

## Histopathological Changes in the Eyes of Mangabey Monkeys with Lepromatous Leprosy<sup>1</sup>

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Leprosy is a chronic systemic infection caused by *Mycobacterium leprae* that frequently damages the eyes. According to the World Health Organization, leprosy blinds up to one million patients (<sup>12</sup>), but ocular complications of the disease get little attention in programs for the prevention of blindness. Although the ophthalmological manifestations of leprosy are well documented clinically, little is known about their pathogenesis. Histopathological studies of eyes of human leprosy patients have been largely limited to specimens obtained at autopsy or from patients whose affected eyes have been enucleated because of complications of advanced lepromatous leprosy. To our knowledge there have been no reports on the histopathologic changes in the early stages of leprosy in human eyes.

There are two main pathogenetic mechanisms for production of ocular damage in leprosy: a) impairment of facial and trigeminal nerve function, causing lagophthalmos with exposure of the cornea, and corneal and conjunctival anesthesia; and b) bacillary invasion of the cornea, conjunctiva, iris, and ciliary body with consequent inflammatory reactions and such complications as loss of corneal transparency, secondary glaucoma, and cataract.

As a first step in our attempt to delineate the pathogenesis of ocular involvement, we

have studied the eyes of several animal species with leprosy and report here our findings in a nonhuman primate model, the sooty mangabey monkey (*Cercocebus atys*).

Naturally acquired lepromatous leprosy was recently described in a sooty mangabey monkey (<sup>11</sup>). Skin lesions showed typical clinical and histopathological features of lepromatous leprosy. As a result of these findings, transmission studies were initiated to reevaluate the susceptibility of nonhuman primates. Subsequently, other mangabeys, rhesus monkeys (*Macaca mulatta*), and African green monkeys (*Cercopithecus aethiops*) were inoculated with *M. leprae*. Although susceptibility among the different species varied, all three species have developed leprosy (<sup>15</sup>).

### MATERIALS AND METHODS

We examined the eyes of three mangabey monkeys. Two of the animals were inoculated with *M. leprae*; the third was not inoculated, but died of pneumonia. All three animals were born at Yerkes Regional Primate Center (YRPC), Emory University, Atlanta, Georgia, U.S.A. The animals inoculated with *M. leprae* were housed at Delta Regional Primate Research Center (DRPC), Covington, Louisiana, U.S.A.

**Inoculum.** The inoculum for animal no. 1 was a suspension of *M. leprae* prepared from cutaneous lepromas of the index mangabey monkey with naturally acquired leprosy. The inoculum for animal no. 2 was from a leproma of a nine-banded armadillo (*Dasypus novemcinctus*) infected with *M. leprae* from a human with lepromatous leprosy. The tissue was homogenized, centrifuged, and acid-fast bacilli (AFB) counted using standard methods (<sup>13</sup>).

Monkey no. 1 was an eight-year-old male obtained from YRPC in January 1980. The animal was healthy and tuberculin negative.

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He was housed in a separate cage in the isolation facility of DRPC and observed for 3 months. Under ketamine HCl (10 mg/kg) anesthesia, this monkey was inoculated with  $1.2 \times 10^9$  *M. leprae* intravenously and  $3 \times 10^8$  *M. leprae* intradermally in each of five sites (brow, lip, calf, and both ears). Clinical examinations and biopsies of the inoculation sites were made at bimonthly intervals.

The animal died 46 months after inoculation following routine ketamine anesthesia for collection of specimens, and a complete necropsy was performed 3 hours postmortem. There was widely disseminated lepromatous leprosy, and the detailed gross and histopathologic findings, except for the eyes, have been described (<sup>2</sup>).

The eyes were enucleated and fixed in 2% glutaraldehyde. For light microscopy, the tissues were embedded in paraffin, sections cut at 5  $\mu$ m, and stained by hematoxylin and eosin (H&E) and Fite-Faraco (FF) acid-fast stains.

For transmission electron microscopy, the tissues were post-fixed in 1% osmium tetroxide, stained en bloc with 1% uranyl acetate, and embedded in Spurr's plastic medium. Thin sections were cut on a Reichert OMU 2 ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Zeiss EM 109 transmission electron microscope. For scanning electron microscopy, the tissues were dried in a Tousimis Samdri PVT 3 critical point dryer, coated with gold in a Technics Hummer I sputtering system, and viewed with a Philips 501 scanning electron microscope.

