Phagocytosis of Splenetic Neutrophils of Mice Enhanced by Orally Administered Peptidoglycan from *Bifidobacterium thermophilum*

Takashi SASAKI, Yasuo SAMEGAI, and Shigeo NAMIOKA
Zen-noh Institute of Animal Health, 7 Ohja-machi, Sakura, Chiba 285, Japan
(Received 2 February 1995/Accepted 14 September 1995)

ABSTRACT. A preparation of peptidoglycan (PG) of swine *Bifidobacterium thermophilum* was orally administered to SPF-BALB/C and ICR mice and its effect on phagocytosis splenetic neutrophils from PG administered mice was measured by chemiluminescent response (CL) and fluorometric analysis and the result was compared with that of non-treated mice. PG stimulated phagocytosis of neutrophils in a dose-dependent manner, whereas dosage exceeding the optimum concentration (500 μg) inhibited phagocytosis. The maximum effect on phagocytosis of neutrophils was observed at 3 days after administration of PG 500 μg. The result of fluorometric analysis was almost similar to that of CL. These results indicate that orally administered PG enhances the activity of the phagocytosis of splenetic neutrophils from mice. — KEY WORDS: Bifidobacterium, neutrophil, peptidoglycan.

J. Vet. Med. Sci. 58(1): 85–86, 1996

Bifidobacterium, the intestinal flora in humans and animals [10], has been reported to possess antitumor activity [21] and play roles in the various biological function especially to exclude the intestinal infections [11]. These effects were considered to be due to the enhancement of immunoactivities by Bifidobacterium: it has been proved that peptidoglycan (PG) of Bifidobacterium [18, 19] has a carcinostatic effect, and that muramyl dipeptide (MDP) purified from PG is the essential component for activating an immune response [2, 9, 12]. PG of *B. thermophilum* enhanced immunoresponse not only of swine [13, 17] but also of chickens [11], mice and guinea pig [12]. It has become clear that PG of *B. thermophilum* possesses the ability of immunopotentiation or sequential stimulator of nonspecific immune responses in a wide variety of hosts. The purpose of this study was to observe the enhancement of activity in phagocyte function of splenetic neutrophil of mice by the oral administration of PG from *B. thermophilum*.

The preparation method of PG precisely followed that of the previous study [12]. The final PG-preparation (1 m*l*) contained the cell wall digestion from 10^{12} native cells of *B. thermophilum* and had non LPS contamination, which was measured by ENDOSPECY ES-200 (Seikagaku Kogyo Co., Ltd., Tokyo).

Splenetic neutrophils of mice were prepared at 4×10^6 cells in 1 m*l* of MEM (Gibco Co., Ltd., U.S.A.) with 5 mM hepes buffer solution (5 mM HEPES-MEM). Phagocyte function of neutrophils was measured by chemiluminescence method with Biolumat LB9505 (Berthold, Germany) and fluorometric analysis with FACScan (BBL. Co., Ltd., U.S.A.). The results were expressed as chemiluminescence (CL) intensity and the number of dots of cytofluorograph. Data were analyzed statistically by Student's paired *t*-test.

The PG solution was prepared at concentrations of 50 μg/0.5 m*l*, 500 μg/0.5 m*l* and 5,000 μg/0.5 m*l* PBS and was administered directly via a canula to SPF-BALB/C mice. Sterile PBS was orally administered to the control group in the same manner. Administration of PG increased the CL response of neutrophils in mice, with the CL intensity of 500 μg PG administered mice being significantly higher than that of non-treated mice ($P<0.01$). The most effective PG dose for CL intensity was 500 μg/mouse of oral administration. In contrast, a high dose (5,000 μg/mouse) actually decreased the activity of CL response (Fig. 1).

CL intensity of splenetic neutrophils at 1, 3 and 7 days after single oral administration of 500 μg PG were significantly higher ($P<0.01$) in PG treated than in non-treated mice, with the value at 3 days post-treatment being particularly elevated. CL response increased at 1 day after treatment, and the difference at this time was also significant compared with that of non-treated control ($P<0.01$). CL intensity at 7 days after PG administration also showed significant difference against the control ($P<0.01$) (Fig. 2).

The fluorometric analysis showed that the results of splenetic neutrophils of 5 ICR mice which had been fed with 500 μg PG were 15,263 ± 225 and non-treated group were 13,969 ± 730. PG-treated group was significantly higher than those of non-treated group ($P<0.01$).

