

Supplementation of Elderly Japanese Men and Women with Fucoidan from Seaweed Increases Immune Responses to Seasonal Influenza Vaccination^{1,2}

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Abstract

The elderly are known to have an inadequate immune response to influenza vaccine. Mekabu fucoidan (MF), a sulfated polysaccharide extracted from seaweed, was previously shown to have an immunomodulatory effect. We therefore investigated antibody production after influenza vaccination in elderly Japanese men and women with and without oral MF intake. A randomized, placebo-controlled, double-blind study was conducted with 70 volunteers >60 y of age. They were randomly assigned to 1 of 2 groups, consuming either MF (300 mg/d) or placebo for 4 wk, and then given a trivalent seasonal influenza vaccine. Serum was sampled at 5 and 20 wk after vaccination to measure the hemagglutination inhibition titer and natural killer cell activity. The MF group had higher antibody titers against all 3 strains contained in the seasonal influenza virus vaccine than the placebo group. Titers against the B/Brisbane/60/2008 (B) strain increased substantially more in the MF group than in the placebo group over the product consumption period. The immune response against B antigen met the European Union Licensure criteria regarding the geometric mean titer ratio in the MF group (2.4), but not in the placebo group (1.7). In the MF group, natural killer cell activity tended to increase from baseline 9 wk after MF intake ($P = 0.08$). However, in the placebo group no substantial increase was noted at 9 wk, and the activity decreased substantially from 9 to 24 wk. In the immunocompromised elderly, MF intake increased antibody production after vaccination, possibly preventing influenza epidemics. *J. Nutr.* 143: 1794–1798, 2013.

Introduction

Japanese were reported to have the longest life expectancy in the world (1). Seaweed, a food material characteristic of Japanese cuisine, has been speculated to have a substantial influence on life expectancy, because several reports have suggested positive effects of seaweed on health. Noting that relatively few patients had herpes in Japan, Cooper et al. (2) conducted a study in patients with herpes. They reported that persons who consumed water extract of wakame (*Undaria pinnatifida*), a seaweed often consumed by the Japanese, experienced improvement in herpes symptoms.

Mekabu fucoidan (MF)⁷, a sulfated polysaccharide extracted from the mekabu, the sporophylls of the edible seaweed (*U. pinnatifida*) (3) and long used as a traditional food in Japan, has attracted

attention as an agent that enhances immune function because various fucoidans reportedly have physiological activities such as antiviral (3–6), anti-inflammatory, anti-angiogenic, anticoagulant (7), and antitumor effects (8). For virus infection in mice, oral administration of MF increased neutralizing antibody production in the mucosa and blood and inhibited viral growth. MF was also proved to enhance immune function through mechanisms involving natural killer (NK) cell activation and increased production of IFN- γ and IL-12 in animal studies (9). The WHO announced a new influenza pandemic on 11 June 2009 (10). Three influenza pandemics (Spanish, Asian, and Hong Kong) in the 20th century, and more recently the extremely pathogenic avian influenza (H5N1) with high lethality reported in Hong Kong in 1997, indicate that humans have long been facing influenza threats (11). The emergence of new influenza viruses and human epidemics is now recognized as a serious global health problem.

In the United States, influenza-associated deaths have increased rapidly in the growing elderly population in recent years (12). As a preventive measure, vaccination effectively attenuates

¹ Project was self-funded. This trial was registered at UMIN Clinical Trials Registry as UMIN000006394. This is a free access article, distributed under terms (<http://www.nutrition.org/publications/guidelines-and-policies/license/>) that permit unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

² Author disclosures: H. Negishi, M. Mori, H. Mori, and Y. Yamori, no conflicts of interest.

