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Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*

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Abstract

Spirulina platensis is a cyanobacterial species that is surmised to potentiate the immune system leading to suppression of cancer development and viral infection. Here, we identified the molecular mechanism of the human immune potentiating capacity of Spirulina by analyzing blood cells of volunteers with pre and post oral administration of hot water extract of Spirulina. NK functions represented by IFN gamma production and cytolysis were enhanced after administration of Spirulina in >50% subjects. IFN gamma was produced in an IL-12/IL-18-dependent fashion. In vitro stimulation of blood cells with BCG cell wall skeleton (CWS) allowed more potent IL-12 p40 production in cells from volunteers given Spirulina than in cells without pre-exposure to Spirulina. As BCG–CWS serves as a ligand for Toll-like receptor (TLR) 2 and 4 to raise the maturation stage of monocytes/macrophages, Spirulina may be involved in the signaling responses through Toll in blood cells even when orally administered. These observations indicated that in humans Spirulina acts directly on myeloid lineages and either directly or indirectly on NK cells. The presence of co-operative IL-12 and IL-18 is critically important for NK-mediated IFN gamma production.

Keywords: IL-12; IL-18; Toll-like receptors; IFN gamma; Cyanobacteria; Macrophages

1. Introduction

The cyanobacterium *Spirulina platensis* here inhabits its carbonate-rich lakes in torrid zones [1]. It has been utilized as a source of protein and vitamin supplements, and has been sold as a health drink or pills in tablet form for more than 10 years without any undesirable effect on humans [2]. Its safety for human consumption has also been established through numerous toxicological studies. Recently, Spirulina has been speculated to be associated with modulation of the host immune system [3]. A hot water extract of Spirulina has been orally administered to patients as an anti-cancer and anti-viral agent although the molecular mechanism by which Spirulina acts on the immune system remains largely undefined.

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The experimental immunomodulatory function of Spirulina was first reported in mice since 1994 [4]. The results are summarized as follows. In mice, Spirulina facilitated antibody production, increased the ratio of activated peritoneal macrophages, and induced spleen cells to grow better in response to Con A [4]. In spleen cells in culture, addition of the hot water extract of Spirulina leads to enhanced IL-1 and then antibody production [5]. These results suggested that the initial target cells for Spirulina could be macrophages, although the mechanism by which these in vivo and in vitro responses are induced again remains elusive.

In this study, healthy male volunteers were given orally with Spirulina every day for several weeks or months to be analyzed for the activity of their natural killer (NK) cells withdrawn at weekly or monthly intervals. The cells originating from the thus collected blood were incubated with IL-12 alone or together with increasing concentrations of IL-18 to observe the release of IFN gamma therefrom in response to these cytokines, which are currently known to be the major ones secreted from activated macrophages or dendritic cells, as an index of the NK cell activity. In some experiments, the cells were incubated with ^{51}Cr-labeled erythroblastoid cells (K562) to see cytolysis as an additional activity of the NK cells. The NK activities, measured as the IFN gamma production in response to IL-12/IL-18, were much higher for the cells taken 2 months after the inset of the Spirulina administration than for the cells taken from the same volunteers before the administration.

2. Materials and methods

2.1. Materials

The functionally active recombinant IL-18 (rIL-18) preparation was a kind gift from Dr. H. Okamura (Hyogo Medical College) and was used for the previously reported functional studies [6]. Recombinant IL-12 (rIL-12) was purchased from Pepro Tech, EC (Rocky Hill, NJ). These samples were stored at -70 °C for >2 days and used within 2 h after thawing followed by centrifugation ($2200 \times g$, 10 min).

The mAb 125-2 H, which recognizes the 'active' form of human IL-18, and the ELISA kit for determination of 'functionally active' IL-18 were purchased from MBL-Immunotech (Nagoya, Japan) [6]. A mAbs against IL-12 receptor beta 1 subunit was purchased from Pharmingen (San Diego, CA). IL-18 receptor consists of alpha and beta chains and only the former is reported to be inducible [7]. pAb against alpha chain of IL-18 receptor was made in our laboratory to assess the level of the alpha chain. Mouse IgG was purchased from Sigma (St. Louis, MO). ELISA kits for determination of human IFN gamma and IL-12 p40 were purchased from Amersham-Pharmacia Biotech., Uppsala, Sweden and Genzyme Techne, respectively. IL-12 p70 was determined by specific ELISA (detection limit <25 pg) (MBL-Immunotech).

Spray-dried powder of *S. platensis* propagated under basic conditions (pH 11) in outdoor open tanks was extracted with water in an autoclave for 1 h at 120 °C. Citric acid was added to the hot water extract to adjust the pH to 4.0 [8]. The water-soluble extract was prepared by removal of insoluble fractions by centrifugation. The soluble extract of Spirulina was condensed for oral administration as described previously [8].

2.2. Administration of Spirulina to volunteers

Fifty milliliters of the Spirulina extract was orally administered every day to 12 healthy male volunteers (No. 1–12) at the age of 40–65 with each subject's informed consent. This trial was approved in the ethic committee in our institute. The appropriate amount of Spirulina for oral administration was determined according to a previous report [9]. The quality and safety of the Spirulina extract used in this trial were published in previous papers [9,10]. Blood samples (20 ml) were drawn before and 1, 2, 4 and 8 weeks after the start of administration. In some experiments, blood samples were prepared after the stop of administration of Spirulina. Blood samples of these of volunteers were collected in our clinic again with each subject's informed consent prior to the investigation procedure.

