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Delayed Hypersensitivity Responses in Mice and Guinea Pigs to *Mycobacterium leprae*, *Mycobacterium vaccae*, and *Mycobacterium nonchromogenicum* Cytoplasmic Proteins

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Antigenic relationships between *Mycobacterium vaccae*, *M. nonchromogenicum*, and *M. leprae* were examined in mice and guinea pigs injected with *M. vaccae* or *M. nonchromogenicum* suspensions. The growth of both organisms in outbred ICR and four inbred mouse strains was followed up to 30 days. *M. nonchromogenicum* persisted in the livers and spleens of the inbred mice substantially better than did the *M. vaccae* population in the same mouse strains. A translucent colony variant of *M. vaccae* isolated from the opossum survived in vivo better than the opaque colony isolated from opossums and cattle. Persistence of *M. vaccae* and *M. nonchromogenicum* was not markedly increased in T-cell-depleted (nude) mice. Normal mice infected with increasing numbers of *M. vaccae* did not develop delayed-type hypersensitivity to the homologous *M. vaccae* cytoplasmic protein antigen. When heat-killed *M. vaccae* were incorporated into Freund adjuvant, both mice and guinea pigs developed delayed hypersensitivity to cytoplasmic antigens prepared from *M. vaccae*, *M. nonchromogenicum*, and *M. leprae*, but not to purified protein derivative. Both *M. nonchromogenicum* and *M. vaccae* vaccines cross-sensitized guinea pigs to the *M. leprae* cytoplasmic antigens.

The development of an effective vaccine for use against human leprosy poses a number of unique practical problems which, so far, have defied solution (19). Live BCG increased resistance to naturally acquired leprosy in some, but not in other field trials (2, 5). BCG is known to possess antigens which result in humoral, rather than cellular, cross-reactions with *M. leprae* (22, 23). Somewhat surprisingly, *M. vaccae* and *M. nonchromogenicum* were found to possess cross-reactive antigens which behave in a manner similar to that of *M. leprae* antigens in lepromatous leprosy patients (31). This antigenic relationship was confirmed by lymphocyte transformation (26) and leukocyte migration inhibition tests (25). The characteristic type of lepromin anergy seen in lepromatous leprosy patients occurred with *M. vaccae* and *M. nonchromogenicum* suspensions, but did not occur with antigens prepared from BCG or other slow-growing mycobacteria (27). Stanford (30) proposed that *M. vaccae* or *M. nonchromogenicum* might therefore function as suitable vaccine strains for use against human leprosy.

The possibility of developing an avirulent cross-reactive vaccine against this important human disease is an attractive one (18, 19). However, preliminary studies indicate that even massive doses of *M. vaccae* or *M. nonchromogenicum* are quickly eliminated from the tissues (14). The present investigation examines the growth characteristics of several recent isolates of *M. vaccae* and *M. nonchromogenicum* in a number of inbred mouse strains in an attempt to develop a better test system. Cross-reactive skin hypersensitivity was determined in both mice and guinea pigs after their inoculation with increasing amounts of live versus heat-killed vaccine.

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MATERIALS AND METHODS

Animals. Specific-pathogen-free outbred ICR; inbred C57BL/6, C3H, BALB/c, and DBA/2; and B6D2 and AB6 F_1 hybrid mice were raised at the Trudeau Animal Breeding Facility. They were kept on sterile bedding under barrier conditions and fed sterile mouse chow and acidified water ad libitum (10, 14). The mice were 5 weeks of age at the time of infection. Small numbers of athymic (nude) mice and their nu/+ littermates (BALB/c backgound) were also used. Inbred strain 2 guinea pigs were housed two to a cage

and fed sterile guinea pig chow and acidified drinking water (9). They were between 300 and 400 g at the time of vaccination.

