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Lentinan ameliorates burn sepsis by attenuating CD4+ CD25+ Tregs

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ABSTRACT

Aim: The aim of our study was to investigate the effect of lentinan on regulatory T cells (Tregs) in sepsis, especially on the generation of interleukin (IL)-10 via regulation of Erk–FoxO1 signaling.

Methods: BalB/c mice were randomized into five groups: sham group, the group with burns plus *Pseudomonas aeruginosa* infection, and the groups with burns plus *P. aeruginosa* infection administered 40, 100, and 250 mg/kg of lentinan. The mice were sacrificed on postburn days 0, 1, 2, 3, and 4, respectively, with eight animals per group at each time point. The peripheral blood CD4+ CD25+ Tregs and CD4+ T cells were isolated using magnetic microbeads. The phenotypes were analyzed by flow cytometry. The cytokine levels were determined with enzyme-linked immunosorbent assay (ELISA). Signal transduction was studied by Western blot, quantitative polymerase chain reaction (qPCR), and luciferase assay.

Results: The IL-10-producing capacity of CD4+ CD25+ Tregs was significantly enhanced in the group with burns plus *P. aeruginosa* infection, compared with the sham group. Administration of lentinan significantly decreased IL-10 production and FoxP3 expression of CD4+ CD25+ Tregs. The proliferative activities of CD4+ T cells, however, were restored. Lentinan decreased lipopolysaccharide (LPS)-induced IL-10 production in the Tregs isolated from burned mice. In addition, lentinan attenuated LPS-stimulated Erk–FoxO1 activation.

Conclusions: Lentinan may improve the outcome of postburn sepsis by suppressing LPS-triggered Erk–FoxO1 activation. Consequently, the hyperfunction of CD4+ CD25+ Tregs is inhibited, leading to a shift in the inflammatory status from Th2 to Th1 in postburn sepsis.

1. Introduction

Lentinan, which is extracted from *Lentinus edodes*, is a (1,3)-beta glucan with beta-(1, 6) branches [1]. It is used as an immunomodulator in various fields, ranging from tumor therapy and host resistance to bacterial, fungal, viral, and parasitic infections [2]. The protective effect of lentinan against infection is mediated by the host; it can be attributed to the enhanced innate and adaptive immune responses [2]. Lentinan has been shown to increase the production of

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interleukin (IL)-1 and tumor necrosis factor alpha (TNF-α) in vivo [3,4]. In vitro studies have shown that lentinan augmented the cytotoxic activity of natural killer (NK) cells and enhanced cytokine production [5,6]. Lentinan can also increase the frequency of T-cell populations and the functions of effector T cells [7,8].

Sepsis accounts for 50–60% of deaths in burn patients [9]. Suppression of the immune response resulting from burn wounds is the leading cause of sepsis [10,11]. Multiple theories have been proposed to explain the immunosuppression in late sepsis, such as the imbalance of Th1/Th2 cells or cytokine profiles, depletion of effector cells, induction of energy, and, most recently, the activation of regulatory T cells (Tregs) [12]. Naive $CD4^+$ $CD25^+$ Tregs are identified as a functionally distinct and mature subgroup of T cells. Their role in modulating immune responses has been established with further understanding of cellular immunity, such as controlling tumor immunity, autoimmunity, and transplantation tolerance [13]. Many reports address the role of $CD4^+$ $CD25^+$ Tregs in acute and chronic infectious diseases, caused by virus, fungi, parasites, and bacteria [14]. In addition, abnormalities of Tregs have also implicated in autoimmune diseases [15]. The activities of $CD4^+$ $CD25^+$ Tregs are either cell contact dependent, which is controlled by the expression of FoxP3, or independent, which is mediated by production of anti-inflammatory cytokines such as IL-10 and transforming growth factor beta (TGF-β) [16,17].

