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Transmission of naturally occurring lymphoma in macaque monkeys

(acquired immune deficiency syndrome/opportunistic infection/tumor)

RONALD D. HUNT*, BEVERLY J. BLAKE*, LAURA V. CHALIFOUX*, PRABHAT K. SEHGAL*, NORVAL W. KING*, AND NORMAN L. LETVIN*†

*New England Regional Primate Research Center, 1 Pine Hill Drive, Southborough, Massachusetts 01772; and †Division of Tumor Immunology, Dana–Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115

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ABSTRACT **Spontaneously occurring rhesus monkey lymphomas were transmitted into healthy rhesus monkeys by using tumor cell suspensions. The naturally arising tumors included an immunoblastic sarcoma and an undifferentiated lymphoma. Recipient animals developed undifferentiated lymphomas, poorly differentiated lymphomas, or parenchymal lymphoproliferative abnormalities suggestive of early lesions of lymphoma. Some of these animals developed such opportunistic infections as cytomegalovirus hepatitis and cryptosporidiosis. They also showed evidence of an abnormal circulating peripheral blood mononuclear cell. These findings, all characteristic of the acquired immune deficiency syndrome (AIDS) of macaques, suggest a link between these transmissible lymphomas and AIDS in macaque monkeys.**

Lymphoma is the most common neoplastic disease of nonhuman primates (1). It has been described in a number of species from each of the major groups of nonhuman primates: prosimians, new world monkeys, old world monkeys, and great apes (2). Although a number of retroviruses and lymphotropic herpesviruses have been isolated from tissues of spontaneously arising lymphomas in macaque monkeys, most of these viruses have not been shown to be pathogenic (3). Thus, no etiologic agent has been associated with lymphoma in macaques nor has the disease been previously shown to be transmissible in healthy monkeys.

The lymphoproliferative abnormalities that we have recently observed associated with an acquired immune deficiency syndrome (AIDS) in macaques are therefore of considerable interest (4). This syndrome in macaques is remarkably similar to AIDS in man (5). Lymphocytes from macaques with this syndrome exhibit depressed *in vitro* responsiveness to lectins, indicating marked abnormalities in cellular immune function in these monkeys. Like humans with AIDS, these animals die of opportunistic infections and unusual tumors. Epidemiologic evidence suggests that this syndrome is due to an infectious agent. Lymphoproliferative disorders, ranging from multiple nodules of lymphocytes in the kidney, liver, and bone marrow to frank lymphoma, have been seen in macaques with AIDS. This relationship between lymphoproliferative changes and AIDS in macaques suggested that lymphomas, or at least a predisposition to developing lymphomas, may be transmissible in macaques.

In this report, we describe the transmission of rhesus (*Macaca mulatta*) lymphomas from spontaneously arising tumors to healthy rhesus monkeys. We, furthermore, discuss the possible relationship of these lymphoma transmissions to AIDS in macaques.

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TRANSMISSION 1

A wild-caught male *M. mulatta* (Mm 510-76) was housed in a primate colony of another institution for 6 years. During that time, he was not used for experimental purposes and required medical attention only for treatment of occasional traumatic injuries. No unusual incidence of lymphoproliferative abnormalities or immune deficiency diseases had been reported at that other facility. At the time of transfer to the New England Regional Primate Research Center (NERPRC), he was noted to have a mass in the jaw. Hematologic data at that time included hemoglobin 10.7 g/dl with hypochromic microcytic indices and a leukocyte count of $10.1 \times 10^3/mm^3$ with a differential count of 67% neutrophils, 15% lymphocytes, 4% atypical lymphocytes, 8% monocytes, 4% eosinophils, and 2% basophils. Blood chemistry values included serum glutamic-oxaloacetic transaminase, 70 units/liter; serum glutamic-pyruvic transaminase, 47 units/liter; alkaline phosphatase, 45 units/liter; lactate dehydrogenase, 976 units/liter; total protein, 8.0 g/dl with albumin of 3.0 g/dl and globulin of 5.0 g/dl. The macaque was sacrificed. At necropsy, a $2 \times 3 \times 4$ cm left mandibular mass was found encompassing the molars and extending into the adjacent muscle. Associated mandibular nodes were enlarged. Smaller masses, similar in appearance to the mass in the jaw, were noted adherent to the left temporal region of the calvarium and in the left temporal lobe of the brain. Meningeal adhesions covered the entire surface of the brain. The animal's spleen was not enlarged.

