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Lysis of Fresh Human Tumor Cells by Autologous Peripheral Blood Lymphocytes and Tumor-infiltrating Lymphocytes Activated by PSK

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The protein-bound polysaccharide PSK was tested for the ability to induce *in vitro* autologous tumor killing (ATK) activity in human cancer patients. Peripheral blood lymphocytes (PBL) and tumor-infiltrating lymphocytes (TIL) demonstrated various levels of cytotoxicity against autologous, freshly isolated tumor cells. When PBL and TIL were cultured overnight with PSK, ATK activity was induced in previously non-reactive cases and augmented in previously reactive samples. The PSK effect was observed with PSK concentrations of 10–100 μg/ml that could be obtained in the blood of cancer patients who received standard oral administration of PSK. The manifestation of PSK-induced ATK required active cell metabolism and RNA and protein syntheses, but not DNA synthesis of lymphocytes. PSK-induced enhancement of ATK was not abrogated by monoclonal antibodies (mAb) directed against interferon (IFN)α or IFNγ. In addition, mAb that neutralized interleukin-2 (IL-2) or mAb reactive with α-chain or β-chain of IL-2 receptors (IL-2R) had no effect on PSK-induced ATK activity. Supernatants from PSK-stimulated lymphocyte cultures did not induce ATK. Cell fractionation experiments revealed that $CD3^-CD16^+$ large granular lymphocytes (LGL) and/or $CD3^+CD16^-$ T lymphocytes were responsible for both spontaneous and PSK-induced ATK. PSK-activated LGL, but not T lymphocytes expressed lysis of fresh allogeneic tumor cells. These results indicate that PSK activates PBL and TIL to exhibit ATK independently of IL-2/IL-2R systems.

Key words: Autologous tumor killing — Tumor-infiltrating lymphocyte — IL-2/IL-2R — PSK

Natural killer (NK)[5] cells are implicated as important anti-tumor effectors because of their killing activity without prior sensitization and major histocompatibility complex.[1,2] It is, however, difficult to interpret the data on cytotoxicity using tumor cell lines as targets since tumor cells alter their characteristics when cultured *in vitro*.[3,4] For better evaluation of the cytotoxic activity of lymphocytes in cancer patients, autologous combinations of fresh effector and target cells have been employed: peripheral blood lymphocytes (PBL) from 10 to 50% of cancer patients, depending on tumor cell types and metastases, lysed tumor cells freshly obtained from the same patients.[5–8] Recent evidence we obtained on the population and single cell levels indicates that $CD3^-CD16^+$ large granular lymphocytes (LGL) of cancer patients lyse autologous, freshly isolated tumor cells,[6,9] and release a novel cytotoxic factor, termed LGL-derived cytotoxic factor (LGL-CF).[10,11] Autologous tumor killing (ATK) was also mediated by $CD3^+CD16^-$ T lymphocytes in patients with localized neoplasms, while it was observed primarily with LGL when patients developed metastasis.[12] Of clinical importance is the fact that ATK is closely associated with the postoperative clinical course of cancer patients,[13,14] indicating that ATK is a meaningful prognostic indicator and providing evidence for immunological control of tumor growth and metastasis.

Considerable attention has been paid to the generation of cytotoxic lymphocytes capable of lysing autologous tumor cells and the use of such activated killer cells for adoptive cellular immunotherapy of cancer patients. *In vitro* culture of PBL with interleukin-2 (IL-2) generated lymphokine-activated killer (LAK) cells that killed autologous and allogeneic fresh tumor cells.[15–17] Recently, tumor-infiltrating lymphocytes (TIL) which are sensitized *in vivo* with autologous tumor cells were shown to be activated *in vitro* with IL-2 to express enhanced ATK.[18–21] Lysis of fresh tumor cells was also observed with autologous PBL activated *in vitro* with IFN,[5,22] lectins,[23] alloantigens,[24] autoantigens,[25] autologous

