Polysaccharide Krestin Is a Novel TLR2 Agonist that Mediates Inhibition of Tumor Growth via Stimulation of CD8 T Cells and NK Cells

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Abstract

Purpose: Polysaccharide krestin (PSK) is a mushroom extract that has been long used in Asia and recently in Western countries as a treatment for cancer due to its presumed immune potentiating effects. Although there have been reports of clinical responses after patients have ingested PSK, the mechanism of action of the agent remains undefined. The current study was undertaken to investigate the mechanism of the antitumor actions of PSK.

Experimental Design: The immunostimulatory effect of PSK was first evaluated in vitro using splenocytes from neu transgenic mice and Toll-like receptor (TLR) 2 knockout (TLR2−/−) mice. Then the immunostimulatory and antitumor effect of PSK was determined using tumor-bearing neu transgenic mice, TLR2−/−, and wild-type C57BL/6 mice.

Results: We demonstrate that PSK is a selective TLR2 agonist, and the activation of dendritic cells (DC) and T cells by PSK is dependent on TLR2. Oral administration of PSK in neu transgenic mice significantly inhibits breast cancer growth. Selective depletion of specific cell populations suggests that the antitumor effect of PSK is dependent on both CD8+ T cell and NK cells, but not CD4+ T cells. PSK does not inhibit tumor growth in TLR2−/− mice suggesting that the antitumor effect is mediated by TLR2.

Conclusion: These results demonstrate that PSK, a natural product commonly used for the treatment of cancer, is a specific TLR2 agonist and has potent antitumor effects via stimulation of both innate and adaptive immune pathways. Clin Cancer Res; 17(1); 67–76. ©2010 AACR.
Translational Relevance

It has been reported that more than half of the cancer patients have tried both conventional and complementary and alternative medicine (CAM) to combat their disease; yet, the mechanism of CAM therapies remain poorly understood. In this study, we demonstrated that polysaccharide krestin (PSK), a highly purified mushroom extract, is a potent and selective Toll-like receptor (TLR) 2 agonist. PSK activates dendritic cells (DC) and T cells in a TLR2-dependent manner. In vivo administration of PSK in a mouse model of breast cancer has potent antitumor effect that is dependent on CD8+ T cells and NK cells. PSK is a natural product with a proven safety profile. Results from this study provide important information for future clinical trials testing the antitumor potential of this novel TLR2 agonist.

Materials and Methods

Animals

A colony of neu transgenic mice harboring the nontransforming rat neu [strain name, FVB/N-TgN (MMTVneu)-202Mull] was established in our animal facilities from breeding pairs (Jackson Laboratory) and maintained as previously described (17). Toll-like receptor (TLR) 2−/− and TLR4−/− mice were originally obtained from Dr. Shizuo Akira. Wild-type C57BL/6 mice were obtained from Jackson Laboratory. Mice were maintained under strict inbreeding conditions. All of the procedures were performed in compliance with the University of Washington Institutional Animal Care and Use Committee guidelines.

Reagents

Reagents used included fetal bovine serum (FBS; Gemini Bioproducts), RPMI 1640, phosphate-buffered saline (PBS), penicillin–streptomycin, and L-glutamine (Invitrogen Life Technologies), fluorochrome-conjugated monoclonal antibodies (mAb) targeting CD3, CD4, CD8, CD11c, NK1.1, Foxp3, and TLR2 (eBiosciences), [3H]-thymidine (PerkinElmer), RNAqueous-4PCR kit for RNA extraction (Ambion), primers and probes and gene expression master mix for real-time PCR (Applied Biosystems). PSK was purchased from Kureha Pharmaceuticals. PSK was dissolved in 96-well plates (200,000 cells per well) in RPMI and incubated with PSK (1–200 μg/mL) for 96 hours. [3H]-thymidine (1 μCi per well) was included for the last 16 hours of culture. For time-course treatment, splenocytes were stimulated with PSK (100 μg/mL) for 24, 48, 72, or 96 hours. Cytokine levels in the culture supernatant were measured using a mouse Th1/Th2 cytokine kit according to the manufacturer’s instructions (Meso Scale Discovery). To measure the percentage of each immune subset (CD4 and CD8 T cells, NK cells, and B cells), PSK or control PBS-treated splenocytes were stained with fluorochrome-conjugated anti-CD4, CD8, NK1.1, and CD19 antibodies using standard procedures as previously described (17). To measure PSK-stimulated IFN-γ production from NK cells, splenocytes from TLR2−/− mice or wild-type (WT) C57Bl/6 mice were treated with PSK (100 μg/mL) for 24 hours. Intracellular staining was used to evaluate IFN-γ production by NK cells, using similar method as described in our previous publication (19). To measure the expression of TLR2 on each immune subset, splenocytes were stained with anti-TLR2-PE mAb (eBiosciences) following the manufacturer’s instructions and the cells were costained with mAbs against CD3, NK1.1, CD19, and CD11c. The expression of TLR2 on mouse mammary carcinoma (MMC) tumor cells was measured using similar method.

