
Effectiveness of immunotherapies from oyster mushroom (*Pleurotus* species) in the management of immunocompromised patients

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Abstract: In the recent years, mushrooms are distinguished as important natural resources of immunotherapy which can be used as immunomodulating and immunostimulating in the management of some immunodeficiency diseases such as cancer, tumour, HIV, tuberculosis etc. Mushroom of the genus *Pleurotus* are good sources of several bioactive compounds which are able to augment or complement a desired immune response. Such bioactive compounds are polysaccharopeptides, polysaccharide-proteins, functional proteins (ubiquinone-9, nebroleolysin, ubiquitin-like peptide and glycoprotein), glucans, proteoglycans and many others. Most of these bioactive compounds follow the immunomodulatory pathway mechanism of polysaccharide (β -glucan) from mushrooms by stimulating activities for both innate and adaptive immune systems. They proliferate and activate innate immune system components such as natural killer (NK) cells, neutrophils, and macrophages, and stimulate cytokines expression and secretion. These cytokines in turn activate adaptive immunity through the promotion of B-cells for antibodies production and stimulation of T-cell differentiation to T helper (Th1 and Th2) cells, which mediate cell and humoral immunities, respectively. In this review, the immunotherapeutic potential of oyster mushroom in relation to bioactive compounds produced is shown and this suggests that the oyster mushrooms are one of the most important natural products and functional foods.

Keywords: Oyster Mushroom, Immunotherapy, Beta Glucan, Lectin, Polysaccharide

1. Introduction

Immunotherapy can be called biologic therapy or biotherapy, which is the treatment that activates certain parts of a patient immune system to fight diseases such as cancer, HIV, tuberculosis etc. This can be done in a couple of ways, such as stimulating the immune system of the patient to work harder or smarter to attack the disease or giving the patient immune system components, such as man-made immune system proteins [1,2]. Immunotherapy treatment can be general or specific. In the general method, the patient immune system is boosted in a general way to attack any disease, while in the specific method, the patient immune system is trained to attack a specific disease. Practices of immunotherapy, for example in treating cancer patient can be traced to William Coley in the late 1800s, when he treated

cancer patients with certain kinds of bacteria, known as *Coley toxins*. Since then, several studies have reported the uses of immunotherapies such as T-cell adoptive immunotherapy for lymphoblastic leukemia [2], cancer regression and autoimmunity by clonal repopulation with antitumour lymphocytes [3], therapeutic antibodies and immunologic conjugate [4], cancer regression by transferring genetically engineered lymphocytes [5], safety and tumour responses with lambrolizumab (anti-PD-1) in melanoma [6], improved survival in patients with metastatic melanoma [7] to mention a few.

Immunotherapy is a way of increasing the survival rate of immunocompromised patients. People who are suffering from different diseases like liver cirrhosis, advanced HIV/AIDS,

cancer, recent bone marrow transplant and other severe immunodeficiency diseases[8] can be immunosuppressed and subjected to increase risks of adverse events (AEs)[9]. In immunocompromised patients, acute respiratory failure (ARF) is both common and severe[10]. ARF is the leading cause of intensive care unit (ICU) admission in patients with hematological or solid malignancies. Few years ago, studies have shown mortality rates of nearly 50% in this population[10,11,12]. Mortality rates are highest in patients with severe respiratory distress requiring invasive mechanical ventilation (IMV). In this situation, mortality can reach 40% in organ transplant recipients and patients receiving immunosuppressive treatments[13,14], and 60% in patients with hematological or solid malignancies[11,12]. Treating or incorporating the diet of these patients with natural products such as mushroom with good potential in immunomodulating activity will increase the survival rate of the patients. Mushrooms (*Pleurotus* species) display *in vivo* and *in vitro* immunomodulatory activity, in particular since fungal compounds exhibit antitumour activity, based on modulation of the immune system[15]. In clinical practice, immunomodulators are usually classified into immunosuppressants, immunostimulants, and immunoadjuvants.

Oyster mushroom (*Pleurotus* species) is commercially important in the world mushroom market, and several species are grown commercially on a large and small scale in many countries[16]. They have long been valued as tasty and nutritional foods, by different societies worldwide. *Pleurotus* species have been recognized as mushroom with dual functions to humans; both as food and medicine [17,18]. They are nutritive with good quantity of proteins, vitamins and minerals. Medicinally, they are been recommended for obese persons and diabetes patients because of low calorie value [17] and very low sugar without starch[19]. Several studies on various *Pleurotus* species have shown a number of pharmacological activities, such as anti-tumour, immunomodulatory, antigenotoxic, antioxidant, antiinflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial and antiviral activities[20,21,18,16]. *Pleurotus* species are good source of immunomodulators, the substances considered as “host defense potentiators” (HDPs) as judged by their immuno-stimulating properties[20]. Several molecules that are able to augment or complement a desired immune response have been isolated from *Pleurotus* spp., such as: water-soluble polysaccharides from *P. citrinopileatus* fermentation broth [22]; glucans from *P. florida* fruiting bodies[23]; proteoglycans, polysaccharides and polysaccharopeptides from *P. ostreatus* mycelia[24,20,25]; a ubiquitin-like peptide from *P. sajor-caju*[26]; a glycoprotein from *P. citrinopileatus* [27]; a Pleurone from *P. eryngii* [28] and DNA from *P. ostreatus* fruiting bodies [29]. These compounds stimulate different cell populations of the immune system, for instance, macrophages, Natural killer (NK) cells, T-cells, and also modulate cytokine system[21]. The objectives of this review are the compilation of the newer achievements in the effectiveness of immuno-

therapies from oyster mushrooms (*Pleurotus* spp.) in health management.

