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Effect of Anti-Cord Factor Antibody on Experimental Tuberculosis in Mice

MASAHIKO KATO

Research Institute of Tuberculosis, Toneyama Hospital, National Sanatorium, Toyonaka, Osaka, Japan 560

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Either active immunization with trehalose-6,6'-dimycolate (cord factor)-methylated bovine serum albumin complex or passive transfer of rabbit anti-cord factor serum induced in mice an enhanced resistance against infection with virulent human *Mycobacterium tuberculosis*. This suggests that the anti-cord factor antibody exerts an infection-protecting effect by neutralizing the toxic action of cord factor during the course of living virulent infection with tubercle bacilli. The protective effect of active immunization with cord factor-methylated bovine serum albumin complex and of anti-cord factor serum was found to be specific for the tuberculous infection among a number of experimental infections in mice with a variety of cytopathogenic microorganisms tested.

A previous paper from this laboratory (12) has described a method for producing a precipitating and neutralizing antibody to trehalose-6,6'-dimycolate (cord factor) of *Mycobacterium tuberculosis* in mice and rabbits by the vaccination with cord factor-methylated bovine serum albumin (MBSA) complex. The accompanying communication (13) gives evidence that this antibody to cord factor resides predominantly in 19*S* immunoglobulin M fraction of serum of rabbits vaccinated with cord factor-MBSA complex, and that no antibody response to cord factor was elicited in man and laboratory animals by the infection with virulent *M. tuberculosis*.

Another series of studies on the biochemical basis of the toxicity of cord factor demonstrated that this toxic glycolipid of *M. tuberculosis* induced either in vivo (8, 15) or in vitro (10, 11) a structural damage of the mitochondrial membrane system and a functional defect in the respiration and accompanying phosphorylation in mouse liver mitochondria. From these observations, it was assumed that cord factor plays a certain role in the virulence of tubercle bacilli by interfering with the basic metabolic activity in host cells.

Since the anti-cord factor antibody neutralizes the toxic action of cord factor both in vivo and in vitro (12), the above hypothetical role of cord factor in the virulence of tubercle bacilli was studied by testing the effect of either active immunization with cord factor-MBSA complex or passive transfer of rabbit anti-cord factor serum on the experimental tuberculosis in mice. The results demonstrated that anti-cord factor antibody protects mice against the infection with virulent tubercle bacilli, which suggests that cord factor plays an important role in the pathogenesis of tuberculosis.

MATERIALS AND METHODS

Animals. Male albino mice of random-bred dd0 stock (8) 4 to 8 weeks of age and rabbits of both sexes weighing 3 kg were maintained as previously described (12).

Cord factor and related compounds. Cord factor, mycolic acid, and acetylated cord factor were prepared from human tubercle bacilli, strain H37Rv, as previously reported (11). Methyl 6-mycoloyl-α-D-glucopyranoside and 6,6'-dimycoloyl sucrose (14) were supplied by J. Asselineau, Laboratoire de Biochimie Structurale et Métabolique, Université Paul Sabatier, Toulouse, France. An aqueous emulsion of these natural and semisynthetic lipids was prepared by the previously reported method (11).

Vaccination. Cord factor-MBSA complex was prepared as reported in a previous paper (12). An aqueous suspension of the complex equivalent to 50 μg of cord factor in 0.05 ml of water was emulsified with an equal volume of Freund incomplete adjuvant and injected subcutaneously into mice twice a week for 3 weeks. The vaccination of rabbits was done in the same manner by using the complex equivalent to 1 mg of cord factor for each injection. Intravenous vaccination of mice was done with the same dose of cord factor-MBSA complex given by the same schedule without adjuvant. For the vaccination of mice

with the combination of the derivatives and semisynthetic analogues of cord factor and either MBSA or BSA, the ratio of lipid to protein was 1:3. Live BCG vaccine (strain 1331) containing 10^7 to 5×10^7 viable units was administered intravenously into mice and rabbits.

