Enhancement of Host Resistance to Bacterial and Viral Infections by *Lactobacillus casei*

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Microorganisms such as *Mycobacterium*, *Corynebacterium*, and *Streptococcus* and their components enhance resistance to bacterial and viral infections (6, 7, 16, 18-23); this effect being caused by the direct activation of macrophage functions or T or B cell-mediated macrophage activation (3, 9, 16). Bloksma et al (4) reported that some lactobacilli, especially *Lactobacillus plantarum*, have a strong adjuvanticity on the antibody production to sheep erythrocyte and on delayed-type hypersensitivity. Kato et al (8) reported that *Lactobacillus casei* may induce macrophages that have potent antitumor activity against sarcoma 180 and L1210 leukemia.

The objective of the present study was to determine if *Lactobacillus*, especially *L. casei* would enhance the host resistance to bacterial and viral infections induced in mice.

ENHANCEMENT OF HOST RESISTANCE TO INFECTION WITH EXTRACELLULAR PARASITE BY *L. CASEI*

The effects of *L. casei* on *Pseudomonas aeruginosa* infections in normal, dexamethasone-treated, and tumor-bearing mice were studied. The details have been reported elsewhere (20, 21).

Normal mice. Mice (ddY strain) were infected intraperitoneally (ip) with *Pseudomonas aeruginosa* ($8.8-11.0 \times 10^6$ cells) on days 1, 7, 14 and 21 after the intraperitoneal injection of heat-killed *L. casei* (2.8×10^8 cells). Survival rates for mice infected with *P. aeruginosa* on days 1, 7 and 14 after the injection of *L. casei* were significantly higher (80, 70 and 50%, respectively) than in the control mice but decreased to 20% in mice injected on day 21. When the same experiment was done by giving an intravenous injection of *L. casei*, the number of survivors did not significantly differ from those in the control group. Seven and 14 days after the infection with *P. aeruginosa*, 10 and 30%, respectively, of the mice had survived. When an intramuscular injection of *L. casei* was given, all of the mice died within 24 hr. *In vivo* killing activity of peritoneal exudate cells (PECs) from mice injected intravenously (iv) with heat-killed *L. casei* (2.0×10^8 cells) against *P. aeruginosa* (1.0×10^5

cells) was determined 3 hr after infection. PECs from *L. casei*-treated mice showed no killing activity on day 1, but there was slight killing activity on day 7, a maximum was reached on day 14, and a reduction was seen on day 21. In contrast, no killing activity of PECs from control mice was nil throughout the experiment. There was no significant difference between *L. casei*-treated mice and control mice with regard to the number of PECs and the percentages of macrophages and polymorphonuclear cells (PMNs) in PECs.

Dexamethasone-treated mice. Mice (ddY strain) were injected ip with dexamethasone in the dose of 0.04 mg once daily for 3 days and 24 hr after the last injection the mice were given ip heat-killed *L. casei* (1.1×10^8 cells) or saline (control). Seven days after the injection, the mice were infected ip with *P. aeruginosa* (1.1×10^6 cells). Seven days after the induced infection, there were no survivors among the dexamethasone-treated mice whereas 50% of the mice given *L. casei* survived. The *in vivo* killing activity of PECs from dexamethasone-treated mice against *P. aeruginosa* (1.0×10^5 cells) at 3 hr after infection was lower than that of PECs from normal mice. When dexamethasone-treated mice were injected ip with heat-killed *L. casei* (2.0×10^8 cells) 7 days before the intraperitoneal infection with *P. aeruginosa*, the reduced killing activity was markedly enhanced. The number of PECs (1.7×10^6 cells/mouse) and the percentages of macrophages in the PECs (49.1%) from dexametha-

Table 1. Effect of LC9018 on the resistance against *P. aeruginosa* in S-180 bearing mice^{a)}

Experiment	Days after tumor graft	Implantation of S-180 cells	Treatment with LC9018	Percentage of survivors
[I] LC9018 (ip) Infection (iv)	2	—	—	50
		+	—	30
		+	+	90
	7	—	—	20
		+	—	20
		+	+	50
	14	—	—	50
		+	—	30
		+	+	70
[II] LC9018 (iv) Infection (ip)	2	—	—	20
		+	—	10
		+	+	30
	7	—	—	20
		+	—	0
		+	+	50
	14	—	—	40
		+	—	10
		+	+	40

^{a)} Mice ($n=10$) were given S-180 (1×10^6 cells) 2, 7 or 14 days before and further intraperitoneally or intravenously LC9018 (0.5 mg) once 3 days before the intravenous or the intraperitoneal challenge with *P. aeruginosa* (7×10^6).

sone-treated mice increased (2.1×10^7 cells/mouse; macrophages 59.5%) with the intraperitoneal administration of *L. casei*. However, there was no difference between the percentage of PMNs from mice given dexamethasone alone (5.8%) and dexamethasone-treated (5.7%) mice injected with *L. casei*.

