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ANIMAL MODELS FOR MULTIBACILLARY LEPROSY

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I. INTRODUCTION

Even though there have been outstanding advances in many areas of research in leprosy in recent years, there remains an urgent need for better animal models for studying the disease. The ideal model would be an immunologically unaltered animal that would manifest the entire spectrum of clinical forms of leprosy, reactional episodes, and peripheral neuritis with the deformities seen regularly in humans. No reported animal model satisfies these requirements. Most studies in the chemotherapy, epidemiology and pathogenesis of leprosy in humans have focused on the patient with multibacillary leprosy. Because of the importance of these areas of interest, this discussion will emphasize studies on those animals that have potential as models for multibacillary leprosy, i.e., borderline-lepromatous (BL) to polar lepromatous (LL) in the Ridley-Jopling (1966) system of the classification of leprosy.

II. HISTORICAL BACKGROUND

On February 28, 1873, Hansen took "magnificent nodules" from the alae nasi of patient Jobs Gil, scraped the cut surface of the nodules with a knife, and observed brown rod-shaped bodies in wet unstained

amounts of the tissue fluid thus obtained (Hansen, 1880). Although a transmissible etiologic agent of no other chronic disease of humans had yet been reported, and contrary to the prevailing view that leprosy was inherited, Hansen believed that leprosy was infectious and courageously announced that these rods were the cause. He undertook two avenues of study on this agent to prove his hypothesis: (1) he tried to grow the organism in artifical media, and (2) he tried to induce the disease in animals by the direct inoculation of animals. He was unsuccessful in both endeavors. During the next eighty years there were numerous additional attempts to transmit leprosy to many animal species, including cats, rabbits, monkeys, dogs, guinea pigs, rats and hamsters. Occasionally success was claimed; for example, Soule and McKinley, in 1932, believed that they had produced rapidly progressive leprosy in monkeys, when, in fact, they had merely elicited a spectacular Mitsuda reaction.

There was no rationale for the selection of species of animals or the site of inoculation of these experimental animals. In 1956, at the First Carville Conference on Progress and Potentials in Leprosy Research, Binford "elaborated the numerous details which support the thesis that in man the leprosy bacillus has a natural preference for anatomic sites of lower body temperatures and he expanded upon methods of applying temperature selection to animal experimentation." On making the observation that temperature might influence the growth of the leprosy bacillus, Binford began large scale studies in the ears and testes of animals, notably hamsters, and his findings were first reported in 1959. Further experiments, reported in 1965, demonstrated that there was regular (20 out of 21 experiments) invasion of nerves in the ears of hamster by *Mycobacterium leprae*; however, dissemination was never observed.

In 1960, Shepard reported the growth of *M. leprae* in the footpads of normal mice. This model, because of its reproducibility, has been highly useful in the detection of the viability of *M. leprae*, screening of antileprosy drugs, the detection of drug-resistant organisms, and studies on the antigenic properties of *M. leprae*. Rees *et al* (1967) produced disseminated leprosy in thymectomized and irradiated mice, but without immunologic manipulation or genetic deficiencies, the infection in mice is localized and self-healing, and does not resemble any of the established forms of leprosy in humans.

III. Animal models of disseminated leprosy

1. Armadillos

A. *Nine-banded armadillos* (Dasypus novemcinctus)

Storrs, while at the Gulf South Research Institute (GSRI), New Iberia, Louisiana, introduced the nine-banded armadillo into leprosy research. Storrs was impressed by the emphasis being placed on the hypothesis that *M. leprae* grew best in the cooler areas of the human body, and in experimental animals. Knowing that the core body temperature of the nine-banded armadillo was 30-35°C, she undertook studies on the transmission of leprosy in this animal (Storrs, 1971). In the initial study, four armadillos were inoculated intradermally in the abdomen and ears with a suspension of *M. leprae*. Approximately 15 months later, infiltrated lesions appeared at the sites of inoculation. Histopathologically, the infiltrations resembled lepromatous leprosy and there was invasion of nerves (Kirchheimer and Storrs, 1971). At necropsy there was widespread disease with extensive involvement of the lymph nodes, liver, spleen, lungs, bone marrow, meninges and other tissues. The infection was much heavier than usually seen in humans (Kirchheimer *et al*, 1972).

In the early studies at GSRI (1970-71), of 59 nine-banded armadillos inoculated, all intracutaneously, 24 (41%) developed disseminated leprosy. The salutary effect that this discovery had on research in leprosy is well known to medical science; however, it is amazing how little systematic research there has been on the development of the nine-banded armadillo as a model for the investigation of the pathogenesis, immunology, epidemiology and chemotherapy of leprosy.