PSK activation of dendritic cell in vitro

Cells were collected from the bone marrow of femurs of mice under aseptic conditions and cultured in 24-well plates in culture medium (RPMI with 10% FBS, 50 μg/mL of β-mercaptoethanol, and penicillin/streptomycin) after lysing red blood cells. Recombinant mouse IL-4 and GM-CSF (granulocyte macrophage colony stimulating factor) was included at a final concentration of 20 ng/mL after removal of nonadherent cells. On day 7, cells were split and cultured in the presence of PSK (200 μg/mL), lipopolysaccharide (LPS; 100 ng/mL, positive control), or PBS (negative control). After a 48-hour treatment, culture supernatant from each well was collected for analysis of IL-12p40 and IL-12p70 using ELISA kits (eBiosciences). The adherent cells were detached and 1 million cells per well were stained with anti-CD11c-APC, anti-CD80-PE, and anti-CD86-PerCP, and analyzed on a FACS Canto analyzer. FACS data analysis was performed using FlowJo software (Treestar).

Assessment of TLR activation using HEK293 cell transfectants

Human embryonic kidney cells (HEK293) expressing TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, or TLR9 (InvivoGen) were cultured in DMEM (Cambrex) containing 4.5 g/L of d-glucose (Sigma-Aldrich) and 10% FBS. To measure NF-kB activation, the TLR-transfected HEK cells were cotransfected with a plasmid that has NF-kB inducible reporter construct secretry alkaline phosphatase (SEAP) activity. The cells were then incubated with different doses of PSK (0.5–1,000 μg/mL) in 1:3 dilution or with serial dilutions of the
known agonist for each TLR as a positive control [heat-killed listeria monocytogenes (HKLM) for TLR2, poly [I:C] for TLR3, LPS for TLR4, flagellin for TLR5, resiquimod for TLR7 and 8, CpG ODN2006 for TLR9]. After overnight incubation, the culture supernatant was collected and the SEAP activity in the supernatant was measured using a Quanti-blue assay (InvivoGen).

**Treatment of tumor-bearing mice with oral PSK**

The antitumor effect of PSK was evaluated in neu transgenic mice in both implanted tumor and spontaneous tumor setting, and in TLR2−/− and WT C57BL/6 mice with implanted tumors. For implanted tumor in the neu transgenic mice, 1 million MMC cells, a syngeneic tumor cell line derived from a spontaneous tumor in these mice (18), was injected to the mice subcutaneously. PSK treatment was started 2 weeks after the implantation when the implanted tumor just became palpable (average size was 50 mm3). For spontaneous tumors, neu transgenic mice with palpable tumors (average size = 50 mm3) were randomly assigned to be treated with PSK (2 mg per mouse; equivalent to 100 mg/kg, 3 times per week for 4 weeks) or control PBS (n = 5 per group). For implanted tumors in C57BL/6 and TLR2−/− mice, the tumor was started by subcutaneous injection of 40,000 TC-1 cervical cancer cells (C57BL/6 background; originally obtained from ATCC). PSK or control PBS treatment was started 10 days after the injection when tumors just became palpable. PSK was dissolved in PBS and given via oral gavage in a 200-μL volume. Mice in the control group received oral gavage of PBS of the same volume. Tumors were measured every other day with vernier calipers and tumor volume was calculated as the product of length × width × height (GraphPad Software). Data were analyzed using the 2-tailed Student’s t test or ANOVA. A value of P < 0.05 was considered statistically significant.