Monkey no. 2, an adult female, was inoculated with  $2.1 \times 10^8$  *M. leprae* intravenously, and a total of  $4.5 \times 10^8$  *M. leprae* intradermally, distributed at the following sites: dorsum of the left forearm, left lateral calf, tips of both ears and nose, left eyebrow, left upper lip, left first knuckle, left third knuckle, and dorsum of the left wrist. Clinical examinations were done bimonthly. Thirteen months after inoculation the animal died following routine ketamine anesthesia. At necropsy the eyes were enucleated, fixed, and processed for light microscopy following the same procedure as described for animal no. 1.

Monkey no. 3 was a 3-year-10-month old female mangabey housed at YRPC. The an-

imal was not inoculated with *M. leprae*, and was well until it developed pneumonia and died at YRPC. At necropsy the eyes were removed and fixed in 10% buffered Formalin and processed, as above, for light microscopy.

## RESULTS

Macroscopic examination of the eyes of the three animals revealed no relevant abnormalities. Microscopic examination of the eyes from the monkeys infected with *M. leprae* showed focal collections of lymphocytes in the superficial stroma of the conjunctiva and in the ciliary body in the region of the pars plana. The eyes of the noninfected animal were normal except for a slight mononuclear infiltration in the conjunctiva. In the monkey inoculated with *M. leprae* 46 months earlier (animal no. 1), there was a mild, chronic, inflammatory infiltrate at the periphery of the cornea and corneoscleral limbus. The infiltrate was composed predominantly of histiocytes mixed with a few lymphocytes and plasma cells. There were no granulomas. There was bilateral involvement, but the cellular infiltrations were more extensive in the right eye, particularly on the temporal side. Proliferation of blood vessels was seen at the limbus in both eyes, with pannus formation.

In Fite-Faraco-stained preparations there were clumps of AFB in the basal layer of the conjunctival epithelium in both eyes (Fig. 1). In the corneal stroma of the right eye, there were occasional single, apparently extracellular AFB and clumps of AFB within histiocytes (Fig. 2). Most of the AFB were well stained. There were AFB in the walls of the blood vessels at the limbus and episclera (Fig. 2). Some of the corneal nerve twigs had lost their normal architecture and contained AFB (Fig. 3). There were AFB in the sclera. No AFB were identified in the eyes of the animal inoculated 13 months previously (animal no. 2), or in the eyes of the animal that had not been inoculated.

The skin of the eyelids of the animal with disseminated leprosy (animal no. 1) showed typical changes of lepromatous leprosy. There were large, predominantly histiocytic infiltrates in the superficial dermis which spared the epidermal zone. The histiocytes

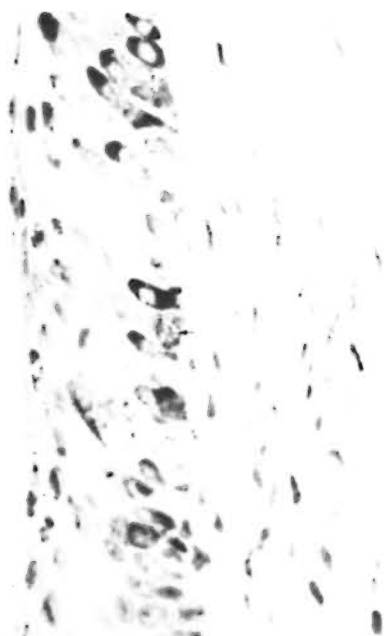


FIG. 1. Photomicrograph of mangabey monkey corneo-scleral junction. Note acid-fast bacilli (arrow) in basal layer of conjunctival epithelium [Fite-Faraco stain (FF)  $\times 250$ ].

