Supplementation with active hexose correlated compound increases survival following infectious challenge in mice

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Active hexose correlated compound (AHCC) is a fermented mushroom extract that is promoted for immune support. This review focuses on results from in vivo studies evaluating the effects of AHCC supplementation on survival and the immune response to a variety of infectious agents, including influenza virus, avian influenza virus, Klebsiella pneumoniae, Candida albicans, Pseudomonas aeruginosa, *and methicillin-resistant* Staphylococcus aureus. *Supplementation with AHCC appears to modulate immunity and increase survival in response to acute infection and warrants further investigation.*

INTRODUCTION

The use of medicinal mushroom preparations for immune support has a long tradition in Asian cultures. More recently, such products have gained popularity in the United States and abroad, such that the estimated world market is now over $6 billion.[1] One such product is active hexose correlated compound (AHCC), an enzyme-fermented extract of the mycelia of *Basidiomycetes* mushroom that is manufactured by Amino Up Chemical Co., Ltd., Sapporo, Japan, and marketed in the United States as a dietary supplement or nutraceutical. This compound contains a mixture of polysaccharides, amino acids, lipids, and minerals. The predominant components of AHCC are oligosaccharides, totaling ~74% of the dry weight. Of these oligosaccharides, nearly 20% are partially acetylated α-1,4-glucans with a mean molecular weight under 5000 Daltons (Figure 1). These α-1,4-glucans are believed to be one of the active compounds in AHCC.[2–4] Alternatively, the activities of β-glucans derived from mushrooms and yeast cell walls have been well characterized and appear to activate innate immunity by binding to C-type lectins, such as Dectin-1, expressed on the surface of macrophages, dendritic cells, natural killer (NK) cells, and γ/δ-T cells.[5] It is unknown whether α-1,4-glucans from AHCC influence innate immunity through a similar mechanism.

Supplementation studies with AHCC have demonstrated positive effects on immune function in rodents[2,3,6–9] and humans,[4,10,11] as well as antioxidant effects.[12,13] These reports further suggest that AHCC is well-tolerated by rodents and humans. Studies to date have suggested that AHCC supplementation may increase innate immunity, most notably as enhanced NK cell activity in humans[10] and rodents[3,9] with malignancy. Additionally, AHCC supplementation may increase T-lymphocyte proliferation[6] as well as alter T-helper (Th) 1 and Th2 cytokine production in response to non-specific stimuli.[6,11] The reported immunomodulatory effects of AHCC supplementation are summarized in Table 1.

This review focuses on the reported effects of AHCC supplementation on the immune response to acute infection in mice. The use of a model organism, such as mice, offers the only ethical, practical, and reliable means of evaluating the in vivo immune response to infectious agents.[19,20] Studies to date examining AHCC supplementation as a potential countermeasure to infectious disease in mice have included the following infectious agents: influenza virus (H1N1), avian influenza virus (H5N1), *Klebsiella pneumoniae*, *Candida albicans*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* (MRSA) as opportunistic infections. Results from these studies are summarized in Table 2 and discussed in detail below.

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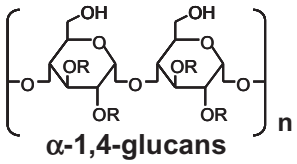

Figure 1 **The chemical structure of the proposed active compound in AHCC.**

INFLUENZA

Influenza viruses are a significant public health threat in the United States and worldwide and a leading cause of infectious disease morbidity and mortality, especially among the elderly, young children, and the immunocompromised.[22–24] Influenza viruses infect the epithelial cells of the respiratory tract, resulting in acute respiratory infections known collectively as influenza or the flu, and are responsible for both seasonal epidemics and occasional pandemics. The influenza viruses belong to the *orthomyxoviridae* family of viruses, which include influenza A, B, and C viruses, as well as the genus *Thogotovirus*, tick-borne viruses that are genetically and structurally similar to the influenza viruses.[25] Influenza A viruses are further differentiated by their surface phenotype, which is comprised of a combination of one of 16 known hemagglutinin (H) glycoprotein subgroups and one of nine neuraminidase (N) subgroups.[26] Utilizing this nomenclature, for example, the common circulating H1N1 subtype of influenza A virus is differentiated from the newly emergent, highly virulent H5N1 avian influenza virus.

Table 1 **Summary of reported immunomodulatory effects of AHCC supplemention.**

Reported effects	References
↓ Tumor formation	Matsushita et al. (2005)[3] Sun et al. (2001)[14]
↓ Infection	Aviles et al. (2008)[2] Ritz et al. (2006)[15] Fujii et al. (2007)[16] Aviles et al. (2003)[17] Ishibashi et al. (2000)[18]
↑ NK cell activity	Ghoneum et al. (1995)[10] Uno et al. (2000)[11] Ritz et al. (2006)[15]
↑ T cell proliferation (ConA, LPS)	Aviles et al. (2004)[6]
Altered cytokine production	Aviles et al. (2008)[2] Aviles et al. (2004)[6] Uno et al. (2000)[11]
↑ Nitric oxide release by peritoneal cells	Aviles et al. (2004)[6]

Abbreviations: NK, natural killer; ConA, concanavalin A; LPS, lipopolysaccharide.