**Evaluation of NK lytic activity using flow cytometry**

Splenocytes from PSK or control PBS-treated mice were isolated and tested for NK-cell lytic activity against NK-sensitive yeast artificial chromosome (YAC)-1 tumor target cells by a standard flow cytometry assay. YAC-1 cells were labeled with 3,3′-dioctadecyloxacarbocyanine [DiOC18(3); Sigma] and then cultured for 4 hours with splenocytes from PSK or PBS-treated mice in triplicate wells at 3 different effector to target (E:T) ratios (50:1, 25:1, and 12.5:1). At the end of 4 hours of incubation, cells were harvested and stained with propidium iodide (PI) and washed. Dead target cells were detected as PI−/DiOC18− cells by flow cytometry. NK-cell activity, defined as percent cytotoxicity of each sample, was calculated at each E:T ratio using the following formula: (% PI−/DiOC18− cells)splenocytes−YAC-1 − (% PI−/DiOC18− cells)YAC-1 alone. Lytic units (LU) values were extrapolated from dose-response curves of percent-specific lysis versus log E:T ratio for each blood sample assayed using a software program provided by Dr. Theresa L. Whiteside, University of Pittsburgh School of Medicine (22, 23). Lytic units of NK-cell activity, defined as the number of cells required to cause 20% target cell lysis relative to 107 effector cells, were determined by the equation: 107/LU20.

**Real-time reverse transcription PCR**

Total RNA from PSK or control PBS-treated tumors was isolated using RNA4Aqueous kit (Ambion). The integrity of RNA was tested using an Agilent BioAnalyzer. Reverse transcription and real-time PCR analysis was done similarly as previously described (17). Primer and probes for CD4 and CD8 were purchased from Applied Biosystems. Data analysis was performed using SDS 2.21 (Applied Biosystems). The mRNA expression level of the target gene was normalized to β-actin using the ΔCT method. Level of expression = 2−ΔΔCT, where ΔΔCT = ΔCTtarget gene − ΔCТactin. CT is the cycle threshold at which the fluorescence signal crosses an arbitrary value.

**Statistical analysis**

Statistical analysis was performed using GraphPad (GraphPad Software). Data were analyzed using the 2-tailed Student’s t test or ANOVA. A value of P < 0.05 was considered statistically significant.
Results

**PSK treatment stimulates T-cell proliferation and Th1 cytokine secretion and induces DC maturation and IL-12 secretion**

PSK treatment (10–200 µg/mL, 48–96 hours) significantly stimulated the proliferation of splenocytes in a dose- (Fig. 1A) and time-dependent manner (Fig. 1B). PSK treatment increased the percentages of CD4^{+} (25.5 ± 1.5% in control vs. 43.2 ± 3.9% after 72 hours PSK at 100 µg/mL; \( P = 0.01 \)) and CD8^{+} T cells (9.9 ± 0.5% vs. 15.5 ± 0.5%; \( P = 0.0015 \)) among total splenocytes and reduced the percentage of B cells (46.8 ± 0.6% vs. 22.4 ± 1.9%; \( P = 0.0003 \); Fig. 1C). B-cell number also decreased, consistent with previous publication that PSK inhibits B-cell growth (24). PSK treatment (10–200 µg/mL, 48–96 hours) also induced secretion of Th1 cytokines in a dose- and time-dependent manner. After 96 hours of PSK treatment (100 µg/mL), the level of IFN-γ was increased by 4.03 ± 0.41-fold (\( P = 0.001 \) from control). TNF-α was induced by 3.21 ± 0.44-fold (\( P = 0.0043 \) from control). IL-2 was induced by 3.40 ± 0.06-fold (\( P = 0.0002 \) from control). The levels of IL-4 and IL-5 were not different from controls (\( P = 0.22 \) and 0.11, respectively; Fig. 1D). None of the innate immune subsets we tested [monocytes (CD14^{+}), macrophage (F4/80^{+}), and DC (CD11c^{+})] were increased among total splenocytes after in vitro PSK treatment (\( P > 0.05 \) as compared with control). Although the number of CD11c^{+} cells was not increased after PSK treatment, we questioned whether the phenotype and functional capacity of DC was impacted by PSK. Using bone marrow-derived dendritic cells (BMDC), we found that PSK treatment (200 µg/mL, 48 hours) resulted in increased percentage of mature DC that that are CD86^{+} MHCIIP^{+} (62.3 ± 3.4% in PBS vs. 80.1 ± 5.0% in PSK group; \( P = 0.04 \); Fig. 2A and B). PSK-treated DC also secreted significantly greater levels of IL-12p40 (441 ± 24 pg/mL in PBS group vs. 689 ± 78 pg/mL in PSK group; \( P = 0.03 \); Fig. 2C), and IL-12p70 (0.4 ± 0.1 pg/mL in PBS vs. 55.3 ± 3.6 pg/mL in PSK group; \( P < 0.001 \)) than controls (Fig. 2 D).

