Coinfection of Macaques with Simian Immunodeficiency Virus and Simian T Cell Leukemia Virus Type I: Effects on Virus Burdens and Disease Progression

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To test the hypothesis that coinfection with human immunodeficiency virus (HIV) and human T cell leukemia/lymphoma virus types I or II (HTLV-I or -II) accelerates progression to AIDS, pig-tailed macaques were inoculated with the simian counterparts, SIV and STLV-I. During 2 years of follow-up of singly and dually infected macaques, no differences in SIV burdens, onset of disease, or survival were detected. However, in the first coinfected macaque that died of AIDS (1 year after infection), >50% of CD4+ and CD8+ lymphocytes expressed CD25. On the basis of the low incidence of HTLV-I- and STLV-I-associated disease during natural infections, this early evidence of neoplastic disease was unexpected. While these results demonstrate that coinfection with SIV and STLV-I has no influence on the development of immunodeficiency disease, they do establish a reliable macaque model of persistent STLV-I infection.

An increasing number of people are being exposed to the two known pathogenic human retroviruses: the human immunodeficiency virus (HIV) and the human T cell leukemia/lymphoma viruses (HTLVs) [1–9]. One population especially at risk is intravenous drug users, among whom the incidence of HIV infection continues to increase, and in certain populations of intravenous drug users, HTLV-II appears endemic [2, 5, 7, 9]. Although no disease syndrome has been definitively linked with HTLV-II, HTLV-I is known to be the etiologic agent of both adult T cell leukemia/lymphoma and a neurologic syndrome identified as tropical spastic paraparesis/HTLV-associated myelopathy [10–13]. Evaluation of some cases of dual infections with HIV and HTLV-I or HTLV-II led to the suggestion that coinfection may accelerate progression to AIDS [14–16]. However, more recent cross-sectional studies concluded that coinfection with HIV and HTLV-I or -II had no impact on HIV load or disease progression [5, 17–20]. In fact, evidence is accumulating that coinfection with HIV-I may increase the virus load in HTLV infections and, in particular, the incidence of neurologic disease in HTLV-II–infected persons [6–8, 21, 22]. Thus, an animal model would be helpful in defining in vivo interactions of these two retroviruses. Moreover, the use of an animal model for a controlled prospective study might allow one to identify the possible consequences of dual infection on development of immunodeficiency, neoplastic, and neurologic diseases.

Infection of macaque monkeys with various isolates of the simian immunodeficiency viruses (SIVs) obtained from macaques or sooty mangabeys is a valuable animal model that reproduces most, if not all, facets of the natural history of HIV infection in humans [23, 24]. Although the STLV-I counterparts of HTLV-I are present as natural infections in many Asian and African species of monkeys and great apes [25], a well-characterized nonhuman primate model of STLV-I infection has not been described. Like HTLV-I, STLV-I infection of many species, including macaques, African green monkeys, baboons, and mangabeys, is highly associated with leukemias and lymphomas in which the STLV-I provirus is clonally integrated [26–30]. In addition, naturally occurring leukemias/lymphomas in monkeys coinfected with SIV and STLV-I have been reported. In one case, a feral African green monkey developed clinical signs typical of SIV-induced immunosuppression and lymphoproliferative disease characterized by enlarged lymph nodes infiltrated with histiocytic-type cells containing an STLV-I provirus integrated at a single site [29]. In another report, two sooty mangabeys presented with symptoms of lymphoproliferative disease—one was diagnosed as a T cell leukemia and the other as disseminated malignant lymphoma—and symptoms normally associated with SIV-induced immunodeficiency, including multinucleated giant cells in lungs, liver, and bone marrow [30]. Although African green monkeys and sooty mangabeys are natural hosts for both retroviruses, these species rarely develop immunodeficiency-like or STLV-I–related neoplastic diseases [25, 31].

