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Effect of Streptococcal Preparation, OK-432, on Experimental Infection due to *Mycobacterium lepraemurium* in Mice of C3H/Jms Strain*)

Hajime SAITO, Haruaki TOMIOKA and Katsumasa SATO

Department of Microbiology and Immunology, Shimane Medical University, Shimane 693, Japan

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ABSTRACT

Streptococcal preparation, OK-432, was examined for its therapeutic effect on experimental infection due to *Mycobacterium lepraemurium* (Mlm) in mice. When C3H/Jms mice (Mlm-sensitive strain) were infected with 10^8 Mlm to the left hind footpad, weekly injections of OK-432 *via* the peritoneal route (0.1 mg dry weight per injection), it considerably enhanced elimination of organisms from the infection site during the first 6 weeks, and thereafter suppressed the growth of organisms during 6 to 23 weeks after infection. In contrast, weekly injections of OK-432 to the infection site (left hind footpad) resulted in the enhanced growth of Mlm in the infection site, indicating that multiple injections of OK-432 to the infection site might generate suppressor cells and/or antagonizing factors against the host defense mechanisms to Mlm.

We previously reported that OK-432, penicillin G-treated *Streptococcus pyogenes* strain Su[7], did not show any significant protective and/or therapeutic effects against infection with *Mycobacterium intracellulare* in mice and that multiple injections of this agent occasionally induced reduction in the host resistance to the organisms, probably due to generation of certain types of suppressor cells[9]. In the present study, we investigated the effects of OK-432 administration to abdominal cavity or to the infection site in mice on the host resistance to the infection due to *Mycobacterium lepraemurium* (Mlm), an obligate intracellular parasite.

Male C3H/Jms (Mlm-sensitive) mice were infected with 10^8 *M. lepraemurium* Hawaiian strain in the left hind footpad (LFP) and were given weekly doses of OK-432 (0.03-0.1 mg) intraperitoneally or subcutaneously to the LFP. At various intervals up to 23 weeks after infection, the mice were sacrificed and the number of Mlm in the LFP was determined according to the method of Shepard[12]. Delayed type hypersensitivity (DTH) in Mlm-infected mice was tested by measuring the swelling of the right hind footpad (RFP) 24 hr after elicitation by 30 μl of Mlm-sonicate (10^8 bacilli equivalent).

As shown in Fig. 1, the number of Mlm in the LFP of C3H/Jms mice without OK-432 treatment markedly decreased (about one log) during the first 6 weeks after infection and thereafter continuously increased over the initial inoculum until the termination of the experiment. This feature resembles that reported by Poulter and Lefford[8]: As they mentioned, it is felt that the elimination of Mlm from the infection site during the early phase is attributable to the expression of anti-Mlm immunity, and the subsequent continuous multiplication of Mlm is a result of generation of some immunosuppressive factors and/or suppressor cells specific to Mlm infection. In fact, anti-Mlm DTH response of C3H/Jms mice was considerably strong at an early phase (6 weeks) but markedly reduced at 14 weeks after infection (Table 1). Multiple intraperitoneal (ip.) injections of OK-432 induced an enhanced elimination of Mlm in the early phase, and sub-

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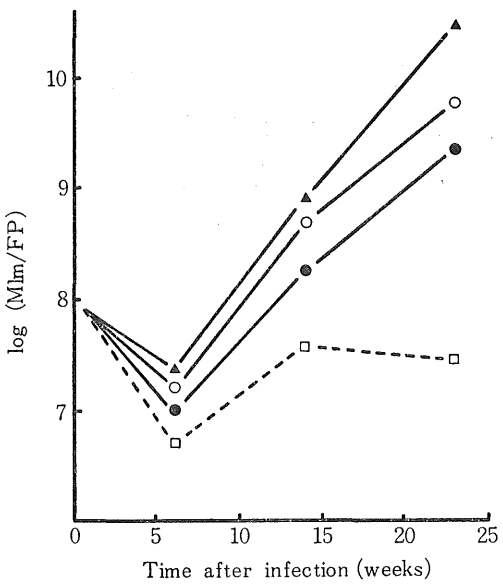

Fig. 1. Effect of OK-432 on the fate of Mlm infected to the hind footpad of C3H/Jms (Mlm-sensitive strain) mice. Mice were infected by 10^8 Mlm to the LFP and were given weekly injections of OK-432 to the LFP (▲) (0.03 mg per injection) or *via* the ip. route (●) (0.1 mg per injection). Open circles and open squares shows the results of untreated C3H/Jms and C3H/He (Mlm-resistant) mice, respectively.

