T Cell Receptor Vβ Repertoire in an Acute Infection of Rhesus Monkeys with Simian Immunodeficiency Viruses and a Chimeric Simian-Human Immunodeficiency Virus

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Summary

Changes in T cell receptor (TCR) Vβ repertoire and their correlation with virologic events were investigated in rhesus monkeys after acute infection with the simian immunodeficiency virus (SIV). 11 genetically defined rhesus monkeys were experimentally infected with SIV_{mac} or a chimeric simian-human immunodeficiency virus (SHIV), and their peripheral blood lymphocytes (PBL) and lymph nodes were prospectively assessed for TCR Vβ gene expression. PBL and lymph nodes of the acutely infected monkeys demonstrated an expansion of selected Vβ-expressing T lymphocyte subpopulations as early as 3 d after infection. These expanded Vβ-expressing lymphocyte subpopulations were comprised predominantly of CD8+ cells. Six of seven infected monkeys sharing a single electrophoretically defined major histocompatibility complex class I allele exhibited a similar expansion of Vβ14-expressing PBL. Sequence analyses of V-D-J segments of TCR-β cDNA indicated that the Vβ-expressing T cell subpopulation expansion can be oligoclonal. SIV_{mac}-specific CD8+ cytotoxic T lymphocytes were demonstrated in both PBL and lymph nodes of the infected monkeys at the time expansion of the selected Vβ-expressing cell subpopulations was seen. Finally, the expansion of the selected Vβ-expressing lymphocytes in PBL coincided with the emergence and clearance of SIV p27 from the plasma of the infected monkeys. These results demonstrate that acute infection of rhesus monkeys with SIV_{mac} or SHIV results in an expansion of CD8+ lymphocyte subpopulations expressing selected Vβ gene families. The selectively expanded T lymphocytes may contribute to early viral clearance after acute SIV_{mac} or SHIV infection.

T lymphocytes play a major role in the antiviral immune response in primary HIV-1 infections. During the acute phase of an HIV-1 infection, individuals often transiently develop a high virus burden in the blood and lymphoid organs (1–5). Virus-specific cytotoxic T lymphocytes (CTL) can be demonstrated in PBL and lymph nodes of AIDS virus–infected individuals as early as a few days after infection; and the evolution of this functional T lymphocyte response correlates temporally with the initial clearance of the AIDS virus from these tissues (6–9). Furthermore, an expansion of selected TCR Vβ–expressing T lymphocyte subpopulations has been observed in PBL of individuals infected with HIV-1 or an acutely lethal variant of simian immunodeficiency virus (SIV)[1] (10, 11). It will be important to elucidate further AIDS viruses–driven TCR Vβ–restricted T cell response and its importance in containing the spread of virus during the acute phase of infection.

SIV-infected nonhuman primates have proven to be powerful models for the study of AIDS. SIVs are similar in sequence

[1] *Abbreviations used in this paper:* 1-D IEF, one-dimensional IEF; 1-D NEPHGE, one-dimensional nonequilibrium pH gradient electrophoresis; SHIV, simian-human immunodeficiency virus; SIV, simian immunodeficiency virus.

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to HIV (12), display a similar tropism for CD4-bearing lymphocytes and macrophages (13), and cause an AIDS-like disease in macaques (14). Early immunologic and virologic events can be studied prospectively in genetically defined macaques experimentally infected with SIV. The unique SIV/macaque model, therefore, provides an important system in which to explore aspects of T lymphocyte interactions with AIDS viruses in vivo. In the present study, we have assessed the changes in TCR Vβ repertoire after acute infection of rhesus monkeys with SIV_{mac} or a chimeric simian-human immunodeficiency virus (SHIV) as well as the temporal association of those changes with virus spread.

Materials and Methods

Viruses and Animals. The viruses used in this study included uncloned SIV_{mac} strain 251 and two molecularly cloned viruses, SIV_{mac} 239 and SHIV. This SHIV was comprised of SIV_{mac} 239 with the *env*, *tat*, *rev*, and *vpu* genes of the HIV-1 clone HXBc2 (15). Three rhesus monkeys were experimentally infected by intravenous inoculation with SIV_{mac} 251 (4 or 400 animal infectious doses per inoculation), four with SIV_{mac} 239 (10^4 $TCID_{50}$) and four with SHIV (4,000, 400, 40, and 4 median tissue culture infectious doses ($TCID_{50}$), respectively). After virus inoculation, the animals were monitored for signs of disease. Infection of these animals was determined by monitoring their plasma for viral antigen using an antigen capture assay for SIV gag p27 protein (Coulter Corp., Hialeah, FL) and their PBL for viral cDNA using the polymerase chain reaction (16). These monkeys were maintained in accordance with the guidelines of the Committee on Animals for the Harvard Medical School and the *Guide for the Care and Use of Laboratory Animals* (Department of Health and Human Services Publication [National Institutes of Health] No. 82-23, revised 1985).

Characterization of MHC Class I and Class II Alleles. The characterization of these monkeys' MHC class I and class II DR alleles was carried out using one-dimensional isoelectric focusing (1-D IEF) and one-dimensional nonequilibrium pH gradient electrophoresis (1-D NEPHGE), respectively (17, 18). Briefly, monkey B lymphoblastoid cell lines were individually established by transforming PBL with *Herpesvirus papio* (19). Cells were ^{35}S *trans*-labeled for 6 h at 37°C. Pelleted cells were lysed on ice in lysis buffer, and lysates were precleared by incubation with protein A-Sepharose CL-4B beads (Sigma Chemical Co., St. Louis, MO) alone and beads saturated with irrelevant antibodies. Immunoprecipitation was performed by incubating the precleared lysates with protein A-Sepharose CL-4B beads saturated with the mAb BB7.7 (for class I) or the mAb L243 (for class II DR). The beads were washed, and then treated with neuraminidase type VIII (Sigma Chemical Co.). BB7.7 immunoprecipitates were analyzed by 1-D IEF, whereas 1-D NEPHGE was used to analyze L243 immunoprecipitates as described previously (17, 18).

Isolation and Fractionation of Lymphocyte Populations. PBL were isolated from heparinized blood of the monkeys using Ficoll/diatrizoate gradient centrifugation. Peripheral lymph nodes were obtained by standard biopsy procedures before and after infection, and were carefully teased to generate single cell suspensions. $CD4^+$ or $CD8^+$ lymphocytes were purified using anti-CD4 or anti-CD8 antibody-conjugated Dynabeads (Dynal, Inc., Great Neck, NY). PBL were incubated with these immunomagnetic beads for 30 min at room temperature, and then selected in two cycles with a magnetic particle concentrator.

mRNA Extraction and cDNA Synthesis. mRNA was extracted from these unfractionated or fractionated lymphocytes using guanidinium thiocyanate and oligo dT spun columns (mRNA extraction kit; Pharmacia Fine Chemicals, Piscataway, NJ). The first-strand cDNA was synthesized in a 20-μl final vol at 42°C for 1 h using 0.2–1 μg of mRNA, 1 μg of random hexanucleotides, and 5 U of reverse transcriptase (Promega Corp., Madison, WI). The samples were heated for 5 min at 95°C to terminate the reaction.