Organisms. *M. vaccae* (TMC 1526) and *M. nonchromogenicum* (TMC 1481) were obtained from the Trudeau Mycobacterial Culture Collection, Saranac Lake, N.Y. Several recent isolates of *M. vaccae* were obtained from P. O'Hara, Wallaceville Animal Research Center, New Zealand. They had been isolated from infected lymph nodes of opossoms and cattle. They were confirmed as *M. vaccae* strains by means of standard cultural and biochemical tests (V. Montalbine, personal communication). Many of the *M. vaccae* and *M. nonchromogenicum* cultures could be dissociated into mucoid, translucent, and smooth opaque colony variants on 7H10 agar (28). The colony variants remained stable when serially cultured on solid media. The separated colony types were grown in gently stirred 7H9 broth at 37°C for 7 days, and the suspensions were stored at −70°C until required (14). The viability of each suspension was checked after thawing and dilution in 0.05% Tween-saline by plating on 7H10 agar incubated at 37°C in sealed plastic bags for 2 to 3 weeks.

Animal inoculation. A frozen ampoule was thawed at 37°C, homogenized mechanically in an equal volume of fresh medium (to break up the clumps), and diluted appropriately in Tween-saline, and 0.2 ml was injected intravenously (14). Guinea pigs were injected subcutaneously into recently shaven mid-flank skin with 0.1 ml of suspension (9). Immediately after inoculation, the viability of each suspension was determined on 7H10 agar plates (10).

Bacterial enumeration of in vivo populations. Groups of five randomly selected mice were sacrificed at weekly intervals, and the inoculation site, the draining lymph node, lungs, liver, and spleen were removed aseptically and homogenized separately in sterile Tween-saline (9, 13). Viable counts were made on 7H10 agar plates incubated at 37°C for 2 to 3 weeks. The counting error for the five replicate determinations varied from 10 to 20%, as reported in earlier studies (10).

Delayed hypersensitivity tests. Mice were footpad tested with 5 μg of cytoplasmic protein antigen (CPA) isolated from sonically disrupted mycobacteria (16, 27) in 0.02 ml of 0.05% Tween-saline or with 10^8 heat-killed whole mycobacterial cells (18). *M. leprae* were prepared from human wedge biopsies obtained by the Leonard Wood Memorial Laboratory, Philippines. The biopsy was trimmed of fatty tissue after autoclaving and homogenized in 0.3 M buffered sucrose by using an Omnimix blender. The cells were centrifuged at 300 × *g* for 5 min to remove tissue pieces, and the supernatant fluid was centrifuged at 15,000 × *g* for 20 min at 4°C. The pellet was resuspended in 1% Triton X-100 in 0.3 M buffered sucrose and washed twice with buffered sucrose before being treated overnight with 100 μg of collagenase per ml (Worthington Biochemicals, Freehold, N.J.) in the presence of 10^{-4} M calcium chloride and 0.05% sodium azide. The bacilli were centrifuged at 15,000 × *g* for 20 min and resuspended in 0.05% Tween-saline. The suspension was counted microscopically (29) and standardized to 5×10^8 bacilli per ml. A small amount of CPA was prepared from the human *M. leprae* suspension by sonic disruption followed by ultracentrifugation at 100,000 × *g* for 90 min. The protein content of the various CPA preparations was checked by the method of Lowry et al. (24) and standardized to 300 μg of protein per ml. The amount of foot swelling to 5 or 10 μg of CPA was measured after 3, 6, 24, 48, and 72 h by using dial gauge calipers (10). The swelling responses to 5×10^7 whole cells were read at twice-weekly intervals for up to 6 weeks. Guinea pigs were skin tested with 5 to 10 μg of purified protein derivative (PPD) or CPA in 0.1 ml of buffered saline injected into clipped flank skin (9). The diameters of skin erythema and skin fold thickness were measured after 3, 24, and 48 h as described earlier (15). An increase of 0.2 mm or more in foot thickness or 1 mm in skin fold thickness (after subtraction of the dilution controls) was significant at the 1% level.

