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Polysaccharide peptide from *Coriolus versicolor* induces interleukin 6-related extension of endotoxin fever in rats

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Abstract

Purpose: Polysaccharide peptide (PSP) extracted from the *Coriolus versicolor* mushroom is frequently suggested as an adjunct to the chemo- or radiotherapy in cancer patients. In previous study we have shown that PSP induced a tumor necrosis factor-α (TNF-α)-dependent an anapyrexia-like response in rats. Thus, PSP appears a factor which modifies number of pathophysiologic responses. Because of the fact, the PSP is suggested as an potential adjuvant used in the cancer therapy during which frequently cancer patients contract a microbial infections accompanied by fever, the aim of the present study was to investigate whether or not the PSP can modulate a course of the fever in a response to the antigen, such as LPS.

Materials and methods: Body temperature (Tb) of the male Wistar rats was measured by biotelemetry system. PSP was injected intraperitoneally (i.p.) at a dose of 100 mg kg^{-1}, 2h before LPS administration (50 μg kg^{-1}; i.p.). The levels of interleukin (IL)-6 and TNF-α in the plasma of rats were estimated 3h and 14h post-injection of PSP using a standard sandwich ELISA kits.

Results: We report that i.p. pre-injection of PSP 2h before LPS administration expanded the duration of endotoxin fever in rats. This phenomenon was accompanied by a significant elevation of the blood IL-6 level of rats both 3h and 14h post-injection of PSP. Pre-treatment i.p. of the rats with anti-IL-6 antibody (30 μg/rat) prevented the PSP-induced prolongation of endotoxin fever.

Conclusions: Based on these data, we conclude that PSP modifies the LPS-induced fever, in IL-6-related fashion.

Running title: *Polysaccharide peptide caused fever extension*

Keywords: endotoxin fever, biotelemetry, polysaccharide peptide, lipopolisaccharide interleukin 6, *Coriolus versicolor*

1. Introduction

Polysaccharide peptide (PSP) isolated from *Coriolus versicolor* strain COV-1, has been widely used as adjunct therapy in cancer patients undergoing chemo- or radio-therapy [1] and its non-toxic properties under acute and chronic conditions have been confirmed [2]. Clinical trials showed that PSP improved the quality of life of patients by decreasing cancer treatment-related symptoms such as fatigue, loss of appetite, nausea, vomiting, and pain [3]. This mushroom-derived polysaccharide exert its activities primarily via immunomodulation [4]. Therefore, it can be classified as a biological response modifier, which is defined as an agent capable of modifying the host's biological response by stimulating the immune system and thereby eliciting various therapeutic effects [5]. Immunostimulatory effect of PSP (*in vitro* and *in vivo*) includes elevation of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) as well as prostaglandin E2 (PGE2) and histamine [3], increase in the production of reactive oxygen and nitrogen intermediates [6], natural killer cells (NK) activity, activation of complement-3, T-cell proliferation [7] and many others.

The above mentioned cytokines and PGE2 secreted by PSP-stimulated cells are important components of the physiological mechanism of fever. This phenomenon is regarded as a part of the acute-phase response to infection, inflammation, injury and trauma [8]. The increase of body temperature (Tb) during fever has several advantages over infections: inhibition of bacterial growth, increase bactericidal activities of neutrophils and macrophages, T cells proliferation and differentiation, B cells proliferation and the production of antibodies or stimulation of acute-phase protein synthesis [9-10]. The initial step in the cascade of events leading to fever is considered to be a stimulation of a large number of various immune types of cells, including monocytes, macrophages and neutrophils by exogenous stimuli, called exogenous pyrogens [11]. These stimuli are represented by bacteria walls components such as lipopolisaccharide (LPS), viral components such as double-stranded RNA and bacterial

DNA (CpG-DNA) [12-13]. Stimulation of the immune cells by the various exogenous pyrogens leads to the synthesis of the pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and interferon-γ (IFN-γ), collectively ascribed as endogenous pyrogens [11, 14-16]. These cytokines trigger liberation of the arachidonic acid from membrane phospholipids, activation of cyclooxygenase (COX), and subsequent production of prostanoids. It is thought, that induction of the expression of COX-2 and generation of PGE2 play a critical role in affecting the thermoregulatory centers to start the fever [17].

As we described previously, PSP provoked an anapyrexia-like response rather than fever in rats, and the response was TNF-α-dependent [18]. Thus, PSP appears a factor which modifies number of pathophysiologic responses. Because of the fact that, the PSP is suggested as an potential adjuvant used in the cancer therapy during which frequently cancer patients contract a microbial infections accompanied by fever, the aim of the present study was to investigate whether or not the PSP can modulate the course of the fever. To the best of our knowledge, this phenomenon has not yet been studied. Moreover, our studies aimed to explore the role of PSP as a modulator of endotoxin fever in a response to the antigen, such as LPS.