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[5] The abbreviations used are: ATK, autologous tumor killing; PBL, peripheral blood lymphocytes; TIL, tumor-infiltrating lymphocytes; mAb, monoclonal antibodies; IFN, interferon; IL-2, interleukin-2; IL-2R, interleukin-2 receptor; LGL, large granular lymphocytes; NK, natural killer; LGL-CF, LGL-derived cytotoxic factor; LAK, lymphokine-activated killer; E:T, effector-to-target.

tumor cells,[14,26] and the streptococcal preparation OK432.[7,27] On the other hand, the protein-bound polysaccharide PSK derived from basidiomycetes has been reported to prolong survival time in some human cancer patients.[28] While the primary effect of PSK has been suggested to be the restoration of host immunity impaired by tumor and/or chemotherapeutic agents,[29] the mechanism responsible for its antitumor activity is not thoroughly understood. The present study was designed to investigate the effect of PSK on the lysis of fresh tumor cells by autologous PBL and TIL from human cancer patients.

MATERIALS AND METHODS

Patients This study included 37 patients with previously untreated, localized neoplasms. Histological diagnosis showed that 8 patients had adenocarcinoma of the lung, 11 had squamous cell carcinoma of the lung, 6 had adenocarcinoma of the colon, 5 had adenocarcinoma of the breast, and 7 had adenocarcinoma of the stomach. The 25 male and 12 female patients ranged in age from 31 to 72 years (median age of 58 years).

Tumor cells Tumor cells were prepared as described elsewhere.[6,7,9-13,25,26] Specimens of tumor tissues were mechanically minced and treated with DNase, collagenase and hyaluronidase. The cells were then washed and centrifuged at 25–40 g for 5–10 min on three-step discontinuous Percoll gradients. Lymphocyte-rich mononuclear cells were collected from the top of the 25% interface, and tumor cells from the bottom. The tumor-enriched fraction was further depleted of macrophages by adherence to plastic dishes. Usually, the nonadherent cells contained mainly tumor cells with less than 10% nonmalignant cells and were more than 93% viable.

The K562 human myeloid leukemia cell line was also used as targets.

Preparation of PBL and TIL Effector cells were prepared from blood and tumor tissues as described in detail elsewhere.[6,7,9-13,25,26] Lymphocyte-rich mononuclear cells were isolated by centrifugation on Ficoll-Hypaque gradients, washed, and suspended in RPMI-1640 medium supplemented with 25 m*M* HEPES, 2 m*M* L-glutamine, 100 U penicillin/ml, 100 μg streptomycin/ml, and 10% heat-inactivated human AB serum (referred to hereafter as "complete medium"). Cell suspensions enriched in lymphocytes were further purified by adherence to serum-coated plastic dishes, Sephadex G10 and nylon-wool columns. The nonadherent lymphocytes with more than 95% purity and viability were fractionated by centrifugation on discontinuous seven-step Percoll gradients. The cells collected from low-density fractions 2 and 3 were further depleted of T-cells by treatment with anti-CD3 monoclonal antibodies (mAb) (OKT3; Ortho Pharmaceutical, Tokyo) and magnetic beads. The fraction contained more than 85% LGL. The cells collected from high-density fractions 6 and 7 contained more than 95% T cells.

Treatment with PSK, IFN, and IL-2 PSK was supplied by Kureha Chemical, Tokyo.[28] Purified human recombinant IFNα (A/D) with specific activity of 1×10^8 U/mg protein was obtained from Japan Roche, Tokyo, and recombinant human IFNγ with specific activity of 1×10^8 U/ml from Daiichi Pharmaceutical, Tokyo. Recombinant human IL-2 was from Shionogi Pharmaceutical, Osaka. Effector cells at 1×10^6/ml were treated alone, with 100 μg PSK/ml unless otherwise stated, or with 3,000 U IFNα/ml, 500 U IFNγ/ml, or 1,000 U IL-2/ml for 20 h at 37°C in serum-free T medium (Daigo, Osaka), as described previously.[7] There were no differences in the recovery of viable cells cultured alone and with PSK. In some experiments supernatants were harvested from 20-h PSK-stimulated lymphocyte cultures and ultracentrifuged at 150,000*g* for 24 h. The resulting supernatant contained virtually no PSK.

