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Immunological Effect of Active Hexose Correlated Compound (AHCC) in Healthy Volunteers: A Double-Blind, Placebo-Controlled Trial

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The aim of this study was to evaluate the effects of active hexose correlated compound (AHCC) intake on immune responses by investigating the number and function of circulating dendritic cells (DCs) in healthy volunteers. Twenty-one healthy volunteers were randomized to receive placebo or AHCC at 3.0 g/day for 4 wk. The number of circulating cluster of differentiation (CD)11c$^+$ DCs (DC1) and CD11c$^-$ DCs (DC2) were measured. Allogeneic mixed-leukocyte reaction (MLR) was performed. Natural killer (NK) cell activity and the proliferative response of T lymphocytes toward mitogen (phytohemagglutinin [PHA]) were measured. We also measured cytokine production stimulated by lipopolysaccharide [interleukin (IL)-2, IL-4, IL-6, IL-10, interferon gamma-γ, tumor necrosis factor-α). The AHCC group ($n = 10$) after AHCC intake had a significantly higher number of total DCs compared to that at baseline and values from control subjects ($n = 11$). The number of DC1s in the AHCC group after intake was significantly higher than at baseline. DC2s in the AHCC group were significantly increased in comparison with controls. The MLR in the AHCC group was significantly increased compared to controls. No significant differences in PHA, NK cell activity, and cytokine production were found between groups. AHCC intake resulted in the increased number of DCs and function of DC1s, which have a role in specific immunity.

INTRODUCTION

Recently, the incidence of malignant tumor has been increasing consistently in Japan (1). The development of imaging modalities has enabled the diagnosis of malignant tumor at an early stage with relative ease. However, it is still difficult to control disease progression of advanced cancer.

Although some current cancer treatments can induce remission, most of these tumors ultimately relapse and cannot be cured. Many attempts have been made to treat cancer by stimulating the patient's immune system. Several biological response modifiers (BRMs) have been developed—such as BCG, Picibanil, polysaccharide-K (PSK), lentinan, interferon (IFN), and interleukin (IL)-12—but the clinical efficacy of these substances has not been clearly confirmed (2–5).

Active hexose correlated compound (AHCC; Amino UP Chemical Co., Ltd., Sapporo, Japan) is a functional food that is extracted from several species of Basidiomycetes mushrooms (6,7). We have shown clinically that AHCC intake resulted in improved liver function, prevented the recurrence of hepatocellular carcinoma (HCC) after resection, and prolonged survival of postoperative HCC patients without any adverse effects (8). However, there has been no report on the functional effect of AHCC on the immune response in humans.

Dendritic cells (DCs) are the most potent antigen-presenting cells (9) capable of priming tumor-specific T cells, and their use in cancer immunotherapy appears to be a promising way to elicit and expand efficient antitumor immune responses (10,11). Herein, we report the results of a randomized controlled trial to evaluate the effects of AHCC intake on immune responses by investigating the number and function of circulating DCs in healthy volunteers.

METHODS

This preliminary study in a double-blinded randomized fashion was approved by the Institutional Review Board at the Kansai Medical University, Osaka, Japan. Informed consent was obtained from each healthy volunteer in accordance with the provisions of the Declaration of Helsinki. Volunteers were excluded if they had malignant tumor, viral hepatitis, uncontrolled diabetes mellitus, and chronic heart dysfunction. Before screening physical and blood examinations, subjects were randomized to receive placebo or AHCC at 3.0 g/day for 4 wk. Blood samples were collected in heparinized syringes in the morning after an overnight fast, and various values were determined at baseline and 4 wk later. The number of circulating CD11c$^+$ DCs (myeloid DC population; DC1), CD11c$^-$ DCs (lymphoid DC population; DC2), natural killer (NK) cells, and CD4$^+$/CD8$^+$

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T lymphocytes were measured in each sample by flow cytometric analysis. To assess immune function, the allogeneic (allo-) mixed-leukocyte reaction (MLR; allo-MLR) was determined. NK cell activity and the proliferative response of T lymphocytes toward mitogen (phytohemagglutinin [PHA]) were measured. We also measured serum hormone levels (thyroid-stimulating hormone, 3,5,3′-triiodothyronine, thyroxine, and estradiol) and cytokine concentrations (IL-2, IL-4, IL-6, IL-10, IFN-γ, tumor necrosis factor [TNF]-α). The duration of the study was 7 mo.

Reagents

The culture medium for all experiments consisted of RPMI 1640 supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-mercaptoethanol (Sigma, St. Louis, MO), and heat-inactivated 10% fetal bovine serum.

The phenotypes of peripheral blood mononuclear cells (PBMCs) were determined by two- and three-color flow cytometric analysis using monoclonal antibodies (mAbs) that were directly conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), or PE cyanin 5.1 (PE-Cy5).