2. Taxonomy of Genus *Pleurotus*

Oyster mushrooms are cosmopolitan, and belong to the genus *Pleurotus* (Fungi: Basidiomycetes). Their cap is normally shell-like (about 5-20 cm in diameter; 1.9-7.8 inches), fleshy, with eccentric or lateral stipe; and their color can be white, cream, yellow, pink, brownish, or dark gray [30]. Oyster mushroom was first cultivated in 1917 at Germany by Flank. Evolutionary connection among species in the genus *Pleurotus* is still not clear and many taxonomic issues remain controversial. The genus *Pleurotus* is one of the most diverse groups of cultivated mushrooms. Fungal populations are established and developed through sexual and asexual reproduction [31]. Conventional methods for classification (fruit body morphology, microscopic observations, mating studies between populations, biochemical analyses) have not provided clear-cut results [30]. This taxonomic confusion has always been associated with the genus *Pleurotus*, especially species belonging to the *P. ostreatus* complex due to morphological variations of different specimens and similarity of isolates belonging to different species [32]. Molecular studies have shown to be more informative: intra and inter-specific heterogeneity was determined using ribosomal and mitochondrial DNA analyses, and phylogenetic studies of ribosomal DNA sequences indicated geographic speciation in several groups. Hilber [33] reported results of mating reactions as well as the microscopic and macroscopic characteristics of many *Pleurotus* isolates and concluded that *P. pulmonarius* and *P. ostreatus* were different species, *P. sapidus* is more likely to correspond to *P. cornucopiae*, and *P. columbinus* is simply a variety of *P. ostreatus*. There are other disagreements concerning the exact taxonomic position of the cultivar *P. florida* (considered either as a *P. pulmonarius* or a *P. ostreatus* strain) and whether *P. cystidiosus* and *P. abalonus* belong to separate species [34,35] or the same species [36,37].

Zervakis and Labarer [38] classified 23 isolates of genus *Pleurotus* with dendrogram of taxonomic distances and demonstrated the suitability of enzyme isoelectric focusing in classification, as it clearly distinguished the four well-defined species *P. cornucopiae*, *P. cystidiosus*, *P. eryngii* and *P. flabellatus*. The *P. ostreatus*, whose taxonomy is controversial, was separated from *P. pulmonarius*, and *P. columbinus* and classified as a distinct taxon. The delimitation of *P. sajor-caju* and *P. sapidus* strains appeared to be more difficult as they seemed to be closely related; the former with *P. pulmonarius* and the latter with *P. ostreatus*. Vilgalys et al. [39] identified three intersterile groups, *P. ostreatus*, *P. pulmonarius* and a new species limited to North America: *P. populinus*. Vilgalys et al. [40] identified 15 intersterility groups of *Pleurotus* (*ostreatus*, *pulmonarius*, *populinus*, *cornucopiae*, *djamor*, *eryngii*, *cystidiosus*, *levis*, *dryinus*, *tuberregium*, *agaves*, *abieticola*, *brazil*, *australis*,

purpureo-olivaceus) of which some groups have been added in the later time. Oyster mushroom are generally classified as follow; Scientific Name:-*Pleurotus spp.*; Phylum:-*Basidiomycotina*; Class:-*Basidiomycetes*; Sub class:-*Holobasidiomycetidae*; Family:-*Polyporaceae*; Genus:-*Pleurotus*; species:- *sajor caju*, *sapidus*, *ostreatus*, *eous*, *membranaceous*, *florida*, *citrinopileatus*, *flabellatus*, *pulmonarius*, *geesteranus*, *ulmarius*, *tuberregium*, *cystidiosus*, *eryngii* and others.

3. Life Cycle of *Pleurotus* (Oyster Mushroom)

Pleurotus mushrooms show the typical life cycle of Basidiomycetes, a major fungal group (Fig. 1a). It begins with the germination of a basidiospore in a suitable substrate, which gives rise to a monokaryotic mycelium containing genetically identical nuclei (n) and capable of indefinite independent growth. When two compatible monokaryotic mycelia are in close contact, they are able to establish a fertile dikaryon by hyphal fusion or plasmogamy. This dikaryon (n+n), having clamp connections and binucleate in each hyphal compartment (Figure 1b), contains two genetically different nuclei (one from each monokaryon) throughout the mycelium [30,41]. When environmental conditions are appropriate (temperature, light, relative humidity), the dikaryotic mycelium will differentiate into fruit bodies having specialized structures called basidia. In these club-shaped, binucleate cells, which are formed in the lamellae (hymenium) of each fruit body, karyogamy (fusion of the paired nuclei; 2n) and meiosis (recombination and segregation) take place. The four resulting haploid nuclei move to the sterigmata on the basidium, to form four new basidiospores. When the fruit bodies are mature, basidiospores are discharged, starting the sexual life cycle again.

4. Production of *Pleurotus* Species

Increase in consumer demand for oyster mushrooms in the world has continued its rapid pace in the last few years. The increase in demand is as result of significant role play in human health, nutrition and disease.

Since the inception of oyster mushroom cultivation by 1917 in Germany, the production kept on increasing annually. Royse[43] reported an increase of more than 18-fold in total mushroom production worldwide in the last 32 years, from about 350,000 metric tons in 1965 to about 6,160,800 metric tons in 1997, with *Pleurotus* species occupy the third position, beside *Agaricus* and *Lentinula* in the first and second position respectively [44]. Annual production increases for *Pleurotus* spp. have averaged 14% (from 880t in 1996 to 1940t in 2002; USDA [45]. International demand for oyster mushrooms has remained steady (at about 900,000t annually) over the last decade. The People's Republic of China is the major producer and consumer of oyster mushrooms,

accounting for nearly 90% of total world production[46]. The available figures have revealed that oyster mushroom production increased 44.2% during the period 1986-1991, reaching about 917,412 tons(fresh weight) in 1991. China, South Korea, Japan, and Indonesia are major producers[30]. Some years ago oyster mushrooms started to occupy the second position among cultivated edible mushrooms worldwide due to their nutritional and medicinal values [47]. In the United States, its cultivation is second only to that of the white button mushroom, *Agaricus bisporus*. In 1995, the United States produced 880t of oyster mushroom, a 94% increase over the previous year [48]. At present, *Pleurotus* spp. has become the second most important cultivated mushroom, accounting for about 22% of the total world production.