Monoclonal antibodies mAb directed against IFNα at 2×10^5 neutralizing activity units/mg protein, mAb against IFNγ at 2×10^4 neutralizing activity units/mg protein, anti-IL-2 mAb containing 1×10^6 neutralizing activity units/mg protein, and control serum were obtained from Cosmo-Bio, Tokyo. These mAbs were added to PSK-stimulated cultures at final concentrations that neutralized 500 U of IFNα, 200 U IFNγ, or 100 U IL-2, as described previously.[7] Tac mAb reactive with α-chain of IL-2 receptor (IL-2R/p55) and 2RB mAb directed against β-chain of IL-2R (IL-2R/p75) were kindly supplied by Dr. T. Uchiyama, 1st Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto.[30] Lymphocytes were treated with saturating amounts (20–50 nmol) of Tac and 2RB mAb for 1 h.[31]

Metabolic inhibitors Effector cells were treated with metabolic inhibitors, as described previously.[7] Lymphocytes at 1×10^6/ml were incubated for 1 h at 37°C alone or with mitomycin C (50 μg/ml), actinomycin D (10 μg/ml), or cycloheximide (100 μg/ml). The above doses of mitomycin C, actinomycin D, and cycloheximide inhibited more than 90% of DNA, RNA, and protein syntheses, as judged by [^{3}H]thymidine-, [^{3}H]uridine-, and [^{3}H]leucine-incorporation assays, respectively.[7]

Cytotoxicity assay A 6-h ^{51}Cr release assay was performed as described.[6,7,9-13,25,26] Briefly, 100 μl of ^{51}Cr-labeled target cells and effector cells were assigned at different ratios (E:T) to each well of microtiter plates and incubated for 6 h at 37°C. Samples were then harvested, and the activity was counted in an autogamma scintillation counter. The percent cytotoxicity was calculated by using the formula: % cytotoxicity = [(experimental release − spontaneous release)/(maximum release −

spontaneous release)] × 100. The spontaneous ^{51}Cr release usually did not exceed 35% of the total isotope count.

Agarose single cell cytotoxicity assay This assay was performed as described.[6] Equal numbers of effector and target cells were mixed, incubated and centrifuged, followed by gentle suspension. One percent agarose was added to the conjugate suspension, and the agar-conjugate mixture was transferred onto microscope slides. The slide was incubated for 4 h at 37°C, then stained with trypan blue and fixed with formaldehyde. Spontaneous target cell death did not exceed 15%.

Statistical analysis All determinations were made in triplicate, and results were calculated as the means plus or minus the standard deviation. Cytotoxicity greater than 10% was always statistically significant at $P<0.05$ by Student's t test and was considered as being positive.

RESULTS

***In vitro* induction or enhancement of ATK activity by PSK** Fresh PBL and TIL from cancer patients exhibited various levels of cytotoxicity against freshly isolated, autologous tumor cells in a 6-h ^{51}Cr release assay. To evaluate whether PSK induces ATK activity, preliminary attempts were made to determine optimal conditions for activation of lymphocytes. Lymphocytes were incubated with various concentrations of PSK for 20 h before they were tested for cytotoxicity. Results of two representative experiments are shown in Fig. 1. The maximum induction of ATK activity was observed when PBL and TIL were treated with 10–100 μg PSK/ml. Such PSK concentrations could be easily achieved in the peripheral blood of human cancer patients by standard oral administration of the drug. Lower doses of PSK had little or no effect on ATK, and higher doses were inhibitory, possibly due to the toxicity of PSK. The ATK-inducing activity of PSK was seen in 10 to 50% of patient's samples, depending on tumor cell types.

PSK-induced ATK as determined in single cell assays In the single cell cytotoxicity assay the number of PSK-treated lymphocytes binding to autologous tumor cells was comparable to that of untreated lymphocytes (Fig.

