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Morphological Changes of an Inflammatory Myopathy in Rhesus Monkeys with Simian Acquired Immunodeficiency Syndrome (42556)

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Abstract. Eleven of 25 rhesus monkeys which died of simian acquired immunodeficiency syndrome (SAIDS) caused by infection with a type D retrovirus related to Mason–Pfizer monkey virus showed evidence of muscle weakness and atrophy and had elevated levels of muscle enzymes. Biopsies of affected muscle studied with enzyme histochemistry showed the characteristic features of polymyositis. Inflammatory cells consisting of lymphocytes, macrophages, and large vacuolated bizarre-shaped cells of undetermined type were surrounding or invading muscle fibers and were present in the perivascular spaces and endomysia septa. Within the perivascular infiltrates, lymphocytes were abundant but very few macrophages were present. Other myopathic features including profound proliferation of fibrous tissue, necrosis, and phagocytosis of muscle fibers were noted to a variable degree. The retrovirus was isolated from affected muscles. The clinical and historical features of polymyositis in rhesus monkeys with SAIDS are very similar to those of human polymyositis. The polymyositis in SAIDS induced by a type D retrovirus related to Mason–Pfizer monkey virus is an excellent primate model to study the mechanism and morphological changes of viral-induced muscle damage.

An immunosuppressive disease, similar in many respects to human acquired immunodeficiency syndrome (AIDS), has been reported to occur enzootically in macaque monkeys housed at several primate centers in the United States (1–8). This immunodeficiency syndrome is caused by different simian retroviruses which include (a) two Type D retroviruses related to Mason–Pfizer monkey virus (MPMV) affecting the genus *Macaca* called simian retrovirus 1 (SRV-1) isolated from monkeys at the California Primate Center (3–11) and SRV-2 isolated from monkeys at the Washington and Oregon centers (2, 10, 11) (Both of these retroviruses are related to MPMV with very closely related genomic base sequence data (3, 8–11). By competition radioimmunoassay and restriction endonuclease cleavage studies, however, some antigenic differences exist between SRV-1 and SRV-2 (10, 11), hence the differences in their subtyping.) and (b) a lentivirus called simian T lymphotropic virus type III (STLV-III) closely related to human HTLV-III/LAV (12).

The clinical spectrum of simian AIDS (SAIDS) induced by the SRV-1 includes lymphadenopathy, splenomegaly, fever, diarrhea, weight loss, pancytopenia, hypogammaglobulinemia, and multiple opportunistic infections (1, 5, 6). Some of these animals also develop Kaposi-like skin lesions indistinguishable from the "patch and plague"-type lesions seen frequently in AIDS patients (5, 6). During our studies of rhesus monkeys with experimentally transmitted SAIDS due to SRV-1 (3, 5), we observed that some animals also developed severe neuromuscular problems characterized by muscular atrophy, weakness, and elevated serum muscle enzymes (5, 7, 13). In this communication, we describe the morphological changes seen in muscle biopsies taken serially from affected animals using enzyme histochemistry in frozen sections.

Methods. *Inocula for studies.* Prior to identification of the causative agent of SAIDS, 25 healthy juvenile rhesus monkeys, housed in pairs in Horsfall isolation chambers at the National Institutes of Health (Bethesda, MD), were experimentally inoculated with various materials from animals with SAIDS to identify the mechanism of disease transmission and its etiologic agent. Materials used in these studies were tissue homogenates, serum, plasma, urine, and saliva from rhesus monkeys with advanced SAIDS. Nineteen animals, 7 males and 12 females, died of SAIDS after receiving these inocula. Later 6 rhesus monkeys, 3 males

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and 3 females, were inoculated with tissue-culture-grown SAIDS retrovirus type 1 (SRV-1) as described (3, 4, 8).

Clinical, virological, and immunological studies. All animals used in these studies were examined daily for manifestations of muscle weakness, muscle atrophy, skin lesions, lymphadenopathy, infections, and fever. Routine tests on blood and urine were performed weekly. Total protein and serum muscle enzymes CK, SGOT, SGPT, and LDH were determined serially in all animals. Titers of antibody to rhesus monkey cytomegalovirus (CMV), SV-40, Epstein–Barr virus (EBV), and SRV-1, IDB strain (8), were determined by previously reported methods (3, 7, 8). Attempts were also made to isolate SRV-1 in tissue culture from homogenates of muscle from five affected animals with weakness and three animals without clinical weakness (3, 7, 13). The functional state of the immune system of animals was evaluated by skin tests for delayed hypersensitivity and by *in vitro* lymphocyte mitogen stimulation tests as described (1, 5, 6). The ratio of lymphocyte surface markers, OKT4/OKT8, and the concentrations of serum immunoglobulins, IgA, IgG, and IgM, were also determined as described (1, 5, 6).