PCR-based Analysis of TCR Vβ Gene Expression. A semiquantitative PCR-based method was used as previously described to determine the relative expression of the 24 Vβ gene families in monkey lymphocytes (11, 16). The cDNA isolated from each lymphocyte sample was aliquoted into 25 tubes, each containing a sense Vβ family–specific and an antisense Cβ primer. As an internal control, each reaction tube also contained a pair of primers that amplified a 105-bp fragment of the constant region of macaque TCR-α chain. A 25-cycle PCR reaction was performed in a 30-μl vol containing 0.3 μM of each Vβ and Cβ primer, 0.03 μM of the 5′ and 3′ Cα primers, 1 U of Taq polymerase (Perkin-Elmer Corp., Norwalk, CT), and ^{32}P end-labeled Cβ and Cα primers (3×10^5 cpm for each reaction). The cycle conditions were 1 min at 95°C, 55°C, and 72°C, respectively. The radiolabeled PCR products were electrophoresed through a 5% polyacrylamide gel, dried, and exposed to x-ray film. The separated Vβ-Cβ and Cα bands were measured for their radioactivity using an Ambis 100 (Ambis Systems, Inc., San Diego, CA). The cpm of individual Vβ bands were normalized by dividing the cpm for each Vβ band by the cpm of its associated internal control Cα band. The relative intensity of individual Vβ gene families was expressed as the number of cpm present in any one of the Vβ families divided by the total cpm present in the repertoire surveyed. As a control, two normal rhesus monkeys were inoculated intravenously with the culture supernatant derived from a virus-free CEMX174 cell line. PBL were obtained from the animals on day 0, 3, 8, and 15 after inoculation and then assessed for TCR-Vβ gene family expression. No perturbation in TCR-Vβ repertoire was seen in PBL derived from the normal monkeys after virus-free supernatant inoculation when compared with the Vβ expression in their PBL obtained before inoculation.

Molecular Cloning and Sequencing of TCR-β cDNA. This was done using a PCR-based cloning technique. cDNA was derived from PBL obtained from monkey 344 on day 14 after infection, a time when Vβ7 expansion was seen. It was amplified by PCR using a Vβ7 family-specific primer containing an EcoRI restriction site and a Cβ primer containing an XbaI restriction site. In an analogous fashion, cDNA derived from monkey L3 PBL obtained on day 9 after-infection was used in the PCR reaction using Vβ14 family-specific and Cβ primers. As a control, cDNA derived from the PBL sampled before infection from each of the two monkeys was amplified by PCR to isolate Vβ7- or Vβ14-bearing cDNA. The sequences for the primers were as follows: Vβ7 EcoRI, GCG CGA ATT CCT GAA TGC TCC AAG AGC T; V7β14 EcoRI, GCG CGA ATT CGT CTC TCG AAA AGA GAA GA; Cβ XbaI, GCG CTC TAG AGT GCT GAC CCC ACT GTG CAC. PCR was performed as previously described (20) for 35 cycles. To minimize PCR-generated misincorporation, PFU DNA polymerase was used in the PCR reactions. The PCR products were digested with EcoRI and XbaI, and ligated into the plasmid pSP65 (Promega Corp., Madison, WI) for cloning and sequencing.

Cytotoxicity Assay. Rhesus monkey B-lymphoblastoid cell lines immortalized with *Herpesvirus papio* served as target cells (19). The B-lymphoblastoid cell lines were infected with recombinant vaccinia viruses carrying the SIV_{mac} *gag* or *env* gene, and control (equine herpesvirus gH) gene (19). Effector cells were Ficoll-diatrizoate–isolated PBL or lymph node cells before and after SIV_{mac} infection of the monkeys. Lymphocytes were cultured for 3 d at 10^6/ml with Con A (5 μg/ml) (Sigma Chemical Co., St. Louis, MO), washed, and then maintained for another 3 d in medium supplemented with human recombinant IL-2 (20 U/ml) (provided by Hoffmann-La Roche, Nutley, NJ). ^{51}Cr-labeled target cells were incubated for 5 h with effector cells at E/T cell ratios of 100:1, 50:1, 25:1, and 12.5:1. Spontaneous release varied from 10 to 20%. Specific release was calculated as [(experimental release − spontaneous release)/(100% release − spontaneous release)] × 100. CD8-depleted or CD8-enriched lymphocytes were prepared from the cultured PBL or lymph node cells using monoclonal anti-CD4 antibodies or anti-CD8 antibodies and immunomagnetic beads (16). These cells were then used in CTL assays as described above.

Results

1-D IEF Characterization of MHC Class I and II Alleles of the Monkeys Used in the Studies. To facilitate interpretation of changes in Vβ repertoires of SIV_{mac}-infected rhesus monkeys, we characterized the MHC class I and class II DR molecules expressed by the studied animals using 1-D IEF and 1-D NEPHGE, respectively (17, 18). Despite the fact that these animals were obtained from several different colonies, they displayed extensive sharing of bands defined by the electrophoretic studies. Four animals (L3, L9, L28, and J28) expressed shared bands in the electrophoretic study of MHC class II DR-β alleles (Fig. 1 *A*). An electrophoretically defined MHC class I band was shared by seven rhesus monkeys (L3, L9, L28, J28, 344, 347, and 290) (Fig. 1 *B*). Other MHC class I alleles defined by 1-D IEF were also conserved among these monkeys (Fig. 1 *B*).

Monkeys Acutely Infected with SIV_{mac} Demonstrated an Expansion of Specific Vβ-expressing Lymphocyte Subpopulations in Both PBL and Lymph Nodes. We sought to determine whether the acute infection of rhesus monkeys with SIV_{mac} would result in the expansion of particular Vβ-expressing PBL subpopulations. Four monkeys were studied after inoculation with molecularly cloned SIV_{mac} 239. TCR Vβ repertoires of these animals were prospectively analyzed using a PCR-based quantitative technique. Expansions of particular Vβ-expressing T cell subpopulations were demonstrated in their PBL after infection. In monkey 344, 2.1- and 3.1-fold increases, respectively, in the amount of Vβ7 and Vβ14 transcript were documented in PBL obtained 17 d after infection when compared to the Vβ signals in their preinfection PBL (Fig. 2, *A* and *B*). Different Vβ-expressing T lymphocyte subpopulations were expanded in PBL of the other three monkeys 14 and 17 d after SIV_{mac} 239 infection (Fig. 2, *A* and *B*). Vβ1- and Vβ14-expressing T lymphocytes increased in number 2.6- and 2.8-fold, respectively, in PBL of monkeys 345; Vβ14- and Vβ23-expressing T lymphocytes increased in number more than 2-fold in PBL of monkeys 347 and 416.

