Limited Susceptibility of Cynomolgus Monkeys (*Macaca fascicularis*) to Leprosy after Experimental Administration of *Mycobacterium leprae*

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Abstract. Cynomolgus monkeys are a useful model for human tuberculosis, but susceptibility to *M. leprae* is unknown. A cynomolgus model of leprosy could increase understanding of pathogenesis—importantly, neuritis and nerve-damaging reactions. We administered viable *Mycobacterium leprae* to 24 cynomolgus monkeys by three routes, with a median follow-up period of 6 years (range = 1–19 years) involving biopsies, nasal smears, antiphenolic glycolipid-1 (PGL-1) antibody serology, and lepromin skin testing. Most developed evanescent papules at intradermal *M. leprae* inoculation sites that, on biopsy, showed a robust cellular immune response akin to a lepromin skin test reaction; many produced PGL-1 antibodies. At necropsy, four monkeys, without cutaneous or gross neurological signs of leprosy but with elevated PGL-1 antibodies, including three with nasal smears (+) for acid fast bacilli (AFB), showed histological features, including AFB, suggestive of leprosy at several sites. Overall, however, cynomolgus monkeys seem minimally susceptible to leprosy after experimental *M. leprae* administration.

INTRODUCTION

Leprosy, caused by *Mycobacterium leprae*, is the second most common mycobacterial infection in man.¹ Improved understanding of leprosy pathogenesis and developing improved therapies have been slowed by the lack of an experimental animal model that replicates the spectrum of features in human disease. Most types of laboratory mice develop localized infection after footpad or intradermal inoculation with *M. leprae*, which is often used as an antileprosy drug screening model.² The nine-banded armadillo is susceptible to *M. leprae* and develops cutaneous and visceral lesions of lepromatous leprosy in naturally acquired leprosy or after experimental administration of *M. leprae*, but it is phylogenetically distant.³

A reliable non-human primate model of human leprosy that developed the spectrum of features in human disease, especially neuritis and nerve-damaging reactions that lead to disability, would be useful to improve our understanding of leprosy pathogenesis. In the late 1980s, reports of naturally occurring leprosy in chimpanzees and sooty mangabey monkeys triggered efforts to develop non-human primate models of leprosy.⁴ The sooty mangabey monkey, from West Africa, seemed most susceptible to leprosy, either naturally acquired or experimental infection, but was not readily available.^{5,6} Leprosy in mangabey monkeys resembles human lepromatous (multibacillary) leprosy, with skin lesions, nerve palsy, immunological abnormalities, reactions, and histological features.^{7–11} The chimpanzee and rhesus monkey seem susceptible to leprosy, but the rhesus monkey tends to develop tuberculoid (paucibacillary) disease only.^{5,12}

The cynomolgus monkey, a commonly available Asian macaque routinely used in pre-clinical drug and vaccine studies, is susceptible to *M. tuberculosis* and serves as a model of

human disease. Cynomolgus monkeys also seem somewhat susceptible to *M. ulcerans*, the causative agent of Buruli ulcer.^{13,14} The susceptibility of cynomolgus monkeys to leprosy after experimental *M. leprae* inoculation is unknown. A report published in 1941 vaguely describes experimental *M. leprae* inoculation of one cynomolgus monkey, and there is one report of naturally acquired borderline lepromatous leprosy in a captive Philippine cynomolgus monkey.^{15,16} Here, as a first step to developing a practical non-human primate model of human leprosy, we experimentally administered *M. leprae* to groups of cynomolgus monkeys and monitored them for signs of leprosy.

METHODS

Protocol and animals. The animal use protocol for this work was approved by the Leonard Wood Memorial Institutional Animal Care and Use Committee. The study was conducted in compliance with the US Animal Welfare Act and Philippine Association for Laboratory Animal Science (PALAS) guidelines, and it adhered to principles stated in the National Research Council publication.

Twenty-four captive-bred, healthy male and female Philippine cynomolgus monkeys (*Macaca fascicularis*), ranging in age from 1 to 7 years old, weighing 1.6–5.5 kg, and having no clinical signs or laboratory values suggestive of *M. leprae* exposure, were used. The latter included nasal smears for acid fast bacilli (AFB) and anti-phenolic glycolipid-1 (PGL-1) immunoglobulin M (IgM) and IgG antibody levels. For all procedures, monkeys were anesthetized with ketamine hydrochloride (20 mg/kg). No paralytics were used.

