WORKSHOP 5: EXPERIMENTAL

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Participants

E. dela Cruz
A. M. Dhople
R. H. Gelber
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R. C. Hastings
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R. D. McDermott-Lancaster
R. W. Truman
Y. Yogi

Progress in the last 5 years

Nude mice. These athymic animals were first reported in leprosy studies in 1976 by Drs. Kohsaka, Colston and colleagues. This model continues to be used in the evaluation of drug regimens for the treatment of leprosy because they can support growth of large inocula to levels of 1010 per foot pad. Dr. McDermott-Lancaster (London) described how rifampin-resistant mutants selected in nude mice with a frequency of 1 to 8 × 10^7 visible organisms grow better in nude than in normal immunologically intact mice. Studies of antileprosy drugs combined with interferon-gamma (IFN-γ) demonstrated a lack of synergy in reducing *Mycobacterium leprae* growth except in combination with a therapeutic dose of rifampin but not with subtherapeutic doses or with dapsone. A new antileprosy agent, ofloxacin, was found to be more effective when administered with daily dapsone than with monthly rifampin. It was found that *M. leprae* exposed to rifampin for 6 hr *in vitro* did not grow in nude mice whereas slight killing by ofloxacin could only be detected after 96 hr of exposure. As few as 101 to 102 organisms inoculated into nude mice but not normal mice showed growth over a 12-month period, demonstrating the value of nude mice in *M. leprae* viability studies. Prof. Ito (Bangkok) reported that human-derived *M. leprae* multiplied as readily in nude as in normal mice. However, a strain of *M. leprae* (Thai-53) that had been passaged for many years in nude mice multiplied more readily in nude than in normal mice. Dr. Hastings (Carville, LA, U.S.A.) reported on a number of current uses for nude-mouse-derived bacilli. As many as 10^9 *M. leprae* could be harvested weekly from nude mouse foot pads for experimental use in metabolic studies, drug screening using radiometric methods, in culture with rodent schwannoma cell lines, and in macrophage culture studies. Adoptive transfer studies using cells from BALB/c-nude heterozygotes immunized with a combination of *M. leprae* plus BCG resulted in development of reversal reactions. In addition, transmission studies found that *M. leprae* applied and abraded (e.g., with thorns) onto cool but not warm skin resulted in growth of the organisms. This finding corresponds with observations made on armadillos caught in the wild that also have had evidence of being infected by *M. leprae*-contaminated thorns. The nasal mucosa appeared to be the primary site of infection in experimental transmission studies in nude mice. In chemotherapy studies, monthly rifampin by gavage was found to be less effective than daily rifampin in mouse food. Future studies in nude mice will evaluate new drug regimens for efficacy against persisting *M. leprae*.

Beige mice. These immunologically deficient [lack of natural killer (NK) cells, defective granulocyte chemotaxis, increased susceptibility to opportunistic infections] mice have been used for a number of years in biomedical research but are new in leprosy studies. Dr. Dhople (Melbourne, FL, U.S.A.) reported that *M. leprae* multiplication in spleen and liver could be detected at least as early as 4 months in mice inoculated i.p. or i.v. Statistically significant enhancement of growth of *M. leprae* in foot pads of beige compared to normal BALB/c mice was also observed. Dr. Dhople also found the model to be suitable for chemotherapy studies.

SCID (severe combined immunodeficient) mice. These mice have an enzymatic defect that results in a lack of functional T and B lymphocytes. They have attracted attention in recent years in infectious disease research due to their tolerance of functional xenogeneic mononuclear cells transplanted into the mice. Dr. Converse (Baltimore, MD, U.S.A.) summarized studies by investigators in Addis Ababa and Tokyo, as well as his own, that have shown that these mice are indeed susceptible to *M. leprae* infection when 5×10^3 to 10^7 bacilli are injected into the foot pad. Dr. Converse found that spread beyond the foot pad to the popliteal lymph node and spleen could occur. In one mouse massive numbers of organisms were found in the nasal turbinates. Growth in SCID mice could not be enhanced by the administration of a single dose of transforming growth factor b to abrogate NK cell function. Coinoculation of *M. leprae* with cell-wall-activated mononuclear cells from an *M. leprae* immune human donor but not a nonimmune donor resulted in a reduction of organisms in foot pad homogenates 3 months after infection. Dr. Yogi (Tokyo) has observed dissemination of *M. leprae* after infection in the foot pad. Dr. Yogi reported that *M. leprae* inoculated i.v. resulted in significantly greater dissemination to foot pads, bone marrow, liver, lips and ears of SCID mice than nude mice 14 months after infection. In addition, it was found by reverse transcriptase/polymerase chain reaction techniques that MPNA of cytokines that influence macrophage function was more detectable in 14-month-infected SCID than nude hindfeet; whereas splenocyte cytokine MRNA was more readily detected in nude mice. In the experience of Dr. Ishaque (Montreal, Canada) initial results also indicated greater susceptibility of SCID mice to *M. leprae* infection in terms of foot pad swelling. However, subsequent experiments enumerating organisms found equivalent growth at 9 months and higher growth in the nude mice at 10 months after infection. Dr. Ishaque was not able to detect bacilli in spleens or livers in SCID mice at this time. Dr. Gelber (San Francisco, CA, U.S.A.) reported that SCID mice reconstituted with immune T cells from BALB/c mice homed to the spleen, maintained long term immune function, and limited multiplication of *M. leprae.*