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⁷ Abbreviations used: B, B/Brisbane/60/2008; CHMP, European Committee for Medical Products for Human Use; GMT, geometric mean titers; H1N1, A/Brisbane/59/2007; H3N2, A/Uruguay/716/2007; MF, mekabu fucoidan; NK, natural killer.

symptoms and reduces the death rate from influenza (13,14). However, antibody production is known to be lower in the elderly than in younger people (15,16). Therefore, a strategy to enhance antibody production after vaccination is needed for influenza prevention in the elderly. In the present study, we evaluated the effect of orally administered MF on the immunogenicity of influenza vaccine and NK cell activity, which are measures of immune function in the elderly, whose antibody production is generally attenuated.

Participants and Methods

Participants. From 1 October 2009 to 16 May 2010, we conducted a stratified randomized, placebo-controlled, double-blind, single-center trial at a nursing home in Osaka, Japan. The participants were male and female volunteers >60 y of age, living in the nursing home. None had autoimmune diseases or cancer, and none used immunosuppressive drugs. All were selected for this study by an attending physician at the facility according to predetermined criteria. This study was conducted in accordance with the Ethical Guidelines for Epidemiological Research established by the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labor and Welfare of Japan, and in accordance with the Declaration of Helsinki. The trial protocol and all relevant documents were approved by 2 ethics committees (at Mukogawa Women's University and the Social Welfare Corporation Toyonaka Aiwakai). Written informed consent was obtained from all potential participants.

Vaccines. All volunteers were vaccinated against seasonal influenza with the same batch of split influenza virus inactivated vaccine, using influenza HA vaccine KAKETSUKEN TF (2009–2010 season), by subcutaneous injection of 0.5 mL into the upper arm. Selected for the 2009–2010 season by the Ministry of Health, Labor, and Welfare in Japan, this seasonal vaccine was a mixture of the following 3 viral strains: A/Brisbane/59/2007, A/Uruguay/716/2007, and B/Brisbane/60/2008. A 0.5-mL dose contained 30 μ g each of hemagglutinin of the individual viral strains.

Study products. Participants in the MF group received granules containing 300 mg of MF (Riken Mekabu Fucoidan, Riken Vitamin) and 300 mg of indigestible dextrin (Fibersol-2, Matsutani Chemical Industry), and those in the placebo group received granules containing only 600 mg of indigestible dextrin. A trace amount of dye was added to the P diet to make the appearance similar to that of MF. Granules of indigestible dextrin with and without MF were prepared by fluidized bed granulation and then packaged. These granules were mixed into lunches and consumed daily. The procedure for isolating fucoidans from sporophylls of *U. pinnatifida* has been described previously (3).

Randomization. Participants were randomly assigned at a 1:1 ratio to either the MF or the placebo group. They received 0.5 mL of seasonal influenza vaccine. From 1 mo before vaccination until the end of the study, MF-group participants consumed granules containing MF plus indigestible dextrin daily, and placebo group participants consumed granules containing dextrin alone daily. The participants were assisted by nurses when taking meals so that the intake of MF and the placebo was confirmed and recorded by the nurses.

Assignments were performed with SPSS for Windows 15.0J by facility staff not involved in any other statistical analysis. Participants were stratified by age, sex, care level, NK cell activity, influenza antibody titer, and protein in baseline blood samples. In the randomization table prepared at the facility, there were no substantial differences in the means of these items between the 2 groups, as was confirmed by the person responsible for random allocation. All data were managed using identification numbers and stored separately from personal information in a sealed box, which was opened only after the intervention study.