Muscle biopsy studies. Open muscle biopsies were performed at least once on all animals in the study. Portions of bicep and/or quadricep muscle which had not been traumatized by needle puncture were removed under ketamine anesthesia. Muscle biopsy specimens were fresh-frozen in isopentane cooled to −160°C in liquid nitrogen according to standard techniques (14, 15). A battery of histological and histochemical reactions commonly used to diagnose human muscle disorders were then employed to study the activity of muscle enzymes present in 6- to 10-μm-thick fresh-frozen sections (14, 15). The battery of reactions included hematoxylin and eosin (H + E); modified Gomori trichrome; myofibrillar ATPase with preincubation at pH 9.4, 4.6, and 4.3 to type or subtype muscle fibers (14, 15); NADH–tetrazolium reductase, an enzyme of mitochondrial and sarcoplasmic reticulum localization; nonspecific esterase to identify activated T cells and macrophages among the inflammatory cell population; succinic dehydrogenase to further study mitochondrial muscle enzymes; acid phosphatase to detect macrophages and possible changes in lysosomes; alkaline phosphatase to highlight regenerating muscle fibers and detect staining of the connective tissue; periodic acid Schiff (PAS) to detect glycogen accumulation; oil red 0 to stain neutral fat; diaminobenzidine to serve as a substrate to stain myoglobin and cytochrome oxidase (pH 6.0) in mitochondria (15); phosphorylase to detect amylophosphorylase; crystal violet to look for amyloid (16, 17); and Alizarin red to detect calcium in the muscle fibers or endomysium. In an attempt to identify the type of the cell predominantly involved in the inflammatory response, serial sections were stained with trichrome, esterase, and acid phosphatase to distinguish monocytes and macrophages from the other lymphocytes. In further experiments, fluorescein-conjugated OKT4 and OKT8 monoclonal antibodies (Ortho, Raritan, NJ) that stain cells with helper or suppressor markers, respectively, were employed in serial muscle biopsy sections using standard direct immunofluorescence techniques (16, 17). Similar studies have been employed to characterize lymphocyte subsets in the biopsies of human polymyositis (18, 19). The OKT4 and OKT8 monoclonal antibodies recognize surface markers in lymphocyte subpopulations of rhesus monkeys similar to those in humans (20).

Results. The gross and microscopic lesions observed at necropsy of the 25 animals which died or were killed in the terminal stages of SAIDS were similar to those reported previously (1, 5). Features common to all these animals were loss of 10 to 36% of maximum weight, diarrhea, generalized lymphadenopathy and splenomegaly. Histologically, both follicular and paracortical areas of lymph nodes, spleen, and thymus of deceased animals showed profound lymphoid depletion. Follicular centers had been replaced with extracellular hyalin material and few plasma cells were seen in medullary areas. Five of the 25 animals developed skin fibrosarcomas located on the arms, legs, and face. Microscopically, they were vascular and composed of interlacing spindle cells with many mitotic figures (5) and resembled the Kaposi-like lesions frequently seen in human AIDS patients.

SRV-1 was isolated in tissue cultures from several tissues including muscles in all animals

studied. The reverse transcriptase of the isolates was characteristic of type D retrovirus and had a Mg^{2+} preference for the synthetic template primers poly(rA)–oligo(dT) 12-18 and poly(rC)–oligo(dG) 12-18 but a Mn^{2+} preference for poly(rCm)–oligo(dG) 12-18 (3, 4). Isolates produced characteristic cytopathology in Raji cells, and were antigenically related to Mason–Pfizer monkey virus as determined by the enzyme-linked immunosorbent assay (ELISA) and competition radioimmunoassay (RIA) (3, 4). Electron microscopy of the virus showed budding particles with type D retrovirus morphology (3, 8, 21). Relatively few opportunistic agents were however isolated from the study group. This is not too surprising because the animals were housed in pairs in isolation chambers. Had our animals been housed in large groups in outdoor corrals, as previously reported (6), they probably would have experienced more opportunistic infections.