Neonatally thymectomized Lewis rats (NTLR). Dr. Gelber described earlier studies in which NTLR were established to be a highly sensitive rodent model for detecting persisting *M. leprae* in patients undergoing initial chemotherapy. More recent studies using NTLR treated with various regimens involving newer antileprosy drugs demonstrated the value of this model in evaluating antipersister drug regimens.

Normal mice. Dr. McDermott-Lancaster reported that sparfloxacin was superior to ofloxacin in infected mice at doses of 25 and 50 mg/kg by daily gavage for 60 days as determined by the proportional bactericidal test.

Armadillos. This model was first described by Dr. E. Storrs and Dr. W. Kirchheimer in 1971. Dr. Dhople related the history of the Florida armadillo colony which has had contracts to supply infected tissues. More than 2300 armadillos have been inoculated for these projects. Until 1988 no problems with cultivable mycobacteria were encountered. Since that time cultivable organisms have been found in approximately 30% of animals with disseminated leprosy. There also has been a decrease in the yields of *M. leprae.* The Workshop participants discussed possible causes of and remedies for these problems in addition to the intensive efforts already undertaken by Dr. Dhople and colleagues. Dr. Dhople also confirmed previous observations of Dr. J. Convit that Venezuelan nine-banded armadillos are not as susceptible to *M. leprae* infection as armadillos from the U.S. None of 25 Venezuelan armadillos developed disease while 70% of U.S. armadillos inoculated with the same suspension developed leprosy. Dr. Dhople found that armadillos metabolize dapsone (DDS) in a manner very similar to humans. Biopsies of cutaneous samples obtained from infected armadillos receiving DDS revealed decreasing ATP levels during the course of treatment.

Dr. Truman (Carville, LA, U.S.A.) presented a comprehensive report on the history, distribution, migration, physiology and husbandry of armadillos. The epidemiology of sylvatic leprosy in armadillos in the United States appears to follow a corridor along

the southern Mississippi River valley and then along the Gulf Coast to the Mexican border. The prevalence of infection in adult armadillos is estimated to be 30% in this corridor. This pattern correlates with the distribution of indigenous human leprosy in the United States. He also reviewed the pathogenesis of *M. leprae* infection in experimentally infected armadillos. As few as 10^3 organisms can result in a successful infection but typically 10^8 are inoculated i.v. in order to shorten the incubation period to an average of 14 months. Early indications of "takes" are a nodule at the injection site, IgM antibodies to PGL-I, and detection of *M. leprae*-specific DNA in PCR. Usually there are few clinical signs of infection. If cutaneous lesions develop and ulcerate, they represent a source of organisms in the environment. Dr. Job (formerly Carville, LA) reported that armadillos whose reactions to lepromin had a histological resemblance to tuberculoid leprosy were usually resistant to subsequent infection; whereas those with a lepromatous type response were the most susceptible. Six weeks after immunization with either 10^7 BCG or 10^7 BCG plus 1.6 $\times$ 10^7 heat-killed *M. leprae,* lepromin conversions were observed in 20% of the armadillos; only 3% of control armadillos converted. Dr. Job also found that foot pad infections were more successful with larger inocula and that infections proceeded from foot pad granulomas and then to regional lymph nodes, the spleen and, finally, to other organs. Dr. Job pointed out that at a preclinical stage *M. leprae* is found in the reticuloendothelial system before invading the nerves. Finally, 2% of "road-kill" armadillos were found to have disseminated disease suggesting the potential for trillions of organisms to be discharged into the environment, thus allowing spread to other hosts by means of skin abrasions.