2. Materials and methods

2.1. Experimental animals and body temperature measurement

Male Wistar rats weighing from 250g to 300g were obtained from the Mossakowski Medical Research Centre Polish Academy of Sciences (Warsaw, Poland). Animals were housed in individual plastic cages and maintained in a temperature/humidity/light- controlled chamber set at 23 ± 1°C, 12:12 h light:dark cycle, with light on at 07:00 a.m. Rodent laboratory food and drinking water were provided *ad libitum*. A week after the shipment, the rats were implanted under sterile conditions with battery-operated miniature biotelemeters (PhysioTel® model TA10TA-F40, Data Sciences International, USA) to monitor deep body temperature (Tb) with accuracy ± 0.1^{o}C as described previously [19]. Described experiments were started

10 days after surgery. All procedures were approved by the Local Bioethical Committee for Animal Care in Bydgoszcz (Poland; permission no. 17/2013).

2.2. Polysaccharide peptide and lipopolisaccharide preparation and administration

Polysaccharide peptide (PSP; extract from the Cov 1 strain of *Coriolus versicolor*; MycoMedica, Czech Republic) was dissolved in sterile 0.9% sodium chloride (saline) and injected intraperitoneally (i.p.) at a dose of 100 mg kg^{-1}. As we described previously, this was the dose of PSP, which modulated the normal Tb in male Wistar rats [18]. In our studies, we also tested the lower dose of PSP (50 mg kg^{-1}) causing the smaller decrease of Tb of rats. However, since the lower dose of PSP did not provoke any significant effect on the LPS-induced febrile response in rats (data not shown), the dose of 100 mg kg^{-1} of PSP was selected for further experiments.

LPS extracted from *Escherichia coli* (0111: B4, Sigma Chemicals) was dissolved in sterile 0.9% sodium chloride. Before injection, the stock solution of LPS (2.5 mg ml^{-1}) was diluted in a warm sterile saline to the desired concentration, and injected i.p. at a dose of 50 μg kg^{-1}, as described previously [19]. All injection solutions were warmed to 37^{o}C before administration. PSP was injected at 7:00 a.m., 2h prior to the LPS administration (9:00 a.m.). The control rats were administered i.p. with an equivalent volume of pyrogen-free saline. The rats were briefly restrained and not anesthetized during the injections. Immediately after the injections, the animals were placed in their home cages.

2.3. IL-6 and TNF-α assays

Blood samples were collected via cardiac puncture onto the solution of ethylenediamine tetraacetic acid disodium salt (Na_2EDTA, Sigma-Aldrich; cat. no. E 5134) at 3h (10:00) and 14h (21:00) post-injection of PSP or pyrogen-free saline from rats anesthetized with a mixture

of ketamine/xylazine (87 mg kg^{-1} and 13 mg kg^{-1}, respectively, intramuscular injection). After centrifugation (20 min, 1500 x g), the resulting plasma was stored at -20°C until assay. Levels of IL-6 and TNF-α were determined by a standard sandwich ELISA kits from R&D Systems (cat. no. R6000B and RTA00, with a detection limit of 21 pg ml^{-1} and 5 pg ml^{-1}, respectively) according to the manufacturer's instructions. Colorimetric changes in the assays were detected using Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, USA).

2.4. Interleukin 6 antibody injection

Interleukin 6 (IL-6) antibody (rabbit polyclonal IgG anti rat IL-6; Invitrogen; cat. no. ARC0062) was injected i.p. at a dose of 30 µg/rat in a volume of 500 µl of phosphate buffered saline (PBS, pH 7.4). This injection was performed 2h (17:00) prior to the earlier observed significant difference in Tb between the examined group of rats (PSP/LPS) and the positive control (saline/LPS). Rabbit IgG (Invitrogen; cat. no. 10500C) at a dose of 30 µg/rat was used as control injection. Rats were restrained and not anesthetized during i.p. injections.

2.5. Statistical analysis

All values are reported as means ± standard error mean (S.E.M.) and were analyzed by analysis of variance (ANOVA) followed by the Student's *t*-test with the level of significance set at $p<0.05$. For the Tb measures, the data were recorded and computed at 5-min intervals using Data Acquisition Programme (Data Sciences International, USA). For data presentation, these 5-min temperature recordings were pooled into 30-min averages. Statistical analyses were performed with GraphPad Prism 5 (USA).

3. Results

3.1. Pre-treatment with PSP expands the duration of endotoxin fever in rats

Effect of PSP on changes of Tb in male Wistar rats during endotoxin fever is illustrated in *Fig. 1*. The rats were injected i.p. with PSP at a dose of 100 mg kg^{-1} at 7:00 a.m., 2h prior to the LPS administration. Pre-treatment of the animals with PSP resulted in a significant alterations of the post-LPS Tb that can be regarded as a protraction of the time-course of fever response to the administration of endotoxin. As can be seen in *Fig. 1*, the rats treated with PSP followed by LPS responded with fever, which started 3,5h post-injection of LPS (12:30), whereas this phenomenon in the saline/LPS-injected animals was observed 1,5h post-injection of LPS (10:30). Moreover, as we described previously [18], PSP administration caused the drop in Tb. However, the Tb of PSP/LPS-treated rats (38.2±0.2^{o}C) was comparable to the Tb of saline/LPS-injected rats (38.3±0.1^{o}C) measured from 13:30 to 18:00 ($p=0.25$). On the other hand, the rats pre-treated with saline 2h prior to LPS administration returned to Tb observed in the non-treated group of animals (NT) 12h post-injection of PSP (19:00), whereas this phenomenon was observed in the PSP/LPS-treated rats only after 21h from injection (04:00). The average Tb of the rats counting from 19:00 to 4:00 for the PSP/LPS-treated animals was 38.3±0.1^{o}C *vs*. 37.8±0.2^{o}C in the saline/LPS treated rats ($p<0.01$). Injection i.p. of sterile 0.9% sodium chloride (solvent for PSP) 2h prior to the i.p. saline administration (solvent for LPS) did not induce alterations in Tb of rats (data not shown).

