Failure of Killed *Corynebacterium parvum* in Induction of Protection in C57BL/6 Mice against *Babesia rodhaini* Challenge Infection

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ABSTRACT. We examined the survival rate of C57BL/6 mice challenged with *Babesia rodhaini* 21 days post-killed *Corynebacterium parvum* (KCP) inoculation in comparison with ICR mice. All KCP-inoculated ICR mice survived the challenge infection, while all KCP-inoculated C57BL/6 mice died. To account in part for the difference in protective effects of KCP, stimulatory effects of KCP on splenic phagocyte chemiluminescence (CL) response were investigated in C57BL/6 and ICR mice. The KCP-inoculated ICR mice exhibited significantly higher CL values than ICR mice without KCP inoculation. No significant difference in CL value was observed between C57BL/6 mice with and without KCP inoculation. These results taken together, the lack of resistance of the KCP-inoculated C57BL/6 mice to *B. rodhaini* infection may be attributed in part to small stimulatory effect of KCP on splenic phagocytes.—**KEY WORDS:** *Babesia rodhaini* infection, C57BL/6 mouse, killed *Corynebacterium parvum*.

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Protection of animals against *Plasmodium* spp. and *Babesia* spp. infections induced by killed *Corynebacterium parvum* (KCP) has been documented [1, 2, 12, 18]. KCP has been reported to exert protective effects on ICR [18], TO Swiss, CBA and CBA nude mice [2] against *B. rodhaini* infection. The KCP-inoculated ICR mice were found to exhibit comparatively high survival rate against *B. rodhaini* challenge infection. C57BL/6 mice are genetically resistant to malaria [19] and used in a variety of infectious experiments. However C57BL/6 mice remain to be examined for resistance to *B. rodhaini* challenge infection post-KCP inoculation. The present study was designed to investigate the degree to which C57BL/6 mice are protected from *B. rodhaini* challenge infection by KCP in comparison with ICR mice, and to estimate splenic phagocyte function by chemiluminescence (CL) method.

B. rodhaini parasites were generously provided by the National Institute of Animal Health of Japan and maintained in our laboratory by passage in ICR mice. Six ICR and 4 C57BL/6 female mice, six weeks of age, were inoculated intraperitoneally (i.p.) with 0.01% thimerosal-killed *C. parvum*, strain CN 6134 (Lot. No. 761, Wellcome Reagents Ltd., England) at a dose of 0.7 mg/head. At day 21 post-KCP inoculation, the mice were challenged i.p. with 1×10^6 *B. rodhaini*-parasitized erythrocytes. Ten ICR and 6 C57BL/6 constituted the control groups and were inoculated i.p. with the same parasite dose. The parasitemia rate was assessed by Wright-Giemsa-stained blood smears prepared from the tail vein of infected mice.

All ICR and C57BL/6 control mice died of *B. rodhaini* infection. The mean survival durations in ICR and C57BL/6 control mice were 6 and 7 days, respectively. All the KCP-inoculated ICR mice survived. Although the mean survival duration of KCP-inoculated C57BL/6 mice (11 days) was 4 days longer than that of the C57BL/6 control mice, all the mice died eventually. Our data demonstrate the high degree of susceptibility of both ICR and C57BL/6 mice to *B. rodhaini*. Interestingly, inoculation of KCP into mice prior to *B. rodhaini* infection provided complete protection to ICR and a prolonged mean survival duration to C57BL/6. In a similar study,

Saeki and Ishii [18] reported 50% survival rate of ICR mice, compared to the 100% survival rate noted in our present study. This difference can be partly attributed to the strain diversity of *C. parvum*.

CL method has been widely used to estimate phagocyte function [6, 10, 12]. Reactive oxygen intermediates generated by activated phagocytes can be detected by CL method. To account in part for such a substantial difference in protective effects of KCP on C57BL/6 and ICR mice against *B. rodhaini* challenge infection, stimulatory effect of KCP on splenic phagocyte function was investigated. Eight ICR and eight C57BL/6 mice inoculated i.p. with KCP (0.7 mg/head) were sacrificed to harvest spleen cells at days 7 and 28 post-KCP inoculation. Four ICR and four C57BL/6 mice were employed as controls without KCP-inoculation. Spleen cells (1×10^7 cells/ml) suspended in Eagle's MEM (pH 7.4) were incubated in a humidified 5% CO₂ incubator at 37°C for 30 min. Zymosan opsonized with mouse serum was used to activate spleen cells to produce luminol-dependent CL. CL response of spleen cells was assayed by means of Biolumat 9500 T (Berthold Co., Germany) following the method of Makimura and Sawaki [11].

CL values noted in the KCP-inoculated ICR mice at days 7 (12.4 ± 1.3 CPM $\times 10^4/10^7$ spleen cells) and 28 (12.9 ± 2.2) post-KCP inoculation were significantly higher than that of the control (3.7 ± 0.4). This result suggests non-specific stimulation of splenic macrophages by KCP. With KCP-inoculated C57BL/6 mice, only a slight increase in CL value was noted at day 7 (5.1 ± 1.5) post-inoculation, although insignificant compared to that of the control (2.3 ± 0.7) and at day 28 (2.8 ± 0.7) post-inoculation.

Complete protection of the KCP-inoculated ICR mice against *B. rodhaini* infection may be attributed in part to a significantly augmented splenic phagocyte activity; while small stimulatory effect of KCP on splenic phagocytes in C57BL/6 mice may account in part for the lack of resistance to *B. rodhaini* infection.

Activated macrophages are known to release parasitocidal reactive oxygen intermediates [9, 13–16], lymphocyte-activating factor [3] and natural killer (NK) cell-

activating interferon [3, 5]. Thus activated macrophages in KCP-inoculated ICR mice could have possibly enhanced specific immune response and NK activity. Important roles of T cells and NK cells in protection against malaria and babesiosis have been reported [7, 8, 20, 21]. It seems reasonable to assume that non-specific and specific immunity against *B. rodhaini* infection could be mounted more quickly in KCP-inoculated ICR mice than in KCP-inoculated C57BL/6 mice presumably because in KCP-inoculated ICR mice, splenic phagocytes could have been activated prior to *B. rodhaini* challenge.

It is well known that KCP stimulates macrophages non-specifically [1, 4, 12, 17]. However this was not the case in C57BL/6 mice. The reason, not clear, may be presumably explained by genetical background unique to C57BL/6 mice.

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