Experimental groups and *M. leprae* inocula. Table 1 summarizes seven experimental groups of cynomolgus monkeys and the characteristics and sources of the *M. leprae* inocula administered. Some studies suggest that *M. leprae* of non-human primate origin may be more pathogenic.^{5,17} We sourced three mangabey monkeys (M 1–3) with leprosy for *M. leprae* inocula, and two of them were also simian immunodeficiency virus serology positive [SIV(+)]. We did not determine SIV serology status for any of the cynomolgus monkeys,

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TABLE 1
Characteristics of *M. leprae* experimentally administered to cynomolgus monkeys by group

Monkey group number	<i>M. leprae</i> inocula source	Number of AFB/mL	Mouse footpad assays (percent mice with AFB+ footpads)	Total AFB/monkey
1	First administration: time 0. From three patients with untreated lepromatous leprosy to cynomolgus monkeys 1–6.	1.4×10^8	29/31 (94%)	IV + ID: $3.2\text{--}3.6 \times 10^8$; IN: $0.5\text{--}1.1 \times 10^8$
1	Second administration: time 0 + 3 years. From armadillo (original <i>M. leprae</i> source was armadillo) to cynomolgus monkeys 3 and 4. Rationale was to assess an alternative <i>M. leprae</i> source.	8.2×10^8	Not done	$1.4\text{--}2.1 \times 10^9$
1	Second administration: time 0 + 4 years. From mangabey monkey 1 SIV(–) (original <i>M. leprae</i> source was human) to cynomolgus monkeys 2, 5, and 6. Rationale was to assess an alternative <i>M. leprae</i> source.	1.2×10^9	Not done	$2.5\text{--}3.1 \times 10^9$
2	From three patients with untreated lepromatous leprosy.	6.5×10^8	11/11 (100%)	$1.7\text{--}1.9 \times 10^9$
3	From mangabey monkey 2 SIV(+).	7.9×10^8	Not done	1.8×10^9
4	From mangabey monkey 3 SIV(+).	8.2×10^8	Not done	IV + ID: 1.9×10^9 ; IN: 8.2×10^8
5	From two subjects with untreated lepromatous leprosy.	3.6×10^6	5/10 (50%)	$6.1\text{--}7.2 \times 10^6$
6	From two subjects with untreated lepromatous leprosy.	2.1×10^8	14/15 (93%)	6.3×10^8
7	From three subjects with untreated lepromatous leprosy.	3.6×10^6	33/35 (94%)	1.1×10^7

AFB = acid fast bacilli; ID = intradermal; IN = intranasal; IV = intravenous; SIV = simian immunodeficiency virus.

including those monkeys inoculated with *M. leprae* obtained from SIV(+) mangabey monkeys with leprosy. For *M. leprae* inocula obtained from patients with untreated lepromatous leprosy, samples were injected into mouse footpads to assess *M. leprae* viability.¹⁸

M. leprae inocula for administration to cynomolgus monkeys were suspended in sterile phosphate-buffered saline, counted, assessed for morphological index (most), and then administered intranasally (IN) to both nostrils or as combined intravenous (IV) and intradermal (ID) inoculations, the latter to one or more adjacent sites on the ear rims, nose tip, upper lip, left lateral arm, and left lateral leg. IV inoculations were in the left saphenous vein near the calf. The concentration of *M. leprae* in inocula of human origin ranged from 3.6×10^6 to 6.5×10^8 AFB/mL, and the concentration of *M. leprae* in inocula of mangabey monkey origin ranged from 7.9×10^8 to 1.2×10^9 AFB/mL. ID and IV inoculation volumes ranged from 0.1 to 1 mL. All inoculations were done with a 1-mL syringe fitted with a 27-gauge needle.

Clinical observations. Inoculation sites were observed monthly for the first year and then every 3–6 months until study completion. Photographs were taken to document lesion status. For 5 years after experimental *M. leprae* administration, monkeys were observed weekly for changes in behavior, including appetite, and monitored for signs of pain, discomfort, neuritis, reversal reaction, and erythema nodosum leprosum (ENL).