RESULTS

Growth of *M. vaccae* in inbred mice. Extensive differences have been reported to exist between the susceptibility of different mouse strains to mycobacterial challenge (6, 20). Previous studies indicated that *M. vaccae* was unable to persist when introduced into normal ICR mice by the intravenous or subcutaneous routes (14). The present study examined the growth of the type strain of *M. vaccae* (TMC 1526) when injected intravenously into eight different strains of mice. The data shown in Fig. 1 indicate that this organism was unable to establish a persisting infection in any of the inbred mouse strains tested, although some quantitative differences were observed in the actual rate of decline. *M. vaccae* failed to persist in the lungs of any of the mice, but their disappearance may have been due to the small initial size of this population within the lungs, rather than to an enhanced local rate of inactivation. The rate of decline by the liver and spleen populations was approximately equal in all of the mice for the first 7 days, but then slowed so that a few viable bacilli were recovered from the BDA-2, C3H, and BALB/c mice even after 60 days.

Similar growth studies were carried out with the translucent and the opaque-domed colony variant of the opossum strain of *M. vaccae*, as well as a bovine isolate which could only be obtained in the mucoid and the opaque colony forms. Both the mucoid and opaque bovine colony types behaved identically in the various mouse strains to the opossum opaque type and were not included for that reason. The translucent opossum variant of *M. vaccae* survived better than the type strain TMC 1526 after intravenous inoculation into the inbred mice (Fig. 2). In fact, the residual population in the C57BL mice after 4 weeks represented nearly 10% of the initial inoculum. When 5×10^8 viable

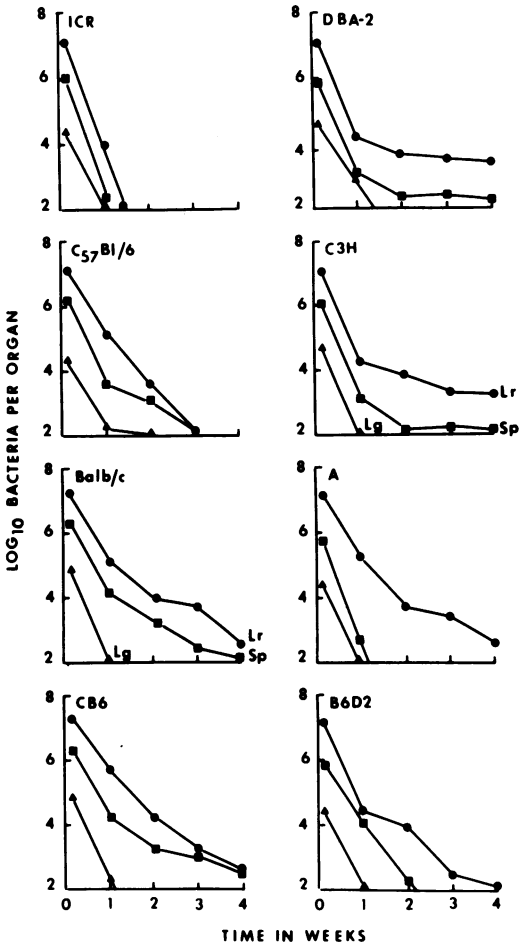

FIG. 1. *Growth curves for M. vaccae TMC 1526 after intravenous inoculation into outbred ICR or inbred mice. Lr, Liver; Sp, spleen; Lg, lung. Each point represents the mean of five determinations. The standard error of the mean was often too small to be accurately represented on the logarithmic scale by vertical bars, which have been omitted for that reason. Counting errors were 10 to 20% of the mean.*

bacilli were injected into B6D2 F_1 hybrid mice, there was no sign of clinical disease even after 90 days, despite the presence of more than 10^5 viable bacilli in the liver. On the other hand, the opaque and the mucoid colony variants of the bovine *M. vaccae* failed to persist in vivo after intravenous inoculation into the various inbred mouse strains, the inoculum being virtually eliminated by day 30 (Fig. 3).

