

# An Evidence-Based Review of a *Lentinula edodes* Mushroom Extract as Complementary Therapy in the Surgical Oncology Patient

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The purpose of this review is to present the currently published evidence regarding the use, efficacy, potential mechanisms of action, and results of published clinical trials regarding the use of a *Lentinula edodes* mushroom-derived extract (active hexose correlated compound) as complementary therapy in patients with cancer. The authors explore the current preclinical and clinical evidence as it relates to this topic and its potential use in the surgical oncology patient. There has been a growing

interest in stimulation of the immune system in trauma, cancer, and surgical patients in general. Little, however, has been written about some of the supplements in widely used in Japan and China, but relatively unheard of in the United States. (*JPEN J Parenter Enteral Nutr.* XXXX-xx:xx-xx)

**Keywords:** AHCC compound; dietary supplement; review; enteral nutrition; oncology; immunonutrition; *Lentinula edodes*

## Clinical Relevancy Statement

Active hexose correlated compound (AHCC) is an enzyme-fermented extract derived from the *Lentinula edodes* mushroom. The published studies reviewed in this manuscript indicate that this compound may have immunostimulatory properties and may be of benefit as an adjunctive therapy in patients with cancer. Although preliminary data suggest its benefit, a number of future studies, including an evaluation of postinjury treatment, mechanisms of action (MOAs), pharmacological and drug interactions, dosing regimens, delivery methods, and prospective randomized trials are necessary before widespread adoption.

AHCC is an enzyme-fermented extract derived from the *Lentinula edodes* mushroom.<sup>1</sup> It is purported to have various therapeutic properties and has been studied as an adjunctive agent for cancer as well as for the amelioration of chemotherapeutic side effects and the reversal of immunosuppression. It is manufactured (Amino Up Chemical Co, Ltd, Sapparo, Japan) and used most predominantly in Japan.<sup>2</sup>

There has been a growing interest in the stimulation of the immune system in cancer and during chemotherapy. However, little has been written on some of the supplements that are widely used in Japan and China, but relatively unheard-of in the United States. The purpose of this review is to critically present the currently published evidence regarding the use, efficacy, potential MOAs, and current clinical trials regarding the use of AHCC as an adjunct in the treatment of cancer and related processes. We explore the current preclinical and clinical evidence as it relates to this topic and highlight requisite future studies needed before initiation of well-controlled clinical trials for specific indications.

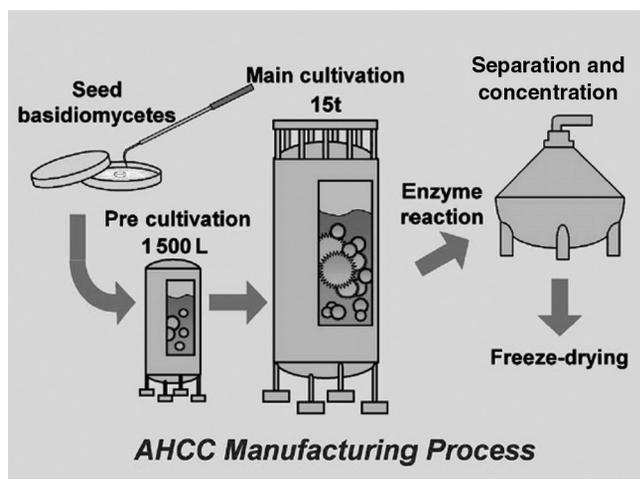
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## Methods

The MEDLINE (1949–present [August 2009]), Cochrane Library, and Scopus (including Em Base) databases were



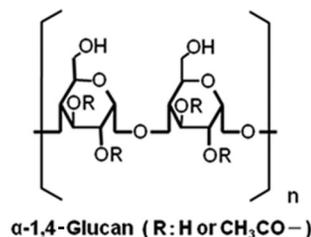
**Figure 1.** This figure depicts the manufacturing of the *Lentinula edodes* extract, active hexose correlated compound (AHCC). After seed culture, the mushroom is cultured in a smaller (1,500 L) followed by a larger (15ton) tank for 45 days. Fermentation is followed by an enzymatic reaction, sterilization, concentration, and freeze drying. From Hirose et al.<sup>11</sup> Reproduced with permission.

searched using the terms *active hexose correlated compound* and *AHCC*. The should be reference list of the articles obtained were searched to identify further potential sources of information. Only published articles were included.

## Background

AHCC is an extract produced during long-term culture of a Basidiomycete mushroom. Although early publications state a variety of Basidiomycete mushrooms are used, more recent literature confirms that *L. edodes*, a type of Basidiomycete mushroom, is the precursor to AHCC.<sup>3</sup> The manufacturing process of AHCC relies on culture of *L. edodes* in liquid media. During long-term culture, saccharolytic and proteolytic enzymes are produced. After a period of fermentation, a process of separation, concentration, sterilization, and freeze-drying leads to the end compound.<sup>3</sup> The manufacturing process is detailed in Figure 1.