There have been many reports that *Lactobacillus bulgaricus* [4, 7, 8, 14], *Lactobacillus casei* [6], kefire grain [3], streptococcal agent [20], *Klebsiella pneumoniae* [1] and bifidobacteria [5] activated the phagocytosis of macrophages

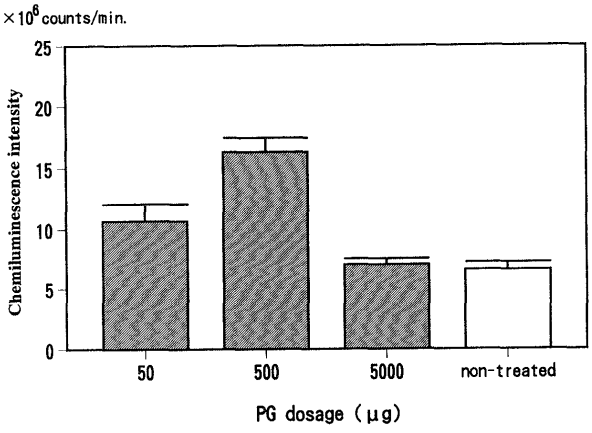


Fig. 1. Effect of PG dose on chemiluminescence intensity (CL intensity) of neutrophils of mice. The phagocytic activity was represented by CL intensity. Data are presented as mean ± standard deviation (S. D.). Five BALB/C mice were treated p.o. with 50 μg, 500 μg or 5,000 μg PG or PBS (non-treated).

NII-Electronic Library Service

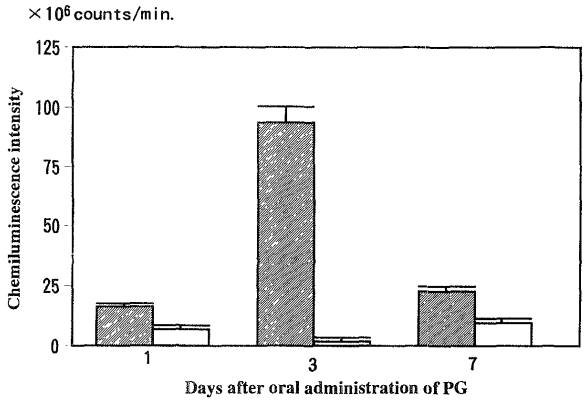


Fig. 2. Time course of the effect of PG administration on CL intensity of neutrophils of mice. The phagocytic activity was represented by CL intensity. Data are presented as mean ± standard deviation (S.D.). Five BALB/C mice were given PG 500 μg (hatched) or PBS (open).

while no details was clarified concerning the phagocytosis of neutrophils in these organism or agents.

In this study, the phagocytic activity was represented by CL response and fluorometric analysis. CL response was applied to the analysis of intra-cellular reaction of phagocytes and fluorometric assay was used to the analysis of the direct phagocytosis of fluorescein-labeled latex particle. We showed that oral administration of PG from *B. thermophilum* promoted the phagocytosis of splenetic neutrophils of mice in which there was a dose-dependent response to PG of the activity of neutrophils. However, when an excessive dose of PG was administered, the phagocytosis of neutrophils was suppressed. This result was the same as the findings reported with β-glycan [21].

We previously confirmed that oral administration of PG from *B. thermophilum* isolated from adult swine activates local immunity response in the small intestine of piglets [13, 18]. It was also found that the defense activity of mice against *Escherichia coli (E. coli)* infection was accelerated by PG treatment, and blast-formation of lymphocytes from the spleen was detected at a higher ratio with PG administration than that of non-treated mice [15]. It was considered that these mechanisms of the defense activity might be the enhancement of phagocytosis in mice with PG treatment. We proved, on the other hand, that the cytocidal activity of natural killer cells and cytotoxic T lymphocytes of mouse was enhanced by orally administered PG [16].

The phagocytic activity of neutrophils was increased 1 day after the oral administration of PG, reached a maximum at 3 days post-treatment, and then followed a decreasing trend. These results were consistent with those obtained from an *E. coli* inoculation study where the survival rate was improved 1 day after giving PG orally, becoming 100% on day 3 post-treatment, and then decreasing thereafter [15]. The results also correspond with those of a study on a lactobacilli preparation [4].

