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An acutely lethal simian immunodeficiency virus stimulates expansion of $V_\beta 7$- and $V_\beta 14$-expressing T lymphocytes

(superantigen/T-cell receptor Vβ repertoire/AIDS/SIVsmmPBj14)

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ABSTRACT **SIVsmmPBj14, a variant simian immunodeficiency virus isolated from a pig-tailed macaque, stimulates the proliferation of macaque T lymphocytes *in vitro* and induces an acutely lethal disease in macaques characterized, in part, by lymphadenopathy and splenomegaly. To determine whether SIVsmmPBj14 exhibits superantigen-like activity, *in vitro* and *in vivo* studies of T-cell receptor V_β repertoire were undertaken using PCR-based quantitative methods. Whereas *in vitro* phytohemagglutinin stimulation of macaque peripheral blood lymphocytes did not cause a perturbation of T-cell receptor V_β repertoire, SIVsmmPBj14 stimulated the expansion of both $CD4^+$ and $CD8^+$ T-lymphocyte subpopulations expressing the $V_\beta 7$ and $V_\beta 14$ gene families. Such $V_\beta 7$ and $V_\beta 14$ expansions could be confirmed by a multiple RNase protection assay. Furthermore, the expansion of the same lymphocyte subpopulations was also detected in peripheral blood lymphocytes and lymph node cells of virus-infected macaques. These observations suggest that SIVsmmPBj14-mediated V_β expansion may contribute to the induction of an acutely lethal disease in macaques.**

T lymphocytes play an important role in the immunopathogenesis of AIDS. Elucidating the molecular interactions of T lymphocytes and the AIDS virus is, therefore, of central importance for understanding the immune sequelae of an AIDS virus infection. Studies undertaken to determine whether superantigen-mediated depletion events (1–5) contribute to the loss of both function and numbers of $CD4^+$ cells in AIDS have been inconclusive (6–9). Further studies are therefore needed to determine whether superantigen-like activity of the AIDS virus may have an impact upon AIDS pathogenesis. Simian immunodeficiency virus (SIV)-infected nonhuman primates are valuable models for the study of AIDS (10–12). Whereas infection with SIV and human immunodeficiency virus usually causes chronic and progressive immunodeficiency, infection of pig-tailed macaques (*Macaca nemestrina*) with a variant isolate, SIVsmmPBj14, results in an acute lethal disease (13–16). The acute disease is characterized by fulminant diarrhea, high circulating levels of inflammatory cytokines, and death within 5–10 days of infection. Moreover, SIVsmmPBj14 has been shown to activate and stimulate proliferation of resting macaque peripheral blood lymphocytes (PBLs) *in vitro* (17). These observations raise the possibility that SIVsmmPBj14-mediated activation of T-lymphocyte subpopulations may contribute to the ability of this virus to induce a lethal disease. Studies were done to assess this possibility.

MATERIALS AND METHODS

Animals. Pig-tailed macaques were experimentally infected with biologically cloned SIVsmmPBj14 (13). These animals 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 no. (National Institutes of Health) 85-23, revised 1985].

***In Vitro* Stimulation of Macaque PBLs.** PBLs were isolated from heparinized blood of 12 normal, uninfected macaques by Ficollsodium diatrizoate (LSM, Organon Teknika, Durham, NC) density gradient centrifugation and then cultured for 5–8 days in the presence of phytohemagglutinin (PHA; 0.1%) or infectious SIVsmmPBj14, a quantity equivalent to 1–5 ng of SIV p27 per ml. For macaques 542 and 543, uninfected PBLs (5×10^6) were viably frozen before SIVsmmPBj inoculation and were subsequently thawed and stimulated with autologous infected lymph node cells (1×10^6).

mRNA Extraction, cDNA Synthesis, and PCR Analysis of T-Cell Receptor (TCR) V_β Expression. mRNA extraction, cDNA synthesis, and PCR-based quantitation were performed as described (7).