5. Bioactive Compounds with Immunotherapeutic Potential from *Pleurotus* Species (Oyster mushroom)

In the recent years, several bioactive compounds have been isolated from genus *Pleurotus*. The like of these bioactives are polysaccharides [22]; polysaccharopeptides [25]; polysaccharide-proteins[18]; functional proteins such as Pleuron [28], glycoprotein [27], ubiquinone-9[49], nebroleolysin[50], ubiquitin-like peptide[26]; glucans [23]; proteoglycans [24] and lectin [51]. Some of these compounds act as immunotherapy by influencing the production of cytokine in human peripheral blood mononuclear cells (hPBMC), which may include mitogenicity, stimulation and activation of immune effector cells[15]. Many other compounds have potent antitumor activity by inhibition of tumor growth and induced apoptosis, while some elicited amitogenic response from murine splenocytes and inhibited HIV-1 reverse transcriptase[51,49]. Generally most of these bioactive compounds follow the immunomodulatory pathway mechanism of polysaccharide (β -glucan) from mushrooms. Mushrooms polysaccharides immunomodulators exhibit stimulating activities for both innate and adaptive immune systems. They proliferate and activate innate immune system components such as natural killer (NK) cells, neutrophils, and macrophages, and stimulate cytokines expression and secretion[52]. These cytokines in turn activate adaptive immunity through the promotion of B-cells for antibodies production and stimulation of T-cell differentiation to T helper (Th1 and Th2) cells, which mediate cell and humoral immunities, respectively[53]. Based on their high molecular weight, mushroom polysaccharides are not able to penetrate the immune cells to activate immune cells directly. Thus, the stimulation mechanism of polysaccharides involves different cell receptors such as dectin-1, Complement receptor 3 (CR3), Lactosyl ceramide (LacCer), and Toll-like receptor (TLR)2. In such cases, the effectiveness of polysaccharides is governed by their binding affinity to immune cell receptors[54].

5.1. Immunotherapy of Mushroom Polysaccharides from *Pleurotus* Species

The polysaccharides (exopolysaccharides and endopolysaccharides) found in mushrooms belonging to the genus *Pleurotus* have revealed important biological activities. Polysaccharides can be isolated from the mycelium, the fruiting body, and the sclerotium, representing three different stages in the fungal life cycle. Polysaccharides differ in their primary structure (type of basic sugar), type of linkage (α , β ,

etc.), degree of branching, and molecular weight, among other parameters. Significant biological activities reported in literature are antitumor, immunomodulatory, antioxidant, hypocholesterolaemic, antihyperglycemic, antimicrobial, antiviral activities and as vaccine adjuvants [21,55,56,57]. In particular, polysaccharides act as a defense mechanism against invasion by foreign bodies to enhance the natural immune system, including activation of macrophages and natural killer (NK) cells (Figures 2 & 3).

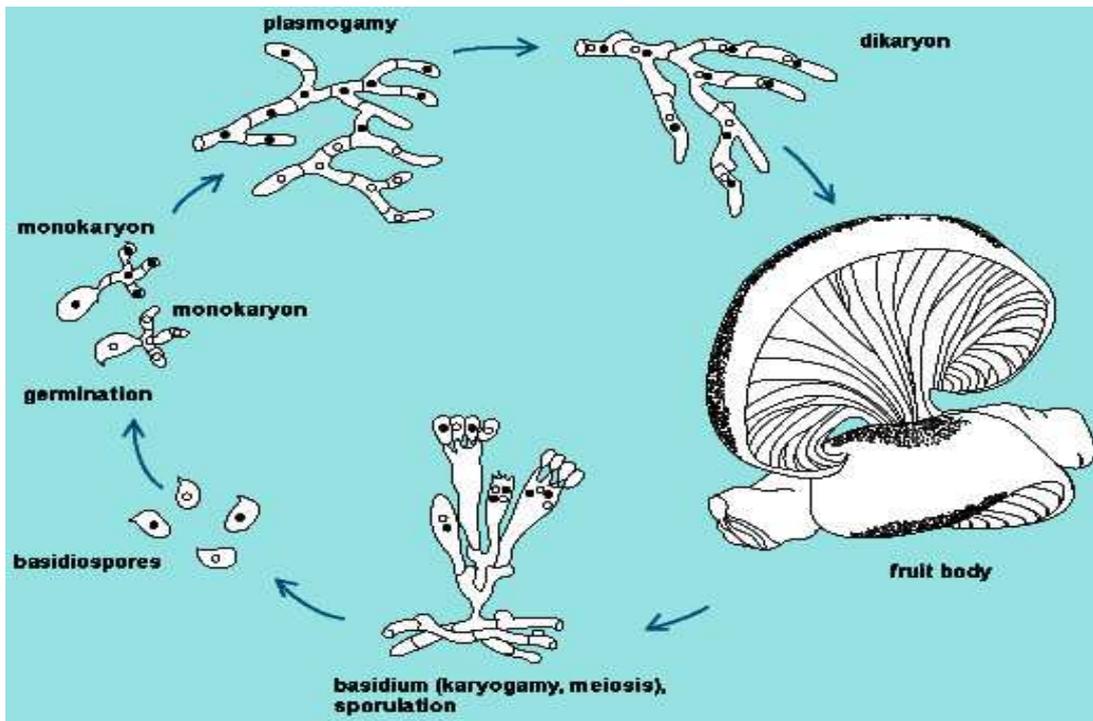


Figure 1a. Life cycle of the oyster mushroom *Pleurotus ostreatus*
Source: Adebayo and Martínez-Carrera [42]