2.3. Cell preparations from volunteers treated or not treated with Spirulina

Healthy volunteers (age 40–65) continued daily drinking of 50 ml of the Spirulina extract for several

weeks or months before the end of the administration. Blood specimens (20 ml) were withdrawn from these volunteers at intervals to give specific populations of blood cells by the experimental procedures described below. Throughout the present communication, the cells collected from the volunteers just before the start of the administration will be referred to, for brevity, as "the cells before Spirulina". Likewise, the cells collected at the decisive time point during the continuation or after the cessation of the administration, will be referred to as "the cells during Spirulina (weeks or months)" or "the cells after Spirulina (weeks or months)", respectively, where the length of the period of continuation or after the cessation of the administration will be shown in the parenthesis.

Fresh human peripheral blood mononuclear cells (PBMC) were prepared from the heparin-supplemented blood (20 units/ml) of the volunteers treated or not treated with Spirulina. Briefly, PBMC were collected by methylcellulose sedimentation followed by centrifugation on a Ficoll-Hypaque cushion as reported previously [11]. The cells at the interface were recovered and washed twice with RPMI 1640/10% FCS [11]. In some experiments, $CD4^+$ T cells or $CD56^+$ NK cells were isolated by high-gradient magnetic cell separation (MACS) using superparamagnetic streptavidin microparticles for labeling CD4- or CD56-positive cells (Miltenyi Biotec), respectively, according to the manufacturer's booklet. The positive cells were eluted from the columns after removal from the magnetic field. $CD8^+$ T cells were negatively selected as a byproduct after removal of $CD56^+$ NK and $CD4^+$ T cells. Purity of the collected cells was >95%, based on evaluation by flow cytometry. Cells were suspended in RPMI 1640/10% FCS.

2.4. Assay for IFNγ-inducing activity

Aliquots (3×10^5 of PBMC, 1×10^5 of $CD4^+$ T or 1×10^5 of NK cells) of the cell preparations were incubated with IL-12 (10 ng/ml) plus 'active' rIL-18 (40 ng/ml) in 96-well plates, and the supernatants were collected 48 h later. The levels of IFN gamma were determined by sandwich ELISA (Amersham Pharmacia Biotech, Buckinghamshire, UK) [12].

For determination of IL-12 p40, heparinized blood (1 ml each) was used as the cell source, and incubated with 15 μl of vehicle (emulsion buffer, PBS containing 1% Drakeol and 1% Tween 80), Con A (15 μg/ml) or BCG–CWS (15 μg/ml) in 24-well plates at 37 °C [13]. After 20 h, plasma was harvested by centrifugation and aliquots were stored at −70 °C until use. The amounts of IL-12 p40 released into the plasma were determined by ELISA (Genzyme Techne). IL-12 p70 could not be detected by ELISA (Genzyme Techne) (data not shown).

2.5. NK-mediated cytolysis

The cytotoxic activity of NK cells was determined by the ^{51}Cr release assay [14]. Effector cells (E), PBMC or NK cells, were stimulated with IL-18 at a concentration of 20 ng/ml in 100 μl of RPMI-1640/10% FCS for 24 h. The human erythroblastoid cell line K562 (T) had been labeled with $Na_2{}^{51}CrO_4$ for 1 h at 37 °C in RPMI medium. After three washes, labeled target cells were suspended in RPMI 1640/10% FCS. A constant amount of PBMC (1×10^4 cells) were incubated with varying numbers of ^{51}Cr-labeled K562 cells at the E/T ratio of 5–50 in 200 μl of RPMI-1640/10% FCS for 4 h. At timed intervals, cells were spun down and the radioactivity in the supernatants was counted with a gamma counter (Pharmacia, Sweden). The percentage of specific ^{51}Cr release was calculated as follows: %lysis = 100 × [(sample release) − (spontaneous release)]/[(maximal release) − (spontaneous release)]. Spontaneous release was obtained by counting the supernatant of ^{51}Cr-labeled target cells with no effector cells. Maximal release was obtained by counting the supernatant of target cells solubilized by 1% NP-40. The assay was performed in 96-well microplates, and all cultures were set up in triplicate.

2.6. Flow cytometry

Cells (2×10^5) suspended in 50 μl of DPBS containing 0.5% BSA and 0.1% NaN_3(BSA/NaN_3/DPBS) were mixed with 50 μl of EDTA-plasma containing 5 μg of mouse IgG, mAb, rabbit IgG or pAb and incubated for 30 min at 4 °C. After washing with BSA/NaN_3/DPBS, the cells were suspended in 90 μl of BSA/NaN_3/DPBS and incubated with FITC-labeled goat $F(ab')_2$ of anti-mouse or anti-rabbit IgG at 4 °C. After 30 min, the cells were washed twice with DPBS and fixed with paraformaldehyde. The samples were

analyzed on an FACSCalibur (Becton-Dickinson, Franklin Lakes, NJ).

