Reports of Congress Committees

COMMITTEE 1: ADVANCES IN EXPERIMENTAL LEPROSY

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This report covers the five years since the last International Leprosy Congress. However, before reviewing these advances and their relevance to leprosy in man, it is necessary to consider, on the one hand, the contributions made from studies on experimental models in the successful fight against other infectious diseases and, on the other hand, the particular difficulties in developing any experimental models for studying leprosy. In no field of medicine has greater progress been made than with the infectious diseases, particularly those caused by bacteria but also some viruses. This progress in knowledge, whether on the microbiologic, pathologic, preventive or therapeutic side, has evolved, in the first instance, from studies on the cultivation and *in vitro* properties of the causative organism and only subsequently on experimental animal models. Unfortunately, leprosy has remained an exception, because *Mycobacterium leprae* has still not been cultured *in vitro* and only since 1960 has an animal model been available. Therefore, once animal models were available for studying leprosy it was reasonable to assume that they would also be applicable to leprosy in man. In the first instance, the mouse foot pad infection was systematically exploited and has enabled the same topics to be studied in leprosy as in other bacterial diseases affecting man. However, the mouse model had also to be adapted for studying the bacteriological characteristics of *M. leprae*, which for other bacteria are studied *in vitro*.

From these general and particular considerations the field of experimental leprosy has been developed and has rapidly advanced, all within the last thirteen years, almost entirely based on animal models using the mouse and more recently, the rat and the nine-banded armadillo. Our report summarizes the relevance, importance and suitability of these animal models in contributing to knowledge of leprosy in man.

ANIMAL MODELS

Mouse. Shepard in 1960 presented unequivocal evidence that infections with *M. leprae* could be transmitted to animals by showing that *M. leprae* multiplied locally when inoculated into the foot pads of mice. This claim has been fully substantiated on hundreds of strains of *M. leprae* in laboratories throughout the world. By applying standardized technics the foot pad infection has provided a sensitive and reproducible *in vivo* mode of bacteriologic studies on *M. leprae*. However, bacterial multiplication in the foot pad is limited to increases of 100-fold and confined to the first 6-8 months following inoculation. Although *M. leprae* infections in other rodents, including rat and hamster, are similar to the mouse, practical reasons favor the mouse as the standard model.

In 1966 Rees introduced the immunologically suppressed mouse model on the assumption that multiplication was limited in the normal mouse by the development of immunity to *M. leprae* infection. Pure-line strains of mice are used (mainly CBA) and made immunologically deficient by adolescent thymectomy (T) followed by total body irradiation (900r), requiring syngeneic bone marrow replacement. In T/900R mice *M. leprae* continue to multiply beyond the six months period, giving eventual yields of

bacilli 10- 1,000-fold higher than in normal mice. These observations in mice have been confirmed in other laboratories. To simplify the T/900R procedure, lead shielding of a limb, or T followed by five fortnightly exposures to 200r, has been used successfully. Both modifications avoid bone marrow replacement and permit the use of outbred mice.

Thus two distinct mouse models were developed initially for bacteriologic studies of *M. leprae*. Subsequently the models were exploited to study the evolution and pathogenesis of experimental leprosy throughout the animals' life-span (2-3 years), following inoculation of *M. leprae* locally into the foot pad or ear, intraperitoneally or intravenously and, in limited experiments, animals exposed to aerosols or nasal drops.

Rat. Foot pad infection with *M. leprae* in the intact rat is similar to that in the mouse. In the neonatally thymectomized Lewis rat, however, the bacillary population reached levels 100-fold higher than in the intact animal. Following intravenous inoculation, spread to peripheral sites (foot pad, ear, tail and nose) occurs. The advantage of these immunologically impaired animals is that they do not develop runting disease. Subtotal body irradiation appears to further depress their immunological capacity.

Armadillo. The nine-banded armadillo (*Dasypus novemcinctus* Linn.), a primitive mammal, possesses some unique biological characteristics, which could make it a valuable animal model for leprosy research. Among the biological features particularly relevant to leprosy are:

1. low body temperature (32°-35°C)
2. long life-span (12-15 years) and
3. regular production of litters of monozygous quadruplets.

Kirchheimer and Storrs reported disseminated infection with *M. leprae* in an armadillo in 1971. Further results in the short period available have shown at autopsy that about a third of dermally inoculated armadillos become systemically and heavily infected before 37 months. In these animals the histology was of the human lepromatous type, including nerve involvement. In the other inoculated animals there was no evidence of infection, including some observed up to 42 months. In addition the two intravenously inoculated armadillos have developed disseminated infection within 30 months.

The evidence for the identification of the organism grown in the armadillo as *M. leprae* is based on:

1. Mouse foot pad inoculation
2. Failure to grow *in vitro*
3. Lepromin testing
4. Dopa-oxidase activity
5. Pyridine extraction
6. Histological picture
7. Immuno-diffusion test

CHARACTERISTICS AND CLINICAL IMPLICATIONS OF ANIMAL MODELS FOR RESEARCH IN LEPROSY

The main work and advances have come from the mouse models because they were the first to be developed.

Unless otherwise stated, the following report is based on the mouse model.

Bacteriologic characteristics. The growth pattern and rate of multiplication of *M. leprae* (mean generation time 13 days) in the mouse foot pad of normal and T/900R mice is completely reproducible for all primary isolates of bacilli from leprosy patients including drug resistant strains, or after serial passage in mice. These characteristics now form a basis for the identification of *M. leprae*.