Corticosteroids strongly suppress various functions of macrophages but do not affect the functions of PMNs (2, 15). Thus, the enhancement of resistance of dexamethasone-treated mice to *P. aeruginosa* infection is probably due to restoration and enhancement of the dysfunctioning macrophage by the administration of *L. casei*.

Tumor-bearing mice. Sarcoma-180 bearing mice were given LC9018 (a heat-killed *L. casei* preparation) once 3 days before challenge with *P. aeruginosa*. The resistance among S-180 bearing mice against *Pseudomonas* infection was lower than among the normal mice. The decreased resistance to infection in the S-180 bearing mice was restored by injecting of LC9018 (Table 1). The number of organisms recovered from the liver of S-180 bearing mice pretreated ip or iv with LC9018 at 6 hr after the infection was relatively less as compared with findings in the liver of S-180 bearing untreated control mice.

The enhancement of resistance of S-180 bearing mice pretreated with LC9018 against *P. aeruginosa* infection is probably due to restoration of depressed functions of the host reticulo-endothelial system in these mice, as induced by the implantation of S-180 tumor cells.

ENHANCEMENT OF HOST RESISTANCE TO INFECTION WITH INTRACELLULAR PARASITE BY *L. CASEI*

The enhancement of host resistance by *Lactobacillus*, particularly *L. casei* on experimental murine infection by *Listeria monocytogenes*, a facultative intracellular parasite was studied.

Role of Macrophages (22)

Protective effects of Lactobacillus spp. on Listeria infection. Mice were injected iv with *Lactobacillus* spp. 2 days before the intravenous challenge with *Listeria monocytogenes* EGD. A significant extension of the time of protection was observed in mice treated with *L. casei* than compared to those treated with other species of *Lactobacillus* (Table 2). Significant protective effects were also found in case of the intraperitoneal administration of *L. casei*, but not by the intramuscular or subcutaneous routes.

Protective activity of L. casei against Listeria infection. The dose dependent effects of *L. casei* on the protective activity against *L. monocytogenes* infection were also studied (Fig. 1). When 5.5×10^7 *L. casei* cells were given to mice (A) the protection was the most potent 2 days after *L. casei* administration and it decreased thereafter. When mice were given 1.1×10^9 *L. casei* cells (C), the enhanced resistance was seen at 3 to 21 days after the administration of *L. casei*. In contrast, when mice were given 2.1×10^8 *L. casei* cells (B) the increased resistance was seen at ca. 2 and 13 days after *L.*

Table 2. Protective activity of *Lactobacillus* spp. against *L. monocytogenes* infection in mice^{a)}

Strain	Expt.	Dose ($\times 10^7$)	Survivors/total (days)		
			3	5	7
Control	I		3/20	0/20	0/20
	II		0/20	0/20	0/20
<i>L. lactis</i> ATCC 12315	I	3.4	5/19	0/19	0/19
	II	5.8	2/10	0/10	0/10
<i>L. bulgaricus</i> ATCC 11842	I	6.4	3/19	0/19	0/19
	II	5.0	1/10	0/10	0/10
<i>L. acidophilus</i> ATCC 19992	I	11.2	8/20	1/20	0/20
	II	2.8	1/10	0/10	0/10
<i>L. salivarius</i> ATCC 11742	I	2.2	6/20	0/20	0/20
	II	2.2	2/10	0/10	0/10
<i>L. plantarum</i> ATCC 8014	I	10.6	2/20	0/20	0/20
	II	3.8	1/10	0/10	0/10
<i>L. fermentum</i> ATCC 9338	I	2.2	1/20	0/20	0/20
	II	5.6	0/10	0/10	0/10
<i>L. cellobiosus</i> ATCC 11739	I	2.2	6/20	0/20	0/20
	II	7.6	1/10	0/10	0/10
<i>L. brevis</i> ATCC 14869	I	11.0	5/19	0/19	0/19
	II	1.8	1/10	0/10	0/10
<i>L. buchneri</i> ATCC 4005	I	11.8	4/20	0/20	0/20
	II	1.3	2/10	0/10	0/10
<i>L. casei</i> YIT 0003	I	15.0	17/19 ^{b)}	8/19 ^{b)}	5/19 ^{c)}
	II	5.8	17/20 ^{b)}	9/20 ^{b)}	3/20
Control			0/10	0/10	0/10
<i>L. casei</i> ATCC 393		12.2	8/10 ^{b)}	2/10	1/10
<i>L. casei</i> ATCC 7469		11.4	10/10 ^{b)}	0/10	0/10
<i>L. casei</i> YIT 0003		10.2	8/10 ^{b)}	2/10	1/10
<i>L. casei</i> YIT 0006		8.4	10/10 ^{b)}	1/10	1/10
<i>L. casei</i> YIT 0091		11.8	9/10 ^{b)}	3/10	0/10
<i>L. casei</i> YIT 0112		7.2	10/10 ^{b)}	2/10	0/10
<i>L. casei</i> YIT 9018		7.6	8/10 ^{b)}	1/10	0/10

^{a)} Optical density (at 540 nm) of indicated *Lactobacillus* suspensions was adjusted to 1.0, and 0.2 ml each was injected into mice 2 days before *L. monocytogenes* (first group of experiments, 1.1×10^6 ; second group of experiments with *L. casei* strains only, 1.0×10^6) infection.