Multiple RNase Protection Assay. PBLs were stimulated as described above and subjected to an RNase protection assay using a lysate derived from *Escherichia coli* as a negative control. Antisense RNA probes were synthesized using the T7 RNA polymerase and PCR-amplified V_β cDNA containing the sequence of the T7 promoter underlined below. The cloned $V_\beta 3$ (one gene), $V_\beta 7$ (three genes), $V_\beta 10$ (one gene), and $V_\beta 14$ (two genes) cDNAs were amplified individually by PCR using the following primers: sense $V_\beta 3$ CCT TTT GTT TCC TGG CTG TA; antisense $V_\beta 3$ AAT TTA ATA CGA CTC ACT ATA GGG ATC AGG GAG AAG CGC TCC; sense $V_\beta 7$ ATG GGC TGC AGG ATC CTC TG; antisense $V_\beta 7$ AAT TTA ATA CGA CTC ACT ATA GGG ATC TTG GAG CAT TCA GGT; sense $V_\beta 10$ CGG TTG GCT CAG ACT TCT C; antisense $V_\beta 10$ AAT TTA ATA CGA CTC ACT ATA GGG ATC TCC AAG GTA CAG G; sense $V_\beta 14$ GCC CCT TGG AAT TCC AAG TG; antisense $V_\beta 14$ AAT TTA ATA CGA CTC ACT ATA GGG ATC AGG GGG AAA TTC C. RNA hybridization and RNase protection were performed following the instructions included with the RNase protection assay (United States Biochemical). The protected RNA fragments were denatured and separated on 5% long ranger sequence gels.

CDR3 Length Analysis. CDR3 length analysis was carried out as described (18). cDNA were amplified by PCR using the $V_\beta 7$ or $V_\beta 14$ and $C\beta$ primers previously employed for V_β repertoire analysis (7). The second round of PCR was performed using nested $V_\beta 7$ ($V_\beta 7$NS) or $V_\beta 14$ ($V_\beta 14$NS) primers and a C_β (C_βR) primer, designed as described (18). The

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Abbreviations: SIV, simian immunodeficiency virus; SIVsmm, SIV of sooty mangabeys; TCR, T-cell receptor; PBL, peripheral blood lymphocyte; PHA, phytohemagglutinin.