3. Results

3.1. Cells responsible for IFN gamma production in response to IL-12 in volunteers having Spirulina

Four volunteers were randomly chosen for pilot studies to test the immune modulatory effect of Spirulina on lymphocytes by monitoring in vitro IFN gamma production. CD56-positive NK, CD4-positive T and CD8-positive T cells were utilized as sources of IFN gamma. In Fig. 1, the NK cell fraction prepared from ''the cells before Spirulina'', i.e., the cells originating from blood specimen collected from volunteers prior to the start of the Spirulina administration, did not respond to IL-12 added in vitro. In contrast, ''the cells during Spirulina (2 months)'', or the same cell preparations from the same volunteers daily treated with Spirulina for 2 months, produced significant amounts of IFN gamma in response to added IL-12. The IL-12-induced IFN gamma was also observed with ''the cells after Spirulina (3 months)'' or with the cells originating from the same volunteers 3 months after the cessation of the Spirulina therapy. $CD4^+$ and $CD8^+$ T cells barely responded to added IL-12 throughout the period we tested except one case (volunteer No. 2), whose $CD8^+$ T cells exhibited a significant level of IFN gamma in ''cells after Spirulina (3 months)'' in response to IL-12. Thus, NK cells are mainly involved in IL-12-dependent IFN gamma production.

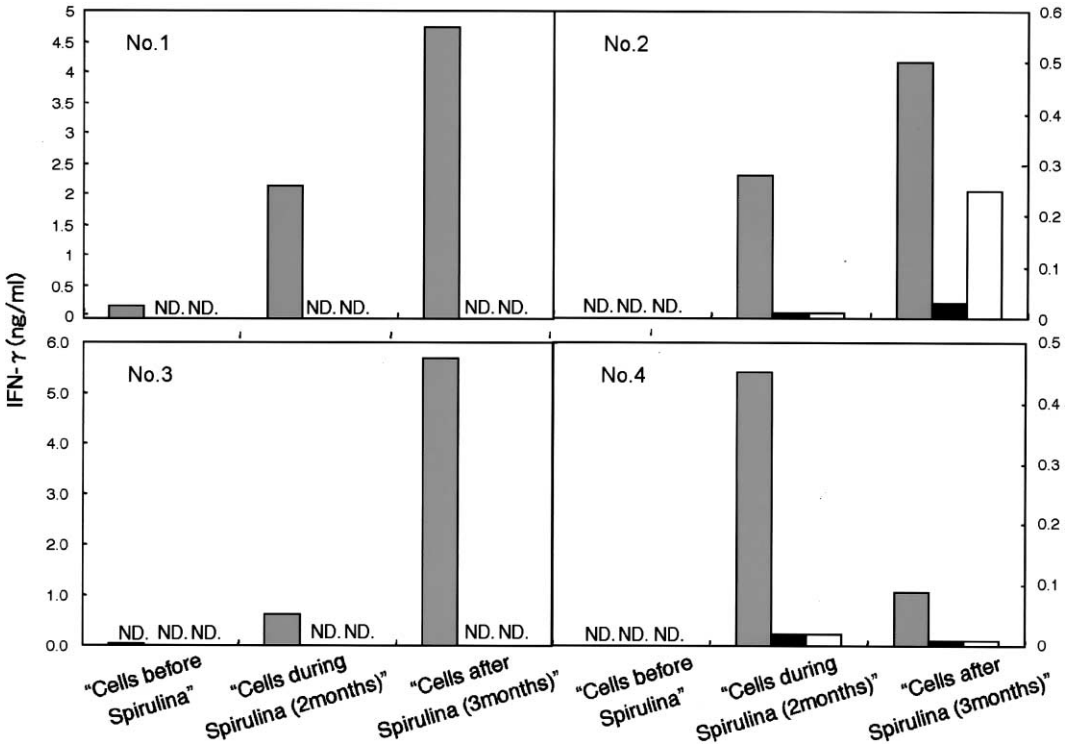

Fig. 1. IL-12-mediated IFN gamma production by NK, CD8-positive, and CD4-positive T cells isolated from Spirulina-treated subjects. Each lymphocyte population was prepared as described in Materials and methods. These lymphocyte subsets were stimulated with 10 ng/ml of IL-12. The levels of IFN gamma in the supernatants were measured by ELISA at the indicated time points. The levels of IFN gamma were calculated and represented as ▒ (NK cells), ■($CD4^+$ T cells) and □($CD8^+$ T cells).

3.2. Effect of IL-18 on IL-12-mediated IFN gamma production in lymphocytes from volunteers having Spirulina

The cooperative effect of IL-18 on IL-12-dependent IFN gamma production by lymphocytes was next examined with variable doses of IL-18 (Fig. 2). Additional four volunteers were chosen for this study. NK, $CD4^+$ and $CD8^+$ cells in "the cells before Spirulina" failed to effectively produce IFN gamma regardless of the amount of IL-18 added. NK cells of "the cells during Spirulina (2 months)" acquired the capacity to effectively produce IFN gamma in response to IL-12 plus IL-18. $CD8^+$ T cells were competent to produce IFN gamma in response to IL-12 plus IL-18, although $CD4^+$ helper T cells were far less or barely responsive to IL-18 compared to $CD8^+$ cells at this stage. In "the cells after Spirulina (3 months)", the IFN gamma-inducing abilities of these lymphocyte subsets had returned to close to the initial levels, except in one case (subject No. 1).

