T-cell lymphoproliferative disorder in an aged rhesus macaque

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Clinical signs in nonhuman primates with lymphoproliferative disorders are usually nonspecific and include lethargy, anorexia, weight loss, pallor, and organomegaly. A diagnosis of leukemia in nonhuman primates is best made by performing serial CBC, bone marrow and lymph node biopsies, and flow cytometry and immunophenotyping of peripheral blood mononuclear cells.

A 23-year-old female rhesus macaque (Macaca mulatta) was evaluated because of a 2-day history of anorexia. The monkey was individually housed indoors in a room maintained at 27 C under a 12-hour light:12-hour dark cycle and was fed a commercially available primate diet and water ad libitum. The monkey had been obtained by the California Regional Primate Research Center (CRPRC) approximately 10 months prior to evaluation; its history prior to arrival at the CRPRC was largely unknown. Virus isolation for months prior to evaluation; its history prior to arrival at the CRPRC was largely unknown. Virus isolation for simian type D retrovirus (SRV) yielded negative results, and SRV antibodies were not detected by enzyme immunoassay and western immunoblot assay.

On initial physical examination, the monkey had mild hepatomegaly and mild left inguinal lymphadenopathy. A CBC revealed severe lymphocytosis (36,828 cells/µl), moderate neutropenia, and normocytic hypochromic anemia (Table 1). Serum biochemical analysis revealed mild hypoalbuminemia (2.5 g/dl). A CBC was repeated 2 days later and revealed an increase in the lymphocytosis (62,856 cells/µl), neutropenia, hypochromic anemia, and thrombocytopenia (Table 1). Moreover, on review of the animal’s record, a CBC obtained on entry into quarantine 10 months previously revealed that lymphocytosis (40,548 cells/µl) was evident at that time.

One week after initial evaluation, bone marrow aspiration was performed on the humerus and revealed slight hypocellularity, severely decreased myelopoietic and erythropoietic cells, no megakaryocytes, and a predominance of mature lymphocytes. A CBC again revealed lymphocytosis (38,220 cells/µl). In-house testing for simian T-lymphotropic virus (STLV) by use of western blot assay was indeterminate; results of radioimmunoprecipitation agar gel electrophoresis for STLV-1 antibodies were negative. Results of radioimmunoassays were positive for herpesvirus saimiri antibodies and negative for herpesvirus tamarinus antibodies.

Because of the persistence of lymphocytosis with no apparent underlying infectious cause, in addition to hepatomegaly, lymphadenopathy, and bone marrow hypoplasia, our presumptive diagnosis was a lymphoproliferative disorder, most likely a leukemia. Serial CBC were performed during the next several weeks and continued to reveal pronounced lymphocytosis, progressive hypochromic anemia, and progressive thrombocytopenia. In addition, CBC revealed an absence of monocytes and eosinophils; on some counts, neutropenia was evident. Serum biochemical analyses were, for the most part, unremarkable, except for intermittent mild hypoalbuminemia (approx 2.3 g/dl; in-house reference range, 3.4 to 4.4 g/dl). The monkey developed severe splenomegaly, had extreme flaccidity of the abdominal musculature, became increasingly pale, and had progressive weight loss, generalized muscle atrophy, and generalized alopecia. Approximately 2.5 months after initial evaluation, the hepatomegaly appeared to partially resolve, although splenomegaly remained. A bone marrow biopsy was performed on the humerus and confirmed the bone marrow aspirate findings obtained previously; fibrosis, hypocellularity, and an increased myeloid:erythroid ratio were evident. Ultrasonography of the abdomen revealed a severely enlarged, diffusely hypoechoic spleen and abnormal increased echogenicity of the liver parenchyma. Computerized tomography of the thorax and abdomen revealed mild dextroposition of the cardiomedastinal structures, splenomegaly, and a mildly enlarged right adrenal gland. An ACTH-stimulation test was performed to rule out hyperadrenocorticism as a cause for the abdominal flaccidity and enlarged right adrenal gland; results were negative.