AHCC is primarily composed of carbohydrates (approximately 70%), protein (13%), ash contents (9%), fats (2%), and fiber (2%).<sup>3,4</sup> A significant portion of the carbohydrates (approximately 20%) is composed of  $\alpha$  1,4-glucans (Figure 2).<sup>2</sup> The relatively high concentration of  $\alpha$ -glucans (15.8/100 g) as compared with  $\beta$ -glucans (0.2/100 g) is believed to be secondary to the production process and is thought to be a major contributor to its pharmacologic effects.  $\alpha$ -glucans are of low molecular



**Figure 2.** Active hexose correlated compound has a relatively high concentration of low-molecular-weight  $\alpha$ -1,4-glucans. The chemical structure is depicted.

weight (approximately 5,000 daltons as opposed to a molecular weight of 10,000–500,000 daltons for  $\beta$ -glucans) and are easily absorbed into the gastrointestinal tract after oral administration. Of note, the production process does not exclude lot-dependent variations in composition.<sup>5-9</sup>

Limited safety studies have been performed with AHCC. This has been based on the assertion that the compound is derived from an edible mushroom.<sup>3</sup> In general, most experimental studies have used the oral and/or intraperitoneal (IP) route (in animal studies) with dose-lethality determinations completed in both male and female rats.<sup>10</sup> The median lethal dose (LD50) in male and female rats is 8.5 g/kg and 9.8 g/kg for oral administration, respectively, and 7.4 g/kg and 8.3 g/kg for IP administration.<sup>11</sup>

## Cancer and Chemotherapy-Related Side Effects

A summary of the potential MOAs of AHCC in cancer and related processes is summarized in Table 1. Universally, the current published literature seems to suggest that AHCC functions as an immunostimulatory compound (Table 2). Whether AHCC has direct static/cidal effects on tumor cells and/or it functions indirectly by upregulating the innate immune response has not been well characterized. In addition, it seems that a potential application of AHCC may be in ameliorating the side effects of chemotherapeutic agents and the side effects of chemotherapy.

## Tumor Immunity

Several basic science studies suggest that the MOA of AHCC may involve augmenting the innate cellular immune response or a more direct role in suppressing tumor growth. Gao et al<sup>12</sup> demonstrated that pretreatment with AHCC delayed tumor development and decreased tumor size in mice injected with B16F0 melanoma tumor cells (subcutaneously) or thymoma EL4 cells (IP). This

**Table 1.** Potential Mechanisms of Action of Active Hexose Correlated Compound (AHCC) in Oncology-Related Applications**Potential Mechanisms of AHCC in Cancer-Related Applications**Direct effect on T-cell-mediated immune function: CD4+, CD8+, natural killer, and  $\gamma$ - $\delta$  T-cell proliferation/activityModulation of cytokine response (IL-2, IL-4, IL-6, IL-10, IL-12, TNF- $\alpha$ , IFN- $\gamma$ )

Effect on nitric oxide synthase

Modulation of chemotherapy-induced side effects

IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

was associated with an increase in CD4+ and CD8+ T-cell proliferation, CD8+ T-cell production of interferon (IFN)- $\gamma$ , and increased numbers of natural killer (NK) and  $\gamma$ - $\delta$  T cells.<sup>12</sup> In addition, when tumor cell lines (K562 and Raji) were incubated with 1 mg/mL of AHCC, growth was suppressed.<sup>13</sup>

### Hepatocellular Cancer

There are few clinical studies examining potential MOAs of AHCC in cancer. It is difficult to glean much useful information because of the small sample sizes and inherent issues with the study design (eg, case reports and cohort studies). In addition, the studies are largely descriptive in nature. The largest studies have been with hepatocellular cancer. Matsui et al<sup>14</sup> examined the effect of AHCC on patients with hepatocellular cancer who had undergone curative liver resection in a prospective, cohort fashion. AHCC was given orally (3 g/d) following surgery. Of note, differences in 2 variables (preoperative serum albumin which was lower in AHCC group and platelet count which was lower in AHCC group) were noted. The only side effect noted was nausea. Rates of recurrence (66.1% vs 34.5%) were higher in the non-AHCC-treated group (20.4% vs 46.8%; median follow-up 30 vs 28 months, while rates of survival were lower in the non-AHCC-treated group. In addition, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and cholinesterase activity were decreased in the AHCC-treated group. A significant limitation of this study involves its uncontrolled nature; also, stage-for-stage recurrence and survival data were not provided. Although the authors demonstrated no statistical differences based on stage of cancer, there were 4 more stage IVA patients (11 vs 7) in the control group, which may have been one potential reason for the worsened outcome in the control group.