To determine whether an SIV_{mac}-specific stimulation of selected Vβ expressing lymphocytes contributed to the lymphadenopathy seen in the infected monkeys, lymph node lymphocytes were also assessed for TCR Vβ repertoire. As shown in Fig. 2 *C*, the same Vβ-expressing lymphocyte subpopulations that expanded in PBL were also increased in the lymph node cells of the SIV_{mac} 239-infected moneksy 344 and 345.

Similar findings were also seen in two monkeys infected with another SIV_{mac} isolate, uncloned SIV_{mac} 251. Vβ7- and Vβ16-expressing lymphocytes were increased in number in PBL of the infected monkey 247; an expansion of Vβ8-expressing lymphocytes was demonstrated after infection in PBL of monkey 250 (Fig. 3).

The Expanded Vβ-expressing Cell Subpopulations Were CD8$^+$ Lymphocytes. We then assessed whether CD4$^+$ or CD8$^+$ lymphocyte populations expressing specific Vβ gene families were expanded after acute SIV_{mac} infection. CD4$^+$ and CD8$^+$ cell subsets in PBL of three acutely infected monkeys were positively selected using monoclonal anti-CD4 or anti-CD8 antibody–conjugated Dynal beads, and these purified cell populations were assessed for the expression of TCR Vβ gene families. Selected Vβ gene family–expressing CD8$^+$, but not CD4$^+$, lymphocyte subpopulations were expanded in PBL sampled at multiple time points after infection of monkeys 344, 345, and 290 (Fig. 4).

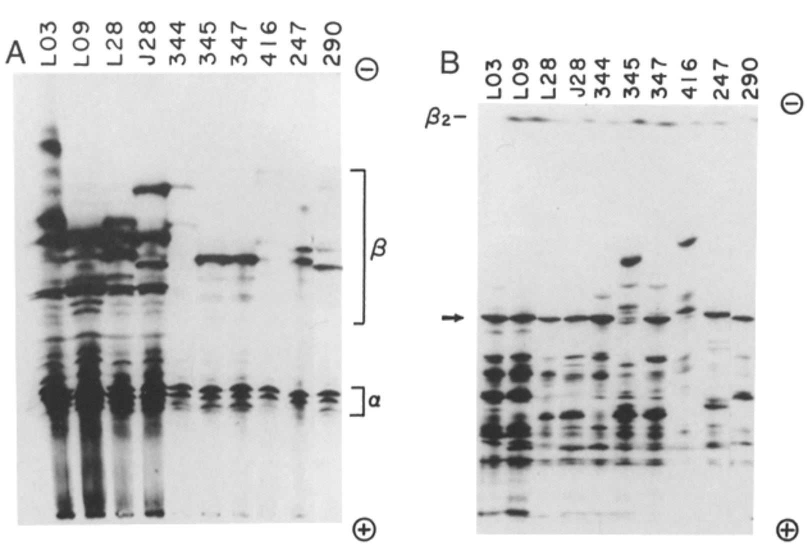
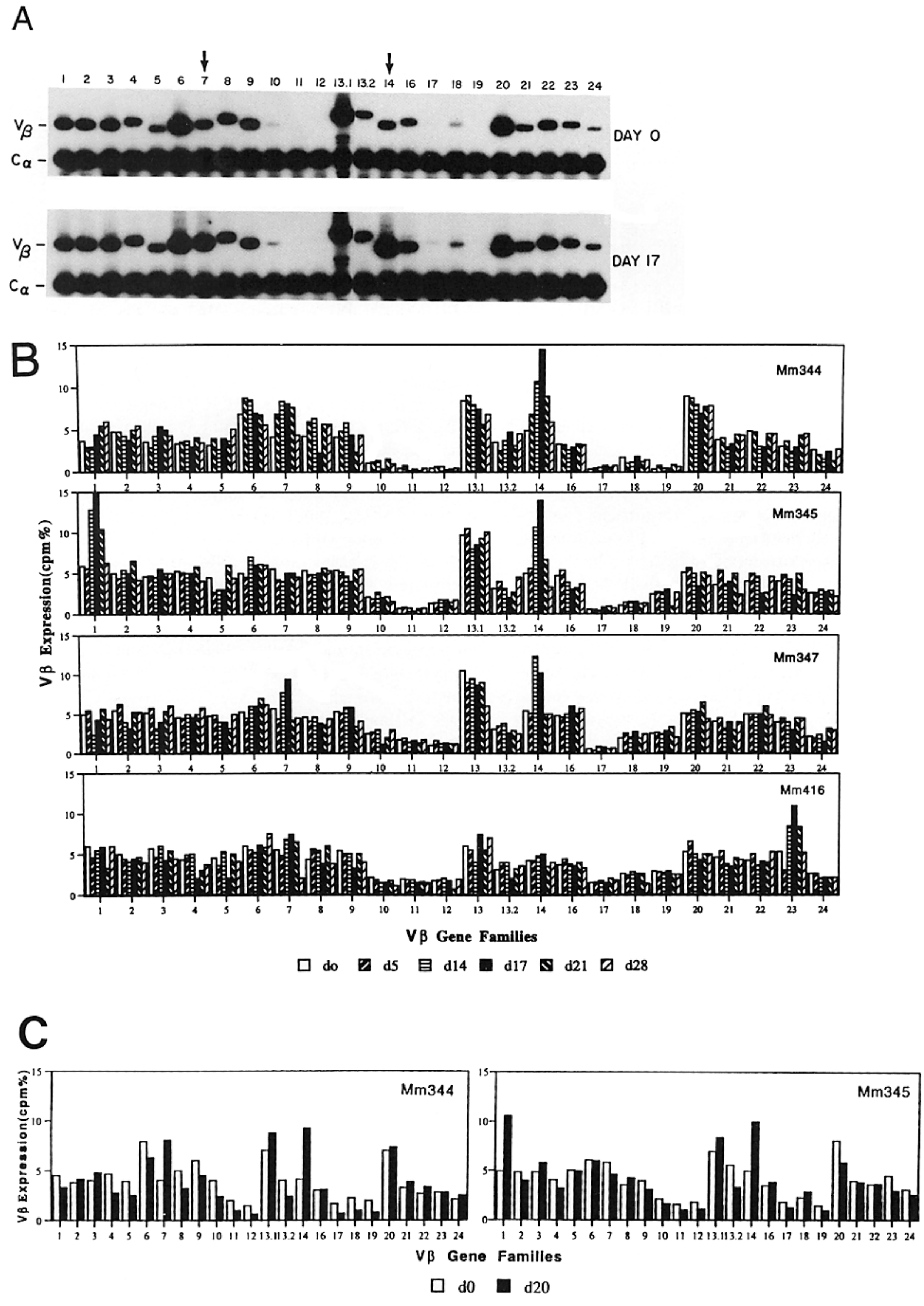


Figure 1. 1-D NEPHGE characterization of MHC class II DR (*A*) and 1-D IEF characterization of MHC class I molecules (*B*) of the rhesus monkeys used in the studies. Animal identification numbers are listed above the lanes of the gels. DR-α and -β gene products are indicated to the right of the gel in *A*. An arrow in *B* indicates an allele shared by six animals. Data from monkey 250 were not shown in these gels.


