Relation of Infection to Tissue Temperature in Mice Infected with *Mycobacterium marinum* and *Mycobacterium leprae*

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Received for publication 17 October 1966

Intravenous and footpad infections with *Mycobacterium marinum* and footpad infections with *M. leprae* were compared in the following mouse strains: A/He, BALB/C, CBA, C3H, C57BL, C57L, DBA, 101, and CFW. The results varied a great deal according to mouse strain used. Intravenous injection of high doses of *M. marinum* resulted in deaths after 28 days of 100% of strain A/He, and none of strain 101; 27 days after injection, the feet and noses of all strain CBA mice, but few of the C57BL, 101, or CFW mice, were involved. Injection of a small dose of *M. marinum* into the footpad produced visible disease in 5 days in all of the C57BL and 101 mice, but in not more than 60% of the A/He, DBA, and CFW mice; the average amount of swelling at 17 days varied from 4.40 mm in strain C57L to 0.92 in strain 101. After footpad injection of *M. leprae*, the average plateau harvests varied from 1.3×10^7 acid-fast bacteria in strain CBA to 6.5×10^8 in strain C57L. The infections in CBA mice extended from the site of inoculation throughout the foot. The temperature was measured rectally, in the footpad, and in the tail. Analysis of all the results revealed little correlation among the three types of infection. There was a strong negative correlation between the tail temperature and the death rate after intravenous injection of *M. marinum*, and a strong positive correlation between footpad temperature and plateau harvest of *M. leprae*.

In this laboratory, the CFW mouse strain has been used in most of the investigations of mice infected with *Mycobacterium leprae*. A comparison was made with two other strains (9, 10), but it had limited value because one strain (C57BL) was simultaneously infected with *Salmonella*, and the other (Chatterjee) was not genetically defined, i.e., it had not been adequately inbred. Rees (8) usually uses the P mouse strain when studying *M. leprae* infection, but he also has limited experience with the Chatterjee strain. Pattyn recently reported (5) that there was little difference in the multiplication of *M. leprae* in strains NMRI, C57BL, C3H, TB, and AKR.

The results of infecting eight inbred strains, as well as CFW, with *M. leprae* and *M. marinum* (Balnei) are presented. These two mycobacteria apparently have a similar temperature optimum that is below 37°C (11, 12), and they grow preferentially in peripheral, glabrous sites. The temperature was measured in the footpad, tail, and rectum of each mouse strain.

MATERIALS AND METHODS

Mice. The inbred strains A/He, BALB/c, CBA, C3H/An, C57BL, C57L, DBA/2, and 101 were pur-

chased from Cumberland Farms, Clinton, Tenn. The CFW strain, originally inbred selectively by Webster for susceptibility to neurotropic viruses, was inbred another 20 generations at Carworth Farms and then maintained by random breeding. It was introduced to the Communicable Disease Center (CDC) in 1950 with about 3,000 mice, and has been maintained at CDC by random breeding. All mice were fed commercial pellets. *M. marinum* was injected into 4-week-old mice, and *M. leprae*, into mice 7 weeks old.

M. marinum. A suspension of the Balnei X strain, grown in Tween-albumin medium and preserved for 7 months at -60°C, was thawed quickly, dilutions were made in Tryptose Broth (Difco), and colony counts were performed on 7H9 medium. Intravenous injections into the tail vein of 0.1 ml containing 1.4×10^6 colony-forming units (CFU) were made in 10 mice of each strain. An additional 10 mice were injected in the right hind footpad with 0.03 ml containing 5.1×10^2 CFU. The mice were inspected at appropriate intervals. For those injected intravenously, the presence or absence of foot, tail, and nose involvement was noted; for those injected in the foot, the degree of foot involvement was noted, as well as the amount of swelling (difference in caliper measurements of thickness of injected and uninjected foot).

M. leprae. Strain N2401, in the 8th mouse passage, was used. By chance, this was the same strain em-

ployed by Pattyn (5). Mice were inoculated in the right hind foodpad with a dose of 5×10^8 acid-fast bacteria (AFB), 32% of which stained solidly (13). Thirty mice of each strain were inoculated. At 1-month intervals during the first 8 months, a mouse was taken from each group for histological sections. When AFB were found in some of the sections, four mice were killed from each group to determine counts of AFB in the inoculated footpads. Counts of AFB were repeated at 3-month intervals from like numbers of mice.