Cowawintaweevat et al<sup>15</sup> also conducted a prospective cohort study to examine the effect of AHCC on patients with hepatocellular cancer not amenable to surgical resection or chemoembolization but with adequate hepatic function. Self-administered treatment with AHCC (6 g/d) or placebo was started after a 1-month period in which the

patients were not receiving any conventional therapy. Oral treatment with AHCC in 34 patients (when compared with 10 patients treated with placebo) prolonged survival. Many comparisons of biochemical values between the 2 groups were not possible because of patient survival in the control group. Whether the differences noted were due to inherent differences in the biological behavior of the tumors or a direct effect of AHCC is unknown, in part, given the small sample sizes and lack of an adequate control group. The drastic differences in the size of groups is important to note when interpreting the results (34 vs 10 in AHCC and control groups, respectively).

### Other Cancers

There are limited data on the utility of AHCC in other cancers. Turner and Chaudhary<sup>16</sup> published a case report of unprescribed oral supplementation with AHCC decreasing serum prostate-specific antigen (PSA) in a patient with androgen-insensitive metastatic prostate cancer. In an uncontrolled observational study, Ghoneum et al<sup>13</sup> studied the effect of AHCC supplementation (3 g/d for 1 month) on 11 patients with a variety of cancers (ie, prostate, ovary, breast, and multiple myeloma). Efficacy of treatment was determined by following specific tumor markers (ie, PSA, CA 125, Bence Jones protein, abnormal protein [multiple myeloma], and CA 15-3) as well as determining NK-cell cytotoxicity. Two of 3 patients with prostate and ovarian cancer had reductions in the respective tumor markers (ie, PSA and CA 125). In the 2 patients with multiple myeloma, 1 patient had a reduction in Bence Jones protein, and 1 had a reduction in abnormal protein. There was no effect on patients with breast cancer (CA 15-3). Nine of the 11 patients had increases in NK-cell cytotoxicity. Whether this had any effect on survival or tumor burden was not mentioned; it would likely be difficult to determine, given the lack of an adequate control group.

Uno et al<sup>17</sup> examined the effect of oral administration of AHCC in 38 patients with various solid tumors. After 6 months of AHCC treatment, levels of interleukin (IL)-12, IFN- $\gamma$ , and NK-cell activity in peripheral blood lymphocytes stimulated with phytohemagglutinin (PHA;

**Table 2.** Summary of the Studies Described

Investigator	Species/Cell Line	Pretreatment/Duration/ Dose	Injury/Intervention	Outcomes
<b>Tumor immunity</b>				
Gao et al (2006) <sup>12</sup>	Mice (C57BL/6); 6–7 weeks	Yes/2 weeks/12 mg/d PO	Melanoma (B16 F0) / lymphoma (EL4/EG7)	Decreased tumor size, increased CD4+ and CD8+ T-cell proliferation, increased NK and $\gamma$ - $\delta$ T cell, and increased CD8+ T-cell IFN- $\gamma$ production
Ghoneum et al (1995) <sup>13</sup>	K562 and Raji	Cultured with 0.5 and 1 mg/mL		Decreased cell growth
<b>Chemotherapy side effects</b>				
Sun et al (2009) <sup>10</sup>	Rats (Sprague-Dawley); 8 days old	Yes/1-hour pre-cytosine arabioside and daily for 7 days/500 mg/kg/d PO or IP or 5% transdermal application	Cytosine arabioside	Protection from alopecia (best in PO-treated group)
Sun et al (2009) <sup>10</sup>	Mice (ddY); 8 weeks	No (concurrent)/28 days/1,000 mg/kg/d PO	6-mercaptopurine plus methotrexate	Decreased liver injury
Matsushita et al (1998) <sup>8</sup>	Rats (congenital T cell depressed/ spontaneously hypertensive); 10–12 weeks	No (concurrent)/35 days/100 mg/kg/d PO	Mammary adenocarcinoma cells (c-SST-2) followed by tegafur/uracil treatment	Decreased primary tumor size, improved NK-cell activity, increased NO production by peritoneal macrophages
Hirose et al (2007) <sup>11</sup>	Mice (BALB/cA SPF); 6 weeks	No (concurrent)/28 days/100 mg/kg/d PO	Colon-26 tumor cells followed by cisplatin treatment	Reduced tumor size, decreased kidney injury, and increased bone marrow cell viability
Burikhanov et al (2000) <sup>19</sup>	Rats (Wistar); 8–10 weeks	Yes/7 days/4% in drinking water	Dexamethasone	Decreased thymic apoptosis
<b>Immunomodulation</b>				
Aviles et al (2006) <sup>23</sup>	Mice (Swiss/Webster); 9–11 weeks	Yes/7 days/1 g/kg/d PO	Intramuscular injection of LD <sub>50</sub> <i>Klebsiella pneumoniae</i>	Increased survival, 10-fold increase in LD <sub>50</sub> , increased bacterial clearance
Aviles et al (2008) <sup>22</sup>	Mice (Swiss/Webster); 9–11 weeks	Yes/7 days/1 g/kg/d PO	Intramuscular injection of LD <sub>20</sub> <i>K. pneumoniae</i>	Increased cytokine response, increased blood monocytes and lymphocytes
Ritz et al (2006) <sup>1</sup>	Mice (C57BL/6); 6–8 weeks	Yes/7 days/1 g/kg/d PO	Intranasal infection of influenza virus	Increased survival, decreased lung viral titers, decreased lung epithelial injury, increased NK-cell activity in lung/spleen
Nogusa et al (2009) <sup>20</sup>	Mice (C57BL/6); 6–8 weeks	Yes/7 days/0.05-1 g/kg/d PO	Intranasal infection of influenza virus	Dose-dependent effect on weight loss and survival
Wang et al (2009) <sup>21</sup>	Mice (C57BL/6); young (6–8 weeks) vs old (21–22 months)	Yes/7 days/600 mg/kg/ every other day PO; AHCC also given days 1 and 3 postinfection	IP injection of approximately LD <sub>100</sub> dose of West Nile virus	Decreased mortality in young mice, increased IgM (young mice) and IgG (young and aged mice) to West Nile virus, decreased viremia in both groups, increase in $\gamma$ - $\delta$ T cells