Procedures. For measuring antibody titers and NK cell activities, blood was collected from individual participants at baseline (before vaccination, 4 wk before study diet intake) and then at 5 and 20 wk after

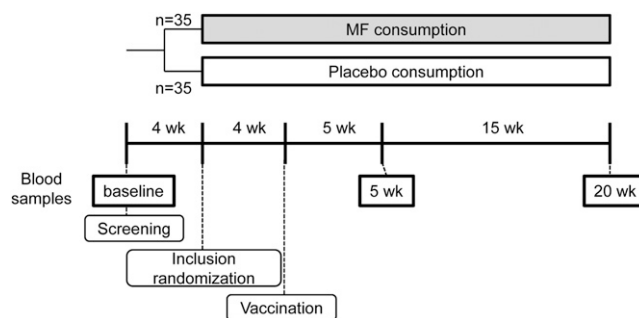


FIGURE 1 Study design. An initial screening visit was conducted 4 wk before enrollment and randomization. At randomization, volunteers started to take mekabu fucoidan or placebo. Vaccination was carried out 4 wk after randomization. Blood samples were collected at 5 and 20 wk after vaccination. Study product consumption lasted 24 wk. MF, mekabu fucoidan.

vaccination (after 9 and 24 wk of study diet intake) (Fig. 1). Serum antibody titers against the viral strains of the vaccine were measured by hemagglutination inhibition with chicken red blood cells, following standard procedures (17). Before assays were performed, all serum samples were treated overnight with a receptor-destroying enzyme. Hemagglutinin inhibition titers against the vaccine seed virus were obtained from Kyowa Synthetic Medical Laboratory. To assess the production of antibodies, we used the European Committee for Medical Products for Human Use (CHMP) evaluation criteria for seasonal influenza vaccine, which was developed for the licensing of influenza vaccination (13). Because a blood sample taken ~3 wk after vaccination was recommended for evaluation, we evaluated blood samples taken 5 wk after vaccination. The CHMP evaluation used for adult participants >60 y is as follows, and at least 1 of the assessments should meet the requirements: 1) the number of seroconversions or substantial (i.e., >4-fold) increase in hemagglutinin inhibition antibody titers should be >30%, 2) the ratio of geometric mean titer (GMT) after and before vaccination (mean geometric increase) should be >2-fold, and 3) the proportion of participants achieving a hemagglutinin inhibition titer >40 should be >60%. NK cell activity was assayed by SRL using the K-562 cell line labeled with ^{51}Cr as target cells and peripheral blood mononuclear cells isolated from blood as effector cells. At the time of blood sampling, a physician interviewed and examined the participants. Temperature and treatment compliance were documented using the participants' medical records.

Statistical analysis. Our objective was to evaluate immune responses to the vaccine 5 and 20 wk after vaccination. To test time and treatment and their interaction (i.e., time \times treatment effects), a 2-factor ANOVA for repeated measures containing 3 time points and 2 groups was used. Comparisons within groups were analyzed using 1-factor ANOVA for repeated measures with Tukey-Kramer multiple comparisons. Comparisons between groups at the same time point were analyzed using unpaired *t* tests. All reported *P* values are 2-sided, and *P* < 0.05 was considered substantial. We used SPSS (version 11.0) and Microsoft Excel software (version 2003).

For the purpose of calculation, hemagglutinin inhibition titers below 10, the limit of detection, were assigned an arbitrary value of 5. The hemagglutinin inhibition titer was transformed into \log_{10} titers for calculation of the GMT and statistical analysis. The hemagglutinin inhibition endpoints were based on criteria established by CHMP for seasonal influenza vaccines for elderly people (>60 y of age) (13).

Results

Recruitment. Participants who did not provide consent were excluded from the 79 volunteers. The remaining 70 elderly people were randomly assigned to 1 of 2 groups with stratification by age, sex, care level, and hematological test results (Fig. 2). There were no differences in age or male-to-female ratio between the 2 groups (Table 1). Three participants