Serum of rabbits was prepared 10 to 14 days after the last injection of cord factor-MBSA complex and 6 weeks after the BCG vaccination as previously described (12).

Infection. Mice were infected by the intravenous inoculation with virulent human *M. tuberculosis* H37Rv grown for 7 days in Tween-albumin liquid medium (Difco Laboratories, Detroit, Mich.). For the inoculation into mice vaccinated with a mixture of cord factor and BSA, bacilli were collected by centrifugation, washed twice with 0.85% saline, and suspended in the initial culture volume of saline containing 0.02% Tween 80. Viable units in the inoculum were determined by plating serial dilutions of the culture with 0.1% BSA on oleic acid-albumin agar (Difco).

Listeria monocytogenes (NCTC 7973) of serotype I was obtained from G. B. Mackaness, Trudeau Institute, Saranac Lake, N.Y. The bacteria were cultered on brain heart infusion agar (Difco) for 17 hr at 37 C. Bacteria were harvested by washing the plate with Hanks balanced salt solution and injected intravenously into mice after appropriately diluting with the same medium. The bacteria contained in the inoculum were counted in a Petroff-Hauser chamber.

Brucella abortus A62 was supplied by K. Kanai, Department of Tuberculosis, National Institute of Health, Tokyo, Japan. The bacteria were cultured on Tryptose agar (Difco) for 48 hr at 37 C. Bacteria were collected by washing the plate with saline, and were appropriately diluted and injected intravenously into mice. Viable units in the inoculum were determined by plating serial dilutions of the bacterial suspension on Tryptose agar.

Salmonella enteritidis no. 11 and *S. typhimurium* LT2 were obtained from K. Saito, Department of Microbiology, Keio University School of Medicine, Tokyo, Japan. The bacteria were grown on nutrient agar (Difco) for 18 hr at 37 C. Bacteria were collected by washing the plates with saline and were brought to an appropriate concentration and injected intraperitoneally into mice. The number of bacteria in the inoculum was determined by plating the increasing dilutions of the bacterial suspension on nutrient agar.

Enumeration of viable units of tubercle bacilli in the infected tissue. Lungs and spleen of mice infected with *M. tuberculosis* H37Rv were aseptically removed and ground with nine volumes (v/w) of sterile 2% BSA in a glass homogenizer of Potter-Elvehjem type. The tissue suspensions were diluted with sterile 0.1% BSA and 25 μliters of appropriate dilutions were plated on oleic acid-albumin agar (Difco). The plates were incubated for 2 and 3 weeks at 37 C, the colonies were counted, and the number of viable units in total organ was calculated from the dilution factor.

Histological procedures. Formalin-fixed mouse lungs were processed by conventional histological techniques and stained with hematoxylin and eosin.

RESULTS

Protective effect of cord factor-MBSA complex on experimental tuberculosis in mice. Figure 1 compares the survival curves of mice vaccinated with either cord factor-MBSA complex or live BCG vaccine with that of nonvaccinated control mice after a massive intravenous challenge with *M. tuberculosis* H37Rv. Mice vaccinated with cord tactor-MBSA complex showed an enhanced resistance to infection which was comparable to that observed in BCG-vaccinated animals. The weight of, and the number of viable units in, the lungs of mice at 30 days after challenge are shown in Table 1. The lungs of nonvaccinated control mice were markedly enlarged and covered with numerous tubercles, whereas the macroscopic lesion in those of cord factor-MBSA- and BCG-vaccinated mice was minimal. Histologically, extensive exudative lesions were observed in the lungs of nonvaccinated control mice, whereas such lesions were hardly visible in the lungs of cord factor-MBSA-vaccinated mice (Fig. 2). In close accord with these findings, the number of viable units in the cord factor-MBSA-vaccinated mouse lungs was 10^2 to 10^3 less than that recovered from the control mouse lungs. An almost identical level of bacterial counts was observed in the lungs of cord factor-MBSA- and BCG-vaccinated mice (Table 1). These results indicate that the protective effect of cord factor-MBSA complex against tuberculous infection is as potent as that of live BCG vaccine.