Temperature measurements. For the duration of the experiments, mice were kept in a room at 20 C. The telethermometer and thermistor probes (Yellow Springs Instrument Co., Yellow Springs, Ohio) were used. Temperature was measured by use of a rectal probe, and by inserting a 22-gauge needle 1 to 2 mm deep into the left hind footpad and the tail. For the latter measurements, only undisturbed mice from huddles were employed (12); of a total of five mice in each cage, not more than one or two could be used each time, and repeated visits to the cages were necessary. The mice were about 8 months old and belonged to the *M. leprae* experiment, i.e., about 6 months post-inoculation.

RESULTS

***M. marinum*.** Footpad infections in strains BALB/C and CFW were compared in a preliminary report. Large differences were seen with all

infectious dilutions of inoculum; for example, at a 10^{-6} dilution of inoculum, the average foot swelling at 22 days was 4.3 mm in BALB/C and 2.0 mm in CFW, and the harvest was 2×10^7 AFB from BALB/C and 2×10^6 AFB from CFW. A more complete study in which all the strains of mice were compared was then carried out.

As described earlier (1, 3), intravenous inoculation led to preferential involvement of the feet, tails, and noses of mice. Deaths occurred owing to pneumonic disease. The frequency of the manifestations and the rates of their development varied considerably in different lines of mice. The rates of the disease manifestations are shown in Fig. 1 for the times at which the differences were very obvious.

At 28 days (Fig. 1) the death rate varied from 100% in strain A/He to 0% in strain 101. More mice died later, but, by 58 days, at least half of the C57BL, C57L, and 101 mice remained alive. Involvement of the feet and nose, which consisted of redness and swelling and frequent abscess formation, was much more frequent in some lines than in others. At 17 days (Fig. 1), all of the CBA mice had diseased feet and noses, but few of the C57BL, 101, or CFW mice showed any changes. By 26 days, all of the surviving CFW

FIG. 1. Manifestations of *Mycobacterium marinum* infections in different strains of mice. The mice were injected intravenously (IV) with a large dose of bacilli or into the right hind footpad (RHF) with a small dose. FF, front feet; HF, hind feet.

mice had characteristic foot disease, but the noses appeared normal. The hind feet were involved more frequently than the front feet or nose in A/He, C3H, and C57L, but the rate was about the same in these locations in BALB/C, CBA, and C57BL mice. The tails were also involved, but metastatic involvement could not be differentiated from that resulting from local deposition during injection.

After footpad injection, all of the mice had local redness and swelling by 17 days, but at 5 days (Fig. 1) not more than 60% of the injected feet had detectable disease in the case of A/He, DBA, and CFW mice. The size of the swelling at 17 days (Fig. 1) varied from an average of 4.40 mm in C57L mice to 0.92 mm in 101 mice. Between 17 and 24 days, the average amount of swelling increased somewhat in some lines, but only in BALB/C and CBA mice was the increase more than 0.5 mm.

M. leprae. The results shown in Fig. 2 should be viewed in light of the normal growth curve of *M. leprae* observed in the CFW and Chatterjee

strains (13). With an inoculum of 5×10^3 AFB, a lag phase of 90 to 120 days occurs first during which AFB are not demonstrable in tissue sections or in the bacterial counts. A logarithmic phase follows, during which bacterial multiplication occurs at a generation time averaging about 12 days. When the bacterial population reaches a level a little over $10^{6.0}$, growth retards quickly and the plateau phase follows. During the plateau phase, the total number of AFB increases slowly to a level sometimes nearing $10^{7.0}$. In the present experiment, all the early tissue sections were negative, but sections from a single mouse contained AFB at 4 months, and those from six mice contained AFB at 5 months. As judged by the subsequent changes in numbers of AFB, the first harvest (5 months) was carried out while the logarithmic phase was still going on in A/He, BALB/C, CBA, DBA, and perhaps, CFW mice. Based on the number of AFB in the first harvest, the appearance of growth was slow with A/He; somewhat faster with BALB/C, CBA, C57BL, C57L, DBA, and CFW; and most rapid with 101

