ELSEVIER

Nutrition 23 (2007) 342–350

NUTRITION

www.elsevier.com/locate/nut

Basic nutritional investigation

Effect of *Lactobacillus johnsonii* La1 on immune function and serum albumin in aged and malnourished aged mice

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Manuscript received October 23, 2006; accepted February 5, 2007.

Abstract

Objective: Protein-energy malnutrition (PEM) is a serious nutritional problem that causes immune dysfunction in elderly people. Probiotic lactic acid bacteria may potentially modify immunity; however, there is little evidence to elucidate the influence of these bacteria on PEM in the elderly.
Methods: The immune modulation effects of lactic acid bacterium *Lactobacillus johnsonii* La1 (La1) were examined in aged mice and aged mice with PEM. Twenty-month-old male 57BL6/n mice ($n = 28$) were divided into four groups and received the following diet for 14 d: a complete diet (20% protein) without Lal (control) or with Lal or a low-protein diet (5% protein) to induce PEM, with or without La1. All mice were immunized with diphtheria toxin (DT) with alfacalciferol at 7 d and sacrificed 14 d after starting the experimental diets.
Results: Serum albumin concentrations and body weight, both of which were reduced by the low-protein diet, were ameliorated by La1 intake and were the same as in mice receiving the control diet. Anti-DT immunoglobulin (Ig) A in fecal extract was increased by La1 intake in mice receiving the complete and low-protein diets. Serum anti-DT IgA, IgG, splenocyte proliferation, and $CD8^+$ T cells were reduced by the low-protein diet and restored by La1 intake.
Conclusion: La1 enhances intestinal IgA production and helps recover nutritional status and systemic immune responses in aged mice with PEM. It is possible that La1 may contribute to immune system recovery in immunocompromised hosts such as elderly humans with PEM.

Keywords: Protein-energy malnutrition; Aging; Immunoglobulin A; Albumin; *Lactobacillus johnsonii* La1

Introduction

Protein-energy malnutrition (PEM) is frequently associated with the occurrence of infection due to a decline in immune function [1]. PEM is a common serious problem that causes an immunocompromised condition and aggravates infection-related mortality in elderly subjects [2,3]. Especially in elderly patients in hospitals, nursing homes, and home care, PEM is a strong independent risk factor for life-threatening morbidity [4]. Elderly people must prevent PEM and improve immune function to protect themselves against infectious diseases. However, amelioration of PEM by nutritional rehabilitation is difficult to achieve in elderly and immunocompromised patients who often show an impaired physiologic decrease in food intake due to problems with digestion or absorption [5,6]. The rapidly aging population in many countries means that ways of preventing and treating PEM in the elderly need to be developed.

Protein-energy malnutrition has been shown to exert diverse influences on host immune systems, including systemic and mucosal immune responses [1,5,6]. Moreover, aging impairs various immune functions [7]. The immune system's effects on aging and PEM are related and might have a cumulative effect. At present, the number and proliferative response of lymphocytes, cytokine production, cytotoxic T-lymphocyte activity, and antigen-specific response to vaccinations are known to be damaged by PEM in

This work was supported in part by Applied Science Board for Lactic Acid Bacteria (ASB-LAB).

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0899-9007/07/$ – see front matter
doi:10.1016/j.nut.2007.02.001

aging [8,9]; however, intestinal immune function and the mechanisms of immunodysfunction have not been sufficiently clarified.

Probiotic lactic acid bacteria (LAB) are an attractive, safe way to regulate and enhance immune function. Among the possible mechanisms of probiotic LAB, the enhancement of the secretory immune response and particularly intestinal immunoglobulin (Ig) A production could be helpful in protecting against infection [10]. Some studies have shown that a few probiotic LAB strains enhance immune function [11] and protect against infection in elderly subjects [12] and malnourished young animals [13]. However, the effect of LAB on the immune system of elderly subjects with PEM is uncertain. In addition, few data are available on the efficacy of LAB in mucosal immunity in elderly subjects. LAB might play a greater role at preventing infectious diseases in elderly subjects with PEM or in those who are immunocompromised than in healthy subjects.

Lactobacillus johnsonii La1 (La1) is a well-characterized probiotic LAB that adheres to human intestinal epithelial cells [14] and activates natural and specific immunities to increased serum IgA [15–18]; it is thought that La1 strengthens the host defense system.

