The basic study of alabinoxlan compound (MGN-3) on the activation of vital defence

Nobuyuki Sudo, Konen Tatoe, Naomi Koyama,
Hideyuki Kanna, Kenji Hirayama, and Chiharu Kubo

Introduction

Arabinoxylan (hereafter called MGN-3) is a plant polysaccharide processed food with a biophylactic action, which mainly contains polysaccharides such as hemicellulose and arabinoxylan and glycoprotein. MGN-3 is known to have various biophylactic actions such as *in vitro* NK cell activity stimulation and anti-HIV effect[1),2)]. In the present study, the vital defense activating effect of MGN-3 was studied in 3 animal experiments: Experiment 1 examined its effect on the survival in a lipopolysaccharide-induced lethal sepsis model, Experiment 2 studied the anti-stress effect on restraint stress, and Experiment 3 examined its effect on the survival of autoimmune disease-prone (NZB × NZW) F1 mice.

I. Methods

1. Experimental procedures

Experiment 1

In the experiment, BALB/c mice (male, 5-7 weeks old) were used. Twenty mg/kg and 200 mg/kg of MGN-3 (provided by Daiwa Pharmaceutical Co., Ltd.) were dissolved into 0.5 ml of PBS, and via an oral zonde, administered every other day for two weeks, in total seven times. Then 0.5 ml of PBS was administered orally in the same interval for the control group. Two hundred μg/mouse of LPS was administered intraperitoneally 12 hours after the final oral administration, and the conditions of the mice were observed over time. In another experiment, 100 μg/mouse of LPS was administered intraperitoneally in the MGN-3 group and control group, the mice were euthanized 0, 2, 4, 8 hr after the LPS administrations, and peripheral blood was collected from the heart. Serum was separated, and IL-6 and TNF were measured. IL-6 activity was measured using the B9 cell line [3)] and TNF activity was measured by bioassay in the WEHI164-13 cell line [4)].

Experiment 2

BALB/c mice (male, 5 weeks old) were fed foods containing 0%, 0.25%, and 0.5% of MGN-3 and subjected to restraint stress in a metal cage for 12 hours, as previously reported[5)]. The thymus and spleen were isolated before and after restraint to study the number of cells and lymphocyte subsets. The number of cells was counted using a hemocytometer and lymphocyte subsets were analyzed by flow cytometry (FACS caliber).

Experiment 3

Autoimmune disease-prone (NZB × NZW) F1 mice (female, 5 weeks old) were given the same foods as in Experiment 2 to observe body weight, proteinuria, and survival rate over time.

2. Statistical analysis

Measurements were expressed as means ± standard deviations. Survival was analyzed using the Kaplan-Meiyer method, and comparison between two groups was performed using Mantel-Cox tests. Others were analyzed by factorial ANOVA and then comparison was made between the two groups using unpaired *t* tests.

II. Results

1. Improvement of survival rates by MGN-3 in a lipopolysaccharide-induced lethal sepsis model

As shown in Figure 1, when 200 μg/mouse of LPS was administered, the survival rate significantly improved in groups where 20 mg/kg or 200 mg/kg of MGN-3 was administered everyday, compared with that in the control group (20 mg/kg MGN-3 group vs. control group, $p = 0.0456$; 200 mg/kg MGN-3 group vs. control group, $p = 0.0232$, by Mantel-Cox test). Also, when 100 μg/mouse of LPS was administered, all the mice survived in groups where 20 mg/kg or 200 mg/kg of MGN-3 was administered everyday, while 3 out of 10 mice died in the control group. Next, to establish the mechanism for improvement of the survival rate by MGN-3, blood concentrations of IL-6 and TNF were measured (Figure 2). In the experimental group where MGN-3 was administered, compared with the control group, the blood IL-6 level was significantly lower two hours after the administration of LPS (control group 702.9 ± 24.7 ng/ml, MGN-3 group 403.1 ± 59.6* ng/ml; $p < 0.01$); however, 8 hr after the administration, it significantly increased (control group 88.5 ± 50.0 ng/ml, MGN-3 group 441.0 ± 115.0* ng/ml; $p < 0.05$). Meanwhile, 4 hr after LPS administration, the blood TNF level significantly increased in the MGN-3 group compared with those in the control group (control group 492 ± 187, MGN-3 group 1816 ± 307* pg/ml; $p < 0.01$).

