Effect of *Corynebacterium parvum* Bacterin on Artificial Immunity against *Babesia rodhaini* Infection in Mice

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We have previously found that the mice intraperitoneally inoculated with a mixture of sonicated *Babesia rodhaini* antigen (S antigen) and formalin-fixed *Corynebacterium parvum* (*Propionibacterium acnes*) bacterin (CPB) developed a relatively high resistance to intraperitoneal (IP) or intravenous challenge infection with homologous parasites (10^6 parasitized erythrocytes: PE) at 3 weeks after booster immunization [7].

In this paper, the duration of immune state in mouse was described. Furthermore, in expectation of the effect of CPB on augmentating the non-specific immunity, single additional IP inoculation of CPB at a dose of 0.5 mg/mouse was carried out at 4 days prior to IP challenges.

The experimental animals consisted of 168 5-week-old female ICR mice. S antigen and CPB were prepared by the method reported previously [8]. After challenge infection, blood was collected daily from the tail vein to investigate

the changes of PE rate, hemoglobinuria and survival rate. The PE rate was assessed under a microscope by counting the number of parasitized cells in 10 fields (about 4000 erythrocytes). Specimens without parasite in at least 50 fields were judged as negative to parasite.

As shown in Fig. 1, mice were immunized initially with a mixture of S antigen (Corresponding to 10^7 PE) and 0.5 mg (dry weight) of CPB. After 2 weeks, the mice were treated with S antigen alone as a booster immunization. At 1 (A, B and C group), 3 (D, E, F) and 5 (G, H, I) weeks after the booster immunization, the mice were then intraperitoneally challenged. Groups J and K showed in the Figure were subjected to IP challenge infection with 10^6 PE at 4 days after 1st or 2nd IP inoculation with CPB alone.

As shown in Table 1, the survival rates in Group A, D and G (intraperitoneally challenged at 1, 3 and 5 weeks respectively after the booster immunization) were 83.3% (20/24), 72.0% (18/25) and 66.7% (16/24) respectively. This might indicated that the resistance tended to diminish with the lapse of time after the immunization.

Fig. 1. Experimental design.

Table 1. Clinical features and survival rate in mice

Mice Group	Prepatent period in days	Number of mice excreted hemoglobinuria/mice used(%)	Mean percentage of maximal PE rate in surviving mice	Mean elapsed days showing maximal PE rate in surviving mice	survival rate(%)	Survival duration of dead mice
A	1.3	11/24 (45.8)	21.4	6.4	20/24 (83.3)	6.3
B	2.1	1/15 (6.7)	11.4	6.5	14/15 (93.3)	12.0 ^{a)}
C	1.3	8/8 (100)	—	—	0/8	6.2
D	1.4	16/25 (64.0)	23.9	5.7	18/25 (72.0)	6.5
E	2.7	2/13 (15.4)	12.8	7.5	11/13 (84.6)	7.0
F	1.3	8/8 (100)	—	—	0/8	6.3
G	1.5	20/24 (83.3)	29.1	7.3	16/24 (66.7)	6.3
H	1.8	6/13 (46.2)	24.7	6.9	12/13 (92.3)	6.0 ^{a)}
I	1.3	8/8 (100)	—	—	0/8	6.2
J	1.5	10/10 (100)	46.4	6.6	5/10 (50.0)	8.0
K	1.4	9/10 (90.0)	34.1	7.0	4/10 (40.0)	7.2

a) Only one mouse died.

Hemoglobinuria was detected in 45.8% (11/24) in Group A, 64.0% (16/25) in Group D and 83.3% (20/24) in Group G. The mean maximal PE rates in surviving mice amounted to 21.4% in Group A, 23.9% in Group D and 29.1% in Group G. The PE rates thus tended to increase with the elapsed time.

These findings distinctly showed that a protective effect is induced to a certain degree against challenge infection which is carried out until at least 5 weeks (7 weeks after the initial immunization).