Growth of *M. nonchromogenicum* in inbred mice. Mice infected with 10^7 viable *M. nonchromogenicum* TMC 1481 (translucent colony type) showed a slower decline in viability over the first 4 weeks of the infection. The

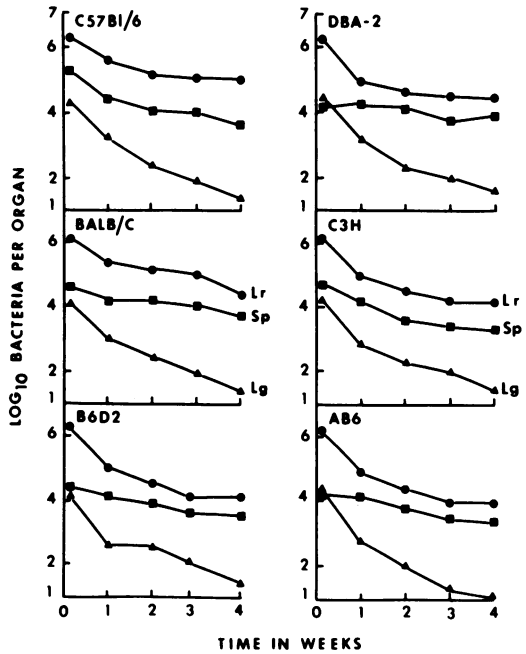

FIG. 2. *Growth curves for the translucent colony variant of M. vaccae opossum (translucent) after intravenous inoculation into inbred and hybrid mice. Lr, Liver; Sp, spleen; Lg, lung.*

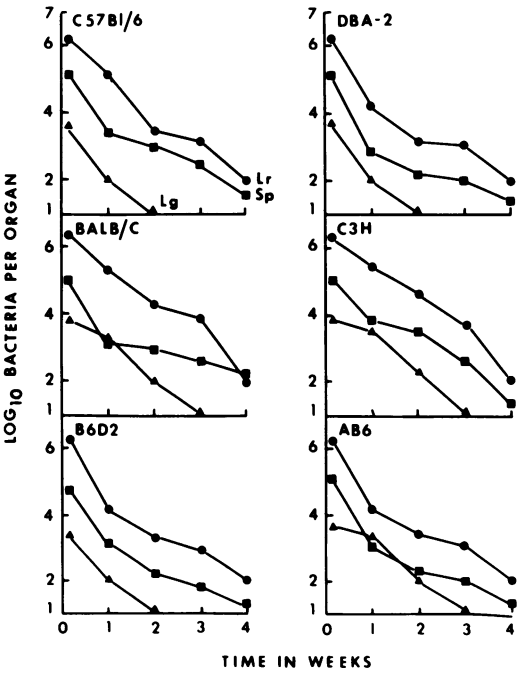

FIG. 3. *Growth curves for the opaque variant of M. vaccae opossum (opaque after intravenous inoculation into inbred mice. Lr, Liver; Sp, spleen; Lg, lung.*

BALB/c and DBA-2 mouse counts tended to stabilize so that many of the animals contained substantial numbers of mycobacteria even after 3 months (Fig. 4).

Growth in athymic (nude) mice. Both *M. vaccae* and *M. nonchromogenicum* exhibited a substantial decline in viability in immunocompetent mice over the first few weeks of the infection. Experiments were carried out in T-cell-depleted mice in an attempt to determine whether this drop was the result of a T-cell-mediated immune response or was due to non-immunological mechanisms. Groups of athymic (nude) mice, together with their heterozygous littermates, were infected intravenously with the various *M. vaccae* and *M. nonchromogenicum* strains, and the resulting growth curves are shown in Fig. 5. Comparisons of the decline rates for these organisms in nude versus heterozygous nu/+ mice (Fig. 1 to 5) show almost identical growth curves with no sign of enhanced survival or disease in the T-cell-depleted host. Liver and spleen viable counts as high as 10^5 viable *M. nonchromogenicum* could be observed after 3 months. In an attempt to determine a mean lethal dose for *M. vaccae* and *M. nonchromogenicum*, normal and THXB B6D2 mice (13) were infected with up to 10^9 viable bacilli, with no deaths occurring up to 12 months.