2. Study on anti-stress effect by MGN-3

Subsequently, we studied the possible inhibitory effect of MGN-3 in lymphocyte reduction in thymus and spleen caused by restraint stress, one of the non-inflammatory stresses. As shown in Figure 3, in the 0.5% MGN-3 group, the number of thymus cells decreased significantly by MGN-3 intake alone. However, in either group, the number of cells in thymus and spleen significantly decreased by loading the restraint stress. Meanwhile, for the lymphocyte subset, although the characteristic fluctuation of subset accompanied by stress loading was observed[6], there was no difference between the MGN-3 group and control group.

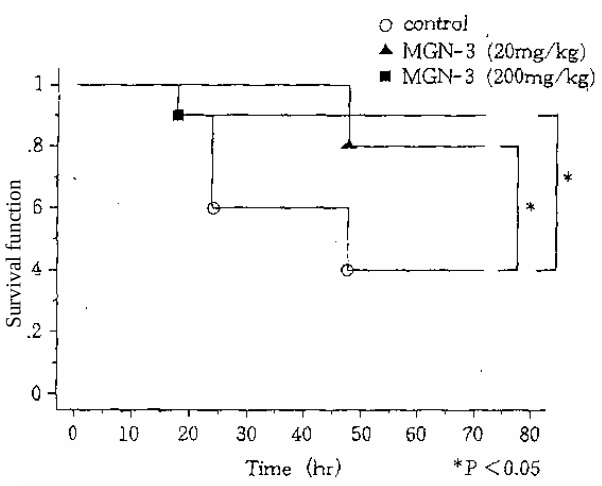

Figure 1 Improvement of survival rates by arabinoxylan

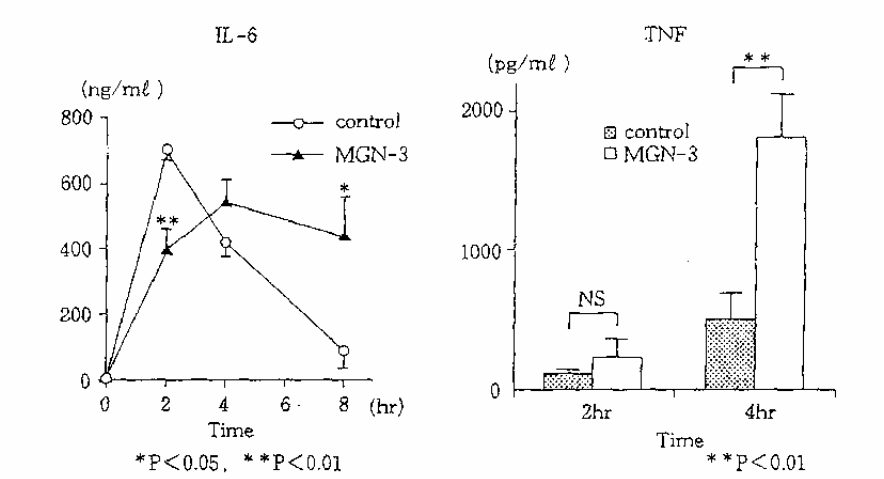

Figure 2 Changes in blood levels of IL-6 and TNF

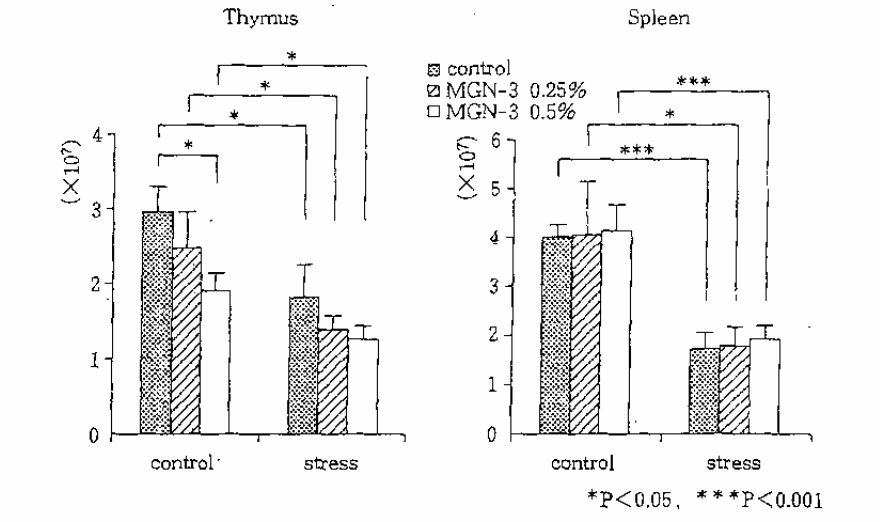