^{b)} Significantly different from controls at $P < 0.01$ (χ^2 test).

^{c)} Significantly different from controls at $P < 0.05$ (χ^2 test).

casei administration, in a two-peak pattern.

When mice were treated with *L. casei* cells 2 days before infection (A) in this series of experiments (Fig. 2), the growth of *Listeria* during 6 to 48 hr after the infection was suppressed by the administration of either 1.1×10^7 or 1.1×10^8 cells of *L. casei*, but was accelerated when 1.1×10^9 *L. casei* cells were given. When *L. casei* was given 13 days before the infection (B), the killing of *Listeria* in the liver during the first 6 hr was significantly enhanced in mice given 1.1×10^9 *L. casei* cells, but not with 1.1×10^7 *L. casei* cells.

A histopathological study was made of the liver of mice injected iv with either

Fig. 1. Appearance and persistency of anti-*L. monocytogenes* action of *L. casei*. Mice were injected iv with (A) 5.5×10^7 , (B) 2.8×10^8 , and (C) 1.1×10^9 of viable *L. casei* cells on various days before *L. monocytogenes* infection and infected with 10^6 *L. monocytogenes* organisms. Survivors were counted at 3 (○) and 7 (●) days after infection.

Fig. 2. Dose dependency of anti-*L. monocytogenes* action upon *L. casei*. Mice were given 1.1×10^7 (■), 1.1×10^8 (▲), or 1.1×10^9 (●) of viable *L. casei* cells 2 (A) or 13 (B) days before infection, after which experimental and control (○) mice were infected with 10^6 *L. monocytogenes* organisms. The fate of *L. monocytogenes* trapped in the liver was studied for up to 48 hr after infection. Each symbol represents the mean $\pm$ standard error ($n=3$ to 5). The asterisks indicate significant difference between controls and *L. casei*-treated mice at levels of $P<0.05$ (*) and $P<0.01$ (**) (Student's *t* test).

1.1×10^7 , 1.1×10^8 , or 1.1×10^9 *L. casei* cells, and challenged with *Listeria monocytogenes* (10^6 cells) 2 and 13 days after injection of *L. casei*. Livers were removed 2 days after listerial infection. In the liver of mice 2 days after administration of 1.1×10^9 of *L. casei* cells, a large number of small to intermediate nodular infiltrations, consisting mainly of monocytes together with a few neutrophils, were observed in the sinusoids. Similar, but milder foci were observed in mice given 1.1×10^7 or 1.1×10^8 *L. casei* cells. On the other hand, in the liver of mice 13 days after administration of 1.1×10^9 *L. casei* cells, intermediate to large epithelioid cell nodules, and a few monocytes were observed only inside or surrounding the nodules (Fig. 3). Histopathological changes after the administration of 1.1×10^7 *L. casei* cells were nil. Forty-eight hours after the *Listeria*-induced infection, a number of bacterial clumps and necrosis were noted in the liver (Fig. 4). In contrast, when mice were infected with *Listeria* 2 days after the administration of *L. casei*, the bacterial clumps and necrosis of the hepatic cells had decreased, regardless of the dose of *L. casei* given. Histopathological manifestations in the liver of mice infected with *Listeria* 13 days after administration of 1.1×10^9 *L. casei* cells were similar to those in the liver of mice treated with 1.1×10^9 *L. casei* cells (non-infected mice), but bacterial clumps were absent (Fig. 5). The hepatic lesions of mice infected with *Listeria* 13 days after administration of 1.1×10^7 *L. casei* cells were similar to those seen in the liver of mice infected with *Listeria*.

Fig. 3. Histopathological findings of the liver of mice 13 days after administration of *L. casei* (1.1×10^9). A large number of nodular infiltrative lesions consisting mainly of monocytes together with a few neutrophils are observed in the sinusoids, with irregular distribution to the lobules. Many epithelioid cells and a few typical monocytes are seen only inside or surrounding the nodules (hematoxylin and eosin stain; original magnification, $\times 200$).

Fig. 4. Liver sample of mice 48 hr after *L. monocytogenes* infection. Note the numerous bacterial clumps and necrosis of the hepatic cells (hematoxylin and eosin stain; original magnification, $\times 200$).

The effects of carrageenan (CAR) on *L. casei*-mediated host resistance against listerial infection were studied (Table 3). Regardless of the timing of *L. casei* administration (-13 or -2 days), a smaller number of *Listeria* was found 10 min after infection in the liver of CAR-treated mice than in that of CAR-nontreated mice.