(continued)

Table 2. (continued)

Investigator	Species/Cell Line	Pretreatment/Duration/ Dose	Injury/Intervention	Outcomes
Tumor immunity				
Ishibashi et al (2000) <sup>24</sup>	Leukopenic mice (cyclophosphamide)	Yes/4 days/1,000 mg/kg/d PO or 50 mg/kg/d IP	<i>Candida albicans</i> , <i>Pseudomonas</i> <i>aeruginosa</i> , MRSA	Increased survival after IP administration for <i>C albicans</i> and MRSA; increased survival after PO administration for <i>C albicans</i> and <i>P aeruginosa</i>
Ikeda et al (2003) <sup>25</sup>	Leukopenic mice (cyclophosphamide, doxorubicin, 5-fluorouracil, prednisolone)	Yes/7 days/2.5% in water PO or 500 mg/kg/d IP	<i>C albicans</i>	Increased survival with IP administration in cyclophosphamide- treated or 5-fluorou- racil-treated mice; minimal effect on doxorubicin-treated mice; no effect of AHCC on prednisolone- treated mice Increased survival with oral treatment in 5-fluorouracil-treatment in mice
Aviles et al (2003) <sup>26</sup>	Mice (Swiss/Webster); 9–11 weeks	Yes/7 days/1 g/kg PO	Hindlimb unloading × 48 hours followed by <i>K pneumonia</i>	Increased survival, increased LD <sub>50</sub> , increased bacterial clearance
Aviles et al (2004) <sup>27</sup>	Mice (Swiss/Webster); 9–11 weeks	Yes/7 days/1 g/kg PO	Hindlimb unloading	Increased splenocyte proliferation and cytokine production in response to LPS or ConA in control animals treated with AHCC; in hindlimb- unloaded animals, increased IL-2 and IFN-γ in splenocytes after ConA, increased splenocyte cytokine and NO production after stimulation with LPS or <i>K pneumonia</i>

ConA, concanavalinA; IFN, interferon; IL, interleukin; IP, intraperitoneal; LD, lethal dose; LPS, lipopolysaccharide; MRSA, methicillin-resistant *Staphylococcus aureus*; NK, natural killer; NO, nitric oxide; PO, per os.

initially all depressed) returned to levels comparable with healthy controls. Won<sup>18</sup> also evaluated the effect of oral supplementation with AHCC in 12 cancer patients and found that AHCC increased the ratio of NK-cells to total lymphocytes by approximately 20% when measured at 3 and 6 months after the beginning of supplementation. However, the levels pre-AHCC and post-AHCC were within the realm of what is considered normal. Similar to the previous studies, there was no mention of whether supplementation affected survival or tumor behavior.