Cells were stained with the following mAbs: PE-Cy5-conjugated anti-human leukocyte antigen(HLA)-DR; a mixture of FITC-conjugated anti-cluster of differentiation (CD)3, CD14, CD15, CD16, CD19 so-called lineage cocktail (Lin) and PE-conjugated anti-CD11c mAbs for DCs; PE-Cy5-conjugated anti-CD3, FITC conjugated anti-CD4, and PE-conjugated anti-CD8 for T lymphocytes; and PE-conjugated anti-CD14 and FITC-conjugated anti-CD56 for NK cells.

All antibodies were obtained from PharMingen (San Diego, CA). The isotype controls, anti-immunoglobulin G1 was also obtained from PharMingen.

Flow Cytometry (FCM)

PBMCs were prepared by Lymphoprep (Nycomed Pharma, Oslo, Norway) gradient centrifugation of heparinized peripheral blood and then washed in phosphate-buffered solution supplemented with 1% fetal bovine serum and 0.1% NaN_3. PBMCs were incubated for 30 min at 4°C with the mAbs. The stained cells, as mentioned above, were analyzed using a FACScan® (Becton Dickinson, Sunnyvale, CA). At least 100,000 events were counted for each mononuclear fraction by FACScan. The typical forward and side scatter gates for DCs and lymphocytes in combination with FITC-, PE-Cy5-, and PE-conjugated mAbs were set to exclude any dead or contaminating cells from the analysis. The following DCs and lymphocyte subsets were analyzed by 2- and 3-color FCM: DC1, myeloid-lineage dendritic cells ($CD11c^+/lin^-/DR^+$); DC2, lymphoid-lineage DCs ($CD11c^-/lin^-/DR^+$); helper T lymphocytes ($CD3^+/CD4^+$); cytotoxic T lymphocytes ($CD3^+/CD8^+$); and NK cells ($CD14^-/CD56^+$). The number of PBMCs per mm^2 was counted under a microscope, and viable cells were determined by the trypan-blue dye exclusion test. Absolute numbers of DCs and lymphocytes were calculated from the number of PBMCs per milliliter of blood multiplied by the percentage of DCs and lymphocytes.

Typical FCM profiles in the AHCC group are shown in Fig. 1. Region R1 includes lymphocytes and monocytes but excludes debris. DCs were detected in region R2 as the population of $Lin^-/HLA\text{-}DR^+$ cells. Two subsets of DCs were identified within the $Lin^-/HLA\text{-}DR^+$ population, which was based on differential expression of CD11c: DC1 ($CD11c^+$ population; region R3) and DC2 ($CD11c^-$ population; region R4). The NK cell fraction was gated in the $CD14^-/CD56^+$ population (region R5). $CD3^+/CD4^+$ T lymphocytes were detected in region R6, and $CD3^+/CD8^+$ T lymphocytes were detected in region R7.

Cell Surface Staining

For surface marker analysis, PBMCs were incubated for 30 min with 4°C with FITC, PE, PE-Cy5, or ECD mAbs conjugated to Lin, HLA-DR, CD11c, and CD40 or CD86. The stained cells, as mentioned above, were analyzed using an EPICS® XL-MCL (Coulter, Hialeah, FL).

DCs Isolation From Peripheral Blood

DCs from peripheral blood were enriched as described elsewhere (12–15). Briefly, PBMCs were incubated with anti-CD3 and anti-CD14 mAbs for 30 min on ice, and cells binding to these mAbs were removed using sheep antimouse Ig-coated magnetic beads (M-450; Dynal, Oslo, Norway). The $CD3^-/CD14^-$ cells were further incubated with CD4-conjugated microbeads (Miltenyi Biotec., Bergisch Gladbach, Germany), and the $CD4^+$ cells were then enriched by passing them through a Mini MACS® magnetic separation column (Miltenyi Biotec.). By using this protocol, the percentage of DCs (originally <1% of total PBMCs) increased up to 20–50%, which was dependent on the individuals.

The resultant DC-enriched population ($CD4^+/CD3^-/CD14^-$ cells) was stained with PE-conjugated anti-CD11c mAb, FITC-conjugated lineage cocktail, and PE-Cy5-conjugated anti-HLA-DR mAb. The stained cells were then analyzed and sorted by an EPICS ELITER® flow cytometer (Coulter, Hialeah, FL). Purity of the sorted cells was always greater than 96% by reanalysis using a FACScan (Becton Dickinson). Consequently, two phenotypically distinct fractions of DC1s and DC2s were collected and used in MLR.