(Insert Figure 1 here)

3.2. PSP increases the level of plasma IL-6 during endotoxin fever in rats

The time of blood collection has been adjusted to the most advanced changes in the course of Tb. The levels of plasma IL-6 were determined at 3h (10:00) and at 14h (21:00) post-injection of PSP or pyrogen-free saline in the all groups of animals. Non-treated rats (NT) as

like as PSP/saline and saline/saline injected animals did not show any significant elevation of IL-6 neither at 3h nor at 14h post-injection of PSP or saline (*Fig. 2*). Moreover, the concentrations of this cytokine in the these three groups of rats were below the lowest standard of ELISA kit, which was 62.5 pg ml^{-1} (respectively 17.3±3 pg ml^{-1}, 16.8±2 pg ml^{-1} and 34.8±1 pg ml^{-1} for the plasma concentration measured 3h post-injection; 15.1±3 pg ml^{-1}, 21.9±2 pg ml^{-1} and 38.1±2 pg ml^{-1} for the level of IL-6 estimated 14h post-injection). In contrast, the levels of IL-6 in the plasma of rats treated with PSP followed by LPS were significantly higher in comparison to the animal's injected i.p. with pyrogen-free saline 2h prior the LPS administration. This phenomenon was observed in both at 10:00 (1694.2±80 pg ml^{-1} *vs*. 315.9±20 pg ml^{-1}; $p<0.001$) and at 21:00 (379.7±7 pg ml^{-1} *vs*. 32.9±9 pg ml^{-1}; $p<0.001$).

(Insert Figure 2 here)

3.3. PSP decreases the level of plasma TNF-α during endotoxin fever in rats

The plasma levels of TNF-α as well as IL-6 were also determined at 3h (10:00) and at 14h (21:00) post-injection of PSP or pyrogen-free saline. As can be seen in Fig. 3, the concentration of this cytokine in the plasma of rats pre-treated with PSP followed by LPS (317.3±40 pg ml^{-1}) was significantly lower in comparison to the animals injected i.p. with pyrogen-free saline 2h prior to the LPS injection (1342.9±310 pg ml^{-1}; $p<0.001$). Moreover, the concentration of TNF-α measured in rats pre-treated with PSP and then injected with LPS (317.3±40 pg ml^{-1}) were significantly higher compared to PSP/saline-treated animals (225.9±4 pg ml^{-1}; $p<0.01$). The plasma levels of this cytokine in the all tested groups of rats measured at 21:00 were below the minimum detectable dose of rat TNF-α in the used ELISA kit, which was 5 pg ml^{-1} (data not shown).

(Insert Figure 3 here)

3.4. Anti-IL-6 antibody prevents the extension of endotoxin fever in rats

As can be seen in Fig. 4, injection of IL-6 antibody prevented the extension of endotoxin fever in rats pre-treated with LPS. The Tb of rats injected with PSP followed by LPS was similar to that observed in the PSP/LPS-treated rats injected at 17:00 with rabbit IgG (38.3 ± 0.1^{o}C *vs.* 38.2 ± 0.1^{o}C; counting from 19:00 to 4:00; p=0.39). On the other hand, the PSP/LPS-injected animals treated i.p. with IL-6 antibody responded with decrease in Tb to a value, which was observed in the non-treated rats (NT) at 12h post-injection of PSP (19:00). The Tb in these two groups of animals (37.8 ± 0.1^{o}C and 37.8 ± 0.1^{o}C, respectively; p=0.35) was significantly lower compared to PSP/LPS-treated rats (38.3 ± 0.1^{o}C) and PSP/LPS-treated animals injected with rabbit IgG (38.2 ± 0.1^{o}C) counting from 12h (19:00) to 21h (4:00) post-injection of PSP ($p<0.01$).

(Insert Figure 4 here)

To determinate whether the dose of an anti-IL-6 antibody used in the experiments affects the course of Tb in rats, separate group of animals was treated i.p. with sterile 0.9% saline at 7:00 and 9:00 (control vehicle for PSP and LPS). Afterwards, the rats were injected i.p. with rabbit polyclonal IgG anti rat IL-6 antibody at a dose of 30 μg/rat or with rabbit IgG (control injection at the same dose) at 10h (17:00) after the first injection of sterile saline. As can be seen in Fig. 5, administration of IL-6 antibody did not effect on Tb in rats. The average Tb of rats treated i.p. with IL-6 antibody, injected i.p. with rabbit IgG and non-treated (control) animals was similar (37.9 ± 0.1^{o}C), counting from 17:00 to 6:00.

