Experimental Leprosy in a Rhesus Monkey: Necropsy Findings[1]

Gary B. Baskin, Bobby J. Gormus, Louis N. Martin,
Robert H. Wolf, Michael Murphey-Corb, Gerald P. Walsh,
Chapman H. Binford, Wayne M. Meyers, and Raga Malaty[2]

Among nonhuman primates, spontaneously occurring leprosy has been observed only in the chimpanzee ([4, 8]) and the sooty mangabey ([10]). For the past 100 years there have been many experimental attempts to infect nonhuman primates with *Mycobacterium leprae*. Successful induction of progressive disease has been described in a chimpanzee ([6]), a gibbon ([13]), sooty mangabeys ([14]), African green monkeys ([14]), and rhesus monkeys ([14]). Detailed postmortem findings have been reported in a naturally infected chimpanzee ([7]), an experimentally infected gibbon ([13]), and an experimentally infected sooty mangabey ([3]).

We have described the early clinical and pathological findings in a rhesus monkey experimentally infected with *M. leprae* derived from a naturally infected sooty mangabey ([9, 14]). Briefly, the monkey developed erythematous plaques and nodules at inoculation sites on the nose and at uninoculated sites on the face, extremities, and scrotum by 14 months post-inoculation. Histologically, the lesions resembled human BL-LL leprosy. The disease regressed at about 2 years post-inoculation, but shortly thereafter exacerbated and again progressed. The lesions histologically resembled human LL leprosy at that time. The disease continued to progress slowly until the monkey was killed for pathological evaluation about 5 years post-inoculation.

This report describes the necropsy findings in this animal.

MATERIALS AND METHODS

A male rhesus monkey (*Macaca mulatta*) (#A125) born at the Delta Regional Primate Research Center (DRPRC), Covington, Louisiana, U.S.A., and raised in the nursery was utilized. The animal was healthy and tuberculin negative. He was individually caged in the isolation facility at the DRPRC, fed a standard laboratory diet, and given water *ad libitum*. The monkey was sedated with ketamine HCl (10 mg/kg) for all procedures requiring handling. An inoculum of *M. leprae* was prepared from cutaneous lepromas removed by biopsy from a naturally infected sooty mangabey. Earlier studies of the donor using microbiological, immunological, histopathological, and biochemical techniques (including DNA homology) had identified the infecting bacillus as *M. leprae* ([10]). The tissue was minced, homogenized in saline, filtered through sterile gauze, and centrifuged at low speed to remove large pieces of tissue. When the monkey was 6 months old, he was inoculated with approximately 1.5×10^8 acid-fast bacilli (AFB) intravenously and 1.5×10^8 AFB at each of four intracutaneous sites (left ear, right ear, lip, brow).

After lesions developed, bacilli were collected from biopsies and inoculated into mouse foot pads and armadillos. The bacilli were also tested for pyridine extractibility of acid-fastness.

The animal was lepromin tested three times with a variety of lepromins, as previously described ([1]), 18, 26, and 42 months

[2] G. B. Baskin, D.V.M., Head, Department of Pathology; B. J. Gormus, Ph.D., Research Scientist; L. N. Martin, Ph.D., Research Scientist; and M. Murphey-Corb, Ph.D., Associate Scientist, Department of Microbiology; R. H. Wolf, Head, Department of Veterinary Science, Delta Regional Primate Research Center, Covington, Louisiana 70433, U.S.A. G. P. Walsh, Ph.D., Chief, Experimental Mycobacteriology; C. H. Binford, M.D., Consultant to the Leprosy Registry; W. M. Meyers, M.D., Chief, Division of Microbiology, Armed Forces Institute of Pathology, Washington, D.C. 20306-6000, U.S.A. R. Malaty, M.D., Department of Ophthalmology, Louisiana State University School of Medicine Eye Center, New Orleans, Louisiana 70112, U.S.A.

after inoculation. He was consistently lepromin negative.