Allo-MLR of Circulating DC1

The cDC1s isolated from peripheral blood were examined for their stimulating capacity against allogeneic T lymphocytes in a standard MLR (13). DC1s were irradiated at 15 Gy (Gamma Cell, Nordion, Ontario, Canada). Graded doses of DC1 were cocultured with 2×10^5 allogeneic T lymphocytes (collected by magnetic beads as $CD3^+$ cells) in 200 μl of culture medium in 96-well culture plates for 4 days.

For the maintenance of DCs, GM-CSF was added to the culture medium for DC1s. Cells were pulsed with 1 μCi of ^{3}H-thymidine during the last 16 h of the culture period. They

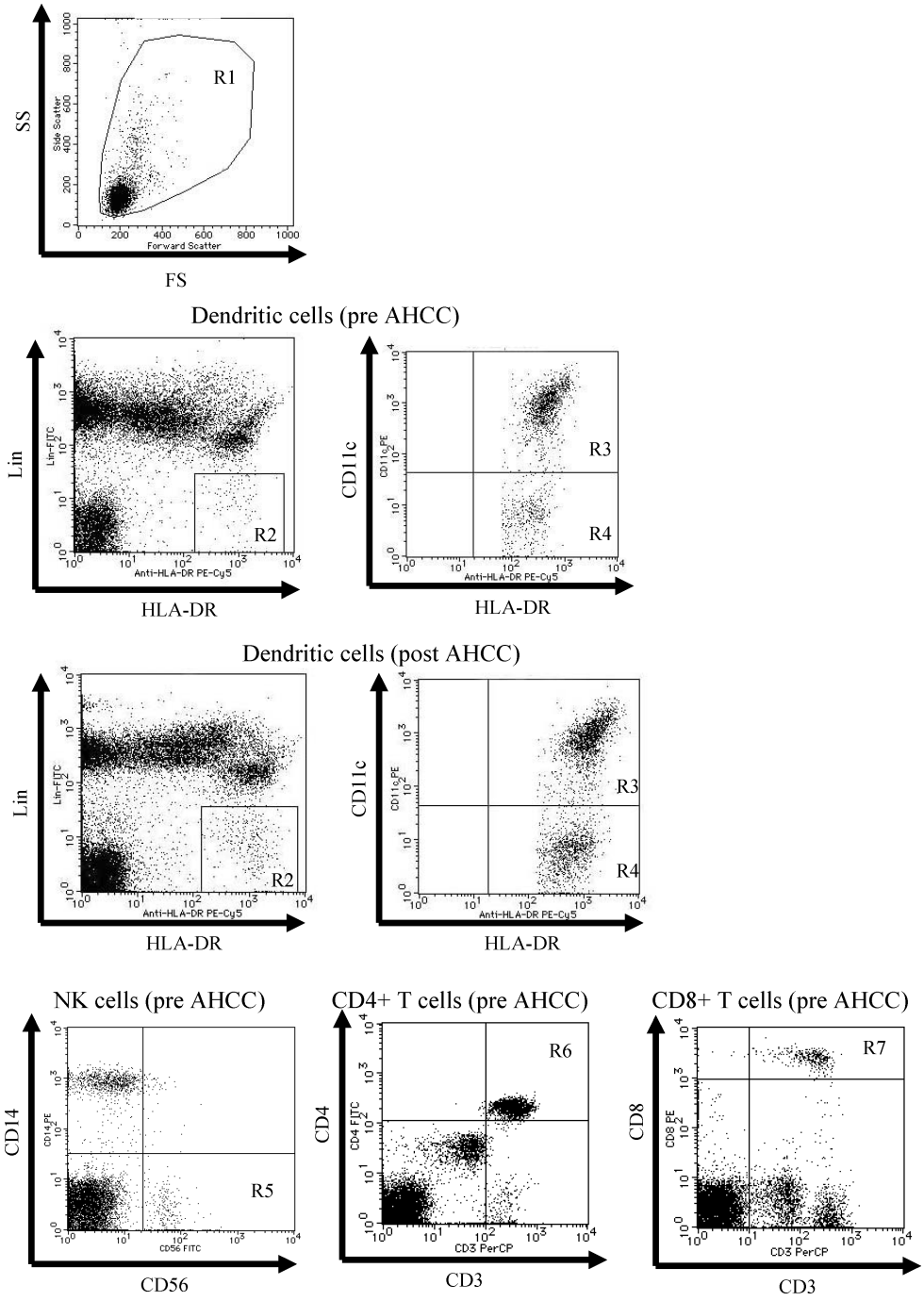

FIG. 1. Flow cytometric analyses of peripheral blood mononuclear cells (PBMCs) by FACScan. In each sample, a total of 300,000 cells were analyzed. Typical profiles of 1 subject in the active hexose correlated compound (AHCC) group are shown. Using light scatter properties, region R1 was defined to include lymphocytes and monocytes and exclude debris. Dendritic cells (DCs) were detected in region R2 as the population of lineage cocktail anti-human leukocyte antigen(Lin)-DR (Lin^-/$HLA\text{-}DR^+$) and divided into 2 fractions by the expression of cluster of differentiation (CD)11c [region R3: $CD11c^+$ DC (DC1); and region R4: $CD11c^-$ DC (DC2)]. The natural killer (NK) cell fraction was gated in the $CD14^-$/$CD56^+$ population (region R5). $CD3^+$/$CD4^+$ T lymphocytes were detected in region R6, and $CD3^+$/$CD8^+$ T lymphocytes were detected in region R7. SS, slide scatter; FS, forward scatter; FITC, fluorescein isothiocyanate; PE, R-phycoerythrin; PE-Cy5, PE cyanin 5.1.

TABLE 1
Characteristics of Study Subjects[a]

	AHCC Group ($n = 10$)	Control Group ($n = 11$)	P Value
Age (yr)	59.3 ± 4.4	60.2 ± 5.5	0.621
Gender (Male:Female)	3:7	5:6	0.659
Height (cm)	158.6 ± 4.4	159.5 ± 8.1	0.901
Weight (kg)	57.1 ± 4.8	60.5 ± 7.3	0.385
BMI (kg/m^2)	22.7 ± 1.0	23.9 ± 2.8	0.099
PNI	54.1 ± 2.9	53.1 ± 5.1	0.870

[a]Continuous variables are expressed as mean ± SD. Abbreviations are as follows: AHCC, active hexose correlated compound. BMI, body mass index; PNI, prognostic nutritional index. BMI equals a person's weight in kilograms divided by height in meters squared (BMI = kg/m^2). PNI = 10 × Serum Alb (g/dl) + 0.005 × total lymphocyte count (/μl).

were harvested onto glass fiber filter papers using an automated harvester, and cell-bound radioactivity was counted in a liquid scintillation counter.

Proliferative Response of T Lymphocytes Toward Mitogen (PHA)

The in vitro proliferative capacity of lymphocytes toward mitogen (PHA) was quantified using standardized assay formats (BAG, Lich, Germany). Lymphocytes were incubated with 1 μCi of 3H-thymidine during the last 6.5 h of the culture period. Cell-bound radioactivity was counted in a liquid scintillation counter. Results were presented as the mean counts per minute (cpm) of triplicate cultures.

