The Lack of Therapeutic Effects in Mice of the Combined Gamma-Irradiated *Mycobacterium leprae* and Viable BCG against *Mycobacterium leprae* Infection

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There are at least two approaches to antileprosy vaccines: One is the use of *Mycobacterium leprae* derived from *M. leprae*-infected armadillos(1) and the other is the use of mycobacteria other than *M. leprae*, which share the antigenic determinants with *M. leprae* such as *M. non-chromogenicum*(2), *M. vaccae*(2), *M. simiae*(2), *M. tuberculosis* H37Ra(4), and *M. bovis* BCG(4). Shepard et al.(4) reported that BCG could sensitize mice against *M. leprae*, on the basis of development of the delayed type hypersensitivity reactions as measured footpad swelling, and that the sensitizing efficacy of BCG was much more satisfactory than those of the other mycobacteria mentioned above. It was of interest to consider another observation(4) that BCG and *M. leprae* could provide the enhanced host with resistance against subsequent *M. leprae* infection. Shepard et al.(4) reported that a vaccine of viable BCG combined with heat-killed *M. leprae* exhibited a superior protective effect over *M. leprae* or BCG alone against *M. leprae* infection, particularly in low doses. The efficacy of the combined vaccine of BCG and killed *M. leprae* in clinical applications was reported by Convit et al.(5), who observed that the Mitsuda reactions converted to positive in patients administered a mixture of the two mycobacteria, but not of either alone. We gave mice a combined vaccine of gamma-irradiated *M. leprae* and BCG and the results are reported herein.

Materials and Methods

**Mice:** Five-week-old male CBA/JN mice purchased from Charles River Co., Kanagawa, were used.

**M. leprae infection:** Suspension of nude mouse-derived *M. leprae* Kurume-Naha was obtained from Dr. K. Kohsaka, Research Institute of Microbial Diseases, Osaka University, Osaka. Mice were given $1 \times 10^4$ *M. leprae* in 0.03ml of saline into left hind footpad.

**Vaccination:** *M. leprae* was purified from gamma-irradiated armadillo liver infected with *M. leprae* (donated by Sasakawa Memorial Health Foundation, Tokyo) by aqueous two phase separation(6). Lyophilized BCG (Japanese strain) was purchased from Nippon BCG Laboratory, Tokyo. One-twentieth ml of a mixture in saline of the equal amount of $1 \times 10^6$/ml of purified *M. leprae* and 4 mg/ml of BCG was given subcutaneously into the lower dorsum of mice. The vaccination was initiated 2 weeks after infection and was repeated once biweekly.
for 26 weeks.

**Estimation of M. lerpae growth in the footpad:** At 118 or 187 days after infection, the tissue of the left hind footpad of mice was scraped off, ground with a mortar and pestle and suspended in one ml of phosphate-buffered saline (pH 7.2) containing 0.2mg/ml of bovine serum albumin. Ten µl of the resultant homogenate was smeared in a circle of 1cm diameter on a microscopic slide. The smear was stained by Ziehl-Neelsen technique (decolorized with 1% HCl in ethanol). The number of acid-fast bacilli was counted in a square \((0.18\text{mm} \times R \text{ mm})\); “0.18mm” was the diameter of the microscopic field at 1,000× magnification and “R” was the diameter of the counted smear). The number of *M. leprae* in the sample homogenate was calculated as:

\[
\text{The number of } M. \text{ leprae per ml} = N \times \frac{3.14 \times (R/2)^2}{0.18 \times R} \times 10^2
\]

(N was the number of *M. leprae* detected in a 0.18×R mm-square).