Figure 3 demonstrates the bacterial counts in lungs and spleen recovered at weekly intervals after the infection of mice with *M. tuberculosis* H37Rv. An apparent prevention of the bacterial multiplication was observed in both lungs and spleen of the cord factor-MBSA-vaccinated mice. In lungs, the bacterial growth was supressed during the first 3 weeks after challenge, and a constant low level of viable in vivo population of tubercle bacilli was observed until week 8 in the vaccinated group. In spleen of the vaccinated mice, the increase in the number of viable tubercle bacilli during the first 3 weeks after challenge was not seen.

Table 2 indicates that the protective potency of cord factor-MBSA complex against both the toxic action of cord factor and the infection with tubercle bacilli becomes appar-

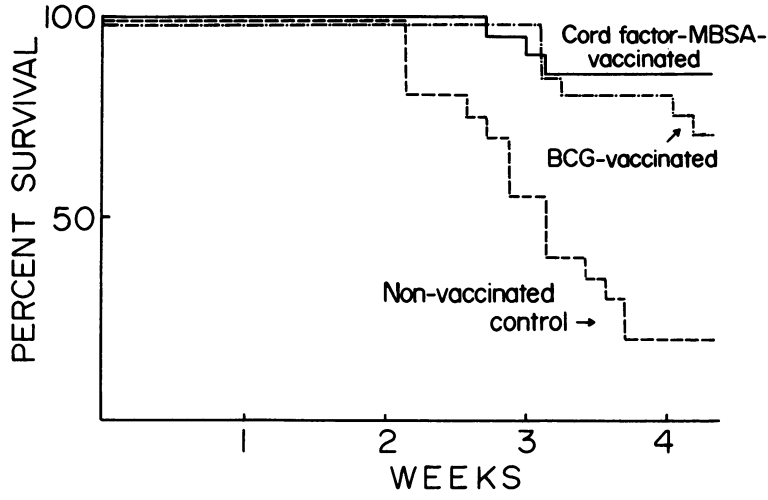

FIG. 1. *Survival curves of mice infected with M. tuberculosis H37Rv after the vaccination with cord factor-MBSA complex. Twenty mice vaccinated with either cord factor-MBSA complex or live BCG and 20 non-vaccinated control mice were infected intravenously with 4.0 × 10⁷ viable units of H37Rv.*

TABLE 1. *Effect of vaccination with cord factor-MBSA complex on tuberculous infection in mice*

Experimental group	Results at 30 days after challenge[a]		
	Wt of lungs[b] (mg)	Viable units in lungs	Survivors[c]
Nonvaccinated control	635 ± 40	3.2×10^{8}[d] 1.1×10^{8} 3.6×10^{7} 2.0×10^{7} 1.2×10^{7}	4/20
BCG-vaccinated control	287 ± 49[e]	2.4×10^{6} 8.8×10^{5} 5.4×10^{5} 2.5×10^{5} 2.2×10^{5}	14/20
Cord factor-MBSA-vaccinated	310 ± 25[e]	1.1×10^{6} 4.0×10^{5} 3.5×10^{5} 2.8×10^{5} 1.4×10^{5}	17/20

[a] Mice were challenged intravenously with 0.1 ml of a 7-day culture of *M. tuberculosis* H37Rv in Tween-albumin medium containing 4.0×10^{7} viable units per inoculum.
[b] Mean ± standard deviation of five mice in each group.
[c] Number of mice survived/number of mice challenged.
[d] Dead mice.
[e] Variance ratio exceeds 1% level of significance.

ent after a repeated vaccinating injection during at least 3 weeks. The previous observation (9) that live BCG vaccine induces an enhanced resistance to the infection with virulent tubercle bacilli but fails to protect mice against the toxicity of cord factor is again confirmed.