Fig. 5. Liver tissue from a mouse infected with *L. monocytogenes* 13 days after *L. casei* (1.1×10^9) administration. Note that changes are similar to those in Fig. 3 (hematoxylin and eosin stain; original magnification, $\times 200$).

Table 3. Antagonistic effect of carrageenan on *L. casei*-mediated enhancement of host resistance^{a)}

<i>L. casei</i> administration at days before infection	Carrageenan treatment	Log ₁₀ no. of viable bacteria/liver at time after infection		
		10 min	6 hr	48 hr
-2				
-	-	3.78 ± 0.15	2.53 ± 0.12	5.33 ± 0.15
-	+	$3.01 \pm 0.12^b)$	3.24 ± 0.26	$6.25 \pm 0.30^c)$
+	-	3.68 ± 0.08	3.10 ± 0.13	$3.23 \pm 0.18^d)$
+	+	3.21 ± 0.20	3.08 ± 0.20	$6.10 \pm 0.24^{e), e)}$
-13				
-	-	3.50 ± 0.02	2.42 ± 0.08	5.18 ± 0.14
-	+	$2.80 \pm 0.11^e)$	3.29 ± 0.29	$6.85 \pm 0.42^e)$
+	-	3.32 ± 0.13	$1.07 \pm 0.14^e)$	$1.94 \pm 0.30^e)$
+	+	2.90 ± 0.15	$2.22 \pm 0.10^b)$	$3.87 \pm 0.40^{b), e)}$

^{a)} Mice were injected iv with 2.5×10^8 viable *L. casei* cells at 1 or 12 days before treatment with carrageenan (4.5 mg). At 24 hr after administration of carrageenan, mice were infected iv with 10^4 *L. monocytogenes* organisms. Values are expressed as mean $\pm$ standard error ($n=4$).

^{b)} Significantly different from carrageenan-nontreated counterpart at $P < 0.05$ (Student's *t* test).

^{c)} Significantly different from carrageenan-nontreated counterpart at $P < 0.01$ (Student's *t* test).

^{d)} Significantly different from controls at $P < 0.05$ (Student's *t* test).

^{e)} Significantly different from controls at $P < 0.01$ (Student's *t* test).

Table 4. Subversion of *L. casei*-mediated enhancement of host resistance by X-ray irradiation^{a)}

<i>L. casei</i> administration at days before infection	X-ray irradiation	Log ₁₀ no. of viable bacteria/liver at time after infection		
		10 min	6 hr	48 hr
-2				
—	—	3.83±0.10	2.44±0.33	5.91±0.29
—	+	3.76±0.07	2.28±0.28	6.90±0.30 ^{b)}
+	—	3.78±0.04	3.12±0.16	4.09±0.24 ^{c)}
+	+	3.81±0.06	3.04±0.23	7.24±0.18 ^{d)}
-13				
—	—	3.71±0.03	2.55±0.04	5.45±0.28
—	+	3.76±0.08	2.26±0.07	6.60±0.51 ^{b)}
+	—	3.80±0.04	1.91±0.27 ^{c)}	2.81±0.18 ^{c)}
+	+	3.73±0.09	2.03±0.07 ^{c)}	5.30±0.47 ^{d)}

^{a)} Mice were injected iv with 2.5×10^8 viable *L. casei* cells 1 hr or 11 days before X-ray irradiation. At 2 days after irradiation (900 rad), mice were infected iv with 10^4 of *L. monocytogenes* organisms. Values are expressed as mean±standard error ($n=4$).

^{b)} Significantly different from X-ray-nontreated counterpart at $P<0.05$ (Student's *t* test).

^{c)} Significantly different from controls at $P<0.05$ (Student's *t* test).

^{d)} Significantly different from X-ray-nontreated counterpart at $P<0.01$ (Student's *t* test).

^{e)} Significantly different from controls at $P<0.01$ (Student's *t* test).

CAR treatment led to growth of *Listeria* 6 hr after the infection (—13 days), and the growth of *Listeria* 48 hr after infection in the *L. casei*-CAR-treated mice was enhanced in the 2- and 13-day groups, over the CAR-nontreated group.

The effects of X-ray irradiation on the *L. casei*-mediated host resistance against *Listeria* infection were studied (Table 4). Trapping time (10 min) of the challenged *Listeria* by the liver and the killing of bacteria in the liver 6 hr after infecting the mice with or without *L. casei* treatment were not affected by X-ray irradiation. On the contrary, the markedly reduced multiplication effect of *Listeria* in the liver 48 hr after infection by *L. casei* given at 2 and 13 days was all but completely abolished by X-ray irradiation.

With respect to the enhancement of host resistance to *Listeria* infection that was seen 2 days after *L. casei* administration, the following considerations were made, based on these results. The administration of 1.1×10^7 or 1.1×10^8 of *L. casei* cells failed to enhance the killing of *Listeria* in the liver at 6 hr after infection but did suppress growth of *Listeria* 48 hr after the infection.