NK Cell Activity

NK cell activity was measured with the standard 4-h ^{51}Cr-release assay. Each 100 μl of PBMCs (effector) and K562 cells (target) was combined at an effector-to-target (E:T) ratio of 50:1 in 96-well microtiter plates. Maximum release was determined by the addition of detergent to K562 cells. Spontaneous release was measured by culturing K562 cells without PBMCs. After a 4-h incubation at 37°C in air with 5% CO2, supernatants were harvested, and radioactivity was assessed using a gamma counter (Aloka, Tokyo, Japan). All assays were performed in triplicate, and the value was calculated as the mean of triplicate cultures. The percentage cytotoxicity was determined as (experimental cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm) ×100. Spontaneous release was less than 10% of the maximum release in all experiments. Because of the limited number of samples, only the measurement at an E:T ratio of 50:1 was performed in this experiment.

Cytokine Production

Briefly, blood samples (2 ml) were drawn directly into heparinized control tubes (15 U/ml final). One ml of blood from each tube was transferred into an lipopolysaccharide (LPS) tube containing 100 EU LPS per tube. The LPS was isolated from Escherichia coli 055B5 (16,17). LPS tubes were incubated for 4 h at 37°C in a programmable incubator on a rocking platform.

Cytokine concentrations were measured with the CBA kit (BD PharMingen, Franklin Lakes, NJ), according to the manufacturer's manual as previously described, with modification of data analysis to use GraphPad Prism software (GraphPad Software, San Diego, CA). These assays are multiplexed such that numerous substances are measured simultaneously in a single well.

The CBA assay consists of a mixture of 6 types of beads uniform in size but containing different fluorescence intensities of a red-emitting dye. Each series of beads is coated with a mAb against a single cytokine (IL-2, IL-4, IL-6, IL-10, IFN-γ, or TNF-α), and the mixture of beads detects 6 cytokines in 1 sample. The captured cytokines are detected via direct immunoassay using 6 different antibodies coupled to phycoerythrin PE. The calibrator's standards ranging from 0 to 5,000 pg/ml for the assay system capture Ab-bead reagent; detector Ab–PE reagent are mixtures of all 6 cytokines. After fluorescence intensity calibrations and electronic color compensation procedures, standard and test samples were analyzed with FACScan. Six standard curves are thus obtainable from 1 set of calibrators; 6 results are obtained from each calibration point. Data were collected using EXPO analysis software (Beckman Coulter, Fullerton, CA).