In vitro experiments have shown that reciprocal virus activation occurs in cell lines coinfected with members of these virus families and, in particular, that the HTLV-I Tax protein can activate the HIV-1 long terminal repeat [32–34]. In addi-
tion, HTLV-I–infected cells readily induce proliferation of both CD4+ and CD8+ lymphocytes in the absence of accessory cells [35, 36], and HTLV-I–mediated proliferation has been shown to induce production of HIV-1 in infected lymphocytes [37]. Moreover, both HIV and HTLV-I induce neurologic diseases [12, 13, 38]; therefore, the potential exists for primary or secondary pathogenic sequelae of either virus to influence disease manifestations of the other one. Because SIV and STLV-I are regulated in the same manner as and have biologic properties similar to those of their human counterparts, it is likely that the simian viruses have the same potentials to promote disease, possibly through synergistic interactions.

We recently reported the isolation of a new STLV-I, designated STLV-I(sm), from a naturally infected sooty mangabey and have established continuous T cell lines with peripheral blood mononuclear cells (PBMC) from normal mangabey and pig-tailed macaque monkeys [39]. These cell lines not only readily transmit STLV-I(sm) to uninfected PBMC in vitro, but also establish in vivo infections in both rhesus and pig-tailed macaques. By use of a mangabey cell line persistently infected with STLV-I(sm) and an SIVsmmPBj14-derived biologic clone, SIV-PBj14-bcl1 [40, 41], a macaque model for co-infection with HIV and HTLV was established. We report here the results of a controlled study to determine whether co-infection with these two retroviruses enhances SIV burdens or progression to immunodeficiency disease and AIDS.

Methods

Animals and virus inoculations. Twenty juvenile pig-tailed macaques (Macaca nemestrina) of either sex that were seronegative for SIV, STLV, and simian type D retrovirus were used in this study. The animals were housed at the University of Alabama at Birmingham according to institutional and Animal Welfare Act guidelines. Macaques were divided randomly into 3 groups and were exposed to virus intravenously as follows: group 1, 4 macaques were inoculated twice 6 weeks apart with STLV-I(sm) only; group 2, 6 macaques were inoculated once with ~104 TCID50 of cell-free SIV-PBj14-bcl1; and group 3, 10 macaques were inoculated twice 6 weeks apart with STLV-I(sm), and then 3 weeks after the second STLV-I(sm) inoculation, they were inoculated with SIV-PBj14-bcl1. The STLV-I(sm) inoculum consisted of 5–10×104 H11 cells, a continuous cell line generated by end-point dilution of FEd-P14 cells [39]. Although the FEd-P14 cells are coinfected with STLV-I(sm) and SIVsmmPBj14, the H11 cloned cells are infected with only STLV-I(sm), as demonstrated by polymerase chain reaction (PCR) analysis for proviral DNA of both viruses and the presence of STLV-I(sm) p19 Gag, but not SIV p27 Gag, in culture supernats (data not shown). Furthermore, none of the macaques inoculated only with the H11 cells seroconverted for SIV, and SIV was not isolated from their PBMC. During the study, blood samples were obtained periodically for hematologic, immunologic, and virologic analyses. Before all virus inoculations and blood collections, the animals were anesthetized with ketamine hydrochloride (10 mg/kg), weighed, and given a physical examination.

Detection of virus. Macaque PBMC were cocultured with normal human PBMC by use of standard culture procedures that were described previously [40]. To assess production of SIV and STLV-I(sm), cell-free culture supernatants were assayed for particulate reverse transcriptase (RT) activity, indicative of SIV replication, and for the presence of STLV-I(sm) p19 Gag antigen. Because STLV-I(sm) has ~95% identity with HTLV-I, an HTLV-I antigen capture kit (Cellular Products, Buffalo, NY) was used. Cultures were maintained 6–7 weeks before being designated as virus-negative. SIV plasma antigenemia and viremia, respectively, were quantified for SIV p27 Gag by use of an SIV core antigen assay kit (Coulter Immunology, Hialeah, FL) and for virion RNA by NASBA (nucleic acid sequence–based amplification) [42].

Serology. Serum antibodies to SIV-PBj14-bcl1 and STLV-I(sm) were detected by use of two commercially available EIA kits: the HIV-2 EIA kit (Sanofi Pasteur Diagnostics, Seattle) and the HTLV-I Microelisa kit (Organon Teknika, Durham, NC). Antibody titers are expressed as the reciprocal of the last serum dilution, to give a reading above the cutoff value recommended by the manufacturers. Antibodies to specific STLV-I(sm) proteins were detected by immunoblot assay with the HTLV Blot 2.4 kit (Genelabs Diagnostics, Singapore).