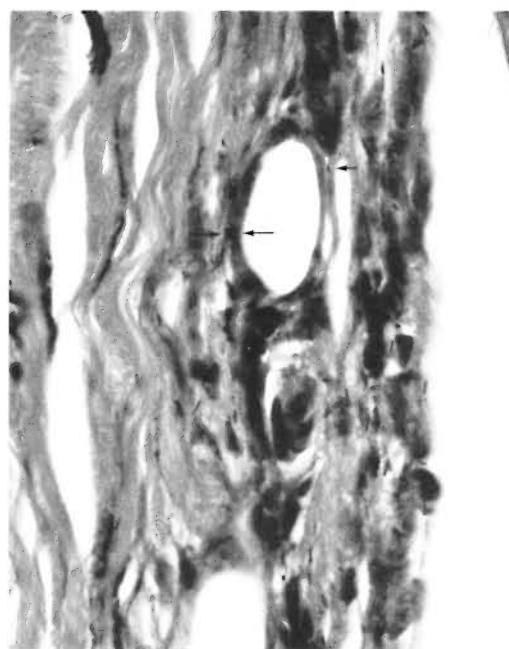


FIG. 2. Photomicrograph of corneal stroma depicts AFB in inflammatory cells and blood vessel walls (arrows) (FF stain  $\times 1000$ ).

around epidermal appendages and dermal nerves contained many AFB.

The eyes from only animal no. 1 were studied by electron microscopy. Transmission electron microscopy revealed many deformed vacuolated keratocytes with intracytoplasmic bacilli (Fig. 4). The bacilli were surrounded by an electron transparent zone, a typical finding in lepromatous leprosy in humans and other experimental animals<sup>(3)</sup>. The macrophages and endothelial cells of the blood vessels also contained bacilli.

Scanning electron microscopy revealed leprosy bacilli lying among the collagen fibers of the corneal stroma and as clumps of bacilli "glued" together within spaces (Fig. 5).

### DISCUSSION

This is the first report of the ocular manifestations of leprosy in any primate, including man, in which the duration of infection is known.

*M. leprae* is an obligate, intracellular, acid-fast bacillus that has not been cultured *in vitro*. Experimental infection of animals has been valuable in the study of the disease;

however, animal models have been underutilized in elucidating the pathogenesis of many important aspects of leprosy. The first immunologically intact animal model of leprosy was the nine-banded armadillo (*Dasypus novemcinctus*)<sup>(7)</sup>. Starting with Hansen, the Norwegian scientist who discovered *M. leprae* in 1873, many investigators have attempted to infect nonhuman primates<sup>(10)</sup>. Until recently, successful transmission has been reported only in a chimpanzee<sup>(9)</sup> and a gibbon<sup>(14)</sup>. Our previous studies of armadillos with disseminated leprosy revealed extensive invasion of the tissues of the anterior segment of the eye<sup>(9)</sup>.

We are aware of only one other study of ocular leprosy in experimental animals: in a single armadillo and in immunologically impaired (thymectomized and irradiated) mice<sup>(5)</sup>. The eyes of a chimpanzee with naturally acquired leprosy showed AFB in the histiocytes infiltrating the sclera, deeper layers of the peripheral cornea, and ciliary body<sup>(8)</sup>. This is the only previous report of ocular leprosy in a nonhuman primate.

The earliest clinical sign of ocular leprosy in humans is the thickening and beading of

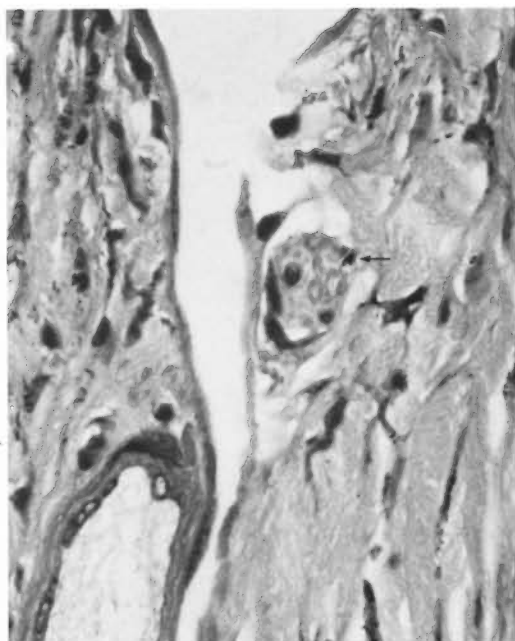


FIG. 3. Photomicrograph of corneal stroma. Corneal nerve is damaged and contains AFB (FF stain  $\times 1000$ ).

nerves in the peripheral cornea, particularly in the upper temporal quadrant (<sup>1,6</sup>). In fact, this finding confirms the diagnosis of leprosy in patients with other minimal clinical manifestations.