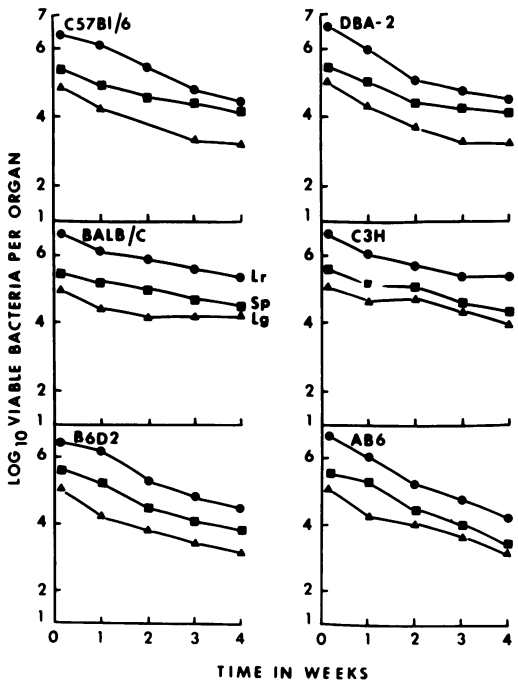

FIG. 4. *Growth curves for M. nonchromogenicum (translucent) after intravenous inoculation into inbred mice. Lr, Liver; Sp, spleen; Lg, lung.*

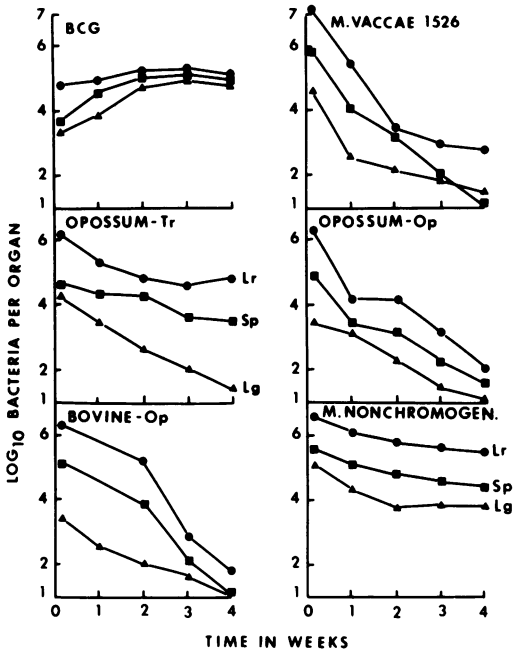

FIG. 5. *Growth curves for BCG, M. vaccae, and M. nonchromogenicum after intravenous inoculation into athymic nude mice. Lr, Liver; Sp, spleen; Lg, lung.*

Delayed hypersensitivity to CPA. The ability of *M. nonchromogenicum* and *M. vaccae* to survive in vivo for several weeks raises the possibility that the infected mice may develop delayed-type hypersensitivity responses to antigens isolated from these organisms. Mice were infected with increasing numbers of viable *M. vaccae* (10^6 to 10^8), and footpads were tested at weekly intervals with 10 μg of the homologous CPA. Although substantial 3-h swelling reactions developed, none of the mice exhibited significant 24-h swelling responses even after 3 weekly doses of 5×10^6 viable *M. vaccae* (Table 1). However, if the *M. vaccae* was emulsified in Freund adjuvant, significant 24-h reactions were observed which were consistently greater than the 3-h swelling responses.

When live *M. vaccae* were injected into strain 2 guinea pigs, no skin reactivity developed unless the organisms were suspended in Freund adjuvant. Injection of *M. vaccae, M. nonchromogenicum*, and *M. leprae* CPA into guinea pig skin after sensitization with heat-killed *M. tuberculosis* or *M. vaccae* resulted in significant delayed-type hypersensitivity responses (Table 2). The *M. leprae* CPA also resulted in a significant delayed-type hypersensitivity reaction in *M. vaccae*-immunized guinea pigs, with no cross-reaction to PPD.