Because of the inadvertent transmission of an acquired immune deficiency syndrome, caused by the retrovirus STLV-III/Delta, to other rhesus monkeys in similar leprosy transmission experiments ([2, 11]), we evaluated this monkey serologically and virologically for evidence of retroviral infection. Sera collected from rhesus #A125 1 month post-inoculation and at necropsy were evaluated. An assessment of STLV-III/Delta antibody was done by Western blot analysis of simian serum using detergent disrupted STLV-III/Delta virions purified from infected cell supernatants as described previously ([11]). Virological assessment was done by culturing lymphoid tissues obtained by biopsy and cultured directly or obtained at necropsy and preserved at −70°C for several months prior to culture. Tissues were Dounce homogenized in culture medium and cultured with 1×10^6 cells/ml of PHA-stimulated human lymphoblasts in RPMI-1640 medium supplemented with 15% fetal calf serum, antibiotics, and human interleukin-2 (Electronucleonics, Silver Spring, Maryland, U.S.A.) at concentrations recommended by the manufacturer. Cultures were maintained for 1 month by subculturing twice weekly. During this time, STLV-III/Delta expression was evaluated at three-day intervals by indirect immunofluorescence of acetone-fixed cells using simian reference serum.

Serum from the mangabey donor of the *M. leprae* inoculum and from rhesus monkey #A125 were tested for antibodies reactive with HTLV-I or HTLV-III using an enzyme-linked immunosorbent assay (ELISA) (Bioenzabead; Litton Bionetics, Charleston, South Carolina, U.S.A.) following the manufacturer's instructions. Sera were tested for antibodies to STLV-III by Western blot assay ([11]).

The animal was killed 56 months after inoculation and a complete necropsy examination was performed immediately after death. Representative samples of all tissues were fixed in 10% neutral buffered Formalin, processed, and embedded in paraffin for routine light microscopy. Sections were cut at 5 μm and stained with hema-

toxylin and eosin (H&E) and Fite-Faraco acid-fast stains. AFB counts were done in tissue samples by the method of Shepard ([12]).

RESULTS

Gross findings

Significant gross lesions were seen in the skin, peripheral nerves, nasal mucosa, and peripheral lymph nodes. The skin over the brow was thickened and had a few small ulcers. The upper lip was diffusely thickened, and the ears slightly thickened and nodular along the margins. The skin of the dorsal surfaces of both hands was dry and scaly. There were patchy infiltrated areas on both forearms, and the skin over the left elbow was thickened and nodular. The skin of the feet was slightly thickened, dry, and scaly. There were patchy infiltrated areas on the distal part of both legs. The toenails were elongated and distorted. The scrotum was diffusely reddened and thickened (Fig. 1), while the skin of the tail was diffusely thickened, dry, and scaly. The skin on other areas of the body was normal.

The major nerve trunks of the right arm were unremarkable. The left median, ulnar, and radial nerves appeared normal until they reached the wrist. As they entered the hand, they were enlarged to several times their normal diameters and were yellow. Both superficial peroneal nerves were enlarged and yellow. The tibial nerves were normal until they entered the feet, where the plantar branches were enlarged and yellow (Fig. 2). The remaining major peripheral nerve trunks were grossly unremarkable.

The nasal mucosa was swollen and partially obstructed the nasal passages.

The inguinal, axillary, and pelvic lymph nodes were moderately enlarged and were dark gray. Other lymph nodes appeared normal.

The remaining viscera, including the eyes and testicles, were grossly unremarkable.

Microscopic findings

Skin. The grossly affected areas were sometimes focally ulcerated. There was often slight hyperkeratosis and diffuse acanthosis. Within the dermis and subcutis was a dense inflammatory infiltrate consisting of histio-

FIG. 1. Thickened nodular skin of scrotum of an experimentally infected rhesus monkey. Several biopsy scars are apparent (AFIP negative no. 82-10302).