Seven of the 25 animals studied had muscle weakness and atrophy in at least one limb. Joints of the involved extremities did not show gross lesions. Abcesses were not present in either the muscles or skin of the involved limbs. Serum muscle enzymes were elevated between 400 and 1100 units (normal 80–180). SRV-1, but no bacteria or other viruses, was isolated from the affected muscle tissue homogenate (3, 8, 13) from all the muscle specimens with polymyositis examined. SRV-1 was not present in three monkeys who did not have histological findings of polymyositis.

Muscle biopsies from all weakened limbs showed evidence of severe polymyositis. Muscle biopsies from limbs of four animals without clinically apparent weakness also showed histological features of polymyositis. Inflammatory infiltrates were present between muscle fibers, interstitially, perivascularly, and in the endomysial spaces (Figs. 1 and 2). Myopathic features characterized by variability in the muscle fiber size, phagocytosed or necrotic fibers, and increased connective tissue were also present (Figs. 1 and 2). The degree of fiber regeneration was small. Within the perimysial inflammatory infiltrates, bizarre-shaped fibroblasts or histiocytes (Fig. 3) or large vacuolated cells, probably macrophages (Fig. 4a), were present. Occasionally, the identity of some cells in the infiltrates could not be ascertained. The subpopulation of cells in the perivascular infiltrates was different from the cells within the interstitial septa, as determined by examining serial sections with trichrome, esterase, and acid phosphatase (Figs. 4 and 5). Within the perivascular infiltrates few cells were esterase positive and much fewer were acid phosphatase positive (Fig. 5). The esterase-positive perivascular cells had a diffuse cytoplasmic staining pattern and not a punctuate staining pattern, representing monocytes rather than activated T cells (22, 23). The fact that few of these cells were acid phosphatase positive (Fig. 5c) indicates that the number of macrophages in the perivascular infiltrates was very small. The perimysial infiltrates, however, which were predominantly fibroblasts or bizarre shaped large cells, were strongly positive for esterase and positive with acid phosphatase (Fig. 4). Based on our preliminary analysis of the findings with immunofluorescence, it ap-

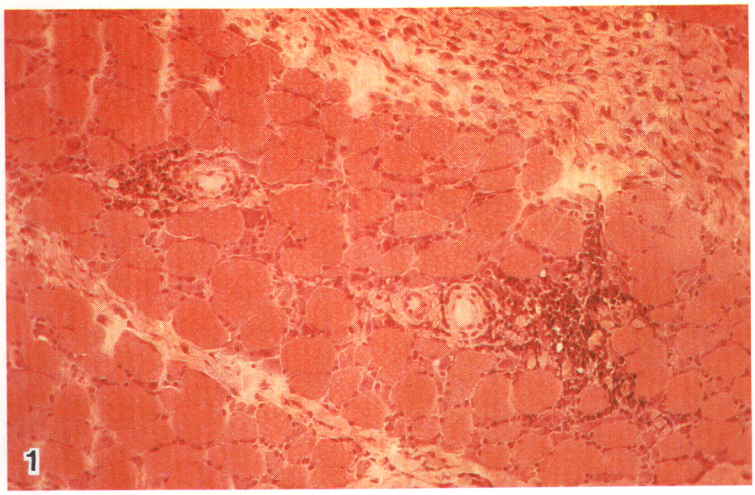
FIGS. 1 AND 2. Transverse frozen sections of muscle biopsies from SAIDS animals stained with H + E (Fig. 1) and trichrome (Fig. 2). Severe perivascular, interstitial, and perimysial inflammatory infiltrate is noted. Myopathic features, phagocytosis and increased connective tissue is prominent in Fig. 2. Fig. 1, ~ ×260; Fig. 2, ~ ×280.

FIG. 3. Muscle biopsy stained with trichrome shows the large, bizarre-shaped cells in the perimysium (×300).

FIG. 4. Serial sections of muscle biopsy stained with trichrome (a), esterase (b), and acid phosphatase (c) shows that most of the perimysial cells, probably fibroblasts and macrophages, are esterase and acid phosphatase positive. Only a few of the perivascular cells are, however, esterase and acid phosphatase positive, indicating that the number of macrophages perivascularly is small (~ ×205).