Approximately 8 weeks after initial evaluation, appearance of lymphocytes was atypical on whole blood smears with cytoplasmic vacuolation, later progressing to both cytoplasmic and nuclear vacuolation and clumping of nuclear chromatin. A CBC was performed approximately 5.5 months after initial evaluation and revealed a few lymphocytes with prominent nucleoli. Bone marrow aspiration of the humerus revealed no megakaryocytes (peripheral platelet count, 49,000 cells/µl), severely decreased myelopoietic...
Table 1—Serial complete blood counts obtained from an aged female rhesus macaque with a T-cell lymphoproliferative disorder

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference range (units)*</th>
<th>1</th>
<th>3</th>
<th>17</th>
<th>35</th>
<th>91</th>
<th>137</th>
<th>165</th>
<th>196</th>
</tr>
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<tbody>
<tr>
<td>HCT</td>
<td>41–47 (%)</td>
<td>24</td>
<td>24</td>
<td>26</td>
<td>25</td>
<td>28.5</td>
<td>27.1</td>
<td>27.6</td>
<td>18.4</td>
</tr>
<tr>
<td>RBC</td>
<td>5.8–6.9 x 10^12/µl</td>
<td>3.38</td>
<td>3.32</td>
<td>3.47</td>
<td>3.38</td>
<td>3.88</td>
<td>3.72</td>
<td>3.6</td>
<td>2.591</td>
</tr>
<tr>
<td>Platelets</td>
<td>2.5–4.4 x 10^11/µl</td>
<td>1.64</td>
<td>0.96</td>
<td>1.8</td>
<td>1.64</td>
<td>0.96</td>
<td>0.71</td>
<td>0.58</td>
<td>0.47</td>
</tr>
<tr>
<td>WBC</td>
<td>5,300–11,300 (cells/µl)</td>
<td>39,600</td>
<td>64,800</td>
<td>145,000</td>
<td>57,200</td>
<td>91,200</td>
<td>122,300</td>
<td>230,000</td>
<td>76,300</td>
</tr>
<tr>
<td>PMN</td>
<td>2,508–5,752 (cells/µl)</td>
<td>1,584</td>
<td>1,296</td>
<td>2,900</td>
<td>572</td>
<td>0</td>
<td>2,646</td>
<td>4,600</td>
<td>1,526</td>
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<tr>
<td>Lymphs</td>
<td>1,335–5,141 (cells/µl)</td>
<td>36,828</td>
<td>62,856</td>
<td>142,100</td>
<td>56,628</td>
<td>129,854</td>
<td>223,100</td>
<td>74,774</td>
<td></td>
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<tr>
<td>Monos</td>
<td>278–848 (cells/µl)</td>
<td>792</td>
<td>648</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eos</td>
<td>0–681 (cells/µl)</td>
<td>396</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1,824</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Based on established in-house reference ranges for geriatric female rhesus macaques (n = 26; mean age, 23.7 years); values represent the mean ± 1 SD.

**Approximately 0.4% of RBC were reticulocytes, indicating nonregenerative anemia. Many of the lymphocytes appeared atypical, with vacuolated cytoplasm and clumping and vacuolation of nuclear chromatin.***


erythrophoietic cells, and a predominance of mature lymphocytes.

To characterize the lymphocytosis, peripheral blood mononuclear cells (PBMC) were isolated by use of a described technique. Cells were stained with fluorochrome-conjugated monoclonal antibodies to CD4, CD20, CD4, CD8, and HLA-DR receptors. Samples (5 x 10^6 cells/panel of antibodies) were gated on the lymphocyte population and analyzed by use of 2-color flow cytometry. Immunophenotyping and flow cytometry of PBMC consistently revealed that 93% of lymphocytes were CD2+ T cells that were also CD8+ cells. These T cells comprise the suppressor-cytotoxic subset of mature T lymphocytes. Approximately 25% of the CD8+ cells were also HLA-DR positive, which indicated cellular activation.

Lymphocytes were cultured, using standard culture conditions; cells were isolated on RPMI 1640 medium supplemented with 10% fetal bovine serum and replaced every 3 to 4 days. Cultures were maintained for 2 weeks. Cultured lymphocytes did not proliferate spontaneously but were stimulated by the mitogen concanavalin A to grow as well as normal rhesus lymphocytes. One proliferating lymphocyte culture developed large-cell cytopathic effect, and electron micrographs of cells from this culture revealed budding and extracellular viral particles with the morphologic characteristics of simian foamy virus; this virus islogic characteristics of simian foamy virus; this virus is present in both the EcorI- and BamHI-digested DNA, suggesting T cell clonality. Although mono- or oligoclonal expansions of CD8+ T cell subsets have been documented in aged mice and humans, they are not associated with derangements in peripheral blood counts or with clinical signs such as anemia, lymphadenopathy, or splenomegaly. Therefore, it was unlikely that this monkey's CD8+ lymphocytosis was caused by benign age-related T cell expansion or by reactive lymphocytosis, which is polyclonal in nature.