The neoplasm, classified as an immunoblastic sarcoma (lymphoma, non-Hodgkin, diffuse histiocytic type), was composed of diffuse sheets of large pleomorphic cells (Fig. 1). The nuclei were usually round, 15 to 20 μm in diameter, and leptochromatic with single prominent eosinophilic nucleoli. Nodules that were noted on the mitral valve had the histologic appearance of bacterial vegetative valvular endocarditis.

Tumor tissue from this animal was homogenized and injected subcutaneously in the neck and thorax of two clinically normal *M. mulatta*. One recipient, a 2-day-old colony-born male (Mm 524-76), was noted to be weak and dehydrated 14 months after inoculation. One week later, he developed right-sided focal motor seizure activity. He was noted at that time to have a holosystolic thrill over his entire precordium. Hematologic data included hemoglobin, 5.3 g/dl with microcytic hypochromic indices, and a leukocyte count of $11.1 \times 10^3/mm^3$ with a dif-

Abbreviation: AIDS, acquired immune deficiency syndrome.

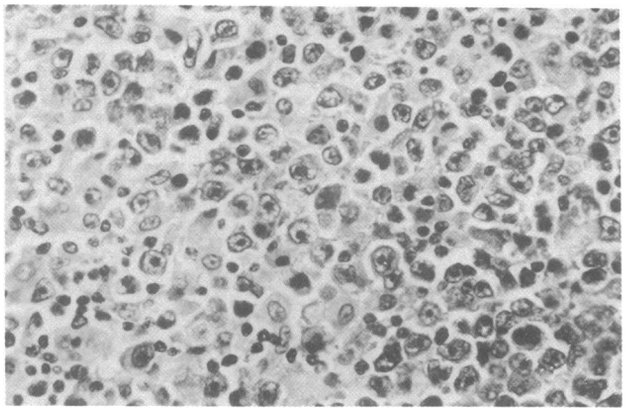

FIG. 1. Spontaneous immunoblastic sarcoma, mandible, Mm 510-76. The lymphoma that affected the mandible and cranium is composed of large cells with prominent nucleoli and abundant cytoplasm. (×1,000.)

ferential count of 71% neutrophils, 6% band forms, 21% lymphocytes, 2% monocytes, and 9 nucleated erythrocytes per 100 leukocytes. A lumbar puncture carried out on the day of death demonstrated crenated erythrocytes.

At autopsy, friable vegetations were noted on the mitral valve and the lungs were edematous with areas of consolidation. Multiple thrombo-embolic infarcts were seen in the brain. A purulent meningitis was also found. Mesenteric nodes were enlarged, but no evidence of lymphoma was seen, even at the sites of tumor inoculation. A small amount of amyloid was present in the Malpighian corpuscles of the spleen. Peripheral and intralobular collections of mononuclear cells, predominantly mature lymphocytes, admixed with a few plasma cells were seen in the liver. Small collections of lymphocytes were also seen in the interstitium of the kidney and beneath the endothelium of the renal arteries. Chronic colitis was also present.

The second *M. mulatta* (442-68) that received tumor tissue was a wild-caught female. A review of her clinical history revealed nothing that might have predisposed her to developing a lymphoma. Fourteen months after receiving this tumor tissue, an abdominal mass was palpated. Hematologic data at that time included hemoglobin at 11.9 g/dl with microcytic hypochromic indices and a leukocyte count of $8.8 \times 10^3/mm^3$ with a differential count of 45% neutrophils, 26% lymphocytes, 4% atypical lymphocytes, 12% monocytes, 11% eosinophils, and 2% basophils. Occasional large bizarre mononuclear cells with vacuolated cytoplasm and prominent nucleoli were seen. A $7 \times 4 \times 5$ cm mass at the ileocecal junction was surgically removed.