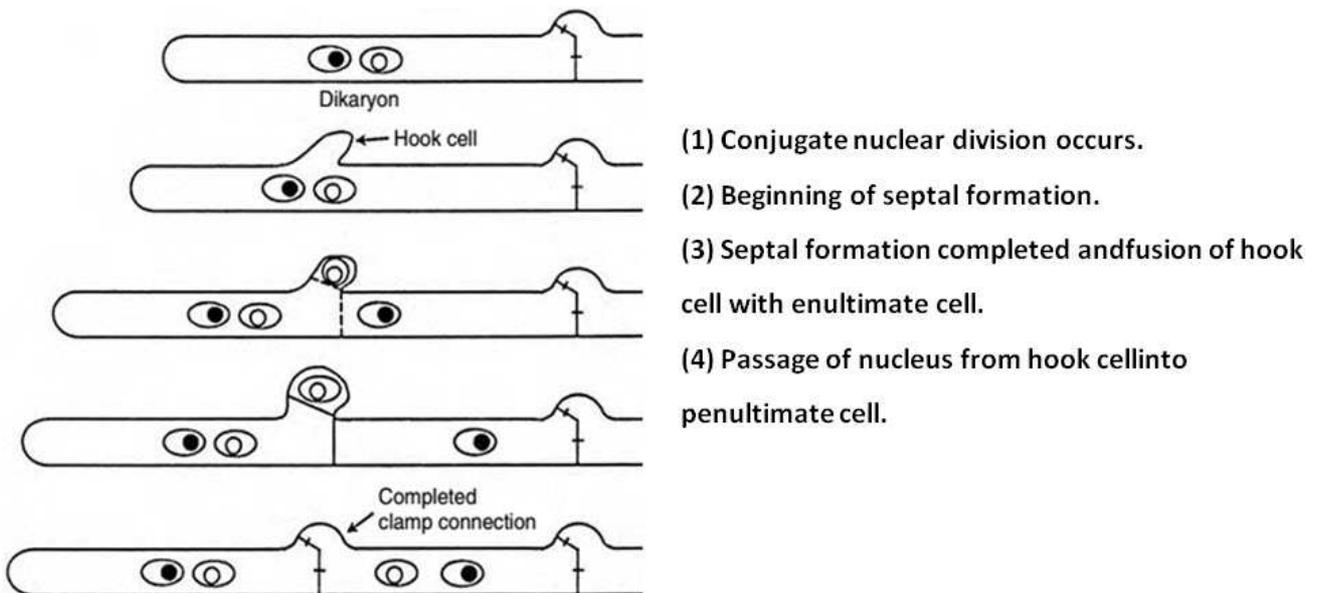


Figure 1b. Diagrammatic representation of clamp connection formation.

The defense mechanism by polysaccharide increases macrophage cytotoxic activity against tumor cells and

microorganisms; activates phagocytic activity; increases reactive oxygen species (ROS) and nitric oxide (NO) production, and enhances secretion of cytokines and chemokines, such as tumor necrosis factor (TNF- α), interleukin (IL)-1 β , IL-6, IL-8, IL-10, IL-12, IFN- γ and IFN- β 2 [25,58]. Several years ago, three antitumor agents of polysaccharide nature i.e. lentinan, schizophyllan and protein-bound polysaccharide (PSK, Krestin), were isolated from *Lentinula edodes*, *Schizophyllum commun* and *Coriolus versicolor* respectively and have since become large market items in Japan [59]. Lentinan and Schizophyllan are pure β -glucans [60,61,62] whereas PSK is a protein-bound β -glucan [63,64]. A polysaccharopeptide (PSP) has also been isolated from a strain of *Coriolus versicolor* in China and become widely used in clinical treatments as an anti-cancer and immunomodulatory agent [65]. PSP manufactured in China is found to be quite similar to PSK in Japan.

Polysaccharide extracts from mycelium and fruit bodies of *P. pulmonarius* exerted a direct antiproliferative effect on cancer cells expressing high galectin-3 concentrations and concomitantly down-regulated the adherence of colon cancer cell lines (HT29, HCT-116), which directly interfere with cancer progression and metastasis [66]. Polysaccharide which contain both α and β linkages obtained from *P. pulmonarius* showed a potent anti-inflammatory effects against carrageenan and formalin-induced paw edema in rats with higher inhibitory percentage in rats writhing; 83.33% and 92.37% inhibition obtained in carrageenan and formalin-induced respectively [18]. Addition of polysaccharide from *P. pulmonarius* into the diet of 4-week old mice containing a carcinogen; Diethylnitrosoamine (DNA) delayed progression of carcinogenesis [67].

Acetone extract from *P. pulmonarius* and *P. ostreatus* exhibited a profound anticancer potential as they offered protection to animals as tumour suppressor [68]. Refaie et al. [25] reported that the intraperitoneal administration of polysaccharopeptides from *P. ostreatus* mycelium at dose "25 mg/kg", thrice weekly, for five consecutive weeks elevated dramatically serum IFN- α level in adult female Swiss albino mice. The water-soluble polysaccharide (POP) with a molecular mass of 2.4×10^4 Da, obtained from the fruiting body of *P. ostreatus*, enhance concanavalin A (ConA)- or induced lymphocyte proliferation on male mice [69].