**PSK is a selective TLR2 agonist and the type I inflammatory response induced by PSK is dependent on TLR2 activation**

It has been shown that fungal pathogens can activate TLRs (25), so we questioned whether PSK, which is a fungal product, may induce type I immunity via TLR activation. PSK specifically activates TLR2 in a dose-dependent manner and demonstrated no activity against TLR3, TLR4, TLR5, TLR7, TLR8, or TLR9 (Fig. 3A and B).
Evaluation of TLR2 expression in different subsets of splenocytes from neu transgenic mice showed that TLR2 is expressed at nondetectable or very low levels in CD3$^+$ T cells and NK1.1$^+$ NK cells. The expression was detectable in CD19$^+$ B cells and highly expressed in CD11c$^+$ DC (Fig. 3C). TLR2 was not expressed in tumor cells from neu transgenic mice (data not shown). Preincubation with an anti-TLR2 mAb but not an anti-TLR4 mAb inhibited PSK-induced IL-12p40 production by BMDC ($P < 0.0001$; Fig. 3D). BMDC from TLR2$^{-/-}$ mice, when stimulated with PSK, secreted significantly less IL-12p40 compared with BMDC from WT or TLR4$^{-/-}$ mice ($254 \pm 8$ pg/mL vs. $787 \pm 21$ pg/mL and $790 \pm 10$ pg/mL; $P < 0.0001$; Fig. 3E). Similarly, splenocytes from TLR2$^{-/-}$ mice did not secrete TNF-α upon PSK stimulation (Supplementary Fig. 2).

In vitro PSK stimulation did not result in the secretion of IFN-γ by splenocytes from TLR2$^{-/-}$ mice (Fig. 3F).

Oral PSK administration significantly inhibits the growth of both implanted and spontaneous breast tumors in neu transgenic mice

PSK was administered via oral gavage to neu transgenic mice bearing implanted or spontaneous tumors. As shown in Figure 4, PSK treatment significantly inhibits the growth of both the tumors. In the implant model, the tumor size after 3 weeks of treatment was 574 ± 26 mm$^3$ in the PSK group and 1,174 ± 41 mm$^3$ in the PBS group ($P < 0.0001$). In the spontaneous tumor setting, the tumor size after 3 weeks of treatment was 95 ± 39 mm$^3$ in PSK group and 825 ± 154 mm$^3$ in PBS group ($P = 0.0006$). We have previously reported that spontaneous tumors are different

Figure 2. In vitro PSK treatment activates DC maturation and secretion of IL-12. A, representative flow graphs of CD11c$^+$ DC from PBS-, PSK-, and LPS-treated BMDC stained with anti-CD86 and anti-MHCII. The number in each graph indicates the percentage of mature DC (CD86$^+$ MHCII high) in each sample. B, the columns represent mean ± SEM of the percentage of mature DC in BMDC treated with PBS (white column), PSK (black column), and LPS (gray column); *, $P < 0.05$; **, $P < 0.01$. Similar results were observed in 3 independent experiments. C and D, the columns represent mean ± SEM of the levels of IL-12p40 and IL-12p70 in culture supernatant of BMDC treated with PBS (white column), PSK (black column), and LPS (gray column); *, $P < 0.05$; **, $P < 0.01$. Similar results were observed in 3 independent experiments.
from implanted tumors in these neu transgenic mice because spontaneous tumors grow slowly, have more infiltrating immune cells, are more immunogenic, and usually responded better to immunotherapy (17, 19). However, as the mice do not develop spontaneous tumors until approximately 5 months old, the implant model is still frequently used, especially when large number of mice is required.