Minimal infective doses of *M. leprae* have not been established, but the route of infection is important. Kirchheimer (1978) reported that 93% of armadillos receiving 10^8 *M. leprae* intravenously had disseminated leprosy within 550 days. Walsh (1978) points out that armadillos inoculated intravenously succumb to disseminated disease much earlier than those inoculated intracutaneously. In our recent experience at the AFIP, animals inoculated with 10^8 organisms intravenously develop heavy infections of the liver, spleen, and lymph nodes, but only small quantities of subcutaneous leproma. Animals

frequently become obtunded at 12 months. Approximately 90% of the animals that survive up to 15 months develop disseminated leprosy.

Apart from the low body temperature, the factors that render this animal susceptible to leprosy are unknown. Morphologically, the lymphoreticular system is intact and well-developed (Purtilo *et al*, 1975); however, lymphocytic function appears to be impaired at the body temperature of the armadillo (Purtilo *et al*, 1974). Lysozyme levels are low (Rea *et al*, 1979).

The Concanavalin-A responsiveness of mononuclear cells from armadillos is suppressed by antigens of *M. leprae* (Shannon *et al*, 1984) in a similar manner to the induced suppressor cell activity in patients with leprosy (Mehra *et al*, 1979). With rare exception (Job *et al*, 1982), only lepromatous leprosy has been observed in nine-banded armadillos. In clinical and histopathologic evaluations of more than 600 infected nine-banded armadillos, we have never observed a delayed-type hypersensitivity reaction to *M. leprae*, nor have we ever seen spontaneous regression of the disease. *D. novemcinctus* in South America appear to be more resistant than those from North America to infection by *M. leprae*. Opromolla *et al* (1980), however, have reported limited success in infecting this species in Brazil.

B. *Seven-banded armadillos* (Dasypus hybridus)

Only one study has been reported in which *Dasypus hybridus* was experimentally infected with *M. leprae*. Storrs *et al* (1975) inoculated each of two animals that originated from Argentina with 3.4×10^7 *M. leprae* intracutaneously at two sites on the abdomen. One of the armadillos developed a nodule at a site of injection at 14 months and the animal was killed at 24 months. There were lepromatous infiltrations in the skin, sciatic nerve, liver, spleen, and bone marrow. The remaining animal was clinically free of disease 13 months post-inoculation.

This armadillo regularly produces 8-16 monozygous offspring and should be an excellent model for the investigation of genetic influences on susceptibility and pathogenesis, but to our knowledge has not been further utilized.

C. *Eight-banded armadillos* (Dasypus sabanicola)

The eight-banded armadillo inhabits the savanna areas of Venezuela. Convit *et al* (1978) have reported the results of inoculation

of 93 *D. sabanicola* with *M. leprae*. A total of 35 animals developed clinical disease. Lesions appeared in several animals, and one armadillo had a hypopigmented area on the abdomen. There was histopathologic evidence of delayed-type hypersensitivity granulomas in some animals; however, in most, the disease was smiliar to that seen in *D. novemcinctus*. The potential of *D. sabanicola* for the study of its ability to produce lesions covering the spectrum of the forms of leprosy has not, to our knowledge, been pursued.

2. Primates

In the late 19th century, there were repeated unsuccessful attempts to infect monkeys and chimpanzees (e.g., Nicolle, 1905; Marchoux and Bourret, 1908). Collier, in 1940, claimed successful transmission of leprosy in monkeys fed a diet of the tuber *Colocassia antiquorum*, but Cochrane (1947) could not confirm this finding. In Malaysia, in 1976, Waters *et al* (1978) necropsied a white-handed gibbon that they had inoculated with *M. leprae* in 1961. Although there was no clinical evidence of disease, histopathologically, there were early disseminated lepromatous infiltrations.

A. *Chimpanzee*

Gunders, in 1958, reported findings in a chimpanzee he had inoculated intravenously with *M. leprae* in Liberia. At 11 months there were nodules rich in acid-fast bacilli in the skin of the extremities and ears. When last observed, 14 months post-inoculation, these lesions were regressing. From our own evaluation of tissues on file at the AFIP, we interpret the disease in this animal as borderline leprosy (BB-BL). We believe this is the first well-documented experimental disseminated infection of an animal by *M. leprae*.

In 1965, Binford began collaborative studies with the Delta Regional Primate Research Center, Covington, Louisiana, on the transmission of leprosy to chimpanzees by the intradermal and intravenous inoculation of large numbers of *M. leprae*. A total of 24 chimpanzees were inoculated. Among these were two young animals born in the chimpanzee colony. These animals received suspensions of *M. leprae* intravenously and intraperitoneally shortly after birth, in an attempt to induce tolerance. After six months the animals were inoculated with *M. leprae*. Approximately one year later, lesions

developed at inoculation sites on the ear and lower forearm. In one animal, borderline leprosy was diagnosed histopathologically, and there were acid-fast bacilli in histiocytes and in small nerves. In the other animal, a histopathologic diagnosis of tuberculoid leprosy was made. There was intraneural involvement. Within six months, the lesions in both animals had healed, and the animals were lepromin positive.