Polysaccharides from hot water extract of *P. geesteranus* showed significant cytotoxicity in human breast cancer cell line MCF-7 [70]. Water-soluble polysaccharides extracted from *P. citrinopileatus* fermentation broth have been shown to reduce the number of metastatic tumour nodules in tumour-bearing mice [22]. The protein-polysaccharide from *P. ostreatus* and *P. sajor-caju* inhibited the growth of a solid Sarcoma 180 tumor implanted in mice [71]. In addition, the hot water extracts of *P. sajor-caju* and *P. pulmonarius* inhibited HIV-1 reverse transcriptase activity [72]. Similar activity was also observed by Choi et al. [73] with hot water and ethanol extracts from fruiting body of oyster mushroom that exerted positive effect on three human solid carcinomas, (A549) and two cervical carcinomas (SiHa and HeLa). Their

report showed that ethanol extracts of *Pleurotus* species had high antitumor activity towards lung cancer cells (A549). Morris et al. [20] reported that *Pleurotus* powder and hot water extract polysaccharides obtained from fruit body, administered orally to cyclophosphamide-treated mice, provides immunological benefits in terms of the recovery of bone marrow cellularity, increase of white blood cell counts, and stimulation of cell-mediated immune responses. Major component behind the anticancer activity of *Pleurotus* mushrooms is its polysaccharide fraction called pleuran, a member of polysaccharide beta glucans, which is an important constituent of *Pleurotus* species.

5.2. β -Glucan as Immunotherapy from *Pleurotus* Species

The major medicinal effects of mushrooms, i.e., immunity potentiation and antitumor activities, are attributed to β -glucans. β -glucans from various fungi are not digested by human enzymes when orally administered, but instead are taken up in the small intestine, which stimulates mucosal and systemic immunity [74]. Uptake of β -glucans stimulates antitumor activities as well as protective activities against fungal and bacterial infections in animals and human. Despite their high molecular weight, β -glucans, when orally administered, are absorbed in the intestine and activate innate and adaptive immunities [75].

β -glucans consist of a backbone of glucose residues usually joined by β -(1-3) linkages, to which glucose side-chain residues are often attached [76,77]. In some fungi β -glucans, no side-chain substitution occurs, as with the bacterial β -glucan, curdlan, which contains only β -(1-3)-glucosidic linkages [77]. No un-branched β -(1-3) or β -(1-6) fungal β -glucans are known, although the extent of the side-chain substitutions can vary considerably [76]. Among the various β -(1-3), β -(1-4) and β -(1-6) β -glucan linkages, only β -(1-3) stimulates immunity and shows antitumor activities [75]. About half the mass of the fungal cell wall consists of β -glucans [78,79,77], with many of them excreted into the growth medium, making their recovery, purification and chemical characterization much simpler [78,76] and they are referred to as extracellular metabolite. β -glucans homeostasis is maintained by β -glucan synthases and β -(1-3) or β -(1-6)-glucanase activities [80,81]. Some fungal β -glucans that markedly stimulate the immune system, offer protection from attack by pathogenic microbes and from harmful effects of environmental toxins and carcinogens [82,83]. β -glucans are not synthesized by humans, so these compounds are recognized as non-self-molecules, inducing both innate and adaptive immune responses [83]. They appear to activate phagocytes, thus leading to elimination of pathogens by phagocytosis [84,85]. Among these, the macrophages preferentially attack dead cells and intracellular pathogens [86]. Natural killer cells (NKs) circulate in blood to lyse cancer and virus-infected cells, whereas neutrophils are effective against pyogenic bacteria. Some β -glucans activates type 3 complement receptors (CR3), which coat bacterial cells to facilitate their engulfment by phagocytes [87,82].

Many β -glucans stimulate macrophages to produce cytokines, local immunomodulators, and these in turn activate adaptive immunity[85,88,89], which can then

stimulate the activity of cytokines such as interleukin (IL)-2, IL-10 and IL-12 (Figure 2) [90,91].

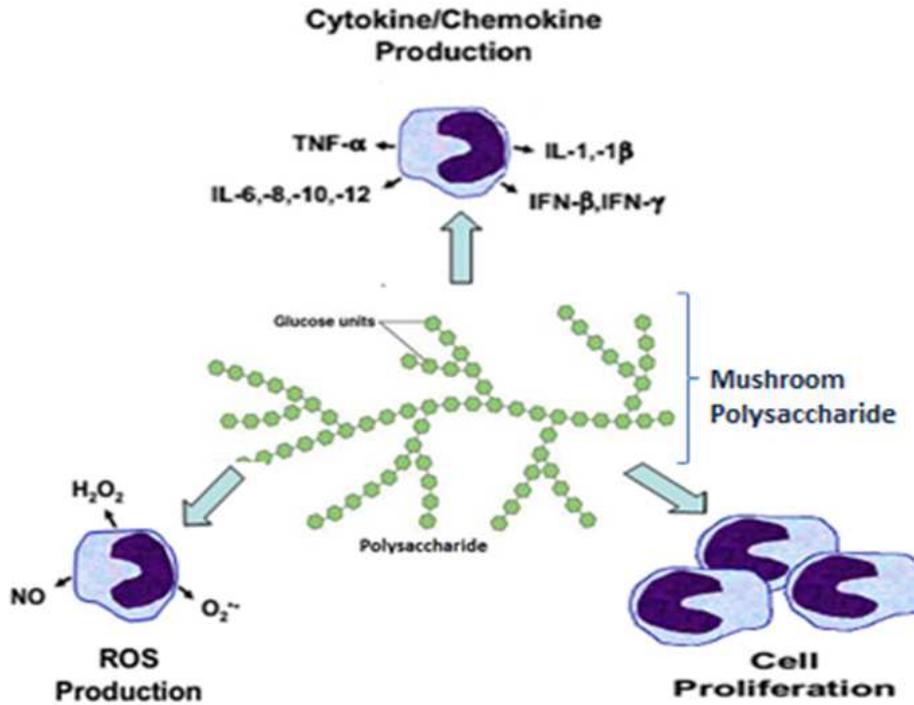


Figure 2. Activation of macrophage host defense responses by mushroom polysaccharides. IL= interleukin; IFN= interferon; $TNF-\alpha$ = tumor necrosis factor α ; NO= nitric oxide; ROS= reactive oxygen species. Source Refaie et al (25).