Figure 3. PSK selectively activates TLR2 and the stimulatory effect of PSK on DC is dependent on TLR2 activation. A and B shown are SEAP activity (y-axis) in the culture supernatant of TLR2- and TLR4-transfected HEK-293 cells when stimulated with serial dilutions of PSK (0.5–1,500 µg/mL, solid line with ●) or the positive control TLR agonists (HKLM for TLR2, and LPS for TLR4, shown as dotted line with *). Each data point represents the mean ± SEM of triplicate culture wells at each concentration. Arbitrary units (AU): PSK 1 unit = 0.5 µg/mL; HKLM 1 unit = 4,500 cells; LPS 1 unit = 0.05 ng/mL. Similar results were obtained from more than 3 experiments. C, histograms showing the expression of TLR2 in T cells, B cells, NK cells, and DC from neu transgenic mice. The filled histogram represents cells stained with anti-TLR2-PE; the unfilled histogram represents cells stained with an isotype control antibody. Experiments were repeated twice with similar results. D, IL-12p40 levels (mean ± SEM) in BMDC isolated from neu transgenic mice pretreated with anti-TLR2 or anti-TLR4 monoclonal antibody (10 µg/mL) for 1 hour prior to PSK treatment (100 µg/mL for 48 hours). **, P < 0.01. E, the columns represent average IL-12p40 levels (mean ± SEM) in PBS-, PSK-, or LPS-treated DC from WT, TLR2−/−, or TLR4−/− mice. ***, P < 0.001 compared with PSK treatment group in WT. Experiments were repeated twice with similar results. F, percentages of IFN-γ-positive NK cells in splenocytes from WT and TLR2−/− mice treated with PSK (100 µg/mL, 24 hours) or control PBS. *, P < 0.05 from PBS control. Results are representative of 3 independent measurements.
Oral PSK administration augments both T-cell and NK cell activity and the antitumor effect of PSK is dependent on CD8\(^+\) T cells and NK cells.

Oral PSK administration augments both T-cell and NK cell activity and the antitumor effect of PSK is dependent on CD8\(^+\) T cells and NK cells (1.6 \(\pm\) 0.5 LU vs. 9.8 \(\pm\) 2.0 LU; \(P = 0.01\); Fig. 5B). Evaluation of tumor-infiltrating lymphocytes (TIL) showed a significantly increased ratio of CD8/CD4 T cells at both the mRNA and protein level in PSK-treated mice (\(P = 0.014\) by RT-PCR and \(P = 0.04\) by FACS as compared with control; Fig. 5C and D). The percentage of CD4\(^+\)Foxp3\(^+\) regulatory T cells among total TIL decreased from 2.73 \(\pm\) 0.60% to 1.02 \(\pm\) 0.16% in PSK-treated mice (\(P = 0.01\); Fig. 5E). To determine the role of different immune subsets in the antitumor effect of PSK, we selectively depleted CD4\(^+\), CD8\(^+\) T cells or NK cells during PSK treatment. As shown in Figure 6A, selective depletion of CD8\(^+\) T cells and NK cells, but not CD4\(^+\) T cells, significantly inhibited the antitumor effect of PSK in mice with implanted breast tumors. Both NK cells and CD8 T cells are required, as depletion of both sets during PSK treatment resulted in larger tumor than depleting either set alone (data not shown). To investigate whether the antitumor effect of PSK is mediated by TLR2 activation, we implanted the same amount of TC-1 tumor cells into TLR2 knockout mice and WT C57BL/6 mice. Then we treated the tumor-bearing TLR2\(^{-/-}\) mice or WT mice with oral PSK or control PBS. As shown in Figure 6B, PSK significantly inhibited tumor growth in WT mice (\(P < 0.0001\) between PBS and PSK group), but not in TLR2\(^{-/-}\) mice (\(P = 0.5\) between PBS and PSK group), indicating that TLR2 is critical in mediating the antitumor effect of PSK.

Discussion

In the current study, we have demonstrated that PSK is a potent and selective TLR2 agonist. Moreover, PSK treatment induces type I T cells potentially via its effect on the DC phenotype. Finally, the antitumor effects observed with PSK treatment are dependent on both T-cell and NK-cell activity.