A
1 2 3 4 5 6 7 8 9 10 11 12 13.1 13.2 14 16 17 18 19 20 21 22 23 24
Vβ
Cα
DAY 0
DAY 17
B
Mm344
Mm345
Mm347
Mm416
Vβ Expression(cpm%)
Vβ Gene Families
do d5 d14 d17 d21 d28
C
Mm344
Mm345
Vβ Expression(cpm%)
Vβ Gene Families
d0 d20

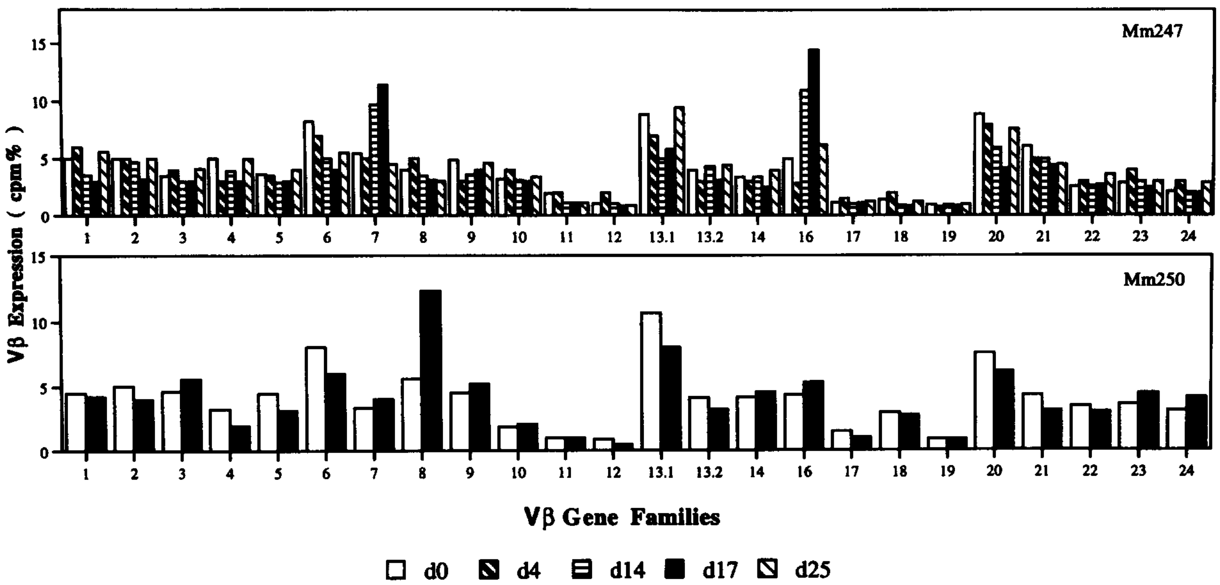


Figure 3. Vβ family expression in PBL of rhesus monkeys obtained before and after infection with SIV_{mac} 251.

Infected Rhesus Monkeys Sharing an Electrophoretically Defined MHC Class I Allele Exhibited an Expansion of Vβ14-expressing PBL. $CD8^+$ lymphocytes recognize viral protein as peptide fragments bound to MHC class I molecules. Since the selected Vβ-expressing T lymphocyte subpopulations in the SIV_{mac}-infected monkeys were predominantly $CD8^+$, there was reason to predict that particular virus-driven Vβ-restricted responses may be associated with certain MHC class I molecules. In fact, Vβ14-expressing T lymphocytes were expanded after infection in PBL of three SIV_{mac} 239– or 251–infected monkeys (344, 347, and 290) that expressed the same electrophoretically defined MHC class I molecule. Another four monkeys in this cohort of experimental animals sharing this same electrophoretically defined MHC class I allele (Fig. 1 *B*) were also examined for an expansion of Vβ14-expressing lymphocyte subpopulations in response to such a viral infection. These monkeys were inoculated with SHIV and their TCR Vβ repertoire was assessed prospectively. After the infection, three of the four monkeys (L3, L9, and L28) exhibited a striking expansion of Vβ14-expressing T lymphocytes in their PBL (Fig. 5). The PBL obtained from monkey L3 showed an initial increase in Vβ14-expressing lymphocytes as early as day 3 after infection, and 3.3- and 2.3-fold expansions of these lymphocyte subpopulations on days 13 and 17, respectively, after infection. 2.8- and 3.6-fold increases in Vβ14-expressing lymphocytes were also seen in PBL obtained from the infected monkeys L9 and L28, respectively. In addition, the Vβ7-expressing T lymphocyte subpopulation was expanded in PBL of monkey L28. We did not see the expansion of Vβ14-expressing lymphocytes in PBL of monkey J28 after infection. This animal was inoculated with the smallest quantity of virus (4 $TCID_{50}$) and, perhaps, did not develop as large an initial burst of viremia as the others in this group of monkeys. Nevertheless, these results suggest that the SIV-mediated expansion of selected Vβ-expressing $CD8^+$ lymphocytes may be associated with specific MHC class I molecules.

The SIV_{mac}-driven Expansion of Selected Vβ-expressing T Lymphocytes Can Be Oligoclonal. To determine the clonality of the selectively expanded Vβ-expressing lymphocyte subpopulations, we examined nucleotide sequences of V-D-J segments of TCR cDNA derived from PBL obtained before and after infection from monkeys L3 and 344. Polyclonal sequences were found in the Vβ14 cDNA clones generated by PCR from the preinfection PBL of monkey L3 (Fig. 6 *A*). In contrast, the cDNA clones derived from this monkey's PBL sampled on day 9 after infection, a time when Vβ14 expansion was detected by PCR quantitation, were oligoclonal. Four predominant clones were seen in the 50 Vβ14-clones sequenced, representing 50, 20, 16, and 10% of the clones (Fig. 6 *B*). Similar results were also seen in the Vβ7 cDNA clones derived from monkey 344 PBL obtained before and after infection (Fig. 7, *A* and *B*). 14 of 30 characterized clones (47%) had an identical junctional sequence which was rearranged with the Jβ 2.7 gene segment. The other predominant clone, D14-03, represented 20% of the total characterized clones.