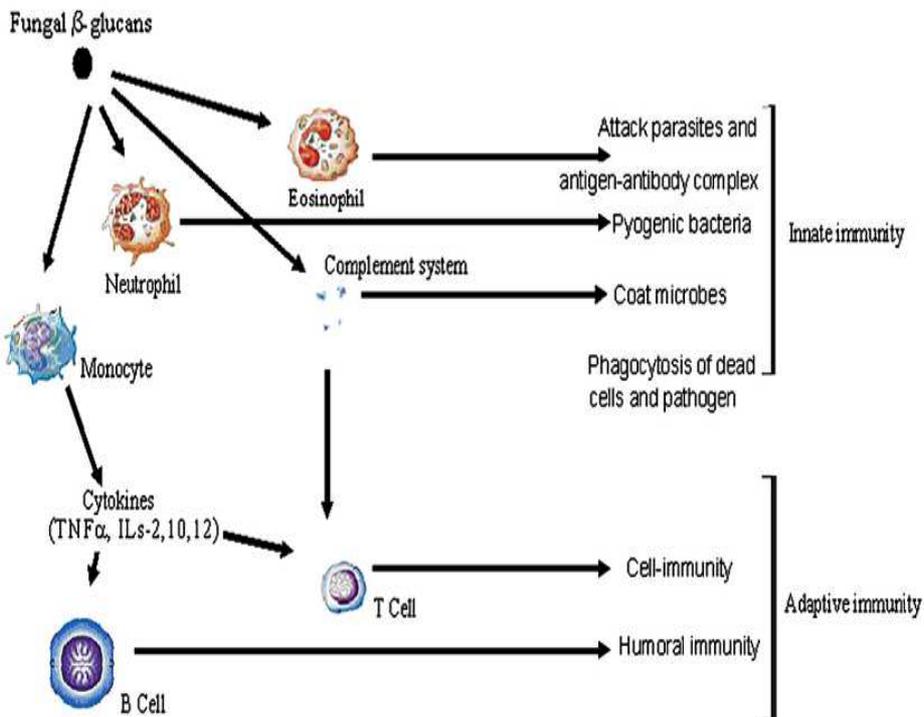


Figure 3. Immunostimulation pathway by fungal β -glucans.

Sources: Chen and Seviour, [54]

β -Glucans of the *P. tuber-regium*, when sulfated, showed an antitumor effect and antiviral activity[92]. Manzi and

Pizzoferrato [93] reported water-soluble and water-insoluble β -glucans from *P. ostreatus*, *P. eryngii* and *P. pulmonarius* that have pronounced effect on humans and animals immune systems. β -glucan with (1-3) and (1-6) linkage, produced by *P. ostreatus* was reported to show strong anti-respiratory infection [94] and activation of natural killer cell (NKs)[95] at clinical trial. The β -((1-3/1-6)-D-glucan isolated from *P. ostreatus* decreased the arthritis development in rats [96]. Jedinak et al. [97] reported that β -glucan from *P. pulmonarius* suppressed lipopolysaccharide (LPS)-induced secretion of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and IL-12p40 from RAW264.7 macrophages. Beta glucan from *P. pulmonarius* inhibited (LPS)-induced production of prostaglandin E2 (PGE2) and nitric oxide (NO) through the down-regulation of expression of COX-2 and iNOS, respectively. β -glucan also inhibited LPS dependent DNA-binding activity of AP-1 and NF- κ B in RAW264.7 cells[97]. Oral administration of β -glucan markedly suppressed secretion of TNF- α and IL-6 in mice challenged with LPS *in vivo*. Anti-inflammatory activity of β -glucan was confirmed by the inhibition of proliferation and secretion of interferon- γ (IFN- γ), IL-2, and IL-6 from concanavalin A(ConA)-stimulated mouse splenocytes[97]. Glucans isolated from *P. florida* fruiting bodies activated the phagocytic response of mouse macrophages *in vitro* [98] and significantly induced the proliferative response as well as phagocytic activity of fish leukocytes *in vitro*[99]. Moreover, Zhang et al.[100] demonstrated that, in contrast to water-insoluble β -glucans isolated from *P. tuber-regium* sclerotia, their corresponding water-soluble sulphated derivatives exerts antiviral activities against herpes simplex virus type 1 and type 2. The effect is presumably elicited by the binding of sulphated β -glucans to viral particles, thus preventing them from infecting the host cells.

5.3. Functional Proteins as Immunotherapy from *Pleurotus Species*

The ability of fungal compounds, like fungal immunomodulatory proteins (FIPs), to alter the cytokine response has been described [101]. Hsu et al.[102] reported the purification of FIP-Vvo and suggested that the immunomodulatory effects might be due to cytokine regulation of human peripheral blood mononuclear cells (hPBMC). FIPs are classified into a distinct family since they are a group of fungal proteins defined by amino acid sequence similarity and their actions on immunological responses [103,104].