Figure 3 Changes in numbers of cells in thymus and spleen

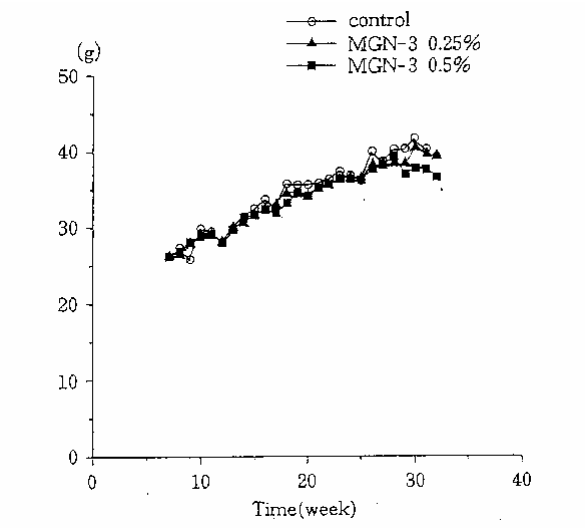

Figure 4 Changes in body weight

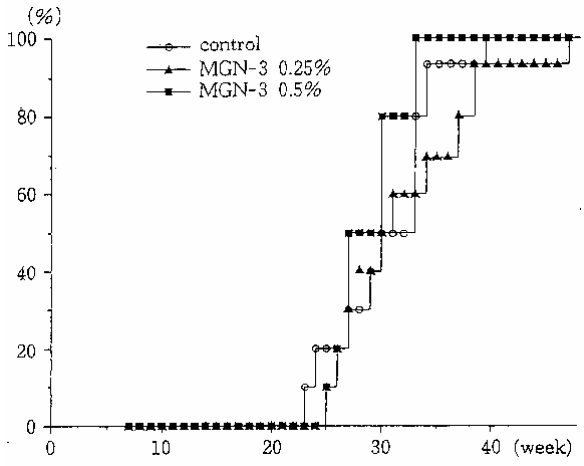

Figure 5 Time-dependent changes in rate of animals with proteinuria

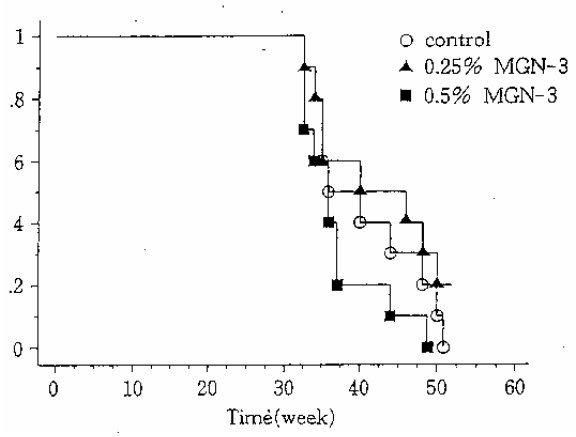

Figure 6 Effect of arabinoxylan on survival rate for (NZB × BZW) F1 mice

3. The effect of MGN-3 on clinical signs in mice with collagen vascular diseases

As shown in Figure 4, changes in body weight were similar for the MGN-3 and control groups. Proteinuria occurred, in increasing order of frequency, in the 0.5% MGN-3, control, and 0.25% MGN-3 groups, and was positive in all mice at 46 weeks (Figure 5). There was no significant difference in the death rate between the 3 groups, but the 0.5 MGN-3 group tended to have a higher death rate than the 0.25 MGN-3 group (Figure 6, 0.5% MGN-3 vs 0.25% MGN-3, p=0.0769).