3.3. IFN gamma-producing profiles in in vitro PBMC from volunteers having Spirulina for short terms

Sequential studies shown in Figs. 1 and 2 suggested that "the cells during Spirulina (2 months)" exert potent activity to produce IFN gamma in an IL-12/IL-18-dependent manner. We then examined the cells with short-term exposure to administered Spirulina. Four alternative volunteers were recruited for this study. PBMC were used in this study since NK cells were

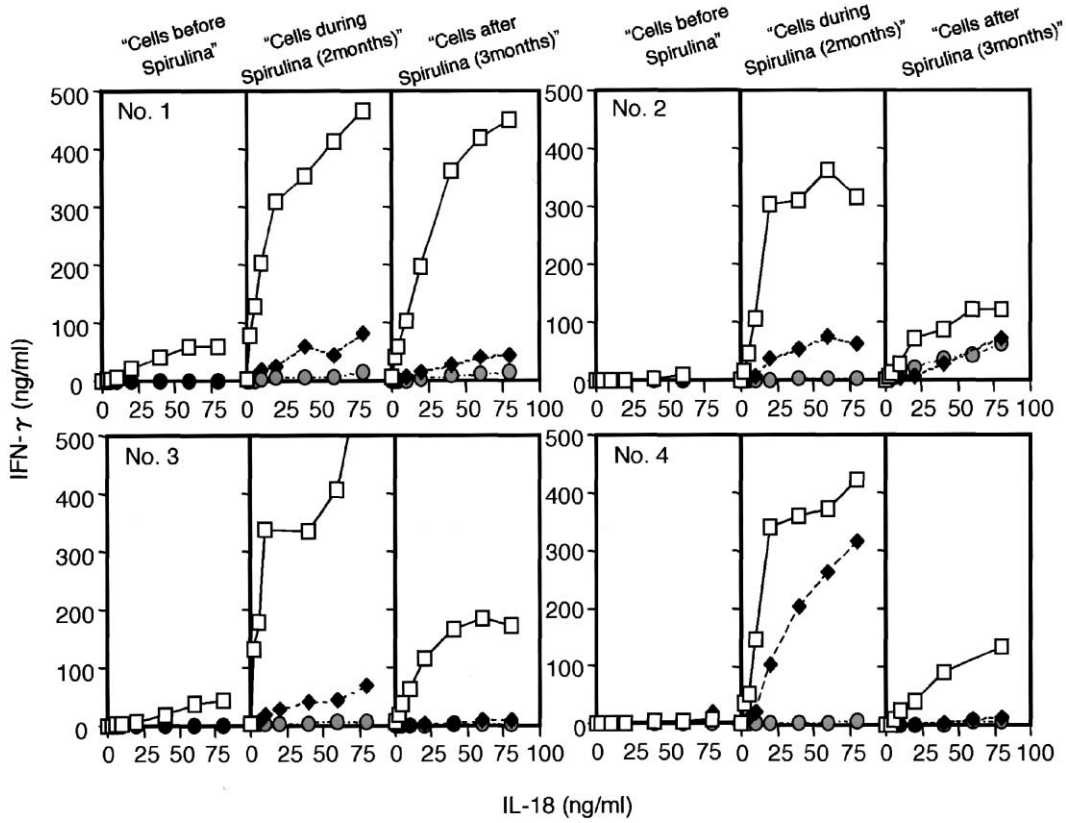

Fig. 2. Dose of IL-18 critically affects IL-12-mediated IFN gamma production by lymphocyte subsets isolated from Spirulina-treated subjects. Blood samples were collected from four subjects treated with Spirulina as indicated. NK cells (□), $CD4^+$ T cells (●) and $CD8^+$ T cells (◆) were stimulated with varying concentrations of IL-18 and 10 ng/ml of IL-12. The cells were allowed to stand for 48 h at 37 °C, and the supernatants were collected for determination of IFN gamma, which was measured by sandwich ELISA as in Fig. 1.

found to be a major source of the generated IFN gamma. The doses of IL-18 were reduced to <20 ng for this study based on the results of Fig. 2 in which linear dose response curves were obtained with 0–25 ng of IL-18. The results are shown in Fig. 3 where "the cells during Spirulina (1, 2 and 4 weeks)" were employed for analysis of IFN gamma-inducing capacity. Increased IFN gamma production was usually observed from 1 week after administration in these cases. It is notable, however, that individual differences were found in IFN gamma-induction by PBMC in response to IL-12/IL-18. Stable and reproducible production of IFN gamma in PBMC by function of IL-12/IL-18 appeared not to be in parallel with the term of Spirulina administration in these volunteers at least until 4 weeks. Hence, >4 weeks administration of Spirulina may be required for sufficient responses to IL-12 and IL-18 to produce IFN gamma in lymphocytes.

Spirulina-mediated augmented IFN gamma production by PBMC usually continued for >4 weeks and then decreased to the background levels >5 weeks after the end of administration (data not shown).