FIG. 5. Serial sections of muscle biopsy stained with trichrome (a), esterase (b), and acid phosphatase (c). A large interstitial and perivascular infiltrate is noted in (a). Among these inflammatory cells few are esterase positive (b) with homogeneous diffuse cytoplasmic patterns suggestive of monocytes. The number of macrophages in this huge infiltrate is very small as suggested by the presence of very few acid phosphatase-positive cells (c) (×240).

FIG. 6. Trichrome stain showing two muscle fibers with vacuole-like structures whose interior walls are lined with a basophilic granular material similar to the structures seen in inclusion-body myositis (×1035).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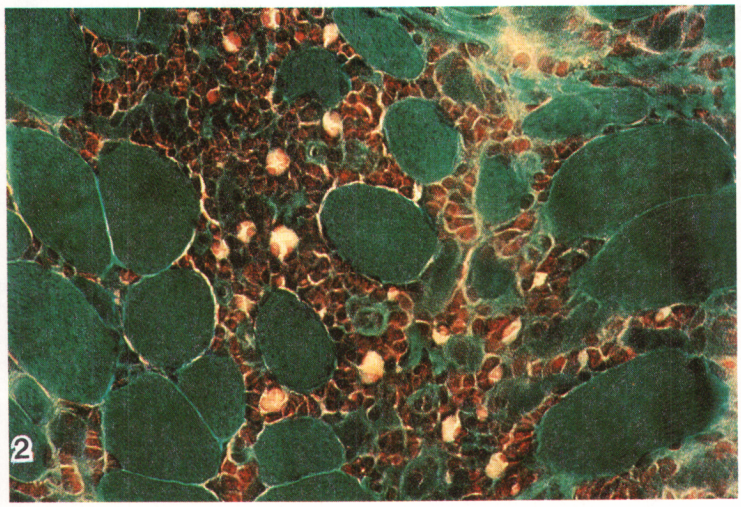
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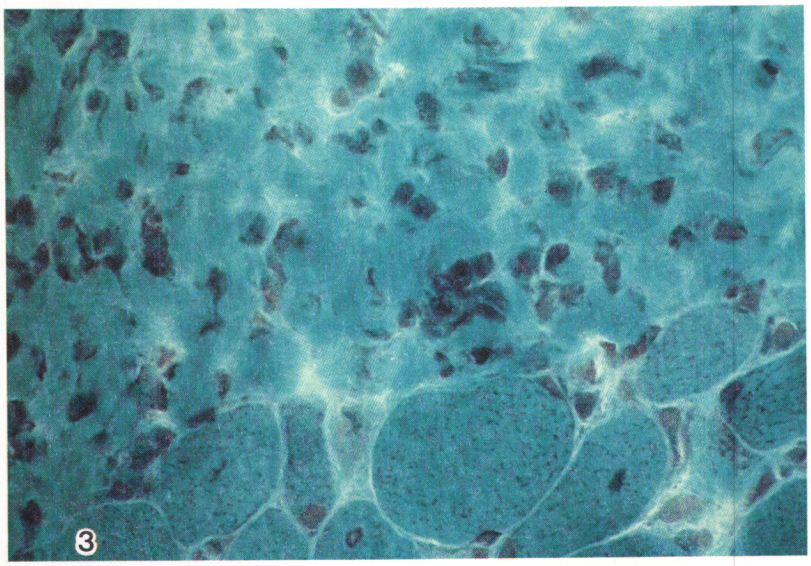
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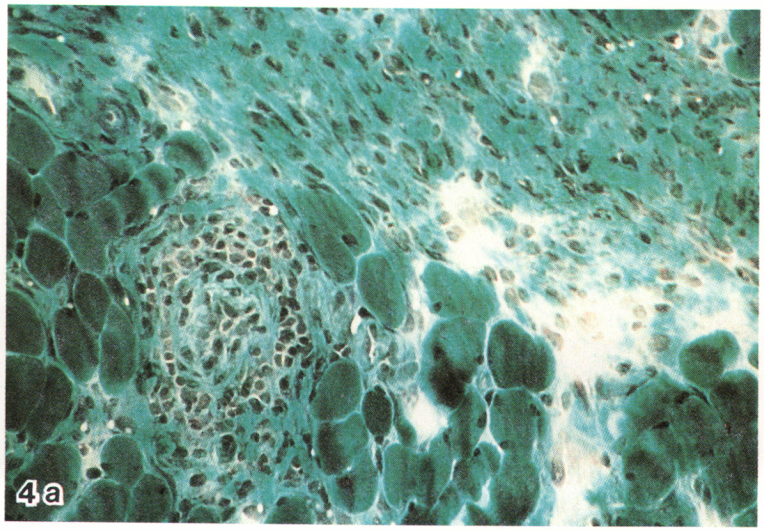

