Actions of *Taimatsu* fermented rice germ solution on human immune responses

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Abstract

In this study, we examined the immunological actions of *Taimatsu* fermented rice germ solution (designated as T-FRGS), containing gamma-aminobutyric acid (GABA) and inositol hexaphosphate (IP6). The morphology of peripheral blood mononuclear cells (PBMCs) from normal healthy donors cultured with T-FRGS was dramatically altered (inducement of cell aggregation) as compared with that of the control cells (cultured without T-FRGS) at 24 hrs. In addition, the PBMCs from normal healthy donors cultured with T-FRGS showed strongly enhanced ability to produce some cytokines, such as interleukin (IL)-1 β ,IL-6, IL-8, tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) but not others, such as IL-2 or IL-12. Taken together, these findings clearly indicate that T-FRGS has stimulatory effects on some human immune responses.

Key words : cytokines, human immune responses, PBMCs, T-FRGS

Introduction

While gamma-aminobutyric acid (GABA) was originally identified as the principal inhibitory neurotransmitter in the mammalian brain [1], it subsequently became clear that GABA and GABA receptors also exist in many non-neuronal peripheral tissues [2]. Recently, GABA has been demonstrated to show additional actions as an immune stimulator that activates immune-related cells and antibody production [3, 4].

Inositol hexaphosphate (IP6) is a polyphosphorylated carbohydrate that is ubiquitous in plants, such as grains and legumes. IP6 is also found in most mammalian cells, and is particularly ubiquitous in cell membranes, where it is found in conjugation with lipids as phosphatidylinositol, and plays biologically significant roles in intracellular signal transduction systems involved in cellular proliferation and differentiation [5, 6]. On the other hand, both *in vivo* and *in vitro* experiments have also demonstrated a novel anticancer action of IP6 against various cancers including cancers of the colon, prostate, breast and liver; this anticancer action is primarily mediated via regulation and suppression of cellular proliferation and cell growth by this compound [7-10].

Several materials that contain both GABA and IP6 elements have been identified, studied and developed for use as supplements in the health food, medical and pharmaceutical industries. However, detailed analyses have yet to be performed of the effects of these compounds on the human immune system, including their actions on the production of various cytokines.

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Recently, we succeeded in preparing a new solution containing both GABA and IP6 from fermented rice germ, designated as T-FRGS, that is now available commercially as a health food supplement [4, 10]. In the present study, to refine and confirm the ability of T-FRGS as an immune stimulator, we conducted an *in vitro* investigation of the effects of this solution on some human immune responses.

Materials and Methods

Reagents

Taimatsu fermented rice germ solution containing GABA and IP6, designated as T-FRGS, was manufactured by Taimatsu Foods Co. (Niigata, Japan) using the latest biological culturing and preparative techniques and is currently available commercially as a health food supplement. Enzyme immunoassay (EIA) kits for interleukin (IL)-1 β, IL-2, IL-6, IL-8, IL-12, tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) were purchased from Cosmo Bio Co. (Tokyo Japan). Phorbol myristate acetate (PMA) was purchased from Sigma Co. (USA).

Donors and preparation of human PBMCs

We prepared human peripheral blood mononuclear cells (PBMCs) from 10 normal healthy donors (mean age, 38.3±8.6 years; 4 males and 6 females) by the Ficoll-Paque density sedimentation method, as previously described [11]. Informed consent was obtained from each of the donors prior to his/her participation in this study.

Culture of PBMCs with T-FRGS

Human PBMCs ($2x10^5$) in RPMI 1640 medium (GIBCO) supplemented with 10mM HEPES buffer, 2mM glutamine and 10% fetal calf serum (FCS) (GIBCO) were cultured in 96-well microplates (Sumitomo Co., Tokyo Japan) with or without T-FRGS (1:100 or 1:50 dilution) or PMA (100ng/mL), at 37°C for 24 hrs. The morphological changes were observed under a phase-contrast microscope. The culture supernatants were then assayed for various cytokines including IL-1 β, IL-2, IL-6, IL-8, IL-12, TNF-α, GM-CSF and G-CSF.

