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Enhanced Humoral Antibody Production and Delayed Type Hypersensitivity Response in Mice by *Lactobacillus casei*[*)]

Hajime SAITO, Katsumasa SATO, Yoshiya HORIKAWA,
Byoung Won JIN, Haruaki TOMIOKA and Takashi WATANABE
Department of Microbiology and Immunology, Shimane Medical University, Izumo 693, Japan
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ABSTRACT

Antibody titers against *Pseudomonas aeruginosa* in sera from mice were markedly elevated by pre-injection of *Lactobacillus casei*. The number of plaque-forming cells against sheep red blood cells (SRBC) in the spleen of mice pretreated with *L. casei* was highter than those in the controls. *L. casei* also enhanced the delayed type hypersensitivity response against SRBC in mice. Therefore, it was revealed that *L. casei* possesses the actions of humoral and cellular immunopotentiation.

Bacterial nonspecific stimulators of host defense (immunostimulators) such as Bacillus Calmette-Guérin (BCG), *Propionibacterium acnes* (*Corynebacterium parvum*), attenuated *Streptococcus pyogenes* (OK-423) and cellular components of these microorganisms activate cell-populations of the reticuloendotherial system. One major site of action of these stimulants is the macrophage[2,4,9]. These agents also stimulate the lymphocytes, granulopoiesis[8], the complement system[11,13] and the production of interferon[12] and humoral antibodies[1,16].

Kato et al.[10] reported the marked antitumor activity of *Lactobacillus casei* YIT-9018 (LC 9018) against allogeneic and syngeneic tumors in mice. We also recently found that the pretreatment of mice with *L. casei* YIT-0003 causes a marked increase in resistance to challenge with *Listeria monocytogcnes* and *Pseudomonas aeruginosa*[14,15]. It is also known that *L. plantarum* and *L. casei* possess adjuvant activities[3,7]. As little is known of the humoral antibody producing effects of *L. casei*, we studied the effects of *L. casei* on the production of antibody and the antibody producing cells against the heat-killed *P. aeruginosa* and sheep red blood cells (SRBC) in mice.

L. casei YIT-0003 cells grown in Rogosa's medium[6] at 37°C for 18 hr were washed and suspended in saline. Each of five-week-old female ddY mice was given intravenously (iv) a 0.2 ml of a 5×10^8/ml saline suspension of live or heat-killed (at 80°C for 30 min) *L. casei* three times at 24 hr intervals. Control mice were given injections of 0.2 ml of saline under the same conditions. Forty-eight hr after the last injection, these mice were immunized subcutaneously (sc) with a 0.1 ml of 3×10^9/ml saline suspension of heat-killed (at 100°C for 60 min) *P. aeruginosa* PAO 3047 three times at 24 hr intervals. After the third immunization, blood was taken from the heart of these animals at various time intervals. The serum was separated by centrifugation and stored at −20°C until use. Sero-agglutination tests were carried out with a microplate. The serum (25 μl) was serially diluted 2-fold with saline and mixed with an equal volume of heat-killed (at 120°C for 90 min) *P. aeruginosa* (1.5×10^9/ml). The reaction mixture was incubated at 37°C for 2 hr and kept at 4°C for 24 hr.

As shown in Fig. 1, the mean values of antibody titers 5 and 8 days after *P. aeruginosa* injection in the *L. casei*-treated group were

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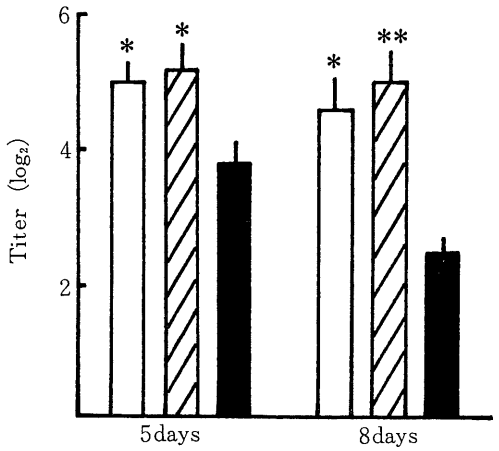

Fig. 1. Agglutinin titers of sera against *P. aeruginosa* in *L. casei*-treated and control mice. Methods are described in the text. Live *L. casei*-treated group (open bar), killed *L. casei*-treated group (hatched bar), control group (shaded bar). Bar: mean±SE (n=5).
* : $P<0.05$, ** : $P<0.01$.

significantly higher than those of the control group. However, there were no differences in titers between the live and the heat-killed *L. casei*-treated group. Time course of the production of *P. aeruginosa* antibody in the heat-killed *L. casei*-treated and control mice was tested for up to 14 days. As shown in Fig. 2, the mean values of antibody titers in *L. casei*-treated mice were higher than titers in control mice throughout the experiment, and the difference in titers on days 5, 7, 10 and 14 between the two groups were statistically significant