Our results have indicated that the oral administration of PG enhanced systemic immunoreaction, and that the defense activity takes place at the systemic level. We thought the activated neutrophils in the PG-orally administered mice eliminated *E. coli* quickly and strongly. As a result, the survival rate of mice might be vastly improved.

Taken together, the findings of this study indicated that the effect of the oral administration of PG reinforced phagocytosis of neutrophils and augmented the initial defense ability against infection. We suggest that the application of the oral administration of PG provides a useful method of prophylaxis against the invasion of bacteria, and that the management of domestic animals/livestock would greatly benefit from the addition of PG as the feed supply.

REFERENCES

1. El Abbouyi, A., Paul, J. L., Roch-Arveiller, M., Sarfati, G., Giroud, J. P., and Raichvarg, D. 1989. *Biol. Response Mod.* 8: 656–664.
2. Ellouz, F., Adam, A., Ciorbaru, R., and Lederer, E. 1974. *Biochem. Biophys. Res. Commun.* 59: 1317–1325.
3. Furukawa, N., Yokokawa, Y., Takahashi, T., and Yamanaka, Y. 1993. *Anim. Sci. Technol. (Jpn.)* 64: 60–67.
4. Gabriela, P., Elena, M. N. M., Alvrez, S., Oliver, G., and Holgado, A. A. P. R. 1986. *Infect. Immun.* 53: 404–410.
5. Kadooka, Y., Fujiwara, S., and Hirota, T. 1991. *Milchwissenschaft* 46: 626–630.
6. Kato, I., Yokokura, T., and Mutai, M. 1983. *Microbiol. Immunol.* 27: 611–618.
7. Khlopovskaia, E. I., Chureeva, L. N., Chernysheva, N. M., Kaplan, V. P., Ogarkov, V, I., and Teleshov, V. A. 1988. *Antibiot.Khimioter.* 33: 524–527.
8. Khlopovskaia, E. I., Chureeva, L. N., Chernysheva, N. M., Kaplan, V. P., Ogarkov, V. I., and Teleshov, V. A. 1988. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 6: 66–68.
9. Kotani, S., Watanabe, Y., Shimono, T.,Narita, T., Kato, K., Stewart-Till, D. E., Kinoshita, F., Yokogawa, K., Kawata, S., Shiba, T., Kusumoto, S., and Tarumi, T. 1975. *Zeitschrift für Immunitätsforschung* 14: 302–319.
10. Mitsuoka, T. 1969. *Zentralblatt fur Bakteriologie 1. Abt.Original B. Hygine* 210: 52–64.
11. Mitsuoka, T. 1990. *J. Int. Micobiol.* 6: 263–268.
12. Morishita, Y., and Mitsuoka, T. 1973. *Jpn. J. Microbiol.* 17: 181–187.
13. Namba, Y., Hidaka, Y., Taki, K., and Morimoto, T. 1981. *Infect. Immun.* 31: 580–583.
14. Namioka, S.,Kumeda, Y., Kawamoto, T., Wang, C. T., Namba, Y., and Murakami, K. 1982. *Br. Vet. J.* 138: 155–167.
15. Robert, L. O., Nathaniel, F. P., Apple, R. T., and William, C. C. Jr. 1986. *J. Infect. Dis.* 153: 1108–1118.
16. Sasaki, T., Fukami, S., and Namioka, S. 1994. *Jpn. J. Vet. Sci.* 56: 433–437.
17. Sasaki, T., Fukami, S., and Namioka, S. 1994. *Jpn. J. Vet. Sci.* 56: 1129–1133.
18. Sasaki, T., Maede, Y., and Namioka, S.1987. *Jpn. J. Vet. Sci.* 49: 235–243.
19. Sekine, K., Tatsuki, T., Watanabe, E., Toida, T., Saito, M., and Kawashima, T. 1990. *Bifidus* 3: 135–141.
20. Sekine, K., Toida, T., Saito, M., Kuboyama, M., Kawashima, T., and Hashimoto, Y. 1985. *Cancer Res.* 45: 1300–1307.
21. Sekine, K., Watanabe-Sekine, E., Ohta, J., Toida, T., Tatsuku, T., Kawashima, T., and Hashimoto, Y. 1994. *Bifidobacteria Microflora* 13: 65–77.

NII-Electronic Library Service