Histopathology. Punch skin biopsies were obtained from *M. leprae* ID inoculation site lesions from five monkeys between 1 and 5 months after inoculation and three monkeys between 6 months and 6 years after ID inoculation (Table 2). In 2004, all of the six monkeys remaining in the study had skin biopsies at or near the original *M. leprae* inoculation sites as well as from the eyelids and scrotum as a final assessment for leprosy; these monkeys were then euthanized at the end of the study. All tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μ m, and then stained with hematoxylin and eosin for routine histology

and by Fite–Faraco method for AFB. Sections with features suggestive of leprosy were classified according to the Ridley–Jopling system to denote leprosy type according to immunity, which for histology, is based on skin lesion mononuclear cell and AFB loads, the latter ranging from 0 (none) to 6+: tuberculoid (TT; 0–1+), borderline tuberculoid (BT; 1–+), borderline (BB; 3–4+), borderline lepromatous (BL; 4–6+), and lepromatous (LL; 5–6+).¹⁹

Nasal mucosa and slit skin smears assessed for AFB. Approximately every 3–6 months after *M. leprae* inoculation for up to 5 years and then less frequently until study completion, nasal mucosa swabs were obtained and stained for AFB by the Zeehl–Neelsen method, examined, and quantified for the amount of AFB on a 0–6+ scale as described elsewhere.²⁰ Slit skin smears from the lower earlobes, stained for AFB, were done periodically, especially if there were other findings suggestive of leprosy.

Anti-PGL-1 antibody serology. Serum for measuring anti-PGL-1 IgM and IgG antibody levels was obtained from monkeys on a schedule similar to the schedule of AFB nasal swabs. Anti-PGL-1 antibody was measured by ELISA on an absorbance scale.²¹ Because of variability of IgM and IgG antibody values in healthy, *M. leprae*-unexposed cynomolgus monkeys, only values in the experimental monkeys more than two times the upper limit of normal (ULN) were considered positive (IgM > 0.1; IgG > 0.14). At time points when anti-PGL-1 IgM, IgG, or both values were above the 2 $\times$ ULN, IgM to IgG ratios calculated as ratios ≤ 1 may be a marker of leprosy resistance.²²

Necropsy. Most monkeys that died on study underwent necropsy, including gross and histological assessments for identifying a possible cause of death. In 2007, six monkeys remaining in the study had final examinations, including chest X-rays, and then, they were euthanized. These six monkeys, along with monkey 24 (group 7), had gross and histological eye examinations.²³

Lepromin skin tests. Lepromin skin tests (Mitsuda) were conducted on 10 monkeys. Up to four concentrations of

TABLE 2
Cynomolgus monkey experimental groups, *M. leprae* inoculation routes, and selected clinical and laboratory outcomes

Group	Monkey number	<i>M. leprae</i> administration route	Cutaneous lesions (time post- <i>M. leprae</i> administration) and biopsy interpretation	Nasal smear scores (0 to 6+; time post- <i>M. leprae</i> administration)	Disposition: years follow-up; necropsy findings*†
1	1	ID + IV	None‡	0 to sporadic 1+	3; lymphoma
1	2	ID + IV	None‡	0 to sporadic 1+	4; ears, nasal septum; lepromatous leprosy
1	3	ID + IV	Arm (5 months): unremarkable‡	0 to sporadic 1+	15; fibrosarcoma
1	4	ID + IV	None‡	0 to sporadic 1+	19; euthanized; gross unremarkable
1	5	IN and later, ID + IV	None‡	2+ to 4+ (1 year)	5; pneumonitis
1	6	IN and later, ID + IV	None‡	2+ to 4+ (1 year)	6; granulomatous pleuritis
2	7	ID + IV	Ear (4 months): lepromin-like‡§	4+ to 5+ (3 years)	5; ears, borderline lepromatous leprosy
2	8	ID + IV	Leg (5 months): lepromin-like‡§	0 or sporadic 1+	18; unremarkable
2	9	ID + IV	Arm (6 years): unremarkable‡	0 or sporadic 1+	19; euthanized; gross unremarkable
2	10	ID + IV	Arm (4 months): lepromin-like‡§	0 or sporadic 1+	19; euthanized; gross unremarkable
3	11	ID + IV; SIV(+) source	None‡	0 or sporadic 1+	2; nasal septum; lepromatous leprosy, pneumonitis
3	12	ID + IV; SIV(+) source	None‡	0 or sporadic 1+	2; fatty liver
3	13	ID + IV; SIV(+) source	Arm (1 month): lepromin-like‡§	0 or sporadic 1+	2; hepatitis
3	14	ID + IV; SIV(+) source	None‡	0 or sporadic 1+	4; cardiopulmonary disease
4	15	ID + IV; SIV(+) source; both IN	Arm (1 month): lepromin-like‡§	0 or sporadic 1+	2; unspecified mycobacteriosis (lung)
4	16	ID + IV; SIV(+) source; both IN	None‡	0 or sporadic 1+	18; euthanized; gross unremarkable
4	17	ID + IV; SIV(+) source; both IN	None‡	0 or sporadic 1+	1; pneumonia
4	18	ID + IV; SIV(+) source; both IN	None‡	0 or sporadic 1+	6; necrotizing rhinitis
5	19	ID + IV	None‡	0	2; unremarkable
5	20	ID + IV	None‡	0	10; mycobacteriosis, lung
5	21	ID + IV	Arm (2 years), shoulder (4 years): unremarkable‡	0	13; unremarkable
5	22	ID + IV	None‡	0	17; euthanized; gross unremarkable
6	23	ID + IV	None¶	0	15; euthanized; unremarkable
7	24	ID + IV	None	2+ to 4+ (8 years)	8; eyelids, nose, scrotum, ulnar nerve, testes; borderline lepromatous leprosy

