



Article

Foods Containing *Pantoea agglomerans* LPS Reduce Eye-Nose Allergies—A Double-Blind, Placebo-Controlled, Randomized, Parallel-Group Comparative Pilot Study

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Abstract: In this study, the effects of foods containing lipopolysaccharide (LPS) from *Pantoea agglomerans* (LPSP) on immunity were preliminarily investigated using a double-blind, placebo-controlled, randomized, parallel-group comparative study design. Thirty healthy subjects aged ≥ 20 years (four males and twenty-six females; mean age 49 ± 9.2 years) were randomly assigned to the LPS-containing food group (488 $\mu\text{g/day}$; LPS) or placebo group. Each food was consumed for 8 weeks, and a subjective survey of cold symptoms (Wisconsin Upper Respiratory Symptom Questionnaire) and allergic symptoms of the eyes and nose were conducted. Phagocytic capacity and lymphocyte counts were measured as indicators of immune function. There were no significant between-group differences with respect to any of the investigated items. On sub-group analysis of eye-nose allergy symptom score, confined only to subjects who reported eye-nose allergic symptoms in previous years, the LPS group showed a trend toward milder symptoms compared to the placebo group. In addition, when the symptom scores were compared only for subjects who developed eye-nose allergies during the study period, the LPS group showed significantly lower overall scores and eye symptom scores compared to the placebo group. These results suggest that the consumption of LPS-containing foods may alleviate or prevent eye-nose allergies. There were no statistically predominant changes in hematology and blood biochemistry tests, indicating that continued consumption of LPS-containing foods is safe. (UMIN000046154).

Keywords: *Pantoea agglomerans*; lipopolysaccharide; immunity; allergy; human clinical trial; double-blind; functional food



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1. Introduction

Lipopolysaccharide (LPS) is a cell wall component of Gram-negative bacteria. Human cells have a receptor for this bacterial component called toll-like receptor 4 (TLR4). Upon invasion of blood vessels or organs by Gram-negative bacteria, the binding of LPS with TLR4 enables the blood monocytes and macrophages [1] to recognize the bacterial infection, triggering the inflammatory cascade to eliminate the invading bacteria. Because of this action, LPS is known as an inflammation-inducing endotoxin.

However, LPS from Gram-negative bacteria is ubiquitous in the environment, including air, soil, and plant surfaces, and does not cause inflammation after oral ingestion or dermal contact. For example, a group of cells in the intestinal tract or skin expressing TLR4 do not secrete inflammatory cytokines in response to LPS stimulation [2,3]. However, these cells are not totally unresponsive, and antimicrobial substances have been reported to be induced in the intestinal paneth cells and skin keratinocytes [4,5]. In addition, regulatory T cells (Tregs), which are inhibitory regulators of inflammation, are present in the skin, and it has been reported that LPS stimulation induces the proliferation of Tregs, which further enhances their anti-inflammatory function [6].

In an in vivo study, oral administration of LPS significantly reduced the fatality rate due to *Toxoplasma* infection in rabbits [7]; in addition, oral administration of LPS in mice was found to increase IgA antibody induction by sublingual influenza vaccine and more strongly suppressed the fatality rate due to infection than the injectable influenza vaccine in experiments using mice [8]. Furthermore, an epidemiological study by Braun–Fahrländer et al. found that children living in rural areas, where there are more opportunities for LPS exposure, are less likely to develop allergies than those living in urban areas [9]. In a double-blind clinical study by Kamijo et al., nasal discomfort caused by cedar pollen was reduced by foods containing acetic acid bacteria extract with LPS content [10].

These results suggest that orally and transdermally administered LPS may help enhance and normalize immune function without inducing inflammation. Therefore, in the present study, we investigated the effects of a continuous intake of LPS-containing foods on the immune function and the related physical symptoms in healthy adults.