Pleurotus have been shown to secrete high levels of proteins and novel enzyme variants, with desirable properties for medicinal and biotechnological applications[105]. A functional protein (PCP-3A)was isolated from the fresh fruit bodies of *P. citrinopileatus*, which inhibited the proliferation of human leukemia cell U937. Flow cytometry analysis revealed that PCP-3A is capable of inhibiting the growth of U937 cells and initiates apoptotic induction[106]. A novel hemolysin was isolated from *P. nebrodensis*, which showed strong cytotoxicity against Lu-04 (lung cancer), Bre-04

(breast cancer), HepG2(liver cancer), L929 (mouse fibroblast), and HeLa cells (cervical cancer) (50). This hemolysin also induced apoptosis in L929 and HeLa cells possesses anti-HIV-1 activity [50]. The protein extracts from *P. ostreatus* exhibited therapeutic efficacy against human colorectal adenocarcinoma cells and human monocytic leukemia cells by induced reactive oxygen species (ROS) production, glutathione (GSH) depletion and mitochondrial trans-membrane potential loss in SW480 cells[107].

Ribonucleases with molecular weight of 10.7 kDa have been isolated and characterized from the *P. ostreatus* with the potential of neutralizing HIV through degradation of viral genetic material[108].

Laccase (66-kDa) with an N-terminal sequence different from those of other mushroom laccases, from fresh fruit bodies of *P. cornucopiae*, which inhibited the proliferation of murine leukemia cell line L1210 and human hepatoma cell line HepG2 has been reported (109). It also reduced the activity of HIV-1 reverse transcriptase with an IC₅₀ of 22 μ M[109].

Bae et al. [49] demonstrated that ubiquinone-9 from *P. eryngii* inhibited mammalian topoisomerase I activity and induced apoptotic cell death of human cancer cells, but not that of normal fibroblast NIH3T3 and 3Y1 cells. An amorphous white powder with different spectral characteristics designated 'pleurone' (4H-1,3-dioxine-2,4-dione) from *P. eryngii*, exhibited inhibitory activity on human neutrophil elastase (HNE) and might be beneficial for the prevention or treatment of skin aging[28]. The inhibitory mechanism of pleurone on HNE is a mixed-type, a combination of noncompetitive and uncompetitive inhibition against HNE. Although pleurone activity has a relatively low HNE-inhibitory activity compared with epigallocatechingallate, it might be more useful as a new HNE inhibitor due to its relatively high water solubility and low molecular weight [28]. A ubiquitin-like peptide with 9.5 kDa and N-terminal sequence extracted from *P. sajor-caju* inhibited cell-free translation with an IC₅₀ of 30 nM and exhibited a ribonuclease activity of 450 U/mg toward yeast tRNA [26].

RNase (mol. wt. 14.5 kDa) was identified from sclerotia of *P. tuber-regium*, exhibited very stable nuclease activity at 100°C for 30 min. with a higher ribonucleolytic activity toward Poly-G[110]. Another ribonuclease, pleuturegin, was also isolated from both fresh and dried sclerotia of *P. tuber-regium* [111]. Similarly a protein contained in hot water extracts of *P. pulmonarius* with molecular weight of 4.5 kDa inhibited HIV-1 reverse transcriptase activity [112]. Maiti et al. [113] assessed the antiproliferative and immunomodulatory properties of a protein fraction, named as Cibacron blue affinity eluted protein (CBAEP), isolated from *P. florida*. The results showed that the isolated protein fraction excreted a stimulatory effect on splenocytes, thymocytes and bone marrow cells. It enhanced the cytotoxicity of mouse natural killer (NK) cells and stimulated macrophages to produce nitric oxide (NO). Moreover, it displayed antiproliferative activity on several tumor cell lines by apoptosis induction.

5.4. Lectin as Immunotherapy from *Pleurotus* Species

Lectins form a diverse group of carbohydrate-binding proteins with specific binding capacities. Lectins are abundant in vegetables, fruits, beans and mushrooms [110,114,115]. However, those derived from mushrooms have been found to possess the following attributes:- immunomodulatory [116], antiproliferative [52], antitumor [117], HIV-1 reverse transcriptase inhibiting [111], cell growth regulating [118], macrophage and lymphocyte activating [119,120] and many more. Lectin purified from *P. ostreatus* (POL) acted as immunogenicity of Hepatitis B Virus (HBV) DNA vaccine, when used as adjuvant [56]. It was demonstrated that low dose of POL (1 µg/mouse) in conjunction with HBVDNA vaccine stimulated stronger HBV-specific delayed-type hypersensitivity (DTH) responses and higher HBV-specific IgG level than that in high dose of POL groups (5 µg/mouse and 10 µg/mouse). POL activated strong Th2 and Th1 cell responses in immunized C57BL/6 and HBVsAg-Tg mice. POL as adjuvant of HBVDNA vaccine effectively enhanced HBV surface protein antibody (HBVsAb) [56].

A dimeric lectin, composed of subunits with a molecular weight of 40 and 41 kDa, respectively, and demonstrating similarity in N-terminal sequence to each other and to *Aleuria aurantia* lectin, was isolated from fresh fruiting bodies of *P. ostreatus* [117]. The lectin exerted potent antitumor activity in mice bearing sarcoma S-180 and hepatoma H-22. Survival in these mice was prolonged and body weight increase reduced after lectin treatment [117].

A novel lectin from *P. citrinopileatus* exerted potent antitumor activity in mice bearing sarcoma 180, and caused approximately 80% inhibition of tumor growth when administered intraperitoneally at 5mg/kg daily for 20 days [51]. It elicited amitogenic response from murine splenocytes *in vitro* with the maximal response at a lectin concentration of 2µM. The lectin inhibited HIV-1 reverse transcriptase with an IC₅₀ of 0.93µM [51]. Lectin produced from *P. florida* has capability of modulating arsenic mediated toxic effects and ameliorating them. This was done by restoration of alterations in the antioxidant enzymes, oxidative stress intermediates and superoxide dismutase (SOD₂) gene expression profile on arsenic exposure [121].