Delayed swelling responses to whole cell antigens. The *M. vaccae*-vaccinated mice were also footpad tested with 10^8 heat-killed whole *M. vaccae* suspension. The increase in footpad thickness was reported at intervals of up to 6 weeks (Table 3). There was a significant swelling response 14 to 21 days after the footpad injection in the mice vaccinated with 10^6 viable *M. vaccae*. As the vaccine dose increased to 10^8 viable units, there was a substantial reduction in the swelling response. The BCG-vaccinated controls also showed a late swelling response, but the peak occurred on day 28, at the same time that the antigen produced a much smaller reaction in the unvaccinated controls. The progressive decrease in the 14- to 21-day swelling responses seen as

TABLE 1. *Increase in foot thickness in M. vaccae-vaccinated B6D2 mice injected in the hind footpad with 10 μg of M. vaccae CPA at increasing time intervals*

Vaccine[b]	Mean increase of foot thickness at time postvaccination:[a]							
	7 Days		14 Days		21 Days		28 Days	
	3 h	24 h	3 h	24 h	3 h	24 h	3 h	24 h
1× Dose live 10^6 i.v.	4.8 ± 0.7	0.8 ± 0.6	4.3 ± 0.2	0	7.8 ± 0.5	3.0 ± 1.0	6.9 ± 0.4	2.1 ± 1.0
1× Dose live 10^7 i.v.	5.2 ± 0.4	1.6 ± 0.6	3.4 ± 0.5	0	6.6 ± 0.5	3.0 ± 0.9	5.7 ± 0.3	2.8 ± 0.7
3× Dose live 10^7 i.v.	5.2 ± 0.7	1.8 ± 0.4	6.8 ± 0.8	2.5 ± 0.7	3.4 ± 0.5	2.0 ± 0.3	4.4 ± 0.4	2.2 ± 0.4
1× Dose live 10^8 i.v.	4.4 ± 0.9	1.6 ± 0.5	3.4 ± 0.6	0	4.0 ± 0.3	2.8 ± 0.4	5.1 ± 0.4	2.9 ± 0.4
1× Dose H-K 10^8 s.c.	3.4 ± 0.2	5.0 ± 0.7	4.4 ± 0.5	6.3 ± 0.9	3.8 ± 0.7	4.5 ± 0.7	1.9 ± 0.4	3.3 ± 0.3

[a] Mean increase (10 U = 1.0 mm) for five determinations ± standard deviations.
[b] i.v., Intravenously; s.c., subcutaneously. H-K, Heat killed.

TABLE 2. *Skin reactions in guinea pigs sensitized with heat-killed mycobacteria suspended in Freund adjuvant*

Skin test antigen (10 μg)	Increase in foot thickness with vaccine dose:[a]											
	10^6 Live BCG				10^8 Heat-killed $H_{37}Rv$				10^8 Heat-killed *M. vaccae*			
	3 h	6 h	24 h	48 h	3 h	6 h	24 h	48 h	3 h	6 h	24 h	48 h
PPD	6 ± 0.7	4 ± 0.8	11 ± 0.4	2 ± 0.5	7 ± 1.0	4 ± 0.5	10 ± 0.2	3 ± 2.0	4 ± 1.3	2 ± 1.4	2 ± 0.7	0
M. vaccae	4 ± 0.5	1 ± 0.2	4 ± 0.7	0	8 ± 0.5	8 ± 0.8	10 ± 0.4	3 ± 0.2	5 ± 0.9	3 ± 1.0	11 ± 0.8	3 ± 1.2
M. nonchromogenicum	5 ± 0.5	3 ± 0.6	13 ± 0.9	0	12 ± 0.9	8 ± 1.1	9 ± 0.5	2 ± 0.6	6 ± 1.1	3 ± 0.7	15 ± 1.1	3 ± 0.4
M. leprae	8 ± 0.6	9 ± 0.8	5 ± 1.4	0	3 ± 0.2	0	3 ± 0.4	0	4 ± 0.9	2 ± 0.3	11 ± 1.0	2 ± 0.4
Saline	2 ± 0.6	1 ± 0.5	0	0	1	0	0	0	2 ± 1.0	1 ± 0.8	1 ± 0.4	0

[a] Increase in skin fold thickness in Schnelltaster units (10 U = 1 mm). Average of four determinations. An increase in 10 U or more is significant at the 1% level.