Histopathological studies revealed that *L. casei* administration increased the number of macrophages in the liver after *L. casei* administration. The killing of *Listeria* 6 hr after infection in *L. casei* (2.5×10^8 cells)-treated mice was not affected by X-ray irradiation or CAR treatment, but the inhibitory effect of *L. casei* on the multiplication of *Listeria* 48 hr after infection was curtailed. Those findings indicate that enhancement of the *L. casei*-mediated host resistance to *Listeria* infection at this

phase is mediated by non-specifically stimulated macrophages newly emigrating from the circulating blood to the liver (12-14) before and after *Listeria* infection but not fixed macrophages in the liver. When 1.1×10^9 *L. casei* cells were given, there was a marked increase in the number of challenged *Listeria*. It seems that macrophages which phagocytosed numerous *L. casei* cells were unable to kill infecting *Listeria*, presumably due to depression of cell functions by overphagocytosis.

Concerning the enhancement of host resistance to *Listeria* infection seen around 13 days after the *L. casei* administration, the following considerations were made. The administration of *L. casei* (1.1×10^9 cells) enhanced the killing of *Listeria* in the liver 6 hr after infection, and the growth of *Listeria* was suppressed by *L. casei* administration (1.1×10^8 or 1.1×10^9 cells) 48 hr after infection. The killing of *Listeria* 6 hr after infection in *L. casei*-treated mice was not affected by X-ray irradiation but was suppressed by CAR treatment. In contrast, the inhibitory effect of *L. casei* on the *Listeria* multiplication 48 hr after infection was inhibited by CAR treatment or by X-ray irradiation. Thus, the enhanced host resistance to *Listeria* infection at ca. 13 days after *L. casei* administration seems to be mediated not only by fixed macrophages but also by macrophages newly emigrating from the blood monocyte pool to the infection site (liver) in response to *Listeria* infection (12, 14). *L. casei* administration accelerated macrophage accumulation into the liver after *Listeria* infection. This may be explained in terms of the following experimental results. The number of peritoneal macrophages in mice given *L. casei* (6.5×10^8 cells) iv. and 13 days later, injected ip with 10^8 UV-killed *L. monocytogenes* was increased. [^3H]TdR incorporations in the liver DNA (Fig. 6) before *Listeria* infection (0 hr) was markedly higher in mice given *L. casei* (2.5×10^8 cells) than in *L. casei*-nontreated controls, but proceeded at almost the same rate in the two groups of mice during the first 6 hr after infection. In contrast, [^3H]TdR incorporation in *L. casei* treated mice during 6 to 48 hr after infection was much higher than in *L. casei*-nontreated mice. Histopathological studies revealed increased monocyte infiltration into the liver by *L. casei* administration 13 days before the *Listeria* infection. Epithelioid granulomas were formed in the liver 13 days after *L. casei* administration. Generally, it is thought that such formation can be ascribed to immunological reactions, and in some cases, delayed type hypersensitivity (DTH) reactions are required for formation (1, 10). Mitsu-yama et al (11) mentioned that macrophages accumulating at the site of DTH reaction are important in the anti-*L. monocytogenes* host resistance. This finding supports the view that the accumulation and subsequent activation of liver macrophages in mice treated with *L. casei* are enhanced by lymphokines produced by *L. casei*-primed T cell.

The lack of increased resistance to *Listeria* infection 13 days after 5.5×10^7 *L. casei* cells can be explained as follows. Viable *L. casei* cells were no longer detectable in the liver (data not shown). Histopathologically, there was evidence of infiltration of macrophages into the liver, and the killing (6 hr) and growth suppression (48 hr) of *L. monocytogenes* in the liver were slight. Therefore, macrophages related to the DTH reaction elicited by *L. casei* antigen do not seem to accumulate before infection.

Fig. 6. Accumulation of macrophages labeled during infection in the liver. Mice were injected iv with 1.0×10^7 (▲) and 2.5×10^8 (■) or without (●) viable *L. casei* cells. After 13 days mice were given 10 μ Ci of [3 H]TdR iv. At 1 hr after labeling, mice were infected iv with 8.4×10^3 *L. monocytogenes* organisms, and the liver of mice was used for radiometry 0, 6, and 48 hr after infection. As controls (○), the liver of mice 30 min and 6 and 48 hr after injection of 10 μ Ci of [3 H]TdR without *L. monocytogenes* infection was used. Each symbol represents the mean $\pm$ standard error ($n=3$). The asterisks indicate significant difference between controls and *L. casei*-treated mice at levels of $P < 0.05$ (*) and $P < 0.01$ (**) (Student's *t* test).

Activation of macrophages. The peritoneal exudate macrophage induced by intraperitoneal injection of *L. casei* was used as a model system to elucidate changes in cellular functions of liver macrophages due to intravenous injections of the agent.

