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Effects of Bacterial Immunopotentiators, LC 9018 and OK-432, on the Resistance Against *Mycobacterium intracellulare* Infection in Mice*)

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ABSTRACT

Bacterial immunopoteniators, *Lactobacillus casei* YIT 9018 (LC 9018) and penicillin G-treated *Streptococcus pyogenes* (OK-432), were examined for their effects on the host resistance against *Mycobacterium intracellulare* infection in mice. When these agents were given intraperitoneally (i. p.) to mice, most of the administration protocols used in the present study failed to show a significant protective or therapeutic effect against the infection. Moreover these bacterial preparations could not exhibit a synergistic effect with minocycline *in vivo*. With a frequent i. p. administration of these agents, there was a tendency toward suppression of the host defense mechanisms to the infection, presumably by induction of some types of suppressor cells. Thus, it is likely that for an expression of more marked protective and/or therapeutic effects of these immunopotentiators against *M. intracellulare* infection, the administration by routes other than i. p. is preferred.

Pulmonary disease due to *Mycobacterium intracellulare*, the most commonly encountered mycobacteriosis other than tuberculosis in Japan[12,17,21] is thought to be an opportunistic infection which is refractory to chemotherapy. Because patients often have impaired defense mechanisms, immunotherapy may be effective treatment for this mycobacterial infection. Bacterial immunopotentiators, such as BCG, *Propionibacterium acnes* (*Corynebacterium parvum*), penicillin G-treated *Streptococcus pyogenes* (OK-432), and *Lactobacillus casei* YIT 9018 (LC 9018) potentiate antitumor immunity of the tumor bearing host[2,3,5,10] and also enhance resistance of the host against certain bacterial infections[1,3,4,11,13,14,16,22]. We examined in mice the protective and/or therapeutic effects of LC 9018 and OK-432 against infection due to *M. intracellulare*.

Six week-old female mice (ddY strain) purchased from the Shizuoka Union for Experimental Animals, Shizuoka were infected intravenously (i. v.) with 5×10^7 of *M. intracellulare* Nakatani grown in Dubos liquid medium. The mice were also given LC 9018 (Yakult Central Institute for Microbiological Research, Tokyo) or OK-432 (Chugai Pharmaceutical Co., Tokyo), as indicated in Tables 1 and 2. At various intervals for up to 3 months after this so-induced infection, the mice were killed and organisms in the lung and the spleen were cultured on 1% Ogawa's egg medium.

Table 1 shows the effect of LC 9018 on the resistance to *M. intracellulare* infection in mice. The number of bacteria in the spleen of untreated control mice (A) gradually decreased during the course of experiment and 3 months after infection, indicating cellular immunity of the host. Some injection protocols (B and G) enhanced considerably the bacterial elimination, while the others showed no such effects. The number of bacteria in the lung of control mice changed little during the observation periods. In this case, none of the protocols used with

*) 斎藤　肇，長島清文，冨岡治明：細菌性免疫促進剤 LC 9018 並びに OK-432 のマウスでの実験的 *Mycobacterium intracellulare* 感染症に対する宿主抵抗性に及ぼす効果

Table 1. Effect LC 9018 on the host resistance against the infection due to *M. intracellulare*[a)]