III Discussion

In the lipopolysaccharide-induced lethal sepsis model, a large amount of inflammatory cytokines (IL-1, IL-6, and TNF-α) released from the whole body reticuloendothelial system induces multiple organ failure, which leads to death of the animal. In this study, MGN-3 significantly improved the survival rate. One hypothetical explanation for this was that MGN-3 may have inhibited the production of tissue-damaging cytokines by macrophages, and blocked reactions leading to damage to target cells. However, although the blood IL-6 level at 2 hours was decreased by MGN-3 ingestion, the level of TNF that actually causes tissue damage was significantly increased in the MGN-3 groups, suggesting that it is unlikely that MGN-3 inhibited the production of inflammatory cytokines. TNF-α is a critical factor determining death or survival in this model. The *in vivo* effect of TNF-α is known to be controlled by competitive soluble TNF-receptors[7]. The mechanism of survival improvement by MGN-3 is as yet unknown. Plausible theories for the mechanism are a contribution of cytokines with a competitive effect on TNF and the decreased sensitivity of target cells to TNF (for example, down-regulation of the receptors).

Non-inflammatory stress (restraint stress) decreases the numbers of cells in the thymus and spleen. As similar decreases in the numbers of cells were observed in the MGN-3 groups subjected to restraint stress, it can be concluded that MGN-3 had no anti-stress effect on the thymus or spleen. However, MGN-3 decreased the number of cells in the thymus. This, together with its mechanism of action, remains to be studied.

SLE-prone (NZB × NZW) F1 mice will develop anti-DNA antibody in the blood and proteinuria with the passage of time, and prematurely die of renal failure. In the present study, proteinuria appeared earlier and the death rate was increased in the 0.5% MGN-3 group compared with the 0.25% MGN-3 group, although the differences were not significant. Administration of MGN-3 at large doses needs careful consideration. IFN-γ is known to worsen renal failure and increase the death rate in (NZB × NZW) F1 mice[8]. It has been reported that MGN-3 promotes the *in vitro* production of IFN-γ by NK cells[1]. Thus, administration of MGN-3 in large doses involves the risk of IFN-γ-induced renal damage. In conclusion, it was suggested that MGN-3 may enhance Th1 type immune reactions.

Conclusion

The plant polysaccharide processed food arabinoxylan was studied for its effect on the vital defense activation in animals. The following results were obtained:

1) The food improved survival rates in an LPS-induced lethal sepsis model.

2) It had no anti-stress effect on restraint-decreased cells in the thymus and spleen.

3) It did not prolong the survival in a mouse model of autoimmune disease.

References

1) Ghoneum, M.: Enhancement of human natural killer cell activity by modified arabinoxylan from Rice Bran (MGN-3). Int. J. Immunotherapy, 14: 89-99, 1998.

2) Ghoneum, M.: Anti-HIV activity in vitro of MGN-3, an activated arabinoxylan from Rice Bran. Biochem. Biophys. Res. Commun., 243: 25-29, 1998.

3) Aarden, L. A., DeGroot, E. R - , Schaap, O.L. et al. Production of hybridoma growth factor by human monocytes. Eur. J. Immunol., 17: 1411-1416, 1987.

4) Espevik, T. and Nissen-Meyer, J.: A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J. Immunol. Methods, 95: 99-105, 1988.

5) Fukui, Y., Sudo, N., Yu, X. N. et al.: The restraint stress-induced reduction in lymphocyte cell number in lymphoid organs correlates with the suppression of in vivo antibody production. J. Neuroimmunol., 79: 211-217, 1997.

6) Sudo, N., Yu, X. N Sogawa, H Kubo, C.: Restraint stress causes tissue-specific changes in the immune cell distribution. Neuroimmunomodulation, 4: 113-119, 1997.

7) Kuhns, D. B Long Priel, D. A Gallin, J. I. et al. Endotoxin and IL-1 hyporesponsiveness in a patient with recurrent bacterial infections. J. Immunol., 158: 3959-3964, 1997.

8) Haas, C., Ryffel, B Le Hir, M.: IFN-γ receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB×NZW) F1 mice. J. Immunol., 160: 3713-3718, 1998.