Listericidal activity of mouse peritoneal macrophages was enhanced by administration of *L. casei* (5×10^7 , 2.5×10^8 , or 1×10^9 cells) 13 days before harvest. In contrast, the activity of macrophages was reduced 2 days after *L. casei* administration, at three different doses, compared with that of resident macrophages. The phagocytic ability of macrophages against latex particles was elevated in peritoneal macrophages from mice treated with *L. casei* cells, irrespective of the timing (—2 of —13 days) of administration of the agent. Phorbol myristate acetate (PMA)-triggered respiratory burst (O_2 and H_2O_2) was elevated in all cases of *L. casei* administration. Lysosomal enzyme activities (acid phosphatase and β -glucuronidase) were significantly elevated in all cases of *L. casei* injections, irrespective of the dose and timing of the *L. casei* administration, except for the β -glucuronidase activity of macrophages harvested 2 days after the *L. casei* administration. In contrast, alkaline phosphodiesterase activity was markedly reduced in all the *L. casei*-elicited macrophages, except for macrophages observed 2 days after the administration of 1×10^9 *L. casei* cells.

The peritoneal macrophages 13 days after *L. casei* administration had a markedly elevated capacity of bactericidal activity against *L. monocytogenes*, PMA-induced respiratory burst and β -glucuronidase. Moreover, these macrophages also showed a remarkably lowered alkaline phosphodiesterase activity. These findings are known to correlate well to an "immunologically activated state" rather than to a "merely stimulated state" of a given macrophage. These results indicate an "immunological activation" of the RES (reticulo-endothelial system), such as the liver and spleen, at 13 days after intravenous administration of *L. casei* cells. There are marked differences in macrophage cellular functions related to their microbicidal mechanisms between macrophage populations induced by *L. casei* at 2 and 13 days after the administration. The former population may belong to "merely stimulated macrophages" with low antilisterial activity, whereas the latter may possess properties as "immunologically activated macrophages" with potent antilisterial activity.

Protective action of L. casei cell fractions against Listeria infection. Cell fractions (cell wall, cytoplasm, polysaccharide, and peptidoglycan), prepared from *Lactobacillus* by Yokokura's method (25), were studied for their efficacies to enhance host resistance against *L. monocytogenes* infection.

Mice were given iv 1 mg of each cell fraction of *Lactobacillus* 2 or 13 days before the intravenous infection of *L. monocytogenes* (1×10^6 cells). A significant extension of survival time occurred in mice injected with the cell wall of *L. casei* (strains YIT 0003 and YIT 9018) 13 days before infection, compared to those given the other fractions of *L. casei* cells and *L. plantarum* and *L. acidophilus* cell fractions. The PMA-triggered respiratory burst of PECs in mice elicited by *L. casei* cell fractions was studied. The chemiluminescence (CL) of PECs (1×10^6 cells) elicited 2, 7, and 13 days after injection of cell fractions of *L. casei* (1 mg each) was higher in PEC induced by the cell wall than in PECs induced by other fractions, whereas the CL of PECs obtained 21 days after injection of those fractions was equal to that of normal PECs (on day 0). The PMN ratio (50%) in PEC induced by the cellular fractions was higher than that in peritoneal cells and the CL in response to PMA was markedly elevated 2 days after injection of the cellular fractions. A considerable part of the apparent CL from the PEC at day-2 is thought to originate from contaminating PMN. The ratios of macrophages and PMN in the PECs at 7, 13, and 21 days after the injection of the cellular fractions were 50% to 80% and 1% to 6%, respectively. The CL on these days is thought to be originated mainly from macrophages. The O_2^- production in response to PMA of peritoneal macrophages obtained 2 and 7 days after intraperitoneal injection of the cell wall was the highest among macrophages elicited by cellular fractions. Such a production in macrophages 13 and 21 days after injection of the cell wall was as high as that in the macrophages on 2 and 7 days, whereas it was much lower with the other cellular fractions. The O_2^- production was elevated in macrophages elicited by the cell wall of *L. casei* and *L. acidophilus*, while the production in macrophages elicited by *L. plantarum*-cell wall was markedly lower than that of the other cell walls. *In vitro* listericidal activity of peritoneal macrophages was enhanced 7 and 13 days but was reduced 2 days after intraperitoneal injection of the cell wall fraction (1 mg) of *L. casei*. The activity of

L. acidophilus cell wall-induced peritoneal macrophages was reduced in all cases, regardless of the timing of the administration of the cell wall. When mice were treated with the cell wall of *L. casei* or *L. acidophilus* 2 days before the infection, growth of the *Listeria* during 6 to 48 hr after infection was not suppressed. Reasons for the lack of anti-listerial action 2 days after the intravenous administration of *L. casei* cell wall (0.1, 0.5 or 1 mg) were not clear. Innate *L. casei* cells might be required for expression of the anti-listerial action.

