

Changes in Viability of Interdermal *M. leprae* Associated with the Histopathological Response of Susceptible and Resistant Armadillos.

Richard Truman Ph.D., and James L. Krahenbuhl Ph.D. Laboratory Research Branch, National Hansen's Programs, HRSA, Baton Rouge, La. 70894, USA.

Introduction

In man and armadillos, leprosy manifests over a broad clinical and histopathological spectrum, ranging from tuberculoid (TT) to lepromatous (LL). The histopathological appearance of the 21day Mitsuda skin test reaction with heat killed *M. leprae* (Lepromin), largely typifies the type of leprosy that a host is likely to develop. While this reaction is useful in classifying a patient's disease type, it has little prognostic value as to whether an uninfected individual is likely to acquire leprosy. Within any given Mitsuda type, individuals exhibit significant variability in their relative susceptibility and resistance towards leprosy.

Armadillos are the only immunologically intact animal models suitable for studies with live *M. leprae*. They are the hosts of choice for *in vivo* propagation of leprosy bacilli, and only Mitsuda negative armadillos are used for that purpose. After experimental intravenous infection with highly viable *M. leprae*, armadillos frequently manifest greater than 1×10^9 *M. leprae* /gram in their livers and spleens within about 20 months. However, incubation periods for individual animals may range from 7 to 48 months and the actual number of bacilli that a given animal might harbor can range from 1×10^{11} *M. leprae*/gram to none. A proportion of Mitsuda negative armadillos successfully resist experimental infection with *M. leprae*.

Results & Design:

To better understand these differences in resistance, we compared the granulomas formed in the skin of armadillos after intradermal inoculation with highly viable *M. leprae*, or killed *M. leprae*. We found that the granulomas formed in response to live *M. leprae* were significantly larger than those produced to *M. leprae* killed by heat, gamma irradiation or by freeze/thaw (Plate 1). Among Mitsuda(-) animals (n=20) granulomas involving viable bacilli ranged 2-12 times larger than those made to killed *M. leprae*. However, their cellular composition was little changed and the bacillary number in the granuloma remained high. Mitsuda(+) animals showed similar enhancement with little qualitative difference in the cellular composition of their granulomas.

To determine if the augmented response to live *M. leprae* could be associated with bacterial killing, we examined the viability of bacilli recoverable from these living Mitsuda reactions. In a cross sectional survey of naive and infected armadillos of different Mitsuda types, we inoculated their skin with 1×10^8 *M. leprae* and harvested the sites by biopsy after 2 to 6 weeks of incubation. We initially used a combination of radiorespirometry and conventional mouse footpad (MFP) technique to assess viability. However, RR was highly correlated with MFP and we concentrated our efforts on metabolic assessments.

Figure 1

Metabolic activity of *M. leprae* recovered from intradermal culture in armadillos after 2 and 4 weeks of host exposure

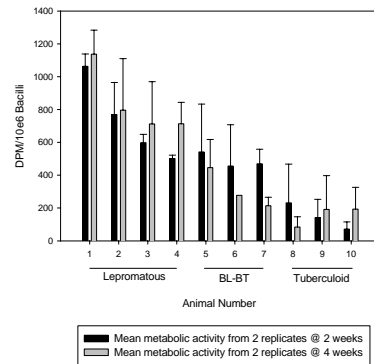
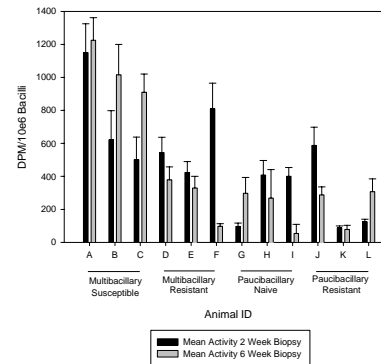


Figure 2

Intradermal Culture of *M. leprae* in Various Armadillos of Known Susceptibility to Experimental Infection



Table

Longitudinal results of experimental infection of individual naive armadillos used in cross sectional study.

Animal ID	Lepromin-A Reaction	AFB in Inguinal Lymph Node @ Death	Days Post Infection to Sustained PGL-IgM Antibody
1	LL	AFB (+)	104
2	LL	AFB (+)	104
3	LL	AFB (+)	104
4	LL	AFB (+)	188
5	BT	AFB (+)	104
6	BL	None	>379
7	BT	None	>379
8	TT	None	>379
9	TT	None	>379
10	TT	None	>379

Plate



Lepromin-A Live *M. leprae*

Skin of normal Lepromatous (LL) armadillo showing 21 day Mitsuda reaction in response to intradermal inoculation with 1.6×10^7 heat killed *M. leprae* prepared as Lepromin-A or an identical number of highly viable *M. leprae* freshly harvested from foot pads of nude mice. Arrows show granulomatous foci.

Results & Design (Cont):

We found that *M. leprae* viabilities fell markedly after initial inoculation into the skin of armadillos but then stabilized. Biopsy bacilli generally showed about a 1-log drop in activity after about 2 weeks in armadillo skin; but they remained significantly more active than uninoculated control bacilli held at 4C for the same time period. Initial viabilities showed a broad range of activities. They varied widely by the Ridley-Jopling classification of the animals. Highest *M. leprae* viabilities were seen among multibacillary spectrum hosts and lowest the among paucibacillary animals. Over a six-week period in the skin, *M. leprae* viabilities among most multibacillary hosts tended to increase, while they decreased or remained very low among the paucibacillary animals (Figure 1). The pattern for viability among leprosy resistant Mitsuda(-) animals (n=3) resembled that seen among paucibacillary hosts, with the higher initial viabilities waning over time (Figure 2). The histopathological response of these animals to Lepromin remained the same. Long term observations following intravenous infection of these naive armadillos showed that the animal's individual ability to resist systemic infection with *M. leprae*, generally correlated with the viability trends seen among their skin biopsy bacilli early in infection. Those LL-spectrum animals that had exhibited high viabilities for their intradermal *M. leprae*, developed signs of fully disseminated disease rapidly, while the resistant and paucibacillary spectrum animals remained free of leprosy (Table).

Conclusions

These data indicate that events occur in the skin of armadillos very early in the course of infection with *M. leprae* that can be correlated with the eventual outcome of their systemic disease. Armadillos can be useful models in the study of resistance to leprosy, but will require development of specific immunological reagents to examine their response.

Intradermal inoculation to assess susceptibility is a cumbersome technique. It requires many days of effort and is difficult to compare results between different groups of animals or preparations of *M. leprae*. In addition, infected animals require special handling.

The etiology of the enhanced histopathological response to live *M. leprae* is unclear. Actively metabolizing bacilli may produce antigens that are not present among killed bacillary preparations, and they may secrete them to the host over a long period. Histopathology is likely too insensitive to reveal the full range of variable resistance across the leprosy spectrum. A better understanding of the *M. leprae* antigens involved in resistance to leprosy by armadillos, and the specific cytokine profile of their response in resisting infection, would be useful in our efforts at *in vivo* propagation, and could benefit our ability to address this issue in man.