5.5. Proteoglycans as Immunotherapy from *Pleurotus* Species

Proteoglycans are group of glyco-conjugates with a special class of glycoproteins that are heavily glycosylated. They consist of a core protein with one or more covalently attached glycosaminoglycan chain(s). Glycosaminoglycan molecules are long unbranched polysaccharides containing a repeating disaccharide unit. Proteoglycans are remarkable for their diversity which include different cores, different numbers of glycosaminoglycans with various lengths and compositions. Many macrofungi have been reported as good producer of proteoglycan with immunomodulating activity. Such fungi are *Ganoderma lucidum*, *Grifola frondosa*,

Lentinula edodes, *Pleurotus ostreatus* [122,24].

Water-soluble proteoglycan fractions from *P. ostreatus* mycelia with immunomodulators and anticancer activity were reported [24]. They were tested for *in vitro* and *in vivo* immunomodulatory and anticancer effects on Sarcoma 180 bearing mouse model. *In vivo* injection of proteoglycans to Sarcoma 180 bearing mice decreased the number of tumor cells. These proteoglycans also elevated mouse natural killer cell cytotoxicity and stimulated macrophages to produce nitric oxide [24].

5.6. Other Immunotherapy Substances from *Pleurotus* Species

The genus *Pleurotus* produced many other bioactive substances that are of medicinal importance. Such compounds include dietary fiber, terpenoids, steroids, phenols, flavonoid, alkaloids, saponins, phlobatannins and anthraquinones [16,123]. A steroid which is the glycosylated form of ergosterol peroxide from the methanol extract of mushroom was found to be an inhibitor of proliferation of tumor cell lines (123; 124).

An extract was obtained from *P. ostreatus* which suppressed proliferation of breast cancer (MCF-7, MDA-MB-231) and colon cancer (HT-29, HCT-116) cells, without affecting proliferation of epithelial mammary MCF-10A and normal colon FHC cells [125]. Flow cytometry revealed that the inhibition of cell proliferation by *P. ostreatus* was associated with the cell cycle arrest at G0/G1 phase in MCF-7 and HT-29 cells.

In yet another experiment hot water extracts of *P. ostreatus* was incubated for 24 h with MCF-7 cells. Cellular proliferation determined by bromodeoxyuridine incorporation was significantly ($P < 0.05$) reduced up to 33% by the extract [126]. Also the extract of *P. ostreatus* (PO) has chemo preventive effect on inflammation-associated colon carcinogenesis induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) promoted by dextran sodium sulfate (DSS) (127). The extract treatment at doses (100 and 500 mg/kg), significantly reduced by 50 and 78% the number of aberrant crypt foci and the multiplicity of colon neoplasms by 43 and 89%, respectively, and reduced the incidence of colon tumors and high grade dysplasia by 50 and 63% only in the dose 500 mg/kg [127].

A water-soluble extract (POE) prepared from the fresh *P. ostreatus* produced the most significant cytotoxicity on PC-3 cells (128). At the same time, POE induced a rapid apoptosis on PC-3 cells detected with annexin V-fluorescein isothiocyanate flow cytometry when the cells were exposed to POE (150 g/mL) for 2 hours. Induced apoptosis was also confirmed by DNA fragment terminal deoxynucleotidyl transferase-mediated by X-dUTP nick end labeling staining, while POE (200 g/mL) was added to PC-3 cells for 6 hours [128].

Methanol extracts of *P. ostreatus* and *P. salmoneostramineus* showed suppressive effect against growth of HT-29 cell line with survival rates of 39.9 and 40.7% at the concentration 500 µg/ml, while survival rate was more

than 50% when *P. cornucopiae* methanol extract was employed [129].

Acetone extracts of *P. cornucopiae* fruiting body completely inhibited growth and induced apoptosis of HL60 cells at a concentration 10.7 µg/ml [130].

Cerevisterol (CE) and ergosta-4,6,8(14),22-tetraen-3-one (EG) were purified and identified from *P. tuber-regium*; and tested for anti-inflammatory effects on RAW 264.7 macrophages (131). Both CE and EG inhibited the production of NO, TNF- α , and PGE₂ in LPS-treated RAW 264.7 cells. The expression of the iNOS and COX2 proteins was inhibited by CE and EG in a dose-dependent manner. In addition, CE and EG repressed the expression of iNOS, COX2, TNF- α , and SOCS3 mRNAs [131].

6. Conclusion

Immune system is the body's ultimate defense against infectious diseases, tumor, immunodeficiency diseases and cancer growth. The immune system is a complex containing many interacting blood cells, protein and chemicals. A healthy immune system contains elements that are in balance with one another but in a compromised immune system, the components are unbalanced and unable to protect the body against harmful agents or processes. As shown in the review, oyster mushroom (*Pleurotus species*) contain a number of bioactive components such as polysaccharopeptides, polysaccharide-proteins, functional proteins, glycoprotein, glucans, proteoglycans and others. Oyster mushroom can be consumed directly as food or the extract in the diet can be used to promote health. The potential immunotherapeutic implications of oyster mushroom are enormous but, detailed mechanisms or pathways of the various health benefits of mushrooms to humans still require intensive investigation, especially with the emergence of new evidence of their health benefit effects. The exploration of newly cultivated oyster mushroom and isolation of their bioactive ingredients with mechanism and pathway based potential immunotherapeutic value remains a challenge and hence mushrooms, especially *Pleurotus* spp. will keep on to be the foremost spotlight of research in the upcoming prospect as well.

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