FIG. 2. Yield of *Mycobacterium leprae* in different strains of mice. For each mouse strain, three bars indicate the number of bacilli harvested at 5, 8, and 11 months, respectively. The broken lines for solid AFB indicate the maximal estimate in cases where the proportion of bacilli that were solidly staining was less than 2%.

and C3H. Since harvests were not made during the logarithmic phase, it is not possible to say whether early appearance of growth arose from a shortened lag phase (due possibly to greater retention of viable AFB in the footpad) or from more rapid multiplication.

The second and third harvests were made during the plateau phase, and they indicated the maximal bacterial population allowed by each strain of mice. The average of the second and third harvests, arranged in numerical order, was as follows: CBA, 1.3×10^7 ; BALB/C, 6.0×10^6 ; CFW, 3.7×10^6 ; C3H, 2.7×10^6 ; DBA, 2.5×10^6 ; 101, 2.0×10^6 ; C57BL, 1.5×10^6 ; A/He, 1.3×10^6 ; and C57L, 6.5×10^5 .

The tissue sections, obtained at 1-month intervals during the first 8 months, were not helpful in estimating numbers of AFB because the area sampled was very limited. No differences in the nature of the cellular response were noted, although all of the A/He mice did have a larger number of mast cells in the footpad tissue.

Two CBA mice remained at 11 months. One of these was killed, and tissue sections of the feet were prepared. All sections of tissue from the feet, including tissues dorsal to the metatarsals, contained many AFB. Thus, there had obviously been extensive spread of infection from the inoculated site in the foot. Tissue sections from hundreds of old infected CFW mice have been examined, and only once has such an extensive spread been observed in the foot. The remaining CBA mouse was killed at 12 months. Half of the foot was processed for tissue sections, and the footpad from the other half was harvested for bacterial counts and passage. The tissue sections had the same extensive bacterial spread seen in the mouse killed at 11 months. There was no evidence of spread to the other tissues, including abdominal viscera, tail, and the other hind foot. There were 9.7×10^6 AFB in the harvested tissue. A dilution of the harvest was inoculated under

usual conditions into CFW mice. Results in the CFW mice (histological picture and bacterial growth rate) identified the AFB in the CBA mice as *M. leprae*, and ruled out the possibility of a contamination with *M. lepraemurium*. Small globi, which are characteristic of *M. leprae* infections in the mouse, were present in the CBA infections as well as in the passages from them in CFW; this also is strong evidence against the possible *M. lepraemurium* contamination.

Experience with other strains of M. leprae. Additional experiments have been carried out to compare more isolates of *M. leprae* in certain strains of mice. Six different isolates in passage in CFW mice were passed one time in CBA mice before being passed back again to CFW mice. Routine laboratory practice (9, 10) was followed. The results of the passage in CBA mice were compared with those of the preceding and following passages in CFW mice. The harvests (AFB per mouse) averaged more in the CBA mouse passages than in the preceding or following CFW mouse passages, and the rate of growth, expressed as generation time [G in (11)], was faster in the CBA passages in each instance. Thus, the growth could be said to be superior in CBA mice.

ICR mice have also been compared with CFW in five comparisons of four isolates in passage in CFW mice, and the CFW mice were found to give superior harvests and rates of growth. An earlier comparison (9) showed that C57BL mice yielded inferior harvests and growth rates for *M. leprae* both in primary isolation and in passage. The earlier, favorable experience with Chatterjee mice (9) was expanded to a total of nine isolates of *M. leprae*, some in primary passage and some in repeated passage up to four times. The rate of growth and the harvest were usually greater in Chatterjee than in CFW mice. Not much difference was observed among strains of *M. leprae* in primary isolation, those in CFW mice, and those in passage in Chatterjee mice.