The results summarized in Table 3 compare the protective effect of cord factor-MBSA complex with those of other related preparations. Cord factor-MBSA complex was found to be most effective when administered with Freund incomplete adjuvant by the subcutaneous route or into footpad. When injected without adjuvant, the complex showed no protective effect against infection even by the same routes. Intravenous vaccination of the complex without adjuvant was less effective. Neither cord factor nor MBSA alone was effective. When mycolic acid or acetylated cord factor was substituted for cord factor and BSA for MBSA, lipid-protein complex was not formed, and no effective protection against tuberculous infection was observed. The vaccination with a mixture of cord factor and BSA provoked in mice a hypersensitive reactivity to BSA which caused an anaphylactic death of mice by the intravenous injection of the bacterial culture in Tween-albumin medium. For this reason, the infection of this group of mice was done with H37Rv cells washed with saline and suspended in saline containing 0.02% Tween 80. The vaccination with the complex formed between two semisynthetic cord factor analogues, either methyl 6-mycoloyl-α-D-glucopyranoside or 6,6′-dimycoloyl sucrose, and MBSA was not effective. Thus, the complex formation of cord factor with MBSA seems to be very essential for the elicitation of protective effect against tuberculous infection. It is also apparent that, except live BCG, the protection against tuberculous infection is intimately connected with the neutralizing activity of each vaccine

against the toxicity of cord factor described in a previous paper (12).

In an attempt to test the specificity of the infection-protecting effect of cord factor-MBSA complex, mice vaccinated with the complex were challenged with a number of cytopathogenic bacteria: listeria, brucellae, and salmonellae. The challenge doses of these organisms were adjusted to cause the deaths of control mice within 3 to 5 days after challenge. Table 4 shows that the infection-protecting effect of cord factor-MBSA complex is highly specific for the infection with *M. tuberculosis*. The vaccination with cord factor-MBSA complex protected mice against the infection with neither listeria, brucellae, nor salmonellae.

Effect of anti-cord factor serum on experimental tuberculosis in mice. The above results demonstrate that the active immunization with cord factor-MBSA complex protected mice against the infection with virulent *M. tuberculosis*. Since it has been shown in a previous paper (12) that the vaccination with cord factor-MBSA complex induced in mice and rabbits an antibody to cord factor which demonstrated a precipitin reaction with cord factor and neutralized its toxicity both in vivo and in vitro, it seems most likely that the anti-cord factor antibody displays the protection of infection by neutralizing the toxic action of cord factor in mice infected with virulent tubercle bacilli. To test this possibility, the pooled sera of cord factor-MBSA-vaccinated rabbits (×160 to ×320 antibody titer by precipitation) were subcutaneously injected into mice everyday starting 1 week before intravenous challenge with *M. tuberculosis* H37Rv. After challenge, the serum was injected every other day for 30 days. Control mice received no serum, nonvaccinated rabbit serum, or BCG-vaccinated rabbit serum.

Figure 4 shows that injection of the serum of rabbits vaccinated with cord factor-MBSA complex produced in mice an enhanced resistance against infection with *M. tuberculosis*. During 30 days after a massive intravenous challenge with H37Rv, no death was observed in the group of mice injected with the serum of rabbits vaccinated with cord factor-MBSA complex, whereas all mice were dead by infection during this period in the groups of animals injected with no sera, control sera, and BCG-vaccinated rabbit sera. After the discontinuation of serum injection at 30 days, 60% of the mice that received the serum of cord factor-MBSA-vaccinated rabbits were dead in 2 weeks, but the remaining 40% of animals survived for more than 60 days post-infection.

