



POLYSACCHARIDE FROM LENTINUS EDODES FOR INTEGRATIVE CANCER TREATMENT: IMMUNOMODULATORY EFFECTS ON LYMPHOCYTE POPULATION

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Abstract – Background: Fungal alpha and β -glucans have been used as therapeutic support for thousands of years in Eastern culture. Lentinan, the backbone of β -(1, 3)-glucan with β -(1, 6) branches, is the main ingredient purified from Shiitake mushrooms and has been approved as a biological response modifier for the treatment of gastric cancer in Japan. Active Hexose Correlated Compound (AHCC) is an alpha-glucan rich nutritional supplement derived from the mycelia of shiitake (*Lentinula edodes*) of the basidiomycete family of mushrooms, a popular integrative medicine in Japan. Lentinan may exert a synergistic action with anti-cancer monoclonal antibodies to modulate complement systems activity through the way of antibody-dependent cellular cytotoxicity and complement dependent cytotoxicity.

Patients and Methods: Seven subjects with adenocarcinoma diagnosis (pancreatic, lung, colorectal) were recruited, and treated with AHCC (3 g/die).

Lymphocyte typing assays were performed by cytofluorometry (Abbott CELL-DYN Ruby) at start (T_0) and after one month from AHCC administration (T_1).

Results: After one month, neutrophils increased from 41% to 54%; lymphocytes and monocytes decreased, respectively, from 45% to 30% and 10% to 1%; lymphocyte population relationships variations: CD3/CD4 increased from 16% to 30%, CD3/CD8 (suppressor) decreased from 53% to 24%; CD4/CD8 increased from 0.3% to 1.3%; CD3+/CD16+/CD56 Natural Killer (NK) cells increased from 113% to 151%; CD8/CD3 (suppressor/cytotoxic) increased from 3% to 5%.

Conclusion: Results showed that immunological effects provided by α -glucans, a possible mode of action of lentinan (not only β -glucan mediated), should enable us to use lentinan more efficiently in the treatment of cancer, and its clinical application, including future potential uses of alpha and beta glucans association.

KEYWORDS: Fungal β -glucans, α -glucans, Shiitake mushrooms, Lymphocyte typing, Anticancer natural remedies.

INTRODUCTION

β -glucans

β -glucans are composed of a diverse group of glucose polymers, with a backbone of β -(1, 3)-linked β -D-glucopyranosyl units with β -(1, 6) linked side

chains. β -glucans may influence the immune system and modulate innate immune responses^{1,2}. Certain categories of glucans, as like as zymosan and lentinan, can anyway significantly stimulate phagocytosis³. Moreover, β -glucans induce macrophages to synthesize cytokines, and modulate adaptive immunity via a complex mechanism that involves both



B and T cells. The immunomodulatory effects of β -glucans in terms of immune response can sometimes be of no significance, probably due to differences in the degree of branching, polymer length, and tertiary structures among β -glucans.

The B and T cells constitute the basic support for the immune system. With the analysis of the subfamilies of T cells and B cells, it is possible to obtain information on the efficiency of the immune system of an individual. B cells produce antibodies to mediate humoral immunity, whereas T cells induce cell-mediated immunity. To the complex system of the adaptive immune response, dendritic cells also participate, presenting antigens to T cells for the activation of immune responses. Several lines of evidence indicate an alteration of dendritic cells in neoplastic disease^{4,5}. β -glucans contribute to improve DCs antigen presentation mechanism⁶, thereby inducing tumor-specific cytotoxic T cells.

Moreover, when the Fc region of an immunoglobulin interacts with receptors for the Fc domain of IgG on leucocytes, numerous biological effects are triggered: phagocytosis, enhancement of antigen presentation and release of inflammatory mediators^{6,7}. β -glucans were reported to enhance the expression of Fc γ R, a meeting point between specific humoral responses and cellular immunity,⁸ and the activation of complements^{9,10}. For this reason, beta glucans may be considered as adjuvants in cancer therapies with monoclonal antibodies^{11,12}.