The safety and efficacy of LPS for oral or transdermal intake have been best studied with LPS from *Pantoea agglomerans* (LPSP) [11]. A food ingredient (Somacy-FP100) containing LPSP as an active ingredient has already been used in health foods and other products since 2007 without adverse events. The standard intake of Somacy-FP100 is 1 mg/kg/day (10 µg/kg/day as LPSP), but a 90-day repeated-dose study in rats conducted by the Organization for Economic Co-operation and Development confirmed a high non-observed adverse effect level of 4500 mg/kg/day of Somacy-FP100 [12]. Therefore, a test food containing LPSP as an active ingredient was used in this study.

In this preliminary investigation of the effects on immunity, as physical outcomes, a subjective survey of cold symptoms (using the Wisconsin Upper Respiratory Symptom Questionnaire) and a subjective survey of eye–nose allergic symptoms (a type of allergy) were conducted. In addition, phagocytosis, as a marker of innate immunity, and changes in lymphocyte counts, as a marker of acquired immunity, were also investigated.

2. Materials and Methods

2.1. Test Foods

The LPS-containing tablets and placebo tablets were manufactured by UMEKEN Co. (Osaka, Japan), and they were identical in taste, appearance, and nutritional composition. Table 1 shows the nutritional composition of the LPS and placebo foods. The LPS formula tablets contain 244 µg of LPSP (*Pantoea agglomerans* derived LPS) per tablet (actual value).

Table 1. Nutritional composition of test foods.

	Placebo Food (/Tablet)	Test Food (/Tablet)
Energy (kcal)	1	1
Protein (g)	<0.1	<0.1
Fat (g)	<0.1	<0.1
Carbohydrates (g)	0.2	0.2
Salt equivalent (g)	0	0

The analysis results of the Japan Food Research Laboratories for the placebo food and the test food are listed in terms of per-grain equivalents.

2.2. Subjects

Subjects were recruited through notification to registered monitors of the non-profit organization (NPO) Natural Immunity Network and through public announcements (posters and newspapers). The sample size was not determined from a statistical standpoint because this study was a preliminary investigation of immune-related physical symptoms and immune cell analysis.

A total of 30 healthy subjects (4 men and 26 women) aged ≥ 20 years (mean age 49 ± 9.2 years) who had received at least 2 doses of the novel coronavirus 2019 vaccine (the most recent dose received at least 4 weeks prior to enrollment) and who had not

received any additional doses of the novel coronavirus vaccine during the study period were enrolled.

None of the following exclusion criteria were met at study entry: (1) patients receiving medication that may affect participation in the study (e.g., antibiotics, immunosuppressive drugs, anti-inflammatory drugs, anti-rheumatic drugs, antihistamines, anti-allergic drugs, or lactic acid bacteria preparations) or receiving medical care for any disease; (2) exercise or diet therapy under the supervision of a physician; (3) individuals potentially allergic to any of the tested foods; (4) pregnant or lactating women; (5) inability to stop consuming foods that may affect participation in the study (e.g., functional foods that may affect immune function); (6) participants in other clinical trials; and (7) individuals who had donated at least 400 mL of blood at least 3 months prior to the date of consent or at least 200 mL of blood at least 1 month prior to the date of consent.

2.3. Study Design

The study was a single-center, double-blind, randomized, placebo-controlled, parallel-group comparative study conducted from January to March 2022 at the NPO Natural Immunity Network, a clinical trial organization. Thirty subjects were randomly assigned to the LPS food group (LPS group) or placebo group in a blinded fashion using Mujinwari (Dolphin Systems, Tokyo, Japan). The LPS group and the placebo group were coded as groups A and B. The group assignment was performed by the registered allocation manager. Because this was a preliminary study for the purpose of screening endpoints, the adequacy of the sample size was not examined from a statistical perspective. All subjects received 2 tablets per day for 8 weeks during the study period, regardless of the timing of intake during the day. Subjects were asked to record their diet, exercise, smoking, and drinking habits as much as possible during the study period, and to note any differences from their usual habits. Intake of any medication that was deemed by the study physicians to not affect the immune system was allowed; it was required for the use of any medication to be recorded in their diaries.