Flow cytometry. Changes in lymphocyte subsets were monitored by use of a panel of mouse anti-human monoclonal antibodies (all reagents were purchased from Becton Dickinson, Mountain View, CA)—CD4, leu3a; CD8, leu2a; CD20, leu16; CD14, leuM3—and the activation marker CD25 (interleukin-2 receptor α chain). EDTA-treated blood samples were stained by a whole blood lysis procedure according to the manufacturer’s instructions. Multitest control, IgG1-fluorescein isothiocyanate/IgG2a-phycocerythrin antibodies were used to identify negative cell populations, and lymphocytes were gated according to forward scatter versus side scatter characteristics. Analyses were done on a FACS-Star flow cytometer (Becton Dickinson).

PCR-based limiting dilution assays. Initially, 1 µg of genomic DNA from macaque PBMC was screened by nested PCR for the presence of STLV-I(sm) proviral DNA. If positive, three to seven independent serial 1:2 and/or 1:3 limiting dilutions into DNA from an uninfected animal were made, after which nested PCRs were done. The conditions and program used for the first- and second-round nested PCR were those described previously by Liska et al. [43]. The number of proviral copies per 1 µg of DNA was calculated by use of the Quality computer program [44]. The outer and inner primers, respectively, amplified 488- and 379-bp fragments and were as follows: NF(2), 5′-CAGCATCTTATTCCAGACCCCTTTGTTCCC-3′ (bases 302–328); NR, 5′-CGTTGGGAGAG-3′ (bases 368–392); NQ, 5′-0′ (bases 789–764); NL, 5′-CAGCATCTTATTCCAGACCCCTTTGTTCCC-3′ (bases 302–328); NR, 5′-TGGAAGGCCTAACAGATGGTTAAAGGA-3′ (bases 789–764); NL, 5′-CAGCATCTTATTCCAGACCCCTTTGTTCCC-3′ (bases 302–328); NR, 5′-TGGAAGGCCTAACAGATGGTTAAAGGA-3′ (bases 789–764); NL, 5′-TGGAAGGCCTAACAGATGGTTAAAGGA-3′ (bases 789–764); NL, 5′-TGGAAGGCCTAACAGATGGTTAAAGGA-3′ (bases 789–764); NL, 5′-TGGAAGGCCTAACAGATGGTTAAAGGA-3′ (bases 789–764); NL, 5′-TGGAAGGCCTAACAGATGGTTAAAGGA-3′ (bases 789–764); NL, 5′-TGGAAGGCCTAACAGATGGTTAAAGGA-3′ (bases 789–764).
the number of virion RNA copies in plasma were quantified. When the maximum levels of p27 Gag antigen present in plasma during the first 4 weeks after SIV-PBj14-bcl1 inoculation were compared, the mean for the dually infected animals in group 3 was ~2-fold higher than that for group 2, which was infected only with SIV-PBj14-bcl1 (figure 1). However, this difference was not significant when tested by a two-tailed Student's t test (Instat 20; GraphPad Software, San Diego, CA). The levels of SIV RNA copies in plasma throughout the first year of infection were virtually superimposable (figure 2). At 2 weeks after SIV-PBj14-bcl1 inoculation, the number of RNA copies per 0.1 mL for group 2 ranged from $1.8 \times 10^5$ to $7.6 \times 10^7$, whereas that for group 3 ranged from $1.5 \times 10^6$ to $>8 \times 10^7$, the latter value being the high-end cutoff for the NASBA assay.

Because STLV-I and HTLV-I generally establish long-term, cell-associated infections with undetectable levels of cell-free virus in plasma, one cannot use levels of viremia as a measure of virus burdens. To obtain an indication of whether there were differences in numbers of STLV-I(sm) proviral copies in PBMC from coinfected animals and those infected only with STLV-I(sm), multiple independent nested PCRs were done on DNA isolated from serially diluted PBMC obtained between 12 and 18 months after STLV-I(sm) inoculation. Total numbers of positive and negative PCR results at each DNA dilution were analyzed with the Quality computer program to determine number of proviral copies in $10^5$ PBMC. Although the number of animals in each group was low, the animals infected only with STLV-I(sm) had remarkably similar levels of circulating PBMC carrying proviruses, whereas those with STLV-I(sm) proviruses in PBMC from the dually infected animals varied over a 5-fold range (table 1).

