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

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Received for publication 14 September 1972

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 19S 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 tuberculous infection 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'-dimycolyol 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 semi-
synthetic 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 $5 \times 10^7$
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 de-
scribed (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
centrifuigation, washed twice with 0.85% saline, and
suspended in the initial culture volume of saline con-
taining 0.02% Tween 80. Viable units in the inocu-
lar were determined by plating serial dilutions of the
culture with 0.1% BSA on oleic acid-albumin agar
(Difco).

*Listeria monocytogenes* (NCTC 7973) of sero-
type I was obtained from G. B. Mackaness, Trudeau
Institute, Saranac Lake, N.Y. The bacteria were cul-
tured 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 intrave-
rously into mice after appropriately diluting with the
same medium. The bacteria contained in the
inoculum were counted in a Petroff-Hausser cham-
ber.

*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 intrave-
rously into mice. Viable units in the inoculum were
determined by plating serial dilutions of the bacte-
rial 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 intra-
peritoneally 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 ster-
ile 2% BSA in a glass homogenizer of Potter-Elveh-
jem type. The tissue suspensions were diluted with
sterile 0.1% BSA and 25 mliters of appropriate dilu-
tions 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. tuber-
culosis* H37Rv. Mice vaccinated with cord
factor-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 nonvacci-
nated 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 mini-
mal. Histologically, extensive exudative le-
sions were observed in the lungs of nonvac-
cinated control mice, whereas such lesions
were hardly visible in the lungs of cord factor-
MBSA-vaccinated mice (Fig. 2). In close ac-
cord with these findings, the number of viable
units in the cord factor-MBSA-vaccinated mouse
lungs was $10^4$ to $10^5$ less than that re-
covered from the control mouse lungs. An al-
most 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 tubercu-
losis infection is as potent as that of live BCG
vaccine.

Figure 3 demonstrates the bacterial counts in
lungs and spleen recovered at weekly inter-
vals after the infection of mice with *M. tuber-
culosis* H37Rv. An apparent prevention of the
bacterial multiplication was observed in both
lungs and spleen of the cord factor-MBSA-vac-
cinated mice. In lungs, the bacterial growth
was suppressed during the first 3 weeks after
challenge, and a constant low level of viable
in vivo population of tubercle bacilli was ob-
served 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 po-
tency of cord factor-MBSA complex against
both the toxic action of cord factor and the
infection with tubercle bacilli becomes appar-

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Cord factor-MBSA-vaccinated control mice were infected intravenously with $4.0 \times 10^7$ viable units of H37Rv.

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

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Results at 30 days after challengea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt of lungsb (mg)</td>
</tr>
<tr>
<td>Nonvaccinated control</td>
<td>653 ± 40</td>
</tr>
<tr>
<td>BCG-vaccinated control</td>
<td>297 ± 49</td>
</tr>
<tr>
<td>Cord factor-MBSA-vaccinated</td>
<td>310 ± 25</td>
</tr>
</tbody>
</table>

Mice were challenged intravenously with 0.1 ml of a 7-day culture of M. tuberculosis H37Rv in Tween-albumin medium containing $4.0 \times 10^7$ viable units per inoculum.

a Mean ± standard deviation of five mice in each group.
b Number of mice survived/number of mice challenged.
c Dead mice.
d Variance ratio exceeds 1% level of significance.

effect 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-mycolyloxy-α-D-glucopyranoside or 6,6'-dimycologlycol sucrone, 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.

**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 $4.0 \times 10^7$ viable units of H37Rv.**
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.

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 10² lower than those in the lungs of control mice. The above protective effect of anti-cord factor antisera 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 antisera 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 × 10^6 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; O, 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

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Weeks</th>
<th>Protective effect (no. of survivors/no. of tested)</th>
<th>Infection with M. tuberculosis* (survivors at 30 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cord factor toxicity* (survivors at 14 days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expt 1    Expt 2    Expt 1    Expt 2</td>
<td></td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>1</td>
<td>3/10 0/10 0/15 1/18</td>
<td></td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>2</td>
<td>2/10 1/10 0/15 0/15</td>
<td></td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>3</td>
<td>9/10 7/10 11/15 14/20</td>
<td></td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>3</td>
<td>7/10 7/10 13/15 15/20</td>
<td></td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>4</td>
<td>8/10 8/10 8/12 17/20</td>
<td></td>
</tr>
<tr>
<td>Live BCG</td>
<td></td>
<td>0/10 1/10 9/10 16/20</td>
<td></td>
</tr>
<tr>
<td>Nonvaccinated control</td>
<td></td>
<td>2/10 1/10 0/15 1/20</td>
<td></td>
</tr>
</tbody>
</table>

* 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.

**Mice were challenged by the intravenous injection with 10^7 to 5 × 10^9 viable units of M. tuberculosis H37Rv 1 week after the last vaccinating injection.

**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.

**Mice were tested for the protecting effects 6 weeks after the intravenous vaccination with 8.6 × 10^4 viable BCG cells.

