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αGlucan (AHCC Compound Liquid derived from Basidiomycetes) Contributes to Macrophage Activation More than βGlucan

~ Promising Outlook for Tumor-Bearing Animals ~

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Photo 1

In recent years, more attention has been given to methods that enhance a host's immunity through immunostimulatory substances as alternatives to surgery or anti-cancer drug for malignant growths (cancer) in small animals.
We focused on AHCC, a basidiomycete-derived culture with an established scientific basis, manufacturing method, and quality management, conducting experiments related to the immune system. First, we produced a liquid for small animals combining arginine, glutamine, nucleic acids (RNA, DNA), etc. with AHCC (Photo 1) to study the activation effects on macrophage (important role in tumor immunity) and lymphocytes. Since we believe the active component of AHCC to be α-glucan, we compared α-glucan with mushroom β-glucan activation, known for its general immune-boosting effects.

Key words : AHCC Liquid, α-glucan, macrophage

What is AHCC

AHCC (Active Hexose Correlated Compound) is a plant polysaccharide compound abundant in α-glucan extracted from a mycelium culture solution of a basidiomycete from the Lentinula edodes. This compound was developed in 1989 as the result of joint research between Amino Up Chemical Co., Ltd. (Sapporo, Japan) and Dr. Toshihiko Okamoto of the faculty of Pharmaceutical Sciences, Tokyo University. AHCC is produced via a liquid culture of basidiomycete mycelium in a large tank over the course of 45 to 60 days. Through preliminary incubation, mycelia form colonies or clusters. After culturing is complete, the substance is made into a commercial product through processes of enzymatic reactions, sterilization, concentration, and freeze-drying (**Fig. 1**). The AHCC manufacturing process and management comply strictly with international quality and safety standards, including ISO9001:2008 and ISO22000:2005.

While β-glucan is generally considered to be a functional component derived from basidiomycetes, AHCC contains only 0.2% of β-glucan. The fact that AHCC contains a higher amount of α-glucan is what makes it different from most basidiomycete (mushroom) or basidiomyce-derived food supplements. Several reports indicated the existence of α-1,4-glucan in which 2'- and 3'-hydroxyl groups have partially esterified, which we believe to be one active component. We surmise that this partial esterification of α-glucan is not simply an extract from basidiomycete culture, but rather stems from normal α-glucan being enzymatically modified in the proprietary production process of AHCC (**Fig. 2**). In addition, AHCC is subject to a number of GLP-conforming safety tests to ensure safety.

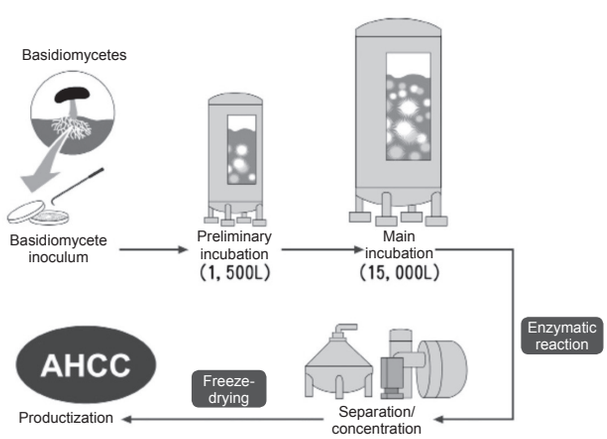
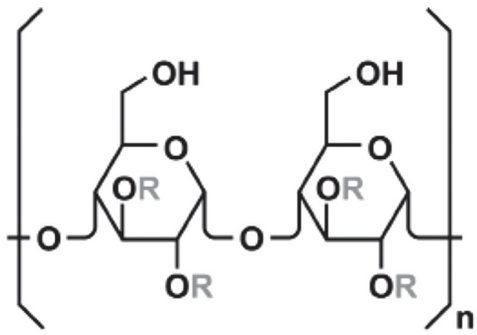