Czuprynski et al (5) reported that increased mobilization of PMN and mononuclear phagocytes at the site of infection is of prime importance to protect the host against *L. monocytogenes* infection. In our experiment, increased numbers of PEC and PMN in PEC and in the resultant augmented O_2^- production of PEC 2 days after injection of *L. casei* cellular fractions were noted, whereas the host resistance to *Listeria* infection was not enhanced by preinjection of the fractions. This observation suggests that PMN are not effective in killing *L. monocytogenes*, in the host mouse. The PMA-triggered O_2^- production of peritoneal macrophages was augmented by the intraperitoneal administration of *L. casei*- and *L. acidophilus* cell wall, but the *in vivo* bactericidal activity and the protective effect against *Listeria* infection was found only in the *L. casei*-cell wall. These findings are in agreement with data that

Fig. 7. Protection of mice pretreated with immunostimulants against HSV-1 infection according to the time of administration of UV-inactivated HSV-1 antigen. Mice ($n=10$) were injected ip with 0.2 ml of each immunostimulant (10 mg/ml) 14 days before and with 0.1 ml of UV-inactivated HSV-1 antigen (5×10^7 PFUs/ml) 3, 7, 10, or 14 days before ip challenge with 0.1 ml of HSV-1 (5×10^6 PFUs/ml). UV-inactivated HSV-1 antigen (UV-HSV-Ag) alone (○), LC9018+UV-HSV-Ag (●), OK-432+UV-HSV-Ag (▲), PSK+UV-HSV-Ag (■), *P. acnes*+UV-HSV-Ag (△). * $P < 0.05$, ** $P < 0.01$.

the protective activity against *Listeria* infection was enhanced only with whole cells of *L. casei*, among 10 species of lactobacilli (see p. 4). Almost all lactobacilli contain the teichoic acid moiety in their cell wall, but *L. casei* lacks such a moiety. Therefore, the anti-*Listeria* action of *L. casei*-cell wall might be mediated by specific components other than teichoic acid.

Enhancement of Host Resistance to Viral Infection by L. casei

The effects of immunostimulants such as LC9018 (8), OK-432 (17), PSK (24) and *Propionibacterium acnes* on fatal infection due to herpes simplex virus type 1 (HSV-1) in adult mice (26).

Protective action of immunostimulants against HSV-1 infection. Mice were given ip 2 mg of LC9018 (a lyophilized preparation of heat-killed *L. casei* YIT9018), OK-432 (attenuated strain of *Streptococcus pyogenes* Su), PSK (a protein-bound polysaccharide extracted from *Coriolus versicolor*), or *P. acnes* (heat-killed at 80°C for 30 min) 14 days before intraperitoneal challenge with HSV-1 (5×10^5 PFUs/mouse). No significant enhancement of resistance to HSV-1 infection in mice was seen after administration of these immunostimulants. However, the survival rate was somewhat higher in mice given LC9018 or *P. acnes* (30–40%) than in the mice given OK-432 or PSK (10–20%).

Effect of UV-inactivated HSV-1 antigen in combination with immunostimulants. Studies

Fig. 8. Correlation between patterns of NT antibody titers and survival rates of mice pretreated with LC9018 in combination with UV-inactivated HSV-1 antigen. Mice were injected ip with 0.2 ml of LC9018 (10 mg/ml) 14 days before and with 0.1 ml of UV-inactivated HSV-1 antigen (5×10^7 PFUs/ml) 7, 14, 21, or 28 days before harvest of the blood samples for titration of serum NT antibody ($n=3$) or ip challenge with 0.1 ml of HSV-1 (5×10^6 PFUs/ml, $n=10$). UV-inactivated HSV-1 antigen alone (○), LC9018+UV-inactivated HSV-1 antigen (●). * $P < 0.05$, ** $P < 0.01$.

were made of mice given immunostimulants (2 mg each) ip 14 days before and UV-inactivated HSV-1 antigen ip 3, 7, 10, or 14 days before infection with HSV-1 (Fig. 7). The survival rates of mice increased depending on the time of administration of the inactivated HSV-1 antigen. Maximum survival rates were noted 10 or 14 days after the administration of inactivated HSV-1 antigen in LC9018 (90% on day 10) and *P. acnes* (90% on day 14), followed by OK-432 (70% on day 14) and PSK (50% on day 10). When mice were injected ip with LC9018 14 days before and with UV-inactivated HSV-1 antigen 3 days before the infection with HSV-1, the survival rate was 40%, whereas serum neutralizing antibodies were not detectable at that time. It may be that the early phase protection of mice against HSV-1 infection after the administration of *L. casei* is related to the increased natural killer cell cytotoxicity mediated by activated macrophages. The correlation between neutralizing antibody levels in serum and survival rates of mice given LC9018 in combination with UV-inactivated HSV-1 antigen was studied (Fig. 8). Neutralizing antibody levels in serum (A, ●) showed maximum levels (64 units) 14 to 21 days after and were slightly reduced 28 days after (32 units) administration of inactivated HSV-1 antigen. The maximum survival rates (B, ●) were observed 14 days after the administration of inactivated HSV-1 antigen (80%); however, they were drastically reduced thereafter. Thus, correlation between patterns of neutralizing antibody titers and the number of survivors was nil. The phenomena may be due to changes in the IgG subclass and/or Fc receptors of effector cells.