Statistical Analysis

Numerical values are given as the mean ± SD. All data were analyzed using the StatView 5.0 statistical software package (Abacus Concepts, Inc., Berkeley, CA). Comparisons of some parameters among AHCC and control groups were made using the Mann–Whitney test and Wilcoxon rank test. Statistical significance was determined at $P < 0.05$.

RESULTS

Twenty-one healthy volunteers underwent a screening physical and blood examination and were randomized to either the placebo (n = 11) or AHCC (n = 10) group. There were no significant differences in age or gender between the two groups (Table 1). None of the subjects withdrew during the study period. Results of blood examinations were within normal

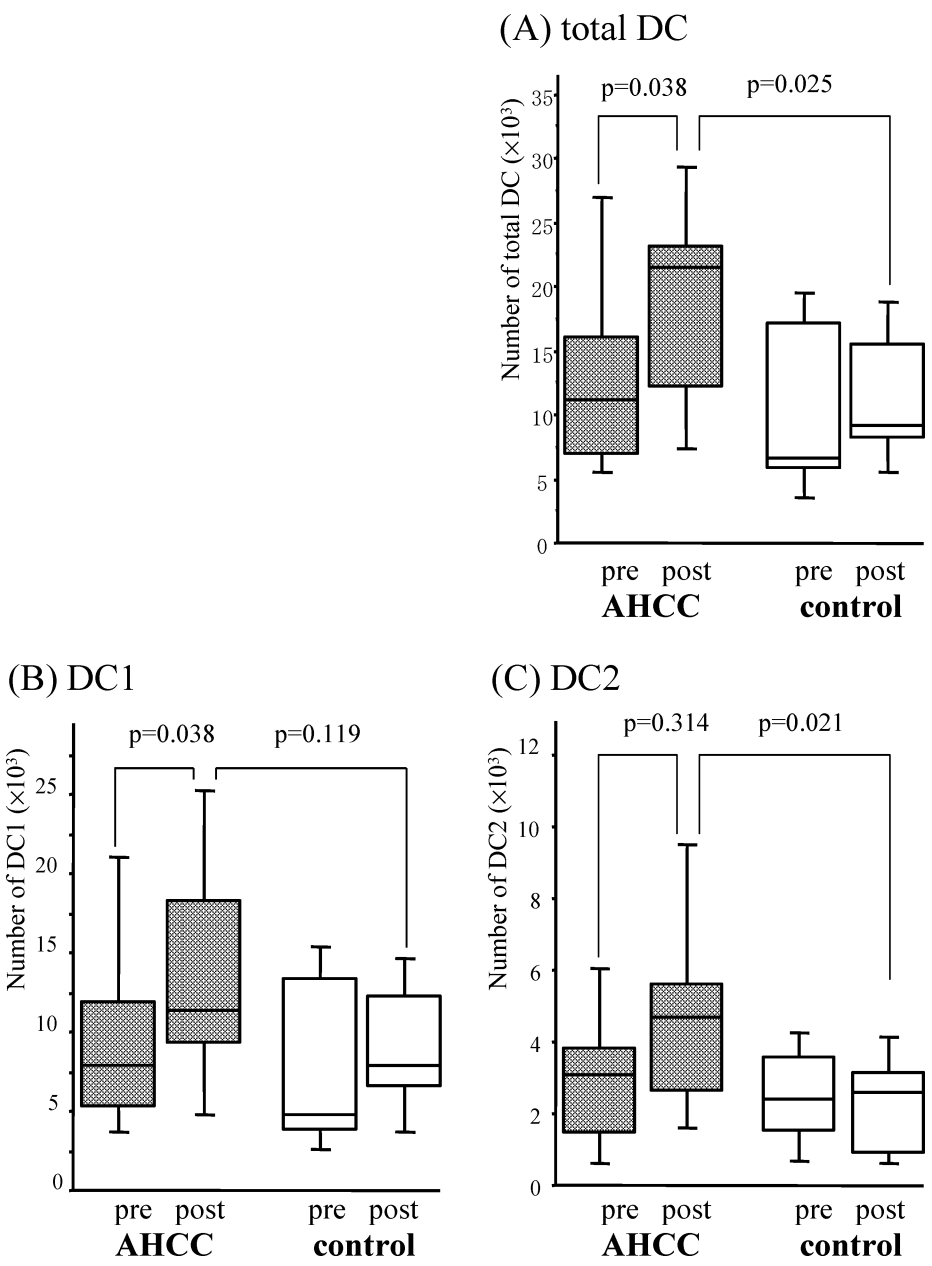

FIG. 2. Comparison of the number of dendritic cells (DCs) between control and active hexose correlated compound (AHCC) groups. The boxes show 75, 50(median), and 25 percentiles; horizontal bar shows 90 and 10 percentiles in box plots. The AHCC group at 4 wk after AHCC intake had a significantly higher number of total DCs in comparison with baseline. A: There were significant differences in the number of total DCs between control and AHCC groups. Significantly higher number of DC1s in the AHCC group after AHCC intake was observed compared with baseline. B: Moreover, number of DC1s in the AHCC group tended to be higher than in controls. C: After AHCC intake for 4 wk, the AHCC group had significantly higher numbers of DC2s than controls.

reference values in both groups (data not shown). Baseline levels of tumor markers (carcinoembryonic antigen, carbohydrateantigen 19-9, alpha-fetoprotein, and protein induced vitamin K absence II) were also within normal reference values in all subjects.

Flow Cytometric Analysis

At baseline, there were no significant differences in the numbers of total DCs, DC1s, and DC2s between the AHCC group and controls (Fig. 2A–2C).

The AHCC group at 4 wk after AHCC intake had a significantly greater number of total DCs than at baseline and than did controls (total DCs: pre-AHCC, 11,932 ± 8,706 cells/ml; post-AHCC, 18,528 ± 7,891 cells/ml; post-controls, 11,116 ± 5,293 cells/ml, Fig. 2A). The number of DC1s was significantly higher in the AHCC group after AHCC intake than at baseline. Moreover, the number of DC1s in the AHCC group had a tendency to be higher than in controls (DC1: pre-AHCC, 9,018 ± 7,046 cells/ml; post-AHCC, 13,061 ± 7,364 cells/ml; post-controls,