β -glucans are recognized via a number of cell surface receptors by the immune system as non-self molecules, inducing both innate and adaptive immune responses^{13,14}. Several receptors have been identified in humans, and these include Dectin-1, the toll-like receptor (TLR), complement receptor type 3 (CR3), scavenger receptors, and lactosylceramide (LacCer).

It is known that, in oncological disease, dendritic cells have functional defects, with down regulation of T and NK cells^{15,16}. Lentinan use was reported to improve the generation of both killer T cells and NK cells^{17,18} and then rebalance killer/suppressor T cells¹⁹. Lentinan up-regulated NK cell-mediated destruction of neoplastic cells^{20,21} probably because of improved FcR expression, which could be related to ADCC augmentation. The addition of Lentinan promotes the activation of both pathways of complement²² and enhances CDC and complement-dependent cell-mediated cytotoxicity via CR3. On the other hand, we could say at this point that immunotherapy with lentinan might be used in conjunction with monoclonal antibodies therapy^{23,24}.

Lentinan, by interacting with specific receptors, activates macrophages and monocytes inducing IL-12 production²⁵, although the process is not completely clear yet. Furthermore, lentinan

decreases serum levels of IL-6 and PGE2 in patients with digestive tract cancer²⁶ and might avert the Th2- predominant balancement. Consequently, lentinan promotes Th1 polarization and rebalances Th1 - Th2 equilibrium. Some studies show that, with the evolution of cancer, the proportion of granulocytes grows in peripheral blood²⁷. Granulocytes decrease the antineoplastic competence of lymphocytes and lymphocyte-activated tumor cell killing^{28,29}; so, the raised numbers of granulocytes facilitate tumor growth by decreasing tumor-suppressing lymphocytes. The cancer-related G-CSF may explain the increase in granulocytes, and the higher granulocytes/lymphocytes ratio (G/L ratio) in the advanced stage, compared to the earlier stage^{30,31}. Other papers show that the administration of lentinan in cancer patients, has effect in G-CSF serum levels reduction, this could also possibly reduce the G/L ratio. Because of the ease with this ratio may be estimated, even in a retrospective analysis, the G/L ratio was chronologically evaluated using as a parameter its immunological action, in a group of subjects treated with chemotherapy alone and another group treated with chemotherapy and lentinan. At the beginning of chemotherapy, the G/L ratio was almost the same with or without lentinan administration. On the other hand, at 1 year after the start of chemotherapy or 1 month before exitus (when the survival time was less than 1 year after starting chemotherapy), the G/L ratio of subjects treated with lentinan was maintained at or below 2, value significantly lower than patients who received chemotherapy alone³² ($p < 0.001$).

α -glucans

AHCC is a cultured mycelium extract obtained from *Lentinus edodes* of Basidiomycete mushrooms fermented in rice bran³³. The main components of AHCC are α -glucans, which are derived from the processed mushrooms. These glucans are thought to provide a carbohydrate that stimulates immune response^{34,35}. Chemical analysis showed that AHCC is composed mainly of low molecular weight oligosaccharides (~5000 MW), of which 20%-30% are α -1,4 hexose linked. The mycelia of basidiomycetes (mushroom root threads) are cultured for 45-60 days and then subjected to enzymatic reaction, sterilization, concentration, and freeze drying sequentially. AHCC is available as a freeze-dried powder, and has been widely used in Japan as a dietary supplement for over 20 years and is currently used in China and the U.S. The immunological effects of AHCC are well documented. For example, murine thymic apoptosis induced by dexamethasone could be prevented by pretreat-

ment with AHCC³⁶. Administration of AHCC resulted in increased cell proliferation and cytokine production in spleen and increased nitric oxide and cytokine production in peritoneal cells of mice in a hindlimb unloading model of spaceflight conditions³⁷. In addition, AHCC could stimulate proliferation of NK cells and killer T-cells, and production of IL-12 and TNF α ^{38,39}. In accordance with its immunomodulatory function, the beneficial effects of AHCC supplementation on survival and the immune response to a variety of infectious agents have been demonstrated in animals⁴⁰⁻⁴². In humans, AHCC has been reported to improve the prognosis of patients with postoperative hepatocellular carcinoma⁴³. The potential of AHCC to act as a biological response modifier has been reported by Cowawintaweewat et al⁴⁴. Previous experiments using rodents have demonstrated that AHCC reduces such chemotherapy side effects as bone marrow suppression, hepatotoxicity, and nephrotoxicity⁴⁵⁻⁴⁷. There have also been several studies with healthy adults that have confirmed the clinical safety and tolerability of AHCC. In these studies, only low incidences of mild and transient side effects have