The intestinal mass, classified as an undifferentiated lymphoma, was composed of diffuse sheets of pleomorphic cells replacing almost all normal structures of the intestinal mucosa, submucosa, and muscularis (Fig. 2). The cells were large and slightly variable in size, with leptochromatic round nuclei and prominent eosinophilic nucleoli (Fig. 3). Each was surrounded by a rim of eosinophilic cytoplasm. There were frequent mitoses.

The animal remained clinically well after this procedure, although a routine hematologic study done 15 months later indicated that she maintained a persistent monocytosis and eosinophilia. This animal died 22 months after the tumor resection with endometriosis, peritonitis, and meningitis. No recurrence of the malignant tumor was seen nor was there evidence of lymphoma at the site of inoculation. Irregularly shaped collections of large and small lymphocytes were present in the bone marrow, renal interstitium, subendothelium of renal arteries, salivary glands, and muscularis and submucosa of the ileum and colon.

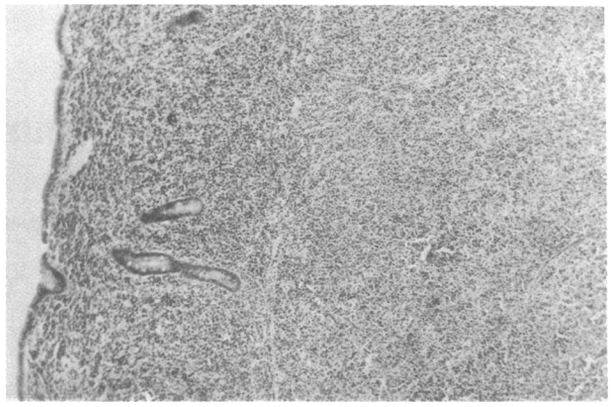

FIG. 2. Transmitted undifferentiated lymphoma, intestine, Mm 442-68. The mucosa and submucosa are significantly expanded and replaced with diffuse sheets of pleomorphic cells. (×60.)

TRANSMISSION 2

A retro-orbital lymphoma was found in a wild-caught female *M. mulatta* (78-72) that had been housed at the NERPRC for 8 years. At the time of diagnosis, hematologic studies showed hemoglobin at 10.7 g/dl and a leukocyte count of $7.8 \times 10^3/mm^3$ with a differential of 53% neutrophils, 2% band forms, 19% lymphocytes, 2% atypical lymphocytes, 2% prolymphocytes, 9% monocytes, 12% eosinophils, and 1% blast forms. Circulating bizarre mononuclear cells with vacuolated cytoplasm and prominent nucleoli were also seen in this animal. Enucleation of the affected eye was performed. A homogenate of the retro-orbital tumor and blood from this animal were injected into two *M. mulatta*. The donor animal was sacrificed 2 months later.

At necropsy, a 2-cm round mass was present in the myocardium and adjacent tricuspid valve. A 5-cm mass was also found adjacent to the posterior pole of the left kidney extending into the pelvic cavity. All lymph nodes were enlarged. Microscopically, the retro-orbital, myocardial, and pelvic tumors were composed of diffuse sheets of a homogeneous population of cells that had round to slightly oval nuclei, prominent nucleoli, and a moderate amount of pink cytoplasm (Fig. 4). Mitotic figures were relatively frequent. The cells had invaded the myocardium and the ocular skeletal muscles and had infiltrated periorbital tissue, the ciliary body, and the iris. Lymph nodes were variably replaced by sheets of similar cells. The tumor was classified as an undifferentiated lymphoma.

There was extensive deposition of amyloid in the interstitium of the cortex of the kidney. *Cryptosporidia* were iden-

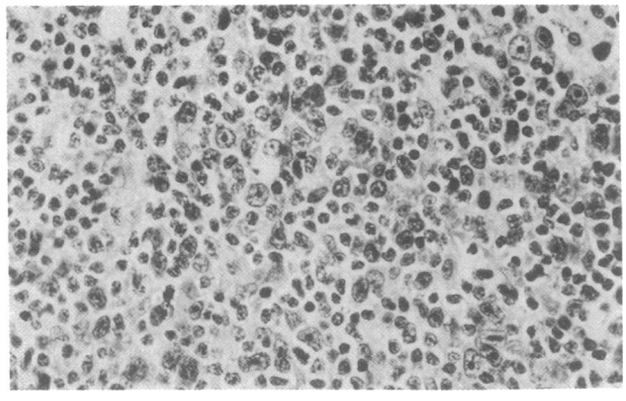

FIG. 3. Higher magnification of Fig. 2 showing pleomorphic cells with prominent nucleoli. More mature lymphocytes are intermixed with the undifferentiated cells. (×1,000.)