Fig. 1 AHCC Production Process



α-1,4 glucan (R:H or CH3CO-)

Fig. 2 AHCC Structure

AHCC
L-Arginine
L-Glutamine
RNA
DNA

Table 1 Basic Formula in AHCC Liquid

We focused on the immunostimulatory activity of AHCC, creating a liquid for use in small animals. The formula for this liquid is shown in Table 1. Of note about this formula is that it is based on enteral alimentation for human usage. In other words, amino acids (arginine, glutamine) and nucleic acids (RNA, DNA) serve to increase immune capacity, and have been used for many years in clinical scenarios for the purpose of post-surgery wound healing. Accordingly, we decided to make use of the functions of these components, making them the core of the AHCC formula.

Methodology

We used C57BL/6J Jcl (7-week, ♂) or ddY (6-week, ♀) mice, administering orally for a continuous seven-day period. We adjusted the product sample to an AHCC-derived glucan or mushroom β-glucan concentration of 350mg/kg.

After this seven-day period, we instituted a 24-hour fast. For the C57BL/6J Jcl mice, we gathered peritoneal cavity macrophage, and performed an assessment of phagocytic capacity for FITC labeled polystyrene latex beads (2μ diameter). For the ddY mice, we harvested the spleens, and measured the cytokine production capacity of IL-12, MCP-1 (monocyte chemoattractant) and other substances from the T lymphocytes using the ELISA method (**Fig. 3**).

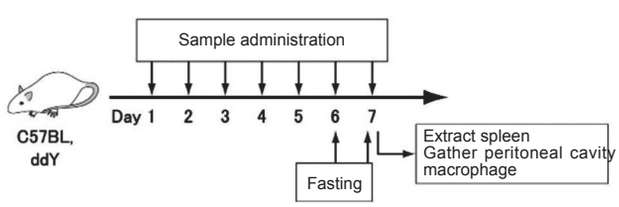


Fig. 3 Experiment Flow

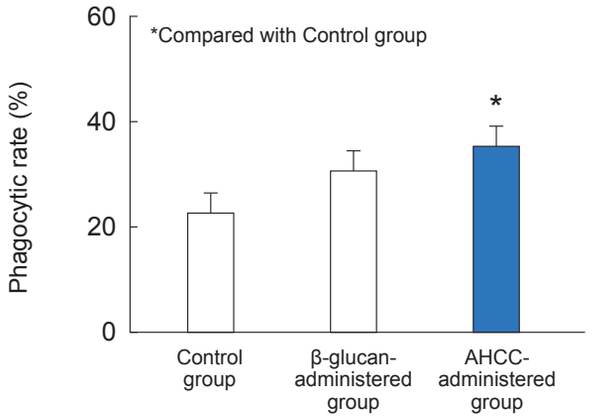


Fig. 4 Macrophage Phagocytic Rate

Result

1) Macrophage Phagocytic Capacity

As shown in Fig. 4, the macrophage phagocytic rate was approximately 1.6 times higher for the AHCC-administered group (group dosed with AHCC compound liquid) in comparison to the control group (Dunnett Method P=0.0002).

Similarly, comparison with the β-glucan-administered group (mushroom β-glucan-administered group) also showed a 1.1 times greater level for the AHCC-administered group.

From these results, we see a significantly increased phagocytic rate in peritoneal macrophage beads for the AHCC-administered group and β-glucan-administered group compared to the control group. We also see that the AHCC-administered group demonstrated higher levels than the β-glucan-administered group. **Photo 2** shows the latex bead macrophage phagocytosis image in each processed group.

(We established LPS stimulation and non-stimulation groups for the cell cultures when taking measurements; however, our data presented here show the non-stimulation group.)