Experimental No.	Protocols for LC 9018 administration[b)]	log (viable units per organ)[c)]					
		Spleen			Lung		
		1 M	2 M	3 M	1 M	2 M	3 M
A	Untreated	6.20[d)] (0.34)	5.72 (0.12)	5.60 (0.42)	4.53 (0.41)	4.24 (0.24)	4.88 (0.17)
B	Once weekly after infection	5.88 (0.28)	5.26 (0.20)	5.44 (0.33)	4.48 (0.44)	4.54 (0.20)	4.99 (0.34)
C	Twice weekly after infection	6.37 (0.06)	6.07 (0.17)	5.47 (0.13)	4.41 (0.32)	4.77 (0.25)	5.06 (0.20)
D	Once daily after infection	6.77 (0.30)	5.75 (0.09)	5.92 (0.14)	4.52 (0.34)	4.97 (0.12)	5.42 (0.21)
E	Once daily for 2 weeks after infection and thereafter once weekly	6.00 (0.23)	6.15 (0.31)	5.43 (0.29)	4.40 (0.13)	5.26 (0.09)	5.12 (0.43)
F	14 and 7 days before infection and then once daily after infection	5.73 (0.19)	6.00 (0.07)	4.92 (0.28)	4.40 (0.28)	4.98 (0.32)	4.84 (0.15)
G	Once daily from 14 days before infection to end of the experiment	5.89 (0.26)	5.45 (0.40)	5.36 (0.14)	4.04 (0.28)	4.90 (0.31)	5.61 (0.48)
H	14 and 7 days before infection, then once daily for 2 weeks and thereafter once weekly after infection	6.20 (0.23)	5.35 (0.11)	5.56 (0.12)	4.25 (0.34)	4.07 (0.23)	4.93 (0.44)

a) Mice were infected i. v. with 5.2×10^7 of *M. intracellulare* strain Nakatani.
b) LC 9018 was given i. p. to mice at the dose 0.1 mg per injection.
c) The number of viable units recovered from organs was counted at months 1, 2, and 3 after infection. The values for the spleen and the lung of untreated mice 1 hr after infection were 2.0×10^6 and 4.3×10^4 (in log, 6.3 and 4.6), respectively.
d) The mean values and SE (n=3) are indicated. The SE values are presented in parentheses.

Table 2. Effect of OK-432 on the host resistance against the infection due to *M. intracellulare*[a)]

Experimental No.	Protocols for OK 432 administration[b)]	log (viable units per organ)					
		Spleen			Lung		
		1 M	2 M	3 M	1 M	2 M	3 M
A	Untreated	6.37 (0.09)	5.63 (0.13)	5.21 (0.08)	4.59 (0.33)	4.20 (0.16)	4.26 (0.36)
B	Once weekly after infection	5.97 (0.09)	6.00 (0.20)	5.71 (0.33)	4.25 (0.21)	4.34 (0.40)	5.08 (0.26)
C	Once daily after infection	5.96 (0.12)	6.32 (0.15)	5.91 (0.34)	4.28 (0.18)	5.60 (0.45)	5.75 (0.17)
D	Once daily for 2 weeks after infection and thereafter once weekly	5.96 (0.07)	6.28 (0.43)	5.85 (0.07)	3.90 (0.21)	6.04 (0.95)	4.92 (0.27)
E	14 and 7 days before infection	5.79 (0.03)	6.06 (0.22)	6.35 (0.19)	4.26 (0.24)	4.78 (0.24)	4.89 (0.28)
F	14 days before infection and then once daily after infection	6.06 (0.18)	6.54 (0.09)	5.92 (0.16)	4.46 (0.51)	4.65 (0.08)	4.90 (0.43)
G	14 and 7 days before infection, then once daily for 2 weeks and thereafter once weekly	5.96 (0.13)	5.76 (0.14)	5.62 (0.25)	4.47 (0.43)	4.44 (0.17)	4.40 (0.65)

a) Mice were infected i. v. with 5.1×10^7 of *M. intracellulare* strain Nakatani.
b) OK-432 was given i. p. to mice in the dose of 1 KE (equivalent to 0.1 mg dry weight of the bacteria) per injection.

LC 9018 had a prophylactic or therapeutic effect, rather there was an enhanced growth of organisms in the lung.

A similar experiment was performed using OK-432. In untreated control mice, organisms in the spleen were gradually eliminated yet they persisted in the lung for up to 3 months after the infection (Table 2). OK-432 enhanced the elimination of organisms from the spleen in most of the protocols, during the first one month after the induced infection. However, the number of bacteria in the spleen at months 2 and 3 was conversely higher in mice given the agent, as compared to the untreated control. This indicates the suppressive action of OK-432 on the expression of anti-*M. intracellulare* immunity in the host spleen. Similar phenomenon has been observed with regard to the fate of organisms in the lung.