## Modulation of Drug Side Effects/ Interaction With Chemotherapy

### Potential Effect of AHCC on Chemotherapy Metabolism

Little is known about the potential effects of AHCC administration on drug metabolism. This is especially important when AHCC is being used as an adjunct to conventional chemotherapy, but we are aware of only 1

study that has been published evaluating potential drug interactions. Mach et al<sup>5</sup> investigated the potential for AHCC to interfere with hepatic cytochrome P450 metabolic pathways. AHCC has shown benefit in hepatitis and hepatocellular cancer, and some investigators theorize that the effect may be due to alteration of hepatic enzyme activity. Because of the fact that many chemotherapeutic agents are metabolized through the cytochrome P450 pathway, it is important to exclude the potential interaction of AHCC with various anticancer drugs. Of the 4 cytochrome P450 isoenzymes tested, AHCC was found not to be a substrate, inhibitor, or inducer of the 3A4, 2C8, and 2C9 isoenzymes; however, AHCC is both a substrate and inducer of the 2D6 isoenzyme. Doxorubicin is the most common chemotherapeutic agent metabolized by the cytochrome P450 2D6 pathway, and caution may need to be exercised with using AHCC as an adjunct to this agent, especially because interaction studies remain sparse.

### **Modulation of Drug Side Effects**

AHCC has been studied as an adjunct to various chemotherapeutic agents (eg, cytarabine, methotrexate [MTX], 6-mercaptopurine [6-MP], tegafur/uracil [UFT], cisplatin, and dexamethasone) as a modulator of various side effects.

#### **Cytosine Arabinoside (Cytarabine)**

Matsushita et al<sup>8</sup> studied the effect of topical (5%), oral (500 mg/kg/d), and IP (500 mg/kg/d) administration of AHCC on alopecia and weight loss induced by the chemotherapeutic agent cytarabine in neonatal rats. Although all 3 routes of administration protected the animals from alopecia, the oral route of administration was more efficacious with regard to loss of hair follicles and preservation of body weight at the end of treatment. Of note, AHCC was administered 1 hour before administration of cytarabine and continued daily for 7 days. In addition, this is the only published study that has evaluated a potential effect of AHCC via an IP or transdermal route in a disease model.

#### **Methotrexate/6-Mercaptopurine**

Matsushita et al<sup>8</sup> studied the effect of 28-day oral administration of AHCC (1,000 mg/kg/d) coadministered with a single concurrent dose of methotrexate MTX and 6-MP. AHCC treatment improved liver histology (decreased vacuolar degeneration/necrosis) and at least partially prevented the observed decrease in serum albumin, red and white blood cell counts, total protein, triglycerides, body weight, and various drug metabolism enzymes (eg, cytochrome P450, glutathione S-transferase, aryl hydrocarbon hydroxylase, p-nitrophenol hydroxylase, and

pentoxoresorufin O-dealkylase). In addition, the increase in liver transaminases (AST and ALT), as well as liver and spleen weights was prevented with AHCC administration.

#### **Tegafur/Uracil**

Matsushita et al<sup>8</sup> examined the effect of AHCC in combination with (UFT) in rats that had congenital T-cell depression and were spontaneously hypertensive. Rat mammary adenocarcinoma cells (SST-2) cells were implanted in the congenitally T-cell-depressed rats. UFT was administered daily starting on day 3 and continued for 35 days. AHCC was given to the rats also beginning on day 3 (35-day course) at a dose of approximately 100 mg/kg/d. In rats treated with AHCC, there was a lower diameter and weight of the primary tumor with less axillary lymph node metastases; however, distant metastases (ie, lung) were not significantly different in the AHCC-treated or AHCC-untreated groups. At day 21, AHCC also reversed the depression of NK-cell activity (by UFT). In addition, analysis showed increased production of nitric oxide by peritoneal macrophages, as well as increased levels of nitric oxide synthase (iNOS) and tumor necrosis factor (TNF)- $\alpha$  mRNA when compared with the UFT-alone group.

#### **Cisplatin**

Hirose et al<sup>11</sup> examined the effect of AHCC on the side-effect profile of the chemotherapeutic agent cisplatin. Six-week-old female BALB/cA SPF mice were inoculated with colon-26 tumor cells. Three days later, IP cisplatin was administered and repeated on days 6, 13, and 20. AHCC (100 mg/kg/d) was administered starting on the first day of cisplatin administration and continued until day 28. AHCC administration in combination with cisplatin significantly reduced tumor weight and size in comparison with cisplatin alone. AHCC in combination with cisplatin significantly reduced serum creatinine as compared with cisplatin alone; however, serum urea nitrogen levels were not significantly different between AHCC + cisplatin and cisplatin alone. Histological examination of the kidney at day 28 revealed no significant differences between control and cisplatin + AHCC groups, suggesting that AHCC was protective against cisplatin-induced nephrotoxicity. Bone marrow cell viability was significantly increased in the AHCC-supplemented group, suggesting amelioration of bone marrow suppression induced by cisplatin.

#### **Dexamethasone**

Burikhanov et al<sup>19</sup> examined the role of AHCC in preventing thymic apoptosis caused by administration of dexamethasone. Male Wistar rats aged 8 to 10 weeks were

given dexamethasone to induce thymic apoptosis. When rats were pretreated with AHCC (4%) for at least 4 days before treatment with Dexamethasone, thymic apoptosis was prevented. Caspase 3 activity was decreased when rats were pretreated with 4% AHCC (in drinking water) for 7 days. There was no difference in melatonin levels or IL-1 $\beta$  levels, 2 substances that may play a role in regulating apoptosis of thymocytes in response to dexamethasone.

