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LEPROSY AS A ZOONOSIS

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Historically, leprosy has been considered a disease confined to humans and the patient with lepromatous leprosy the sole source of *Mycobacterium leprae*. In the last ten years we have studied naturally-acquired leprosy in three different animal species: the nine-banded armadillo (*Dasypus novemcinctus*), a chimpanzee (*Pan troglodytes*), and a mangabey monkey (*Cercocebus atys*). These studies demonstrate unequivocably that animal reservoirs of the disease exist and enzootic infection could be a significant factor in epidemiologic studies in areas where human leprosy is endemic.

NATURALLY-ACQUIRED LEPROSY IN NINE-BANDED ARMADILLOS

Background and present status

The discovery in 1971 of the susceptibility of the nine-banded armadillo to leprosy provided a laboratory animal model for the study of experimental lepromatous leprosy [28]. Armadillos inoculated with

M. leprae develop lesions as early as 6 months after inoculation, and by 18 months, dissemination to all major tissues and organs has occurred [29]. The bacillary load, the extent of the infection, and the absence of any detectable cell-mediated immune response in the majority of inoculated animals indicate that armadillos are more susceptible to leprosy than humans [32]. We believe that the hypersusceptibility of the armadillo is responsible to a large degree for the development and perpetuation of naturally-acquired leprosy in wild-caught armadillos.

The first armadillo with naturally-acquired leprosy was found in 1974 while collecting tissues from normal, recently captured animals. The only gross pathologic change in this animal was enlarged inguinal lymph nodes. Tissue smears of these nodes contained large numbers of acid-fast bacilli (AFB) which were later found to be non-cultivable on mycobacterial media. Histopathologic examination of formalin-fixed tissues revealed a disseminated mycobacteriosis indistinguishable from that seen in armadillos experimentally infected with *M. leprae*.

In the ensuing 12 months, a total of 7 armadillos captured in three different locations in southern Louisiana were found with the disease [30]. Later studies of large numbers of animals from Louisiana showed prevalence rates ranging from 4% to 29.6% [31].

To date we have found a total of 105 armadillos with naturally-acquired leprosy, 104 of which were captured in Louisiana and one was found in northeast Texas. Most of the positive sites in Louisiana are swampy areas which have dense foliage and harbor large numbers of insects.

Fourteen of the animals had clinically apparent disease at the time of capture. The remaining 91 were detected by ear specimen examination.

Naturally-infected armadillos have been reported by four other centers. In 1976 a naturally-infected armadillo was found near Natchez, Mississippi [11]; Smith *et al* in 1978 reported the disease in two armadillos in southern Louisiana [26]; in 1978 two armadillos with naturally-acquired leprosy captured near College Station, Texas, were reported [1, 5]; and Smith *et al* in 1983 [27] completed an extensive survey of armadillos captured along the Texas Gulf Coast and found 21 naturally-infected armadillos among 451 animals examined, for an overall prevalence of 4.6%. The local prevalence ranged from 1.0% to 15.4%, and the authors concluded that because the rates of infectivity were highest in southern Texas, it was unlikely that the disease had originated from any of the research centers in Louisiana where armadillos were being used in leprosy studies.

Because naturally-acquired leprosy had never been diagnosed in any animal species, great emphasis was placed on confirming the identification of the etiologic agent isolated from infected armadillos. In 1968 at the 8th International Leprosy Congress, several criteria were proposed for identifying an isolate as *M. leprae*, and since then more have been added. These criteria have been used to identify the agent causing natural armadillo leprosy. These studies have been reported in detail and are only summarized here:

a. *Histopathology:* Large numbers of AFB, frequently arranged in packets or globi, staining more intensely with the Fite-Faraco stain than with the Ziehl-Neelsen method, and the invasion of peripheral nerves, all are consistent with the identification of the organism as *M. leprae* [3].

b. *Microbiology:* The etiologic agent is not cultivable on standard mycobacterial media even though several cultivable species of mycobacteria (*M. avium-intracellulare, M. scrofulaceum*, and *M. gordonae*) were isolated, primarily from the lymph nodes of a small number of animals [3, 27]. Acid-fastness is pyridine extractable [3]. Base ratios and DNA homology with *M. leprae* show that the etiologic agent is identical to the leprosy bacillus [27]. The natural agent, like *M. leprae*, is unusually sensitive to Dapsone [12].

c. *Immunology:* Lepromin prepared from tissues of naturally-infected armadillos, and standard human lepromin gave similar reactions in leprosy patients [19]; concentrations of antibodies to mycobacterial antigens 2, 5, and 7 in sera of naturally-infected armadillos are similar to those seen in experimentally-infected armadillos and in lepromatous patients [13]. Fluorescent antibody studies show that the natural agent is indistinguishable from *M. leprae.*

d. *Animal passage:* Normal armadillos inoculated with the natural agent developed disseminated leprosy [33]. The growth patterns in the mouse footpad were similar to *M. leprae* [20].

e. *Ultrastructural studies:* Freeze-etch and thin-section preparations revealed no differences between tissues from armadillos with the natural infection and those with experimental leprosy [18].