TABLE 3. *Increase in foot thickness in B6D2 mice vaccinated with increasing doses of live M. vaccae given intravenously (i.v.) and their footpads tested with 10^8 heat-killed whole M. vaccae*

Time (days)	Increase in foot thickness[a]					
	Nil	*M. vaccae* i.v.				BCG i.v.
		10^6	10^7	10^8	3×10^7	10^6
3 h	3.0 ± 0.5	12.4 ± 0.7	11.5 ± 0.8	10.6 ± 2.6	16.0 ± 2.6	7.8 ± 0.6
6 h	4.4 ± 0.5	13.8 ± 0.8	13.4 ± 0.6	14.0 ± 0.6	14.0 ± 0.6	12.0 ± 1.0
1	2.8 ± 0.5	10.0 ± 0.5	9.9 ± 0.8	7.6 ± 0.6	7.6 ± 0.6	10.2 ± 1.5
2	2.2 ± 0.6	3.2 ± 0.7	4.9 ± 0.5	4.0 ± 0.7	4.0 ± 0.7	7.8 ± 0.4
3	0.4 ± 0.2	2.0 ± 0.3	1.9 ± 0.5	1.2 ± 0.6	1.2 ± 0.6	
4	0.0	2.0 ± 0.6	1.0 ± 0.4	0.4 ± 0.4	0.4 ± 0.4	3.0 ± 0.6
7	0.0	2.6 ± 0.2	2.2 ± 0.2	1.8 ± 0.7	1.8 ± 0.7	2.0 ± 0.4
14	2.0 ± 0.5	8.0 ± 0.6	6.1 ± 0.2	4.8 ± 1.3	4.0 ± 0.6	2.0 ± 0.6
21	1.2 ± 0.5	5.0 ± 0.3	4.3 ± 0.4	2.6 ± 0.8	4.2 ± 0.6	2.5 ± 0.4
28	4.8 ± 0.7	5.4 ± 0.2	3.5 ± 0.6	1.8 ± 0.7	5.4 ± 0.4	4.0 ± 0.2
35	0.0	3.9 ± 0.8	1.8 ± 0.4	0.0	3.8 ± 0.6	2.5 ± 0.4
42	0.0	1.4 ± 0.2	0.0	0.0	2.4 ± 0.4	1.6 ± 0.4

[a] Mean increase (10 U = 1.0 mm) for five determinations ± standard deviation.

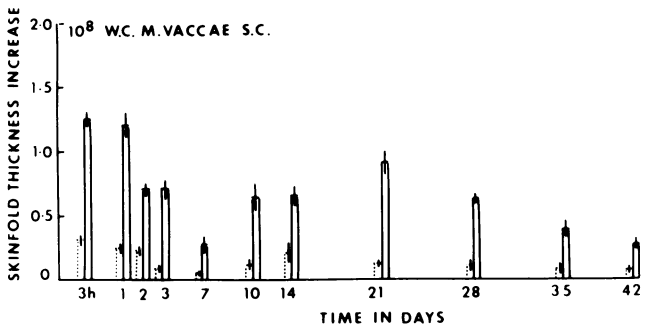

FIG. 6. *Increases in skin fold thickness in strain 2 guinea pigs vaccinated 2 weeks previously with 10^7 live M. vaccae and skin tested with 10^8 whole cell antigen (heat-killed M. vaccae). The dotted vertical bars represent the unvaccinated controls. The solid vertical bars represent ± standard error of the mean for four determinations. W.C., Whole cell; s.c., subcutaneous.*

the vaccination dose was increased from 10^6 to 10^8 *M. vaccae* appeared to be a type of overload anergy, since increasing the vaccine dose further to 10^9 viable *M. vaccae* completely ablated the footpad response.

A similar swelling response was observed in *M. vaccae*-infected guinea pigs skin tested with 10^8 heat-killed *M. vaccae* 2 weeks later. There was an early peak (3 to 24 h) followed by a decline to nonsignificant levels and a second peak about day 21 (Fig. 6). A decreasing response was also seen as the vaccinating dose was increased from 10^6 to 10^8 viable units.