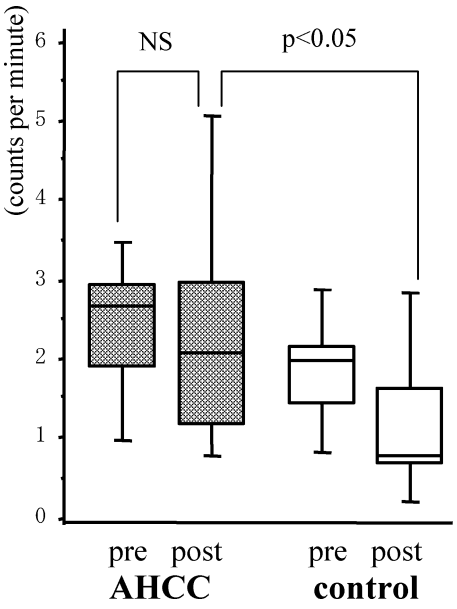

FIG. 3. Comparison of the allogeneic (allo-) mixed -leukocyte reaction (MLR; allo-MLR) of dendritic cells (DC1s) between control and active hexose correlated compound (AHCC) groups. Freshly prepared DC1s from blood samples were examined for stimulating capacity against allogeneic T lymphocytes in a standard MLR. The allo-MLR of DC1s after AHCC intake was significantly higher than in controls ($P = 0.044$). NS, nonsignificant.

8,877 ± 4,169 cells/ml, Fig. 2B). After AHCC intake for 4 wk, DC2s in the AHCC group had significantly increased after 4 wk compared to control values (DC2: pre-AHCC, 3,018 ± 1,997 cells/ml; post-AHCC, 5,467 ± 3,664 cells/ml; post-controls, 2,239 ± 1,373 cells/ml, Fig 2C).

Allo-MLR

DCs isolated from peripheral blood were tested for the ability to stimulate allogeneic T lymphocytes in a standard MLR. At baseline, there was no significant difference in the MLR of DC1s between the AHCC group and controls. After AHCC intake for 4 wk, the MLR in the AHCC group was significantly increased in comparison with the control value (AHCC, 2.5 ± 2.2 cpm: 1×10^3 cells/DC1; controls, 1.1 ± 1.1 cpm; $P < 0.05$, Fig. 3). There was no significant difference in MLR between baseline and after AHCC intake.

Cell Surface Expression Levels of Costimulatory Molecules in DC1s

Phenotypic analysis of $Lin^-DR^+11c^+$ (DC1) was performed for both groups. Results are expressed as mean fluorescence intensity. The expression of CD40 and CD86 did not differ significantly after AHCC intake in the AHCC group and after 4 wk in the control group (data not shown).

Other Parameters of Immune Function

There were no significant differences in the number of PBMCs and lymphocyte subset distribution at baseline between the AHCC group and controls. Changes in the count of each fraction of PBMC, $CD4^+$:$CD8^+$ ratio or DC1:DC2 ratio after AHCC intake were not significant (Table 2). Also, no significant differences in PHA and NK cell activity were found between the AHCC group and controls (Table 2).