Detection of cytokines in the culture supernatants

EIA kits were used for the detection of the cytokines, including IL-1 β, L-2, IL-6, IL-8, IL-12, TNF-α, GM-CSF and G-CSF, in the culture supernatants of the PBMCs cultured with or without T-FRGS (1:100 dilution). Each experiment was repeated three times.

Statistical analysis

The statistical analysis was performed using the Student *t*-test. Differences were considered significant when the P value was less than 0.01.

Results and Discussion

In this study, we investigated the effects of *Taimatsu* fermented rice germ solution containing GABA and IP6 (designated as T-FRGS) on human immune responses. First, dramatic morphological changes of PBMCs from normal healthy donors cultured with T-FRGS (1:100 or 1:50 dilution) were demonstrated. As shown in Fig. 1, the typical morphologies of the PBMCs cultured with T-FRGS were dramatically altered (inducement of cell aggregation) at 24 hrs compared with those of the control cells cultured without T-FRGS. In addition, PMA also altered morphology of the PBMCs (Fig. 1). PMA is a protein kinase C (PKC) activator that activates intracellular second messengers and influences various human immune responses by regulating the expression of several functional receptors in immune related cells [12]. The morphological changes induced by T-FRGS strongly suggest possible immunostimulatory effects of this solution.

To examine the biological actions of T-FRGS, we next measured the levels of various cytokines, such as IL-1 β, IL-2, IL-6, IL-8, IL-12, TNF-α, GM-CSF

and G-CSF, in the culture supernatants of the PBMCs from normal healthy donors cultured with or without T-FRGS, using EIA kits. Table 1 shows that T-FRGS strongly induced the production of IL-1 β , IL-6, IL-8, TNF- α , GM-CSF and G-CSF, but not of IL-2 or IL-12 from the PBMCs, as determined by the cytokine levels as measured at 24 hrs of culture, and the differences in the levels between the culture supernatants of the PBMCs cultured in the presence or absence of T-FRGS were significant ($P<0.001$).

IL-1 β is secreted by myeloid cells that participate in the regulation of various immune responses, and also plays an important role in the central nervous system [13]. IL-6 is secreted by T cells and macrophages to stimulate some immune responses, and supports the growth of B cells. Furthermore, IL-6 is also secreted by macrophages in response to specific microbial molecules, referred to as pathogen-associated molecular patterns (PAMPs), through toll-like receptors (TLRs) in the innate immune system [14]. IL-8 is a chemokine produced by macrophages and other cell types. The receptors for IL-8 are the G protein-coupled serpentine receptors CXCR1 and CXCR2. The production of IL-8 also appears to depend on NF-κB activation and intracellular signaling mediated by TLR-2 [15]. TNF- α produced by activated macrophages regulates the activities of immune cells, and in particular, induces apoptotic cell death [16]. GM-CSF is secreted by macrophages, T cells, mast cells, endothelial cells and fibroblasts, and stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes [17]. G-CSF is produced by a number of different tissues, and stimulates stem cells in the bone marrow to produce granulocytes, and influences the survival, proliferation, differentiation and functions of neutrophil precursors and mature neutrophils [18]. Taken together, the findings of this study suggest that T-FRGS strongly enhanced the production of IL-1 β , IL-6, IL-8, TNF- α GM-CSF and G-CSF from human PBMCs, as determined by measurements of the cytokine levels in the culture supernatant at 24 hrs of culture, thereby indicating that T-FRGS may have stimulatory actions on various human immune responses.

The mechanisms underlying the actions of T-FRGS observed in this study are not yet well understood at the present time. However, we have provided evidence to indicate that GABA also acts as an immune stimulator in addition to being an inhibitory neurotransmitter [4]. Furthermore, T-FRGS also contains IP6 in addition to GABA. The basic carbohydrate moiety “inositol” in IP6 and its other phosphate derivatives (IP1-IP5) is physiologically interconvertible and regulates vital cellular functions. The immunological actions of IP6 are based on the hypotheses that exogenously administered IP6 may be internalized, dephosphorylated to IP3, and this IP3 may affect the immunological actions [5]. In fact, IP3 is known to have important roles in signal transduction for proliferation/differentiation of various cells [6, 19-21]. Thus, T-FRGS is a very useful material for the regulation of various human immune responses, based on the synergic effects of GABA and IP6.