There were no further reported studies on leprosy in the chimpanzee until 1977, when Donham and Leininger detected naturally-acquired leprosy in an animal imported from Sierra Leone. They studied this chimpanzee extensively, and the clinical, microbiologic and histopathologic features were those of borderline-lepromatous leprosy. The etiologic agent could not be differentiated from *M. leprae* (Leininger *et al*, 1978), and at necropsy there was wide dissemination of the disease (Leininger *et al*, 1980). Acid-fast bacilli from this animal were inoculated into a number of other chimpanzees in 1976 and 1977. These animals remain under observation, but have no lesions (Leininger, 1983).

B. *Mangabey monkey*

In December 1979, in collaboration with George Imes, D. V. M., of the Veterinary Pathology Department of the AFIP, we made a histopathologic diagnosis of lepromatous leprosy (LL-BL) in a biopsy specimen of skin from the muzzle of a young adult female sooty mangabey monkey (*Cercocebus atys*), then housed at GSRI (Walsh *et al*, 1981; Binford *et al*, 1982). The animal, imported in 1975, originated in West Africa and was on dietary cholesterol studies. She had never been experimentally inoculated with *M. leprae*. Thirteen months after diagnosis, there was extensive progression of the cutaneous lesions over the face, ears, limbs and tail, and there were paralytic deformities of the hands and feet.

The etiologic agent in this index animals was undistinguishable from *M. leprae* (Meyers *et al*, 1980). The animal's general health deteriorated, and combined therapy with rifampin and dapsone was started 14 months after diagnosis. Clinical and histopathologic responses to therapy were good, and the animal is alive and well today.

We transmitted this disease to other sooty mangabey monkeys. Initially, we inoculated two male mangabeys with suspensions of organisms separated from tissues of the index monkey. Each animal received 3×10^8 bacilli into each of 5 sites in the skin of the ears and

muzzle, and 1.2×10^9 bacilli intravenously. Nodules were first noted at the inoculation sites 4 months later. By 17 months, these nodules had enlarged and there was dissemination to uninoculated surfaces of the body, including the limbs and tail, and particularly the scrotum. Histopathologic changes in the scrotum included extensive infiltrations of macrophages containing large numbers of acid-fast bacilli. There were occasional patches of lymphocytes, but most of these were associated with intracutaneous lymphoid nodules rather than delayed-type hypersensitivity granulomas. Acid-fast bacilli invaded the smooth muscle of the scrotal wall and nerves.

One of these animals died unexpectedly at 46 months post-inoculation. Death followed anesthesia. At necropsy there were extensive lepromatous infiltrations of the skin of the face, ears, front and hind limbs, tail, scrotum and testes. Peroneal nerves were enlarged. Histopathologic analysis revealed extensive lepromatous infiltrations at all these sites, including nearly complete replacement of the peroneal nerve. Inguinal lymph nodes contained large numbers of acid-fast bacilli in histiocytes, and the paracortical areas of peripheral lymph nodes were largely replaced by bacilli-laden histiocytes. The liver and spleen showed minimal infiltration. Thus, the pathologic changes in this animal closely resemble those seen in humans with early advanced lepromatous leprosy. The second mangabey monkey, inoculated in March 1980, is now under chemotherapy, and is responding favorably. Two sooty mangabey monkeys were inoculated in December 1980 with suspensions of *M. leprae* of human origin passaged once in armadillos. Inoculations were by the intravenous and intracutaneous routes. Thirty-two months after inoculation, there is active progressive disease at all inoculation sites, and both animals have acid-fast bacilli in nasal smears. Histopathologically, the nodules in the skin of both animals are in the subpolar lepromatous (LLs) area of the spectrum of the disease.

Twenty-two mangabey monkeys have been inoculated with, or otherwise exposed to, *M. leprae*. Clinical, histopathologic and immunologic observations indicate that leprosy in this species simulates lepromatous leprosy in humans in most respects tested. Mangabey monkeys with advanced lepromatous leprosy show declining responses to Concanavalin-A, and there is an associated increase in suppressor T-cells (OKT8).

The sooty mangabey monkey seems to offer the best promise as an ideal model for multibacillary leprosy. Although only preliminary observations are available, the following clinical features are noted:

bacteremia, dissemination to cool area of the body, variable clinical forms of the disease, and neuropathic deformities. There has been a favorable response to chemotherapy. Harboe (1981) has suggested that the mangabey monkey may serve as a suitable model for testing the efficacy of candidate vaccine for leprosy, but these studies have not yet been possible. The longevity of 20.5 years (Napier and Napier, 1967) for mangabey monkeys in captivity would make long-term observations possible. Mangabey monkeys breed readily in captivity.