Since the frequency of virus isolation from PBMC by use of standard culture conditions is directly related to virus burdens [45–47], these results were also used as a measure of virus burdens. Cocultures of macaque PBMC with normal human PBMC were established routinely and monitored for replication of SIV-PBj14-bcl1 by RT assay and STLV-I(sm) by p19 Gag.
Table 1. Quantification of STLV-I(sm) provirus burdens by polymerase chain reaction (PCR)–based limiting dilution assay.

<table>
<thead>
<tr>
<th>Group, animal</th>
<th>Weeks infected with STLV-I</th>
<th>STLV-I proviral copies/10^5 PBMC</th>
<th>SE</th>
<th>No. of assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA7</td>
<td>65</td>
<td>6.2</td>
<td>4.34</td>
<td>3</td>
</tr>
<tr>
<td>TB7</td>
<td>80</td>
<td>6.2</td>
<td>4.37</td>
<td>3</td>
</tr>
<tr>
<td>TC1</td>
<td>65</td>
<td>6.2</td>
<td>4.34</td>
<td>5</td>
</tr>
<tr>
<td>P010</td>
<td>66</td>
<td>18.2</td>
<td>5.04</td>
<td>7</td>
</tr>
<tr>
<td>P9E</td>
<td>71</td>
<td>11.4</td>
<td>7.59</td>
<td>3</td>
</tr>
<tr>
<td>P007</td>
<td>75</td>
<td>14.1</td>
<td>7.59</td>
<td>3</td>
</tr>
<tr>
<td>P8G</td>
<td>66</td>
<td>6.2</td>
<td>4.29</td>
<td>5</td>
</tr>
<tr>
<td>P9Ba</td>
<td>55</td>
<td>21.2</td>
<td>2.22</td>
<td>7</td>
</tr>
</tbody>
</table>

NOTE. Estimates of mean proviral copy numbers and SEs were determined with Quality program and results from multiple independent limiting dilutions and PCR assays done on a total of 1 µg of genomic DNA. PBMC, peripheral blood mononuclear cells.

Table 2. Status of macaques 2 years after infection.

<table>
<thead>
<tr>
<th>Group, animal</th>
<th>CD4⁺ T cells/µL a</th>
<th>Frequency of isolation b</th>
<th>Status c</th>
</tr>
</thead>
<tbody>
<tr>
<td>P8V</td>
<td>115</td>
<td>13/13</td>
<td>Died week 50, end-stage AIDS</td>
</tr>
<tr>
<td>TD1</td>
<td>184</td>
<td>14/14</td>
<td>Chronic diarrhea</td>
</tr>
<tr>
<td>P002</td>
<td>930</td>
<td>20/20</td>
<td>Healthy</td>
</tr>
<tr>
<td>P008</td>
<td>254</td>
<td>27/27</td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>P014</td>
<td>444</td>
<td>26/26</td>
<td>Severe rash, lymphadenopathy</td>
</tr>
<tr>
<td>P021</td>
<td>870</td>
<td>15/15</td>
<td>Healthy</td>
</tr>
<tr>
<td>P03</td>
<td>13/15</td>
<td>14/14</td>
<td>Died week 55, end-stage AIDS</td>
</tr>
<tr>
<td>P9E</td>
<td>974</td>
<td>22/22</td>
<td>Healthy</td>
</tr>
<tr>
<td>P003</td>
<td>189</td>
<td>22/22</td>
<td>Sporadic diarrhea, low platelet counts</td>
</tr>
<tr>
<td>P007</td>
<td>659</td>
<td>24/24</td>
<td>Healthy</td>
</tr>
<tr>
<td>P018</td>
<td>1393</td>
<td>16/16</td>
<td>Died week 93, severe thrombocytopenia</td>
</tr>
<tr>
<td>P020</td>
<td>668</td>
<td>23/23</td>
<td>Healthy</td>
</tr>
<tr>
<td>P023</td>
<td>545</td>
<td>16/16</td>
<td>Low platelet counts</td>
</tr>
</tbody>
</table>