Histopathological studies of human eyes have been limited to those with extensive advanced leprosy. Clinical manifestations in the cornea of leprosy patients include avascular keratitis, pannus formation, interstitial keratitis, and corneal leproma (<sup>1</sup>). Corneal involvement in leprosy can be classified according to the extent and type of lesions, and this is of practical importance in assessing the status of ocular disease. AFB may be seen in or between the axis cylinders of corneal nerves and may be accompanied by an inflammatory infiltrate and, later, by more severe damage to the nerves. In avascular keratitis, there are focal inflammatory infiltrates just below Bowman's layer with cells forming small clusters. In more advanced stages, corneal opacification extends centrally, and AFB may be found in the keratocytes, histiocytes and epithelial cells, and free between cells.

The mangabey monkey with ocular involvement had developed generalized lep-

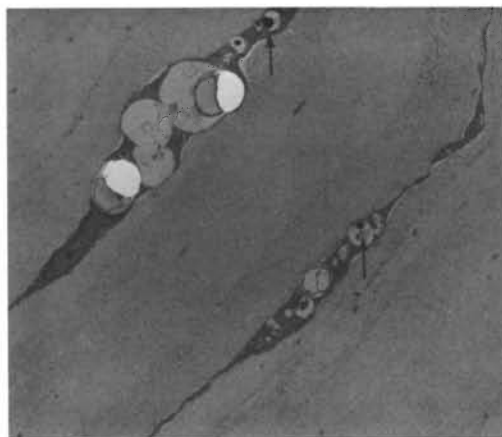


FIG. 4. Electron micrograph of corneal stroma. Keratocyte cytoplasm is vacuolated and distended. Remnants of *M. leprae* (arrows) surrounded by electron-transparent zone ( $\times 7000$ ).

romatous leprosy. At necropsy, there were lepromatous lesions mainly in the skin, nasal mucosa, peripheral nerves, and testicles—sites that are frequently affected in human lepromatous leprosy (<sup>2</sup>). The corneal findings, namely, the minimal subepithelial infiltrate in the limbal region and the localization of AFB in the nerves and in blood vessels, suggest that the ocular disease was in its early stages. By electron microscopy, however, the keratocytes were markedly damaged, and there were bacilli in the cytoplasm of keratocytes and among the collagen fibers of the corneal stroma.

The focal inflammatory cellular exudates in the ciliary body of the two monkeys inoculated with *M. leprae* were mainly lymphocytes and showed no invasion with AFB. Similar focal aggregates have been observed in the skin of mangabey monkeys with lepromatous leprosy, but their association with the disease has been neither consistent nor clarified.

The dissemination of *M. leprae* infection may be hematogenous, which is suggested here since endothelial cells of the limbal and episcleral blood vessels harbored AFB. However, because of the corneal nerve involvement, the organisms may also travel along the nerves either by intra-axonal or Schwann cell-to-Schwann cell spread.

Our studies have demonstrated early lesions of leprosy in the cornea of a mangabey monkey with advanced lepromatous lep-