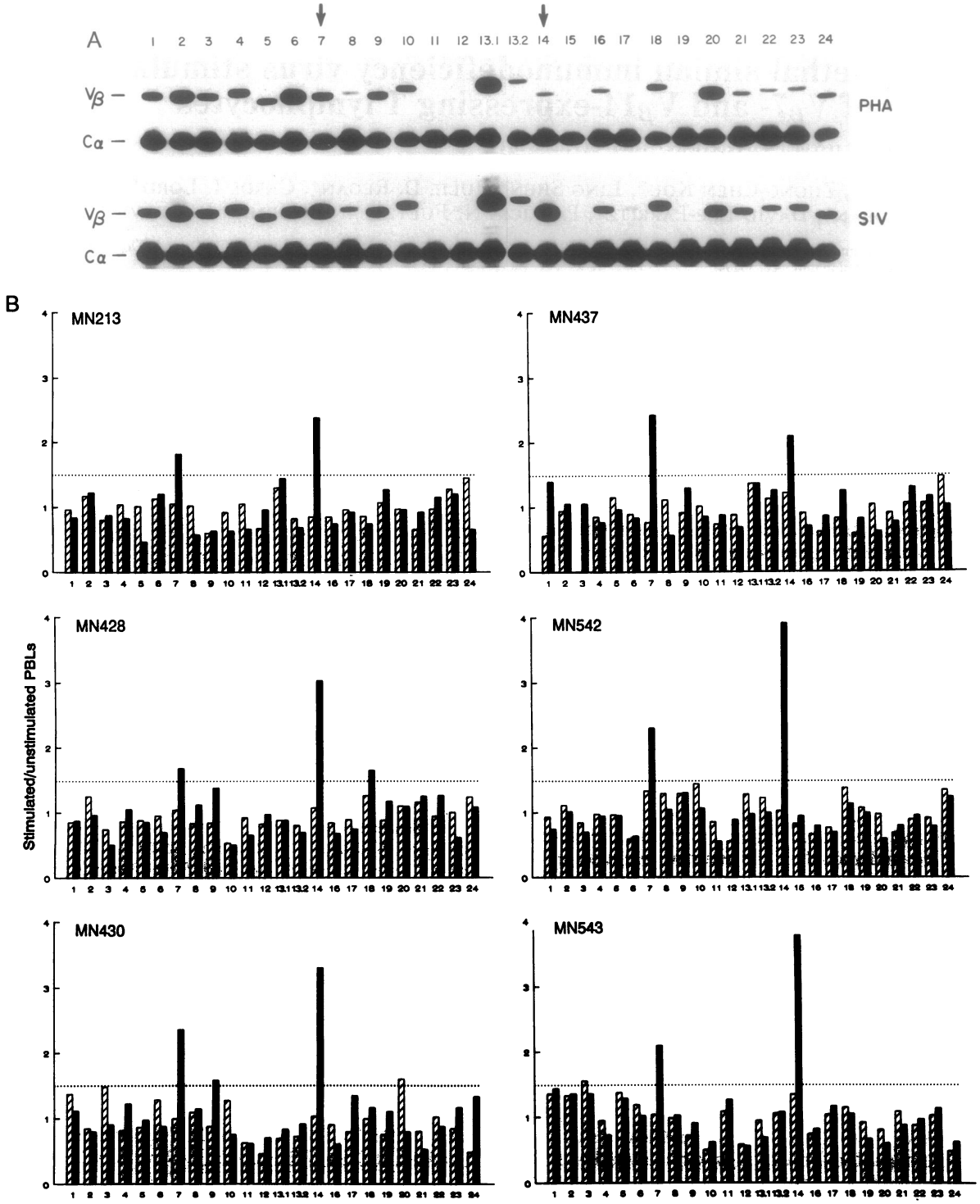

FIG. 1. PCR analysis of TCR V_β gene expression in unstimulated and PHA- and SIVsmmPBj14-stimulated PBLs. (*A*) Autoradiogram of TCR V_β transcript of PHA- (upper panel) and SIVsmmPBj14-stimulated PBLs (lower panel) obtained from a normal macaque (Mn542). The numbers indicate lanes in which the noted V_β gene families are present. (*B*) Comparison of V_β expression in PHA- or SIVsmmPBj14-stimulated PBLs with those in unstimulated PBLs. Individual V_β expression was estimated, as described (7), as the percentage of total V_β cpm of each stimulated or unstimulated PBL population. Thus, values for each V_β gene family represent the ratio of the % of total cpm obtained from PHA- (▨) or SIVsmmPBj14-(■) stimulated PBLs relative to the corresponding % from unstimulated PBLs. A value of 1.5 (dotted line) for V_β comparison was defined based on the mean + 2.2 SD of representative V_β ratios derived from multiple amplifications of cDNA generated from lymphocytes of a normal macaque.

sequences for the primers were as follows: V_β7NS, CTC AGC CCT GTA TCT CTG CG; V_β14NS, GAC CTC TCT GTA CCT CTG TG; and C_βR, CTT CTG ATG GCT CAA ACA C with its 5′ end ^{32}P labeled. The amplified TCR β chains of various CDR3 lengths were visualized as series of radiolabeled bands 3 bp apart on a 6% polyacrylamide sequencing gel.

RESULTS AND DISCUSSION

In vitro studies were initiated to determine whether SIVsmmPBj14 can stimulate a large fraction of particular V_β-expressing lymphocyte subpopulations. SIVsmmPBj14-stimulated PBLs exhibited a selective expansion of T-lymphocyte subpopulations expressing TCR employing the V_β7

