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VACCINATION OF MICE AGAINST M. LEPRAE INFECTION

Charles C. Shepard

Center for Disease Control
Public Health Service
U. S. Department of Health, Education, and Welfare
Atlanta, Georgia 30333

We have continued our studies of vaccination against M. leprae infections in mice. In the earlier studies our supplies of M. leprae for vaccine were limited, so we were unable to investigate some of the areas adequately. The coming availability of M. leprae from armadillos and the IMMLEP program made it important to study these areas now.

In the first experiment to be described we sought explanations for the apparent discrepancy between the consistent protection afforded by intradermal BCG vaccination and the apparently inconsistent protection afforded by an M. leprae infection in the opposite foot pad. We were fortunate to receive from Dr. Russell a skin biopsy specimen that contained an especially large number of M. leprae (4.6×10^9 M. leprae/gram).

The design of the experiment is shown in Figure 1. Vaccine, consisting of suspensions of M. leprae or BCG, was given intradermally in the right flank or subcutaneously into the left foot pad. The dose per mouse was 3×10^6 M. leprae and 1×10^7 BCG. The M. leprae were purified by the trypsin method, described previously, which provides a high degree of purification without detectable loss in viability. The M. leprae vaccine very likely contained some viable M. leprae because 3 days earlier it had had apparently normal infectivity for mice. Probably the number of bacilli was low, though, since the specimen had been about 4 days en route from New Guinea. The BCG was our usual culture that originated from a Rosenthal vaccine; the suspension was prepared from a young culture grown in Tb-Tween medium, washed centrifugally in phosphate buffered saline with Tween 80 and kept at -60°C. The challenge, a mouse passage strain of M. leprae, was given into the right hind foot pad 28 days after the vaccine. The growth curve was typical of a "fast" strain and reached plateau at about 187 days, at which time counts of M. leprae in the right hind foot pads were carried out on eight mice from each group. Counts were repeated 90 days later. In Figure 1, the results for individual mice are shown as dots, and the average for the group is shown by the bar. There were two control groups, marked A and A'. Groups F and G received mixtures of M. leprae and BCG. Group J received M. leprae killed by freezing and thawing in Hanks balanced salt solution containing 0.1% bovine plasma albumin and 0.05% Tween 80, and this was the diluent for all the vaccines.