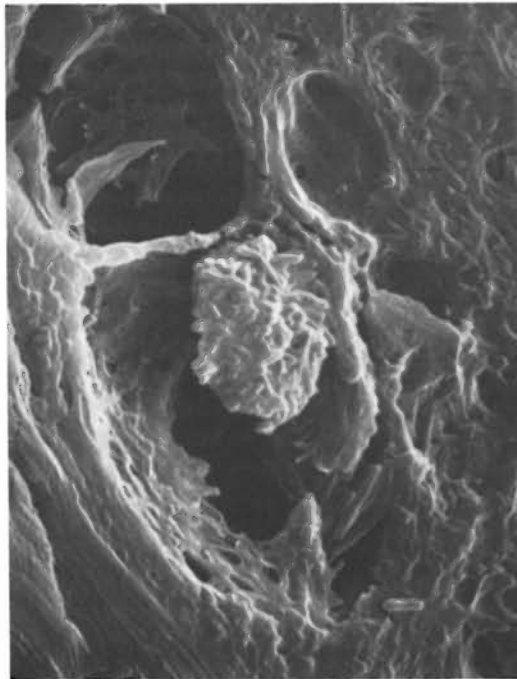


FIG. 5. Scanning electron micrograph of corneal stroma. Bacilli (arrows) are scattered among collagen fibers and a clump of bacilli surrounded by a space suggestive of *M. leprae* lipid antigen removed during processing ( $\times 5000$ ).

rosy. However, further clinical and histopathological studies of the eyes of primates at different stages following inoculation with *M. leprae* would be of great value in determining more precisely the pathogenesis of the ocular complications of leprosy, and may help in the management of this potentially blinding disease.

#### SUMMARY

Leprosy is the third leading cause of preventable blindness; however, little is known about the spread of infection to the eye. We have studied the eyes of three sooty mangabey monkeys. Two were experimentally infected with *Mycobacterium leprae*; the third was not infected. In one of the infected animals there was histopathological evidence of lepromatous leprosy as evidenced by a chronic inflammatory infiltrate at the limbus, and detection of acid-fast bacilli in the corneal stroma, blood vessel walls, and corneal nerves. The latter were damaged as a result of the bacillary invasion. Electron microscopy revealed involvement and dis-

tortion of keratocytes with *M. leprae* and invasion of the corneal stroma by macrophages containing bacilli. Both infected animals showed focal collections of lymphocytes in the superficial stroma of the conjunctiva and in the ciliary body. This is the first report of the ocular manifestations of leprosy in any primate, including man, in which the duration of infection is known.

#### RESUMEN

Lepre es la tercer causa de ceguera prevenible; sin embargo, poco se sabe sobre la diseminación de la enfermedad en el ojo. Nosotros hemos estudiado los ojos de 3 monos mangabey tiznados. Dos fueron infectados experimentalmente con *Mycobacterium leprae*; el tercero no fue infectado. En uno de los animales infectados hubieron evidencias histopatológicas de lepra lepromatosa tales como un infiltrado crónico inflamatorio en el limbus y la presencia de bacilos ácido resistentes en el estroma corneal, en las paredes de los vasos sanguíneos y en los nervios corneales. Estos últimos resultaron dañados por la invasión bacilar. La microscopía electrónica reveló afección y distorsión de los queratocitos con *M. leprae* e invasión del estroma corneal por macrófagos conteniendo bacilos. Los dos animales infectados mostraron colecciones focales de linfocitos en el estroma superficial de la conjuntiva y en el cuerpo ciliar. Este es el primer reporte de las manifestaciones oculares de la lepra en primates, incluyendo al hombre, en donde se conoce la duración de la infección.

#### RÉSUMÉ

Parmi les causes de cécité qu'il est possible de prévenir, la lèpre est au troisième rang. Néanmoins, on connaît fort mal le mécanisme qui détermine la dissémination des infections oculaires. Nous avons étudié les yeux de trois singes mangabey. Deux avaient été infectés expérimentalement avec *Mycobacterium leprae*. Le troisième n'était pas infecté. Chez l'un des animaux infectés, on a décelé des signes histopathologiques de lèpre lépromateuse, qui se manifestaient par un infiltrat inflammatoire chronique du limbe, la présence de bacilles acido-résistants dans le stroma cornéen, dans la paroi des vaisseaux sanguins et dans les nerfs de la cornée. La cornée, par ailleurs, était entamée suite à l'invasion bacillaire. Les études menées au microscope électronique ont révélé une atteinte des kératocytes par *M. leprae*, leur bouleversement, et l'invasion du stroma cornéen par des macrophages contenant des bacilles. Les deux animaux infectés présentaient des amoncellements en foyers de lymphocytes dans le stroma superficiel de la conjonctive et dans le corps ciliaire. Ceci est le premier rapport de manifestations oculaires de la lèpre chez un primate, y comprise l'homme, chez lequel la durée de l'infection est précisée.

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