been found⁴⁸. Matsui et al⁴⁹ have found AHCC reduces the recurrence of hepatocellular carcinoma and adverse events associated with chemotherapy in a human prospective cohort study. However, the mechanisms of action of AHCC are still poorly understood, and conflicting evidence suggests the need for further investigation⁵⁰.

PATIENTS AND METHODS

Seven subjects with adenocarcinoma diagnosis were recruited and treated with AHCC (3 g/die) for one month, with no apparent toxicity. Patients were treated with paclitaxel and gemcitabine (2 pancreatic cancer), paclitaxel/alimta (2 lung cancer) FOLFOX (3 colorectal cancer).

Lymphocyte typing assays were performed by cytofluorometry (Abbott CELL-DYN Ruby) at start (T_0) and after one month from AHCC administration (T_1).

100% of participants reported various lymphocyte population alterations at T_1 .

Values expressed in percentage (Figures 1-5), are the average of the seven patients.

Fig. 1.

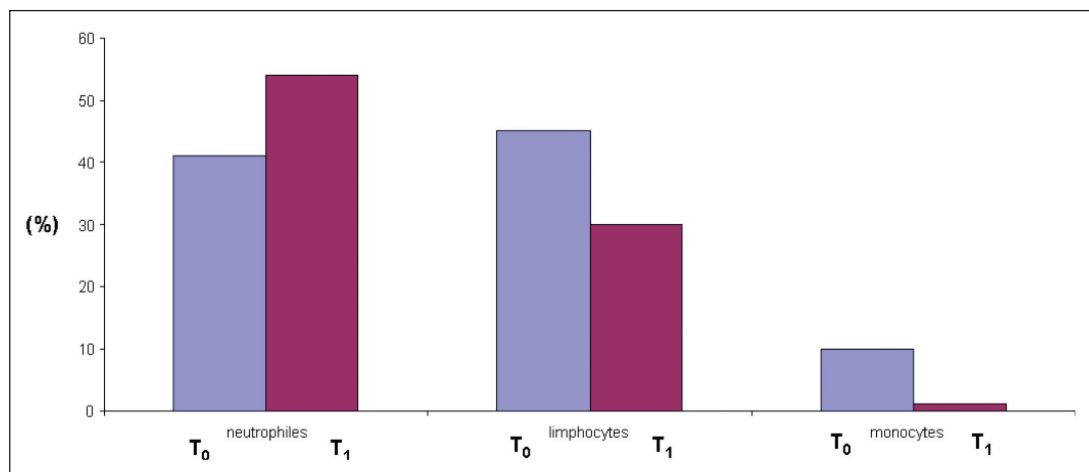
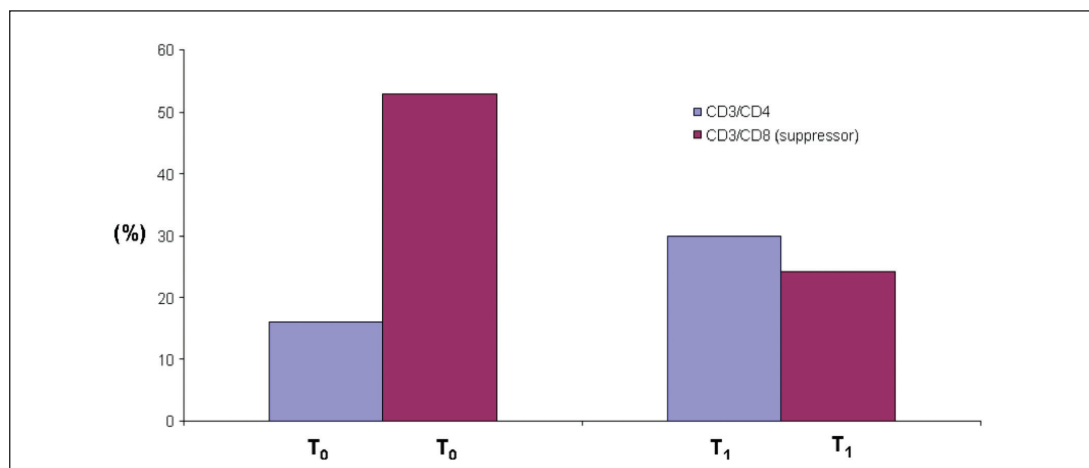