Cells from a healthy aged donor monkey were used as a control for the cell phenotyping, NK cell, and T cell clonality assessment assays. For our purposes, a healthy monkey was one that did not have clinical signs of disease, and aged was defined as a monkey 20 years or older. Because we had not performed these assays on a large number of healthy, aged macaques, we cannot define this control as normal.

Three days prior to euthanasia, hematocrit was noticed. Treatment with antibiotic (enrofloxacin) and nonsteroidal anti-inflammatory (flunixin meglumine) drugs was initiated, pending bacteriologic culture results from a rectal swab (this culture later grew Shigella flexneri and Campylobacter coli). Euthanasia (overdose of sodium pentobarbital, administered IV) was elected on day 196 because of the monkey's declining condition and poor prognosis. Results of a CBC obtained just after the monkey's lymphocytes in this assay, which indicated a lack of NK activity.

Detecting clonal T-cell populations for the diagnosis of T-cell leukemias is possible by use of molecular probes to detect T-cell receptor gene rearrangements. Total cellular DNA was extracted from 10^7 PBMC, digested with 3 restriction enzymes, electrophoresed, hybridized with a Jβ1/JβII T-cell receptor probe cocktail, and analyzed by use of Southern blot, according to manufacturers' instructions. A rearranged pattern was present in both the EcoRI- and BamHI-digested DNA, suggesting T cell clonality. Although mono- or oligo- clonal expansions of CD8+ T cell subsets have been documented in aged mice and humans, they are not associated with derangements in peripheral blood counts or with clinical signs such as anemia, lymphadenopathy, or splenomegaly. Therefore, it was unlikely that this monkey's CD8+ lymphocytosis was caused by benign age-related T cell expansion or by reactive lymphocytosis, which is polyclonal in nature.

On gross evaluation, necropsy revealed splenomegaly, gastric ulcers, colitis, hepatic atrophy, pulmonary acariasis, and emaciation. Histologic evaluation of the bone marrow confirmed lymphocytic hyperplasia with an increased number of mature lymphocytes throughout the marrow and foci of lymphocytic hyperplasia.
phoblastic cells with a moderate mitotic index. The spleen contained scattered depleted lymphoid follicles, and the red pulp contained a large number of mature lymphocytes. Lymph nodes appeared hyperplastic and reactive, with a depletion of lymphoid follicles and moderately cellular medullary cords containing large numbers of plasma cells as well as lymphocytes, erythrocytes, and histiocytes. Hepatic sinusoids contained large numbers of lymphocytes. Other incidental findings included chronic lymphoplasmyastic gastritis, enterocolitis, and bronchiolar-alveolar pneumonitis attributed to Pneumonyssus simica infestation.

At necropsy, immunophenotyping and flow cytometry of lymphocytes from blood, bone marrow, spleen, and lymph nodes were performed (by use of the aforementioned methods). The dominant lymphocyte population in blood, bone marrow, and spleen was the CD8⁺ T lymphocyte. Both B cells and CD4⁺ T cells were detected in a peripheral lymph node. This distribution of lymphocyte populations is consistent with T-cell leukemia and with clinical and pathologic findings of marrow infiltration, splenomegaly with leukemic infiltration, and mild to no lymphadenopathy.

Several lymphoproliferative disorders have been reported in captive nonhuman primates. Commonly, leukemias and lymphomas in nonhuman primates are associated with STLV, a nonhuman primate leukemia virus, oncogenic herpesviruses, and types C, D, and E retroviruses. However, the rhesus macaque of this report was not infected with any of these viruses typically associated with lymphoproliferative disorders in Old World primates; therefore, its CD8⁺ T-cell lymphoproliferative disorder most resembled human T-cell leukemia.