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

<table>
<thead>
<tr>
<th>Vaccine*</th>
<th>Adjuvant</th>
<th>Route of vaccination</th>
<th>Protection (no. of survivors/no. of tested at 30 days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord factor-MBSA</td>
<td>+</td>
<td>Subcutaneous</td>
<td>9/10 17/20</td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>+</td>
<td>Footpad</td>
<td>7/10 ND</td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>−</td>
<td>Subcutaneous</td>
<td>1/10 0/20</td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>−</td>
<td>Footpad</td>
<td>0/10 ND</td>
</tr>
<tr>
<td>Cord factor-MBSA</td>
<td>−</td>
<td>Intravenous</td>
<td>4/10 7/20</td>
</tr>
<tr>
<td>Cord factor only</td>
<td>+</td>
<td>Subcutaneous</td>
<td>1/10 0/20</td>
</tr>
<tr>
<td>MBSA only</td>
<td>+</td>
<td>Subcutaneous</td>
<td>1/10 0/20</td>
</tr>
<tr>
<td>Mycolic acid +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylated cord fac-tor + MBSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl 6-mycoloyl-α-α-glucopyranoside MBSA</td>
<td>+</td>
<td>Subcutaneous</td>
<td>1/10 ND</td>
</tr>
<tr>
<td>6,6′-dimycoloyl sucrose-MBSA</td>
<td>+</td>
<td>Subcutaneous</td>
<td>0/10 ND</td>
</tr>
<tr>
<td>Live BCG</td>
<td>−</td>
<td>Intravenous</td>
<td>8/10 18/20</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td>0/10 1/20</td>
</tr>
</tbody>
</table>

* 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 × 10^4 viable units) was intravenously vaccinated 6 weeks before challenge.

**Mice were challenged by intravenous inoculation with 10^3 to 5 × 10^3 viable units of M. tuberculosis H37Rv 1 week after the last injection of vaccine.

** Not done.
TABLE 4. Specificity of the protective effect of cord factor-MBSA complex against various experimental infections in mice

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Infecting units in inoculum</th>
<th>Survival times of mice (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nonvaccinated control</td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td>$2.5 \times 10^7$</td>
<td>15, 15, 17, 18, 19, 20, 21, 23,</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>$5.0 \times 10^7$</td>
<td>1, 1, 1, 1, 1, 1, 2, 2, 2</td>
</tr>
<tr>
<td><em>Brucella abortus</em></td>
<td>$4.0 \times 10^4$</td>
<td>3, 3, 3, 3, 3, 3, 3, 4, 4</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>$2.0 \times 10^4$</td>
<td>1, 2, 2, 2, 3, 3, 3, 4, 5</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>$1.0 \times 10^4$</td>
<td>2, 2, 2, 2, 3, 3, 3, 4, 5</td>
</tr>
</tbody>
</table>

*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 $1.2 \times 10^7$ 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
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 suppression 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 Bekier-kunst 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

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

<table>
<thead>
<tr>
<th>Serum*</th>
<th>Viable units recovered from mouse lungs at various time after infectionb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 Hr</td>
</tr>
<tr>
<td>Normal serum</td>
<td></td>
</tr>
<tr>
<td>5.8 × 10⁴</td>
<td>8.0 × 10⁴</td>
</tr>
<tr>
<td>5.1 × 10⁴</td>
<td>5.6 × 10⁴</td>
</tr>
<tr>
<td>4.6 × 10⁴</td>
<td>4.9 × 10⁴</td>
</tr>
<tr>
<td>4.4 × 10⁴</td>
<td>4.7 × 10⁴</td>
</tr>
<tr>
<td>3.5 × 10⁴</td>
<td>2.7 × 10⁴</td>
</tr>
<tr>
<td>Anti-cord factor serum</td>
<td></td>
</tr>
<tr>
<td>7.8 × 10⁴</td>
<td>6.8 × 10⁴</td>
</tr>
<tr>
<td>4.3 × 10⁴</td>
<td>5.0 × 10⁴</td>
</tr>
<tr>
<td>4.0 × 10⁴</td>
<td>3.6 × 10⁴</td>
</tr>
<tr>
<td>3.8 × 10⁴</td>
<td>2.0 × 10⁴</td>
</tr>
<tr>
<td>1.7 × 10⁴</td>
<td>1.2 × 10⁴</td>
</tr>
</tbody>
</table>

* 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 × 10⁴ 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.
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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.

ACKNOWLEDGMENTS

I thank G. B. Mackaness, K. Kanai, and K. Saito for kindly supplying the bacterial strains and J. Asselineau for the supply of semisynthetic cord factor analogues. The performance of histopathological examinations of mouse lungs by Y. Ogawa of this laboratory and the encouragement and valuable discussions of Yuichi Yamamura, Osaka University Medical School, are also gratefully acknowledged.

LITERATURE CITED