### Prevention of Infections/Protection in Setting of Immunosuppression

Agents/therapies to afford protection against infections in an immunocompromised state may be of particular value in the oncology patient.

#### Infections (Viral)

Several studies have examined the role of AHCC in models of viral and bacterial infection. Ritz et al<sup>1</sup> studied the effect of AHCC in pathogen-free C57BL/6 mice (6–8 weeks old) infected with influenza (H1N1). Mice were pretreated with 1 g/kg administered orally for 7 days before infection and continued during and after infection. AHCC administration increased survival (95% vs 75%); decreased lung viral titers at days 5, 7, and 10; and decreased epithelial erosion (day 10), lymphocyte infiltration (day 7), and macrophage infiltration in the lungs (day 7). In addition, there were increased levels of NK cells (day 2) in the lungs and increased NK-cell activity in the lung (days 1–4) and spleen (day 2). Nogusa et al<sup>20</sup> also examined the administration of AHCC in C57BL/6 mice infected with influenza using a similar design (ie, pretreatment for 7 days and intranasal infection of H1N1 influenza A) with varying doses (0.05–1 g/kg). Recovery from influenza was increased in animals supplemented with  $\geq 0.1$  g/kg of AHCC with an apparent dose-dependent effect. At day 7, mice supplemented with 0.1 g/kg of AHCC had undetectable levels of lung viral titers. However, AHCC at this low dose did not increase NK-cell cytotoxicity or the number of cells in the lung or spleen. At day 2, AHCC-treated mice had a greater NK-cell lytic efficiency.

Wang et al<sup>21</sup> examined the effect of AHCC on young (ie, 6–8 weeks old) and old (ie, 21–22 months) C57BL/6 mice infected with West Nile virus. Before infection, mice were pretreated (600 mg/kg) with AHCC on alternate days for 1 week. After infection, the mice received AHCC on days 1 and 3. Mortality was decreased in young but not old mice. In both groups, virus levels (blood) were decreased. In young mice, Immunoglobulin (Ig)M and IgG antibodies specific to West Nile virus were increased at day 4. There were no differences seen in young mice with regard to cytokines, including IFN- $\alpha$ , TNF- $\alpha$ , IL-6,

and IL-12. AHCC administration did increase the expansion of  $\gamma$ - $\delta$  T cells at day 3 with an increased number of  $\gamma$ - $\delta$  IFN- $\gamma$  (+) T cells.  $\gamma$ - $\delta$  T cells are believed to play a protective role, especially in the prevention of West Nile virus encephalitis. In older mice, IgG but not IgM antibodies specific to West Nile virus were increased. Although the number of  $\gamma$ - $\delta$  T cells was increased, certain subsets of  $\gamma$ - $\delta$  T cells (specifically V $\gamma$ 4 (+)) exhibited a decreased response. AHCC was only partially protective in older mice.

#### Infections (Bacterial)

Aviles et al<sup>22</sup> have examined the use of AHCC in a mouse model of intramuscular infection. Before infection, female Swiss/Webster mice (9–11 weeks) were orally pretreated with AHCC for 1 week (1 g/kg). After a 24-hour fast, *Klebsiella pneumoniae* (LD<sub>20</sub>) was injected intramuscularly into the thigh. At day 6, there were no bacteria that could be detected in the lung, liver, or blood in the AHCC-treated mice. Levels of cytokines, including monocyte chemoattractant protein-1 (MCP-1) (day 2), IL-12 (day 3), IL-6 (day 3), and TNF- $\alpha$  (day 3), were increased as compared with vehicle-treated mice. The increase in cytokines in these mice did not occur until day 5. Interestingly, levels of polymorphonuclear cells (neutrophils) were lower in AHCC-treated mice; however, levels of monocytes (day 2) and total white blood cells and lymphocytes (day 3) were increased. There were no significant differences between the groups with regard to levels of IgG or IgM antibodies specific to *K pneumoniae*. Aviles et al<sup>23</sup> conducted a similar earlier experiment evaluating the use of AHCC in a mouse model of wound infection. The animals (Swiss/Webster mice, 9–11 weeks) were pretreated similarly for 1 week with oral administration of 1 g/kg of AHCC followed by intramuscular injection of *K. pneumoniae* at a higher dose (LD<sub>50</sub>). There was increased survival in AHCC-treated mice as well as a higher LD<sub>50</sub> (10-fold increase). There was no difference in IgG or IgM antibody production, but bacterial clearance was greater in AHCC-treated mice.