2) IL-12 and MCP-1 Production Effects in Spleen Lymphocytes

We assessed IL-12 production volume from spleen cells under LPS (1μg/mL) stimulation and non-stimulation. Comparing the AHCC-administered group and β-glucan-administered group, we noted that the AHCC-administered group demonstrated stronger production increases.

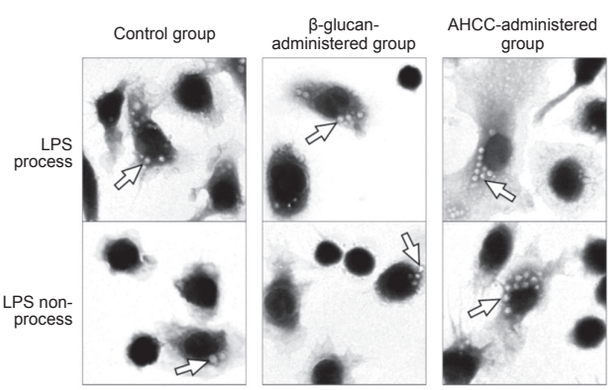


Photo 2 Macrophage Phagocytosis Image
(Arrows: Beads)

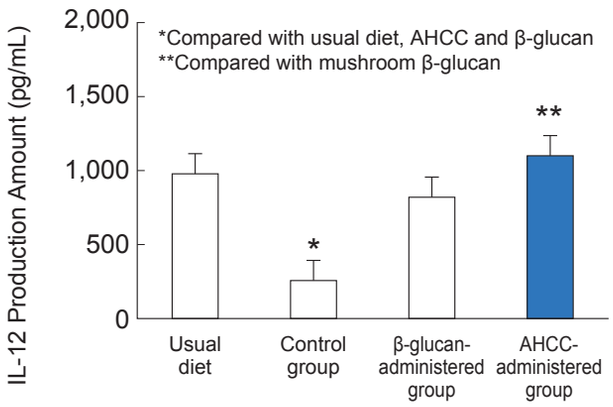


Fig. 5 IL-12 Production in Spleen Lymphocytes

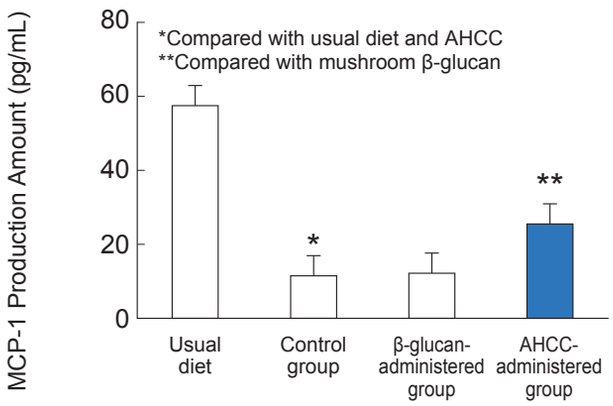


Fig. 6 MCP-1 Production in Spleen Lymphocytes

In particular, we confirmed a significant difference under LPS stimulation (**Fig. 5**). We observed a similar trend for MCP-1 production under LPS (1μg/mL) stimulation and non-stimulation. At the same time, IL-12 production increased for the β-glucan-administered group, but we did not observe any effects for MCP-1. Both showed lower levels than for the AHCC-administered group (**Fig. 6**).

Observations

It is known that by adjusting natural immunity and acquired immunity, AHCC can strengthen immunological surveillance, providing phylactic effects against fungi, bacteria, and the influenza virus[1]. Recently, research into AHCC and natural immunity has suggested that IL-6 response induced through α-glucan fraction (the primary active component in AHCC) and NK cell activation are dependent on TLR2, and that one receptor of α-glucan fraction is TLR2[2].