C. *Rhesus monkeys*

In one of two rhesus monkeys (*Macaca mulatta*), leprosy developed 14 months after the intravenous and intradermal inoculation of large numbers of *M. leprae*. The disease in the early stages resembled borderline leprosy, but now, at 30 months post-inoculation, the disease is near to polar lepromatous with wide dissemination. Eighteen rhesus monkeys have now been inoculated with *M. leprae*. Two of the additional animals show early dissemination of the disease.

D. *African green monkeys*

Three African green monkeys (*Cercopithecus aethiops*) were inoculated intravenously and intradermally with large doses of *M. leprae*. Nodules developed on the ears of all three animals, beginning approximately 3-25 months after inoculation. Smears from the nasal mucosa of all the animals contain acid-fast bacilli. The histopathologic changes in the nodules on the ears are those of lepromatous leprosy. One of the animals has widely disseminated disease.

3. ATHYMIC RODENTS

A. *Nude mouse*

Prabhakaran *et al*, in 1975, were the first to study the growth of *M. leprae* following inoculation into the footpad of the nude mouse (nu/nu). They concluded that such mice did not develop generalized infection, but had observed the animals for only six months post-inoculation. Colston and Hilson (1976), however, continued their observations on the growth and dissemination of *M. leprae* for up to 322 days. At the end of this period the inoculated hind footpads contained 10^9 organisms and there was dissemination to testes, nose,

tail, forepaws, liver and spleen. There was no dissemination in nu/+ littermates and the footpads contained only 10^6 organisms. Kohsaka *et al*, and Nakamura and Yogi, in 1979, further developed the nude mouse model under specific pathogen-free conditions and were able to maintain the animals for up to 22 months. They noted dissemination to lung, liver and spleen.

Hastings *et al*, in 1980, showed the regular spread of *M. leprae* infections in nu/nu mice to the liver and spleen, between approximately 100 and 280 days. Job *et al* (1982) reported their observations in nu/nu mice for up to 565 days after inoculation of *M. leprae* into the hind footpad. There was dissemination from day 273, and at 565 days there were lepromatous infiltrations in all organs except the brain. Despite high body temperatures, there is marked proliferation in the viscera, even in the parenchymatous cells of liver, kidney and other organs and tissues. Preliminary studies suggest that the therapeutic efficacy of DDS in nude mice may be variable (Kohsaka *et al*, 1981).

B. *Nude rat*

Fieldsteel and coworkers (1971, 1976, 1980, 1981) have developed the neonatally thymectomized Lewis rat as a model of multibacillary leprosy, and later extended their studies to congenitally athymic rats. *M. leprae* infections in thymectomized rats proved to be unpredictable; however, infections in congenitally athymic rats were more uniform. There was dissemination of the infection beginning about 8 months after inoculation into the footpad (Dawson *et al*, 1983). Dissemination was limited to the cooler parts of the rat (tail, footpads, snout and ears), peripheral lymph nodes and bone marrow. There were a few AFB in the liver, but these disappeared at about 15 months post-inoculation. Cutaneous nerves contain small numbers of organisms, but the sciatic nerve is not affected. Of particular interest is the limitation of the infection in the congenitally athymic rat, even though thymic-dependent T-cell function is lacking. This host has been relatively little studied, and further observations are needed.

IV. Summary

Disseminated multibacillary leprosy has been reported in unaltered subjects in three species of armadillos, chimpanzees, sooty mangabey monkeys, rhesus monkeys, African green monkeys, nude rats and nude

mice. The chimpanzee has not been shown to be regularly susceptible, and, thus, requires much more study to establish its potential usefulness. Armadillos, by virtue of their accessibility, at least in the Western Hemisphere, have great potential for experimentation, but their usefulness in nearly all areas of experimentation remains almost untested. Nude mice and nude rats have the advantage of being readily available to appropriately equipped laboratories; nevertheless, husbandry is tedious, maintenance is expensive, and these animals are relatively short lived. Infections in nude mice appear to be overwhelming and are, like those in the armadillo, usually more severe than in most leprosy patients. Moreover, these rodents, although not artificially altered, have an established genetic immunologic deficiency, in contrast to the immune system of individuals susceptible to leprosy. The mangabey monkey, although still in an early stage of experimentation, appears to offer great promise today as a model of many of the clinical manifestations of leprosy. The most important disadvantages are: the relatively short supply of this animal and the expense of maintenance. An overriding advantage is the close species comparability to humans.

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