The results of all these studies thus demonstrate that the organism isolated from armadillos with naturally-acquired leprosy is *M. leprae.*

Origin and significance

Armadillos have not been domesticated and it is unlikely that they acquired leprosy directly from lepromatous patients; however, contact with contaminated fomites such as clothing and bandages must be considered as a possible source of the infection. Such contact would have been possible in the pre-sulfone era, when patients living at home in rural areas would have discarded such items. Leprosy bacilli have been shown to remain viable in dried nasal secretions for up to 7 days [6] and in moist soil at room temperature for up to 46 days [25]. Because armadillos commonly encounter insects, these also must be considered as sources of the infection in armadillos.

Once introduced into the armadillo population, animal-to-animal transfer of leprosy, either by direct contact or by inhalation of contaminated soil in burrows, could have resulted in the disease becoming endemic in this species. In addition, we have detected *M. leprae* in the milk and mammary glands of lactating armadillos, so transmission via mother's milk to newborn armadillos is a definite possibility [21].

Leprosy in humans has been endemic in Louisiana for more than 150 years [9]. Armadillos migrated into the state in 1926 and therefore are not responsible for introducing leprosy into Louisiana. A study by the Centers for Disease Control published in 1977 failed to show any association between human leprosy patients and contact with armadillos [10]. However, a report in 1983 by researchers at Baylor College of Medicine implicated armadillo contact as the most likely source of the infection in five patients diagnosed with the disease [16]. A sixth patient has been found since the publication of that report [17]. In the United States, indigenous human leprosy is concentrated in two states: Louisiana and Texas. In Louisiana, of the 38 cases reported in the period 1967-1976, 34 (or 89%) were native-born individuals [8]. In 1980, of the 32 cases reported in Texas, 15 (or 47%) occurred in native-born persons [2]. By contrast, California reported 91 cases in 1980, of which only 1 was from the indigenous population.

The prevalence of the disease in armadillos in Louisiana, and the more recent report of prevalences as high as 15.4% in armadillos captured in Texas, require that armadillos be considered as a reservoir of the disease in these areas.

Naturally-Acquired Leprosy in a Chimpanzee

Background

Naturally-acquired leprosy in a nonhuman primate, a chimpanzee, was first reported in 1977 [7]. The animal was obtained from a primate import company who had purchased it from local trappers in Sierra Leone. Two months after arriving at the University of Iowa, the animal was inoculated, along with 7 other chimpanzees, with bovine leukemia virus. Two months after the inoculation, lesions appeared and spread over the entire trunk and limbs of the animal and within 6 months, the ears had become markedly thickened. A biopsy specimen of the ear revealed a diffuse infiltration of macrophages containing large numbers of AFB. In the 14 months that followed, lesions developed on the lower lip, nares, eyebrows, and scrotum. AFB were present in peripheral nerves, and nasal smears contained large numbers of acid-fast organisms.

Thirty-three months after the appearance of the initial lesions, the animal died following anesthesia for a routine surgical procedure. Necropsy revealed disseminated leprosy involving the liver, spleen, lymph nodes, testes, lungs, eyes, and nasal mucosa [15]. Microbiological studies confirmed the identification of the AFB as *M. leprae* [14].

Source and significance

We speculate that this chimpanzee acquired the infection from a patient with multibacillary leprosy. In some African countries, chimpanzee mothers are killed and the young animals are raised by villagers until they are sold to animal exporters. Human leprosy is highly endemic in Sierra Leone and it is possible that this animal was in close contact with patients with untreated lepromatous disease prior to export to the United States. It is very unlikely that exposure to leprosy occurred after arrival in the United States.

It has not been possible to establish any relationship between the inoculation of bovine leukemia virus and the development of leprosy in this animal. All of the chimpanzees in the study had serological evidence of infection by the virus. It is possible that the experimental virus infection could have compromised the animal immunologically, permitting a latent leprosy infection to progress.

The frequency of naturally-acquired leprosy in chimpanzees in the wild is not known, and therefore it is not possible to determine the potential role of chimpanzees in the epidemiology of leprosy in humans.

Chimpanzees are found throughout the tropical rain forests of Africa, where there are numerous leprosy-endemic areas, and the information that natural leprosy can occur in this species could be of great importance. In earlier studies, chimpanzees inoculated with *M. leprae* did not develop progressive disease [4]. The discovery of naturally-acquired leprosy in a chimpanzee suggests that the susceptibility of this species should be re-evaluated, using inocula containing large numbers of viable *M. leprae* such as those prepared from leprosy-infected armadillo tissues.