Fig. 2.



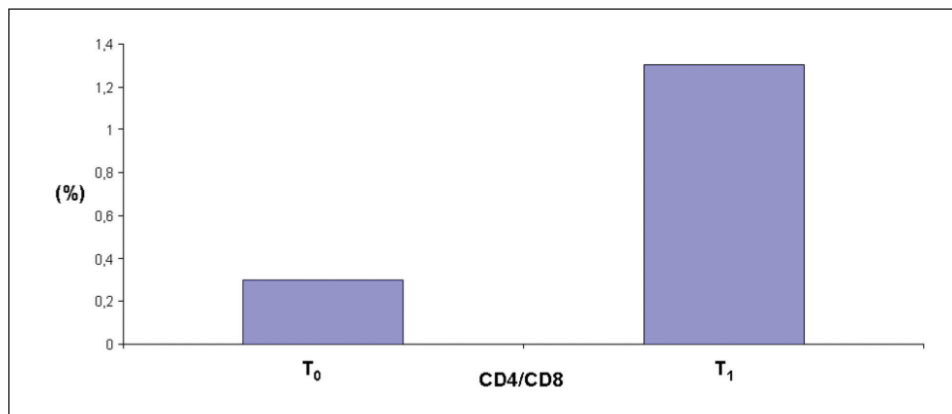


Fig. 3.

Reagents used: unconjugated and PE-conjugated anti-CD8 (UC-Leu-2a, PE-Leu-2a), PE-conjugated anti-CD4 (PE-Leu-3a), unconjugated and FITC-conjugated anti-CD3 (UC-Leu-4, FITC-Leu-4), PE-conjugated anti-CD 16 (PE-Leu-11), PE-conjugated anti-CD56 (PE-Leu-19), and FITC-conjugated anti-CD45 (FITC-HLE), were purchased from Beckman Coulter Italia.

The total number of white blood cells is calculated by Cytofluorimetric assay using automated counting equipment (PE and FITC Fluoro-labeled Cluster Differentiation). For a white blood cell count with differential, 100 white blood cells are counted and the proportion of each type is calculated. Since T cells and B cells have similar appearances, a differential can only give the proportion of lymphocytes in the blood, not the proportion of specific lymphocyte types⁵¹.

RESULTS AND DISCUSSION

After one month of AHCC-based therapy (T₀-T₁), neutrophils increased from 41% to 54%; lymphocytes decreased from 45% to 30% and monocytes decreased from 10% to 1% (Figure 1); T-lymphocyte sub-pop-

ulation relationships: CD3/CD4 increased from 16% to 30%, CD3/CD8 (suppressor) decreased from 53% to 24% (Figure 2); CD4/CD8 increased from 0.3% to 1.3% (Figure 3); CD3+/CD16+/CD56 NK increased from 113% to 151% (Figure 4); CD8/CD3 (suppressor/citotoxic) increased from 3% to 5% (Figure 5). Furthermore, for the entire duration of the treatment, none of the subjects had used immunostimulants drugs (Filgrastim, Pegfilgrastim, etc.).

Alpha glucans had more effect on innate immunity (CD8, CD56) than acquired immunity (CD4). All seven subjects showed a low CD4/CD8 ratio. This ratio could be related to “immunoediting escape” effect (increasing of immunological tolerance). After one month CD4/CD8 ratio was increased in all subjects. Alpha glucans also showed a clear modulation of natural killer levels (NK, CD56). NK is crucial into the direct destruction of the neoplastic cells, but also in down-regulation of CD8.