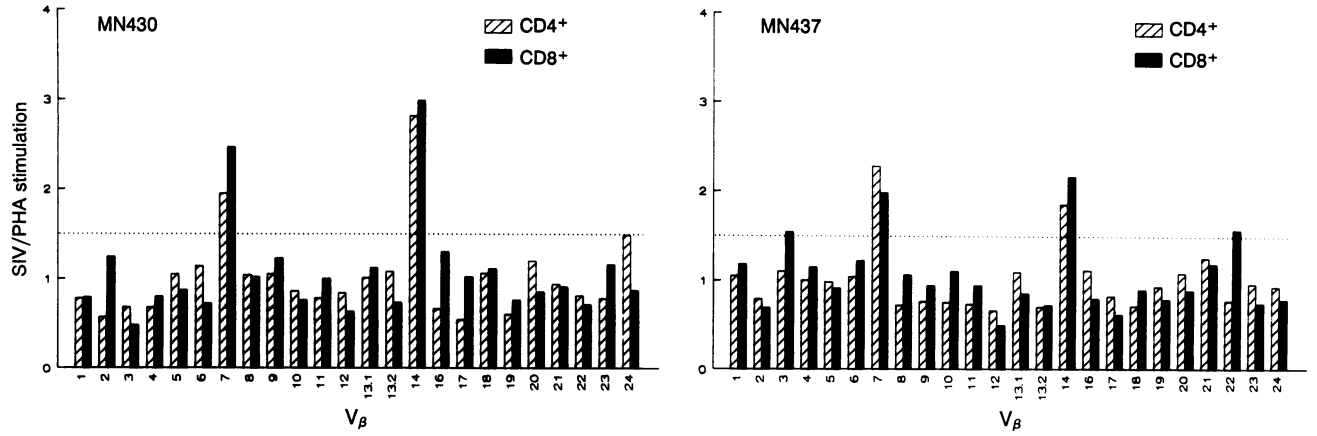

FIG. 2. TCR V_β expression in SIVsmmPBj14-stimulated $CD4^+$ or $CD8^+$ cells compared with those in PHA-stimulated $CD4^+$ and $CD8^+$ cells. Values are ratios of individual V_β gene expression (% of total V_β cpm) in SIVsmmPBj14-stimulated $CD4^+$ (▨) or $CD8^+$ (■) cells relative to % of the corresponding V_β gene families in PHA-stimulated $CD4^+$ or $CD8^+$ cells.

and $V_\beta 14$ gene families (Fig. 1). No significant increase in $V_\beta 7$ or $V_\beta 14$ expression was detected in PHA-stimulated macaque PBLs. In contrast, 1.86- to 3.96-fold increases in $V_\beta 7$ and $V_\beta 14$ expression were seen in SIVsmmPBj14-stimulated PBLs obtained from six macaques. Statistical analysis indicated that the selective expansion of lymphocytes expressing TCR $V_\beta 7$ and $V_\beta 14$ was highly significant ($P < 0.01$). To determine whether this apparent SIVsmmPBj14-mediated V_β stimulation simply reflected a virus-induced alteration in the relative number of $CD4^+$ and $CD8^+$ cells present in the PBL population, cDNAs from the $CD4^+$ and $CD8^+$ cell populations isolated using immunomagnetic beads (7) were subjected to the PCR-based V_β analysis. SIVsmmPBj14 selectively stimulated $V_\beta 7$ and $V_\beta 14$ gene family expression in both $CD4^+$ and $CD8^+$ lymphocytes (Fig. 2).

To confirm that lymphocytes expressing selected TCR V_β gene families were being expanded upon exposure to SIVsmmPBj14, $V_\beta 7$ and $V_\beta 14$ RNA expression in virus-stimulated macaque PBLs was assessed employing the multiple RNase protection assay. Even in the absence of PCR amplification, $V_\beta 7$ and $V_\beta 14$ expansions were detected by RNA hybridization in SIVsmmPBj14-stimulated PBLs obtained from macaques 542 and 543 (Fig. 3). An analysis of the V_β signals using a densitometer revealed 1.3- and 1.9-fold increases for $V_\beta 7$ and 1.9- and 2.8-fold increases for $V_\beta 14$ mRNA, respectively, in the SIVsmmPBj14-stimulated PBLs when compared to the PHA-stimulated PBLs.

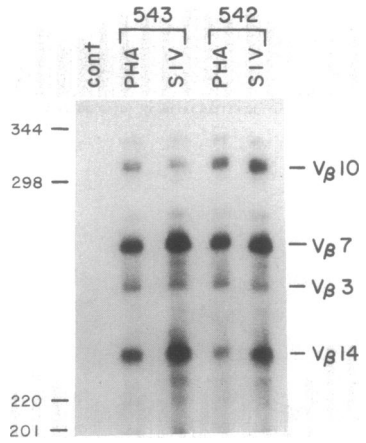

FIG. 3. Multiple RNase protection analysis of $V_\beta 3$, $V_\beta 7$, $V_\beta 10$, and $V_\beta 14$ mRNA in PHA- and SIVsmmPBj14-stimulated PBLs. Indicated are animal number (at top), molecular markers (left), and the noted V_β RNAs (right). cont, Control.