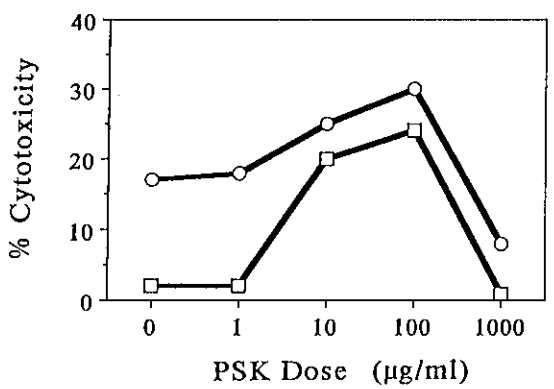

Fig. 1. PSK dose-dependent augmentation of ATK activity. PBL from patient #1 (□) and TIL from patient #2 (○) were treated for 20 h alone or with various concentrations of PSK. They were then washed and tested for lysis of autologous tumor cells in a 6-h ^{51}Cr release assay at an E:T of 40:1. Similar results were obtained in 3 different experiments.

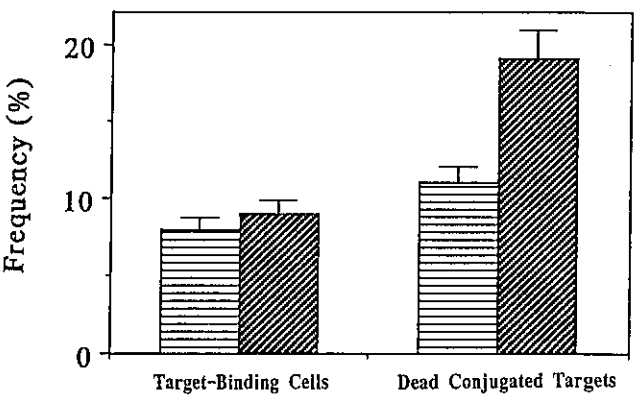

Fig. 2. Augmentation of ATK activity by PSK determined in single cell cytotoxicity assay. PBL and TIL were treated with 100 μg PSK/ml and were tested for binding (TBC; target binding cells) and killing (LC; lytic conjugates) of autologous tumor cells in a single cell cytotoxicity assay. Similar results were obtained in 2 different experiments. ▤ Control; ▨ PSK.

Table I. Effects of PSK on Cytotoxicity against Autologous and Allogeneic Tumor Cells

Patient	Effectors[a]	PSK treatment	% Cytotoxicity against		
			Tumor #7	Tumor #8	K562
#7	LGL	−	3±0.8	7±3.4	13±1.4
		+	15±2.2[b]	21±2.3[b]	23±2.2[b]
	T	−	4±1.2	1±0.8	3±1.5
		+	14±1.9[b]	2±1.1	3±1.3
#8	LGL	−	8±1.6	25±2.5	34±3.1
		+	20±2.6[b]	41±3.3[b]	48±3.0[b]
	T	−	2±0.4	10±2.0	0±0.7
		+	4±2.0	23±1.7[b]	4±2.3

a) Blood $CD3^-$ LGL and $CD3^+$ T lymphocytes from patients #7 and #8 were each treated with 100 μg PSK/ml for 20 h. They were then tested for cytotoxicity against fresh tumor cells from patients #7 and #8 and K562 at an E:T of 40:1 in a 6-h ^{51}Cr release assay.

b) Value is significantly higher than that of untreated lymphocytes at $P<0.05$.

Similar results were obtained in 3 different patients.

2). By contrast, the frequency of dead conjugated target cells among effector-target conjugates was higher in the PSK-activated lymphocytes than in the corresponding untreated ones. Thus, the augmentation by PSK of ATK activity is unlikely to be due to an enhancement of their target binding capacity but may rather be a result of an elevated ability to produce lethal hits.

Lymphoid subsets involved in PSK-induced ATK Since spontaneous ATK activity was mediated by $CD3^-CD16^+$ LGL and/or $CD3^+CD16^-$ T lymphocytes, lymphocytes were fractionated into $CD3^-CD16^+$ LGL and $CD3^+CD16^-$ T cells prior to stimulation with PSK. When purified LGL were treated with PSK, They exhibited higher levels of cytotoxicity against autologous tumor cells than did the corresponding untreated LGL (Table I). Similarly, purified T cells responded *in vitro* to PSK to express augmented ATK. These results indicate that both LGL and T lymphocytes could be activated by PSK, which does not require other cell components.