FIG. 2. Enlarged, discolored plantar nerve (◀) in left foot of experimentally infected rhesus monkey.

cytes intermixed with a few lymphocytes and rare neutrophils (Fig. 3). There was usually a narrow but distinct subepidermal clear zone. In less-severely affected areas, the infiltrate was concentrated around neurovascular bundles and dermal adnexae, and often had a lobulated appearance. The histiocytes had a moderate amount of pink foamy or slightly vacuolated cytoplasm. Many contained large globi. Nuclei were large, irregular, and vesicular. Occasional multinucleate cells containing giant globi were present. There were a few small focal accumulations of lymphocytes in some areas. Many small dermal and subcutaneous nerves contained histiocytes and other mononuclear inflammatory cells. The walls of some small arteries were infiltrated. The Fite-Faraco stain revealed many well-stained AFB, singly and in clumps, within the infiltrate (Fig. 4). The nerves, walls of arteries, and arrector pili muscles contained AFB. Skin from the proximal extremities and the abdomen was histologically normal.

Nervous system. The central nervous system was normal except for rare AFB in the meninges. The following peripheral nerve trunks were examined microscopically: left and right radial, right median, left and right palmar, left and right tibial, left and right superficial peroneal, right plantar, and right saphenous. Only the right saphenous nerve was normal. The other nerves had various degrees of inflammatory cell infiltration. Less affected areas had only a few histiocytes and lymphocytes scattered between nerve fibers. The most severely affected nerves were completely replaced by a lymphohistiocytic infiltrate. There were sometimes focal accumulations of lymphocytes and numerous multinucleated cells with giant globi (Fig. 5). In the most severely affected nerves there was a small amount of fibrosis. The perineural connective tissue was focally infiltrated with inflammatory cells. There were numerous AFB, singly and in clumps, in histiocytes and Schwann cells in the nerves and in the perineurium.

Respiratory tract. The nasal epithelium was focally ulcerated, and the mucosa was heavily infiltrated with histiocytes, lymphocytes, and neutrophils (Fig. 6). In most areas the submucosal glands were destroyed.
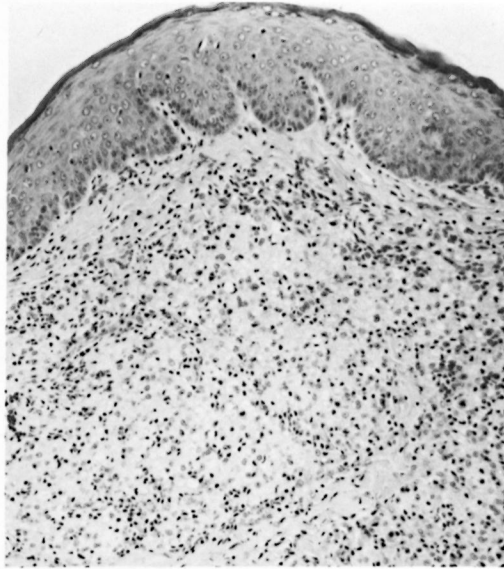
FIG. 3. Scrotum from experimentally infected rhesus monkey. Diffuse dermal infiltrate of histiocytes and lymphocytes (H&E ×100).
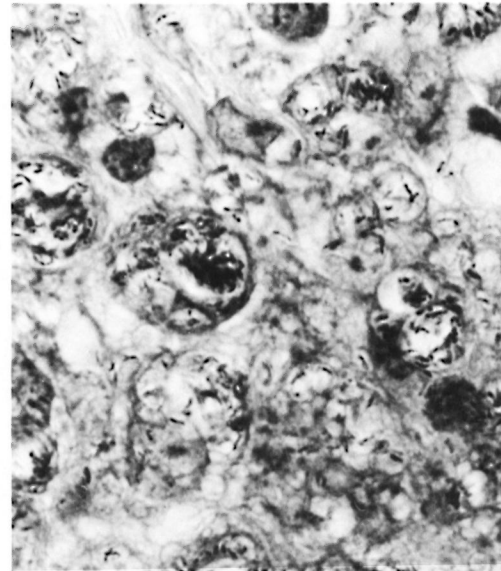

FIG. 4. Acid-fast bacilli within cutaneous infiltrate of experimentally infected rhesus monkey (Fite-Faraco ×630).