Figure 2. PCR analysis of TCR Vβ expression in PBL or lymph nodes obtained from SIV_{mac} 239–infected rhesus monkeys. (*A*) Autoradiogram of TCR Vβ transcripts in PBL derived from monkey 344 on day 0 (*upper panel*) and day 17 (*lower panel*) after infection. (*B*) Comparison of Vβ expression in postinfection PBL with that in preinfection PBL. Individual Vβ expression was expressed as the percentage of total Vβ cpm in PBL observed at each time point as previously described (11, 16). A >1.5-fold increase in the ratio was considered significant (16). (*C*) Comparison of the Vβ expression in lymph node lymphocytes of two monkeys obtained before and after infection. Shown are individual Vβ expressions in lymphocytes from lymph nodes obtained preinfection and on day 20 postinfection.

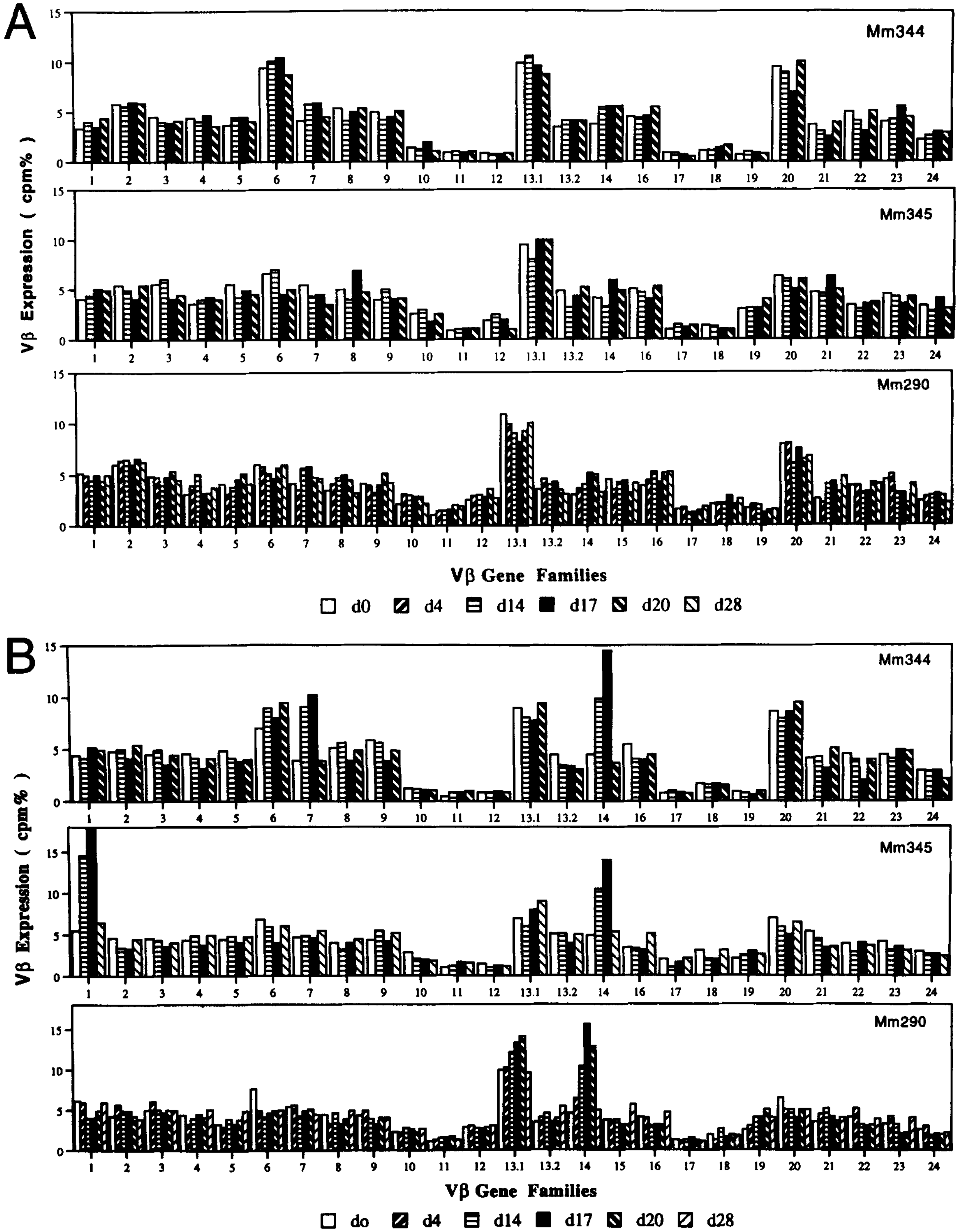


Figure 4. Vβ family expression in CD4+ (*A*) or CD8+ (*B*) lymphocytes of rhesus monkeys before and after infection with SIV_{mac} 239 or SIV_m 251. CD4+ or CD8+ lymphocytes were purified using anti-CD4 or anti-CD8 antibody-conjugated magnetic beads.

These results suggest that the expansion of selected Vβ-expressing T lymphocyte subpopulations soon after infection with SIV_{mac} or SHIV is oligoclonal.

The Expansion of Selected Vβ-expressing Lymphocytes Coincided with the Emergence of SIV_{mac}-specific $CD8^+$ CTL. We sought to determine whether the expansion of these selected Vβ-expressing $CD8^+$ lymphocyte subpopulations is temporally associated with the emergence of functional SIV_{mac}-specific CTL. For this purpose, PBL or lymph node cells obtained from two acutely infected monkeys were assessed for SIV_{mac}-specific CTL responses. At the time the Vβ14-expressing $CD8^+$ lymphocyte subpopulation was expanded in PBL of monkey 290 on day 18 and 20 after SIV_{mac} infection, SIV_{mac} Gag- and Env-specific CTL were readily demonstrated in PBL obtained at these same time points (Fig. 4, Tables 1 and 2). Similarly, SIV_{mac}-specific CTL were also detected in lymph node cells obtained from monkey 344 on day 20 after-infection, the time when the Vβ14- and Vβ7-expressing lymphocyte subpopulation expansions were seen (Fig. 2 *C*, Tables 1 and 2). These virus-specific CTL were $CD8^+$, but not $CD4^+$ cells, since depletion of $CD8^+$ cells in PBL resulted in the abrogation of specific CTL activity (data not shown). Furthermore, SIV_{mac} Gag-specific CTL from the monkeys 344 and 290 consistently lysed SIV_{mac} Gag-expressing autologous targets and targets expressing this shared electrophoretically defined MHC class I allele, but not fully MHC class I mismatched allogeneic targets. Therefore, there appeared to be a correlation between the expression of this electrophoretically defined MHC class I allele, the expansion of a Vβ14-expressing $CD8^+$ lymphocyte subpopulation, and the emergence of SIV_{mac} Gag-specific CTL response.