### Protection in Setting of Immunosuppression

Ishibashi et al<sup>24</sup> pretreated leukopenic mice (secondary to cyclophosphamide) with either oral (1,000 mg/kg/d) or IP (50 mg/kg/d) administration of AHCC for 4 days. IP administration prolonged survival after infection with *Candida albicans* and methicillin-resistant *Staphylococcus aureus*. Oral administration prolonged survival after infection with *C albicans* and *Pseudomonas aeruginosa*. Similar results were seen in leukopenic mice (secondary to 5-fluorouracil or doxorubicin, but not prednisolone) in response to *C albicans* with IP administration of AHCC. In addition, in the 5-fluorouracil-treated group, there

were increased levels of peripheral leukocytes in oral AHCC-treated leukopenic and infected mice.<sup>25</sup>

Aviles et al<sup>26</sup> examined the role of AHCC in a female mouse hindlimb-unloading model of spaceflight. In this model, there is altered resistance and response to infection. Nine- to 11-week-old female pathogen-free mice were administered oral AHCC (1 g/kg) for 1 week before hindlimb unloading. Forty-eight hours after hindlimb unloading was started, IP *K pneumoniae* was injected. AHCC was continued throughout the entire hindlimb-unloading period (10 days). Mice that had undergone hindlimb unloading and treatment with AHCC had increased survival and LD<sub>50</sub> (CFU/animal) as compared with untreated animals; there was no difference with control animals (ie, not subjected to hindlimb unloading) treated with AHCC. IgG antibodies against *K pneumoniae* were increased in AHCC-treated groups with an associated decrease in bacteremia. It is important to note that AHCC administration did not affect IgM antibody production, which is more important in the early, acute reaction to infection.

In subsequent work, Aviles et al<sup>27</sup> characterized in more detail the effect of AHCC on the immune response. Splenocytes isolated from AHCC-treated mice not subjected to any intervention underwent increased proliferation in response to treatment of concanavalinA (ConA) and lipopolysaccharide (LPS). In addition, cytokine production was increased in cells treated with Con-A (IFN- $\gamma$ , IL-2) and LPS (IL-4, IL-6, IL-10, and IFN- $\gamma$ ). Splenocytes harvested from AHCC-treated mice subjected to hindlimb unloading behaved differently. There was no increase in proliferation in response to ConA or LPS treatment. When splenocytes were treated with ConA, there was a noted increase in IFN- $\gamma$  and IL-2 levels. There was no difference in cytokine levels in LPS-treated cells. To further investigate the effect of AHCC treatment on inflammatory cytokine production, peritoneal cells were harvested from mice (with or without hindlimb unloading) after IP AHCC. Subsequent cytokine profiling showed increased levels of TNF- $\alpha$  and IL-1 $\beta$ . Further investigation using peritoneal cells harvested from AHCC-treated animals after hindlimb unloading showed increased nitric oxide (with and without LPS stimulation) and IL-6 (with LPS stimulation alone), as well as elevation in TNF- $\alpha$  and IL-1 $\beta$  levels.

There are few studies that examine the particular component of AHCC that is responsible for the noted immunostimulatory properties. Kulkarni et al (unpublished data, ) suggest that the  $\alpha$ -glucan portion may be responsible for at least part of the observed effects. AHCC was separated with a DIAION HP-20 column, and the separated fraction was subsequently treated with ethanol and run through a cation exchange column to obtain the water fraction ( $\alpha$ -glucans). When murine T cells were cultured in the presence of this fraction, there

was significantly increased proliferation when stimulated with PHA, ConA, or LPS as compared with cells cultured in the absence of this fraction.

### Potential Side Effects: Healthy Volunteers

The effect of AHCC on humans has not been widely explored, specifically in relation to adverse effects. Spierings et al<sup>6</sup> conducted an open-label phase I trial of 26 human volunteers using a dose of 9 g/d in 3 divided doses for 14 consecutive days. The adverse events reported were transient and included nausea, vomiting, headache, cramps, bloating, diarrhea, and fatigue. There was no change in a variety of laboratory parameters (ie, hematologic, thyroid function, electrolytes, liver and kidney function, and coagulation parameter), blood pressure, urin analysis, or electrocardiogram. In the other clinical trials evaluating the use of AHCC (mostly in cancer), nausea has been the most commonly reported side effect.

Terakawa et al<sup>28</sup> also examined the effect of AHCC administration on various immunological parameters in 21 healthy human volunteers in a double-blind, placebo-controlled fashion. AHCC was administered daily at a dose of 3 g/d for a total of 4 weeks. Both CD11c-positive and CD11c-negative dendritic cells were increased after treatment with AHCC. In addition, the allogenic mixed leukocyte reaction was increased in AHCC-treated individuals.