2.4. Number of Days of Cold and Hay Fever Onset and Measurement of Symptom Severity

Every day during the study period, the participants recorded symptoms of runny nose, plugged nose, sneezing, sore throat, cough, hoarseness, headache congestion, chest congestion, and feeling tired on an 8-point scale in a daily logbook according to the Wisconsin Upper Respiratory Symptom Scale (WURSS-21), and rated the total score. Eye–nose allergic symptoms were also recorded on an 8-point scale for runny nose, plugged nose, sneezing, and itchy eyes, and rated with a total score.

2.5. Phagocytosis Assay

Peripheral blood phagocytic capacity was measured before and 8 weeks after the consumption of the test food. Heparinized whole blood samples were stored at 25 °C and phagocytosis was measured within 4 h of blood collection. A total of 500 µL of pHrodo™ Green Zymosan Bioparticles™ Conjugate for Phagocytosis (Thermo Fisher Scientific Inc., Tokyo, Japan), adjusted to 20 µg/mL, was added to 30 µL of blood and incubated at 37 °C for 2 h while the vessel was rotated on a rotator. Negative targets were allowed to stand on ice for 2 h. Then, 3 µL of APC-labeled anti-CD11b antibody (APC anti-mouse/human CD11b Clone M1/70, bio legend) was added and allowed to stand on ice for 20 min. The number of CD11b-positive and Green Zymosan positive cells was measured using a flow cytometer (Gallios, Beckman Coulter Inc., Tokyo, Japan). The ratio of phagocytic cells was indicated by the difference from that measured in the negative target. The measurements were outsourced to the Control of Innate Immunity, Collaborative Innovation Partnership (Kagawa, Japan).

2.6. Lymphocyte Count

T-cell count, CD4 T-cell count, CD8 T-cell count, CD4/CD8 T-cell ratio, naive (N) T-cell count, memory (M) T-cell count, N/M T-cell ratio, CD8⁺CD28⁺ T-cell count, B-cell count, NK cell count, and CD4⁺CD25⁺ cell count were all measured before and also 8 weeks after the intake of test foods. The measurements were performed at the Institute for Health Life Science Co. (Tokyo, Japan).

2.7. Hematology and Blood Biochemistry Tests

Hematology (white blood cell count, red blood cell count, hemoglobin, hematocrit, platelet count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count) and blood biochemical tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and C-reactive protein (CRP)) were performed before and then at 4 and 8 weeks after the intake of test foods, using samples obtained after fasting for at least 12 h. The measurements were contracted to Shikoku Chuken Inc. (Kagawa, Japan).

2.8. Statistical Analysis

Results are presented as mean $\pm$ standard error or standard deviation, with a risk rate of <5% considered as a significant difference.

3. Results

3.1. Subject Background

The flow of subject selection for analysis is shown in Figure 1, and the background characteristics of subjects are summarized in Table 2. There were no significant differences between the LPS and placebo groups with respect to sex or age. Eight subjects in the placebo group and nine in the LPS group had an exercise habit. Thirteen subjects each in the placebo group and the LPS group were habitual smokers. Eight subjects in the placebo group and five in the LPS group reported hay fever. None of the subjects described any diet, exercise, or smoking habits as being unusual during the study period. The study was conducted in compliance with the study protocol. A total of 30 patients were evaluated for analysis during the study period with no adverse event reports or dropouts.

Table 2. Background of subjects.

	Placebo Group (<i>n</i> = 15) Mean $\pm$ SD	LPS Group (<i>n</i> = 15) Mean $\pm$ SD	<i>p</i> -Value
Male: Female (<i>n</i>)	2:13	2:13	
Age (years)	50.3 $\pm$ 9.3	47.8 $\pm$ 9.2	0.43
Smoking habitue (<i>n</i>)	2	2	
Hay fever (<i>n</i>)	8	5	

Statistical comparisons were made between the placebo and LPS groups by Mann–Whitney’s U test.