(Insert Figure 5 here)

4. Discussion

In the present report we demonstrate for the first time the effect of polysaccharide peptide (PSP) on the endotoxin fever in rats. Pre-treatment with PSP provoked a significant alterations of the Tb in LPS-injected rats that can be regarded as a prolongation of fever

response to the administration of endotoxin (Fig. 1). This effect was accompanied by a significant elevation of the LPS-induced blood IL-6 level of both 3h and 14h (Fig. 2). Plasma levels of TNF-α (Fig. 3) and IL-6 suggest that PSP-induced extension of endotoxin fever in rats is related rather to IL-6 concentration than TNF-α. The extension of fever was prevented by an i.p. injection of anti-IL-6 antibody (Fig. 4). The dose of this antibody (30 μg/rat) used in the experiments affected neither normal Tb nor circadian rhythm of Tb (Fig. 5). In our studies, we also examined the plasma concentration of IL-1β (one of the key cytokine that contributes to induction of fever) in the all tested groups of rats, which was, however, below the minimum detectable concentration of IL-1β in the used ELISA kit (less than 5 pg ml^{-1}; sandwich ELISA kits from R&D Systems, cat. no. RLB00) both 3h and 14h post-injection of PSP (data not shown).

Immunostimulatory effects of PSP (*in vitro* and *in vivo*) include elevation of pro-inflammatory cytokines, such as IL-6 and TNF-α [3]. Similarly, it is well-known, that stimulation of immune cells by exogenous stimuli such as LPS leads to synthesis of pro-inflammatory mediators, among which the most important are cytokines such as IL-6 and TNF-α [11, 16, 20]. Experimental data strongly suggest important role of IL-6 as endogenous mediators in LPS-induced fever. The presence of IL-6 is critical for fever, as seen by the absence of the febrile response to peripheral immune challenge in IL-6 knock-out (*KO*) mice as well as in animals treated with IL-6 antiserum [20-21]. In the present data, we showed that the pre-treatment of the rats with PSP expands the duration of LPS-induced fever, and the response is IL-6-related. Therefore, we suppose that PSP may intensify the production of IL-6 by the immune cells such as monocytes, macrophages and neutrophils. However, further *in vitro* studies are needed to investigate the reactivity of peripheral blood mononuclear cells (PBMCs) isolated from the rats pre-treated with PSP and then injected with LPS. This

reactivity can be measured as the production of pro-inflammatory cytokines (IL-6, TNF-α) by PBMCs.

As we described previously, PSP derived from the mushroom *Coriolus versicolor* induced a TNF-α dependent drop of Tb in rats [18]. In the present studies, the results of measurement of the plasma concentration of TNF-α showed that the pre-injection of PSP prevented the LPS-induced elevation of plasma TNF-α. In contrast, the concentration of this cytokine in rats pre-treated with PSP and then injected with LPS was significantly higher compared to PSP/saline-treated animals (Fig. 3). Moreover, PSP demonstrates an additive effect on the synthesis of IL-6 during the LPS-induced fever (Fig. 2). Potential explanation of this phenomenon may be related to Toll-like receptor 4 (TLR4) signal transduction pathway. It is well-known that LPS constitutes a pathogen-associated molecular pattern (PAMP) recognized by TLR4 [22-24]. In contrast, there are only few reports presenting that PSP acts via TLR4. Li et al. (2010) showed that PSP up-regulated expression of 22 genes, including five members of TLR family: LY64, TLR5, TLR6, TLR7 and finally TLR4 in PBMCs stimulated with PSP [25]. Moreover, these authors also observed the increase in an expression of genes related to nuclear factor-κB (NF-κB) pathway - one of the most important transcription factor, which is necessary for the induction of the synthesis of pro-inflammatory cytokines, including IL-6 and TNF-α [26]. It is well-known that a common downstream pathway operates in the signal transduction via TLRs involving the myeloid differentiation factor 88 (MyD88)-dependent and MAPK-dependent up-regulation of the NF-κB [27]. Similarly, Wang et al. (2013) demonstrated that PSP has an immunoregulatory effect through regulation of the TLR4-TIRAP/MAL-MyD88 signaling pathway in PBMCs from breast cancer patients [28]. There are also reports indicating that the compounds derived from *Coriolus versicolor* and having a similar structure as PSP are recognized by TLR4. Yang et al. (2015) showed that *Coriolus versicolor* mushroom polysaccharides (CVP), which as like as PSP exert a broad range of

biological effects, including anti-tumor and immunoregulatory activities [29-30] can bind and induce B cell activation using membrane Ig and TLR4 as potential immune receptors. Consequently, CVP activates mouse B cells through the MAPK and NF-κB signaling pathway [31]. Based on these results we presume that PSP may constitute the PAMP recognized by TLR4.

It has been accepted that TLR4 signal transduction pathway could be divided into two sub-pathways including myeloid differentiation factor 88 (MyD88)-dependent and TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent (MyD88- independent) according to the different adaptors. MyD88 adaptor-like protein (Mal) is an essential adapter protein together with the MyD88. Activated MyD88/Mal activates, i. a. transforming growth factor-β-activated protein kinase 1 (TAK1), which activates also members of the mitogen-activated protein kinases (MAPK) to activate an alternative closely related pathway that phosphorylates, i.e. p38 MAPK. The p38 MAPK is regarded as the essential regulators of pro-inflammatory molecules in the cellular responses that occur following induction of inflammatory gene transcription [32-33].