Next, the target specificity of PSK-activated killer LGL and T lymphocytes was evaluated by the use of allogeneic fresh tumor cells and K562 cells as targets. The cytotoxicity mediated by the PSK-activated LGL was not restricted to autologous tumor cells, since they were also cytotoxic to allogeneic fresh tumor cells and K562. By contrast, the PSK-activated T lymphocytes killed only autologous tumor cells, with no lytic effect on allogeneic tumor cells and K562.

Requirement of active cell metabolism for PSK-induced cytotoxicity Lymphocytes were preincubated alone or with PSK either at 4°C or 37°C. Cytotoxicity against autologous tumor cells of lymphocytes treated with PSK at 37°C was higher than that of the untreated cells, whereas lymphocytes treated at 4°C showed no increase in ATK (Fig. 3). Pretreatment with mitomycin C did not abrogate spontaneous or PSK-induced cytotoxicity. In

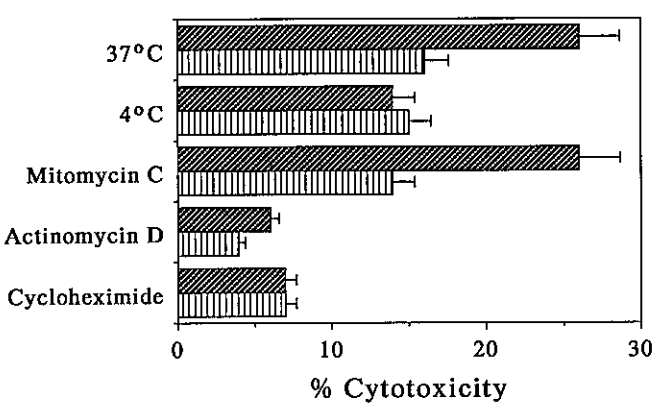

Fig. 3. PBL were incubated for 1 h with medium, mitomycin C, actinomycin D, or cycloheximide. They were then washed and were further incubated for 20 h either at 4°C or 37°C, after which they were washed and tested for cytotoxicity against autologous tumor cells at an E:T of 40:1. Similar results were obtained in 2 different experiments. ▥ Control; ▨ PSK.

Table II. Possible Role of IFN in PSK-induced ATK

Treatment[a]	% Cytotoxicity by	
	LGL	T
None	23±2.4	13±2.5
PSK	41±3.9[b]	24±2.3[b]
PSK+control serum	38±2.4[b]	25±1.8[b]
PSK+anti-IFNα	37±2.0[b]	27±2.6[b]
PSK+anti-IFNγ	39±2.3[b]	22±2.9[b]
PSK+anti-IFNα + anti-IFNγ	38±1.6[b]	22±1.7[b]
Supernatants	24±3.8	14±2.0
IFNα	31±1.2[b]	12±1.6
IFNγ	29±1.0[b]	11±1.5
IFNα+IFNγ	33±2.2[b]	14±2.8
IFNα+IFNγ+anti-IFNα+anti-IFNγ	21±3.5[b]	13±2.2

a) Blood LGL and T lymphocytes were treated with medium, 100 μg PSK/ml in the presence or absence of anti-IFNα mAb and/or anti-IFNγ mAb, or with IFNα and/or IFNγ or supernatants from PSK-stimulated lymphocyte cultures (50%) for 20 h. They were then washed and tested for cytotoxicity against autologous fresh tumor cells at an E:T of 40:1 in a 6-h ^{51}Cr release assay.

b) Value is significantly different from that of "none" at $P<0.05$. Similar results were obtained in 3 different patients.