ID = intradermal; IN = intranasal; IV = intravenous.

* Among the six monkeys remaining in the study, biopsies of eyelids, earlobes, lips, nose tips, scrotum, and left arm inoculation sites were all unremarkable; the monkeys were then euthanized.

† Ridley-Jopling classification described in the text.

‡ ID inoculation sites: nose tip thickening and papules.

§ Lepromin-like indicates histological features of a robust cellular immune response akin to a lepromin (Mitsuda) skin test reaction (shown in Figure 2C).

¶ Nose tip and inoculation site papules.

|| No lesions.

lepromin were used to test each monkey. Lepromin was derived from armadillo (three concentrations; obtained from George W. Long Hansen's Disease Center, Carville, LA) or mangabey monkey (one concentration; prepared by Tulane National Primate Research Center, Covington, LA) isolates ranging from 1.6×10^8 to 5.4×10^8 AFB/mL. Lepromin was administered ID to four abdominal sites in 0.1-mL volumes. Each reaction site was measured 3 weeks later. Reaction (induration only) scores, based on the longest diameter of each reaction, were ± 0 (1–3 mm; equivocal), 1+ (4–5 mm), and 2+ (≥ 5 mm).

Testing was done before and after experimental *M. leprae* administration as follows: five monkeys were tested 1–4 years before *M. leprae* inoculation, and three of these monkeys were retested up to 6 years after *M. leprae* inoculation. Another

group of five other monkeys was tested after *M. leprae* inoculation only 4–8 years later. Skin reactions from 7 of 10 tested monkeys were biopsied for histological assessment.

RESULTS

Clinical observations. Table 2 summarizes findings in 24 cynomolgus monkeys after experimental administration of *M. leprae*. Median follow-up time was 6 years/monkey and ranged from 1 to 19 years. Most monkeys (21/22) inoculated with *M. leprae* ID + IV developed evanescent thickening and papules at ID inoculation sites up to 2 cm in diameter within 1–3 months of inoculation (Figure 1). All skin lesions resolved within 1 year. Skin or mucous membrane lesions distant to

FIGURE 1. Skin papules that developed at sites inoculated with *M. leprae* ($\sim 10^8$ AFB/site) in monkey 7 (group 2) 2 months later. The papules remained localized and resolved by 12 months. (A) Papules on the lip, arm, and leg (arrows). (B) Papules on ears (arrows).

M. leprae inoculation sites were not noted. There were no signs of discomfort, pain, neuritis, or reactions. Among four monkeys receiving IN *M. leprae*, none developed any clinical signs or laboratory values suggestive of *M. leprae* exposure.

Histopathology. Table 2 summarizes skin lesion biopsy findings. Among six skin punch biopsies of *M. leprae* inoculation site lesions obtained within 6 months of *M. leprae* administration, five biopsies were characterized as lepromin-like, showing features analogous to a lepromin (Mitsuda) reaction, which included a robust cellular immune reaction and presence of AFB (Figure 2A and C). Three *M. leprae* inoculation site biopsies, obtained 2–6 years after ID inoculation, were unremarkable.