Fig. 9. Protection of mice pretreated with UV-inactivated HSV-1 antigen against HSV-1 infection according to the time of administration of LC9018. Mice ($n=10$) were injected ip with 0.1 ml of UV-inactivated HSV-1 antigen (5×10^7 PFUs/ml) 14 days before and with 0.2 ml of LC9018 (10 mg/ml) 3, 7, 10, 14, or 21 days before challenge with 0.1 ml of HSV-1 (5×10^6 PFUs/ml). LC9018 alone (○), LC9018 + UV-inactivated HSV-1 antigen (●). * $P < 0.05$, ** $P < 0.01$.

Effects of immunostimulants in combination with UV-inactivated HSV-1 antigen. Mice given UV-inactivated HSV-1 antigen 14 days before infection were injected with 2 mg of *L. casei* at various intervals before the intraperitoneal infection with HSV-1 (Fig. 9). The survival rates of mice treated with inactivated HSV-1 antigen in combination with LC9018 were higher than those of mice given LC9018 alone and the highest rate was noted on day 14 (90%). The correlation between neutralizing antibody levels in serum and survival rates of mice given UV-inactivated HSV-1 antigen in combination with *L. casei* was studied. Similar patterns were noted, as shown in Fig. 8. There was no correlation between the patterns of neutralizing antibody titers and the number of survivors 21 days after administration of *L. casei* (Fig. 10). There is probably a lack of function of the macrophage as a result of maturation or to the appearance of suppressor cells and/or suppressor factors.

Effects of transfer of PECs or spleen cells on HSV-1 infection in mice. Protection against on HSV-1 infection could be transferred to syngeneic recipient mice by the intraperitoneal injection of PECs from C3H/He mice treated with LC9018 alone and in combination with UV-inactivated HSV-1 antigen or with thioglycollate broth 18 hr before intraperitoneal challenge with HSV-1. In contrast, there was no protective effect of spleen cells from mice treated with thioglycollate broth alone or with LC9018 in combination with thioglycollate broth (Tables 5 and 6). PECs induced by LC9018 alone also showed HSV-I focus formation-inhibiting ability

Fig. 10. Correlation between patterns of NT antibody titers and survival rates of mice pretreated with UV-inactivated HSV-1 antigen in combination with LC9018. Mice were injected ip with 0.1 ml of UV-inactivated HSV-1 antigen (5×10^7 PFUs/ml) 14 days before and with 0.2 ml of LC9018 (10 mg/ml) 7, 14, 21, or 28 days before harvest of the blood samples for titration of serum NT antibody ($n=3$) or ip challenge with 0.1 ml of HSV-1 (5×10^6 PFUs/ml, $n=10$). * $P<0.05$, ** $P<0.01$.

Table 5. Effects of transfer of PECs from mice treated with LC9018 alone or in combination with UV-inactivated HSV-1 antigen on HSV-1 infection^{a)}

	No. of cells transferred ($\times 10^7$)	Percentage of survivors
None	—	0
PECs from		
Untreated mice	1.5	0
LC9018-treated mice	1.0	40
(LC9018+UV-inactivated HSV-1 antigen)-treated mice	1.0	40

^{a)} PECs were harvested 14 days after the simultaneous administration (ip) of LC9018 (2 mg) and UV-inactivated HSV-1 antigen (5×10^6 PFUs), and transferred into the peritoneal cavities of syngeneic recipient C3H/He mice. Mice were injected ip with HSV-1 (5×10^5 PFUs) 18 hr after transfer.

Table 6. Effects of transfer of spleen cells or PECs from mice treated with thioglycollate alone or in combination with LC9018 on HSV-1 infection^{a)}

	No. of cells transferred ($\times 10^7$)	Route	Percentage of survivors
None	—	iv	0
	—	ip	0
Thioglycollate alone	Spleen cells 16	iv-iv	0
	PEC 0.9	ip-ip	0
LC9018+thioglycollate	Spleen cells 16	iv-iv	0
	PECs 0.9	ip-ip	40

^{a)} Syngeneic recipient mice were injected iv or ip with spleen cells or PECs harvested from syngeneic donor mice 14 and 4 days after ip administration of LC9018 (2 mg) and thioglycollate broth (2 ml), and injected iv or ip with HSV-1 (5×10^5 PFUs) 18 hr after transfer.

in vitro, whereas focus formation was not inhibited by thioglycollate-elicited PECs, which exhibited high phagocytic activity. These findings suggest that the enhancement of resistance to HSV-I infection in mice by the administration of *L. casei* in combination with UV-inactivated HSV-I antigen is mediated mainly by activated macrophages.

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