NOTE. NA, not available.

a Absolute nos. of CD4⁺ lymphocytes at 2 years after first inoculation of either STLV-I(sm) or SIV-PBJ14-bcl1.

b No. of times STLV-I(sm) or SIV was detected in cell-free supernatants of cocultures of macaque and human PBMC/no. of cocultures evaluated during first 2 years after infection.

c Status at 2 years after initial infection or time of death.
Of interest, Beilke et al. [48] detected HTLV-I p19 Gag in PBMC supernatants from only 46% (11/24) of HIV/HTLV-I dually infected people. Taken together, the quantitative results demonstrated that coinfection of macaques with SIV-PBj14-bcl1 and STLV-I(sm) had little apparent impact on virus burdens.

**Serologic responses to retroviral infection.** All SIV-PBj14-bcl1–infected animals, irrespective of STLV-I(sm) status, rapidly generated high levels of antibodies to SIV antigens. At 4 weeks after inoculation of SIV-PBj14-bcl1, antibody titers in group 2 ranged from 1:3200 to 1:51,200 and were 4-fold lower than those in the coinfected group 3 animals, which ranged from 1:12,800 to 1:204,800 (data not shown). When the serum antibody titers to STLV-I(sm) in groups 1 and 3 were compared, it was of interest that the coinfected macaques in group 3 tended to have higher antibody titers to STLV-I(sm) than did those animals infected only with this virus (figure 3). Immunoblot analysis of antibodies to STLV-I(sm) in serum obtained from macaques at 1 year after the first inoculation of H11 cells revealed essentially no differences between the 2 STLV-I(sm)–infected groups in the patterns of reactivity (figure 4). All animals inoculated with STLV-I(sm) had antibodies to gag-encoded antigens, but only 4 macaques clearly had antibodies

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**Figure 3.** Serum antibody titers to STLV-I(sm) in groups 1 (A) and 3 (B, C). All macaques were inoculated with STLV-I(sm) at weeks 0 and 6. Arrow indicates time (week 9) at which group 3 macaques were inoculated with SIV-PBj14-bcl1. Values below 5 are negative.
that recognized the external glycoprotein, gp46. The serum sample least reactive, with only a faint band at the gp46 position, was from macaque P9B, a coinfected animal that was euthanized 4 weeks after this serum sample was taken because of increasing morbidity due to an AIDS-like disease. It is possible, therefore, that the lack of antibodies to STLV-I(sm) in serum from P9B was a result of immunodeficiency.

Hematologic changes. Macaques infected only with SIV-PBJ14-bcl1, as well as those infected with both retroviruses, exhibited loss of CD4+ lymphocytes in association with increased levels of virus and deteriorating health. This loss of CD4+ T cells was manifest as decreases in both percentages of total lymphocytes and absolute numbers of cells (figure 5). Animals that progressed to immunodeficiency disease also exhibited increases in percentages and numbers of CD8+ T cells, which, in association with loss of CD4+ T cells, contributed to decreased CD4:CD8 ratios (figure 6). Animals that remained healthy or had transient episodes of diarrhea or rash maintained relatively stable levels of CD4+ and CD8+ T cells. With the exception of macaque TB7, hematologic values of the animals infected only with STLV-I(sm) did not change appreciably. TB7 maintained stable CD4:CD8 ratios but experienced recurrent episodes of lymphopenia characterized by low levels of both CD4+ and CD8+ lymphocytes.