To confirm the administration terms for the effect of Spirulina, we next use the major target, purified NK cells, instead of PBMC, and compare the results with those in Figs. 1 and 2. Reproducible IFN gamma production was observed with "the NK cells after Spirulina (4 weeks)" (Fig. 4a) and to a lesser extent with "the NK cells after Spirulina (2 weeks)" (data

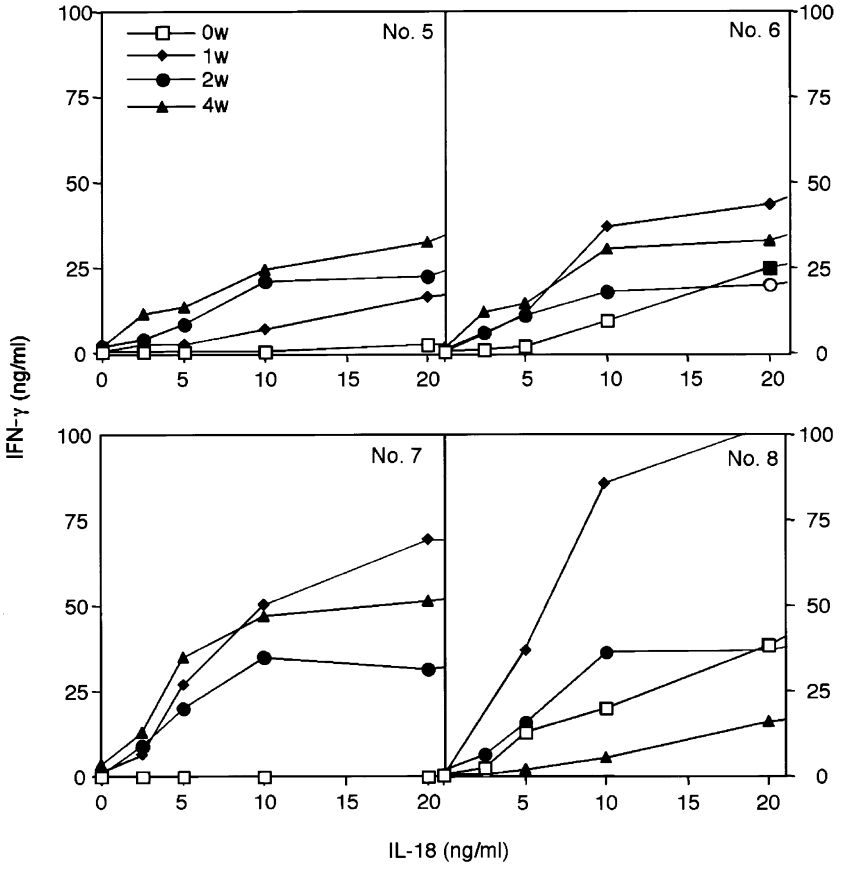

Fig. 3. IL-12/IL-18-dependent IFN gamma production by human PBMC prepared from Spirulina-treated subjects. Blood samples were prepared from volunteers treated with Spirulina for the indicated length of periods. PBMC were isolated as described in Materials and methods. PBMC (1×10^4 cells) were stimulated with the indicated concentrations of IL-18 together with 10 ng/ml of IL-12 in 96-well flat-bottomed plates for 48 h at 37 °C. The concentrations of IFN gamma released into the culture medium are plotted as a function of IL-18 concentrations used.

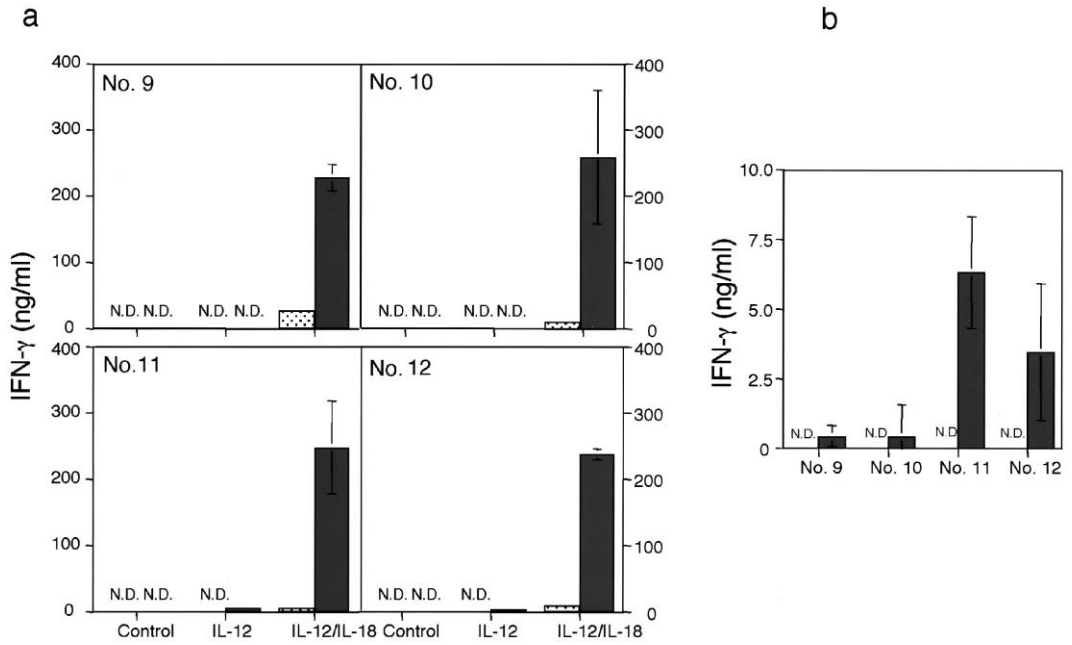

Fig. 4. IL-12/IL-18-dependent IFN gamma production by purified NK cells of Spirulina-administrated volunteers. Blood samples were prepared from volunteers having Spirulina for 4 weeks. NK cells were purified as described in Materials and methods. NK cells (1×10^4 cells) were stimulated with 20 ng/ml of IL-18 and 10 ng/ml of IL-12 (Panel a) or 10 ng/ml of IL-12 alone (Panel b) in 96-well flat-bottomed plates for 48 h at 37 °C. The IFN gamma released into the culture medium are measured by ELISA. Control, NK cells with no stimulation; N.D., not detected. ⬚, "NK cells before Spirulina";■, "NK cells after Spirulina".