3.2. Cold Symptoms

Of the 30 subjects, 7 in the placebo group and 8 in the LPS group developed cold symptoms during the intake period. The symptoms and overall scores of the 30 subjects, including those who had no symptoms, are shown in Table 3. Intention to Treat (ITT) analysis of each symptom and overall score during the intake period showed no significant differences between the two groups.

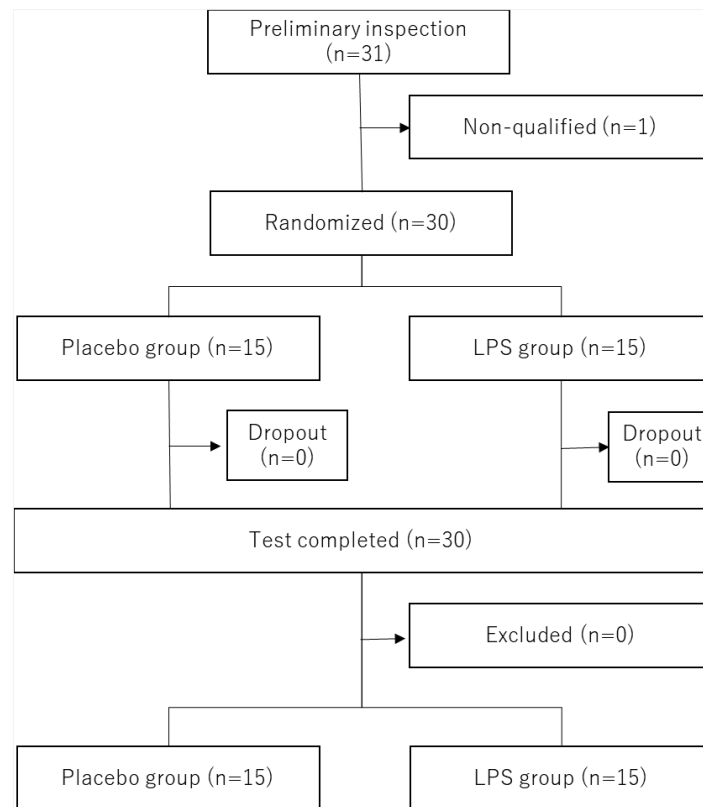


Figure 1. Flow of subject selection.

Table 3. Cumulative cold symptom scores.

	Placebo Group (n = 15) Mean ± SE	LPS Group (n = 15) Mean ± SE	p-Value
Number of people with symptoms (n)	7	8	
Overall Score	12.40 ± 6.49	20.20 ± 11.64	0.74
Runny nose	0.93 ± 0.64	0.67 ± 0.37	0.51
Plugged nose	1.40 ± 0.97	1.40 ± 1.33	1.00
Sneezing	0.53 ± 0.41	2.13 ± 1.99	0.68
Sore throat	0.47 ± 0.40	1.53 ± 0.86	0.31
Cough	0.33 ± 0.27	7.80 ± 6.03	0.27
Hoarseness	0.13 ± 0.09	2.33 ± 1.53	0.50
Head congestion	4.73 ± 2.40	3.80 ± 2.37	0.68
Chest congestion	0.93 ± 0.93	0.07 ± 0.07	0.96
Feeling tired	2.93 ± 2.93	0.47 ± 0.29	0.34

Statistical comparisons were made between the placebo and LPS groups by Mann–Whitney’s U test.

3.3. Eye–Nose Allergic Symptoms

Seven subjects in the placebo group and five in the LPS group experienced eye–nose allergy symptoms during the intake period. The symptoms and overall scores for each of the 30 subjects, including those who did not develop symptoms, are shown in Table 4(A). Although there were no significant between-group differences in symptom scores during the intake period in the ITT analysis, the LPS group tended to have lower overall scores and lower mean value for each symptom score compared to the placebo group (Figure 2A).