In addition, a group at Yale University reported that AHCC contributes to both natural immunity and acquired immunity, showing the potential that in addition to cellular immunity, AHCC may complement the activation of anti-cancer drugs by strengthening natural immunity. In other words, the tumor propagation in mice implanted with either B16F0 melanoma cells or EL4 lymphoma cells slowed significantly with AHCC administration, and AHCC treatment resulted in the enhanced activation and propagation in antigen-specific CD4+ and CD8+T cells. The number of tumor antigen-specific CD8+T cells also increased. Further—and importantly—the number of tumor antigen-specific IFN-γ produced CD8+T cells increased. Interestingly, AHCC treatment increased NK cell and γδT cell numbers, leading to the conclusion that AHCC plays a role in promoting lymphocyte activation in connection with natural immunity[3].

Further, the Kansai Medical University Surgery Group 1 took 21 healthy individuals and divided them into a 10-person AHCC-administered group (AHCC daily 3g, 4-week protocol) and an 11-person control group (placebo). The group conducted a double-blind randomized study to evaluate the number of dendritic cells in peripheral blood. The results of the study showed an increase in total dendritic cells and myeloid dendritic cells (DC1), as well as stronger MLR (mixed lymphocyte reaction) in the experimental group. DC1 cells have an important anti-cancer effect mediated through the naive T lymphocytes, and the study suggests that AHCC may improve immune response in cancer hosts[4].

Arginine, which is one amino acid used in this liquid formulation, is a precursor of polyamine and nucleic acid, important in protein synthesis. Arginine is also a precursor of nitric oxide. This promotes lymphocyte mitosis, increasing T cell numbers and promoting their function. It is known that the effects on individuals with depressed immune systems in particular are remarkable. Arginine is also known to be important in the synthesis of hydroxyproline, which is a collagen precursor encouraging wound healing.

Glutamine contains molecular nitrogen in single molecules of amino groups and amide groups, serving as a main energy source for lymphocytes and gut mucosa cells. This is vital for elevating lymphocyte function and maintaining the structure of gut mucosa. Experiments have shown that glutamine administered at the time of invasion improves intestinal immunity function and inhibits bacterial translocation. Exogenous glutamine promotes post-surgery muscle protein synthesis, improving nitrogen balance and increasing cellular immune capacity[5].

With respect to nucleic acid (DNA, RNA), nucleic acid de novo synthesis during elevated catabolism is subject to damage, which leads us to conclude there is significance in exogenous nucleic acid administration. In particular, there have been reports that dietary RNA intake is effective in expressing macrophage and small intestinal cell growth and function[6].

In our study, we created a formulation involving immunity components, looking into the activation of macrophage and lymphocytes. As shown in our results, the macrophage phagocytic capacity (phagocytic index) and IL-12, MCP-1 production activation from lymphocytes were all significantly greater than the control group or the mushroom β-glucan group.

Macrophage has a deep relationship to natural immunity. In particular, its phagocytic effects prey on bacteria and other malignant forms that have undergone apoptosis, digesting them and providing antigens to Helper T Cells.

Further, MCP-1 (Monocyte Chemotactic Protein-1) from the lymphocytes is known as a monocyte chemoattractant. The effect on this monocyte is not only chemotactic elevation, but it also

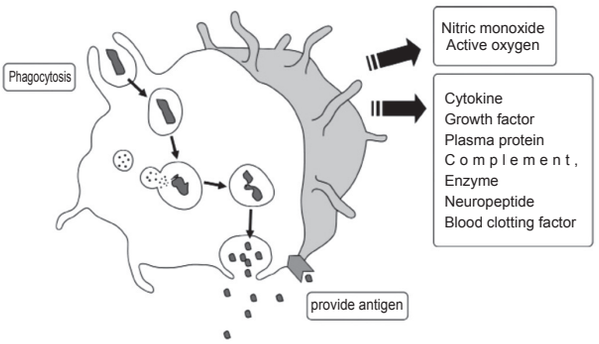


Fig. 7 Macrophage Action

clearly has a role as monocyte activator, elevating delivery of lysosomal enzymes and active oxygen, increasing anti-tumor activation, and inducing the production of IL-1 and IL-6. In addition to monocytes, there are also basiphilic leukocytes leading to chemical mediator liberation and T cell chemotactic activation. Further, IL-12 is a strong IFN-γ inducer, activating natural killer (NK) cells (**Fig. 7**). These results suggest that the AHCC liquid is effective in tumor-bearing animals due to its activation of the immune system.