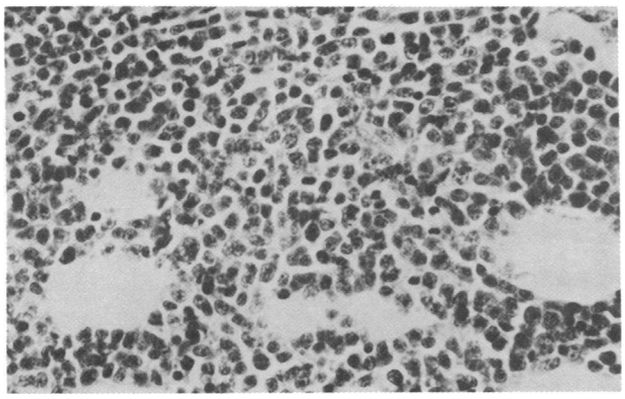

FIG. 4. Spontaneous undifferentiated lymphoma, Mm 78-72, invading the myocardium. Diffuse sheets of a homogeneous population of cells can be seen. (×1,000.)

tified attached to biliary epithelial cells of several of the larger hepatic bile ducts (Fig. 5), which were surrounded by slight fibrosis and lymphocytic infiltration. An extensive enteritis involving the jejunum and ileum was characterized by focal necrosis and dysplasia of the surface epithelium. Numerous eosinophilic intranuclear inclusion bodies were present.

The first recipient of tumor tissue from this animal was an 8-month-old colony-born male (Mm 434-79) with no significant previous medical history. This animal was maintained in isolation from the time of inoculation until its death; it was never exposed to the adult macaque colony of the NERPRC. Five months after inoculation, the animal was noted to have an enlarged spleen. Hematologic data showed hemoglobin at 6.0 g/dl and a leukocyte count of $2.9 \times 10^3/mm^3$ with a differential of 1% neutrophils, 85% lymphocytes, 6% prolymphocytes, 4% monocytes, and 4% blast forms. Again, circulating bizarre mononuclear cells were seen. The animal was sacrificed.

At necropsy, there was an irregularly shaped mass in the anterior mediastinum. Microscopically, the mass was composed of solid sheets of a relatively homogeneous population of cells (Fig. 6) with round nuclei, distinct basophilic nucleoli, and a small amount of eosinophilic cytoplasm. Occasional histiocytes were intermixed with these tumor cells. Most lymph nodes were enlarged and variably replaced by similar sheets of cells that sometimes extended through the capsule. The bone marrow was diffusely replaced by such cells. Masses of these cells were also present in the ciliary body, retina, and choroid of one eye and under the entire length of the capsule of the spleen. Many Malpighian corpuscles in the spleen had irregular zones of necrosis. Small foci of hepatocellular necrosis containing neutrophils and occasional cells bearing large intranuclear inclusion bodies typical of those produced by cytomegalovirus were seen (Fig. 7). Similar inclusion-bearing cells were found in endothelial and smooth muscle cells of the intrahepatic arteries. An extensive viral enteritis identical to that seen in the donor animal was also present. In addition, cryptosporidia were seen in the small intestine. Morphologically, the tumor did not clearly fit standard classifications of human lymphomas but most closely resembled undifferentiated lymphoma.