Clinical course after infection. On the basis of the original hypothesis that coinfection with SIV and STLV-I would enhance disease progression, we chose a strain of SIV that was not likely to induce immunodeficiency disease in pig-tailed macaques for at least 1 year. Such a strain would enable us to determine more readily whether progression of SIV-related disease was accelerated. The virologic and serologic results revealed no major differences in any parameter when comparing the coinfected animals with those infected only with SIV-PBJ14-bcl1. Survival of animals in these 2 groups was also virtually identical (figure 7). Among those macaques infected only with SIV-PBJ14-bcl1, 1 animal (P8V) was euthanized 50 weeks after inoculation. In the coinfected group, 2 animals were euthanized, 1 at 46 and another at 84 weeks after SIV-PBJ14-bcl1 infection, or 55 and 93 weeks, respectively, after STLV-I(sm) inoculation. Among those animals still surviving, the most common prob-
Figure 5. Absolute numbers of peripheral blood CD4⁺ lymphocytes in macaques infected with only STLV-I(sm) (A), with only SIV-PBj14-bcl1 (B), or with both STLV-I(sm) and SIV-PBj14-bcl1 (C, D). Dashed lines indicate time at which dually infected macaques were inoculated with SIV-PBj14-bcl1.
lems have been rash of varying severity, intermittent or chronic diarrhea, and abnormalities in platelets, ranging from low platelet counts to severe thrombocytopenia (table 2). At 2 years after infection, 2 of the macaques in group 2 and 3 macaques in group 3 appear to be healthy.

P8V, infected only with SIV-PBj14-bcl1, and the 2 dually infected macaques (P9B and P018) that were euthanized exhibited various signs of disease characteristic of AIDS before their deaths. Of the 3 animals, only P018 maintained normal percentages and numbers of lymphocyte subsets in PBMC (ta-

**Figure 6.** CD4/CD8 ratios for macaques infected with only STLV-I(sm) (A), only SIV-PBj14-bcl1 (B), or both STLV-I(sm) and SIV-PBj14-bcl1 (C, D). Dashed lines indicate time at which dually infected macaques were inoculated with SIV-PBj14-bcl1.
ble 3). However, this animal had experienced persistent severe thrombocytopenia for ~7 months, which was the primary reason for euthanasia. Histopathologic evaluation of tissues from this animal taken at necropsy included mild follicular hyperplasia in lymph nodes, focal lymphocyte infiltrates in lung and large intestine, and lymphocytic infiltrates in the lamina propria of the submucosa of the small intestine and large numbers of eosinophils. In addition, the liver appeared congested, with multifocal centriflobular necrosis, which was suggestive of cardiac insufficiency. Both P9B and P8V had severe depletion of peripheral blood CD4+ T cells and elevated numbers of both CD4+ and CD8+ T cells expressing HLA-DR (table 3). In addition to areas of lymphoid depletion with increased numbers of epithelioid macrophages in lymph nodes from P9B, histopathology of other tissues revealed plasmacytid infiltrates and increased numbers of eosinophils in the lamina propria of the large intestine and significant congestion in the spleen. In the lung, focal parabronchiolar lymphoid infiltrates with interstitial pneumonitis and proteinaceous exudates in many alveoli were observed. That P9B may have been in a preleukemic state was supported by the presence in the peripheral lymph node lymphocytes [49±51], but use different cell surface receptors [52], it is theoretically possible that superinfection could occur in vivo. Moreover, in vitro studies demonstrated not only that HIV and HTLV could infect the same cell but also that dual infection resulted in the formation of pseudotyped viruses [53–56]. These results led investigators to postulate that chimeric viruses with the genome of HIV encapsidated in a virion bearing the HTLV surface glycoprotein might broaden HIV’s cellular and tissue tropism, thus enhancing disease progression.

Although it is clear that pseudotyped retroviruses can be produced during coinfection in vitro, such virions have not been demonstrated in vivo. Relative to HIV and HTLV, one reason to suppose that pseudotypes do not exist in vivo is that the production of HTLV/STLV is limited and the transmissibility of cell-free HTLV/STLV is inefficient [57–59]. However, since SIV and HIV replicate continuously and to high titers in infected individuals [60–64], in our model the likelihood of SIV-PBj14-bcl1 superinfecting a CD4+ cell harboring STLV-I(sm) or of a STLV-I(sm)–infected cell fusing with a cell infected with

