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Experimental Transmission of Human Leprosy Bacilli in Foot Pads of Severe Combined Immunodeficient Mice

TO THE EDITOR:

After the discovery of *Mycobacterium leprae* as the etiologic agent of human leprosy, it soon became clear that this mycobacterium cannot be grown *in vitro*. Hence, the search for a suitable animal model began. Animal models of leprosy used by investigators between 1879 and 1986 have been reviewed by Johnstone ([1]). Of the several animal models so far employed, only armadillos and nude mice are currently used for the production of *M. leprae* to be used in all fields of leprosy research. After infection, the maintenance of these animals for 12–18 months under controlled conditions is quite expensive. Recently, a mouse with severe combined immunodeficiency (SCID) reconstituted with human peripheral blood leukocytes has been developed ([4]). In an attempt to determine if SCID mice are susceptible to human leprosy and whether higher yields of *M. leprae* can be obtained in a relatively shorter period of time, studies on the transmission of human leprosy to SCID mice were carried out.

A bacillary suspension of *M. leprae* containing 1×10^8/ml acid-fast bacilli (AFB) was prepared from a foot-pad lesion of nude mice previously infected with human leprosy bacilli. Three groups of 10 SCID mice (females, 6 weeks of age) were inoculated in the hind foot pads with a 20 μl bacillary suspension containing 1×10^5, 1×10^6 and 1×10^7 bacilli. In parallel, three groups of 10 nude mice (as controls) were also infected the same way. Both types of mice were kept at 22°C in the same specific pathogen free vinyl plastic isolator. Food, water (*ad libitum*) and bedding after sterilization were provided under aseptic conditions. Following the inoculation of the foot pads with *M. leprae* both SCID and nude mice were sacrificed at various time intervals and AFB were counted according to the method of Shepard and McRae ([5]).

Regardless of the number of bacilli used in the inocula, about 5 months' postinfection a slight swelling in all foot pads of both types of mice started to appear; although more visible in SCID mice. The swelling gradually continued and became quite apparent after 7 to 8 months of infection. Our results have shown that in the foot pads of SCID mice infected with 1×10^5, 1×10^6 and 1×10^7 AFB maximum yields of 1.2×10^8, 4.3×10^8 and 9.0×10^8 bacilli were found after 11, 9 and 8 months of infection, respectively. Thereafter, the number of bacilli gradually decreased upon further incubation, and only some degenerated bacilli were found at the inoculation site after 15 months of incubation. In the foot pads of nude mice infected with 1×10^5 and 1×10^6 bacilli, at 10 months' postinfection 7.8×10^7 and 2.5×10^8 bacilli/foot pad were obtained, respectively. These results show that up to 10 months postinfection the total number of bacilli in the foot pads of nude mice were lower than estimated in the foot pads of SCID mice. However, in the foot pads of nude mice multiplication of *M. leprae* continued progressively at all three inocula used and about 12 months postinfection remarkable swelling of the infected foot

pads was noted. In the foot pads of nude mice infected with 1×10^5, 1×10^6 and 1×10^7 bacilli, maximum yields of 1.7×10^{10}, 2.0×10^{10} and 2.1×10^{10} bacilli per foot pad were estimated, respectively, after 13, 12 and 11 months of infection. Dissemination of *M. leprae* in foot pads of nude mice is well established ([2, 3]). These results of a comparative study show that, like nude mice, SCID mice were also susceptible to *M. leprae* infection and the onset of the lepromatoid lesions in the foot pads of SCID mice occurred earlier than that observed in the nude mice. However, an interesting aspect of this study is that the progress of *M. leprae* infection in SCID mice is different than the progress observed in the nude mice. Although a rapid multiplication of *M. leprae* occurred in the foot pads of SCID mice after reaching a maximum of about 4 to 9 $\times 10^8$ bacilli/foot pad, the number of bacilli decreased upon further incubation and eventually, about 15 months' postinfection, only a few degenerated bacilli were found at the site of infection and there was no sign of dissemination. On the other hand, the number of bacilli in the foot pads of nude mice after reaching about 1.5 to 2.0×10^{10}/foot pad remained nearly the same up to 15 months of incubation. Since the total number of bacilli in the foot pads of nude mice is considerably higher, such mice should continue to be used for the production of *M. leprae* for leprosy research. SCID mice possibly could be used for screening the antileprosy drugs in a relatively shorter period of time.

The infection of *M. leprae* in SCID mice has not been investigated extensively. The phenomenon of decline and eventual clearing of millions of bacilli in the foot pads of SCID mice in a relatively shorter time period is not clear and is worthy of investigation. Additional studies such as histopathology and the status of the immune response of SCID mice at various stages of infection, lepromin reactivity as well as DNA homology, should be carried out.

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Accurate Diagnosis of Tuberculosis Meningitis Using Polymerase Chain Reaction

TO THE EDITOR:

Tuberculous meningitis (TBM) is unique and important in the pediatric age group and happens to be the most common cause of death in children suffering from tuberculosis. Favorable prognosis depends upon the early diagnosis of tuberculous meningitis, for which reliable methods based on serology are not available. The ultimate diagnosis for TBM depends on isolation and identification of mycobacterial species, which is time-consuming and often gives negative results in spite of clinical disease. We previously have reported the presence of a repetitive sequence on 5.6-Kb AluI restricted *Mycobacterium tuberculosis* DNA