We then sought to determine whether SIVsmmPBj14 is able to stimulate the expansion of these specific V_β-expressing lymphocyte subpopulations *in vivo*. Following infection with SIVsmmPBj14, PBLs from four of the six macaques exhibited selective expansions of $V_\beta 7$- and/or $V_\beta 14$-expressing T lymphocytes (Fig. 4*A*). An increased representation of these T-cell subpopulations was most evident in PBLs obtained on days 4 and 6 after inoculation, with up to 2.5- and 3.8-fold increases, respectively, in the expression of $V_\beta 7$ and $V_\beta 14$ transcripts. For example, in macaque 543, expression of $V_\beta 7$ increased from 4.1% in PBLs obtained before infection to 10.4% in PBLs obtained on day 4 after infection. Similarly, in macaque 542, expression of $V_\beta 14$ increased from 3.1% to 11.5% in PBLs obtained on day 6 after infection. We did not see striking $V_\beta 7$ and $V_\beta 14$ expansion in PBLs of the infected macaques 275 and 276, perhaps due to the lack of multiple blood samples obtained early after infection. In addition, $V_\beta 10$ and $V_\beta 23$ expansions were seen in the lymphocytes from macaque 541; a possible $V_\beta 22$ expansion in PBLs of macaque 542 was also documented (Fig. 4*A*). These variations may reflect genetic influences on lymphocyte subpopulation responses to the viral antigen. Such influences are likely to be manifested in an outbred population (19).

We also assessed the TCR V_β repertoire in lymphocytes derived from lymphoid tissue of the SIVsmmPBj14-infected macaques to explore the possibility that profound V_β stimulation by the virus may play a role in the lymphoid hyperplasia that occurs following infection. Lymphocytes isolated from peripheral and mesenteric lymph nodes were examined for V_β expression using lymphocytes from peripheral lymph nodes obtained prior to infection as control cell populations. Lymph node cells from macaques 541 and 542, the animals euthanized on day 6 after infection, exhibited TCR $V_\beta 7$ and $V_\beta 14$ expansions (Fig. 4*B*). Macaque 543 still displayed TCR $V_\beta 7$ and $V_\beta 14$ expansion in lymph node cells at the time it was moribund, similar to findings in this animal's PBLs. These results indicate that SIVsmmPBj14 infection resulted in expansion of $V_\beta 7$- and $V_\beta 14$-expressing lymphocytes in peripheral blood and lymph nodes.

In fact, the $V_\beta 7$ and $V_\beta 14$ expansions induced by SIVsmmPBj14 appeared to be polyclonal, since a TCR CDR3 length analysis (18, 20) revealed a lack of clonal predominance in the $V_\beta 7$- and $V_\beta 14$-containing cDNA derived from the virus-infected PBLs (Fig. 5).

Thus, we have shown a selective expansion of T-lymphocyte subpopulations that expressed TCR employing the $V_\beta 7$ and $V_\beta 14$ gene families in SIVsmmPBj14-stimulated PBLs both in culture and in virus-infected macaques. The mecha-