Most cases of PEM in elderly people involve marasmic kwashiorkor, which is typified by wasting and lower serum albumin concentrations [4]. In our preliminary study (data not shown), a low-protein diet for mice was shown to induce PEM with marasmic kwashiorkor. The present study used a low-protein diet, with or without of La1 intake, to analyze intestinal and systemic immune functions in aged mice with experimentally induced PEM.

Materials and methods

Animals

The study used C57BL6/n male mice (Japan SLC Inc., Shizuoka, Japan) kept in a temperature- and light-controlled environment. Three or four mice were kept in each cage. As a model of aging, we used 20-mo-old mice. The mean life span of C57BL6/n male mice is about 26 to 28 mo. The animals were allowed free access to a non-purified MF diet (Oriental Yeast Co. Ltd., Osaka, Japan) and water until the experiment began. The experiment was performed according to the Guide for the Care and Use of Laboratory Animals (1985) of the National Institutes of Health.

Diets

The complete diet, containing semi-purified 20% protein diet, followed the American Institute of Nutrition (AIN-93) protocol [19] in terms of composition. The low-protein contained semi-purified 5% protein, prepared by substituting egg white protein in the control diet with an equal weight of cornstarch. PEM was induced using this low-protein diet. Egg white protein containing 86.5% crude protein was used as the protein source. Table 1 lists the compositions of the complete and low-protein diets.

Table 1
Composition of experimental diets

Ingredient (g/kg)	Complete diet	Low-protein diet
Egg white protein*	200.0	50.0
Cornstarch	399.5	512.5
α-Cornstarch	133.0	170.0
Sucrose	100.0	100.0
Soy oil	70.0	70.0
Cellulose	50.0	50.0
Mineral mix†	35.0	35.0
Vitamin mix‡	10.0	10.0
Choline bitartrate	2.5	2.5
Tert-butyl-hydroquinone (mg/kg)	14.0	14.0
Energy (MJ/kg)	15.08	15.08
Protein (g/kg)	173.5	43.9
Lipid (g/kg)	74.5	75.0

* Contains 86.5% crude protein.

† American Institute of Nutrition 93G; mineral mix provided (g/kg diet): calcium 5.0, phosphorus 1.6, sodium 1.0, potassium 2.3, magnesium 0.5, iron 0.03, zinc 0.03, and copper 0.01.

‡ American Institute of Nutrition 93; vitamin mix provided (mg/kg diet): nicotinic acid 30.0, calcium pantothenate 16.0, pyridoxine-HCl 7.0, thiamin-HCl 6.0, riboflavin 6.0, folic acid 2.0, biotin 2.0, cyanocobalamin 25.0, α-tocopherol 150.0, retinyl palmitate 8.0, cholecalciferol 2.5, and phylloquinone 0.75.

Intake of Lactobacillus johnsonii *La1*

Freeze-dried *L. johnsonii* La1 (La1; NCC533, Nestle Culture Collection, Nestle Ltd., Lausanne, Switzerland) powder was dissolved 3×10^8 colony-forming units/mL in distilled water as drinking water; it was given to mice using a sterilized water pack (AN pack; Musashi Co. Ltd., Saitama, Japan) and water supply nozzle (SE nozzle; Musashi Co. Ltd.). Each diet group was given distilled water with or without La1. Water was replaced daily along with a new sterilized pack and nozzle. Intake of drinking water was recorded per cage daily, and intake per mouse was obtained by dividing by the number of mice in the cage. Each mouse consumed $\geq 1 \times 10^9$ colony-forming units of La1 per day. We had previously confirmed that intake of drinking water per mouse was not significantly different between group housing and single housing.

Experimental protocol

Twenty-eight aged mice received the complete diet for 1 wk during the acclimation period. The animals were then allocated to the following four experimental groups: 1) mice were fed the complete diet as a control group ($n = 6$, C group); 2) mice fed the complete diet in addition to drinking water containing La1 ($n = 6$, CL group); 3) mice fed a low-protein diet to induce PEM ($n = 8$, LP group); and 4) mice fed a low-protein diet in addition to drinking water containing La1 ($n = 8$, LPL group). The C and LP groups