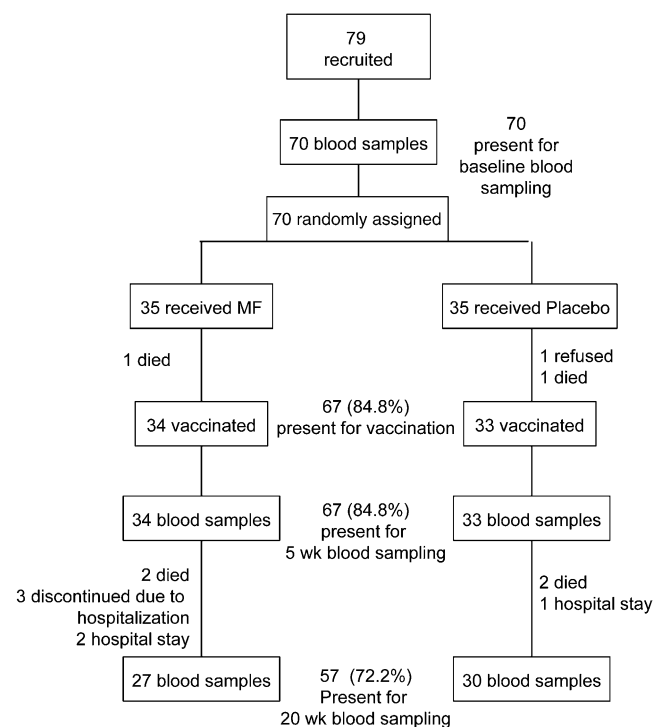


FIGURE 2 Profile of elderly participants in the trial at recruitment and randomization, and reasons for losses to follow-up at different stages of the trial in the mekabu fucoidan group and the placebo group. MF, mekabu fucoidan.

in the MF group and 2 in the placebo group died of senility or illness before completion of the study. In the placebo group, 1 person refused to undergo testing, and another discontinued the study diet because of hospitalization. These participants were excluded from the analyses. From 5 to 20 wk after vaccination, 1 person in MF group, who had difficulty swallowing because of senility, and 4 who were hospitalized, were unable to continue the study. In the placebo group, 1 person dropped out because of hospitalization. Data on these participants at 20 wk were excluded. The reasons for hospitalization (fracture, head injury, jaundice, and bladder tumor) were considered by the attending physician to be unrelated to the study diet. Deaths were also considered to be unrelated to the study diet. The data from the participants who died were excluded from the analyses. No participants experienced acute allergic reactions to the vaccinations or the study diet. Throughout the study period, no participants or facility personnel were infected with influenza viruses.

Increased antibody responses in the MF group. Results of measuring hemagglutination inhibition titers were obtained (Fig. 3). At baseline, there were no differences between the MF and the placebo groups in antibody titers against the 3 vaccine strains. As expected, 5 wk after vaccination, antibody concentrations were higher than baseline in both groups for all strains. Antibody concentrations for all 3 strains were higher in the MF group than in the placebo group. MF consumption increased B strain antibody titers ($P = 0.038$), with differences between the groups at 5 wk ($P = 0.038$) and 20 wk ($P = 0.047$). The high antibody titers were maintained over 20 wk after vaccination in the MF group.

Evaluation according to the CHMP evaluation. At 5 wk after vaccination, the MF group in all participants had higher seroprotection against all vaccine strains than the placebo group (Table 2). For the H1N1 virus, the CHMP evaluation criteria were not met in the MF and placebo groups. For the H3N2 virus, however, the 2 variables of the GMT and the 2 of seroprotection met the criteria in both groups. In contrast, the GMT ratio for B/Brisbane/60/2008 (2.4 in the MF group and 1.7 in the placebo group) met the evaluation criteria only in the MF group.

Participants were stratified for evaluation by prevaccination antibody titers (<40) against each viral vaccine strain (Table 2). An antibody titer

of 1:40 or higher is generally considered to be protective against a virus strain (13,17). In the case of antibody titer <40, the MF group had higher GMT ratios against all vaccine strains at 5 wk than the placebo group. In individuals with antibody titers <40, the GMT ratios against all vaccine strains in the MF group were >2-fold (2.8 for B, 3.4 for H3N2, 2.1 for H1N1), meeting the CHMP criteria. In the placebo group, the criteria were met only for H3N2 (GMT ratio, 2.5). For all vaccine strains, the seroconversion rate and seroprotection at 5 wk were higher in the MF group than in the placebo group.