To determine the potential effects of AHCC supplementation on the immune response to H1N1 influenza virus, young (6–8-week-old) male C57BL/6 mice were supplemented orally with 1 g/kg of body weight/d of AHCC or water as a control for 7 days prior to and throughout the course of infection.[15] Supplemented and control mice were infected intranasally (i.n.) with 100 hemagglutination units (HAU) of mouse-adapted influenza virus (Influenza A/Puerto Rico/8/34). The mice supplemented with AHCC exhibited increased survival and maintained body weight during the infection compared to controls, indicative of a less severe infection. Supplementation with AHCC also resulted in enhanced NK cell activity in the lungs and spleen and rapid virus clearance from the lungs. Finally, the AHCC-supplemented mice demonstrated improved lung epithelial integrity following influenza infection compared to controls, as well as reduced lymphocyte and macrophage infiltration in lung tissue. Taken together, these data suggest that AHCC supplementation enhanced NK cell activity in response to influenza infection, which was associated with a decrease in lung virus titers, a less severe infection, and increased survival.

Avian influenza

Influenza A viruses undergo continuous genetic variations that periodically result in the emergence of new strains. New strains result from either antigenic drift (the accumulation of point mutations) or antigenic shift (reassortment of the viral genome resulting from the mixing of two or more viral subtypes). Antigenic drift is principally responsible for yearly variations in the common circulating strains of influenza virus. Experts study antigenic drift to make predictions on which viruses will be in circulation during flu season, because the effectiveness of the trivalent flu vaccine depends on the accuracy of the match between viruses in the vaccine and viruses circulating in the community. Antigenic shift results in a more dramatic change in the virus, and examples include the emergence of H1N1 in 1918 (Spanish Flu), the emergence of H2N2 in 1957 (Asian Flu), the emergence of H3N2 in 1968 (Hong Kong Flu), and the re-emergence of H1N1 in 1977 (Russian Flu).[27] More recently, the H5N1 influenza A virus subtype emerged in poultry. The first cases of human infection with H5N1 virus were reported in Hong Kong in 1997, following direct contact with infected poultry. Eighteen confirmed human infections resulted in six deaths.[28] An outbreak of H5N1 among poultry followed in Asia in late 2003–2004, killing approximately 150 million birds. Since 2003, confirmed human infections with highly virulent H5N1 avian influenza virus have totaled 365, with a combined mortality rate of over 60%.[29] In addition to countries reporting human

Table 2 **Outcomes from in vivo studies evaluating the effects of AHCC supplementation on survival and immune response following challenge with infectious agents.**

Agent (route of infection)	Mice			AHCC supplementation		Outcome	Reference
	Strain	Age	No.	Dose (route of admin.)	Duration		
Influenza – H1N1 (i.n.)	C57BL/6	6–8 wk	20	1 g/kg/d (oral)	7 d + during infection	↑ Survival ↓ Severity (as weight loss)	Ritz et al. (2006)[15]
	C57BL/6	6–8 wk	3	1 g/kg/d (oral)	7 d + during infection	↓ Lung virus titer ↑ NK cell activity	Ritz et al. (2006)[15]
Avian influenza – H5N1 (i.n.)	Balb/c	6–8 wk	40	0.5 g/kg/d (gavage)	7 d	↑ Survival	Fujii et al. (2007)[16]
Klebsiella pneumoniae (i.p.) + stress (hindlimb-unloading)	Swiss/ Webster	9–11 wk	12–16	1 g/kg/d (gavage)	7 d + during infection	↑ Survival ↓ Susceptibility to infection ↑ Bacterial clearance	Aviles et al. (2003)[17]
Klebsiella pneumoniae (i.m.)	Swiss/ Webster	9–11 wk	4–8	1 g/kg/d (gavage)	7 d + during infection	↑ Survival ↑ Bacterial clearance Altered cytokine expression	Aviles et al. (2006)[21] Aviles et al. (2008)[2]
Candida albicans (i.v.) + immunosuppressed (cyclophosphamide)	CD-1 (ICR)	4 wk	15–23	1 g/kg/d (oral)	4 d	↑ Survival	Ishibashi et al. (2000)[18]
Pseudomonas aeruginosa (i.p.) + immunosuppressed (cyclophosphamide)	CD-1 (ICR)	4 wk	8–9	1 g/kg/d (oral)	4 d	↑ Survival	Ishibashi et al. (2000)[18]
Methicillin-resistant *Staphylococcus aureus* (i.v.) + immunosuppressed (cyclophosphamide)	CD-1 (ICR)	4 wk	8–9	0.5 g/kg/d (i.p.)	4 d	↑ Survival	Ishibashi et al. (2000)[18]

Abbreviations: No., number of mice per treatment group; i.n., intranasal; i.p., intraperitoneal; i.m., intramuscular; i.v., intravenous; NR, not reported.

infections, poultry infections have occurred in Malaysia, Iran, India, Greece, Italy, Austria, Germany, and France.[30]

The potential effects of AHCC supplementation against infection with H5N1 avian influenza virus were evaluated in young (6–8-week-old) female BALB/c mice.[16] Mice received AHCC at a dose of 0.5 g/kg body weight/d or an equal volume of phosphate buffer solution (PBS) by gavage for 7 days. At 21 or 28 days post-treatment, mice were infected i.n. with 100 times the 50% lethal dose (LD_{50}) of H5N1 avian influenza virus. The control mice that did not receive AHCC demonstrated 100% mortality within 12 days post-infection. In contrast, those mice that were supplemented with AHCC for 7 days and infected 21 or 28 days post-treatment demonstrated 20% and 30% survival, respectively. These data are highly intriguing and justify additional studies on the use of AHCC in combination with vaccination practices.