<table>
<thead>
<tr>
<th>Lymphocyte subset</th>
<th>% for macaque</th>
</tr>
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<tbody>
<tr>
<td>CD4+</td>
<td>12.0</td>
</tr>
<tr>
<td>CD8+</td>
<td>61.6</td>
</tr>
<tr>
<td>CD4:CD8 ratio</td>
<td>0.2</td>
</tr>
<tr>
<td>HLA-DR CD4+</td>
<td>33.7</td>
</tr>
<tr>
<td>HLA-DR CD8+</td>
<td>26.0</td>
</tr>
<tr>
<td>CD25:CD8+</td>
<td>4.8</td>
</tr>
<tr>
<td>CD25</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of T cell subsets in peripheral blood of macaques at death.
SIV-PBj14-bcl1 may be high. If pseudotypes were formed in the dually infected macaques described above, these viruses had no apparent impact on virus burdens or progression to immunodeficiency disease.

Our results using the macaque model agree with recent cross-sectional studies of humans coinfected with HIV and either HTLV-I or -II that showed no significant differences in virus burden or disease [17–20]. In one study in which more severe disease in coinfected persons was indicated, when the results were adjusted for age, this disparity was no longer apparent [65]. Despite the lack of disease enhancement, several investigators have noted that coinfected persons with more advanced HIV disease tend to have higher numbers of CD4+ T cells than do those infected only with HIV [9, 19, 20]. Furthermore, it appears that HTLV-I and -II lead to different sequelae in HIV-infected persons, indicating that investigators should distinguish between these two viruses and not extrapolate results from studies of persons coinfected with HIV and one HTLV type to those coinfected with the other HTLV type [9, 19]. This differential effect, if real, might be related to the fact that HTLV-I preferentially infects CD4+ T cells, whereas HTLV-II preferentially infects CD8+ T cells [66]. Since STLV-I–associated leukemias/lymphomas are phenotypically CD4+ or CD8+ in different simian species [25], dually infected macaques could be analogous to persons coinfected with HIV and either HTLV-I or -II. Although all simian transformed cell lines we generated with STLV-I(sm) have had CD2+, CD4+, and CD8+, the observation that in macaque P9B 50% of both lymphocyte subsets expressed CD25 suggests that neoplastic cells in pig-tailed macaques can be derived from either T cell subset.

Because of the low incidence of disease associated with both HTLV infection of humans and STLV infection of nonhuman primates, it was surprising that macaque P9B had evidence of abnormal lymphoproliferation, with >50% of its lymphocytes expressing CD25, at the time of its death 55 weeks after STLV-I(sm) inoculation. It is intriguing to speculate that SIV may potentiate STLV-related disease. This possibility is supported by the observation that, as a group, coinfected macaques had higher titers of antibodies to STLV-I(sm) than did those animals infected only with this virus (figure 3). It has been shown that there is a direct association between HTLV-I provirus load and antibody titers [67, 68]; therefore, if, as is true for HIV/SIV infections, HTLV/STLV disease progression is related directly to virus burden, then enhancement of STLV-I–related disease by SIV is a real possibility. In the present study, the macaques were inoculated with SIV after STLV-I(sm) infections were established. Whether preexisting SIV infection would have a different impact on STLV-I(sm)–induced disease has not been evaluated. Studies are in progress to determine STLV-I(sm) burdens throughout infection in PBMC and at necropsy in tissues from singly and dually infected macaques.

Results of various studies of humans coinfected with HIV and HTLV have led to disparate conclusions, primarily because it is almost impossible to perform prospective studies that are dependent on individuals being infected by two different retrovirus families, one of which is a large group of genotypic and phenotypic variants. The advantages of using the macaque model are multiple: the exact dates of virus infection are known; the animals are age-matched; the effects of antiviral therapies are not an issue; and the study subjects are not infected with different HIV/SIV strains that might vary in pathogenicity. In addition to demonstrating unequivocally that coinfection of macaques with SIV and STLV-I (and by analogy HIV and HTLV) does not enhance lentivirus burdens or progression to AIDS, we have shown that pig-tailed macaques can be persistently infected with STLV-I(sm). Thus, we have established a reliable model system for identifying factors that may influence induction of STLV-I–related disease. Long-term evaluation of the singly and dually infected macaques in the present study may allow us to determine whether dual infection enhances development of neurologic or neoplastic disease or elicits novel pathologies.

Acknowledgments

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