There were numerous intracellular AFB in the infiltrate and small nerves. The soft palate, larynx, trachea, and lungs were normal and contained no AFB.

Lympho-reticular system. The spleen had a few small focal histiocytic granulomas with a few AFB. The spleen contained 1.1×10^7 AFB/g with a morphological index (MI) of 0%.

The axillary, submandibular, inguinal, and pelvic lymph nodes were enlarged and had hyperplastic lymphoid follicles with large, well-defined germinal centers. Sinusoids were heavily infiltrated with histiocytes, and there were numerous multinucleated cells with giant globi (Fig. 7). Numerous AFB were in the histiocytes. Bronchial and mesenteric lymph nodes had hyperplastic follicles, but the sinusoids contained no histiocytes and AFB were not observed.

An inguinal lymph node contained 5.15×10^8 AFB/g with a MI of 0%, a submandibular lymph node contained 1.6×10^8 AFB/g with a MI of 1%, and a mesenteric lymph node contained no AFB.

All elements of the bone marrow were slightly hyperplastic with rare beaded or fragmented AFB.

Liver. There was slight fatty change and vacuolation of hepatocytes. There were numerous small focal accumulations of vacuolated histiocytes in the sinusoids. Histiocytes and Kupffer cells contained a few AFB. The AFB count of the liver was 2.6×10^8 AFB/g with a MI of 0%.

Reproductive tract. There were no lesions in the penis, testicles, prostate, or seminal vesicles, and no AFB were seen.

Eye. A few mononuclear cells were at the limbus and in the trabecular meshwork, but no AFB were found.

Other tissues. There were no changes related to leprosy in any other organs or tissues.

Bacteriological findings

Laboratory studies including pryidine extraction and mouse foot pad and armadillo inoculation confirmed that the etiologic agent of the disease in rhesus #A125 was *M. leprae.*

Virological findings

No antibodies reactive with HTLV-1, HTLV-III, or STLV-III were detectable in serum from rhesus #A125 by the ELISA or Western blot assays. The donor mangabey sera lacked antibody reactive with STLV-III and HTLV-III, but had strong reactivity with HTLV-I in the ELISA test.
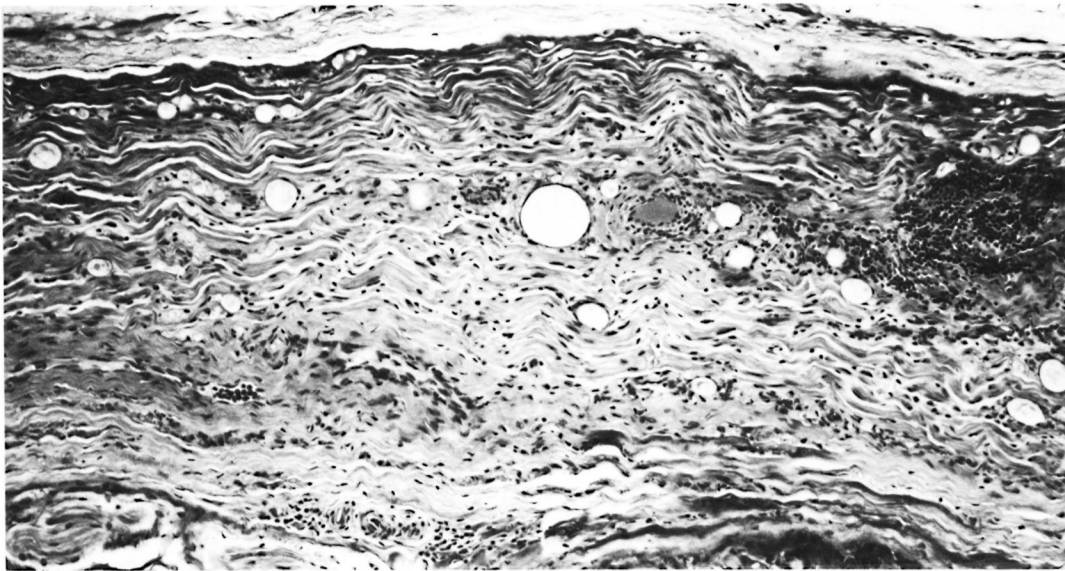
FIG. 5.   Right peroneal nerve from an experimentally infected rhesus monkey. Note inflammatory infiltrate, large globi, and focal lymphocytic infiltrate (H&E ×100).