In addition to the above-mentioned signal transduction pathways, among the many inflammatory mediators induced by the LPS, which signals via TLR4, IL-6 trans-signaling via STAT3 is a critical modulator of LPS-driven pro-inflammatory responses through cross-talk regulation of the TLR4/Mal signaling pathway [34]. IL-6 mediates its biological activities through a receptor complex composed of the specific signal-transducing receptor subunit gp130. After ligand binding, the gp130 recruits transcription factors of the STAT family (i.e., STAT3). Activated STATs translocate to the nucleus, and bind to enhancer elements of target genes [35].

The hyperresponsiveness of gp130F/F mice to LPS involved the specific up-regulation of IL-6 in a gp130/STAT3- and TLR4/Mal-dependent manner, suggesting both pathways synergize

to promote the production of IL-6 in response to LPS. Moreover, there is the preferential up-regulation of IL-6 after LPS stimulation compared with TNF-α in an *in vivo* disease model (i.e., gp130F/F mice) [36]. Although the mechanism of this phenomenon remains unclear, it is likely to reflect subtle differences in the transcriptional regulation of specific pro-inflammatory genes produced via TLR4 signaling cascades. For instance, activation of p38 MAPK is required for the LPS/TLR4-induced expression of TNF-α, but not IL-6 [37-38]. Moreover, *in vitro* studies have shown that blocking STAT3 activity preferentially inhibits LPS-mediated IL-6 production, but not TNF-α in RAW264.7 cells [39], and STAT3 activation does not directly regulate LPS-induced TNF-α production in human monocytes [40]. Based on these results, it can be concluded that the LPS/TLR4-induced production of TNF-α, but not IL-6, requires the activity of p38 MAPK. On the other hand, signaling pathway via STAT3 is a critical for increasing the expression of IL-6, but not TNF-α. In the present studies, we have shown that PSP alone (without LPS) induces TNF-α, but not IL-6 expression in rats. Therefore, we suppose that PSP may act via TLR4/p38 MAPK signaling pathway. Our assumptions are consistent with the observations of Yang et al. (2015), who demonstrated that *Coriolus versicolor* mushroom polysaccharides induced, in a time-dependent manner, the increase of phosphorylation of p38 MAPK [31].

Our results also demonstrated that PSP and LPS showed the additive effect on the IL-6 expression, whereas the injection of PSP alone (without LPS) did not induce the secretion of IL-6 (plasma level measured 3h post-injection of PSP). Based on these results we presume, that PSP alone is not able to activate the both TLR4-induced signal transduction pathways, involving p38 MAPK and STAT3. On the other hand, the simultaneous activation of the TLR4 signaling pathway by LPS and PSP causes the additive effect on IL-6 production. This phenomenon may result due to the fact, that PSP as well as LPS induces TLR4 signaling pathway, which leads to the activation of NF-κB [25; 31-32]. Moreover, the both inducers

may also active signaling pathway via STAT3. The other potential explanation of this phenomenon may result from the fact that in our experiment PSP was injected in rats 2h prior to the LPS administration. As we described previously, PSP induced a significant elevation of the blood TNF-α level 2h post-injection [18]. It can be assumed that raised concentration of TNF-α causes the increase of LPS-induced IL-6 production. Ghezzi et al. (2000) showed that the anti-TNF-α antibodies inhibited LPS-induced IL-6 production in three different models: IL-6 production by mouse peritoneal macrophages *in vitro*; serum IL-6 levels induced by an i.p. injection of LPS, and brain IL-6 concentration induced by an intracerebroventricular (i.c.v.) administration of LPS [41]. Similarly, Benigni et al. (1996) demonstrated that i.c.v. injection of LPS into TNF receptor-deficient mice produces lower brain IL-6 levels than in wild type mice [42]. To the best of our knowledge, this phenomenon has not yet been examined. Therefore, detailed studies on the TLR4 signal transduction pathway, involving p38 MAPK and STAT3, in the PSP/LPS-treated rats are required.

PSP is considered as a useful adjuvant especially combined with chemotherapy in clinical treatment of cancer patients [1-2]. For this reason, it is important to examine the effect of PSP in these patients who may experience fever during microbial infections. Moreover, there are clinical reports suggesting a decreased frequency of fever, or even the lack of capability of generating fever within certain groups of patients, especially amongst cancer patients [43]. It is also well documented that fever directly activates defense against various dangers, including cancer cells [44-45] and the endogenous mediators of fever play a significant role in defense against tumor cells [46-47]. The observation that cancer patients who experienced a feverish period after surgery survived significantly longer than patients without fever, and the fact that spontaneous tumor remission was observed mostly after a fever, confirms the significant meaning of this mechanism for a patient's recovery [48]. A large fraction of spontaneous regressions and remissions of tumors described in the literature was preceded by

acute infections especially when accompanied by high fever [49-51]. Based on recent observations in the clinic together with the improved understanding of tumor immunology, it is believed that fever, being a part of innate response, can induce and facilitate an efficient anti-tumor response, and may improve anti-tumor efficacy of immunotherapy [52-53]. However, the mechanisms of this phenomenon has not yet been fully elucidated. It is well-known that following fever, especially in relation to an acute infection, an increase in pro-inflammatory cytokines levels, stimulation of the differentiation of T cells and enhancement of cytotoxic potential of neutrophils, NK cells, and dendritic cells are observed [9, 11]. In addition to the immunologic effects of fever, there is also the thermal aspect. Tumor cells are more fragile and vulnerable to heat with apoptosis taking place at lower temperatures compared to normal cells [49, 54].