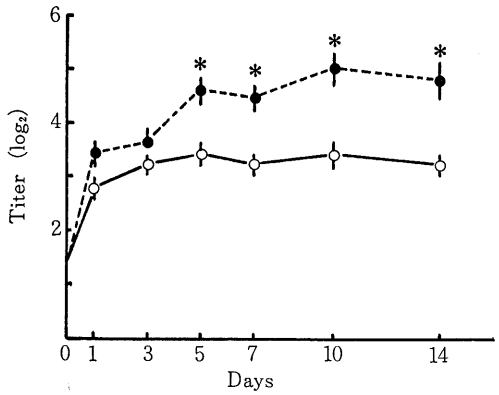

Fig. 2. Time course of agglutinin production in *L. casei* (heat-killed)-treated and control mice. Methods are described in the text. *L. casei*-treated group (●), control group (○). Bar: mean±SE (n=5). * : $P<0.01$.

($P<0.01$).

To determine whether or not there was an increase in the production of *P. aeruginosa* antibody producing cells by *L. sasei*, the numbers of plaque-forming cells (PFC) against SRBC were counted. Mice were injected with the heat-killed *L. casei* as mentioned above, and were immunized iv with a 0.2 ml of a 1×10^9/ml saline suspension 48 hr after the last injection. PFC in spleen cells from experimental and control mice were determined by the method of Cunningham and Szenberg[5]. The mean numbers of IgM-PFC in spleen cells from experimental mice were significantly higher than those from control mice on days 5 and 7 (Table 1). Hemagglutination titers of sera from

Table 1. Production of IgM-plaque-forming cells in spleen cells against SRBC in *L. casei*-treated and control mice.

Groups	PFC ($\times10^2$)/Spleen		
	3d[a]	5d	7d
Control	3.8±1.2[b]	442.0± 36.2	87.6±14.7
L. casei-treated	3.1±1.1	1172.0±238.6*	256.0±66.6**

[a] : Days after immunization. [b] : mean±SE (n=5)
* : $P<0.05$, ** : $P<0.01$.

mice treated with *L. casei* were much the same as those of sera from control mice (data not shown). It may be that hemagglutination titers of sera from experimental mice did not increase because of the single immunization with SRBC. In fact, increases in pseudomonas-agglutination titers of sera were nil when the mice injected with *L. casei* were immunized once with *P. aeruginosa*.

To determine whether or not the delayed type hypersensitivity (DTH) response is enhanced by *L. casei*, the following experiment was carried out. Mice were injected three times with the live and the heat-killed *L. casei* (1×10^8) as mentioned above, and were immunized intraperitoneally with SRBC (2×10^8) 48 hr after the last injection. Five and 8 days after immunization, 50 μl of 2×10^9/ml saline suspension of SRBC were inoculated sc into the left hind footpad of the mouse and the footpad thickness was measured 24 hr later. Calculation of the footpad thickness against SRBC was carried

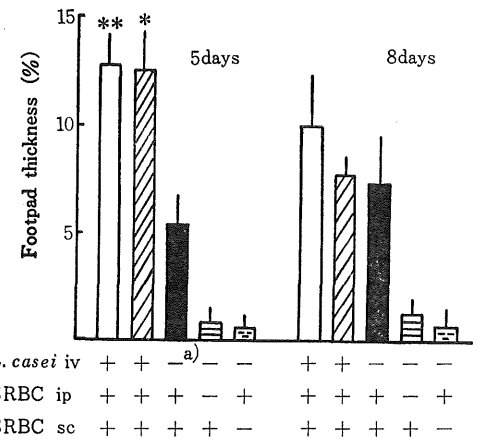

Fig. 3. Delayed type hypersensitivity response to SRBC in *L. casei*-treated and control mice. Methods are described in the text. Live *L. casei*-treated group (open bar), killed *L. casei*-treated group (hatched bar), control group (shaded bar). a) Mice were injected with saline. Bar: mean± SE (n=5). $*P<0.05$, $**: P<0.01$.

out according to the method of Wing[17]: [(A-B)/] ×100; A=thickness of footpad injected with SRBC or saline and B=thickness of the opposite footpad. As shown in Fig. 3, the footpad thickness in mice injected with the live or the heat-killed *L. casei* on day 5 after immunization with SRBC was gignificantly increased as compared with that in control mice ($P<0.01$ or $P<0.05$). No differences were observed in the degree of thickness between the live and the heat-killed *L. casei*-treated groups.