The Expansion of Selected Vβ-expressing Lymphocytes Coincided with the Emergence and Clearance of SIV Antigen in Plasma. Finally, we sought to determine whether there is a correlation between viral burden and the expansion of selected Vβ-expressing lymphocytes in rhesus monkeys after infection with SIV_{mac}. Plasmas were sequentially obtained from the infected monkeys and assessed for SIV p27 antigen. The expansion of selected Vβ-expressing lymphocytes appeared to coincide

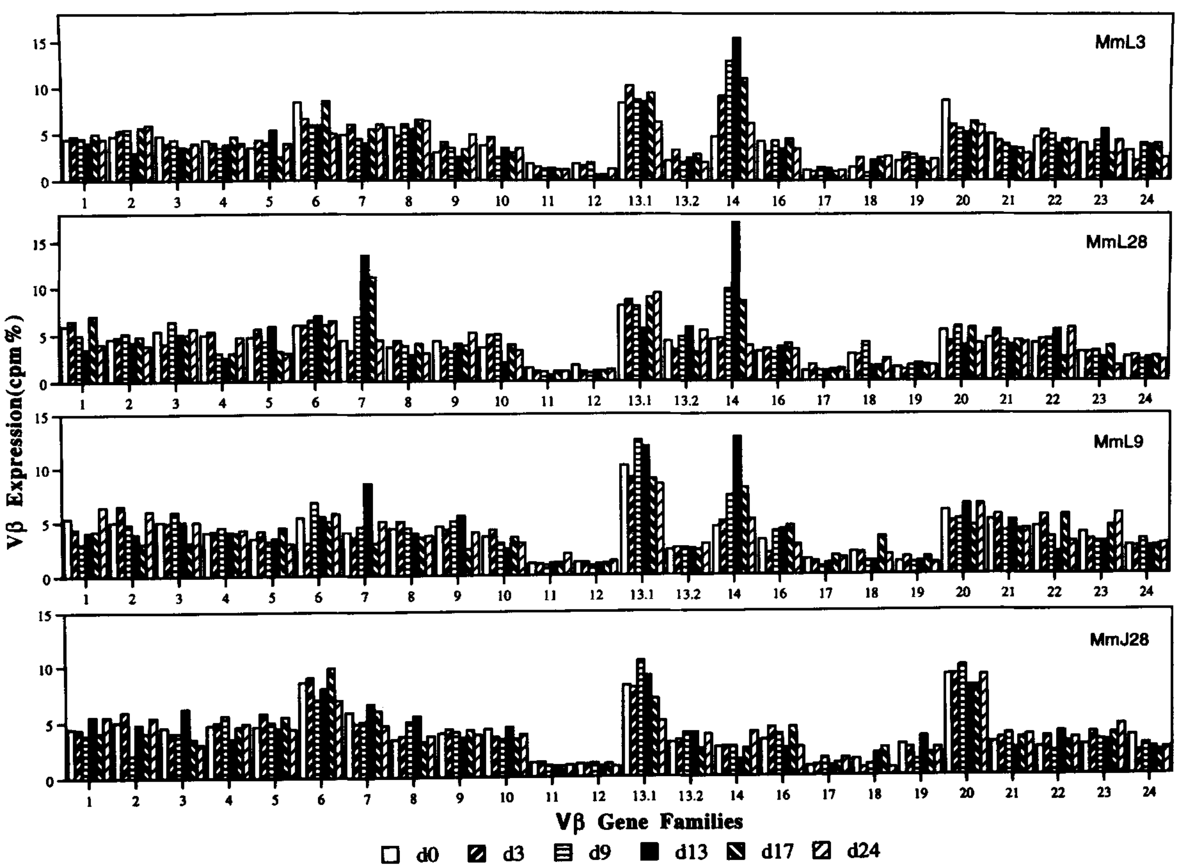


Figure 5. Vβ family expression in PBL of rhesus monkeys obtained before and after infection with SHIV.

A

CLONE	Vß	D+N	Jß		FREQUENCY
		CDR3			
D0-16	CASS	GTRD	YDYTFG	Jß1.2	1/20
D0-10	----	ITGGSN	------	Jß1.2	1/20
D0-04	---	RWVEN	------	Jß1.2	1/20
D0-22	----	LSGGAS	------	Jß1.2	1/20
D0-23	----	PRQIN	------	Jß1.2	1/20
D0-30	----	LAVSEP	------	Jß1.2	1/20
D0-05	----	REPG	------	Jß1.2	1/20
D0-17	----	KQGEDD	EKLF--	Jß1.4	1/20
D0-18	----	FQGSD	EKLF--	Jß1.4	1/20
D0-26	---	GRRVW	EKLF--	Jß1.4	1/20
D0-33	---	RTGTGANRG	EKLF--	Jß1.4	1/20
D0-12	----	LEGQGD	NQPQY--	Jß1.5	1/20
D0-14	---	QGT	NQPQY--	Jß1.5	1/20
D0-31	----	LLSGGG	NQPQY--	Jß1.5	1/20
D0-24	----	SRPGA	AQLF--	Jß2.2	1/20
D0-06	----	RSADA	TDPQY--	Jß2.3	1/20
D0-15	----	YRDS	TDPQY--	Jß2.3	1/20
D0-25	----	LSRDS	TDPQY--	Jß2.3	1/20
D0-03	----	YGRRKAD	NTQY--	Jß2.4	1/20
D0-01	----	IGAAE	NTQY--	Jß2.4	1/20

B

CLONE	Vß	D+N	Jß		FREQUENCY
		CDR3			
D9-09	CASS	LDRG	WDYTFG	Jß1.2	8/50
D9-05	----	TRDIN	EKLF--	Jß1.4	10/50
D9-15	----	-GDRN	TEAF--	Jß1.1	5/50
D9-16	----	-RDRN	NQPQY--	Jß1.5	1/50
D9-03	----	FYGGAR	GQPQY--	Jß1.5	25/50
D9-02	----	-EGQSDT	AQLF--	Jß2.2	1/50

Figure 6. Amino acid sequences in the TCR-β junctional region of Vβ14 recombinant clones derived from PBL collected from monkey L03 before (*A*) and on day 9 (*B*) after infection. The frequency of each recombinant in the total cDNA clones characterized is indicated. *V*, variable; *N*, nontemplated; *D*, diversity; *J*, joining; *CDR3*, complementary determining region (21, 22).

with the emergence and clearance of SIV viral protein from the plasma of the infected monkeys (Fig. 8). Plasma antigenemia was maximal in monkeys 344, 345, 347, and 416 on day 14 after infection; an expansion of selected Vβ-expressing T cells in PBL was detected in these animals on day 14 and was maximal on day 17 after infection (Fig. 8 *A*). A similar correlation between antigenemia and Vβ subpopulation expansion was also seen in the other two rhesus monkeys studied (290, 247) after acute infection with SIV$_{mac}$251 (Fig. 8 *B*).