Table 4. (A). Cumulative hay fever symptom scores (ITT analysis). (B). Cumulative hay fever symptom scores (analysis with only those who developed the disease).

(A)			
	Placebo Group (<i>n</i> = 15) Mean ± SE	LPS Group (<i>n</i> = 15) Mean ± SE	<i>p</i> -Value
Number of people with symptoms (<i>n</i>)	7	5	
Overall Score	54.67 ± 22.55	9.27 ± 5.24	0.20
Nasal symptom score	37.87 ± 18.29	6.07 ± 4.36	0.25
Runny nose	15.13 ± 7.81	2.87 ± 2.18	0.52
Plugged nose	13.00 ± 9.39	1.73 ± 1.42	0.20
Sneezing	9.73 ± 4.61	1.47 ± 0.83	0.40
Eye symptom score (itchy eyes)	16.8 ± 7.17	3.20 ± 2.20	0.13
(B)			
	Placebo Group (<i>n</i> = 7) Mean ± SE	LPS Group (<i>n</i> = 5) Mean ± SE	<i>p</i> -Value
Overall Score	117.14 ± 36.38	27.80 ± 12.63	0.04
Nasal symptom score	81.14 ± 32.97	18.20 ± 12.00	0.12
Runny nose	32.43 ± 14.54	8.60 ± 6.14	0.56
Plugged nose	27.86 ± 19.29	5.20 ± 4.07	0.31
Sneezing	20.86 ± 8.23	4.40 ± 2.01	0.21
Eye symptom score (itchy eyes)	36.00 ± 11.91	9.60 ± 5.95	0.04

Statistical comparisons were made between the placebo and LPS groups by Mann–Whitney’s U test.

In the current study, the subjects were not screened for eye–nose allergic disposition but were asked in a pre-test questionnaire whether they had experienced any symptoms of hay fever in the preceding years. Eight subjects in the placebo group and five in the LPS group reported having hay fever symptoms in the pre-test questionnaire. Among these 13 subjects, the number who experienced allergic symptoms in the eyes and nose during the intake period was 7/8 in the placebo group and 4/5 in the LPS group. On the other hand, one subject in the LPS group who did not report hay fever symptoms in the pre-test questionnaire developed eye–nose allergy symptoms during the intake period. Comparison of symptom scores of only those subjects who developed eye–nose allergies showed that the LPS group had significantly lower overall scores and eye symptom scores than the placebo group, as shown in Table 4(B) and Figure 2B.

3.4. Phagocytic Capacity

Table 5 shows the change from baseline at the end of the study in the phagocytic capacity of blood phagocytes as an indicator of innate immunity. There were no significant differences between the two groups in this respect.