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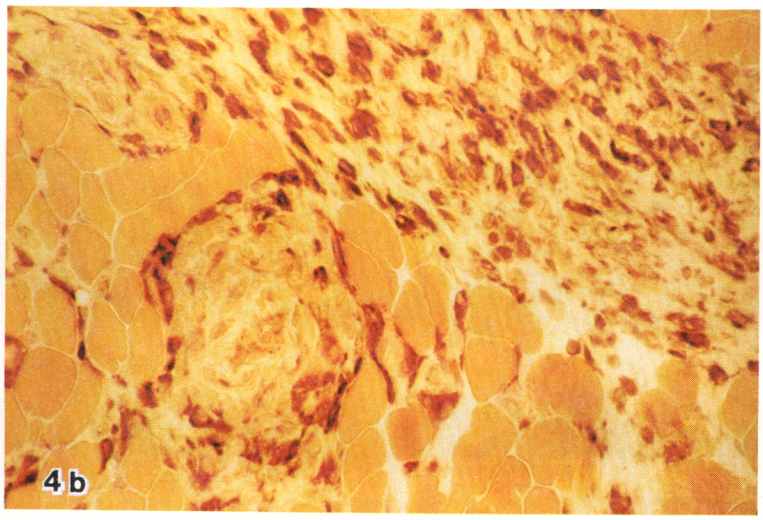

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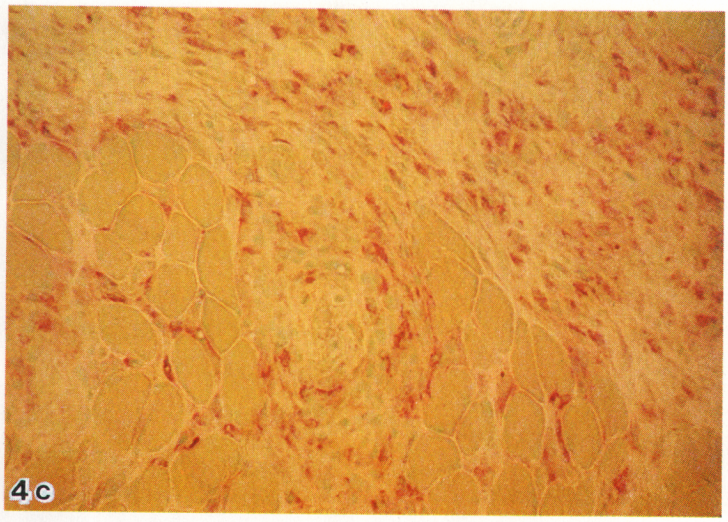

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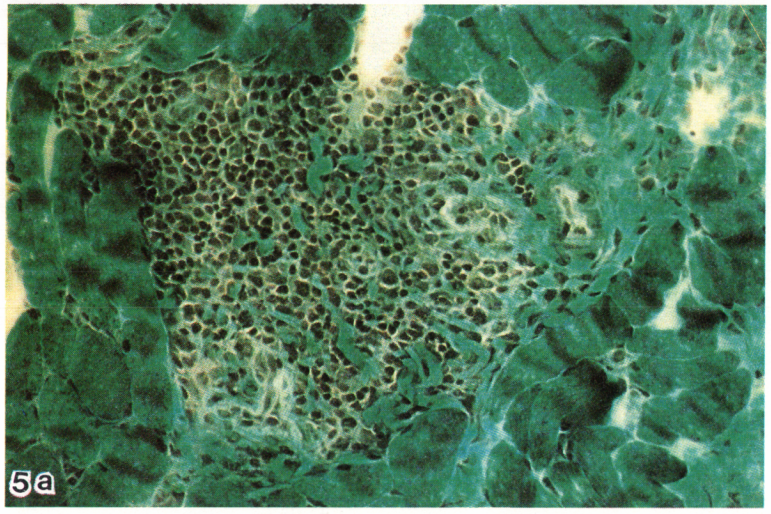