Klebsiella pneumoniae

The effects of AHCC supplementation on resistance to infection with *Klebsiella pneumoniae*, a pathogen principally associated with bacterial pneumonia, urinary tract infections, and opportunistic or hospital-acquired infections, were evaluated in two experimental models: hindlimb-unloading[17] and surgical infection.[2,21] Hindlimb-unloading, or 15–20° head-down tilt, is a model of stress-associated immunosuppression and, due to a lack of load-bearing on hindlimbs and a fluid shift to the head, is utilized to approximate microgravity conditions experienced during spaceflight. Groups of Swiss/Webster mice were supplemented with 1 g/kg of body weight/d of AHCC or PBS vehicle control by gavage for 7 days before hindlimb-unloading and throughout the suspension and infection period.[17] Mice were suspended for 2 days prior to i.p. infection with one 30% lethal dose of *K. pneumoniae*. Following infection, AHCC-supplemented mice demonstrated increased survival, increased mean time until death, decreased susceptibility to infection (as determined by an increase in the infectious dose required for 50% lethality), and increased bacterial clearance from the blood among those mice that survived until 10 days post-infection.

Next, in a mouse model of surgical intramuscular infection and trauma, female Swiss/Webster mice were supplemented with 1 g/kg of body weight/d of AHCC or PBS control by gavage for 7 days and food-deprived for 24 h prior to infection and 6 h post-infection with *K. pneumoniae*.[2,21] AHCC supplementation induced resistance to intramuscular infection with a 20% lethal dose of *K. pneumoniae*, as indicated by enhanced bacterial clearance from the blood, lungs, and liver by 5–6 days post-infection.[2] In a similar study, AHCC-supplemented mice challenged with a 50% lethal dose of *K. pneumoniae* exhibited increased survival compared to controls.[21]

Opportunistic infections

The effectiveness of AHCC against experimental opportunistic infections was further evaluated in groups of immunosuppressed mice infected with lethal doses of *Candida albicans*, *Pseudomonas aeruginosa*, or MRSA.[18] Four days prior to infection, leukopenia was induced in young (4-week-old) female CD-1 (ICR) mice by the intraperitoneal administration of 200 mg/kg cyclophosphamide; the mice were then supplemented orally with 1g/kg body weight/d of AHCC or received the vehicle control. Alternatively, AHCC was administered at a dose of 0.5 g/kg body weight by intraperitoneal injection (MRSA group). All three groups of AHCC-supplemented mice exhibited increased survival compared to controls. Therefore, together with the results from the *K. pneumoniae* studies above, these data suggest that AHCC increases survival in immunocompromised mice in response to infectious challenge.

CONCLUSION

The study of bioactive compounds as potential countermeasures to infectious disease is particularly important in this era of bioterrorism threats as well as the continued emergence of new or increasingly common infectious agents, including H5N1 avian influenza and community-acquired MRSA, respectively. Further, the study of agents that modify the host response to acute infection has become increasingly attractive given the limitations of vaccine and antiviral strategies. For example, it is unknown whether traditional vaccines will be effective against H5N1 avian influenza virus, and although governments race to stockpile antiviral drugs, analysis of H5N1 influenza viruses isolated from both poultry and human samples have already demonstrated some resistance against currently available antiviral agents.[31] Importantly, AHCC supplementation may show some benefit as a vaccine adjuvant, as in a mouse model of avian influenza infection, which requires further study.

These early results in both immunocompentent and immunosuppressed mice suggest that AHCC may enhance survival in response to a broad spectrum of infectious agents. Thus, it is imperative to both continue and expand the evaluation of AHCC as a potential agent to modulate the immune response to acute infection. Several items must be addressed in future studies. Notably, the majority of available studies were conducted using a supplemental dose of 1g of AHCC per kg of body weight per day, or an approximate equivalent dose of

5 g/d in a 60 kg human.[32] Therefore, dose-response data is urgently needed. Further, in each study, AHCC was administered prophylactically, so it remains unknown whether AHCC would increase survival if given at the time of infection or post-infection. Finally, additional studies are needed to determine a mechanism of action for the generalized improvement in the immune response and increased survival observed in AHCC-supplemented mice during acute infection as compared to controls. Given the abundance of preliminary positive results across different infections in mice, AHCC supplementation demonstrates valuable and clinically relevant potential as an immune-enhancing agent that merits further study.

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