Although, there is lack of research focused on the direct effect of fever on the various aspects of immune system in the cancer patients or/and tumor bearing animals, the results of studies using a fever-range whole-body hyperthermia (FR-WBH) demonstrate a beneficial activity of the temperature in the range of 39.5°C – 40.5°C, lasting for 4 – 6 hours (physiological status similar to the fever). Fever-range temperature is associated with enhancement of the innate and adaptive arms of the immune response through augmentation of T-cell proliferation and cytotoxicity, bioactivity of inflammatory cytokines and neutrophil motility and chemotaxis [11, 55-56]. It also promotes the egress of blood-borne lymphocytes across high endothelial venules (HEV) in lymph nodes and Peyer's patches [57]. Moreover, FR-WBH regulates adhesion molecule expression on select vascular endothelial sites. It increases the expression of intercellular adhesion molecule 1 (ICAM-1) and strongly increases the intravascular display of CCL21, a key homeostatic chemokine, which mediates lymphocyte trafficking across high endothelial venules. FR-WBH also enhances L-selectin/α4β7 integrin affinity and/or avidity for endothelial adhesion molecules, ultimately leading to improved homing to

lymphoid tissues [58-59]. The studies using tumor bearing animals revealed that the FR-WBH resulted in a significant lymphoid infiltrate and tumor cells apoptosis due to the activity NK cells. Moreover, Burd et al. (1998) showed also that a single treatment of Balb/c mice bearing human breast tumor xenografts with a low-temperature, long-duration, and whole-body hyperthermia for 6–8h caused a temporary reduction of tumor volume and/or a growth delay. This inhibition was correlated with the appearance of large numbers of apoptotic tumor cells. The authors also suggested that this type of mild heat exposure, comparable to a common fever, is not itself directly cytotoxic, but it stimulates some component(s) of the immune response, which results in increased antitumor activity. In support of this hypothesis, Burd et al. observed the increase in numbers of lymphocyte-like cells, macrophages, and granulocytes in the tumor vasculature and in the tumor stroma immediately following this mild hyperthermia exposure [60]. Similarly, Matsuda et al. (1997) demonstrated that the FR-WBH procedure applied alone using a rat tumor model, without any other additional therapy, delayed a tumor growth together with a significantly (50%) reduced incidence of lymph node metastases [61].

In addition, fever-range thermal stress can also activate processes involved in the killing of tumor cells. FR-WBH enhances antigen presentation by dendritic cells and promotes dendritic cell maturation, activates immune effector cells (making the tumor cells more sensitive to lysis by NK and lymphocyte CD8+ T cells) and switches the activities of the IL-6 to a predominantly anti-tumorigenic function that promotes anti-tumor immunity by mobilizing T cell trafficking in the recalcitrant tumor microenvironment [53, 62-66].

Based on these results it seems to be an interesting to use the immunomodulatory properties of PSP as a factor stimulating the organisms of cancer patients to feverish response.

5. Conclusion

We concluded, that PSP isolated from *Coriolus versicolor*, which is a bioactive component exhibiting antitumor and immunomodulatory properties, expands the duration of LPS-induced fever, and the effect is IL-6-related. Moreover, our results also suggest the compensatory effect of PSP-induced hypothermia on LPS-induced fever during this early stage of the febrile response. Finally, it seems to be an interesting to use the immunomodulatory properties of PSP as a factor stimulating the organisms of cancer patients to feverish response.

Declaration of interest

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The authors report no declarations of interest.

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Figure legends

Figure 1. Changes of body temperature (^{o}C) over time (h) of rats treated intraperitoneally (i.p.) with PSP (100 mg kg^{-1}) or 0.9% sterile saline at 7:00 (black arrowhead) and then injected i.p. with LPS (50 μg kg^{-1}) or 0.9% sterile saline at 9:00 (white arrowhead) in comparison to non-treated animals (NT). Values are means ± S.E.M. at 30-min averages. Letter n indicates sample size in a respective group. Asterisk indicates significant differences between PSP/LPS and saline/LPS groups; hash denotes significant differences between examined groups (PSP/LPS and saline/LPS) and control groups (NT and PSP/saline) at defined time intervals (**p<0.01; ###p<0.001, respectively).