TLR4, an essential pattern recognition receptor (PRR), is expressed not only on monocytes, macrophages, and dendritic cells (DCs) but also on T cells [18]. In their study, Dai et al. showed that the significant suppressive effect of Tregs was TLR4 dependent [19]. TLR4 can be bound by β-1, 3-glucan or lipopolysaccharide (LPS), thus initiating nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, and promoting pro-inflammatory cytokine secretion [20,21]. LPS-triggered Erk and p38 activation is essential for *IL-10* transcription [22,23], as both pathways were found to particularly phosphorylate forkhead box protein O1 (FoxO1), the transcriptional factor of IL-10 [24,25].

Although previous studies have demonstrated some effects of lentinan on specific immune functions, its role in modulating Tregs in burn wound sepsis is unclear. The aim of our study was to investigate whether lentinan could improve the prognosis of sepsis by modulating Tregs in a murine model of postburn *Pseudomonas aeruginosa* (*P. aeruginosa*) infection, by exploring the underlying mechanisms.

2. Materials and methods

2.1. The extraction of lentinan

To 100 g of frozen cap tissue, 300 ml of boiling water was added. The samples were then homogenized with an Ultrathurax for 1 min and boiled for 3 h while continuously stirring. After cooling down, the mixture was precipitated by adding an equal volume of 95% ethanol followed by incubation at 4 °C for 16 h. The samples were centrifuged at 5000 rpm (Beckman) for 20 min at 4 °C. The pellet was snap-frozen in liquid nitrogen and lyophilized in a freeze dryer. One volume of hot water (60 °C) was slowly added to the lyophilized pellet and the solute was homogenized with an Ultrathurax for 1 min. Subsequently, the homogenate was boiled for 8 h under continuous stirring, stored overnight at 4 °C, and centrifuged at 5000 rpm for 20 min at 4 °C. The supernatant was collected and precipitated overnight with one volume of 95% ethanol. The precipitate was collected by two centrifugation steps at, respectively, 5000 and 7000 rpm for 20 min at 4 °C and subsequently lyophilized. The obtained crude lentinan powder was further dried in an oven at 60 °C for 1 day. The samples were weighted (concentration lentinan/g tissue) and stored in a desiccator before further analysis.

2.2. Mice

Male BALB/c mice (aged 6–8 weeks) were purchased from the Laboratory Animal Center of Chinese Academy of Medical Sciences (Beijing, P.R. China). Before the experiment, the mice were housed in a temperature-controlled room with a 12-h light/dark cycle to acclimatize for at least 7 days. All experiments were carried out according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.3. Experimental design

Male BALB/c mice (6–8 weeks old) were randomized into four groups as follows, each containing 32 mice:

Group 1 comprised mice with burns plus *P. aeruginosa* infection (the mice were burned and then infected with *P. aeruginosa*).
Group 2 included mice with burns plus *P. aeruginosa* infection treated with lentinan (40 mg/kg) (the mice received 40 mg/kg lentinan daily by intraperitoneal injection based on group 1).
Group 3: comprised mice with burns plus *P. aeruginosa* infection treated with lentinan (100 mg/kg) (the mice received 100 mg/kg of lentinan intraperitoneally based on group 1 daily).
Group 4 included mice with burns plus *P. aeruginosa* infection treated with lentinan (250 mg/kg) (the mice received 250 mg/kg of lentinan intraperitoneally based on group 1 daily).

The four groups were further divided into four subgroups, each comprising eight animals. They were sacrificed on postburn days (PBD) 1, 2, 3, and 4, respectively. Eight anesthetized mice were considered as the sham control and sacrificed on PBD 0.

To evaluate the effect of lentinan on the mortality rate of mice with thermal injury and *P. aeruginosa* infection, 60 mice were grouped using the same strategy, but with each group comprising 15 animals. The survival rate was monitored for 5 days.

2.4. Model of burn plus P. aeruginosa infection

A full-thickness skin dorsal scald burn injury was reproduced in a mouse model. Mice were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital sodium (Sigma–Aldrich,