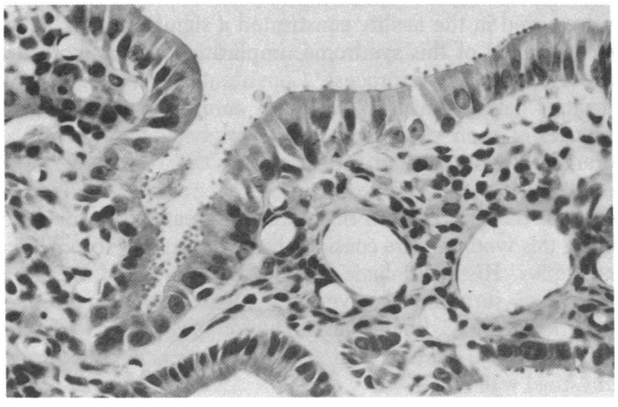

FIG. 5. Cryptosporidiosis, bile duct, Mm 78-72. (×1,000.)

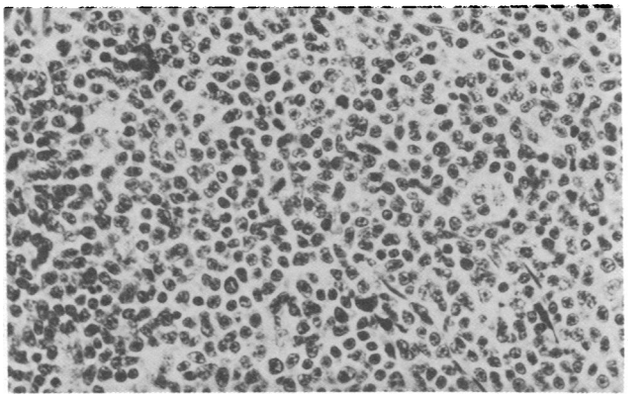

FIG. 6. Transmitted lymphoma, Mm 434-79. The tumor most closely resembled an undifferentiated lymphoma. (×1,000.)

The second recipient was a colony-born 10-month-old male *M. mulatta* (251-79) with no significant previous medical history. Twenty-six months following inoculation the macaque was noted to have hepatosplenomegaly and generalized lymphadenopathy. His hemoglobin was 11.4 g/dl and leukocyte count was $6.0 \times 10^3/mm^3$ with a differential of 46% neutrophils, 40% lymphocytes, 1% atypical lymphocytes, 1% prolymphocytes, 11% monocytes, and 1% eosinophils. Circulating bizarre mononuclear cells were also seen in this animal's peripheral blood. Blood chemistry values included serum glutamic-oxaloacetic transaminase, 77 units/liter; serum glutamic-pyruvic transaminase, 238 units/liter; alkaline phosphatase, >999 units/liter; lactate dehydrogenase, 398 units/liter; and a total protein of 8.5 g/dl with an albumin of 2.9 g/dl and globulin of 5.6 g/dl. The macaque was sacrificed.

At necropsy, there was splenomegaly, hepatomegaly, and enlargement of almost all lymph nodes. Microscopically, there were variably sized nodules of lymphocytes with leptochromatic nuclei and prominent nucleoli in the kidney and bone

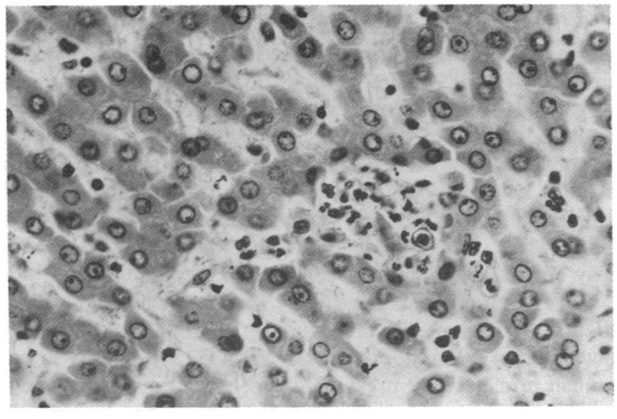

FIG. 7. Liver, Mm 434-79, containing a small focus of necrosis and an inclusion bearing megalocyte typical of cytomegalovirus infection. (×1,000.)