Cynomolgus monkeys. Dr. dela Cruz (Cebu, The Philippines) described experimental studies in Philippine cynomolgus monkeys. Thus far, 4 out of 22 animals have developed AFB-positive nasal smears after inoculation with *M. leprae* and the positivity of the nasal mucosa correlates well with PGL-I antibody levels in these animals. PCR examination of nasal smears were positive in the 3 animals with available specimens as well as in 2 additional inoculated animals that were AFB negative by conventional methods. Sooty mangabey monkey isolates containing the simian immunodeficiency virus (SIV) were used to infect several groups of cynomolgus monkeys, and the presence of SIV appears to enhance the susceptibility of this species to leprosy. Surveys of feral cynomolgus monkeys revealed serological evidence of natural leprosy in 3 out of 596 monkeys. Additional studies of these 3 animals as well as more feral monkeys are in progress.

Chimpanzees. Dr. Gormus (Covington, LA, U.S.A.) reviewed naturally acquired leprosy in three chimpanzees. The first chimp with leprosy was from Sierra Leone and had been in the U.S. only a short time prior to diagnosis. The remaining two chimps had been in the U.S. for more than 10 years prior to diagnosis, and retrospective serological studies revealed that the disease was probably incubating in these animals when they were imported from Africa. Current efforts involve serological testing of chimps maintained in various colonies for other biomedical investigations.

Other Old World monkeys. Naturally acquired leprosy was described in two sooty mangabey monkeys (SMM). It is likely the first mangabey acquired the disease in Africa and was the source of infection for the second animal with which it was caged for a number of months. This appears to be the first case of monkey-to-monkey transmission. Experimental leprosy studies in SMM revealed that this species was very susceptible to leprosy. Although dose-response studies demonstrated a variety of individual responses in terms of time and extent of disease, the SMM is undoubtedly the most susceptible nonhuman primate species studied to date. Variations in the course of leprosy in inoculated animals may be reflected in the cyclic variations in lymphocyte responses to mitogens observed in normal and *M. leprae*-infected SMM. Experimental studies also demonstrated that rhesus monkeys were susceptible and developed BB to LL leprosy after infection with *M. leprae.* Captive SMMs are asymptomatic carriers of SIV but rhesus monkeys inoculated with SMM isolates develop simian AIDS (SAIDS) together with leprosy.

Many succumb to SAIDS-related opportunistic infections. SIV appears to enhance the susceptibility of rhesus monkeys to leprosy. Experimental studies in African green monkeys demonstrate that they will develop a BB/BL form of leprosy primarily involving the nerves.

Future directions

Recognizing the contribution of animal models and their continuing role in providing information on the epidemiologic, chemotherapeutic, immunologic, microbiologic, and pathogenic aspects of leprosy, the following aims for future work are recommended:

1. Additional studies in SCID mice should be carried out to resolve questions concerning *M. leprae* dissemination, reproducibility, and the overall utility of the model, including vaccine and cell-transfer experiments.

2. Nude mice continue to have value a) as a source of viable *M. leprae* for *in vitro* and other experimental studies (e.g., transmission and animal inoculations), b) as a relatively inexpensive model for chemotherapy studies, and c) as a means of detecting small numbers of viable bacilli.

3. Future studies in beige mice will involve histopathology and additional experiments on the pathogenesis of leprosy infection.

4. The normal mouse foot pad assay remains the most readily available model for viability, drug screening, and drug-sensitivity testing.

5. The NTLR will continue to be used in *M. leprae* persister studies.

6. The armadillo has nearly untapped potential in studies of the transmission, epidemiology, chemotherapy, and immunology of leprosy. Investigations of these various aspects of leprosy in wild-caught animals should be continued. This model's potential for assessing candidate vaccines and new drugs may be particularly worthwhile areas to investigate. New techniques in assisted reproduction may overcome the present barriers to breeding in captivity. Understanding the differences in susceptibility of Venezuelan and North American nine-banded armadillos may be a rewarding avenue of investigation.

7. In nonhuman primates, studies should: a) ascertain natural infection in feral and captive primates and investigate the contribution of retroviruses to the development of leprosy in these species; b) continue studies evaluating the susceptibility of cynomolgus monkeys to experimental infection with *M. leprae*; c) continue to evaluate the relevance of the SMM vaccine model; d) continue to study the African green monkey as a model for neuritic leprosy; e) investigate *M. leprae* strain differences in the pathogenicity of infection in rhesus monkeys as a model to determine if strain differences play a role in the spectrum of human leprosy.