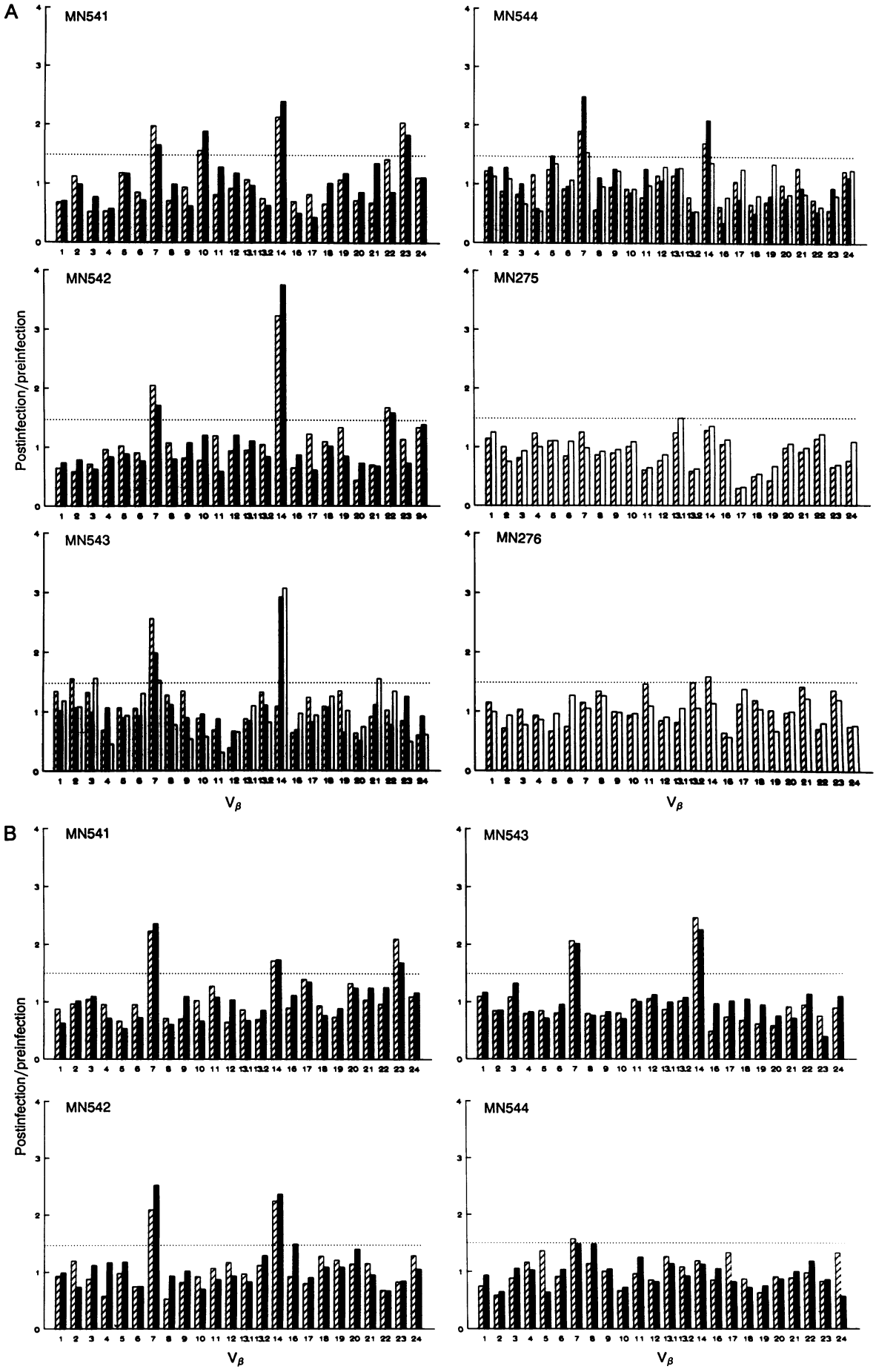

FIG. 4. *(Legend appears at the bottom of the opposite page.)*

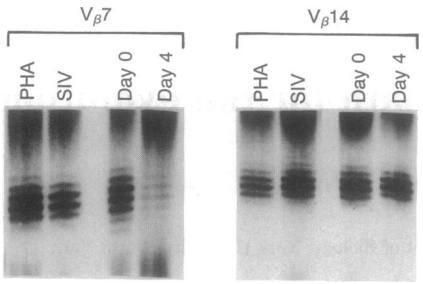

FIG. 5. CDR3 length analysis of $V_\beta 7$- and $V_\beta 14$-containing cDNA derived from SIVsmmPBj14-stimulated PBLs. No predominance of CDR3 lengths was observed in the virus-stimulated (SIV) and *in vivo* infected (Day 4) PBLs when compared to those in PHA-stimulated (PHA) and uninfected (Day 0) PBLs. Data were generated from lymphocytes of macaques 543 (for $V_\beta 7$) and 542 ($V_\beta 14$).

nism by which this virus mediates V_β expansion remains to be elucidated. Nevertheless, stimulation of a large fraction of T lymphocytes may amplify SIVsmmPBj14 infection, facilitating its rapid spread throughout the lymphoid system. In fact, a recent study demonstrated that the ability of SIVsmmPBj14 to stimulate proliferation of resting PBLs *in vitro* is predictive of its acutely lethal pathogenicity *in vivo* (15). In concert with these data, the results of the present study suggest that SIVsmmPBj14-mediated V_β stimulation and expansion may contribute to the acutely lethal disease that the virus induces. The pathogenesis of acute syndromes in primates induced by human immunodeficiency virus or SIV_{mac} infection may have important parallels to the acutely lethal syndrome induced by SIVsmmPBj14 in macaques (21–24). The definition of the immune changes in the SIVsmmPBj14-infected macaques, therefore, may help to clarify the immunopathogenesis of all acute AIDS virus infections.