CONCLUSIONS AND FUTURE OUTLOOK

Strategies previously described allowing physicians to improve the efficacy of integrative cancer

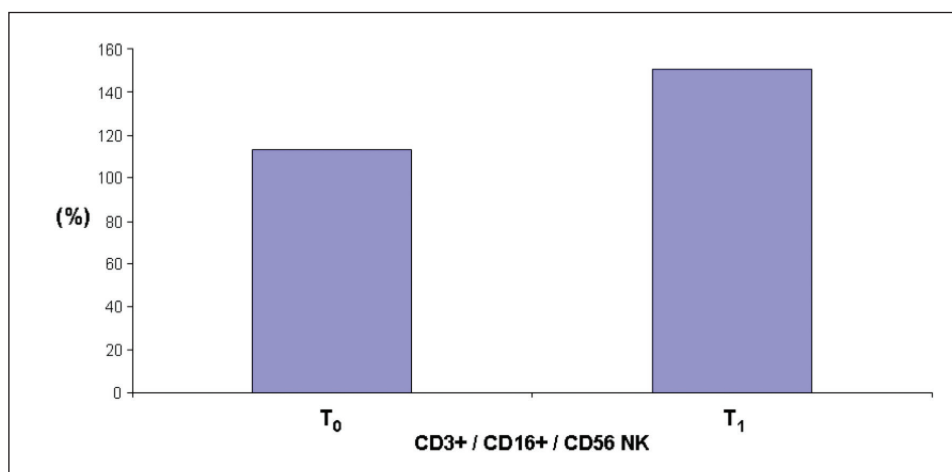
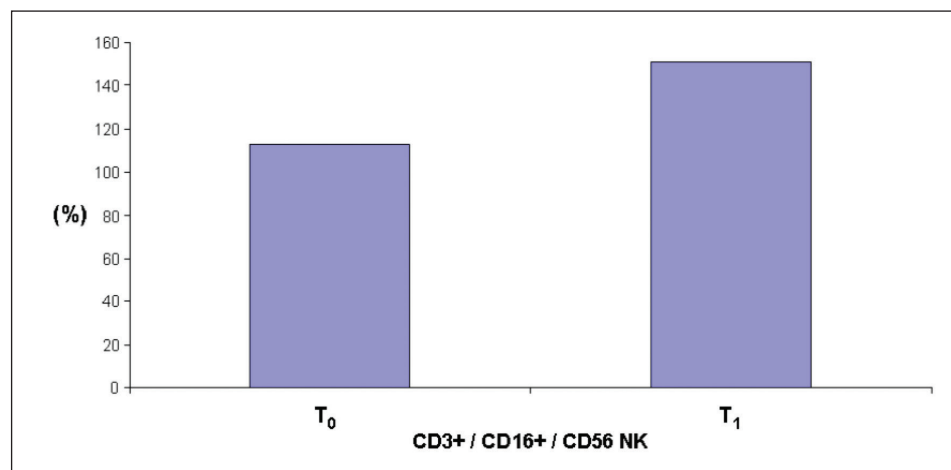


Fig. 4.

Fig. 5.



therapy. It is now clear that bio-behavioural factors, as nutrition, not only affect cellular immunity but both, directly and indirectly, modulate fundamental processes in cancer growth, including inflammation, angiogenesis, invasion, metastasis, chemo/radio effectiveness and immunological tolerance^{52,53}.

Lentian and other alpha and beta-glucans have already demonstrated immunomodulatory properties in animal experiments and *in vitro* studies. Multiple pathways have been described for the effects observed in the immune system, including up regulation of T-cell, cytokine, monocyte, tumor necrosis factor, natural killer cell, complement activation and other macrophage responses. However, the number of quality clinical trials and studies published in the English language, peer-reviewed journals is limited, so further studies are needed to assess current outcome indicators.

CONFLICT OF INTERESTS:

The Authors declare that they have no conflict of interests.

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