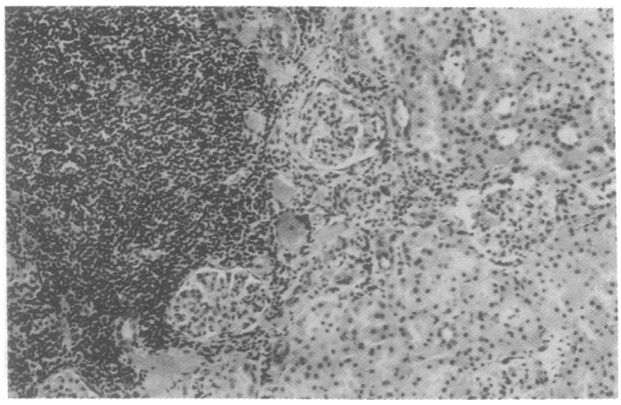

FIG. 8. Transmitted poorly differentiated lymphocytic lymphoma, kidney, Mm 251-79. A neoplastic nodule is seen compressing and invading renal tubules. (×250.)

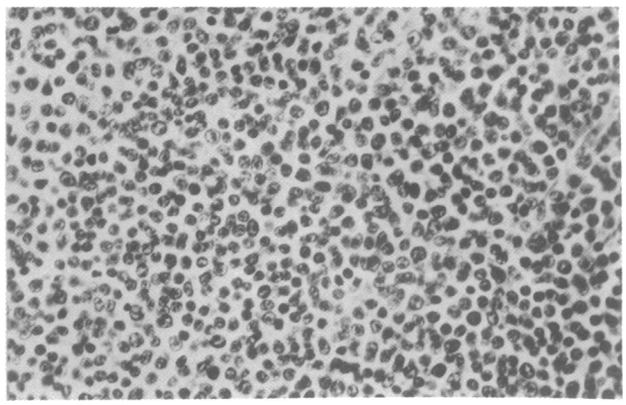

FIG. 9. Lymph node, Mm 251-79, replaced by a nearly uniform population of cells. (×1,000.)

marrow (Fig. 8). The nodules in the kidney, some of which mimicked normal follicles, compressed adjacent tissues. Lymph nodes varied in appearance; some were hyperplastic while others were replaced by diffuse sheets of immature-appearing lymphocytes that in some areas extended through the capsule of the node (Fig. 9). The splenic Malpighian corpuscles contained amyloid and, while enlarged, were not clearly lymphomatous. The histologic features of this tumor suggested that it was either a poorly differentiated lymphocytic or mixed lymphocytic/histiocytic lymphoma. Karyotypic analysis of phytohemagglutinin-stimulated lymphocytes from affected lymph nodes of the animal showed both a normal appearance and number of chromosomes and that these cells were of male origin. Since the tumor inoculum was derived from a female rhesus, this indicates that the lymphoma was transmitted rather than transplanted.

DISCUSSION

Lymphomas in nonhuman primates have attracted considerable attention from investigators as models for these neoplasms in man. Most of this interest has focused on the role of various herpesviruses in the induction of lymphoproliferative syndromes in new world primates (6). In spite of the fact that lymphomas are the most common tumors in such old world monkeys as macaques (1), little has been done to characterize them in these species. The physiologic similarities between man and old world primates suggest that the study of these neoplasms in macaques should lead to a greater understanding of lymphomas in man.

A review of spontaneously occurring lymphomas in macaques at the NERPRC over the past 12 years indicates that the histologic characteristics of these tumors resemble those seen in man (unpublished observations). They include well-differentiated, poorly differentiated, and histiocytic histologies. While lymphomas have occurred only sporadically at the NERPRC, a single epidemic outbreak of lymphomas was reported at the University of California at Davis National Center for Primate Biology (7). Such an epidemic suggests that an infectious agent may be etiologic in at least some macaque lymphomas.

Several viral agents have been shown to cause lymphoproliferative syndromes in some nonhuman primates. Yet, the only documented example of a virally induced lymphoma in nonhuman primates that presumably occurs in a natural setting is gibbon lymphoma. A retrovirus, the gibbon ape leukemia virus, has been shown to cause these lymphomas in gibbons under experimental and naturally occurring conditions (8). Certain herpesviruses can be used to experimentally induce lymphomas in new world primates. After inoculation with Epstein–Barr virus, cotton-topped marmosets develop diseases ranging from lymphoid hyperplasia to fatal lymphomas (9). Marmosets, owl monkeys, spider monkeys, and howler monkeys develop fatal lymphoproliferative syndromes after inoculation with herpesvirus saimiri (10). These are not thought to occur in a natural setting.