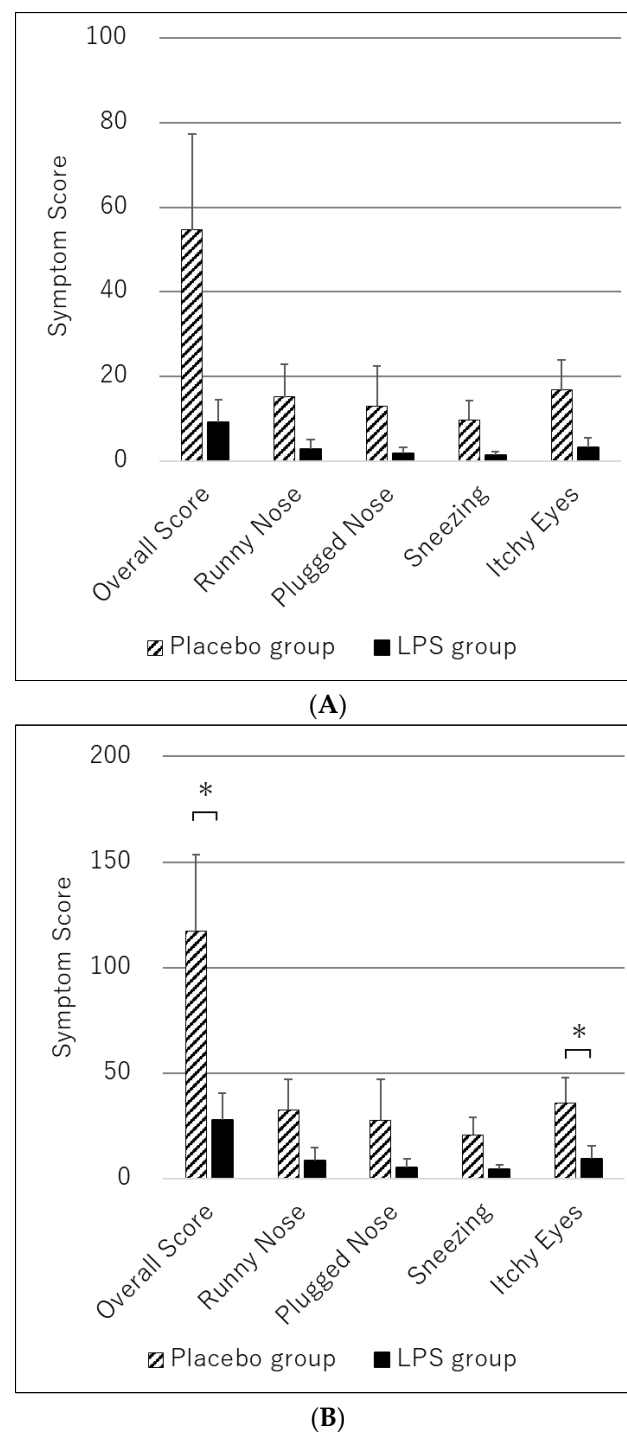


Figure 2. (A). Cumulative eye–nose allergic symptom score (ITT analysis). (B). Cumulative eye–nose allergic symptom score (analysis with only those who developed the disease) * $p < 0.05$.

Table 5. Change from baseline in phagocytotic ability.

	Change from Baseline		<i>p</i> -Value
	Placebo Group (<i>n</i> = 15) Mean ± SE	LPS Group (<i>n</i> = 15) Mean ± SE	
Phagocytosis (%)	−8.4 ± 2.7	−8.5 ± 2.7	0.88

Statistical comparisons were made between the placebo and LPS groups by Mann–Whitney’s U test.

3.5. Lymphocyte Count

Table 6 shows the change from baseline at the end of the study in the blood lymphocyte count as an indicator of acquired immunity. There were no significant differences between the two groups in this respect.

Table 6. Change from baseline in lymphocyte counts at end.

	Change from Baseline		<i>p</i> -Value
	Placebo Group (<i>n</i> = 15) Mean ± SE	LPS Group (<i>n</i> = 15) Mean ± SE	
White blood cell	40 ± 279	113 ± 247	0.74
Neutrophil	−30 ± 239	174 ± 253	0.37
Monocyte	8 ± 12	−12 ± 12	0.25
Lymphocyte	48 ± 75	−59 ± 108	0.55
T-cell	37 ± 53	−4 ± 88	0.65
CD4 T-cell	48 ± 39	45 ± 52	1.00
CD8 T-cell	−12 ± 18	−37 ± 30	0.66
CD8 ⁺ CD28 ⁺ T-cell	−3 ± 14	9 ± 16	0.76
B cell	19 ± 12	−26 ± 18	0.06
NK cell	−14 ± 20	−34 ± 13	0.59

Statistical comparisons were made between the placebo and LPS groups by Mann–Whitney's U test.

