance of T cell receptors within the $CD8^+$ $CD45RO^+$ subset in normal human subjects. *J. Immunol. 151: 5762.*

8. Fitzgerald, J. E., N. S. Ricalton, A.-C. Meyer, S. G. West, H. Kaplan, C. Behrendt, and B. L. Kotzin. 1995. Analysis of clonal $CD8^+$ T cell expansions in normal individuals and patients with rheumatoid arthritis. *J. Immunol. 154: 3538.*
9. Callan, M. F. C., N. Steven, P. Krausa, J. D. K. Wilson, P. A. H. Moss, G. M. Gillespie, J. I. Bell, A. B. Rickinson, and A. J. McMichael. 1996 Large clonal expansions of $CD8^+$ T cells in acute infectious mononucleosis. *Nat. Med. 2:906.*
10. Mato, T., K. Masuko, Y. Misaki, N. Hirose, K. Ito, Y. Takemoto, K. Izawa, S. Yamamori, T. Kato, K. Nishioka, K. Yamamoto. 1997. Correlation of clonal expansion with disease activity in systemic lupus erythematosus. *Int. Immunol. 9:547.*
11. In-Hong, C., C. Yong-Jun, S. Woo-Sup, K. Dong-Soo, D.-H. Kwon, K. Joo-Deuk, and K. Se-Jong. 1997. Clonal expansion of CD8 T cells in Kawasaki disease. *J. Immunol. 159:481.*
12. Panteleo, G., J. F. Demarest, H. Soudeyns, C. Graziosi, F. Denis, J. W. Adelsberger, P. Borrow, M. S. Saag, G. M. Shaw, R. P. Sekaly, and A. Fauci. 1994. Major expansions of CD8 T cells with a predominant Vβ usage during the primary immune response to HIV. *Nature 370:463.*
13. Panteleo, G., J. F. Demarest, T. Schacker, M. Vaccarezza, O. J. Cohen, M. Daucher, C. Graziosi, S. S. Schnitman, T. S. Quinn, G. M. Shaw, et al. 1997. The qualitative nature of the primary immune response to HIV infection is a prognosticator of disease progression independent of the initial level of plasma viremia. *Proc. Natl. Acad. Sci. USA 94:254.*
14. Gorochov, G., A. U. Neumann, A. Kereveur, C. Parizot, T. Li, C. Katlama, M. Karmochkine, G. Raguin, B. Autran, and P. Debre. 1998. Perturbation of CD4 and CD8 T-cell repertoires during progression to AIDS and regulation of the CD4 repertoire during antiviral therapy. *Nat. Med. 4:215.*
15. Halapi, E., D. Gigliotti, V. Hodara, G. Scarlatti, P. A. Tovo, A. DeMaria, H. Wigzell, and P. Rossi. 1996. Detection of CD8 T cell expansions with restricted T cell receptor V gene usage in infants vertically infected by HIV-1. *AIDS 10:1621.*
16. Silvestri, G., H. Soudeyns, F. Denis, N. Lapointe, and R. P. Sekaly. 1996. T cell receptor Vβ-specific expansions in children from HIV-infected mothers. *AIDS 10:549.*
17. Garderet, L., N. Dulphy, N. Chalumeau, V. Schaeffer, M.-T. Zilber, A. Lim, J. Even, N. Mooney, C. Gelin, E. Glukman, D. Charron, and A. Toubert. 1998. The umbilical cord blood $\alpha\beta$ T-cell repertoire: characteristics of a polyclonal and naive but completely formed repertoire. *Blood 91:340.*
18. 1994. Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Morb. Mortal. Wkly. Rep. 43:1.*
19. Monteiro, J., R. Hingorani, I.-H. Choi, J. Silver, R. Pergolizzi, and P. K. Gregersen. 1995. Oligoclonality in the human $CD8^+$ T cell repertoire in normal subjects and monozygotic twins: implications for studies of infectious and autoimmune diseases. *Mol. Med. 1:614.*
20. Borrow, P., H. Lewicki, B. H. Hahn, G. M. Shaw, and M. Oldstone. 1994. Virus-specific $CD8^+$ CTL activity associated with control of viremia in primary HIV type 1 infection. *J. Virol. 68:6103.*
21. Levy, J., C. Mackewicz, and E. Barker. 1996. Controlling HIV pathogenesis: the role of the non-cytotoxic anti-HIV response of $CD8^+$ T cells. *Immunol. Today 17:217.*
22. Luzuriaga, K., D. Homles, A. Hereema, J. Wong, D. L. Panicali, and J. L. Sullivan. 1995. HIV-1 specific CTL responses in the first year of life. *J. Immunol. 154:433.*
23. Pollack, H., M.-X. Zhan, J. T. Safrit, C.-H. Chen, G. Rochford, P.-Z. Tao, R. Koup, K. Krasinski, and W. Brokowski. 1997. CD8 T-cell-mediated suppression of HIV replication in the first year of life: association with lower viral load and favorable early survival. *AIDS 11:F9.*
24. Finkel, T. H., G. Tudor-Williams, N. K. Banda, M. F. Cotton, T. Curiel, C. Monks, R. M. Ruprecht, and A. Kupfer. 1995. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. *Nat. Med. 2:129.*
25. Puppo, F., S. Brenci, O. Bosco, L. Lanza, S. Barocci, A. Nocera, M. Ghio, P. Contini, M. Setti, M. Scudeletti, and F. Indiveri. 1997. Downregulation of HLA class I antigen expression in $CD4^+$ T lymphocytes from HIV type 1-infected individuals. *AIDS Res. Human Retroviruses 13:1509.*
26. Rosenberg, E., J. M. Billingsley, A. M. Caliendo, S. L. Boswell, P. E. Sax, S. A. Kalams, and B. Walker. 1998. Vigorous HIV-1-specific $CD4^+$ T cell responses associated with control of viremia. *Science 278:1447.*
27. Ogg, G. S., X. Jin, P. Bonhoeffer, R. Dunbar, M. A. Nowak, S. Monard, P. S. Jeremy, Y. Cao, S. L. Rowland-Jones, V. Cerundolo, et al. 1998. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science 279:2103.*