Cytokine Production and Serum Hormone Levels

All cytokine production stimulated by LPS did not differ significantly between the AHCC group and controls before and after AHCC intake (Table 3). No significant changes in thyroid and adrenal gonadal hormones after AHCC intake were found.

DISCUSSION

In this double-blind, randomized controlled trial, we investigated whether AHCC is as useful in improving immunological competence as BRM. AHCC is an extract obtained from several species of Basidiomycetes mushrooms. AHCC is a mixture of polysaccharides, amino acids, lipids, and minerals derived from fungi. It is obtained by hot water extraction after culturing mycelia of several Basidiomycetes in a liquid culture media and then treating them with some enzymes. The chemical analysis has revealed that oligosaccharides are the major components of AHCC, consisting about 74%, among which nearly 20% of the oligosaccharide are a-1, 4-glucan and their acetylated forms with an average molecular weight of approximately 5,000, which may be responsible for its biological activities. In contrast to conventional active components such as the β-1,3-glucan structural component found in PSK and lentinan, the glucose oligomer in AHCC has an α-1,4-linkage structure and some esterified hydroxy groups (18). However, AHCC may function as a BRM in the same manner as PSK and lentinan. A food is considered functional if it has been satisfactorily demonstrated to have a beneficial effect on one or more target functions in the body as a BRM in a way that is beyond adequate nutritional effects and is relevant to either the state of well-being and health or to a reduction in the risk of a disease (19).

We have reported that AHCC intake resulted in improved liver function, the prevention of recurrence of HCC after resection, and the prolonged survival of postoperative HCC patients without any adverse effects (8). Therefore, AHCC treatment could be a valuable adjuvant therapy as a BRM in these patients. AHCC has been successfully used as a BRM in various disorders, but little is known of its mechanism of action. There have been only a few published, well-controlled studies of the effect of AHCC on immune function. Therefore, we thought it would be of interest to test AHCC for its immunomodulating effects in a clinical trial.

DCs are highly specialized antigen-presenting cells able to efficiently induce immune responses. During migration, they acquire professional antigen presenting capacity, upregulate the

TABLE 2
Immunological Parameters[a]

	Baseline			4 Wk		
	AHCC	Control	*P* Value	AHCC	Control	*P* value
PBMC ($\times 10^6$/ml)	2.35 ± 1.03	2.15 ± 0.71	0.820	2.95 ± 0.81	2.35 ± 0.76	0.184
CD4 (%)	32.2 ± 12.2	20.2 ± 12.8	0.137	32.7 ± 6.4	26.2 ± 15.7	0.184
CD8 (%)	8.5 ± 4.3	8.3 ± 8.6	0.470	8.9 ± 3.5	8.5 ± 6.4	0.790
CD4:8	4.2 ± 1.1	3.9 ± 2.6	0.271	4.0 ± 1.4	4.2 ± 3.3	0.382
NK (%)	6.9 ± 3.1	9.2 ± 7.4	0.704	7.6 ± 3.9	8.9 ± 5.3	0.514
NK activity (%)	30.8 ± 15.8	35.7 ± 14.8	0.526	31.3 ± 12.5	35.3 ± 13.0	0.704
PHA (cpm)	39,304 ± 16,570	51,221 ± 20,812	0.324	41,768 ± 13,517	56,117 ± 18,783	0.157

[a]Abbreviations are as follows: PBMC, peripheral blood mononuclear cell; CD, cluster of differentiation; CD4:8, CD4:8 subset ratio; NK, natural killer; PHA, phytohemagglutinin; cpm, counts per minute. Continuous variables are expressed as mean ± SD. PHA = proliferative response of T lymphocytes towards mitogen.

major histocompatibility complex and costimulatory molecules, and become competent to activate both T and B cells (9). DCs play a central role in the initiation and modulation of immune system responses.

We have investigated that both OK432 and KP-40, as BRMs, were found to upregulate the activity of DC1 (13). The immunological monitoring of DCs may be a useful therapeutic strategy in the treatment of pancreatic cancer (14,15). Two major subsets of DC precursors have been identified in human peripheral blood (20): the $CD11c^+$ subset belongs to the myeloid lineage, whereas the $CD11c^-$ plasmacytoid subset (21–24) is of lymphoid lineage. Both subsets express high levels of HLA-DR and lack the lineage markers CD3, CD14, CD15, CD16, and CD19. Two DC subsets were shown to regulate immune responses via the polarization of Th1, Th2, or even Th3/Tr1 differentiation through the production of cytokines (25). DC1s are an essential part of protection against cancer through the strong stimulation of naive T lymphocytes. When tissues are damaged by malignant transformation, DCs migrate to these sites. After capturing antigens there, DCs produce a high amount of IL-12 as they mature and migrate into the draining lymph nodes where they present processed antigens to T lymphocytes to initiate an immune response against the tumor (26).