In vitro cultures of lymph node homogenates obtained by biopsy early in the course of the disease and of those obtained at necropsy failed to result in the isolation of STLV-III/Delta.

DISCUSSION

The lesions of experimental *M. leprae* infection in the rhesus monkey described here are similar to those seen in generalized lepromatous leprosy in humans. The cooler parts of the body (distal extremities, face, upper respiratory tract, superficial segments of peripheral nerves) were preferentially involved, while internal organs were relatively spared due to the temperature selectivity
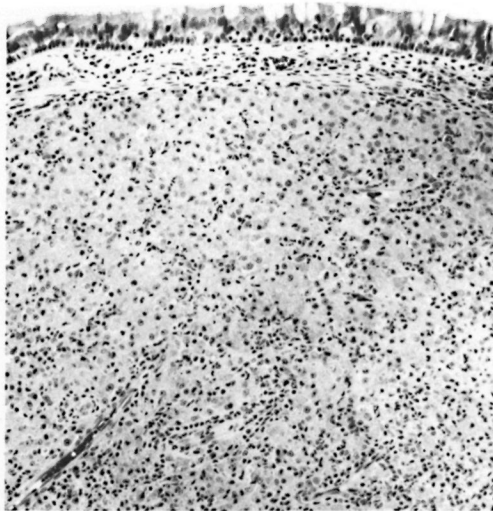

FIG. 6.   Diffuse lympho-histiocytic infiltrate in nasal mucosa of experimentally infected rhesus monkey (H&E ×100).
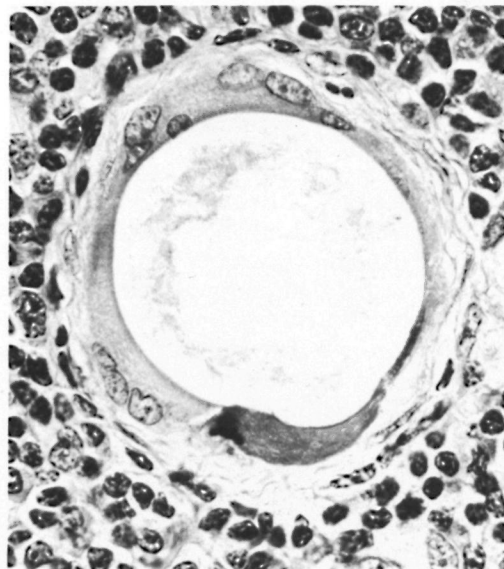

FIG. 7.   Multinucleated cell containing large globus in lymph node of experimentally infected rhesus monkey (H&E ×630).

of *M. leprae*. The inflammatory infiltrate was also similar histologically to human lepromatous leprosy.

The lesions in this animal were also similar to those in the experimentally infected sooty mangabey monkey previously described ([3]), although there are some differences. The skin lesions in the rhesus were less nodular than those of the mangabey. In addition, the affected skin had a dry, scaly appearance which was not seen in the mangabey. This may be due to more extensive autonomic nerve damage in the rhesus. The histiocytes in the rhesus were more vacuolated, and globi were larger and more numerous than in the mangabey. Nerve involvement was more extensive in the rhesus. The liver and spleen of the rhesus contained more AFB than those of the mangabey, although the normal core temperature of both species is about the same.

The testicles of the mangabey were severely involved, whereas the testicles of the rhesus monkey were normal. This may have occurred because the rhesus monkey was not sexually mature and the testicles not completely descended into the scrotum at the time of inoculation. Sufficient time for dissemination may not have elapsed after the testicles descended into the cooler scrotum. More animals of both species must be examined before we will know whether these differences are merely individual variations or reflect species differences in reactivity to *M. leprae*.