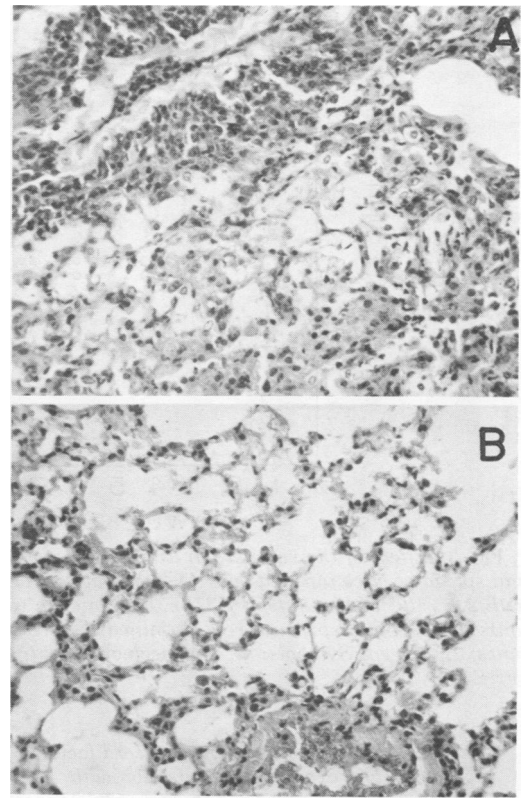

FIG. 2. *Section of lungs of nonvaccinated and cord factor-MBSA-vaccinated mouse 30 days after the intravenous inoculation with 4.0 × 10^7 viable units of M. tuberculosis H37Rv. A, Nonvaccinated mouse lung; B, cord factor-MBSA-vaccinated mouse lung. Extensive exudative lesions are present in A; barely visible pathologic change is seen in B. Hematoxylin and eosin staining, ×365.*

Table 5 shows that the viable bacterial counts recovered from the lungs of mice injected with the anti-cord factor serum were on the average 10^2 lower than those in the lungs of control mice. The above protective effect of anti-cord factor antiserum was specific for the infection with *M. tuberculosis*, since mice were not protected against the infection with listeria, brucellae, or salmonellae organisms by the injection of antiserum to cord factor.

DISCUSSION

The primary aim of the present study was to establish the role of cord factor in the pathogenesis of tuberculosis, which has not been fully defined despite a number of experimental

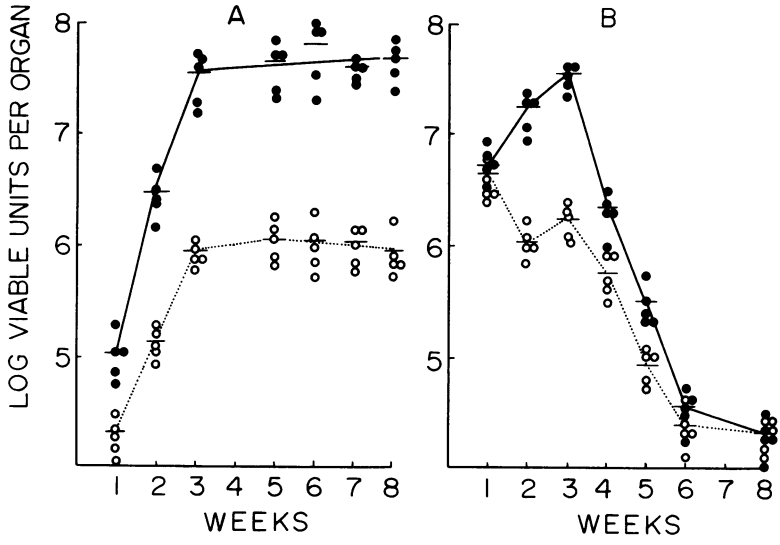

FIG. 3. *Effect of the vaccination with cord factor-MBSA complex on the bacterial multiplication in lungs and spleen of mice infected with M. tuberculosis H37Rv. Mice were infected by the intravenous inoculation with 2.2 × 10⁶ viable units of H37Rv. Five mice in each group were sacrificed at weekly intervals, and viable units in lungs and spleen were determined. Figures represent the number of viable units in whole organ. A, lungs; B, spleen. Symbols: ●, nonvaccinated control; ○, vaccinated with cord factor-MBSA complex.*

TABLE 2. *Protection of mice against cord factor-toxicity and experimental infection with M. tuberculosis by vaccination with cord factor-MBSA complex*