Data presented here are the first evidence that PSK selectively activates TLR2. TLRs are a family of evolutionarily conserved pathogen recognition receptors that play a pivotal role in host defense by regulating both innate and adaptive immune responses (26, 27). TLR agonists have been tested intensively in tumor immunotherapy in recent years, and almost all of the studies exploring the antitumor effect of TLR agonists have focused on the ligands for TLR3, TLR7, TLR8, and TLR9 (28–30). TLR2 is a transmembrane protein receptor and can form a heterodimer with TLR1 or TLR6. The expression of TLR2 on T cells is shown to be upregulated following T-cell activation and can act as a costimulatory receptor (31). Recently, it has been found that this costimulation via TLR2 is more responsible for proliferation and survival of CD8\(^+\) T cells than for that of CD4\(^+\) T cells (32). It has also been reported that TLR2 engagement on CD8\(^+\) T cells enables generation of functional memory cells in response to a suboptimal TCR (T-cell receptor) signal, such as that seen against self-tumor antigens (33). Moreover, engagement of TLR1/2 on ova-specific OT-1 CTLs increased cell proliferation and the expression of various effector molecules on T cells (34). These previous reports concerning TLR2 activation are
consistent with our finding that the ratio of CD8/CD4 in TIL increased in PSK-treated mice (Fig. 5) and CD8+ T cells, together with NK cells, mediate the antitumor effect of PSK. In addition to the effect on CD8+ T cells, recent literature suggests that TLR2 can abolish the suppressive capacity of regulatory T cells or make effector T cells resistant to the suppression of regulatory T cells (35, 36). We observed a decrease in regulatory T cells in tumor after PSK treatment that could also have contributed to the antitumor effect of PSK.

The finding that TLR2 is minimally expressed on T cells but highly expressed on DC (Fig. 3C) suggests that the effect of PSK on T cells could be indirect via activation of DC, secretion of IL-12, and the polarizing of a tumor-specific Th1 response. The ability of TLR agonists to augment antitumor immunity via stimulation of DC has been well described (37–39). For example, it has been shown that TLR8-primed DC can generate high-avidity antitumor T cells via IL-12 production (37). Ligation of TLR9 by CpG converts tolerogenic DC into antigen-presenting cells capable of stimulating antitumor immunity via activating Th1/Th17 and NK-cell response (38). Our results suggest the potential of using PSK, a natural product with a demonstrated favorable safety profile, to augment antitumor immunity via stimulating DC. A recent study showed that a combination of 3 TLR ligands (TLR2/6, TLR3, and TLR9) greatly enhanced IL-15 production from DC and increased the generation of high-avidity T cells after vaccination (40). Whether combining PSK with other TLR agonists will have enhanced antitumor effect remains to be investigated.

NK cells are also activated by PSK treatment and play an important role in the antitumor activity of PSK. It is noted that TLR2 expression on NK cells is very low, although significantly higher than the expression on T cells. Thus, the question remains whether the activation of NK cells by PSK is via direct activation of TLR2 on NK cells or indirect via activation of antigen-presenting cells. It is reported that NK-cell priming requires the presence of CD11c+ high DC (41). However, direct TLR2 signaling on NK cells has also been reported (42). The crosstalk between NK cells and DC has also been well described (43, 44). The finding that NK cells are activated by PSK indicates the potential of using this...
PSK Activates TLR2

The major mechanism of the antitumor effect of trastuzumab is antibody-dependent cell-mediated cytotoxicity (ADCC) in which the tumor cells are coated with the mAb and lysed by NK cells via binding of the Fc receptor. NK cell–stimulatory cytokines, such as IL-12 and IL-21, have been shown to enhance ADCC (45). The potential of using TLR agonists to enhance ADCC has also been suggested in publications (46–48). Our results suggest the potential of using PSK to augment the therapeutic effect of trastuzumab in breast cancer patients. Preliminary analysis using human PBMC showed that PSK has similar stimulatory effect on human immune cells and augments the ability of NK cell to lyse tumor targets (Lu et al., unpublished data).

It is noted that PSK-induced IL-12 production from BMDC was not completely abrogated in TLR2−/− mice. Thus, it is possible that receptors other than TLR2 may also be stimulated by PSK and have contributed to IL-12 production in the knockout mice. It has been reported that C-type lectins, such as dectin-1 and dectin-2, are involved in recognition of some fungal pathogens (49, 50). Whether PSK could also activate these lectin receptors remains to be investigated in future studies.

In summary, results from the current study demonstrate that PSK is a selective TLR2 agonist. The effect of PSK on DC and T cells is dependent on TLR2 activation. Oral PSK inhibits breast cancer growth in neu transgenic mice and the antitumor effect is dependent on both CD8+ T cells and NK cells. Results from this study elucidate the mechanism of action of this mushroom-based natural product and may lead to more effective methods of therapeutically exploiting the antitumor effects of PSK.

Disclosure of Potential Conflicts of Interests

No potential conflicts of interests were disclosed.

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