**Results**

As shown in Fig. 1, the number of challenged *M. leprae* recovered from the footpad of
mice vaccinated with a mixture of *M. leprae* and BCG was considerably higher at 118 days (A), but slightly less at 187 days after infection (B) compared to findings in non-vaccinated controls. The thickness of the left hind footpad (*M. leprae* infected site) and the right hind footpad (*M. leprae* uninfected site) of the animals was as follows: 118 days after infection; non-vaccinated control mice, 2.76±0.02mm (left) (mean±SE, n=5) and 2.76±0.02mm (right), vaccinated mice, 2.82±0.06mm (left) and 2.79±0.02mm (right), and 187 days after infection; non-vaccinated control mice, 2.89±0.03mm (left, n=10) and 2.89±0.04mm (right), vaccinated mice, 2.91±0.02mm (left) and 2.89±0.03mm (right). Thus, there was no footpad swelling at the *M. leprae* infected site, indicating that the inflammatory reactions by *M. leprae* infection itself or by the delayed type hypersensitivity specific for *M. leprae* were not produced in the infection sites, of both the non-vaccinated control and *M. leprae*-BCG vaccinated mice.

**Discussion**

We examined a combined vaccine of gamma-irradiated *M. leprae* and live BCG for therapeutic effects on *M. leprae* infection in mice. As indicated in Fig. 1, multiple vaccinations once biweekly from 2 weeks after infection up to the end of the experiment (187-days) caused no significant inhibition of the growth of *M. leprae* at the infection site. Shepard et al.(3,4) reported that the vaccinations of heat-killed *M. leprae* in combination with BCG or either alone 4 weeks before challenge infection with *M. leprae* showed a marked protection against the infection and that the combined vaccine of *M. leprae* and BCG exhibited the highest protection, particularly at low doses. The discrepancy of these findings is attributed to differences in the experimental designs, that is, we studied the therapeutic effect, whereas they studied the protective effect of the combined vaccine against *M. leprae* infection. Differences in the BCG-strains used may also account for these different findings.

Shepard et al.(4) reported that any of the above vaccines rendered little or no protection in mice with *M. leprae*-tolerance produced by a high-dose of *M. leprae* given intravenously, although BCG alone or in combination with *M. leprae* did sensitize mice against *M. leprae* antigens, determined on the basis of delayed type hypersensitivity. This may explain why the combined vaccine of *M. leprae* and BCG was therapeutically ineffective. Certain microenvironmental changes related to the unresponsiveness to *M. leprae* antigens may have occurred around the infection site of *M. leprae*, even in an early phase of infection. If so, this may be the cause of the ineffectiveness of therapeutic administration of the combined vaccine, as in the case of the *M. leprae*-tolerant mice(4), because vaccination against a given bacterium is totally dependent upon the ability of the host immune system to respond to its antigens, in a local as well as systemic manner.

**Summary**

Gamma-irradiated *M. leprae* in combination with BCG given once biweekly to mice from 2 weeks for up to 187 days after infection with *M. leprae* caused no significant growth inhibition of *M. leprae*, at the site of the infection.
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References

マウスの実験ライに対するガンマ線照射ライ菌と
BCG 生菌の混合ワクチンの治療効果の検討

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CBA/JN 系雄マウスの左足趾皮下に Mycobacterium leprae Kurume-Naha 株の 10⁴ を接種し、その 2 週後より M. leprae (M. leprae 感染アルマジロ肝 (γ 照射) より MMLEP の 2 相分配法により精製) 及び凍結乾燥 BCG (日本 BCG 研究所) より調製した混合ワクチン (1 × 10⁷/ml M. leprae (1 容) + 4 mg/ml BCG (1 容)) の 50 μl 宛をマウスの背部皮下に 2 週に 1 回の割で 26 過に亘って投与した。ワクチン効果の判定は感染マウスの足趾当たりの M. leprae 菌数を Shepard の方法に準じて計測することによってなされた。その結果、感染 118 日目では、ワクチン投与群の足趾内菌数は対照群に比べて若干多かったのに対して、感染 187 日目で逆にワクチン投与群において対照群におけるよりもや少な傾向がみられたが、何れの場合を問わずワクチン投与群と対照群との間には有意差は見出せなかった。って、今回の実験の限りでは、BCG 生菌と M. leprae 死菌よりなる混合ワクチンはマウスの実験ライに対し治療効果を示さないものと結論づけられよう。