The survival rates in Groups B, E and H which had been treated by additional inoculation of CPB at 4 days before challenge infection were 93.3% (14/15), 84.6% (11/13) and 92.3% (12/13) respectively. These rates exceeded those in Group A, D and G which had not been treated with additional CPB. It was especially noteworthy to find that the survival rate in Group H was improved, compared to that in Group G (66.7%). Besides, the survival duration of dead mouse in Group B extended to 12 days. These facts might demonstrate the effect of additional CPB inoculation on artificial immunization in mice. Hemoglobinuria was not found in Groups B and E except dead animals, and that the mean maximal PE rates in surviving mice were relatively low in Group B (11.4%) and E (12.8%). In Group H, the survival rate was high (92.3%) but hemoglobinuria (46.6%) and the mean maximal

PE rate (24.7%) were also somewhat higher than those in other 2 groups. This seemed to suggest that even if additionally inoculated, the effect of the previous immunization on inhibiting multiplication of parasite is gradually diminished with elapsed time.

The series of above-mentioned improvements, which were achieved by additional inoculation of CPB, seems to be attributable to the formation of more solid resistance due to the enhancement of CPB-induced non-specific immunity in the mice in which specific immunity against *B. rodhaini* was already established to a certain extent.

In Groups J and K which were subjected to single or twice treatment with CPB, 5 of 10 mice and 4 of 10 mice respectively survived from the challenge infection with *B. rodhaini*. However, when compared with the groups treated with S antigen and CPB, the mean maximal PE rates in surviving mice were both higher (46.4% in Group J and 34.1% in Group K). Hemoglobinuria appeared in all mice of Group J and in 9 of 10 mice of Group K. The mean survival durations of dead mice in Group J and K were 8.0 and 7.2 days respectively. These were slightly longer but were not significantly different compared to the control groups (C, F and I).

Clark *et al.* [3] have described that all the mice treated with CPB at doses of 0.4–1.0 mg/mouse resisted to the challenge with 10^6 PE at 30–105 days after the CPB-treatment.

In consideration of the differences in the source of CPB, pathogenicity of *B. rodhaini* and strain of experimental mice, simple comparison of their experimental results with our ones may not be always justifiable. The results obtained in this study lead to the fact that once (Group J) or twice (Group K) treatment with 0.5 mg/mouse of CPB alone provided a non-specific resistance to *B. rodhaini* infection and permitted approximately half of mice to survive and also to the conclusion that the presence of a specific antigen is fundamentally indispensable to obtain a high survival rate against challenge infection with *B. rodhaini*. Even if CPB was inoculated twice, its effect was not augmented as seen in Group K. This seems to suggest that the resistance against *B. rodhaini* challenge accrues from the non-specific enhancement of the immune system and does not represent the specific responses to cross-reacting CPB and *B. rodhaini* antigens.

The action of CPB to activate macrophages may accelerate their phagocytic activity [1] and induce concomitantly the release of reactive oxygen intermediates [2, 4]. Furthermore, the production of monokine may evoke a diversity of biological responses [2, 5, 6]. We consider that

these non-specific factors may result in manifestation at a certain degree of resistance to *B. rodhaini*. Further investigation should be carried out to make it clear.

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要 約

Corynebacterium parvum ホルマリン死菌体 (CPB) 投与による *Babesia rodhaini* 感染発症防御の増強 (短報) : 佐伯英治・石井俊雄 (日本獣医畜産大学獣医寄生虫学教室) —— *B. rodhaini* 超音波処理抗原と CPB で初回免疫したマウスに対し, その 3, 5 および 7 週 (追加免疫 1, 3 および 5 週) 後に 10^6 の虫体で攻撃したところ, 生残率はそれぞれ 83.0, 72.0 および 66.7% と逐次低下した. しかし, 攻撃 4 日前に CPB を追加接種するとマウスの抵抗性は回復し, 初回免疫 7 週間後の攻撃に対して 92.3% が耐過した. 一方, CPB 単独接種マウスも約半数が耐過した. 以上, CPB の特異および非特異的免疫増強効果が確認された.