Cited Texts:

1) Shoko Nogusa, Jeffrey Gerbino, Barry W. Ritz (Department of Biology, Drexel University, USA) “Low-dose supplementation with active hexose correlated compoundimproves the immune response to acute influenza infection in C57BL/6 mice” Nutrition Research, 29: 139-143 (2009)
2) Yusuke Akao (Hokkaido University Graduate School of Medicine), Hiroshi Nishioka, Koji Wakame (Amino Up Chemical Co. Ltd.) “Potentiating of AHCC on natural immunity” Medical Science Digest, 35 (6): 241-244 (2009)
3) Yunfei Gao, Zhinan Yin, et al. (Yale School of Medicine)”Active hexose correlated compound enhances tumor surveillance through regulating both innate and adaptive immune responses” Cancer Immunol Immunother, 55 (10):1258-1266 (2005)
4) Naoyoshi Terakawa, Yoichi Matsui, Sohei Satoi, Hiroaki Yanagimoto, Kanji Takahashi, Tomohisa Yamamoto, Jun Yamano, Soichiro Takai, A-Hon Kwon, Yasuo Kamiyama (Department of Surgery, Kansai Medical University, Osaka, Japan) “Immunological Effect of Active Hexose Correlated Compound (AHCC) in Healthy Volunteers: A Double-Blind, Placebo-Controlled Trial” Nutrition and Cancer, 60 (5): 643-651 (2008)
5) Yamauchi K, Yamamoto S, et al. “Glutamine and arginine affect Caco-2 cell proliferation by promotion of nucleotide synthesis.” Nutrition. 18 (4): 329-333 (2002)
6) Yamauchi K, Hales NW, et al. “Dietary nucleotides prevent decrease in cellular immunity in ground-based microgravity analog.” J Appl Physiol. 93 (1): 161-6 (2002)

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* Control of Innate Immunity Technology Research Association
A METI-certified technology research association based in Shikoku, Japan. The association is engaged in activities promoting the research of materials that control natural immunity and literacy improvement regarding health strategies via natural immunity control.

◆ References

The Course of Integrated Immune System Study of Kagawa University Faculty of Medicine / the Control of Innate Immunity Technology Research Association invites noted researchers in various specialties to lecture at symposia (such as the following) for the purpose of publicizing the latest natural immunity research trends in an easy-to-understand format. The Course of Integrated Immune System Study of Kagawa University Faculty of Medicine / the Control of Innate Immunity Technology Research Association is supported by METI, the Japan Bioindustry Association, and other organizations. We hope to include those involved in animal medicine in the future.

Name: Symposium “Natural Immunity, and its New Development”

Lecture
Chairperson: Ko Okumura (Professor of Juntendo University Faculty of Medicine / Director of Atopy Research Center)

1st Subject Tomotari Mitsuoka (Emeritus Professor of Tokyo University)
“History and Evolution of Probiotic Study”

2nd Subject Genichiro Soma (Professor of the Course of Integrated Immune System Study, Kagawa University Faculty of Medicine)
“Pantoea agglomerans and Immunopotentiative Action”

3rd Subject Shizuo Akira (Director of Immunology Frontier Research Center, Osaka University)
“New Natural Immunology”

* We wish to convey our condolences to all those so deeply affected by the March 11 earthquake and tsunami. You have our heartfelt prayers for your safety and a quick recovery.
Due to the circumstances, we unavoidably postponed the symposium scheduled for March 14. We apologize for the late date of our announcement.