The significance of the differences between groups was estimated by

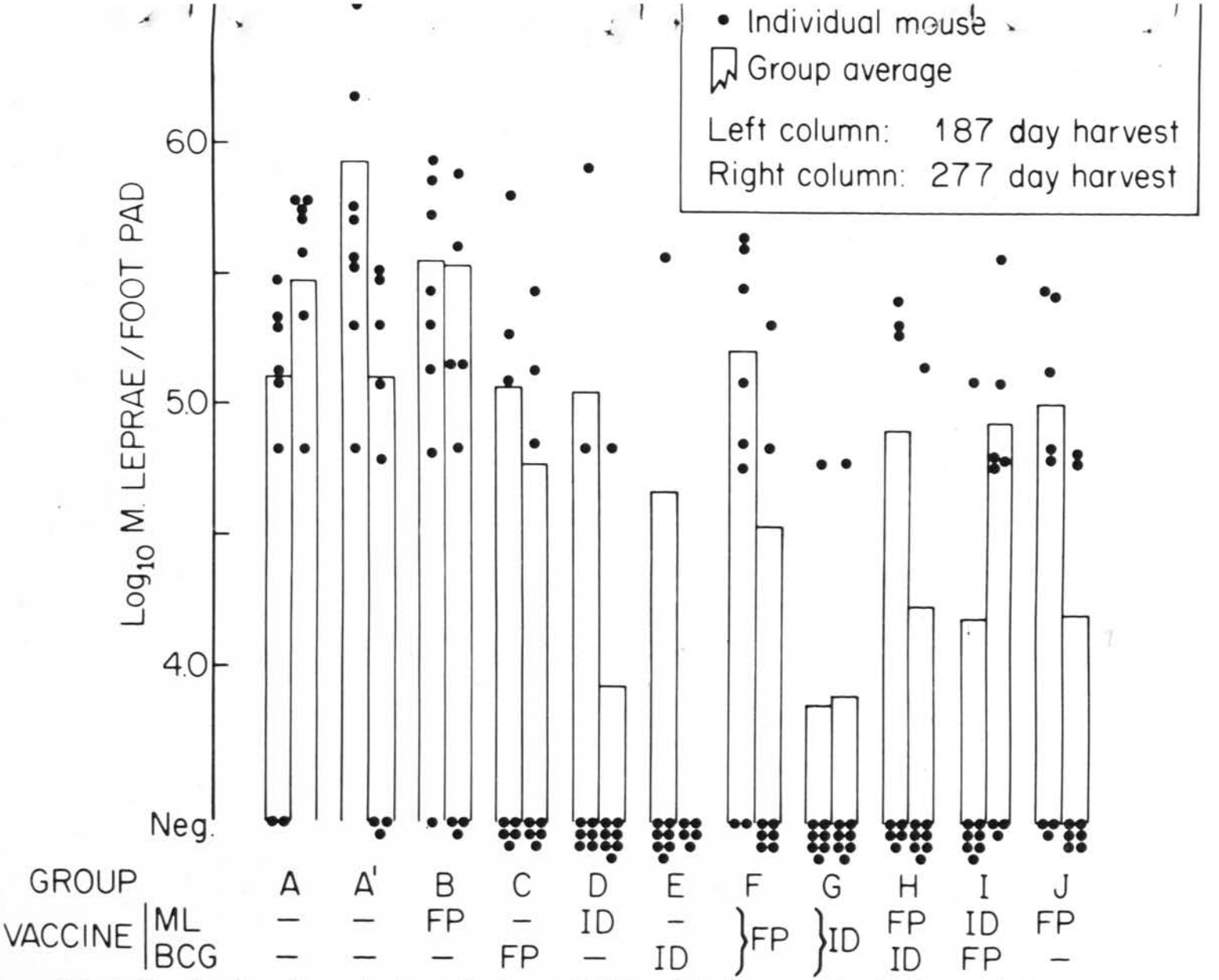

Figure 1. Results of vaccination of mice with BCG and M. leprae. The M. leprae (ML) vaccine dosage was 3 x 10^6 bacilli; the BCG dosage was 1 x 10^7 bacilli. The vaccines were given into the foot pads or intradermally, 28 days before challenge.

the two-sample rank test (Table 1) with the two control groups considered as a single group. The following conclusions could be drawn.

a) M. leprae given into the foot pad gave no significant protection (B vs. A), but it did when given intradermally (D vs. A). In an earlier experiment described at the meeting last year in Kyoto, a suspension of M. leprae purified from mouse foot pads had given protection by the foot pad route and somewhat more protection when given intradermally.

b) The intradermal route was more effective than the foot pad route for all three vaccines -- M. leprae (D vs. B), BCG (E vs. C) and the mixture of M. leprae and BCG (G vs. F).

c) M. leprae was significantly less effective than BCG when given in the foot pad (B vs. C) but not when given intradermally (E vs. D), perhaps because when given intradermally both M. leprae and BCG exerted a nearly maximal effect. Since the M. leprae suspension contained a far smaller fraction of viable bacilli, it would not be justified to conclude that viable M. leprae is less effective than equal numbers of viable BCG.

d) BCG provided no adjuvant effect on M. leprae; the mixtures were not any more effective than the more effective member of the mixture, namely BCG, itself (F vs. C and G vs. E).

e) Unexpectedly, freezing and thawing M. leprae increased its activity (J vs. B). The difference was present at both harvests, and the combined P values indicate a high degree of significance to the increase in activity resulting from freezing and thawing (P=0.0019). You may also note that there is no significant difference between the protection provided by viable BCG and that by frozen and thawed M. leprae. We had included the frozen and thawed vaccine because experimentation with the armadillo-grown bacilli will be very much facilitated if frozen tissues can be used as a source of bacilli for vaccines. We had not expected an increase in activity. A repeat experiment is in progress.

Because M. leprae infections in mice can be interfered with by graft-versus-host reactions, we had thought that some of the protective effect of BCG might be exerted through similar non-specific mechanisms, for example, through activation of macrophages. The results suggested that such non-specific effects were manifested against the M. leprae challenge itself. An earlier study of the effect of BCG given after the M. leprae challenge had found fluctuations in the protective effect that suggested that pre-challenge BCG also exerted at least part of its effect against the challenge (J. Immunol. (1966) 96:279). Consequently we began a new experiment on the effect of post-challenge BCG. BCG was given intradermally at -28, +28, +56, and +91 days in relation to challenge. The experiment is still in progress, but the early results are given in Table 2.