Vaccine	Weeks	Protective effect (no. of survivors/no. tested)			
		Cord factor toxicity[a] (survivors at 14 days)		Infection with *M. tuberculosis*[b] (survivors at 30 days)	
		Expt 1	Expt 2	Expt 1	Expt 2
Cord factor-MBSA[c]	1	3/10	0/10	0/15	1/18
Cord factor-MBSA	2	2/10	1/10	0/15	0/15
Cord factor-MBSA	3	9/10	7/10	11/15	14/20
Cord factor-MBSA	3	7/10	7/10	13/15	15/20
Cord factor-MBSA	4	8/10	8/10	8/12	17/20
Live BCG[d]		0/10	1/10	9/10	16/20
Nonvaccinated control		2/10	1/10	0/15	1/20

[a] Cord factor toxicity was tested by injecting mice intraperitoneally with 10 μg of cord factor in 0.1 ml of Bayol F at 2-day intervals for 14 days.

[b] Mice were challenged by the intravenous injection with 10^7 to 5 × 10^7 viable units of *M. tuberculosis* H37Rv 1 week after the last vaccinating injection.

[c] Mice were vaccinated with 50 μg of cord factor equivalent cord factor-MBSA complex emulsified with an equal volume of Freund incomplete adjuvant twice weekly for 1 to 4 weeks.

[d] Mice were tested for the protecting effects 6 weeks after the intravenous vaccination with 8.6 × 10^6 viable BCG cells.

TABLE 3. *Protective effect of various vaccines against experimental infection with M. tuberculosis in mice*

Vaccine[a]	Adjuvant	Route of vaccination	Protection (no. of survivors/no. of tested at 30 days)[b]	
			Expt 1	Expt 2
Cord factor-MBSA	+	Subcutaneous	9/10	17/20
Cord factor-MBSA	+	Footpad	7/10	ND[c]
Cord factor-MBSA	–	Subcutaneous	1/10	0/20
Cord factor-MBSA	–	Footpad	0/10	ND
Cord factor-MBSA	–	Intravenous	4/10	7/20
Cord factor only	+	Subcutaneous	0/10	1/20
MBSA only	+	Subcutaneous	0/10	0/20
Cord factor + BSA	+	Subcutaneous	1/10	3/20
Mycolic acid + MBSA	+	Subcutaneous	0/10	ND
Acetylated cord factor + MBSA	+	Subcutaneous	1/10	ND
Methyl 6-mycoloyl-α-D-glucopyranoside-MBSA	+	Subcutaneous	0/10	2/20
6,6'-dimycoloyl sucrose-MBSA	+	Subcutaneous	0/10	ND
Live BCG	–	Intravenous	8/10	18/20
None			0/10	1/20

[a] All vaccines except BCG were injected twice a week for 3 weeks with or without adjuvant by the designated route of vaccination. BCG (1.5 × 10^7 viable units) was intravenously vaccinated 6 weeks before challenge.

[b] Mice were challenged by intravenous inoculation with 10^7 to 5 × 10^7 viable units of *M. tuberculosis* H37Rv 1 week after the last injection of vaccine.

[c] Not done.

TABLE 4. *Specificity of the protective effect of cord factor-MBSA complex against various experimental infections in mice*

Organisms	Infecting units in inoculum	Survival times of mice (days)	
		Nonvaccinated control	Cord factor-MBSA-vaccinated
Mycobacterium tuberculosis	2.5×10^7	15, 15, 17, 18, 19, 19, 20, 21, 21, 23, 23, 24, 25, 25, 25, 25, 26, 27, 28, 28	20, 29, 30, S, S, S, S, S, S, S, S, S, S, S, S, S, S, S, S, S[a]
Listeria monocytogenes	5.0×10^7	1, 1, 1, 1, 1, 1, 2, 2, 2, 2	1, 1, 1, 2, 2, 2, 2, 3, 3, 3
Brucella abortus ..	4.0×10^6	3, 3, 3, 3, 3, 3, 3, 3, 4, 4	2, 3, 3, 3, 3, 3, 3, 4, 4, 4
Salmonella enteritidis	2.0×10^5	1, 2, 2, 2, 3, 3, 3, 4, 4, 5	2, 2, 2, 3, 3, 3, 3, 3, 4, 4,
S. typhimurium ...	1.0×10^6	2, 2, 2, 2, 3, 3, 3, 4, 5, 5	1, 1, 2, 2, 2, 3, 4, 4, 4, 5

[a] S indicates that mice survived at 30 days after infection when the experiment was discontinued.