Fleck et al.[7] reported that *L. casei* stimulate the DTH response. However, the effect of *L. casei* on the production of humoral antibody has not been reported. In the present study, it was demonstrated that both the live and the heat-killed *L. casei* effectively enhance the production of antibody against *P. aeruginosa* in mice. This enhancement seems to be due to the activation of antibody producing cells by *L. casei* because the PFC response to SRBC in mouse spleen cells was markedly enhanced by the administration of *L. casei*. Bloksma et al.[3] reported that viable *L. plantarum* stimulate exclusively the DTH response, whereas heat-killed bacterium have an adjuvant effect on the antibody production. In the present study, not only live cells but also heat-killed cells of *L. casei* stimulated the DTH response to SRBC. The difference in the mechanism of action between *L. casei* and *L. plantarum* remains to be determined.

The present study revealed that *L. casei* possesses the effect of humoral and cellular immunopotentiation. Opportunistic infections are one of the most important problems facing cancer patients as their immune defense system is markedly reduced. *L. casei*, one of the members of normal flora in the animal intestine, is used to make yogurt. The possibility that *L. casei* may also protect against opportunistic infections in clinical applications has to be considered.

REFERENCES

1. **Adlam, C. and Scott, T.** 1973. Lymphoreticular stimulatory properties of *Corynebacterium parvum* and related bacteria. J. Med. Microbiol. **6**: 261-274.
2. **Bast, R. C., Zbar, B., Borsos, T. and Rapp, H. J.** 1974. BCG and cancer. N. Eng. J. Med. **290**: 1413-1420.
3. **Bloksma, N., De Heer, E., Van Dijk, H. and Willers, J. M.** 1979. Adjuvanticity of lactobacilli I. differential effects of viable and killed bacteria. Clin. exp. Immunol. **37**: 367-375.
4. **Buck, G. E. and Kelly, M. T.** 1980. Investigation of the component of *Propionibacterium acnes* (*Corynebacterium parvum*) responsible for macrophage activation. Infect. Immun. **27**: 620-627.
5. **Cunningham, A. J. and Szenberg, A.** 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. Immunology **14**: 599-600.
6. **Eftymiou, C. and Hansen, P. A.** 1962. An antigenic analysis of *Lactobacillus acidophilus*. J. Infect. Dis. **110**: 258-267.
7. **Fleck, J., Mock, M., Tytgat, F., Nauciel, C. and Minck, R.** 1974. Adjuvant activity in delayed hypersensitivity of the peptidic part of bacterial peptidoglycans. Nature **250**: 517-518.
8. **Furusawa, S., Hazama, S., Enokihara, H., Saito, K., Hirose, K., Komatsu, H. and Shishido, H.** 1977. Effect of a streptococcal preparation (OK-432) on granulopoiesis. Igaku no Ayumi (Tokyo). **103**: 215-217.
9. **Glasgow, L. A., Fischbach, J., Bryant, S. M. and Kern, E. R.** 1977. Immunomodulation of host resistance to experimental viral infections in mice: effects of *Corynebacterium acnes*, *Corynebacterium parvum*, and Bacillus Calmette-Guérin. J. Infect. Dis. **135**: 763-770.
10. **Kato, I., Kobayashi, S., Yokokura, T. and Mutai, M.** 1981. Antitumour activity of *Lactobacillus casei* in mice. Gann **72**: 517-523.

11. **Kondo, M., Kato, H., Takemura, S., Yoshikawa, T., Yokoe, N., Ikezaki, M. and Imanishi, H.** 1977. Activation of cellular immunity and complement system in immune-deficient aged subjects by streptococcal preparation OK-432 (Picibanil). Jpn. J. Clin. Oncol. **7** : 21-26.
12. **Matsubara, S., Suzuki, F. and Isida, N.** 1979. Induction of immune interferon in mice treated with bacterial immunopotentiator, OK-432. Cancer Immunol. Immunother. **6** : 41-45.
13. **McBride, W. H., Weir, D. M., Kay, A. B., Pearce, D. and Caldwell, J. R.** 1975. Activation of the classical and alternate pathways of complement by *Corynebacterium parvum*. Clin. exp. Immunol. **19** : 143-147.
14. **Saito, H., Tomioka, H. and Sato, K.** 1981. Enhanced resistance of *Lactobacillus* against *Listeria* infection in mice. Med. Biol. (Tokyo) **102** : 273-277.
15. **Saito, H., Watanabe, T. and Horikawa, Y.** 1981. Enhanced resistance of *Lactobacillus* against the opportunistic infection in mice. Med. Biol. (Tokyo) **102** : 309-314.
16. **Watanabe, T., Adachi, K., Horikawa, Y. Sato, K., and Saito, H.** 1981. Enhanced humoral antibody production in mice against influenza virus by streptococcal preparation OK-432. Microbiol. Immunol. **25** : 205-208.
17. **Wing, E. J.** 1981. Bacillus Calmette-Guérin (BCG) decreases resistance to *Listeria monocytogenes* infection in mice. Immunology **44** : 509-515.