Enhancement of NK cell activity. In the MF group, NK cell activity tended to increase ($P = 0.08$) from baseline to 9 wk after the intake of the study product. The activity at 24 wk was not substantially different from that at 9 wk and at baseline (Table 3). In the placebo group no substantial increase was noted at 9 wk from baseline, and the activity at 24 wk substantially decreased from that at 9 wk ($P = 0.006$).

Discussion

The present study showed MF intake to enhance immune responses to seasonal influenza vaccine in the elderly. Based on the CHMP criteria, no enhancement of the responses was observed against B (B/Brisbane/60/2008) antigen in the placebo group. In the MF group, however, the responses were sufficiently enhanced to meet the criteria. Increasing antibody production is important for preventing infections and serious conditions such as pneumonia in the immunocompromised elderly, now a growing population in developed countries.

MF is a fucose-rich sulfated polysaccharide present in seaweed. Continuous oral administration of MF was reported to inhibit the growth of tumor cells in mice, increase NK cell activity, and augment the production of the related cytokines, such as IFN- γ and IL-12 (8,9). Antiviral effects of MF on influenza and herpes viruses were also reported: MF (5 mg) given orally enhanced antibody production, inhibited viral growth, enhanced antibody production, and relieved symptoms compared with a placebo in Balb/c mice infected with H1N1 influenza virus (4). These benefits of MF are provided partly by acting on T-cells, because their activity decreases with age along with NK cell activity (18,19). As T-cell functions decrease, so do the functions of B cells, which are directed by T-cells. Bernstein et al. (20) demonstrated that after influenza vaccination the production of IFN- γ was substantially lower in the elderly than in younger adults. IFN- γ production and cytotoxic T-lymphocyte activity were enhanced by oral MF administration in herpes-infected mice (5). These findings in animals support the present findings that MF increases antibody production and NK cell activity, probably by activating T-cell functions in the elderly.

Our study is the first to demonstrate the immunostimulatory effect of MF in humans. The results confirmed the applicability to humans of the findings, just cited, of antibody titer elevation

TABLE 1 Age and sex distribution of elderly participants in the mekabu fucoidan and placebo group

	Mekabu fucoidan group	Placebo group
Total randomly assigned, <i>n</i>	35	35
Age, <i>y</i>		
Mean \pm SD	86.6 \pm 7.7	87.34 \pm 8.3
Range	70–98	67–102
Median	89	91
Sex, <i>n</i> (% of total)		
Female	32 (91.4)	32 (91.4)
Male	3 (8.6)	3 (8.6)