For the last six monkeys remaining in the study ≥ 15 years after *M. leprae* administration, biopsies from all monkeys obtained from or near original inoculation sites as well as eyelids and scrotums showed no features of leprosy. Additional histopathology results are described below.

Nasal mucosa smears for AFB. Table 2 summarizes the nasal smears assessed for AFB in 20 monkeys; they were generally unremarkable, with most being 0 or sporadic 1+. Sporadic 1+ scores were considered indeterminate, because these smears contained only 1 or 2 AFB and never occurred in consecutive fashion; 1+ AFB nasal smears also occur occasionally in healthy cynomolgus monkeys. Four monkeys developed AFB+ nasal smears in study, and two monkeys had AFB+ nasal smears detected at necropsy (the latter is described below).

Anti-PGL-1 antibody serology. Anti-PGL-1 antibodies were elevated in 16 (66%) monkeys at one or more time points. Among a total of 640 observation points, IgM and IgG values were $> 2 \times$ ULN in 21% of points. For IgM to IgG ratios, 237/368 (64%) were ≤ 1 , suggestive of a leprosy-resistant phenotype.²² Group 5, inoculated IV + ID with the lowest concentration of *M. leprae*, had the weakest and most sporadic anti-PGL-1 antibody responses.

As depicted in Figures 3 and 4, anti-PGL-1 antibody levels followed one of four general patterns, including (1) within normal limits throughout the study ($N = 8$; including all four monkeys inoculated IN); (2) sporadic increases of IgM and IgG within 3 months of *M. leprae* inoculation ($N = 6$); (3) sporadic increases of IgM, IgG, or both ($N = 6$); and (4) persistent increases in IgM, IgG, or both ($N = 4$; all with histological features suggestive of leprosy at necropsy as described below).

FIGURE 2. (A and B) Histological examination of the ear papule in Figure 1B. Mononuclear cells (macrophages and lymphocytes) stain blue, and AFB appear as red rod-like structures. (A) Dermis with numerous mononuclear cells and multinucleated giant cells (arrows; hematoxylin and eosin, $\times 100$). (B) Numerous mononuclear cells (single-tail arrow) and AFB scattered (double-tail arrow) and in small clumps (box; Fite–Faraco, $\times 1,000$) characterized as lepromin-like, which is akin to the lepromin (Mitsuda) skin test reaction shown in C. (C) Lepromin (Mitsuda) skin test reaction. The dermis shows numerous mononuclear cells (notable within the square) and scattered multinucleated giant cells (arrows), reflecting the robust cellular immune response of a lepromin (Mitsuda) skin test reaction (hematoxylin and eosin, $\times 100$).

FIGURE 3. Among monkeys inoculated with *M. leprae* that developed no clinical signs of leprosy and at necropsy, lacked histological findings suggesting leprosy, three patterns of anti-PGL-1 antibody responses were observed. A representative example of each is shown. Y-axis scales differ among graphs. ULN = upper limit of normal. (A) Within normal limits throughout the study. (B) Early spikes in IgM and IgG and then normalizing. (C) Sporadic increases in IgM or IgG throughout the study.

Necropsy findings. Table 2 summarizes monkey deaths on study and necropsy findings. Eighteen monkeys died while in the study from various or undefined causes, but no deaths were attributed to leprosy. Some monkeys in groups 3 and 4 inoculated with *M. leprae* obtained from SIV+ mangabey monkeys died relatively sooner; some had conditions associated with immunosuppression. Monkey 11 (group 3), one of eight monkeys inoculated with *M. leprae* from an SIV+ source, died 2 years after *M. leprae* inoculation and had abundant nasal mucosa AFB on necropsy (Figure 5). Assays to determine mycobacterial species were not done.

Table 3 summarizes findings in four monkeys that died in the study and developed laboratory values consistent with leprosy; on necropsy, they showed histological features suggestive of leprosy, including AFB. No monkeys had detectable skin lesions or gross neurological deficits at death.

Table 4 summarizes gross and histological findings of the eyes in seven monkeys; all findings were non-specific and had no evidence of *M. leprae* invasion.

Lepromin skin tests. Mitsuda lepromin skin test results among all five monkeys tested before *M. leprae* inoculation were reactive (1+ or 2+) (Figure 6). All reaction sites were biopsied; four sites were consistent with lepromin reactivity. Monkey 24 (group 7) had a 1+ clinical reaction

but lacked features of lepromin reactivity. Three of these five monkeys lepromin retested after *M. leprae* inoculation were reactive; one biopsy of one reaction was consistent with lepromin reactivity.