As shown in Fig. 1, minocycline which has anti-*M. intracellulare* activity *in vitro*[18] had no significant therapeutic effects on this mycobacterial infection. LC 9018 or OK-432 in combination with minocycline showed no significant synergistic effects on the elimination of organisms from the spleen and the lung.

Our findings suggest that neither LC 9018 nor OK-432 has a substantial ability to potentiate the host defense mechanisms against *M. intracellulare* infection in mice, when these agents were given i. p. However, both these immunopotentiators enhanced elimeination of organisms from the spleen and the lung in the early stage after infection under conditions of appropriate protocol. On the other hand, with a frequent administration of these preparations there was a tendency toward enhancement of the growth of organisms, as compared to untreated controls. This negative effect on the host defense mechanisms to *M. intracellulare* infection seems to depend on the induction of certain types of suppressor cells which reduce the anti-*M. intracellulare* immunity. It has been reported that BCG as well as *Propionibacterium acnes* induce suppressor cells against the host cellular immunity[6-9,19,20]. In our preliminary experiments, OK-432 administered i. p. to mice once daily for 2~3 weeks induced suppressor cells which depress the concanavalin A-mediated T cell proliferative response (data not shown).

In summary, the present study showed that

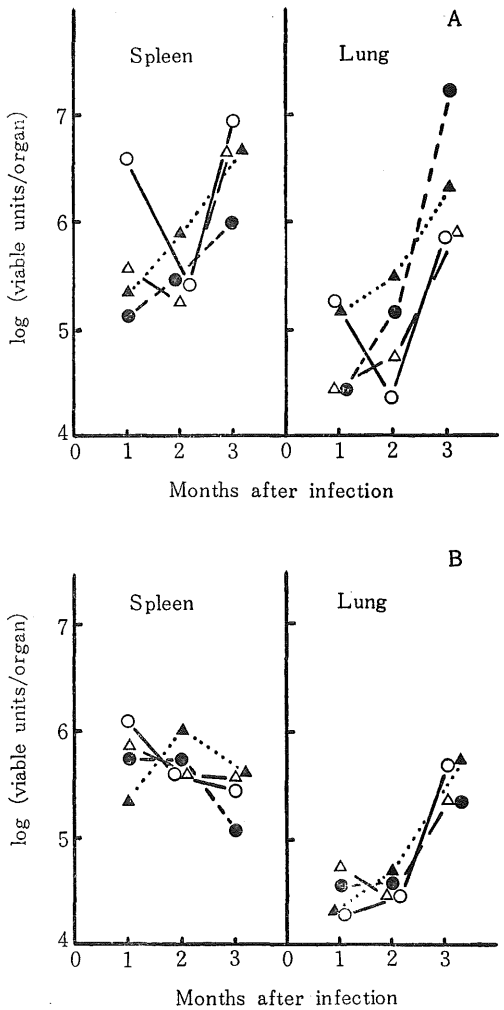

Fig. 1. Effects of LC 9018 or OK-432 in combination with minocycline on *M. intracellulare* infection mice. Mice were infected i. p. with 5.8×10^7 of *M. intracellulare* strain Nakatani. Minocycline (5 mg/kg) was given subcutaneously to mice 3 times weekly from 2 weeks after infection to the end of the experiments. LC 9018 (Fig. A) and OK-432 (Fig. B) were given intramuscularly in doses of 0.1 mg and 1 KE per injection, respectively, to mice 6, 4, and 2 days before infection and then twice a week after infection. Untreated (○), minocycline alone (△), LC 9018 or OK-432 alone (●), minocycline in combination with LC 9018 or OK-432 (▲).

LC 9018 and OK-432 which enhance the host resistance to Listeria monocytogenes[11], *Pseudomonas aeruginosa*[13], and *Candida albicans*[14, 15] infections did not potentiate the host defense mechanisms against *M. intracelluare* infection in mice. The protective and therapeutic effects

of these agents given by routes other than i. p. are now being studied using a more virulent strain of *M. intrecellulare*.

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