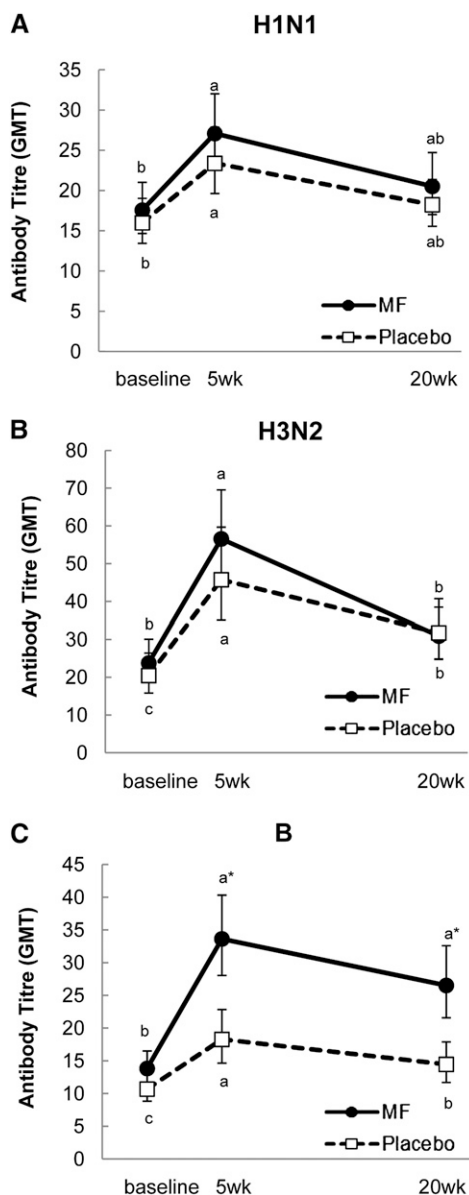


FIGURE 3 Hemagglutination inhibition antibody response in elderly participants given MF or placebo. Antibody titers were measured in response to A/Brisbane/59/2007 (H1N1) (A), A/Uruguay/716/2007 (H3N2) (B), and B/Brisbane/60/2008 (B) (C). Values are geometric mean titers \pm geometric SEMs, $n = 27$ – 32 . Within a group, means without a common letter differ, $P < 0.05$. *Different from placebo at that time: $P < 0.05$. There was a substantial product \times time interaction on B strain antibody titers ($P < 0.05$, repeated-measures ANOVA) and a substantial effect of time on all strains (H3N2 and B, $P < 0.001$; H1N1, $P < 0.01$, repeated-measures ANOVA). GMT, geometric mean titer; MF, mekabu fucoidan.

by MF against herpes and influenza vaccines (5,6). MF is derived from the widely used edible seaweed “wakame,” one of various seaweed types traditionally eaten in Japan, where the average daily consumption is 14.5 g per adult (21). Cooper et al. (2) reported that persons consuming wakame water extract experienced improvement in herpes symptoms.

Adjuvant treatments have been developed with the aim of enhancing vaccination efficacy. These treatments are designed to induce high concentrations of antibodies in response to small amounts of antigens, in preparation for when vaccines are needed for a new worldwide influenza pandemic. Although the usefulness

of Microfluidized Emulsion 59 (Novartis Vaccines and Diagnostics) adjuvant for the H5N3 virus has been reported (22), vitamin E, a component of this adjuvant emulsion, might enhance antibody production through T-cell activation (23), because immunomodulatory effects of vitamin E, such as increased antibody titers after hepatitis B or pneumococcal vaccination in the elderly, were observed with intake of 200 mg of vitamin E for 4 mo (24). Intake of zinc and selenium was also reported to increase antibody titers after influenza vaccination (25), indicating that not only adjuvant compounds but also nutrients might exert an immunomodulatory effect of enhancing antibody production after vaccination. In addition to nutrients, some studies report the contribution of probiotics in enhancing the effects of influenza vaccination. For example, in a human clinical trial, Olivares et al. (26) demonstrated that oral administration of lactic acid bacteria induces an increase in antigen-specific immunoglobulin A.

In the present study, MF intake substantially increased antibody titers in individuals with titers < 40 . This efficacy was particularly evident in the stratum in need of increased antibody concentrations after vaccination. The clinical efficacy of using dietary constituents in a coadjuvant approach to viral infections has been demonstrated, and it has been reported that dietary constituents increase antibodies reasonably for each person. We noted neither allergic nor undesirable excessive immune responses even in persons with antibodies before vaccination.

In this study, efficacy was most evident against B (B/Brisbane/60/2008) antigen among the 3 viral vaccine strains. This is partly explained by the influenza A strains in the 2008–2009 season being antigenically identical to those in the 2009–2010 season. Consequently, antibody titers against the 2 influenza A strains at baseline were higher than those against B antigen, and there were fewer persons with an antibody titer < 10 , and these are the persons that would most likely benefit from MF (14/65 persons for A/H1N1, 19/65 for A/H3N2, 30/65 for B). This background may account for the lack of appreciable between-group differences for influenza A compared with influenza B.