In a group of five other monkeys tested only after *M. leprae* inoculation, four monkeys were reactive (data not shown). One biopsy of one reaction was consistent with lepromin reactivity.

DISCUSSION

We administered *M. leprae*, obtained from human and mangabey monkey sources, to 24 cynomolgus monkeys by three routes using inocula sizes similar to earlier experimental leprosy studies in non-cynomolgus monkeys.^{4,12,22,24,25} A median observation period of 6 years/monkey after *M. leprae* administration, notably longer than many earlier studies, along with periodic laboratory assays and histological assessments, including necropsy, likely contributed to the discovery of *M. leprae* of subclinical-like infection in four monkeys. Nonetheless, cynomolgus monkeys generally showed negligible susceptibility to experimental leprosy, including a lack of neuropathies or reactions, arguing against additional efforts to develop this model.

FIGURE 4. Four monkeys (2, 7, 11, and 24) that died 2–6 years after *M. leprae* administration, with no skin lesions or gross neurological deficits suggestive of leprosy, showed histological features on necropsy suggestive of leprosy at various anatomic sites (Figure 5). All developed persistently increased anti-PGL-1 antibody levels, and three monkeys had AFB+ nasal smears. Y-axis scales differ among graphs. ULN = upper limit of normal. (A) Monkey 2 416-B (group 1). (B) Monkey 7 108-B (group 2). (C) Monkey 11 230-B (group 3). (D) Monkey 24 1166-A (group 7).

M. leprae from mangabey monkeys may be more pathogenic than *M. leprae* from other sources.^{9,25} Here, two of three mangabey monkeys sourced for *M. leprae* were SIV(+), which being pathogenic for cynomolgus monkeys, could potentially increase leprosy susceptibility.^{26–29} However, there was no indication that mangabey monkey-sourced *M. leprae*, whether from SIV(+) or SIV(–) monkeys, was more pathogenic than *M. leprae* from human or armadillo sources. Indeed, only one of four cynomolgus monkeys with subclinical leprosy at necropsy had been inoculated with *M. leprae* from an SIV(+) mangabey monkey source. Of note, six of eight cynomolgus monkeys (groups 3 and 4) inoculated with *M. leprae* from SIV(+) mangabey monkeys died 1–4 years after *M. leprae* inoculation, some with signs of immune suppression.

IV + ID and IN administrations and the amounts of AFB used generally followed earlier reports in chimpanzees and rhesus and mangabey monkeys, some with encouraging outcomes.^{4,12,22,24,25} Rhesus and mangabey monkeys show great variability in susceptibility to experimental leprosy, and some studies suggest that ID administration alone triggers sensitization, robust immunity, and resistance to leprosy. Our IV + ID inocula, ranging from $> 10^6$ to $> 10^9$ AFB/administration

site, may have induced some degree of immunity. Conversely, group 5 received the smallest inocula and developed the weakest, most sporadic PGL-1 antibody responses, partly supporting the rationale for using large inocula.

M. leprae administered IN to four monkeys mimicked a possible mode of human leprosy transmission through nasal droplet.¹ However, no monkeys developed any clinical sign or even a consistent laboratory result indicating exposure to *M. leprae*. In human untreated lepromatous leprosy, nasal mucosa harbors massive numbers of AFB, but AFB involved in transmission may be relatively small, with magnitudes less than we administered IN.³⁰ The rationale for a large number of AFB administered IN was to increase the odds of developing leprosy. Paradoxically, it may have induced a robust innate or mucosal IgA response or some degree of immune tolerance,³¹ potentially resulting in weak anti-PGL-1 IgM and IgG antibody production.

