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Protective Effects of Bacterial Immunostimulants, OK-432 and LC 9018 on *Pseudomonas aeruginosa* Infection in Tumor-Bearing Mice

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ABSTRACT

Survival rates among sarcoma-180 bearing mice against *Pseudomonas aeruginosa* infection were fewer than those among normal mice. However, the mortality of tumor-bearing mice against the infection was reduced in case of administration of bacterial immunostimulants such as OK-432 and LC 9018.

It is known that attenuated *Streptococcus pyogenes* Su (OK-432) and heat-killed *Lactobacillus casei* YIT 9018 (LC 9018) preparations have an ability to cause intense stimulation of reticuloendothelial system (RES) in terms of the activation of macrophages[2,8], antibody-producing cells[7,17], natural killer cells[3], interleukin-producing cells[16], in addition to having an antitumor activity[1,5].

In our previous studies[6,11-15], we found that *L. casei* and OK-432 had a markedly enhanced resistance to several bacterial infections in normal and dexamethasone-treated mice. The objective of the present study was to determine whether or not OK-432 and LC 9018 would enhance the resistance to *Pseudomonas aeruginosa* infection in tumor-bearing mice implanted by sarcoma-180 (S-180) cells.

OK-432 and LC 9018 were donated by Chugai Pharmaceutical Co., Tokyo and Yakult Central Institute for Microbiological Research, Tokyo, respectively. *P. aeruginosa* PAO 3047 grown in heart infusion broth at 37°C for 18 hr were washed twice with saline and suspended in saline. The number of colony-forming units (CFU) in bacterial suspension of serial 10-fold dilutions was determined on a nalidixic acid-cetrimide agar plate. Tumor-bearing animals were prepared as follows. Seven to eight-week old female ddY mice, purchased from the Shizuoka Union for Experimental Animals, Shizuoka, were inoculated subcutaneously (sc) with S-180 cells (1×10^6) at the dorsum 2, 7 or 14 days before the intraperitoneal or intravenous challenge with *P. aeruginosa*.

Fig. 1 shows the effect of OK-432 on *P. aeruginosa* infection in S-180 bearing mice. The values of survivors among normal mice (n=10) against the intraperitoneal challenge with *P. aeruginosa* (5×10^6) were 30%. In contrast, the survival percentage among S-180 bearing mice was 20% 2 days after the implantation of S-180 cells, and there were no survivors among them 7 or 14 days after the implantation. When S-180 bearing mice were given intraperitoneally (ip) OK-432 (0.2 mg) once daily for 3 days before the intraperitoneal challenge with *P. aeruginosa*, the survival rates increased and were much the same as compared with those of normal mice administered OK-432. Mice (n=10) were given S-180 cells 2, 7 or 14 days before and further ip or intravenously (iv) LC 9018 (0.5 mg) once 3 days before the intravenous or the intraperitoneal challenge with *P. aeruginosa* (7×10^6). As shown in Table 1, the survival rates among S-180 bearing mice against *P. aerugino-*

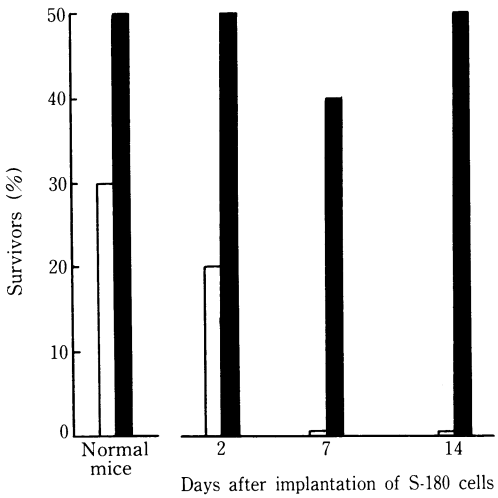

Fig. 1. Effect of OK-432 on the resistance against *P. aeruginosa* infection in S-180 bearing mice.
Normal and S-180 bearing mice (n=10) were injected ip with (■) or without (□) OK-432 (0.2 mg) once daily for 3 days before the intraperitoneal challenge with *P. aeruginosa* PAO 3047 (5×10^6). The survivors were recorded 7 days after infection.

sa infection were lower than those among normal mice. However, the decreased resistance to the infection in S-180 bearing mice was restored by the administration of LC 9018. To clarify whether or not the functions of RES in S-180 bearing mice was stimulated by the administration of LC 9018, the following test was done. S-180 bearing mice were treated once ip or iv with or without LC 9018 (0.5 mg) and infected iv or ip with *P. aeruginosa* (7×10^6) 3 days after the treatment with LC 9018. These mice (n=3) were decapitated 6 hr after infection. The livers were removed, homogenized in 5 ml of saline with a glass homogenizer and serially diluted 10-fold with saline, and then the number of CFU was assayed. As shown in Table 2, the number of CFU of organisms recovered from the liver of S-180 bearing mice pretreated ip or iv with LC 9018 was relatively less as compared with those from the liver of untreated control (S-180 bearing) mice.

It has been reported that the immunosuppression of tumor-bearing hosts is induced by an immunosuppressive factor produced and/or suppressor cells, and consequently the host resistance to opportunistic infections is markedly reduced[4,9,10]. In the present study, it was found that survivors among S-180 bearing mice against *P. aeruginosa* infection were fewer than those among normal mice. However, the mortality of S-180 bearing mice was reduced in case of ad-

Table 1. Effect of LC 9018 on the resistance against *P. aeruginosa* infection in S-180 bearing mice

Experiment	Days after tumor graft	Implantation of S-180 cells	Treatment with LC 9018 [a]	Percentage of survivors
I LC 9018 (ip) Infection (iv)	2	−	−	50
		+	−	30
		+	+	90
	7	−	−	20
		+	−	20
		+	+	50
	14	−	−	50
		+	−	30
		+	+	70
II LC 9018 (iv) Infection (ip)	2	−	−	20
		+	−	10
		+	+	30
	7	−	−	20
		+	−	0
		+	+	50
	14	−	−	40
		+	−	10
		+	+	40

[a] LC 9018 (0.5 mg) was given to mice (n=10) 3 days before challenge.

Table 2. Effect of LC 9018 on the bacterial growth in the liver during the early phase of infection in S-180 bearing mine

Experiment	Implantation of S-180 cells	Treatment with LC 9018	Days after tumor graft	log CFU [a] per organ
I LC 9018 (ip) Infection (iv)	+	−	2	4.9 ± 0.1
			7	5.0 ± 0.2
			14	4.4 ± 0.1
	+	+	2	4.2 ± 0.1
			7	3.7 ± 0.2
			14	4.2 ± 0.4
II LC 9018 (iv) Infection (ip)	+	−	2	5.4 ± 0.1
			7	5.3 ± 0.1
			14	5.8 ± 0.1
	+	+	2	4.9 ± 0.1
			7	4.7 ± 0.2
			14	4.6 ± 0.2

[a]The number of CFU in the liver was determined 6 hr after infection.

ministration of OK-432 or LC 9018, and further the number of *P. aeruginosa* in the liver during the early phase of infection (after 6 hr) was lower in S-180 bearing mice pretreated ip or iv with LC 9018 than in untreated S-180 bearing mice.

These findings suggest that the reduction of susceptibility of S-180 bearing mice administered OK-432 or LC 9018 against *P. aeruginosa* infection is probably due to the restoration of depressed functions of RES in these mice induced by the implantation of S-180 cells.

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