3.6. Safety Assessment

No adverse events were reported during the study. Changes in blood indices before and after the study are shown in Table 7. There were no significant changes in any of the hematological and blood biochemical indices in both the placebo and the LPS groups, and the investigator judged that the 8-week continuous intake of LPS-containing food was safe.

Table 7. Blood test values.

Evaluation Item	Placebo Group (<i>n</i> = 15) LPS Group (<i>n</i> = 15)	Before Intake Mean ± SE	After 8 Weeks of Intake Mean ± SE	<i>p</i> -Value (before vs. after Ingestion)
White blood cell (×10 ⁴ /uL)	Placebo group	4587 ± 263	4627 ± 288	0.98
	LPS group	4707 ± 453	4820 ± 379	0.68
Red blood cell (×10 ⁴ /uL)	Placebo group	441 ± 10	432 ± 11	0.34
	LPS group	450 ± 13	442 ± 13	0.80
Hemoglobin (g/dL)	Placebo group	13.0 ± 0.4	12.7 ± 0.4	0.21
	LPS group	13.3 ± 0.5	13.0 ± 0.5	0.65
Hematocrit (%)	Placebo group	40.2 ± 1	38.9 ± 0.9	0.18
	LPS group	40.9 ± 1.1	40.0 ± 1.1	0.51
Platelets (×10 ⁴ /uL)	Placebo group	26.0 ± 1.1	25.8 ± 1.1	0.92
	LPS group	25.5 ± 1.2	24.2 ± 1.1	0.42
AST (GOT) (U/L)	Placebo group	20.4 ± 1.4	18.6 ± 0.6	0.44
	LPS group	20.6 ± 1.1	19.1 ± 0.9	0.37
ALT (GPT) (U/L)	Placebo group	18.8 ± 2.5	16.3 ± 1.7	0.45
	LPS group	20.1 ± 2.4	17.1 ± 1.5	0.41
Creatinine (mg/dL)	Placebo group	0.633 ± 0.038	0.631 ± 0.038	0.85
	LPS group	0.612 ± 0.032	0.609 ± 0.031	0.98
CRP (mg/dL)	Placebo group	0.053 ± 0.009	0.047 ± 0.01	0.46
	LPS group	0.047 ± 0.008	0.066 ± 0.017	0.77

Statistical comparisons were made between the placebo and LPS groups by Mann–Whitney's U test.

4. Discussion

This was a preliminary study of the effects of LPS intake on immunity. An 8-week, double-blind, placebo-controlled, randomized, parallel-group comparative study was conducted in healthy subjects. The key outcome measures were cold symptoms, eye–nose allergic symptoms, phagocytic capacity, and changes in lymphocyte counts.

The total number of subjects who developed cold symptoms in this study was 15/30 (LPS and placebo groups combined), corresponding to an overall incidence rate of 50%. There was no significant difference between the two groups in ITT analysis regarding the scores of cold symptoms that developed during the test food intake period. The mean symptom scores of the two groups were higher in the LPS group. Another pre-survey with a larger number of subjects is warranted in order to investigate the effect of LPS in suppressing cold symptoms as a marker of immunity.

The total number of subjects who developed nasal allergic symptoms was 11/30 (LPS and placebo groups combined), corresponding to an incidence rate of 37%. ITT analysis showed no significant difference between the two groups in terms of the scores of eye–nose allergic symptoms that developed during the intake of the test foods, but there was a trend toward symptom suppression in the LPS group. LPS has been shown to improve Th1/Th2 balance [13] and to activate Treg cells [14]. Epidemiological studies conducted in Europe have shown that LPS is a key factor in the suppression of allergies [9], and it may contribute to the prevention and alleviation of eye–nose allergies.