Table 1. P values for the differences between the groups shown in Figure 1, as calculated by the two-sample rank test. A refers to group A and A' in Figure 1.

Mouse Group	Vaccine	Route	A	B	C	D	E	F	G	H	I
			187-day harvests								
A	Nil		-								
B	ML	FP	<.10	-							
C	BCG	FP	.02-.05	.028	-						
D	ML	ID	.002-.02	.019	.360	-					
E	BCG	ID	.002-.02	.006	.236	.360	-				
F	ML + BCG	FP	>.10	.139	.178	.065	.025	-			
G	ML + BCG	ID	<.002	.001	.178	.323	.500	.010	-		
H	ML + BCG	FP + ID	.02-.05	.025	.088	.360	.270	.117	.178	-	
I	ML + BCG	ID + FP	<.002	.001-.002	.206	.360	.500	.019	.500	.178	-
J	ML*	FP	.05-.10	.046	.305	.152	.088	.221	.029	.270	.046
			*Frozen and thawed								
			277-day harvests								
A			-								
B			>.10	-							
C			.05 -.10	.139	-						
D			.002-.01	.032	.191	-					
E			<.002	.004	.022	.072	-				
F			.002-.02	.088	.342	.342	.041	-			
G			.002-.02	.029	.179	.500	.072	.323	-		
H			.001-.02	.036	.439	.500	.072	.360	.500	-	
I			>.10	.206	.342	.058	.004	.164	.041	.072	-
J			.002-.02	.041	.253	.399	.041	.439	.342	.399	.107

Table 2. Influence of time of BCG vaccine relative to M. leprae challenge.

Day BCG given [a]	Log_{10} (M. leprae/mouse)[b] on:						
	105d	118d	147d	185d	211d	240d	268d
Nil		<4.15	5.56	5.94	6.29	5.89	6.04
-28d		<4.15	4.18	4.96	4.77	4.95	<4.18
+28d		4.18	4.70	4.49	4.71	5.13	<4.18
+56d		5.26	5.10	4.80	4.97	4.48	4.48
+91d		4.18	4.65	4.88	5.50	6.30	5.15
Nil	4.78		5.22	5.68	6.07	5.95	5.98

[a]Relative to day of challenge with M. leprae.
[b]In pool of four mice.

All the vaccinations were effective, but the vaccine given at +91 days, just at about the time the logarithmic phase began, was the least effective. The results with the vaccine given at -28 days were very similar to those with the vaccine given at +28 days. There was no ineffective period just after challenge this time, and there was no suggestion of a special degree of growth delay as a result of activity exerted against the challenge inoculum itself. It would appear that if one wishes to see a consistent effect, he should not wait too long to give the vaccine. Judging by regional lymph node enlargement and by results of others with challenge with tubercle bacilli, BCG vaccine takes about a month to achieve its ful effect, so the M. leprae may have been definitely in their logarithmic phase before the +91-day vaccine could exert its full activity.

In another experiment we have given BCG vaccine at -168, -119, -70, and -28 days relative to challenge. Very early results indicate activity with all schedules. Thus there is no evidence so far in these experiments that the protective effect of BCG deteriorates with time. We have also observed that regional lymph node enlargement persists unabated for at least 178 days after intradermal BCT vaccine.

In summary, good vaccine protection was seen with suspensions of BCG and M. leprae, much better when given intradermally than when given into the foot pad (contralateral to the foot pad to be challenged). Mixtures of BCG and M. leprae were no more effective than the more effective member of the combination; there was no evidence of adjuvancy or inhibition. Freezing and thawing the M. leprae suspension increased its activity. Early results of studies of the timing of BCG vaccine indicate that vaccine given before or after challenge is about equally effective, and they give no ground for fears that pre-challenge vaccine exerts its protective effect only non-specifically. My feeling is that we should continue to test new vaccines by giving them before challenge. The second harvest, 90 days after plateau is reached, should detect instances of simple growth delay, and in case of doubt the vaccine can be given after challenge as well in a repeat experiment.

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