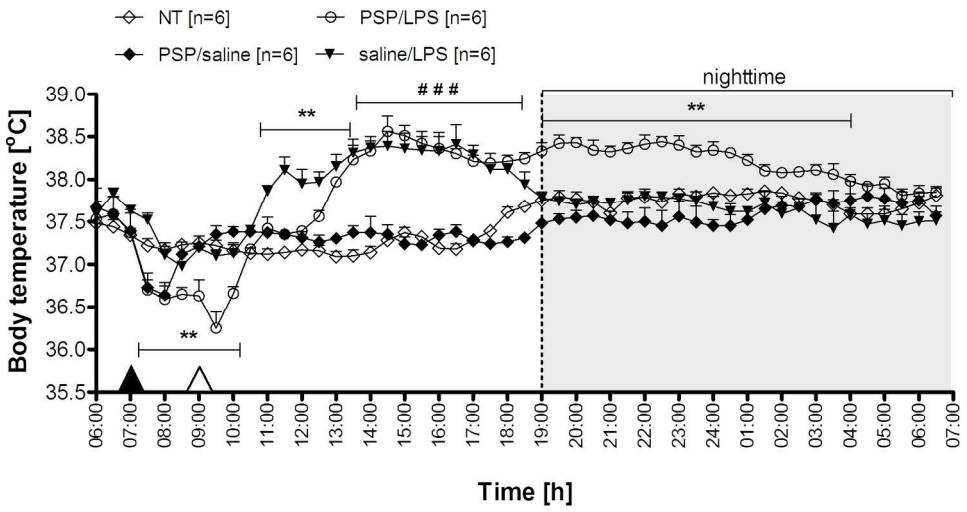
Figure 2. Plasma levels of IL-6 (pg ml^{-1}) estimated at 3h and 14h post-injection of PSP or saline in the rats injected i.p. with PSP (100 mg kg^{-1}) or saline 2h prior to the LPS administration (50 μg kg^{-1}) in comparison to non-treated animals (NT) and rats pre-treated with PSP followed by sterile saline. Values are expressed as means ±S.E.M. Assays were performed on four individuals in each group. Asterisk indicates significant difference (***p<0.001).

Figure 3. Plasma levels of TNF-α (pg ml^{-1}) estimated at 3h post-injection of PSP or saline in the rats injected i.p. with PSP (100 mg kg^{-1}) or saline 2h prior to the LPS administration (50 μg kg^{-1}) in comparison to non-treated animals (NT) and rats pre-treated with PSP followed by sterile saline. Values are expressed as means ±S.E.M. Assays were performed on four individuals in each group. Asterisk indicates significant difference (**p<0.01 and ***p<0.001, respectively).

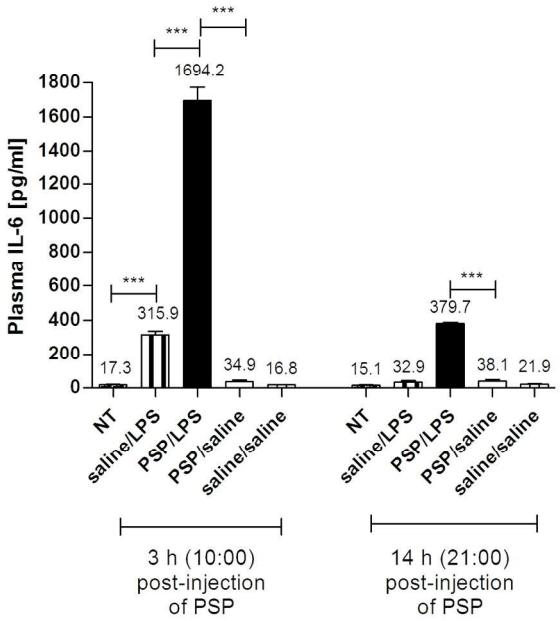
Figure 4. Changes of body temperature (^{o}C) over time (h) of rats treated intraperitoneally (i.p.) with PSP (100 mg kg^{-1}) or 0.9% sterile saline at 7:00. (black arrowhead), then injected i.p. with LPS (50 μg kg^{-1}) or 0.9% sterile saline at 9:00 (white arrowhead) and finally administrated i.p. with rabbit polyclonal IgG anti rat IL-6 or rabbit IgG at 17:00 (30 μg/rat;

gray arrowhead). Values are means ± S.E.M. at 30-min averages. Letter n indicates sample size in a respective group. Asterisk indicates significant differences between PSP/LPS + IgG and PSP/LPS + anti-IL-6 groups; hash denotes significant differences between examined groups of rats and non-treated animals (NT) at defined time intervals (**p<0.01; ##p<0.01; ###p<0.001, respectively).

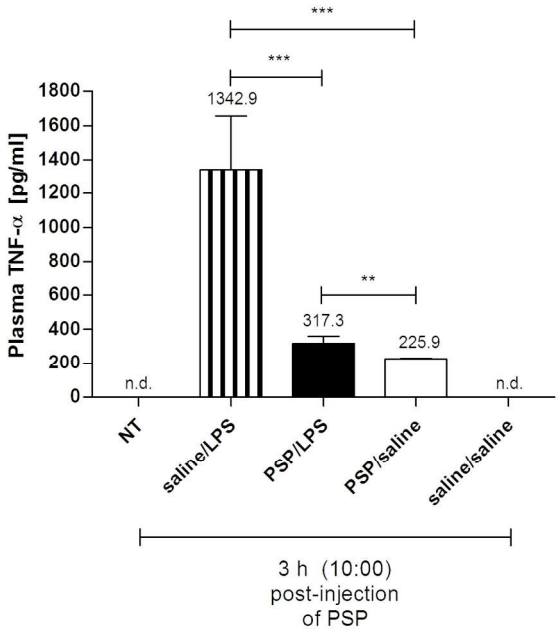
Figure 5. Changes of body temperature (^{o}C) over time (h) of rats injected intraperitoneally (i.p.) with sterile 0.9% saline at 7:00 (control vehicle for PSP injection; black arrowhead) and at 9:00 (control vehicle for LPS administration; white arrowhead), and finally treated i.p. with rabbit polyclonal IgG anti rat IL-6 or rabbit IgG at 17:00 (30 μg/rat; gray arrowhead) in comparison to non-treated animals (NT). Values are means ± S.E.M. at 30-min averages. Letter n indicates sample size in a respective groups.