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1. Kotzin, B. L., Leung, D. Y., Kappler, J. W. & Marrack, P. (1993) *Adv. Immunol.* **54**, 99–166.
2. Kawabe, Y. & Ochi, A. (1990) *Nature (London)* **349**, 245–248.
3. Rammensee, H. G., Kroschewski, R. & Frangoulis, B. (1989) *Nature (London)* **339**, 541–544.
4. Webb, S., Morris, C. & Sprent, J. (1990) *Cell* **63**, 1249–1256.
5. Rellahan, B. L., Jones, L. A., Kruisbeek, A. M., Fry, A. M. & Matis, L. A. (1990) *J. Exp. Med.* **172**, 1091–1100.
6. Imberti, L., Scottini, A., Bettinardi, A., Puoti, M. & Primi, D. (1991) *Science* **254**, 860–862.
7. Chen, Z. W., Kou, Z., Shen, L., Reimann, K. A. & Letvin, N. L. (1993) *J. Immunol.* **151**, 2177–2187.
8. Bahadoran, P., Rieux-Laucat, F., LeDeist, F., Blanche, S., Fischer, A. & de Villartay, J. (1993) *Eur. J. Immunol.* **23**, 2041–2044.
9. Laurence, J., Hodsteve, A. S. & Posnett, D. N. (1992) *Nature (London)* **358**, 255–259.
10. Letvin, N. L. (1990) *Immunol. Today* **11**, 322–326.
11. Desrosiers, R. C. (1990) *Annu. Rev. Immunol.* **8**, 557–578.
12. Letvin, N. L., & King, N. W. (1990) *J. AIDS* **3**, 1023–1040.
13. Fultz, P. N., McClure, H. M., Anderson, D. C. & Switzer, W. M. (1989) *AIDS Res. Hum. Retroviruses* **5**, 397–409.
14. Dewhurst, S., Embretson, J. E., Anderson, D. C., Mullins, J. I. & Fultz, P. N. (1990) *Nature (London)* **345**, 636–639.
15. Novembre, F. J., Johnson, P. R., Lewis, M. G., Anderson, D. C., Klumpp, S., McClure, H. M. & Hirsch, V. M. (1993) *J. Virol.* **67**, 2446–2474.
16. Lewis, M., Zack, P. M., Elkins, W. R. & Jahrling, P. B. (1992) *AIDS Res. Hum. Retroviruses* **8**, 1631–1639.
17. Fultz, P. N. (1991) *J. Virol.* **65**, 4902–4909.
18. Hingorani, R., Choi, I., Akolkar, P., Gulwani-Akolakar, B., Pergolizzi, R., Silver, J. & Gregerson, P. K. (1993) *J. Immunol.* **151**, 5762–5769.
19. Cole, B. C., Balderas, R. A., Ahmed, E. A., Kono, D. & Theofilopoulos, A. N. (1993) *J. Immunol.* **150**, 3291–3299.
20. Candeias, S., Waltzinger, C., Benoist, C. & Mathis, D. (1991) *J. Exp. Med.* **174**, 989–1000.
21. Sinicco, A., Palestro, G., Caramello, P., Gaicobbi, D., Guilianl, G., Sciadra, M. & Giannini, P. (1990) *J. Acquired Immune Defic. Syndr.* **3**, 260–265.
22. Tindall, B. & Cooper, D. A. (1991) *AIDS* **5**, 1–14.
23. Graziosi, C., Panteleo, G., Butini, L., Demarest, J. F., Saag, M. S., Shaw, G. M. & Fauci, A. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6405–6409.
24. Reimann, K. A., Tenner-Racz, K., Racz, P., Montefiori, D., Yasutomi, Y., Lin, W., Ransil, B. & Letvin, N. L. (1994) *J. Virol.* **68**, 2363–2370.

FIG. 4. PCR analysis of TCR V_β gene expression in PBLs and lymph nodes obtained from SIVsmmPBj14-infected macaques. (*A*) Comparison of V_β expression in SIVsmmPBj14-infected PBLs with those in uninfected lymphocytes. Values are ratios of individual V_β gene expression (% of total V_β cpm) in infected PBLs sampled on day 4 (□), on day 6 (■), or at the time of death (▨) to % of cpm of the corresponding V_β gene families in preinfection PBLs. (*B*) Comparison of V_β expression in lymph nodes of four macaques before and after SIVsmmPBj14 infection. Values are the ratios of individual V_β mRNA expression in infected lymphocytes from peripheral (▨) and mesenteric (■) lymph nodes obtained at the time of their death relative to the corresponding V_β gene family mRNA expression in peripheral lymph node cells obtained before infection.