DISCUSSION

Previous studies of the growth of *M. vaccae* in intravenously infected ICR mice (14) showed that this organism was unable to survive in vivo for more than 1 week, even when introduced into the tissues in large numbers. Examination of the various growth curves shown in Fig. 1 indicates that the outbred ICR mouse had been an unfortunate choice for the earlier studies (14), since persistence was noticeably better in several of the inbred mouse strains infected with the various *M. vaccae* substrains tested during the present investigation. The differences in survival shown by the inbred mice were relatively small, but there was no question that the colonial form shown by the challenge strain was a highly important factor in determining the relative survival of the inoculum in vivo. The opossum strain of *M. vaccae* persisted in vivo much better than the type strain (TMC 1526) or the bovine isolate. The translucent to opaque colony variation seems to be essentially the same as that described for *M. avium* and *M. intracellulare* (17, 28). In the latter group, this colonial variation was associated with changes in virulence, both in chickens and mice (1). Although it is not possible to refer to *M. vaccae* or *M. nonchromogenicum* in terms of mouse virulence, the translucent colony variants did persist in vivo far longer than the opaque colony type (cf. Fig. 2 and 3). Thus, any attempt to develop a living *M. vaccae* vaccine would have to employ the translucent variant, and further study of this strain in C57BL, DBA-2, or the B6D2 F_1 hybrid mice would seem to be worthwhile.

M. nonchromogenicum persisted in the inbred mice far better than did *M. vaccae*. However, none of the heavily infected mice developed obvious signs of disease, even after many months. When *M. nonchromogenicum* was introduced into the athymic mice, there was no sign of a progressive infection and none of the animals died as a result of the infection. This was taken as evidence that *M. vaccae* and *M. nonchromogenicum* are fully avirulent, even for immunosuppressed mice. However, the question of their potential virulence as human vaccines is still unanswered. Stanford (30) cites a single case of *M. nonchromogenicum* infection in humans. The usual source of this organism is soil and water, and most workers consider it to be completely innocuous for humans (32). On the other hand, *M. vaccae* is frequently isolated from bovine lymph nodes and from milk (4), but whether this organism can cause infections in humans has not yet been established. It is not clear whether the opossum isolates were primarily responsible for the lymph node infections seen in these animals or whether they were present merely as secondary invaders or adventitious contaminants (P. O'Hara, personal communication).

Anti-tuberculous immunity is a T-cell-mediated response developing as a result of the introduction of living *Mycobacteria* into the tissues (7, 8). On the other hand, killed bacilli fail to induce significant levels of cellular activation unless first incorporated into a Freund-type ad-

juvant (3, 10). However, not all living attenuated mycobacteria are capable of inducing significant levels of cell-mediated immunity (10, 13). This inability on the part of the nonpersisting mycobacterial inoculum to sensitize the host occurs despite the presence of the relevant antigens in the bacterial cell (12). Active sensitization requires a persistent, metabolically active population of bacteria in the spleen or the draining lymph node (13). Thus the injection of living *M. vaccae* (or *M. nonchromogenicum*) may still fail to induce detectable levels of cellular hypersensitivity, if they are unable to persist within the vaccinated host (Table 1). In this regard, the development of delayed-type hypersensitivity to the CPA preparation is merely a convenient parameter of the cellular immune response to the living *M. vaccae* or *M. nonchromogenicum*. The inability of live *M. vaccae* to induce delayed hypersensitivity responses to the *M. vaccae* CPA, unless the organisms are incorporated into Freund adjuvant, makes it unlikely that this organism will prove to be a practical anti-leprosy vaccine for use in humans. However, until its growth behavior has been established in human volunteers, it is difficult to make a final assessment on this point. Some skin reactivity to *M. vaccae* CPA and *M. leprae* CPA was seen in guinea pigs immunized with heat-killed *M. vaccae* (Table 2). These same animals displayed no cross-reactivity when the skin test antigens were tested in BCG-vaccinated controls. Interestingly, the guinea pigs receiving live *M. vaccae* developed substantial late skin responsiveness to the *M. vaccae* whole-cell antigen (Fig. 6). A similar response was observed in actively infected mice footpad tested with the same whole-cell preparation (Table 3). This same cross-reactivity developed in the *M. vaccae*-infected mice and guinea pigs tested with the *M. leprae* antigens. Such reactivity was quite significant and seems to justify further study of these attenuated mycobacteria as potential anti-leprosy vaccines for use in humans.

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