Table III. Unlikely Involvement of IL-2/IL-2R Systems in PSK-induced ATK

Treatment[a]	% Cytotoxicity		
	Pt #11	Pt #12	Pt #13
None	10±1.6	2±1.6	6±3.3
PSK	29±1.7[b]	14±1.3[b]	4±0.8
PSK+control serum	26±2.7[b]	15±1.8[b]	3±1.5
PSK+anti-IL-2	27±2.0[b]	17±2.6[b]	1±0.7
PSK+anti-IL-2R/p55	29±1.5[b]	13±1.8[b]	5±2.4
PSK+anti-IL-2R/p75	25±1.8[b]	12±1.7[b]	3±1.6
PSK+anti-IL-2R/p55 +anti-IL-2R/p75	29±2.7[b]	14±1.8[b]	2±0.8
IL-2	28±4.1[b]	11±2.8[b]	21±2.1[b]
IL-2+anti-IL-2	12±3.2	3±2.0	5±2.5

a) PBL (Patient #11 and #13) and TIL (Patient #12) were treated with medium, 100 μg PSK/ml in the presence or absence of anti-IL-2 mAb, anti-IL-2R/p55 mAb, anti-IL-2R/p75 mAb, or with IL-2 for 20 h. They were then tested for cytotoxicity against autologous fresh tumor cells at an E:T of 40:1 in a 6-h ^{51}Cr release assay.

b) Value is significantly different from that of "none" at $P<0.05$.

addition, PSK did not induce the proliferation of lymphocytes (data not shown). In contrast, treatment with actinomycin D or cycloheximide inhibited both spontaneous and PSK-induced ATK. There were essentially no differences in the recovery of viable cells treated with medium and the metabolic inhibitors. Thus, RNA and protein syntheses, but not DNA synthesis of lymphocytes are required for the induction of ATK by PSK.

Possible involvement of IFN in PSK-induced cytotoxicity To determine whether PSK-activated lymphocytes produce factors that have ATK-enhancing capacity, lymphocytes were precultured for 20 h alone, with supernatants produced by PSK-stimulated lymphocyte cultures, or with PSK. The supernatant did not induce cytotoxicity, while PSK-treated lymphocytes exhibited the enhanced ATK (Table II). Next, we compared the ATK-enhancing effect of PSK with that of IFN. Overnight treatment with IFNα or IFNγ of purified $CD3^-$ LGL augmented ATK activity, the level of which was, however, different from that seen with PSK-activated LGL. By contrast, T lymphocytes did not respond to IFNα or IFNγ, while they were activated by PSK. Furthermore, neither anti-IFNα mAb nor anti-IFNγ mAb abrogated the induction of ATK activity by PSK even when used in combinations. In addition, the supernatant of PSK-stimulated lymphocyte cultures contained no detectable amount of IFN (data not shown).

Possible involvement of IL-2/IL-2R in PSK-induced cytotoxicity When the ATK-inducing effect of PSK was compared with that of IL-2, each lymphocyte sample responded differently to PSK and IL-2 (Table III). Then, PBL and TIL were activated with PSK in the presence or absence of IL-2-neutralizing mAb. The level of ATK induced in the presence of anti-IL-2 mAb was comparable to that seen in its absence. No detectable amount of IL-2 was produced by PSK-stimulated lymphocytes (data not shown). Next, Tac and 2RB mAb were added to PSK-stimulated lymphocyte cultures. Neither anti-IL-2R/p55 mAb nor anti-IL-2R/p75 mAb abrogated the generation of ATK potential by PSK. The mixture of both mAbs produced no inhibitory effect. Thus PSK may induce ATK activity independently of IL-2/IL-2R systems.

DISCUSSION

The present study is, to our knowledge, the first to demonstrate that the protein-bound polysaccharide PSK activates human PBL and TIL to express augmented lysis of autologous, freshly isolated tumor cells. Of clinical importance is the finding that the induction of ATK activity by PSK is achieved at PSK concentrations which are seen in the peripheral blood of PSK-treated cancer patients. A similar dose of PSK was previously shown to activate murine lymphocytes.[29] Several attempts have been made to generate cytotoxic lymphocytes capable of lysing fresh autologous tumor cells, and IL-2 has been implicated as an important cytokine in the induction of killer cells. PSK, however, appears to induce or augment the ATK activity of PBL and TIL independently of the IL-2/IL-2R system for the following reasons: 1) mAb that neutralized IL-2 did not abrogate generation of ATK activity by PSK, 2) Tac and 2RB mAb that blocked binding of IL-2 to α-chain and β-chain of IL-2R, respectively, did not affect the ATK-inducing ability of PSK, 3) supernatants produced by PSK-stimulated lymphocyte cultures contained no detectable amount of IL-2 and did not induce ATK activity, 4) PSK-activated T cells lysed only autologous tumor cells, while LAK cells had broad cytotoxicity, 5) responsiveness of individual patients to PSK differed from that to IL-2, 6) stimulation of lymphocytes with PSK did not induce Tac antigens (data not shown). The above findings are supported by the recent evidence that no mRNA of IL-2 is induced by stimulation of lymphocytes with PSK.[31] Thus, there exists an ATK system that could be up-regulated by PSK independently of the IL-2/IL-2R system.

