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# Attenuated *Mycobacterium lepraemurium* Vaccine Non-Protective against *Mycobacterium intracellulare* Infection in Mice

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## ABSTRACT

Since *Mycobacterium lepraemurium* (Mlm) is closely related to *Mycobacterium intracellulare*, with respect to its antigenicity, attenuated Mlm obtained by passages on 1% Ogawa egg medium was examined for possible effects as a vaccine against *M. intracellulare* infection. Four weeks after subcutaneous injection of Mlm ($1.4 \times 10^7$ bacilli), mice were infected intravenously with $2.4 \times 10^7$ organisms per mouse of *M. intracellulare*. During the first 12 weeks, no difference was noted in the rate of progression of *M. intracellulare* infection between mice given or not given Mlm-vaccine, when severity of the infection was measured on the basis of the number of viable *M. intracellulare* in the lungs and spleen. Therefore, Mlm-vaccine is probably not efficacious in protecting mice against *M. intracellulare* infection.

*Mycobacterium lepraemurium* (Mlm) is related to *M. intracellulare* in antigenicity[4]. If the shared antigens between the two involve protective antigens to *M. intracellulare* infection, which can efficiently induce an immune response responsible for specific resistance to the organism, such as generation and expansion of sensitized T lymphocytes and development of delayed type hypersensitivity. We examined the effect of attenuated live Mlm against *M. intracellulare* infection.

## MATERIALS AND METHODS

*Mice.* Five-week-old female ddY mice were purchased from the Sizuoka Union for Experimental Animals, Shizuoka, Japan. At the time of experiment the mice were 6 weeks of age.

*Vaccination and Infection. M. lepraemurium* Hawaiian had been serially transferred 87 times on 1% Ogawa egg yolk medium[5] by M. Matsuoka, National Institute for Leprosy Research, Tokyo, and following acquisition of this organisms, 2 additional passages were made. The organisms were harvested, ground with mortar and pestle by adding 3 drops of 1% Tween 80® and suspended in distilled water. Bacterial suspension was sonicated with Handy Sonic (model UR-20P, Tomii Seiki Co., Tokyo) for 10 sec. One-tenth ml of bacterial suspension ($1.4 \times 10^8$ Mlm/ml) were given subcutaneously to mice into the left inguinal region. *M. intracellulare* 31F093TD obtained from F. Kuze, Kyoto University, Kyoto, was cultured in Dubos Tween-albmin liquid medium at 37 C for 10 days, and the optical density was adjusted to 1.0 at 540 nm on a Spectrophotometer (Model 10-100 Hitachi, Tokyo), using the same culture medium. Mice were infected intravenously with 0.2 ml of bacterial suspension of $1.2 \times 10^8$ per ml 4 weeks after vaccination with Mlm.

*Colony-forming units from visera.* The mice were decapitated 4-week-intervals for up to 16 weeks after the infection. The spleen and lungs were homogenized in 5 ml of saline in glass homogenizer with the aid of a mortor (SM EH-20, Toyo Co., Tokyo). The homogenate was mixed with 0.5 ml 1% NaOH, allowed to stand

for 30 sec and serially dilluted 10-fold with saline, and then, 0.1 ml of each dilution was inoculated onto 1% Ogawa egg slant. The colony-forming units (CFUs) were counted after incubation for 6 weeks at 37 C.

## RESULTS

Fig. 1 shows the CFU of *M. intracellulare* recovered from lungs (A) and spleen (B) of mice. For up to 12 weeks after the induced infection, the number of organisms in both organs did not differ between unvaccinated and vaccinated mice: In animals of both groups, the number of organisms transitorily decreased during the first 4 weeks after infection and thereafter increased almost 2 orders during weeks 8 to 12. From weeks 12 to 16, the number of organisms in the vaccinated mice decreased whereas that of control mice continued to increase. As shown in Table 1, weights of the liver and spleen of the host increased 4 weeks after *M. intracellulare* infection. On the contrary, the weights of those organs were not affected by Mlm-vaccination. Thus, *M. intracellulare* infection but not Mlm-vaccination is though to enhance the reticulo endothelial system of the host animals.