TABLE 1. *Temperatures of strains of mice*^a

Strain of mice	Rectal	Hind footpad	Tail
A/He	36.03 $\pm$ 0.97 ^b	29.61 $\pm$ 0.72	23.00 $\pm$ 1.72
BALB/C	34.70 $\pm$ 0.77	30.20 $\pm$ 1.45	23.30 $\pm$ 1.95
CBA	35.38 $\pm$ 0.71	30.66 $\pm$ 1.37	23.51 $\pm$ 1.56
C3H	34.97 $\pm$ 0.95	29.95 $\pm$ 0.92	23.83 $\pm$ 1.46
C57BL	35.35 $\pm$ 0.90	29.40 $\pm$ 1.31	28.63 $\pm$ 1.58
C57L	34.77 $\pm$ 0.81	29.56 $\pm$ 1.27	26.31 $\pm$ 1.67
DBA	34.84 $\pm$ 0.88	29.81 $\pm$ 1.91	23.83 $\pm$ 1.85
101	34.98 $\pm$ 0.97	30.29 $\pm$ 1.52	25.83 $\pm$ 1.29
CFW	35.85 $\pm$ 0.57	30.55 $\pm$ 1.53	25.05 $\pm$ 2.07

^a Fifteen measurements were made for each entry.

^b Results expressed as mean and standard deviation in degrees centigrade.

TABLE 2. Rank correlation coefficients among results according to strain of mice^a

Determination	a	b		d	e	f	g	h	i	j	k	l	m
<i>Mycobacterium marinum</i> , intravenous injection													
a. Dead, 28 days (%)		+0.63	+0.59	+0.68	-0.60	+0.34	-0.47	+0.30	+0.30	+0.07	+0.08	+0.01	-0.89
b. HF involved, 17 days (%)			+0.62	+0.67	-0.02	+0.52	-0.17	+0.20	+0.03	+0.08	+0.07	+0.10	-0.50
c. FF involved, 17 days (%)				+0.95	+0.19	+0.04	-0.05	+0.44	+0.56	+0.46	+0.21	+0.34	-0.57
d. Noses involved, 17 days (%)					+0.16	+0.18	-0.12	+0.39	+0.63	+0.29	+0.25	+0.28	-0.55
<i>M. marinum</i> , RHF injection													
e. RHF involved, 5 days (%)						-0.32	+0.48	-0.22	+0.03	+0.07	+0.13	+0.03	+0.57
f. RHF swelling, 17 days (mm)							-0.49	+0.14	-0.06	-0.14	-0.59	-0.30	-0.15
<i>M. leprae</i> , RHF injection													
g. AFB/mouse, 5 months								-0.13	+0.13	-0.12	+0.13	+0.30	+0.56
h. AFB/mouse, 8 months									+0.68	+0.44	+0.03	+0.82	-0.55
i. AFB/mouse, 11 months										+0.08	+0.07	+0.62	-0.33
j. Solid ratio, 5 months											+0.45	+0.30	-0.31
Temperatures													
k. Rectal												+0.32	-0.15
l. HF													-0.17
m. Tail													

^a Abbreviations: AFB, acid-fast bacteria; FF, front feet; HF, hind feet; RHF, right hind feet. For each correlation, the significance level (*P* value) is 0.05 for a coefficient of 0.60 and 0.01 for a coefficient of 0.78.

FIG. 3. Strong correlations of Table 2. Left: (a) death rate 28 days after intravenous injection of *Mycobacterium marinum* versus (m) average tail temperature. Middle: (c) front foot involvement 17 days after intravenous injection of *M. marinum* versus (d) nose involvement at same time. Right: (h) harvest of *M. leprae* at 8 months versus (l) average footpad temperature.

Thus, different isolates of *M. leprae* behaved uniformly regarding their preference for strains of mice, and such preference seemed the same for *M. leprae* in primary isolation and in repeated passage.

Temperatures. Measurements of the rectal, hind-footpad, and tail temperatures are given in Table 1. The footpad temperatures for CFW agree with earlier measurements (12); the rectal temperatures are a little lower, but within one standard deviation.

Correlation among the results. Rank correlation coefficients (14) among the results for each strain of mice are given in Table 2, and they are discussed below. The strongest correlations are illustrated in Fig. 3; the probability values indicate that it is extremely unlikely that these correlations arose by chance.