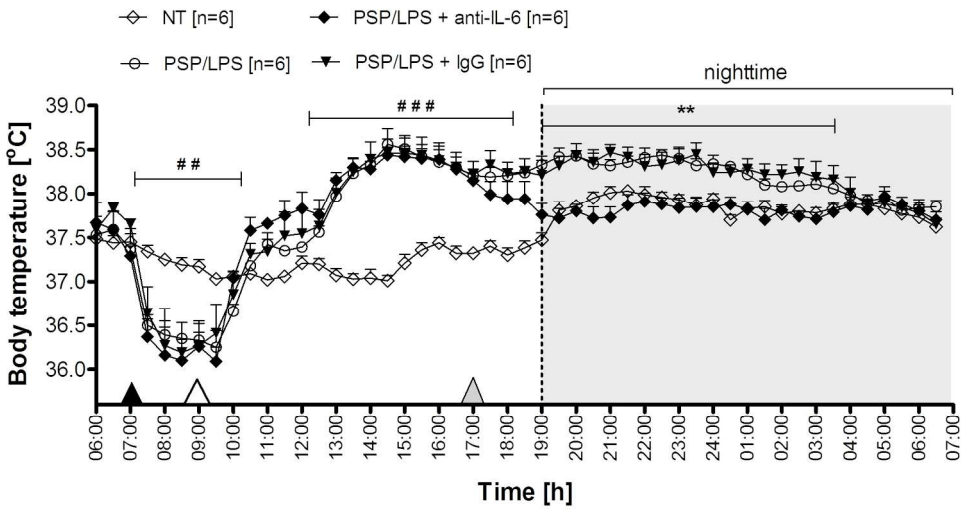
Changes of body temperature (°C) over time (h) of rats treated intraperitoneally (i.p.) with PSP (100 mg/kg) or 0.9% sterile saline at 7:00 (black arrowhead) and then injected i.p. with LPS (50 µg/kg) or 0.9% sterile saline at 9:00 (white arrowhead) in comparison to non-treated animals (NT). Values are means ± S.E.M. at 30-min averages. Letter n indicates sample size in a respective group. Asterisk indicates significant differences between PSP/LPS and saline/LPS groups; hash denotes significant differences between examined groups (PSP/LPS and saline/LPS) and control groups (NT and PSP/saline) at defined time intervals (**p<0.01; ###p<0.001, respectively).



Plasma levels of IL 6 (pg/ml) estimated at 3h and 14h post injection of PSP or saline in the rats injected i.p. with PSP (100 mg/kg) or saline 2h prior to the LPS administration (50 µg/kg) in comparison to non treated animals (NT) and rats pre treated with PSP followed by sterile saline. Values are expressed as means ±S.E.M. Assays were performed on four individuals in each group. Asterisk indicates significant difference (***p<0.001).



Plasma levels of TNF-α (pg/ml) estimated at 3h post-injection of PSP or saline in the rats injected i.p. with PSP (100 mg/kg) or saline 2h prior to the LPS administration (50 μg/kg) in comparison to non-treated animals (NT) and rats pre-treated with PSP followed by sterile saline. Values are expressed as means ±S.E.M. Assays were performed on four individuals in each group. Asterisk indicates significant difference (**p<0.01 and ***p<0.001, respectively).



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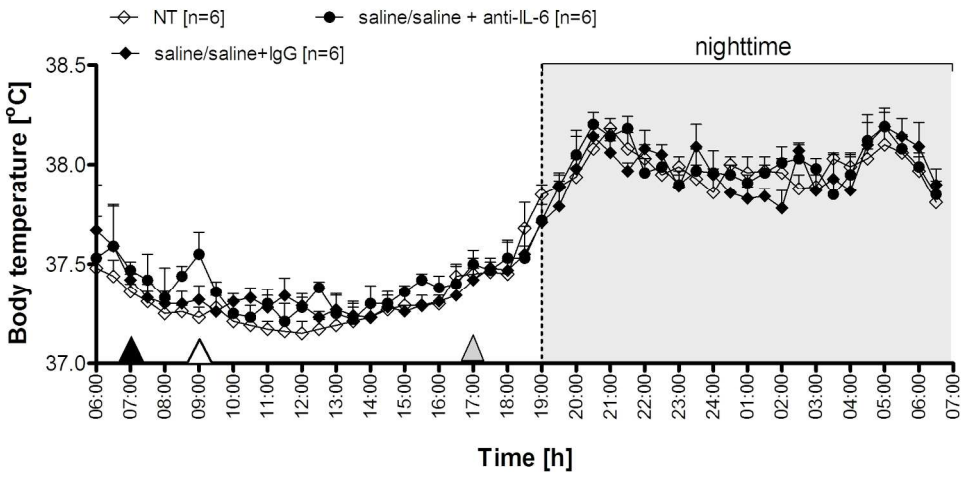

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