not shown). The IL-12 (without IL-18)-mediated augmentation of IFN gamma production was subtly observed in "the NK cells after Spirulina (4 weeks)" (Fig. 4b), also. These results again reinforced the finding that >4 weeks administration of Spirulina should be the essential requisites for Spirulina-mediated immune activation and that IL-18 markedly potentiates the IL-12-mediated IFN gamma production in "the NK cells after Spirulina".

3.4. Enhancement of NK cytolytic activity by Spirulina

NK cytotoxic activity was determined using ^{51}Cr-labeled K562 as the target cells (Fig. 5). PBMC were collected from the four volunteers shown in Fig. 3 before and after taking Spirulina. %Cytotoxicity was determined at E/T ratios of 5–50 as previously described [14]. NK cytolytic activity was enhanced in "cells during Spirulina" in two cases, while in the remaining cases (Nos. 11 and 12) virtually no enhancement was observed. The levels of NK activity are known to vary among individuals. NK cells with high cytotoxic activity (Nos. 11 and 12) may not respond to administered Spirulina. Unexpectedly, neither IL-18 (Fig. 5) nor IL-12 (not shown) significantly increased cytotoxicity in either case, which is inconsistent with a previous report using mouse IL-12/IL-18 [7]. NK cytotoxity may be another marker of the effects of Spirulina.

3.5. Spirulina-mediated augmentation of IL-12 p40 production by BCG–CWS-treated PBMC

BCG–CWS is a ligand for TLR2 and TLR4 in myeloid cells. In vitro addition of BCG–CWS to monocytes (expressing TLR2/4) or PBMC results in induction of IL-12 p40, which can be detected in the culture medium of cells [13]. The effect of Spirulina on BCG–CWS-mediated IL-12 p40 production was tested using PBMC prepared from the volunteers (Fig. 6). Throughout the pre- and post-administration periods, no IL-12 p40 was detected in control samples, which contained vehicle only. Con A often induced the minimal production of IL-12 p40 in PBMC, but in