Naturally-Acquired Leprosy in a Mangabey Monkey

Background

This animal, commonly known as a "sooty" mangabey monkey, was imported to the United States from West Africa in 1975. The first lesions of leprosy were seen in September 1979, consisting of firm nodules on the face and ears. It was never inoculated with *M. leprae* and was on a cholesterol metabolism study at the time the disease became apparent.

Four months later, following a histopathologic diagnosis of lepromatous leprosy made at the Armed Forces Institute of Pathology in Washington DC, the animal was transferred from the Gulf South Research Institute in New Iberia, Louisiana, to the Delta Regional Primate Research Center, Covington, Louisiana. At that time, the face was heavily infiltrated and many of the lesions were ulcerated. There was nodular thickening of the ears and on the extensor surfaces of the forearms. Peripheral nerves were not enlarged, and no paralytic deformities were detectable at that time.

Microbiologic, immunologic, histopathologic, and electron microscopic studies have shown that the infecting organism is *M. leprae*. Details of these studies have been published [22, 23, 24] and are only summarized here.

a. *Microbiology:* Non-cultivable AFB with loss of acid-fastness after treatment with pyridine; oxidation of D-DOPA; 100% homology with DNA from *M. leprae*.

b. *Immunology:* Presence of antibodies to mycobacterial antigens 2, 5, and 7 in concentrations similar to those seen in lepromatous patients and experimentally-infected armadillos.

c. *Histopathology:* Presence of histiocytes containing AFB frequently arranged in packets or globi, with invasion of dermal nerves by AFB; the AFB stain more intensely with Fite-Faraco stain than with the Ziehl-Neelsen technique.

d. *Electron microscopy:* Demonstration of macrophages containing foamy structures composed of bacilli surrounded by spherical droplets.

Paralytic deformities

Approximately 16 months after the cutaneous lesions were first seen, paralytic deformities developed in this mangabey monkey. The intrinsic muscles of the foot and hand, as well as the peroneal muscles of the foot, were paralyzed, with accompanying deformities. To our knowledge, this is the first leprosy-infected animal that has manifested paralytic deformities like those seen in human leprosy.

Response to therapy

Soon after the deformities were observed, the general health of the mangabey deteriorated. Treatment was started with Rifampin (RFM) at 10 mg/kg *per os* for a period of 28 days, followed by injection of a repository form of Dapsone (DADDS) at a dosage of 20 mg every 77 days. Within 30 days after the start of treatment, the general health of the animal had markedly improved, and smears from skin lesions and the nasal mucosa contained no solidly-staining AFB. Bacilli taken from lesions at this time failed to multiply in the mouse footpad, confirming effective chemotherapy. Over the ensuing 12-16 months, there was gradual resolution of the lesions. After that time, however, there was a gradual re-exacerbation of the disease, and organisms isolated from a persistent lesion below one of the nares multiplied in the mouse footpad. RFM therapy was re-instituted, with a favorable response. Drug sensitivity studies have shown that the mangabey isolate is partially resistant to Dapsone [24].

Passage studies in normal mangabeys and other nonhuman primates

Leprosy has been transmitted to normal mangabeys inoculated with either the mangabey isolate or with human *M. leprae* passaged in

the armadillo. Rhesus monkeys and African green monkeys have also been found to be susceptible to leprosy. It appears at this time, however, that mangabeys may prove to be the most suitable model of all the species studied thus far.

Significance of spontaneous leprosy in a mangabey monkey

The discovery of naturally-acquired leprosy in a mangabey provides additional evidence that leprosy can exist in nonhuman species and is not exclusively a disease of humans. While it is reasonable to assume that this animal was infected following contact with an untreated lepromatous patient, the possibility that it became infected following contact with another mangabey with lepromatous leprosy cannot be excluded. The results of the passage studies showing that mangabeys as a species are susceptible to leprosy lend credence to this possibility.

The finding that normal mangabeys as well as other nonhuman primates are susceptible to leprosy is of great significance. Studies of the susceptibility of mangabeys and other nonhuman primate species were prompted by the discovery of naturally-acquired leprosy in a mangabey monkey, and the information obtained so far indicates that primates can now be used as experimental models of this disease.

SUMMARY

We have studied naturally-acquired leprosy in three animal species. The prevalence of the infection in wild armadillos in the southern United States necessitates that armadillos be designated as reservoirs of the disease in this area. Although the prevalence of leprosy in chimpanzees and mangabey monkeys in the wild is not known, the discovery of naturally-acquired leprosy in these species requires that they be considered as possible reservoirs of the disease in geographic areas where these species are found. The infection in all three species has been of the lepromatous or near-lepromatous type and therefore is highly bacilliferous and contagious. The role that each species may play in the transmission of leprosy to man must now be ascertained.

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