In our study, the AHCC group had a significantly higher number of total DCs than did controls, and the number of DC1s in the AHCC group had a tendency to be higher than in controls. Moreover, the allo-stimulatory activity of DC1s was also increased. These results suggest that AHCC could be an effective modulator of immunological function in patients with cancer. AHCC is as useful in improving immunological competence as BRM. It might be that AHCC has an influence to immune function from DCs. AHCC was reported to enhance the activity of NK cells in cancer patients (27). Also, AHCC reduced the metastasis rate of rat mammary adenocarcinomas (18). Therefore, this AHCC effect may be mediated by natural host immunity, which is restored or activated by AHCC. These results suggest that AHCC acts as a promising BRM.

Results of an in vivo study showed that AHCC restores the NK cell activity that was depressed by an anticancer agent and stimulated peritoneal macrophage cytotoxicity and nitric oxide

TABLE 3
Cytokine Production Stimulated by LPS[a]

	Baseline			4 Wk		
	AHCC	Control	*P* Value	AHCC	Control	*P* Value
IFN-γ (pg/ml)	774.3 ± 329.7	915.8 ± 299.4	0.205	844.3 ± 395.6	701.5 ± 283.7	0.481
TNF-α (ng/ml)	64.4 ± 44.6	63.4 ± 28.4	0.573	60.8 ± 43.0	61.7 ± 21.8	0.725
IL-10 (pg/ml)	281.1 ± 87.9	317.7 ± 252.6	0.944	288.0 ± 142.5	251.0 ± 162.4	0.526
IL-6 (ng/ml)	148.1 ± 111.8	140.8 ± 71.2	0.833	142.7 ± 91.8	150.0 ± 75.5	0.778
IL-4 (pg/ml)	601.6 ± 246.3	744.1 ± 382.1	0.204	598.6 ± 225.2	455.7 ± 457.5	0.205
IL-2 (pg/ml)	290.0 ± 72.7	420.8 ± 197.1	0.058	285.4 ± 94.2	192.2 ± 219.0	0.398

[a]Abbreviations are as follows: LPS, lipopolysaccharide; AHCC, active hexose correlated compound; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin. Continuous variables are expressed as mean ± SD.

and cytokine production (18). The ratio of NK cells to total lymphocytes increased after intake of AHCC for 3 mo in patients with solid cancer (26). Uno et al. (27) reported that the basal level of NK cell activity in cancer patients with solid tumors was lower than in normal controls, but NK cell activity increased to normal levels after AHCC intake at 6.0 g/day for over 4 mo. There was no obvious change in the ratio of $CD4^+$:$CD8^+$ after AHCC intake (26). We also examined PHA and NK cell activity to assess the function of T lymphocytes and NK cells. Impaired NK activity or PHA was observed in patients with lung, esophageal, head and neck, or breast cancer (28–30). However we found no significant difference in the number of NK cells or $CD4^+$:$CD8^+$ T lymphocytes or NK cell activity or PHA after AHCC intake. Further study will be needed to determine the effect of longer and higher doses of AHCC on PHA and NK cells.

It was reported that IFN-γ and IL-12 production in patients with solid cancers was lower than in normal controls, and both cytokines were increased to normal levels after AHCC intake (27). IL-12 preferentially induced Th1 cells from naïve T cells (31). It was suggested that AHCC might induce Th1 differentiation. However, in our small study, there were no significant differences in Th1 cell product cytokines; IL-2, IFN-γ, and Th2 cell product cytokines; and IL4, 6, and 10.

AHCC intake might improve subjective symptoms such as lack of sleep, poor appetite, and feeling unwell in patients with advanced cancer (32). Adrenocortical hormone has been used to relieve some symptoms in many patients with advanced cancer. It could be speculated that AHCC has adrenocortical hormonal effects and psychotropic action**.** However, we found no alterations in adrenocortical and thyroid hormones and those stimulating hormones in healthy volunteers after 4 wk of intake of AHCC.

In conclusion, AHCC intake for 4 wk in healthy volunteers resulted in the improved number of DCs and function of DC1s, which is a part of specific immunity but not innate immunity such as NK cell activity and PHA. AHCC may be useful to protect against cancer progression as well as microbial infection. These observations need to be confirmed in longer, randomized-controlled, double-blind trials. In addition, the dose-response relationship should be investigated, and more detailed studies are required to elucidate the mechanisms responsible for the effects of AHCC.

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