The study of an immune deficiency syndrome transmitted to rhesus monkeys in similar leprosy transmission experiments ([2]) resulted in the isolation of a retrovirus, STLV-III/Delta, from affected rhesus monkeys and from healthy sooty mangabeys ([11]). We have found that many sooty mangabeys carry this virus without apparent ill effects. It is now known that STLV-III is endemic in the colony from which the donor mangabeys in these studies came ([5]). Two rhesus monkeys inoculated with *M. leprae* from a mangabey donor acquired STLV-III infection, developed the immune deficiency syndrome, and had disseminated lepromatous leprosy (unpublished observations). Although the mangabey which served as a donor for rhesus #A125 did not originate from the infected colony and is serologically and virologically negative for STLV-III, we nevertheless evaluated rhesus #A125 virologically and serologically for evidence of STLV-III/Delta infection. We were unable to find any evidence that this monkey was infected. In addition, rhesus #A125 was born in a colony which has less than a 5% incidence of antibody to the type-D retrovirus also associated with SAIDS, and in which no SAIDS-like disease has ever been recognized. Therefore, we believe that the development of leprosy in this animal was unrelated to the simian acquired immune deficiency syndrome.

The successful induction of progressive generalized lepromatous leprosy in a rhesus monkey establishes another nonhuman primate model of leprosy, in addition to the sooty mangabey. Rhesus monkeys are available commercially; mangabeys are not. There are vast amounts of biological data available for the rhesus monkey; such data are limited for the mangabey. We have established that the lepromin test, with some modifications, is useful in evaluating cell-mediated immunity to *M. leprae* in rhesus monkeys ([1]). Normal uninoculated rhesus monkeys are lepromin negative. Those which are inoculated with *M. leprae* and do not develop disease become lepromin positive; those which develop lepromatous leprosy are lepromin negative. These factors make the rhesus monkey a valuable addition to the list of animal models available for the study of leprosy.

SUMMARY

A 6-month-old male rhesus monkey (*Macaca mulatta*) was inoculated intravenously and intracutaneously with *Mycobacterium leprae* obtained from a naturally infected mangabey monkey. The animal developed generalized lepromatous leprosy, and was killed for pathological examination 56 months after inoculation. Lesions were observed in the skin, nasal mucosa, peripheral nerves, and peripheral lymph nodes, with relative sparing of viscera. The monkey was carefully evaluated for the retrovirus STLV-III infection and was found negative. The rhesus monkey thus provides another animal model for the study of leprosy.

RESUMEN

Un mono rhesus (*Macaca mulatta*) macho de 6 meses de edad se inoculó intravenosa-e intracutáneamente con *Mycobacterium leprae* obtenido de monos mangabey infectados de manera natural. El animal desarrolló lepra lepromatosa generalizada y fue sacrificado 56 meses después de la inoculación para efectuar su examen patalógico. Se observaron lesiones en la piel, en la mucosa nasal, en nervios periféricos, y en ganglios linfáticos periféricos con poca afección de las vísceras. El mono fue cuidadosamente estudiado en cuanto a su infección con el retrovirus STLV-III y resultó negativo. El mono rhesus constituye otro modelo animal útil en el estudio de la lepra.

RÉSUMÉ

On a inoculé par voie intraveineuse et par voie intracutanée un singe rhésus (*Macaca mulatta*) âgé de 6 mois et de sexe masculin, avec *Mycobacterium leprae* recueilli chez un singe Mangabey infecté de manière naturelle. L'animal a développé une lèpre lépromateuse généralisée. On l'a sacrifié 56 mois après l'inoculation afin de procéder à des examens pathologiques. Des lésions ont été observées dans la peau, le mucus nasal, les nerfs périphériques, et les ganglions lymphatiques périphériques; les viscères étaient relativement épargnés. On a soigneusement étudié ce singe en vue de déceler éventuellement une infection par le rétrovirus STLV-III; les investigations ont cependant été négatives. Les singes rhésus fournissent dès lors un modèle animal supplémentaire pour l'étude de la lèpre.

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