No specific virus has yet been implicated as an etiologic agent in lymphomas in macaques. Only one successful transmission of a macaque lymphoma has been reported in the past (7). In that case, rhesus tumor cells were inoculated into a rhesus neonate that was immunosuppressed with azothioprine. Our evidence from karyotype analysis of tumor cells of recipient animals that the sex of the tumor cells were that of the recipient and not the donor macaque argues that this is a transmitted, not a transplanted, lymphoma.

It is noteworthy that animal Mm 524-76, the only recipient discussed in this report that did not develop frank lymphoma, did evidence lymphoproliferative changes in the liver and kidney. These changes may represent early lesions of lymphoma in this monkey. It may be possible to use immunoperoxidase staining techniques to determine the phenotype of the cells seen in early lesions of this type as well as in frank lymphomas.

A number of aspects of the clinical and pathologic findings in these cases of transmissible lymphoma seem to parallel findings in cases of a naturally occurring AIDS in macaques that we have recently described (5). Monkeys with AIDS have died with unusual opportunistic infections and tumors. Hematologic data indicate that these animals are anemic and neutropenic. Furthermore, peripheral blood smears on the macaques with AIDS reveal an immature circulating mononuclear cell. Epidemiologic studies indicated that the location in which the animals were caged in the facility constituted a significant risk for the development of this syndrome, implicating a common source agent in the disease process. Consistent immunologic abnormalities have been noted in these animals. Proliferative responses of their peripheral blood lymphocytes to lectins are dramatically diminished. Furthermore, the T4 (helper/inducer):T8(suppressor/cytotoxic) ratios of circulating T lymphocytes of the macaques in the colony at greatest risk for developing this syndrome are considerably less than that seen in other macaques. Histopathologic findings on autopsy of these animals have, surprisingly, revealed evidence, not only of opportunistic infections, but of unusual lymphoproliferative changes, retroperitoneal fibrosis, and amyloid deposits confined to the intestinal wall (4).

The immune status of the macaques into which lymphomas were transmitted was not studied. It is therefore impossible on

the basis of this data to unequivocally link macaque AIDS with these cases of lymphoma transmission in monkeys. Yet striking similarities do exist between these processes. The frequent occurrence of lymphoproliferative syndromes in macaques with AIDS is itself worthy of note in light of the cases of experimentally transmitted lymphomas. In a carefully analyzed cohort of 16 macaques with AIDS, 4 evidenced lymphoproliferative abnormalities at necropsy ranging from unusual lymphoid hyperplasia to frank lymphoma (4).

A similar array of opportunistic infections has also been seen in these two groups of monkeys. Macaques that have died with AIDS at the NERPRC have shown evidence of opportunistic infections including cytomegalovirus mononucleosis, Noma (necrotizing gingivitis), hematogenous *Hexamita*, *Pneumocystis carinii* pneumonia, and *Mycobacterium avium-intracellulare* adenitis (11). The animals with lymphomas described here also had CMV hepatitis, viral enteritis, and a *Cryptosporidia* infection. Furthermore, the pathologic changes of colonic submucosal fibroplasia seen in Mm 524-76 at necropsy were reminiscent of the retroperitoneal fibrosis observed in 3 of the cohort of 16 carefully studied macaques with AIDS (unpublished data).

The remarkably similar hematologic profile in these two groups of macaques, those with a transmissible lymphoma and those with AIDS, provides what is perhaps the strongest link between these apparently separable syndromes. The macaques with AIDS were anemic with a marked neutropenia and monocytosis (5). They, furthermore, all had a circulating bizarre immature mononuclear cell. Strikingly, four of the six animals described here showed a bizarre circulating mononuclear cell on peripheral smears during their illness that was morphologically identical to that seen in the macaques with AIDS.

These cases, therefore, are important for two reasons. They represent a transmission of lymphomas into normal macaques. This suggests an infectious etiology to a grouping of lymphomas with histopathologic findings remarkably similar to human lymphomas. Moreover, these cases suggest a link between this transmissible lymphoma and AIDS in macaque monkeys.

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