For IV + ID *M. leprae* administration, skin papules developed at ID inoculation sites that, on biopsy within 6 months, showed features of lepromin-like (Mitsuda) skin test hypersensitivity reactions. Resolution of all papules within 12 months also supported lepromin-like reactions rather than

FIGURE 5. Four monkeys (2, 7, 11, and 24) described in Figure 4 that died 2–6 years after *M. leprae* administration, with no skin lesions or gross neurological deficits suggestive of leprosy, showed histological features on necropsy suggestive of leprosy, including AFB, at various anatomic sites. Mononuclear cells (macrophages and lymphocytes) stain blue, and AFB appear as red rod-like structures. BI = bacterial index. (A) Monkey 2 (group 1). (Upper) This section of nasal septum mucosa shows scattered mononuclear cells (arrows) and a moderate number of AFB, especially prominent within the boxes (BI = 3+). These features suggested lepromatous leprosy (Fite–Faraco stain, $\times 1,000$). (Lower) This section of earlobe skin shows scattered mononuclear cells (short arrows) and scattered AFB (long arrows; BI = 4+). Some AFB appear beaded, suggesting that these bacilli are dead. These features suggested lepromatous leprosy (Fite–Faraco stain, $\times 1,000$). (B) Monkey 7 (group 2). A section of earlobe shows a focal, perivascular collection of mononuclear cells (short arrows) and a focal, intracellular collection of AFB (long arrows); some are beaded, suggesting dead bacilli (BI = 5+). These features suggested borderline lepromatous leprosy. The asterisk inside a clear, irregularly bordered circular space may represent a blood vessel (Fite–Faraco stain, $\times 1,000$). (C) Monkey 11 (group 3). A section of nasal septum mucosa. (Upper) In the dermis, scattered mononuclear cells (short arrows) and AFB in small clumps. (Lower) Abundant AFB, many in clumps (long arrows; BI = 4+). Features in these photomicrographs suggested lepromatous leprosy (Fite–Faraco stain, $\times 100$ and $\times 1,000$, respectively). (D) Monkey 24 (group 7). (Upper Left) A section of the scrotal sac shows scattered AFB, some in small clusters (arrows; BI = 5+). These findings suggested borderline leprosy (Fite–Faraco stain, $\times 1,000$). (Upper Right) A section of testes shows mononuclear cells (short arrows) and a small cluster of AFB (box; BI = 5+). These features suggested borderline lepromatous leprosy (Fite–Faraco stain, $\times 1,000$). (Lower Left) A section of ulnar nerve (denoted by the asterisk) shows several AFB (arrows), suggesting lepromatous leprosy (Fite–Faraco stain, $\times 1,000$). (Lower Right) A section of eyelid skin shows mononuclear cells (short arrows) and AFB in an intracellular cluster (long arrow) and scattered (two boxes; BI = 5+). These features suggested borderline lepromatous leprosy (Fite–Faraco stain, $\times 1,000$).

TABLE 3

Notable findings in four cynomolgus monkeys inoculated with *M. leprae* that developed laboratory values consistent with leprosy and on necropsy, showed histological features suggestive of leprosy

Group	Monkey number	<i>M. leprae</i> route	Cutaneous sign (biopsy site and interpretation)	Nasal smear scores (0 to 6+): on study or at necropsy	Necropsy histological findings* and supplementary assessments
1	2	Time 0: ID + IV; time 0 + 3 years: ID + IV	ID inoculation sites: nose tip thickening, papules; biopsy: none	0	Died 1 year post-second <i>M. leprae</i> administration; ears, nasal septum: lepromatous leprosy; lung tissue smears: AFB(-)
2	7	ID + IV	ID inoculation sites: nose tip thickening, papules; biopsy: ear (4 months), lepromin-like†	3.5 years after <i>M. leprae</i> administration: 5+; at necropsy: 1+	Died 5 years post- <i>M. leprae</i> administration; earlobes: borderline lepromatous leprosy; slit skin smear of earlobe: 1 + AFB (5 AFB/100 fields); lung tissue smears: AFB(-)‡
3	11	All ID + IV [SIV(+)]	ID inoculation sites: nose tip thickening, papules; biopsy: none	At necropsy: 4+	Died 2 years post- <i>M. leprae</i> administration; nasal septum: lepromatous leprosy; lung histology: AFB(-)‡
7	24§	ID + IV	No lesions; biopsy: none	8 years after <i>M. leprae</i> administration: 2+; at necropsy: 4+	Died 8 years post- <i>M. leprae</i> administration; eyebrows, nose, scrotum (including nerves), axillary lymph nodes, right ulnar nerve, testes (including nerves): borderline lepromatous leprosy; lung histology: AFB(-)‡

* Ridley-Jopling classification as described in the text.

† Lepromin-like indicates histological features of a robust cellular immune response akin to a lepromin (Mitsuda) skin test reaction (shown in Figure 2C).

‡ Assays to assess for tuberculosis Ridley-Jopling classification, described in Methods. AFB = acid fast bacilli.