TABLE 2 Stratified analysis by using CHMP criteria at 5 wk after vaccination in elderly participants given MF or placebo in all participants and in stratified participants by prevaccination antibody titers (< 40) against all viral vaccine strains¹

	All participants ²		Stratified participants ³	
	Placebo	MF	Placebo	MF
Mean geometric increase, ratio ⁴				
H1N1	1.5	1.5	1.6	2.1 ⁵
H3N2	2.2 ⁵	2.4 ⁵	2.5 ⁵	3.4 ⁵
B	1.7	2.4 ⁵	1.7	2.8 ⁵
Seroconversion rate, %				
H1N1	6.5	15.6	8	21.7
H3N2	29	21.9	31.6 ⁵	35.3 ⁵
B	6.5	18.8	8.7	25
Seroprotection, %				
H1N1	29	40.6	16	30.4
H3N2	61.3 ⁵	71.9 ⁵	36.8	47.1
B	41.9	56.3	21.7	41.7

¹ B, B/Brisbane/60/2008; CHMP, European Committee for Medical Products for Human Use; H1N1, A/Brisbane/59/2007; H3N2, A/Uruguay/716/2007; MF, mekabu fucoidan.

² Baseline and 5 wk: MF, $n = 32$; placebo, $n = 31$.

³ Baseline and 5 wk: MF, $n = 23$, H1N1; $n = 17$, H3N2; $n = 24$, B, and placebo, $n = 25$, H1N1; $n = 19$, H3N2; $n = 23$, B.

⁴ Mean geometric increase, ratio of geometric mean titer after and before vaccination.

⁵ Met CHMP licensing criteria.

TABLE 3 Changes in NK cell activity in elderly participants in mekabu fucoidan or placebo group¹

	Group			
	Mekabu fucoidan		Placebo	
	NK cell activity	Cases, <i>n</i>	NK cell activity	Cases, <i>n</i>
Baseline	41.2 ± 14.0 ^a	32	41.7 ± 14.2 ^{ab}	31
9 wk	44.9 ± 13.1 ^a	32	43.5 ± 12.6 ^a	31
24 wk	41.9 ± 14.8 ^a	27	38.8 ± 14.7 ^b	30

¹ Values are means ± SEs. Means without a common letter differ, $P < 0.01$. There was a substantial effect of time ($P < 0.05$, repeated-measures ANOVA). Throughout the treatment period, there was no substantial product effect or product by time interaction ($P > 0.05$, repeated-measures ANOVA). NK, natural killer.

NK cell activity was reported to decrease in the elderly (19). In the present study, NK cell activity decreased in the placebo group 24 wk after the intake of the placebo product. However, in the MF group NK cell activity tended to increase at 9 wk after the intake of the product, and the activity did not substantially decrease further by 24 wk after intake, whereas the activity substantially decreased in the placebo group. MF was reported to augment NK cell activity in immunosuppressed mice treated by 5-fluorouracil (5), and the present study suggested a beneficial effect of MF on the aging-related suppression of NK cell activity. Increase in antibody titers (acquired immunity) and the maintenance of NK cell activity, or the tendency for this activity to increase (natural immunity), appear to result from the activation of overall immune functions by MF intake.

Our study showed a possible adjunctive role of MF in antibody production in the elderly, although further studies on the underlying immunomodulatory mechanisms are needed. It is hoped that the popular seaweeds eaten daily in Japan, though almost unknown around the world as a nutritional source, will be consumed outside Japan for possible immunopotential and for attenuating the burden of infectious diseases in the elderly.

Acknowledgments

Y.Y. and H.M. designed the research; H.N., H.M., and H.N.'s staff conducted research; M.M. analyzed data; H.N. and Y.Y. wrote the paper; Y.Y. had primary responsibility for the final content. All authors read and approved the final manuscript.

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