### Discussion

Published research regarding AHCC indicates that it may have immunostimulatory effects; whether this may be of translational benefit in infectious and neoplastic processes remains to be determined. As these are disease processes that are components of many surgical diseases, specifically related to the oncology patient, the potential utility of this compound may be of interest to surgeons and surgical scientists. There are, however, several significant issues that require further exploration.

The majority of the studies have involved pretreatment or concurrent treatment (in studies examining effect when administered with various chemotherapeutic drugs). Although this has potential use in circumstances that can be anticipated—that is, ischemia-reperfusion injury after planned major cardiac/vascular surgery (eg, cardiopulmonary bypass, abdominal aortic aneurysm repair), radiation/surgery as a component of multimodal cancer treatment, and so on—it is important to elucidate potential MOAs and benefit with postinjury treatment. Animal studies that examine posttreatment with AHCC in relevant disease models are necessary to demonstrate

increased clinical relevance and before well-designed controlled clinical trials.

Potential MOAs of AHCC are poorly understood, especially in relation to modulation of chemotherapy-induced side effects preservation of body weight (potential of AHCC representing an easily absorbed calorie source), and tumor inhibitory functions. Much of the published literature suggests that AHCC functions as an immunostimulatory compound. However, there are also conflicting appearing MOAs (effects on decreasing oxidative stress, inflammation, and iNOS activity) that suggest require further investigation.<sup>4,29-33</sup> It is possible that components of AHCC (besides  $\alpha$ -1,4-glucans) may be responsible for these effects; however, this is not known. In addition, whether minor variations in production<sup>9</sup> affect the biological activity of this compound is not known. It would be important to determine what factors are responsible for these seemingly contradictory MOAs, as certain attributed MOAs may not be beneficial in the setting of cancer.

Before any widespread recommendation for the use of AHCC as an adjunctive agent in patients with cancer can be made, detailed studies into the pharmacological properties (eg, biodistribution, metabolism, breakdown products) are necessary. Secondary to the fact that AHCC is derived from an edible mushroom, the impetus for rigorous safety testing is somewhat lacking. It is important to note that the production process of AHCC alters the biological content of the mushroom (ie, minimal  $\beta$ -glucan content); whether this also affects the safety profile therefore, needs to be more fully examined. The only study to date evaluating potential drug interactions indicated that AHCC may interfere with the pathways used for metabolism of certain chemotherapeutic agents, which may pose issues with some chemotherapeutic regimens.

Dosing of AHCC varies widely between preclinical studies. The only published preclinical study that examined a dose response (approximate human equivalence dose of 500 mg to 5 g/d) demonstrated that the immunostimulatory effects of AHCC were dose-dependent.<sup>20</sup> Further study to optimize dosing regimens for particular indications is necessary.

An important component of mechanistic studies would also involve determining specific molecular components of AHCC that are responsible for the beneficial effect. Although the MOA of AHCC is widely attributed to  $\alpha$ -glucans, laboratory studies to date do not exclude an effect from the  $\beta$ -glucan component or the other purported components of this compound. Certain investigators have demonstrated some of the same beneficial (ie, immunostimulatory) effects attributed to AHCC to differing preparations of glucans.<sup>34-36</sup> An important study to determine whether the increased concentration of  $\alpha$ -glucans is important to the biological activity may consist of a comparison of the biological activity of AHCC to that of the mushroom from which it originates. Another

area of required investigation is in routes of administration. The majority of the studies are with oral administration. Although this is useful for many cases, in patients with significant intestinal dysfunction (postoperative or disease-related feeding intolerance), other routes of administration need to be more fully developed, including intravenous, IP, transdermal, and topical (ie, enema) routes. There has been one study that we referenced in the text that studied the transdermal route of administration and several that have used the IP route with varying effects. In accordance with drug-delivery methods, biodistribution and pharmacokinetic studies as well as further investigation into potential drug interactions and side effects are necessary before widespread adoption.

In addition, the varying effects of AHCC based on age and/or genotype need to be explored further. One study<sup>21</sup> evaluated the difference in age-based responses to AHCC in a model of West Nile virus; whether this also applies to other disease settings is unknown. In addition, the specific genetic makeup of an individual may affect or predict a response to various mycelial extracts, as has been shown by Yagita et al<sup>37</sup> using PSK (another substance derived from a Basidiomycete mushroom) to increase IL-12 levels in tumor-bearing mice.

It is important to note that the vast majority of the published research on AHCC has been supported by the manufacturer. Although a healthy relationship with industry is often beneficial and has resulted in significant scientific advancement, an independently funded research base to validate and add to the current published literature is imperative. Clinical trials involving the use of AHCC have been limited to observational studies and small, poorly controlled clinical trials. There are no prospective, randomized studies evaluating the effects of AHCC. Larger and more definitive clinical trials are likely to follow the development of a more comprehensive and diversely funded basic science literature base.

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