Important recent applications of these criteria are:

1. Monitoring the viability of *M. leprae* used to inoculate other animals and the identification of the acid-fast organism subsequently recovered.
2. Identification as *M. leprae* of acid-fast bacilli in nasal discharges and their survival up to 1.75 days in discharges allowed to dry outside the body.
3. Identification of *M. leprae* in various arthropods fed on leprosy patients or recovered from arthropods in the vicinity of cases with untreated leprosy.
4. Monitoring *in vitro* attempts to cultivate *M. leprae*.

Until *M. leprae* are cultured *in vitro*, the only, but relatively small, laboratory source of *M. leprae* has been from mice. Susceptible armadillos can now provide large yields of bacilli which will be of the

greatest importance for future studies of *M. leprae*.

Clinical implications. Although there are small variations in the growth pattern of leprosy bacilli in mice, the same variations are seen in bacilli obtained from bacilliferous patients in different parts of the world. There is no evidence from these observations that the geographical variation in the clinical form of leprosy is caused by variations in the virulence of different strains of *M. leprae*.

Chemotherapeutic applications. Very great advances have been made in chemotherapy entirely based on the mouse models. These are reported in detail by the Committee on Experimental Chemotherapy. However, it is important to make clear that drug-resistant variants (to dapsone and thiambutosine) have the same infectivity and pathogenecity in the mouse as sensitive strains. All studies on the significance and incidence of drug resistance in leprosy should be based on tests using the mouse foot pad model. On the other hand, basic studies on the frequency of drug resistant mutants in populations of *M. leprae* could only be studied in highly susceptible animals with bacillary populations comparable to those found in man. The susceptible armadillo is the animal model most likely to provide this important information.

Pathologic characteristics. A detailed picture of the pathology and pathogenesis of *M. leprae* infections in the mouse models has evolved from histopathologic studies of tissues taken at regular intervals throughout the life-span of the animals (based on CBA mice). To correlate the histology with the bacteriology during the evolution of the infections, paired organs or tissues divided equally were used for the respective assessments.

The main findings are summarized:

1. Although a lesion is first localized to the site of inoculation, systemic spread eventually occurs, with overwhelming evidence that it is hematogenous in origin, since bacilli are found in the lining cells of capillaries haphazardly throughout the body. There are, however, sites of predilection, including the dermis of foot pads, ears and tail, the nose, the testes and dermal and peripheral nerves. Although nerves become infected later than the other sites they are always involved by 20 months. The nose and testes are the sites most frequently and heavily infected. These same sites of predilection follow intravenous or intraperitoneal inoculation of *M. leprae*.
2. The late cellular and bacteriological patterns of response to *M. leprae* mimic those seen in human leprosy as defined by the Ridley-Jopling classifications. Thus by 20 months in the normal mouse, there is a well-developed epithelioid granuloma resembling BB to BT type leprosy and in T/900R mice the lesions resemble BB to LL type leprosy. Cellular changes in nerves mimic the complete spectrum of human disease from TT to LL.
3. Nasal involvement, particularly in T/900R mice, is associated with positive nasal smears and histopathologically shows, unlike the dermis, the juxtaposition of the granuloma to the surface epithelium giving exit of bacilli to the exterior.
4. Histopathologic studies on immunological models have shown that established lepromatoid leprosy in T/900R mice changes to a BB or BT picture when the mice are given syngeneic lymph node cells or thymus grafts. These changes are associated with an influx of lymphocytes into the lesions, destruction of bacilli, edema and later collagen deposition. Similar changes are seen in nerves and are followed by destruction of axons.
5. *M. leprae* has the same predilection for nerves in the mouse as in man, a characteristic shared by no other species of mycobacterium. Early nerve infections show bacilli in Schwann and perineurial cells, later axons and perineurial cells are destroyed and at both sites this is followed by deposition of collagen. Special studies have shown that leprosy neuritis in mice is associated with a defect in the blood-nerve barrier, since markers such as trypan blue and ferritin readily diffuse through the endoneurial capillaries. This defect plus

the destruction of the perineurial sheath would seriously change the endoneurial environment, thus diminishing nerve conductivity and also allowing the entry of macrophages and lymphocytes.

6. In all the mouse models, striated muscle fibers were frequently seen to contain bacilli. In human leprosy although smooth muscle such as the arrector pili and dartos muscles are frequently infected, striated muscle is less so.

Preliminary histopathologic studies in susceptible armadillos also show the importance of hematogenous spread, including infection of nerves, nose with positive nasal discharges and most other sites common to man and mouse. In the armadillo the cellular picture at autopsy resembles LL type leprosy. However, in the armadillo, atypical sites, including the lung, are heavily infected, possibly because of lower body temperatures.

Clinical implications. The significance of these models for studying clinical leprosy is that they reproduce, or can be adapted to reproduce, many of the features of leprosy in man. They particularly provide models for studying early phases of the evolution and pathogenesis of leprosy that can never be undertaken in man. These features are of particular importance for studying the pathogenesis of leprosy neuritis and possible routes of infection via the lungs, nose or gastrointestinal tract. Mice provide precise models for unravelling the immunological complexities of leprosy, bearing in mind that the majority of patients with leprosy are in the TT to BB range and these are the type of leprosy seen in normal mice. The armadillo, on the other hand, may in addition provide models for studying innate susceptibility and resistance and their possible genetic bases.

CONCLUSIONS

The report summarizes the considerable advances that have been made in experimental leprosy using animal models in a period of only 13 years. Thus, animal models are proving to be as valuable in leprosy as they have been for studying other human infections. The particular merits of the various animal models available for studying leprosy are discussed.

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