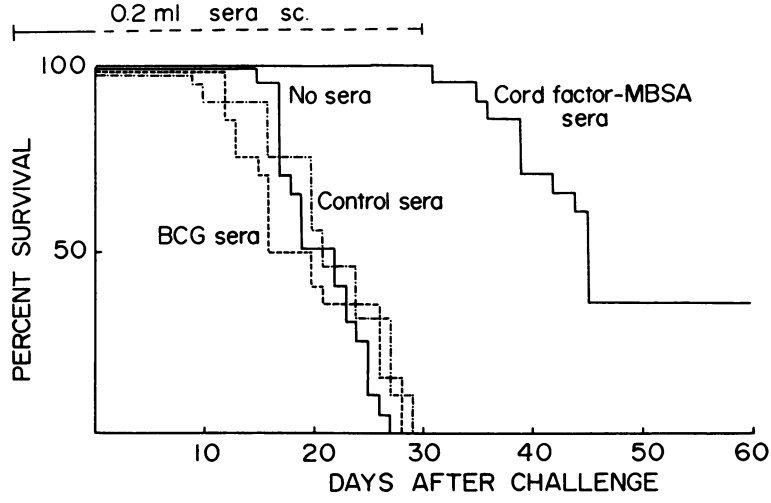

FIG. 4. *Effect of anti-cord factor rabbit serum on the survival of mice infected with virulent M. tuberculosis. The subcutaneous injection of serum was started 7 days before the intravenous challenge with 1.2×10^7 viable units of M. tuberculosis H37Rv and continued thereafter every other day for 30 days. A 0.2-ml sample of serum of nonvaccinated, BCG-vaccinated, and cord factor-MBSA-vaccinated rabbits was injected into 20 mice each. Twenty mice receiving no serum injection were used as controls. The injection of serum was discontinued at 30 days after challenge, and the survival of mice was observed for 60 days.*

approaches by several investigators (5–7, 16). Our previous study (16) of the effect of the direct addition of cord factor-in-oil on the tuberculous infection in mice failed to confirm the claim of Bloch and Noll (6) that cord factor enhances the multiplication of virulent tubercle bacilli in vivo. Recent development of a method of producing a neutralizing antibody against cord factor by the vaccination of animals with cord factor-MBSA complex (12) enabled us to reexamine this subject by using an immunological technique.

The facts that either the active immunization with cord factor-MBSA complex or the passive transfer of anti-cord factor serum protected mice against both the toxic action of cord factor (12) and the infection with virulent *M. tuberculosis*, that the bacterial multiplication in the organs of mice infected with tubercle bacilli was prevented by the vaccination with cord factor-MBSA complex or by the injection with anti-cord factor serum, and that the infection-protecting effect of cord factor-MBSA complex and of anti-cord factor serum was specific for the tuberculous infection indicate that cord factor plays an important role in the pathogenesis of tuberculosis. An earlier paper from this laboratory (7) presented indirect evidence that tubercle bacilli produce toxic substance(s) identical to cord factor during their multiplication in vivo. The present results that both the vaccination with cord

TABLE 5. *Effect of rabbit anti-cord factor serum on the multiplication of virulent M. tuberculosis in mouse lungs*