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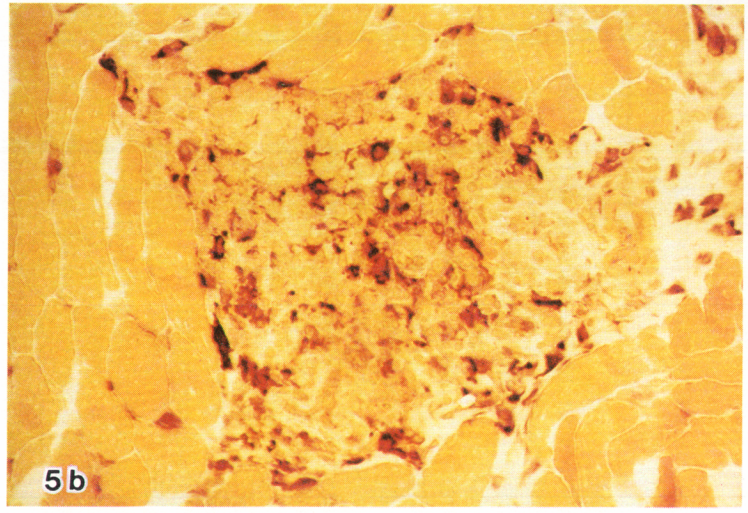

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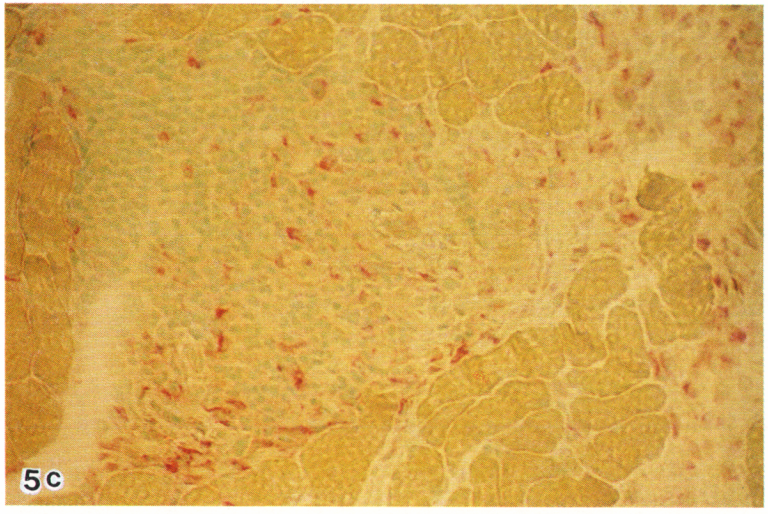

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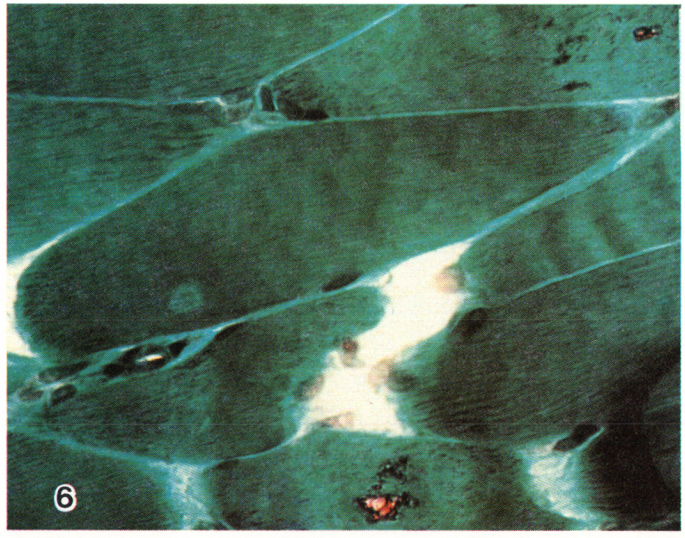

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pears that many of the perivascular infiltrates were composed of OKT4- and OKT8-positive cells but it was difficult to ascertain which was the predominant cell subset. The cell type or subtype number that predominated in the interstitial infiltrates or between muscle fibers is under investigation.