The possibility that PSK-activated killer cells are identical to lectin-activated killer cells cannot be ruled out in the present study. However, the maximum appearance of lectin-activated lytic cells was shown to require 3 days of culture and to coincide with the maximum appearance in the culture of cells with blastoid morphology,[23] whereas overnight culture with PSK was sufficient for the ATK induction, which was not accompanied with appearance of cells with blastoid morphology (data not shown). The binding of PSK to the surface of cytotoxic cells appears to activate or augment their lytic function independently of IFN and IL-2. An alternative possibility is that PSK stimulates the production of another lymphokine in the cytotoxic cells that in turn is responsible for the ATK induction.

The mechanism responsible for the induction of ATK activity by PSK is not clearly understood. Our finding that DNA synthesis of lymphocytes is not necessary for an induction of ATK by PSK suggest that the PSK-induced ATK is not a result of a clonal expansion of lymphocytes with ATK activity. In fact, PSK did not increase the number of lymphocytes binding to autologous tumor cells, but it enhanced the postbinding lethal hit stage. This is further confirmed by our preliminary experiments in which PSK-activated LGL released higher amounts of LGL-CF upon stimulation with autologous tumor cells. On the other hand, the streptococcal preparation OK432 elevated both binding and postbinding steps of ATK,[7] suggesting that PSK and OK432 augment ATK systems through different mechanisms.

Considerable attention has been paid to adoptive cellular immunotherapy with the use of IL-2-activated TIL in cancer patients.[18-21] The therapy is based on the concept that TIL which are sensitized *in vivo* with autologous tumor could be differentiated *in vitro* to mature cytotoxic lymphocytes with ATK by re-stimulation with autologous tumor cells and IL-2 and that those effector cells might be responsible for the clinical benefit observed in the patients. We have reported that both Tac (IL-2R/p55) and non-Tac (possibly IL-2R/p75) IL-2 receptors are involved in IL-2-dependent proliferation of TIL.[21] Human TIL have now been found to be activated with PSK to exhibit ATK activity, and the activation of TIL appears to be independent of the IL-2/IL-2R system. Since PSK-activated TIL exhibited cytotoxicity to autologous tumor cells but not to autologous nonmalignant cells or allogeneic tumor cells, they could be used for adoptive immunotherapy in human cancer patients.

Previous studies indicated that *in vitro* exposure to IFNα failed to activate PBL to kill autologous tumor cells.[32] Other investigators, however, found that treatment with high doses of IFNα or IFNβ of PBL augments lysis of autologous tumor cells.[5, 22] The present study has extended these findings to demonstrate that both IFNα and IFNγ activate $CD3^-$ LGL, but not T lymphocytes, to express augmented ATK activity. By contrast, both subsets were activated by PSK. Thus, the effect of PSK on ATK systems may not be mediated through IFN induction. This is supported by experimental data that neither anti-IFNα mAb nor anti-IFNγ mAb abrogated the PSK effect.

In conclusion, the data presented in this manuscript clearly indicate that PSK activates *in vitro* autotumor reactive lymphocytes, resulting in augmented ATK activity. Since the presence of lymphocytes with ATK activity in human cancer patients may predict long-term survival,[13] our data suggest that the anti-tumor activity of PSK may at least in part be mediated through activation of the ATK system. We are conducting a study to determine whether induction of ATK before surgery by treatment with PSK can improve the clinical outcome in patients who do not naturally have this potential. In preliminary clinical trials some cancer patients responded to preoperative therapy with PSK, showed massive infiltration of TIL capable of lysing autologous tumor cells and marked reduction of tumor cells at the site of tumor growth.

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