Serum[a]	Viable units recovered from mouse lungs at various time after infection[b]			
	48 Hr	1 Week	2 Weeks	3 Weeks
Normal serum	5.8×10^4	8.0×10^5	1.2×10^7	1.8×10^8
	5.1×10^4	5.6×10^5	9.4×10^6	9.4×10^7
	4.6×10^4	4.9×10^5	7.3×10^6	7.6×10^7
	4.4×10^4	4.7×10^5	5.2×10^6	5.1×10^7
	3.5×10^4	2.7×10^5	3.1×10^6	5.0×10^7
Anti-cord factor serum	7.8×10^4	6.8×10^4	1.4×10^5	1.9×10^6
	4.3×10^4	5.0×10^4	1.0×10^5	1.8×10^6
	4.0×10^4	3.6×10^4	8.3×10^4	1.0×10^6
	3.8×10^4	2.0×10^4	6.2×10^4	7.4×10^5
	1.7×10^4	1.2×10^4	2.9×10^4	4.6×10^5

[a] A sample (0.2 ml) of normal and anti-cord factor rabbit serum was injected subcutaneously every day for 3 weeks after the infection with tubercle bacilli.

[b] Mice were intravenously infected with 5.0×10^7 viable units of human virulent *M. tuberculosis* H37Rv. Lung homogenates of five randomly selected mice from each group were cultured on Tween-albumin medium, and the number of viable units in whole lungs was determined.

factor-MBSA complex and the injection with anti-cord factor serum induced in mice an enhanced resistance against tuberculous infection add further and more substantial support to this view. It appears from the present study that cord factor enhances the multiplication of tubercle bacilli in vivo by affecting the basic metabolic activity of host-cell mitochondria (8, 10, 11, 15). Anti-cord factor antibody seems to compete with this toxic action of cord factor and interfere with the in vivo proliferation of tubercle bacilli, resulting in a supression of the overall infectious processes in host tissues. Relevant to this assumption is the fact that vaccines, except live BCG, which were found to be ineffective for inducing antitoxic immunity to cord factor in vivo (12) showed no effective protection against the infection with virulent tubercle bacilli.

Although cord factor-MBSA complex proves to be an artificial immunogen as potent as live BCG vaccine against the experimental tuberculosis in mice, the mechanisms of the immunity induced by these agents are essentially different (12). As reported in previous papers (9, 12) and confirmed in this study, BCG can neither protect mice against the toxic action of cord factor nor induce the production of anti-cord factor antibody in rabbit serum, despite its potent immunogenic activity against the tuberculous infection. It is also shown in the accompanying paper (13) that the infection with, or sensitization to, virulent *M. tuberculosis* failed to produce the precipitin antibody to cord factor in man and animals, indicating that this humoral antibody against cord factor is associated with neither the immune state elicited by live BCG vaccine nor the infection with virulent tubercle bacilli.

It has been reported recently by Bekierkunst et al. (1) that the intravenous injection of cord factor-in-oil emulsified in Tween-saline induced a granuloma formation in the lungs of mice, as did live BCG vaccine, and also a certain level of resistance to the infection with tubercle bacilli. This effect of cord factor was attributed to an elevated phagocytic activity of macrophages in the granulomatous tissue and therefore considered to be of nonspecific nature (3). Later communication from these authors indicated that cord factor, as well as BCG cells, injected into footpads of mice induced in the draining lymph nodes the hyperplasia of lymphoid tissue (2) and an enhanced immune response to an unrelated antigen as sheep red blood cells (4). However, the mechanism of the protection of tuberculous infection by the vaccination with cord factor-MBSA complex is entirely different from this case. First, the aqueous emulsion of cord factor was completely ineffective to protect mice against both the toxic action of cord factor (12) and the infection with tubercle bacilli when it was injected subcutaneously into mice with Freund incomplete adjuvant. Secondly, a precipitin antibody to cord factor was produced in animals by the repeated subcutaneous vaccination with cord factor-MBSA complex which could neutralize the toxicity of cord factor and display an infection-protecting

effect when passively transferred to mice. And lastly, the immunogenic effect of cord factor-MBSA complex and the protective action of anti-cord factor serum are highly specific for the tuberculous infection.

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