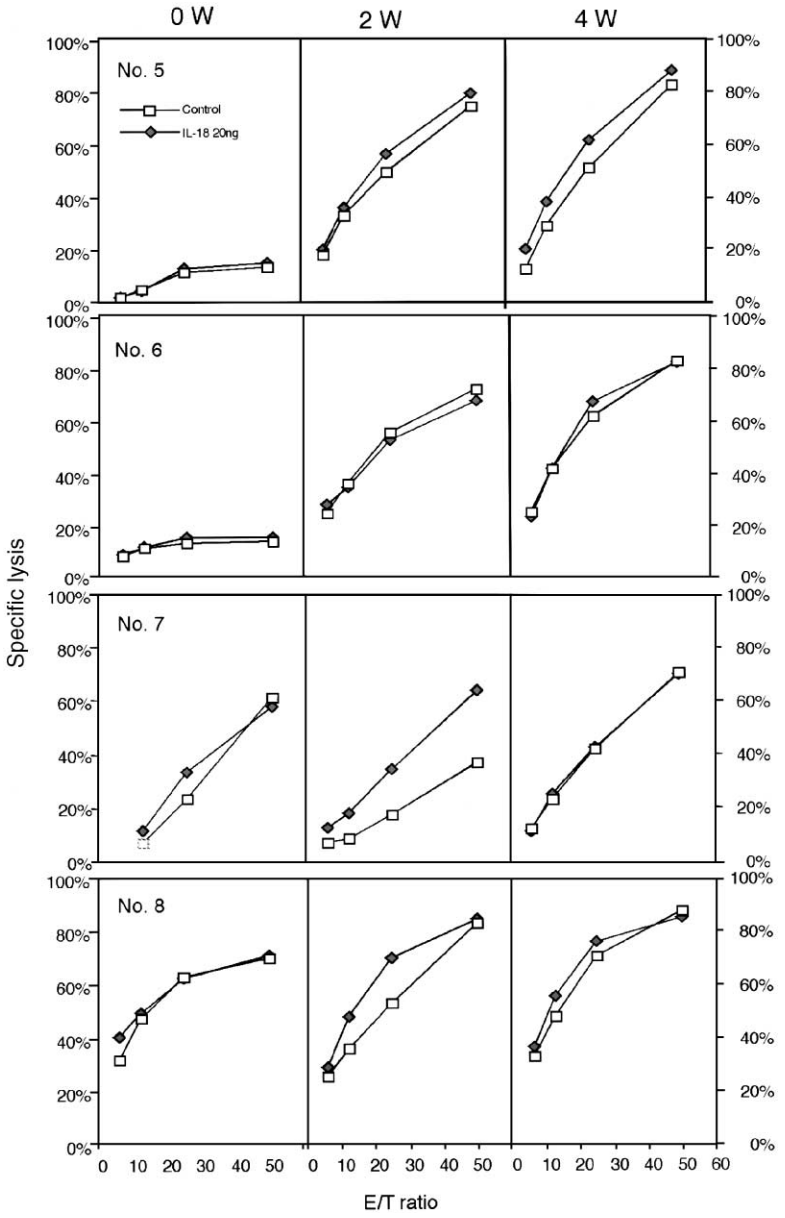

Fig. 5. Administration of Spirulina extract enhances NK cytotoxicity. PBMC were prepared as a source of NK cells (E, effector cells). PBMC (1×10^4 cells/0.1 ml) were plated in 96-well plates in triplicate, and cultured with or without IL-18 (20 ng/ml) for 24 h at 37 °C. Then, ^{51}Cr-labeled K562 cells (T, target cells) were added to each well at the indicated E/T ratios, and the plates were incubated for an additional 4 h at 37 °C. The levels of ^{51}Cr released from the target cells were measured with a gamma-counter. Data represent the means of triplicate observations.

most cases no up-regulation of IL-12 by Spirulina administration was observed. In contrast, IL-12 p40 production by BCG−CWS was markedly augmented by administration of Spirulina. However, we could not detect IL-12 p70 by specific ELISA (detection limit <25 pg) under any condition (data not shown), suggesting that a p40 complex such as IL-23 or p40 dimer is produced in immune competent cells. The p40

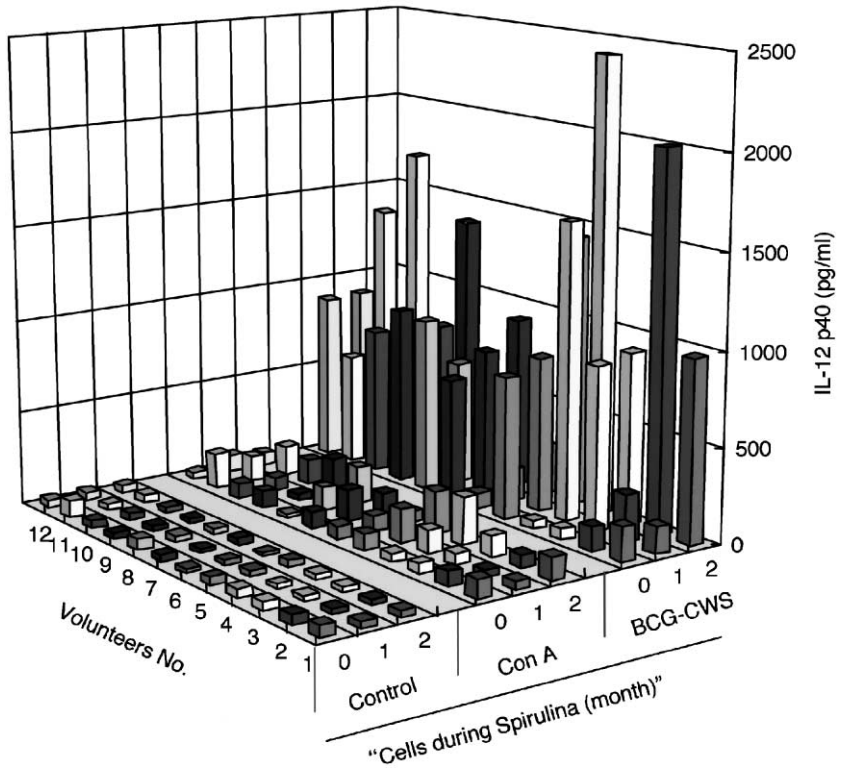

Fig. 6. Administration of Spirulina extract enhances BCG–CWS-mediated IL-12 p40 production from peripheral blood cells. Peripheral blood samples were collected from volunteers as indicated. Aliquots (1 ml) of samples were stimulated with vehicle only (indicated as Control), Con A (15 μg/ml) (indicated as Con A) or BCG–CWS (15 μg/ml) (indicated as BCG–CWS) for 24 h at 37 °C. Then, the levels of IL-12 p40 in the plasma supernatants were determined by ELISA as in Materials and methods.

complexes are produced in mature myeloid cells involved in innate immune responses [13]. Thus, myeloid lineage cells, most likely monocytes or macrophages, should be first targets of Spirulina, which enhances TLR-mediated cytokine induction in innate immune competent cells.

3.6. The receptor levels of IL-12/IL-18 do not contribute to Spirulina-augmented IFN gamma production by NK cells

To see the molecular basis for enhanced IFN gamma production by NK cells after Spirulina administration, the expression levels of IL-12 receptor (beta1 subunit) and the alpha chain (inducible element) of IL-18 were tested with NK cells by flow cytometry. Representative profiles of two samples are shown in Table 1. Both IL-12 receptor and IL-18 receptor were not inducible through Spirulina administration for 4 weeks. Thus, we hypothesized that the assembly of receptor complexes rather than up-regu-

Table 1
Flow cytometric analysis of IL-12/IL-18 receptors on "NK cells before and after Spirulina"

Receptors	Volunteers	Mean fluorescence shift[a]	
		NK cells before Spirulina[b]	NK cells after Spirulina[b]
IL-12R[c]	No. 9	0.75	0.89
	No. 10	0.24	0.32
IL-18R[d]	No. 9	39.06	42.06
	No. 10	37.10	40.66

[a] Determined by flow cytometer.
[b] NK cells were harvested from blood samples of volunteers before and 4 weeks after administration of Spirulina.
[c] Determined with mAb against IL-12 receptor beta 1 subunit.
[d] Determined with pAb against IL-18 receptor alpha subunit.

lation of receptor components would be important for the receptor functions of these initial cytokines [15]. Spirulina may participate in reconstitution of the functional receptor complex formation.