§ Monkey #24, only monkey among five lepromin skin-tested, pre-*M. leprae* administration, with skin reaction site biopsy graded as "non-reactive".

disease. Most monkeys produced anti-PGL-1 antibody in response to *M. leprae* administration, especially by IV + ID. However, antibody levels, with the exception of levels in four monkeys with subclinical infection at necropsy (discussed

below), were generally sporadic, perhaps consistent with a lack of susceptibility to *M. leprae*. IgM to IgG ratios of ≤ 1 in 64% of all measurements supported a generally leprosy-resistant phenotype.^{9,32,33}

TABLE 4

Eye findings at necropsy of seven cynomolgus monkeys inoculated with *M. leprae*

Group	Monkey	Sample (eye)	Years post- <i>M. leprae</i> inoculation	Gross	Histology	
					Globe	Orbit
1	4	One	19	Normal	Normal	Follicular conjunctivitis; small foci chronic inflammation in extraocular muscle, some surrounding peripheral nerves
2	9	Two	19	Right: corneal opacity, cataract; left: normal	Right: cornea scarred, neovascularized; chronic inflammation in ciliary body, cornea, iris, anterior sclera, posterior choroid; plasma cells in ciliary body and iris pigment epithelium; iris neovascularization with angle closure; one iris leaflet necrotic; chronic retinal detachment; left: normal	Right: normal; left: chronic non-specific inflammation of conjunctiva; small foci of chronic, non-specific perivascular orbital inflammation
2	10	One	19	Normal	Normal	Chronic non-specific inflammation of conjunctiva
4	16	One	18	Normal	Small foci of chronic non-specific inflammation in iris, choroid body, and anterior choroid; modified Zeehl-Neelsen AFB(-) stain	Chronic non-specific inflammation of conjunctiva; focal fat necrosis scattered throughout
5	22	Two	17	Right and left: normal	Right and left: normal	Right and left: chronic non-specific inflammation of conjunctiva and dermis
6	23	One	15	Normal	Normal	Normal
7	24	One	8	Poorly fixed, no interpretation	Mixed chronic inflammation of iris, iris sphincter muscle, ciliary body; modified Zeehl-Neelsen AFB(-) stain	Chronic non-specific dermal inflammation; focal fat necrosis scattered throughout

FIGURE 6. Lepromin (Mitsuda) skin test reactivity in five monkeys conducted before *M. leprae* administration (black bars); three of five monkeys were retested up to 6 years after *M. leprae* inoculation (red bars). All eight tests were reactive. Among six reaction site biopsies, all except monkey 24 (group 7) were histologically consistent with lepromin reactivity, which is denoted as Bx+ and Bx-. Monkey 24, on necropsy at 8 years after *M. leprae* administration, showed histological features of borderline leprosy at multiple anatomic sites (described in Figures 4D and 5D).

Cynomolgus monkeys 2, 7, 11, and 24 (groups 1, 2, 3, and 7, respectively; all IV + ID) developed evanescent papules at ID *M. leprae* inoculation sites and died 2–8 years after *M. leprae* administration with no skin lesions or gross neurological deficits but persistently elevated anti-PGL-1 antibody; for three of the monkeys, AFB(+) nasal smears suggested a colonized-like status with *M. leprae*. On necropsy, all four monkeys showed histological features suggestive of leprosy in various organs, including AFB in earlobes, a scrotal sac, and an ulnar nerve. In three of four monkeys, AFB(+) nasal smears ($\geq 2+$), noted 3–8 years after *M. leprae* administration, and necropsy results suggested dissemination to nasal mucosa, akin to what may occur in human leprosy.¹ The significance of a colonized, subclinical-like state in these monkeys was unclear.

Lepromin skin test reactivity in monkeys may be a marker for leprosy susceptibility. Lepromin reactivity in 8 of 10 tested cynomolgus monkeys, suggesting a leprosy-resistant phenotype, paralleled our findings. Among monkeys 2, 7, 11, and 24 (groups 1, 2, 3, and 7, respectively), all with subclinical-like infection after *M. leprae* administration, monkey 24 lacked histological features consistent with reactivity at lepromin test sites; this finding suggested that lepromin reactivity in cynomolgus monkeys may have some usefulness in predicting leprosy susceptibility. Monkeys 2, 7, and 11 were not lepromin tested, precluding additional comment.

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