Table 1. Anti-Mlm delayed type hypersensitivity in the Mlm-infected mice with or without injection of OK-432

OK-432 injection[a]	Footpad reaction[b]	
	6-week	14-week
None	0.45±0.09	0.14±0.10
Intraperitoneal injection	0.50±0.11	0.03±0.03
Subcutaneous injection to the LFP	0.22±0.02	0.19±0.05

[a]OK-432 was weekly injected into mice in doses of 0.1 mg and 0.03 mg per injection *via ip.* and sc., respectively.
[b]Footpad swelling at 24 hr after antigen-elicitation is indicated (the mean±SE, n=3). Footpad reaction testing was performed 6 and 14 weeks after infection.

sequently a considerable suppression of Mlm growth (Fig. 1, closed circles). Thus, it can be regarded that ip. injection of OK-432 has therapeutic activity against the infection with Mlm, which seems to be due to the immunopotentiating activity of OK-432[11,13]. It is known that OK-432 enhances host resistance against infection due to *Candida*, one of the facultative intracellular parasites[14]. We observed that OK-432 stimulates macrophage H_2O_2 releasing function[10], which is important for the expression of antimicrobial activity of the phagocytes[2].

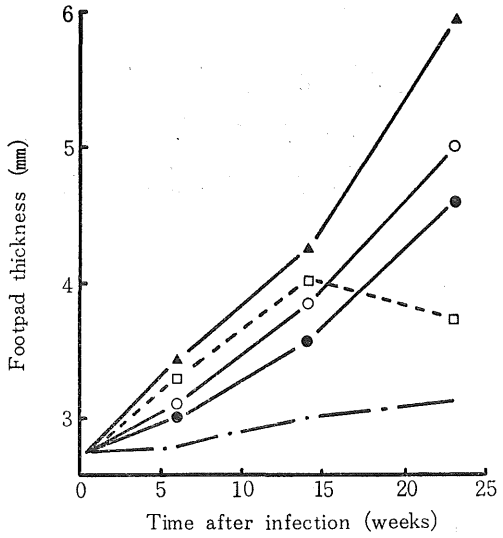

Fig. 2. Changes in the footpad thickness of Mlm-infected mice after infection. Mice were infected with Mlm and treated with OK-432 as described in Fig. 1. Thickness of the LFP (Mlm-infection site) was recorded up to 23 weeks after injection. The details and the symbols are the same as described in Fig. 1. Broken and dotted lines indicate footpad thickness of normal mice.

When OK-432 was injected subcutaneously (topically) into the Mlm-infection site (LFP), the early elimination of Mlm was suppressed and the subsequent Mlm growth was enhanced (Fig. 1, closed triangles). This indicates that immunosuppressive reaction occurred around the infection site due to interactions between the biological reactions elicited by OK-432 and by Mlm. It should be noted that anti-Mlm DTH reactivity at the early phase (6 weeks) after infection was markedly reduced by local injection of OK-432 to the infection site as compared to untreated control mice (Table 1). As shown in Fig. 2, the footpad swelling at the infection site (LFP) of Mlm was most remarkable in the animals given OK-432 to the LFP, while it was considerably more depressed in mice injected intraperitoneally with OK-432 than untreated control mice. Thus, a good

correlation was noted between the number of bacteria at the infection site and the extent of chronic inflammatory reaction, especially edema due to the infection.

When Mlm-resistant C3H/He mice were infected with the same inoculum size (10^8) as that for C3H/Jms, the early elimination of organisms during the first 6 weeks, and the subsequent suppressed growth of Mlm was remakable as compared with those of Mlm sensitive C3H/Jms mice (Fig. 1, open squares). It should be noteworthy that the LFP-swelling in C3H/He mice was more intensive than that in C3H/Jms during the first 14 weeks and weak 23 weeks after infection (Fig. 2, open square). This is remarkably different from the pattern observed in C3H/Jms mice, indicating that the footpad swelling due to Mlm-infection involves at least two types of inflammatory reactions: one is related to the host anti-Mlm resistance such as anti-Mlm DTH reaction, and the other, although not functioning in the host defense mechanisms against Mlm, may antagonize the host immune response against Mlm in some cases. In fact, we previously observed that phorbol myristate acetate (a potent tumor promoter) which elicits inflammatory reactions, promoted the growth of Mlm, when mice were infected by 10^4 organisms[15].

In this study, it was proved that a streptococcal preparation, OK-432, exhibited dual effects against Mlm infection depending on the route of injection: When it was administered intraperitoneally into mice infected with Mlm to the LFP, it acts therapeutically, whereas when it was injected into the infection site the agent antagonized the host anti-Mlm resistance. Because weekly injections of OK-432 to the LFP induced severe DTH reaction specific to OK-432 antigens (data not shown), it is likely that inflammatory reactions due to repeated DTH responses by multiple injections of OK-432 may abrogate the expression of anti-Mlm immune responses by unknown mechanisms. Induction of certain types of suppressor cells or immuno-suppressive factors may be the reason for this phenomenon, as described by other investigators[1,3-6,16,17].

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