Human chronic lymphocytic leukemias (CLL) are characterized by a preponderance of mature-appearing lymphocytes in peripheral blood, with WBC counts ranging from 15,000 to 200,000 cells/µl. These cells usually infiltrate bone marrow, spleen, and lymph nodes to some extent. Human CLL is most common in adults > 50 years old and in 95% of cases is comprised of a clonal expansion of B lymphocytes; in < 5% of cases, the lymphocytes are identified as T cells.³ True T-cell CLL is one of the major recognized T-cell proliferative disorders, which are generally differentiated by physical examination findings and clinical course, cell morphologic characteristics, and cell surface markers. These disorders include T-γ lymphocytosis syndrome, T-cell prolymphocytic leukemia, adult T-cell leukemia/lymphoma, and true T-cell chronic lymphocytic leukemia. The T-γ lymphocytosis syndrome is characterized by a proliferation of large granular lymphocytes and is usually a benign disease. Large granular lymphocytes are easily distinguishable from small lymphocytes on peripheral blood smears. T-cell prolymphocytic leukemia is characterized by a proliferation of prolymphocytes (also morphologically distinct from mature lymphocytes) and is an aggressive disease. Adult T-cell leukemia/lymphoma is linked to infection with the retrovirus human T-lymphotropic virus-1.

True T-cell CLL is much more rare than the more common B-cell CLL. Patients are often asymptomatic but may have signs of lethargy, anorexia, or weight loss, and they commonly have splenomegaly, lymphadenopathy, and severe lymphocytosis. Most cases reported in humans involve proliferation of CD4⁺ T cells, but there are reports of CD8⁺ T-cell CLL.³ The disease course varies from insidious but progressive to fulminant with rapid progression.

A diagnosis of CLL is made on the basis of physical examination findings, which are often related to infiltration of neoplastic cells in other tissues (e.g., lethargy, weight loss, anorexia, pallor, lymphadenopathy, hepatomegaly, and frequent or opportunistic infections) and on examination of whole blood smears. T cells can be differentiated, using cell surface marker identification. In advanced CLL, there is anemia, granulocytopenia, and thrombocytopenia because of bone marrow infiltration. All CLL must be differentiated from reactive lymphocytosis; in the latter case, there is polyclonal T-cell expansion, as opposed to the monoclonal B-cell or T-cell proliferation seen with CLL.

The T-cell lymphoproliferative disorder in the monkey of this report resembled human T-cell CLL in several ways; the monkey had profound lymphocytosis, which consisted uniformly of mature small lymphocytes with evidence of cellular infiltration of the bone marrow and spleen. The monkey was an aged animal, equivalent to a human in the third trimester of life, and did not have any of the viruses that have been linked to lymphoproliferative disorders in other Old World primates, including the simian equivalent of human T-lymphotropic virus, STLV. The monkey did have profound anemia and granulocytopenia, indicating that the disease was advanced. The lymphocytosis was clearly not reactive, because the T cells were monoclonal and most likely did not represent an age-related benign expansion of clonal CD8⁺ T cells, because this monkey had peripheral blood count abnormalities, bone marrow hypoplasia, and splenic infiltration compatible with a neoplastic process.

Although this rhesus macaque did not have any of the viruses that have been linked to lymphoproliferative disorders in Old World primates, the presence of antibodies against herpesvirus saimiri in this monkey is noteworthy. Although herpesvirus saimiri does not cause disease in its natural host, the squirrel monkey (Saimiri sciureus), infections have been linked to lymphoproliferative disorders in other New World primate species and transform human CD8⁺ T lymphocytes in vitro. However, it is quite likely that the positive herpesvirus saimiri result in this monkey represented cross-reactivity of the assay with rhesus rhadinovirus (RRV) antibodies rather than indicating a true herpesvirus saimiri infection. Rhesus rhadinovirus is highly prevalent in some captive primate colonies and was first described in rhesus macaques that had positive test results for herpesvirus saimiri by use of ELISA.¹² Rhesus rhadinovirus is a gamma herpesvirus related antigenically to herpesvirus saimiri and to Kaposi’s sarcoma-associated herpesvirus (KSHV), which have been linked to lymphoproliferative disorders in primates and humans, respectively. Moreover, an oncogene was recently identified in the RRV genome that is equivalent in position to the herpesvirus saimiri and KSHV oncogenes and that trans-
forms mouse fibroblasts in vitro. At present, however, the association of RRV infection with disease in primates is unknown.

References