DISCUSSION

Several manifestations of the intravenous infection with *M. marinum* (a-d, Table 2) were positively correlated with each other. The front foot involvement and nose involvement (c and d, Table 2) were especially closely related. However, there was little correlation between the manifestations of the intravenous infection and those of right hind foot infection (e and f, Table 2). Intravenous *M. tuberculosis* infections have also been studied in several of the strains of mice used here (2, 6, 15), but there is no apparent correlation of those results with the results of this study.

Light is thrown on the mechanisms involved in the intravenous *M. marinum* infections by the negative correlations between the manifestations of intravenous infection and the tail temperature. The tail temperatures probably did not reflect the actual temperatures during injection because the tails were warmed in water and wiped dry

before the injection. Also, since the mycobacteria would have been exposed to tail temperature only briefly during their passage up the tail veins, it is unlikely that this would have affected their future behavior. However, the tail temperature is a function of the amount of blood being passed through it (see below), and it seems probable that the governing factor was the circulation of the tail. The exact mechanism is not clear, but it seems likely that the venous development, the amount of anastomotic connection, or the rate of dilution of the inoculum with venous blood could be important.

The results with *M. leprae* were not forecast by any aspects of the *M. marinum* infections. However, the footpad temperatures (1) were strongly correlated with the plateau level of *M. leprae*, as measured by the harvest at 8 months (h, Table 2). It is unlikely that the temperature difference itself could be the responsible factor, since the difference was small, only 1.26 C between the highest and lowest average. Such a large decrease in multiplication of *M. leprae* for a temperature decrease of 1.26 C in this temperature range would require that the temperature relationships of *M. leprae* be much different from other bacteria. It seems more probable that the responsible mechanism was the circulation through the foot.

The tail and feet, especially the hind feet, are important organs in heat regulation of the mouse (4) and rat (7), and body (core) temperature is regulated by variation in the amount of blood circulated through tail and feet, as well as by variation in metabolic rate and by changes in physical attitude (huddling, etc.). Although the tail surface is larger, the amount of heat loss is proportionately greater from the foot because the temperature difference from the environment is greater (9.4 to 10.7 C for foot versus 3.0 to 8.6 C for tail). Warmer footpad temperatures would

signify greater exposure of those tissues to the conditions of arterial blood, that is, increased supply of nutrients and oxygen and decreased exposure to catabolites and carbon dioxide.

The preferred sites of *M. leprae* are peripheral sites of heat loss that average about 30 C in the mouse (12) and in man (11). These sites of heat loss are characterized by rich circulation when required by the body's heat load, whether from increased metabolism or warm environment. That a good blood supply is important for the multiplication of *M. leprae* in the tissues has also been suggested by the normal vascularity and the lack of necrosis in the lesions of *M. leprae* in mouse and man. In contrast, the lesions of *M. marinum* and most other mycobacteria characteristically contain necrotic areas, especially in the chronic stages. One interpretation of this histological situation is that *M. leprae* dies before tissue necrosis can appear.

We have avoided use of the terms "susceptibility" and "resistance." It would have been traditional to arrange the strains of mice in order of the degree of involvement in a particular infection and to speak of the arrangement as an order of susceptibility. There would be a connotation that the differences were caused by some general tissue property. The results obtained, however, illustrate how poorly the outcome of one mycobacterial infection can correlate with another, even when caused by the same organism administered by another route. To explain differences in a particular infection in different animals on the basis of differences in "susceptibility," when in fact the "susceptibility" itself is measured only by the differences in the infections, seems to us unsound and obfuscating.

There has been considerable interest in the influence of heredity on the outcome of leprosy infections in humans. Although the results obtained here illustrate that genetic differences in the host can play a role in the mouse infection, it is not possible to say that the pertinent genetic differences in the mouse have any similarity to those that might be concerned in the human infection. Since integumentary pigmentation is often mentioned in this regard, it should be noted that the present results were not at all correlated with coat color.

ACKNOWLEDGMENT

We are indebted to Elmer C. Hall of the Communicable Disease Center for advice on the use of rank correlation coefficients.

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