In conclusion, T-FRGS altered the morphology of human PBMCs and enhanced the production of some cytokines closely associated with human immune responses. These findings strongly suggest that T-FRGS may significantly influence immunological responses. Further analyses are needed to demonstrate other soluble factors (soluble formed CD molecules; sCD molecules), such as sCD25, sCD31, sCD54, sCD93, sCD95, etc., involved in the regulation of various human immune systems at the cellular and molecular levels.

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Table 1. Enhancement of the production of some cytokines from human PBMCs cultured with T-FRGS

Cytokine		T-FRGS(-)	T-FRGS(+)	P value*
IL-1β	(pg/mL)	7.2±1.9	176.8±76.2	P<0.001
IL-2	(U/mL)	0.5±0.1	0.5±0.08	P=0.792
IL-6	(pg/mL)	2.6±1.0	4493.4±1479.7	P<0.001
IL-8	(pg/mL)	5262.5±2239.9	28412.9±9639.6	P<0.001
IL-12	(pg/mL)	6.5±0.8	6.1±1.3	P=0.564
TNF-α	(pg/mL)	0.3±0.1	75.8±11.8	P<0.001
GM-CSF	(pg/mL)	12.3±1.9	337.4±105.5	P<0.001
G-CSF	(pg/mL)	6.2±0.8	2085.7±883.7	P<0.001

EIA kits were used for the detection of IL-1β, L-2, IL-6, IL-8, IL-12, TNF- α , GM-CSF and G-CSF in the culture supernatants of human PBMCs cultured with or without T-FRGS (1:100). Each experiment was repeated three times.

* T-FRGS(-) vs. T-FRGS(+)

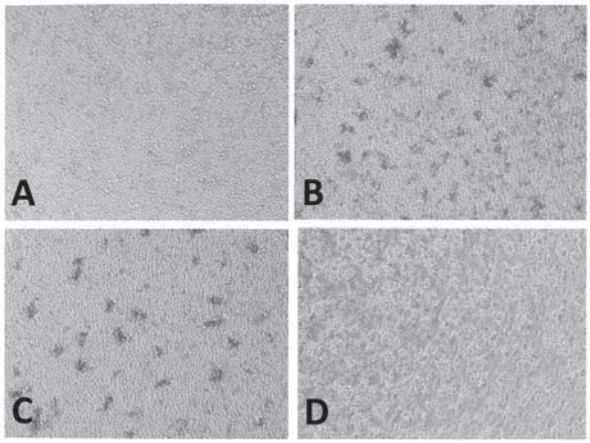

Figure 1. T-FRGS induced morphological changes in human PBMCs. Human PBMCs were cultured with or without T-FRGS (1:100 or 1:50 dilution) or PMA (100ng/mL) for 24 hrs (see Materials and Methods section for details). The morphological changes were observed by phase-contrast microscopy (100x). A: control (cultured without T-FRGS), B: cultured with T-FRGS (1:50), C: cultured with T-FRGS (1:100), D: cultured with PMA (100ng/mL).

たいまつ米胚芽発酵液のヒト免疫応答に与える作用

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要　旨

本研究では、我々が独自に開発した GABA および IP6 含有たいまつ米胚芽発酵液（T-FRGS）のヒト末梢血単核球 (PBMCs) に対する形態変化およびサイトカイン産生能を解析した。その結果、T-FRGS は PBMCs に形態変化（細胞間凝集）を誘導した。さらに、Enzyme immunoassay (EIA) を用いた解析から、T-FRGS は PBMCs から IL-1β、IL-6、IL-8、TNF-α、GM-CSF、G-CSF の産生を有意に増強させた（$P<0.001$）。以上の結果から、T-FRGS にはヒト免疫応答を増強させる作用があることがわかった。

キーワード：サイトカイン、ヒト免疫応答、末梢血単核球 (PBMCs)、たいまつ米胚芽発酵液 (T-FRGS)