Discussion

The present study indicates that acute infection of rhesus monkeys with SIV$_{mac}$ and SHIV resulted in an oligoclonal expansion of selected Vβ-expressing CD8$^+$ T lymphocytes. These findings are consistent with the recent demonstration that PBL of humans acutely infected with HIV-1 exhibited an oligoclonal expansion of selected Vβ-expressing CD8$^+$ T cells with HIV-1–specific cytotoxic function (10). Our study extends those findings in HIV-1–infected humans by demonstrating that lymphocytes expressing selected Vβ families were

A

CLONE	Vß	D+N	Jß		FREQUENCY
		CDR3			
D0-22	CASS	LTGVG	YDYTFG	Jß1.2	1/16
D0-15	----	QAIG	EKLF--	Jß1.4	1/16
D0-04	----	GQRGPN	EKLF--	Jß1.4	1/16
D0-13	----	QGRTS	NQPQY--	Jß1.5	1/16
D0-11	---	RRAQGD	HQPQY--	Jß1.5	1/16
D0-09	----	HHL	DQPQY--	Jß1.5	1/16
D0-01	----	RRAD	NSPLH--	Jß1.6	1/16
D0-06	----	TGQAG	NSPLH--	Jß1.6	1/16
D0-02	----	QGLGGAD	NEQF--	Jß2.1	1/16
D0-14	----	QEGSPSSS	NEQF--	Jß2.1	1/16
D0-10	----	AYG	NEQF--	Jß2.1	1/16
D0-07	----	EQGTL	TDPQY--	Jß2.3	1/16
D0-04	----	QDADS	TDPQY--	Jß2.3	1/16
D0-03	----	HPGLG	PNTQY--	Jß2.4	1/16
D0-05	----	QDGGQGG	DNTQY--	Jß2.4	1/16
D0-12	----	LSQV	INTQY--	Jß2.4	1/16

B

CLONE	Vß	D+N	Jß		FREQUENCY
		CDR3			
D14-02	CASS	QDPGGR	GEQYFG	Jß2.7	14/30
D14-20	----	--T--VL	------	Jß2.7	2/30
D14-04	----	--NR-L	NQP----	Jß1.5	6/30
D14-10	----	--R-S	NQP----	Jß1.5	4/30
D14-07	----	--RVS	NQP----	Jß1.5	3/30
D14-08	----	-EKVS	NQP----	Jß1.5	1/30

Figure 7. Amino acid sequences in the TCR-β junctional region of Vβ7 recombinant clones derived from PBL obtained from monkey 344 before (*A*) and on day 14 (*B*) after infection. The frequency of each recombinant in the total cDNA clones characterized is indicated.

Table 1. *Lymphocytes of Acutely SIV$_{mac}$-infected Rhesus Monkeys Lysed SIV$_{mac}$ Gag- and Env-expressing Autologous Target Cells at the Time of Peak Expansion of Vβ T Cell Subpopulations*

Effectors*	E:T Ratio	Percent lysis with‡		
		Cont	Gag	Env
Mm290	100:1	2	37	17
	50:1	0	30	13
	25:1	0	18	0
	12.5:1	0	6	0
Mm344	100:1	2	27	16
	50:1	2	16	12
	25:1	0	7	9
	12.5:1	1	5	3

* Effector cells from the monkey 290 were PBL sampled on day 18 after SIV$_{mac}$ infection; those from the monkey 344 were lymph node lymphocytes obtained on day 20 after viral infection. Lymphocytes were cultured for 3 d with Con A, and then maintained for another 3 d in IL-2–containing medium before CTL assay.

‡ Target cells were autologous B-lymphoblastoid cell lines infected with recombinant vaccinia viruses carrying the SIV$_{mac}$ gag, env, and equine herpesvirus gH (Cont) genes. Target cells were labeled with ^{51}Cr and incubated for 5 h with effector cells at the indicated E/T ratios.

Table 2. *SIV_{mac} Gag-specific Lysis by CD8-enriched Lymphocytes from SIV_{mac}-infected Monkeys Appeared to Be Associated with the Expression of an Electrophoretically Defined MHC Class I Allele*

Effectors*	E:T Ratio	Percent lysis of target cells from‡							
		Mm290		Mm347		Mm344		Mm416	
		Cont	Gag	Cont	Gag	Cont	Gag	Cont	Gag
Mm290	100:1	12	53	0	24	nd§	nd	5	7
	50:1	11	46	3	22	nd	nd	9	5
	25:1	8	39	4	21	nd	nd	9	5
	12.5:1	3	28	1	7	nd	nd	12	7
Mm344	100:1	nd	nd	8	29	7	38	8	5
	50:1	nd	nd	3	19	3	26	2	3
	25:1	nd	nd	1	8	1	9	4	4
	12.5:1	nd	nd	0	6	0	8	2	5

* CD8-enriched effector cells of monkey 290 were derived from PBL sampled on day 20 after infection; those of monkey 344 were from lymph node lymphocytes obtained on day 20 after infection.
‡ Target cells were prepared from *Herpesvirus papio*–transformed B-lymphoblastoid cell lines derived from the animals indicated. 1-D IEF showed that cell lines from the monkeys 344, 347, and 290, but not the monkey 416, expressed a shared MHC class I band.
§ nd, not done.

expanded not only in PBL, but also in lymphoid tissues of infected monkeys. The present findings also suggest that the virus-driven expansion of selected Vβ-expressing lymphocytes may be associated with specific electrophoretically defined MHC class I molecules. Furthermore, these studies show that the expansion of the selected Vβ-expressing lymphocytes coincided with the appearance of SIV_{mac}-specific CTL responses as well as the emergence and clearance of SIV antigen in plasma after infection. Thus, these studies may implicate the expanded subpopulations of $CD8^+$ T cells in the immune response that contains early AIDS virus spread.

The SIV_{mac}-mediated expansion of selected Vβ-expressing lymphocytes in PBL of the monkeys was less dramatic than the Vβ-expressing lymphocyte subpopulation expansions seen in HIV-1–infected humans. This may simply reflect differences in the virologic events observed in HIV-1 and SIV_{mac} infections. The period of early, high level viral antigenemia appears to last longer in primary HIV-1 infection of humans than in acute SIV_{mac} infection of rhesus monkeys (1–5). This more persistent antigenemia may drive a greater clonal expansion of $CD8^+$ T cells.

We have recently shown that SIV_{smm}PBj14 mediates the expansion of pig-tailed macaque T lymphocytes expressing TCR Vβ7 and Vβ14 gene families in vitro and in vivo (11). It is interesting that we have observed not only an expansion of selected Vβ-expressing lymphocyte subpopulations in both the SIV_{smm}PBj14–infected pig-tailed macaques and the SIV_{mac}- and SHIV-infected rhesus monkeys, but that expression of Vβ14-expressing T cell subpopulations occurred preferentially in both settings. An explanation for this particular preferential Vβ expansion is not readily apparent. This finding is not likely to represent an artifact in the PCR-based Vβ repertoire analyses, since we did not observe such a skewing in Vβ expression in PBL obtained from control animals inoculated with virus-free supernatant from the cell line CEMX174 (see Materials and Methods). Moreover, we saw oligoclonal Vβ14 sequences in cDNA derived from the PBL of monkeys which exhibited this increase in Vβ14 expression, but not from the PBL of a monkey which displayed only Vβ23 expansion (data not shown).