4. Discussion

The goal of this study was to establish the immune-potentiating function of the hot water extract of Spirulina in humans. The targets of Spirulina, although not specified as primary or secondary, are monocytes and NK cells. In NK cells, both IFN gamma and cytolysis are up-regulated by Spirulina in 50% of subjects, and IL-18 levels critically affect the yield of IFN gamma. In myeloid cells, Spirulina may exhibit an additive effect on TLR-mediated cytokine production pathways.

It is generally accepted that IL-12 is secreted from myeloid cells to induce IFN gamma in NK cells [3,4]. If this is the case, Spirulina might first act on monocytes to induce IL-12, which in turn drives NK cells to produce IFN gamma. It is noteworthy, however, that purified NK cells depleted of myeloid (CD14-positive) cells still exhibited the high capacity to produce IFN gamma in vitro only after oral administration of Spirulina. Thus, certain Spirulina components should be internalized via the intestine/colon into the blood stream for prestimulation of monocytes in these volunteers. Studies are currently in progress in our group to identify these components, which is the most tantalizing issue in this context.

Structural investigation of Spirulina indicated that it contains glycolipids, such as O-β-D-galactosyl-(1-1′)-2′,3′-di-O-acyl-D-glycerol, which possess fatty acid moieties, palmitic acid and linoleic or linolenic acids [16]. It is currently accepted that lipid moieties of microbes often serve as ligands of Toll receptors. Thus, it is not surprising that Spirulina glycolipids serve as Toll ligands for stimulation of TLR2 and TLR4 together with BCG–CWS [11,17]. Although most cell wall components of microbes are insoluble in water, that of Spirulina can be disrupted mechanically [5]. It remains to be tested that some fragmented cell wall components are readily extractable in the hot water-soluble fraction. Furthermore, absorption efficacy of the relevant components in the hot water extract of Spirulina has not been determined. These points again need to be addressed.

There have been a number of reports concerning the functions of Spirulina in rodents. Zhang et al. [18] reported that the hot water extract of Spirulina showed significant hydroxy radical scavenging activity in mice. In another study, the methanolic extract of Spirulina showed weak anti-oxidant activity in rats [19]. Several papers suggested that the relevant substance is phycocyanin in Spirulina [19,20]. Using an experimental squamous cell cancer model of hamsters, administration of Spirulina extract has been reported to result in total tumor regression in 30% of animals [21]. Intraperitoneal injection of a polysaccharide extract of Spirulina was shown to inhibit proliferation of ascitic hepatoma cells in mice [22]. Calcium Spirulan, a polysaccharide isolated from *S. platensis*, inhibited lung metastasis of mouse B16 melanoma cells by i.v. administration [23]. Hence, phycocyanin and water-soluble components, presumably polysaccharides, may be responsible for anti-oxidant and anti-cancer effects in rodents.

In humans, rough Spirulina was reported to alleviate oral leukoplakia in pan tobacco chewers [24]. Water-soluble Spirulina components also inhibited the replication of human viruses, herpes simplex, cytomegalo, measles, mumps and influenza A viruses [25]. The water extract also inhibited HIV replication in human T cells, T cell lines and Langerhans cells [26]. Again, water-soluble polysaccharides appear to participate in the anti-oxidant, anti-cancer and anti-viral effects of Spirulina. These reports, together with our finding of modulation of Toll signaling by the water extract in concert with immune modulation (Hazeki, Matsumoto and Seya, unpublished), imply that the target of Spirulina-mediated immune activation is the innate immune system.

Plants have unique constituents from the viewpoint of animals, all of which including vertebrates and invertebrates possess a host defense system consisting of bacterial receptors [27]. Toll is a representative of such receptors. Spirulina is a cyanobacterium species, which is regarded as a foreign material in the side of animals. Chlorella [28], mushrooms [29,30] and agarics [31] are also categorized as plants and have been suggested to have immune potentiating abilities similarly to Spirulina. Although there is little scientific background or results of physicochemical analyses to support these activities, it is becoming clear that animal cells, particularly those of the myeloid lineage,

are equipped with a repertoire of microbe-recognizing receptors [32]. It is likely that some components of these materials can stimulate certain microbe receptors. Immune potentiation is representative of the anticancer and anti-viral effects of Spirulina. In fact, we have obtained evidence that a partially purified material of Spirulina provokes NF-κB and MAPK in human and mouse macrophages (Hazeki, Hirahashi, Masuda, Matsumoto and Seya, unpublished observation). Clarification of the mechanism of immune potentiation by Spirulina would help to complete the outline of the primary or ancient host defense system and to understand its significance with regard to maintenance of health in humans.

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