With the modified trichrome stain, occasional fibers contained one or two vacuole-like structures whose interior was lined with a basophilic granular material (Fig. 6), similar to those seen in patients with inclusion-body myositis (15, 24). With ATPase histochemical reaction, some animals had a selective and severe atrophy of type II muscle fibers which could partially account for the muscle weakness in those animals who did not have severe signs of inflammatory myopathy. The intramuscular nerve twigs appeared normal and no histochemical findings of neurogenic atrophy were noted. With alkaline phosphatase, the fibroblasts and perimysial connective tissue were positive, as seen in human polymyositis (25). No perifascicular atrophy or signs of vasculitis were noted. Similarly, no signs of endothelial necrosis were noted on frozen sections.

Discussion. We have found that 44% (11/25) of juvenile rhesus monkeys which died of SAIDS following experimental inoculation with various materials containing SRV-1 had evidence of polymyositis. Clinically, affected animals showed evidence of muscle weakness with or without induration or atrophy. The serum sarcoplasmic enzymes were elevated and muscle biopsy revealed severe inflammatory myopathy with necrosis and phagocytosis but few regenerating fibers. The inflammatory infiltrates contained lymphocytes, macrophages, and bizarre-shaped cells of uncertain identity, similar to the cells found in the inflammatory infiltrates in muscle biopsies of human polymyositis (15). A profound proliferation of fibrous tissue which often reacted strongly with alkaline phosphatase, as seen in severe cases of human polymyositis (15, 25), was also noted. These suggest that polymyositis in SAIDS has morphological features very similar to the human disease.

Vacuole-like structures whose interior was lined with a basophilic granular material was observed in few fibers. At the light-microscopy level, these structures were similar to those seen in inclusion-body myositis (15, 25), an inflammatory muscle disease of suspected viral etiology (15, 25, 26). SRV-1, a Mason–Pfizer-like type D retrovirus, has been firmly established as the cause of SAIDS (3, 9, 21, 27). By use of immunofluorescence and virus isolation techniques, we have previously showed that SRV-1 was associated with the involved muscle (13), suggesting that such a family of viruses (retroviruses and lentiviruses) may play a role in the etiology of human inflammatory myopathies including the inclusion body myositis. Future *in situ* hybridization and electromicroscopic studies are now planned to test this hypothesis.

Polymyositis associated with the retrovirus HTLV-III has been reported in patients with AIDS (28–30). In two such patients that we studied, we found the HTLV-III antigen on the $OKT4^+$ cells, immunohistochemically (30). This, along with our present study, indicates that retroviruses can be associated with polymyositis and may account for some cases of human disease.

The mechanisms of polymyositis in SAIDS is currently unknown. It may result (a) from invasion of muscle cells by the retrovirus, (b) by an inflammatory immune response to viral antigens present in the SRV-1-positive interstitial fibroblasts (13) that may serve as cross-reacting antigens, or (c) as a consequence of the immunodeficiency state. The subpopulation of cells within the infiltrates was different perivascularly and perimysially. Perivascularly, the predominant cell appeared to be a lymphocyte with very few monocytes and macrophages; in contrast perimysially, histiocytes, macrophages, or monocytes predominated (Figs. 4 and 5). Although macrophages can be present in nonspecific myopathies behaving as scavenger cells to clear debris, their presence in areas without an associated muscle fiber necrosis or phagocytosis may suggest that macrophages or activated T cells (both antigen-presenting cells) may play a primary role in initiating muscle damage by presenting antigen (virus?) to intact muscle cells.

The lymphocyte subset that predominated in the interstitial infiltrates could not be conclusively determined from our present study. Preliminary observations of the phenotypic

expression of these cells—some of which carry viral antigens (13)—suggest that in SAIDS muscle fiber involvement may be mediated via a functionally heterogeneous subpopulation of cells with different regional distribution. Further study is now in progress to accurately identify and quantitate the subsets of lymphocytes that predominate in the perivascular exudates or interstitially and to characterize with our SRV-1 antiserum (13) those cells that carry viral antigen and determine their proximity to muscle fibers.

SAIDS caused by a well-characterized retrovirus provides the first highly reproducible animal model of viral polymyositis in primates. Because the disease is similar to human polymyositis, this model may prove to be very useful in elucidating the role of viruses or immunodeficiency in inducing inflammatory muscle disease including the inclusion-body myositis. It gives us an opportunity to study the direct effect of SRV-1 retrovirus infection in muscle and examine the role of immune effector cells in facilitating muscle damage.

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