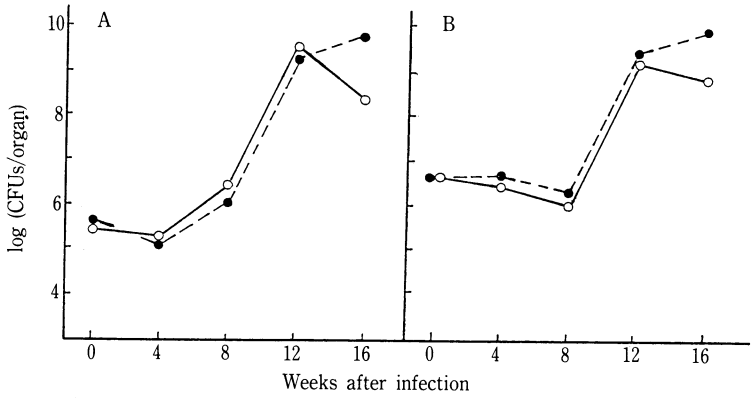


**Fig. 1.** Effect of Mlm-vaccination on the growth of *M. intracellulare* in the lungs (A) and spleen (B) of mice infected with *M. intracellulare*. ●, untreated; ○, vaccinated with 1.4 × 10 of Mlm. Ten mice per regimen were used through the experiment.

**Table 1.** Changes in organ weights of *M. intracellulare* infected mice with or without Mlm-vaccination

| Organs | Weeks after infection | *M. intracellulare* infection | Weight of organs | |
|---|---|---|---|---|
| | | | Mlm-vaccination | |
| | | | − | + |
| Liver | 0 | − | 1.46 ± 0.05[a)] | 1.28 ± 0.04 |
| | 4 | − | N.D. | 1.54 ± 0.10 |
| | 4 | + | 2.80 ± 0.14 | 2.96 ± 0.15 |
| | 16 | − | N.D. | 1.80 ± 0.14 |
| | 16 | + | 2.50 ± 0.22 | 2.46 ± 0.14 |
| Spleen | 0 | − | 0.15 ± 0.01 | 0.12 ± 0.01 |
| | 4 | − | N.D. | 0.12 ± 0.01 |
| | 4 | + | 0.56 ± 0.06 | 0.74 ± 0.11 |
| | 16 | − | N.D. | 0.13 ± 0.01 |
| | 16 | + | 0.61 ± 0.09 | 0.66 ± 0.09 |
| Lungs | 0 | − | 0.26 ± 0.02 | 0.24 ± 0.01 |
| | 4 | − | N.D. | 0.26 ± 0.02 |
| | 4 | + | 0.30 ± 0.01 | 0.32 ± 0.02 |
| | 16 | − | N.D. | 0.29 ± 0.01 |
| | 16 | + | 0.53 ± 0.03 | 0.51 ± 0.05 |

a) The mean ± SE (n = 10)

## DISCUSSION

We examined the efficacy of live Mlm as a possible vaccine against *M. intracellulare* infection, for the following reasons. Firstly, Mlm and *M. intracellulare* are closely related, with regard to antigenicity[4], and biological an biochemical characteristics[7]. Secondary, only live organisms can serve as a efficacious vaccine against infections due to mycobacteria[8]. Therefore, protection of the animals against *M. intracellulare* infection may be feasible, when the animals are vaccinated with attenuated Mlm which fail to cause a progressive infection in the host[2]. As indicated in Fig. 1, the Mlm-vaccination in the present protocol did not suppress the proliferation of *M. intracellulare* in the lungs and spleen of the host animals, thereby indicating little immunizing activity of the Mlm vaccine against *M. intracellulare* infection. It is thought that a sufficient degree of delated type hypersensitivity (DTH) developed in the host due to Mlm vaccination, because the same number of Mlm Hawaiian strain given mouse footpads developed substantial DTH as early as 25 days after infection[6]. Live Mlm given subcutaneously into the footpad of mice enhanced the host resistance against *M. intracellulare* and *M. bovis* BCG[3]. This effect is mediated by sensitized T lymphocytes specific to Mlm, which also recognize *M. tuberculosis* and BCG though crossreactive antigens shared by Mlm and *M. tuberculosis* or BCG[1]. The present Mlm vaccine was not efficacious against the infection by *M. intracellulare*, possibly for the following reasons; the differences may have been in virulence between Mlm strains used and those we used. They immunized host animals using virulent Mlm harvested from infected animal tissue. It may also be that the shared antigens between Mlm and *M. intracellulare* involve no protective antigen which can induce antigen-specific cellular immunity to *M. intracellulare* to cause an essential enhancement of host resistance to the infection. Suppressor cells were probably induced by Mlm vaccine although the vaccine might actually induce effector T cell subsets which can act on *M. intracellulare*. The second possibility is currently being investigated by examining whether or not the present Mlm-vaccine has a protective effect against *M. avium* infection, because *M. avium* is more closely related to Mlm than *M. intracellulare*[9].

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