The effect size (Cohen's *d*) calculated from the mean and standard deviation of the ITT analysis for the eye–nose allergic symptom score was 0.724. Assuming an effect size of 0.724, a dominance level of 0.05, a detection rate of 80%, and a dropout rate of 20%, a sample size of 50 is required, as calculated by G*Power. Factoring a dropout rate of 10%, a total of 55 subjects is required for efficacy evaluation. The study did not screen for allergies prior to enrollment. In this study, 13/30 (43%) subjects reported a history of hay fever symptoms in the preliminary questionnaire, and 11/13 (85%) of these subjects developed nasal and eye symptoms during the study period. Statistical analysis of symptom scores for those who developed symptoms showed that the LPS group had significantly lower symptom scores than the placebo group. This result suggests that LPS may suppress the symptoms of eye–nose allergies in individuals with a predisposition towards allergies, but further study in an allergic population is required to confirm this finding. In this study, two subjects in the LPS group and one subject in the placebo group used anti-allergy medication during the study period. Since allergic subjects often use over-the-counter medicines to control their symptoms during the hay fever season, it is necessary to analyze the frequency of use of antiallergic medicines in the study of symptom suppression of eye–nose allergies. Sex differences in immunology have been reported to exist [15], and future trials will need to eliminate gender bias in the number of men and women as much as possible, and further stratified analysis by gender will be necessary.

The LPS content in the test food used in this study was 488 µg/day. Significant or improving trends in fasting blood glucose and low-density lipoprotein (LDL) [16], inhibition of bone density loss [17], and an increase in the number of capillaries in fingertips [18] have been reported in double-blind human studies on LPS-containing foods. The LPS content in LPS-containing foods used in these human studies ranged from 400 to 600 µg/day.

In addition to physical symptoms, changes in phagocytic capacity (marker of innate immunity) and changes in lymphocyte counts (marker of acquired immunity) were also investigated, but no significant between-group differences were observed with respect to the changes in cell counts from baseline to the end of the study. Since there were no significant differences in physical symptoms between the two groups, it was not possible to determine whether changes in immune function correlated with changes in phagocytic activity or lymphocyte counts. Further modification of the experimental system is required because phagocytic function must be measured while the cells are still fully bioactive after blood collection.

These results suggest that it is difficult to examine the effect of 488 µg/day of LPS on immunity using the immune indices and measurement methods used in this study, and it is also possible that the daily LPS intake in this study was insufficient. Therefore, although it is certain that oral administration of 400–600 µg/day of LPS has various effects, further studies are required to clarify whether these effects of LPS are mediated via its effect on the

immune function. It is necessary to consider the test method, the number of subjects, the amount of LPS, and other aspects to obtain more robust evidence in this regard.

On the other hand, there were no adverse events or dropouts, and no changes were observed in hematological and blood biochemical indices, which demonstrates the safety of LPS intake. Based on this preliminary study, we will next investigate the suppression of eye–nose allergy symptoms by LPS intake with a sufficient number of subjects and with the addition of new endpoints.

5. Conclusions

This study confirms that continuous LPS intake is safe and suggests that it may improve eye–nose allergies. However, further confirmation with a sufficient number of subjects for statistical analysis is required.

Author Contributions: G.-I.S., C.K. and H.I. conceived and designed the study. M.Y. conducted the phagocytosis test. M.U. analyzed the data. C.K. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in compliance with the “Declaration of Helsinki (1964)” and the “Ethical Guidelines for Life Science and Medical Research Involving Human Subjects (23 March 2021)” issued by the Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labor and Welfare, and Ministry of Economy, Trade and Industry, and approved by the NPO Natural Immunity Network Ethics Review Committee (approval number LSIN 2021-001, approval date 15 November 2021), (Approval date: 15 November 2021). The study protocol was pre-registered in the clinical trial registration system (UMIN-CTR) (UMIN000046154).

Informed Consent Statement: The study was fully explained and written informed consent was obtained from all of the subjects who participated. Written informed consent was also obtained from the subjects for the publication of this paper.

Data Availability Statement: Not applicable.

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Conflicts of Interest: G.-I.S., H.I., M.U. and C.K. are employees of Macrophil Inc. The authors declare no competing interest.

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