The expansion of a particular $CD8^+$ Vβ-expressing T lymphocyte subpopulation might be expected if the monkeys shared an MHC class I allele and were responding to the same epitope of the infecting virus. However, lymphocytes expressing the same Vβ-gene families can also be driven by different MHC class I–viral peptide complexes (10, 23). In fact, Vβ14-expressing T lymphocytes were expanded in $CD8^+$ PBL obtained after infection from monkey 345, an animal that did not appear to express the electrophoretically defined MHC class I allele shared by the other seven monkeys. Such a selectivity in Vβ responsiveness may explain why two different macaque species, each infected with a different SIV isolate, might demonstrate expansions of similar T lymphocyte subpopulations.

SIV_{smm}PBj14, unlike SIV_{mac} or SHIV, stimulates the in vitro proliferation and expansion of both $CD4^+$ and $CD8^+$ lymphocyte subpopulations, and induces an acute lethal disease in pig-tailed macaques. The ability of SIV_{smm}PBj14 to stimulate proliferation and activation of resting PBL in vitro is predictive of its acute lethal pathogenicity in vivo (24). These properties of SIV_{smm}PBj14 appear to be unique among primate immunodeficiency viruses. While we cannot exclude the possibility that the SIV_{smm}PBj14-induced immunologic events in infected pig-tailed macaques may simply reflect an

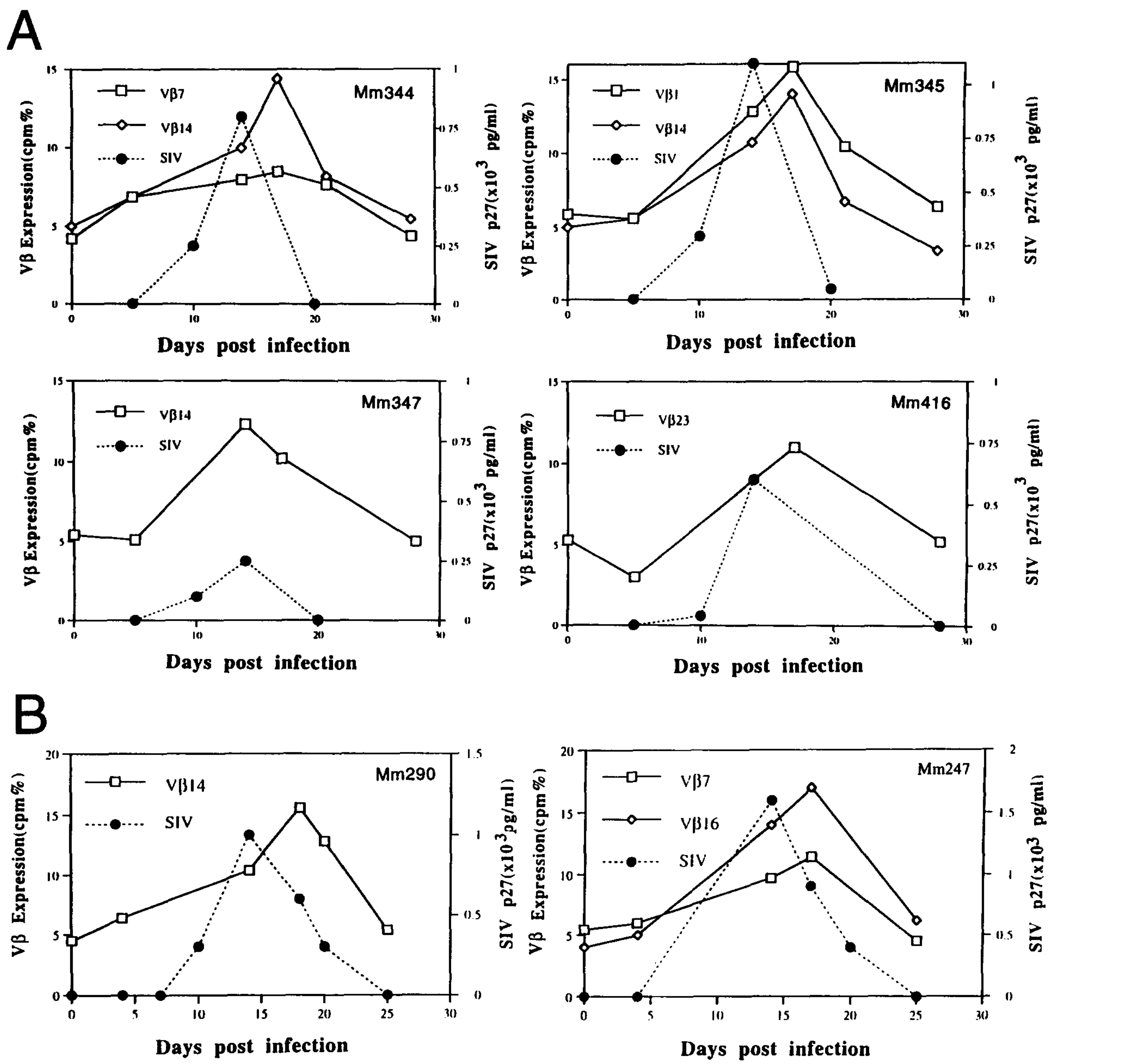


Figure 8. Correlation between SIV antigenemia and the expansion of selected Vβ-expressing lymphocyte subpopulations in SIV_{mac} 239- (*A*) and SIV_{mac} 251- (*B*) infected monkeys. Indicated are SIV p27 protein concentrations in plasma and the expansion of selected Vβ gene families.

exaggeration of the SIV_{mac}- or SHIV-mediated expansion of selected Vβ-expressing T lymphocytes of the infected rhesus monkeys, these events may be driven by a superantigen-like property of this unusual virus.

The oligoclonality of the expanded Vβ-expressing cells in PBL of the SIV_{mac} -infected monkeys is likely to reflect a virus-specific T lymphocyte response. It is of interest to determine the viral antigen(s) that are eliciting this immune response. At least some of the antigen(s) stimulating the expansion of Vβ-expressing lymphocytes may be encoded by viral gene(s) other than the envelope-coding region of SIV_{mac}. This possibility is supported by the demonstration that both SIV_{mac}- and SHIV-infected rhesus monkeys sharing a single MHC class I allele exhibited the same expansion of Vβ14-expressing T lymphocyte subpopulations in their PBL (Figs. 2 and 5).

The present study indicates a correlation between the expansion of selected Vβ-expressing $CD8^+$ lymphocyte subpopulations, the emergence of AIDS virus–specific CTL responses, and the appearance and clearance of SIV antigen in plasma of the monkeys. Elucidating the molecular aspects of the interactions of the selected Vβ-expressing T cells with SIV_{mac} will further define the role played by these lymphocyte subpopulations in containing an SIV_{mac} infection.

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