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ANTI-TUMOR ACTIVITY OF MURINE PERITONEAL MACROPHAGES INDUCED BY PORCINE SKIN GELATIN

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***Aim*: To study the induction of anti-tumor activity of murine peritoneal macrophages *in vitro* by porcine skin gelatin. *Methods:* Anti-tumor activity of the macrophages was evaluated with tritium thymidine uptake by target tumor cells. ELISA was used to measure amounts of cytokines secreted in culture medium. *Results:* The ability of the gelatin to induce anti-tumor activity of the macrophages was stronger than that of lipopolysaccharide of *E. coli*. Combination of the lipopolysaccharide and interferon-γ synergistically stimulated the macrophages but that of the gelatin and interferon-γ additionally did. The culture supernatant of the macrophages incubated with the gelatin also showed higher anti-tumor activity than that with the lipopolysaccharide though the lipopolysaccharide was more excellent than the gelatin in stimulating secretion of anti-tumor cytokines (IL-1, IL-6, TNF-α, IFN-γ) by the macrophages. Anti-TNF-α antibody partially suppressed the anti-tumor activity of the culture supernatant of the macrophages incubated with the lipopolysaccharide but not with the gelatin. The gelatin induced anti-tumor activity of the macrophages of C3H/HeJ as well as C3H/HeN mice whereas the lipopolysaccharide did only in C3H/HeN mice. The macrophages stimulated *in vitro* by the gelatin exerted anti-tumor activity *in vivo*. Moreover, the gelatin stimulated peritoneal exudates cells *in vivo* when subcutaneously administered with them. *Conclusions:* Porcine skin gelatin induces anti-tumor activity of macrophages in mice and its magnitude is greater than that of lipopolysaccharide of *E. coli*. Its mechanism is different from that of the lipopolysaccharide but not fully clarified.**
***Key Words:* porcine skin gelatin, macrophages, cytotoxicity.**

We have observed that different gelatins exhibit different biological activities. Bovine bone (BB) gelatin stimulated proliferation of murine spleen cells *in vitro* [1]. BB and porcine skin (PS) gelatins stimulated murine peritoneal macrophages (mPMs) to secrete several cyokines, e.g., interleukin (IL)-6, -12, TNF-α and MCP-1 [2]. Alternatively PS gelatin suppressed proliferation of cells of human as well as murine cell lines [1, 3, 4]. In this study, we investigated whether or not PS gelatin induced anti-tumor activity of mPMs. There are many reports about stimulants that induce anti-tumor activity of macrophages. Mantovani [5] described that activated mononuclear phagocytes killed transformed target cells via different mediators, e. g., cytokines (IL-1 [6], TNF [7], IL-6 [8]), reactive intermediates of oxygen [9], or nitrogen [10]. It was documented that neutral serine protease [11] secreted by activated macrophages was involved in the effector function. The present study demonstrated that PS gelatin induced anti-tumor activity of mPMs and that its mechanism was mostly different from all described above.

MATERIALS AND METHODS

Mice. Five to eight week-old female C3H/HeN and C3H/HeJ mice were purchased from Chubu Kagaku Shizai Co. Ltd. (Nagoya, Japan). All the experiments were performed accoding to the approved animal-care protocols of the Ethical Committee of institution.

Cell Lines. A murine hepatic cell carcinoma cell line, MH134, a murine fibrosarcoma cell line, Meth A and a murine T cell lymphoma cell line, BW5147 have been maintained in suspension culture in our laboratory.

Reagents. PS gelatin and Interferon-γ(IFN-γ) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lipopolysaccharide (LPS) derived from *E. coli* 026: B6 was purchased from Difco Laboratories (Detroit, MI, USA). Anti-mouse TNF-α antibody was purchased from upstate biotechnology (Lake Placid, NY 12946, USA).

Peritoneal exudate cells. A mouse was injected with 3 ml of 3% thioglycollate medium (Sigma Chemical Co.) intraperitoneally (i.p.). Three days after, about 8 ml of physiological buffered saline (PBS) was injected into the peritoneal cavity and the PBS containing peritoneal exudate cells (PECs) was aspirated. PECs were washed with modified MEM twice. Modified MEM was composed of 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 25 mM HEPES and Eagle's minimum essential medium(Nissui Pharmaceutical Co. Ltd., Tokyo, Japan).

In vitro anti-tumor activity assay using tritium thymidine. PECs (1×10^5/well) were incubated in wells of a 96-well round-bottomed microtiter plate, each containing 0.1 ml of 10% FCS modified MEM at 37 °C for 90 min. The PECs equally adhered to the round bottoms, which was confirmed microscopically. The adherent PECs were used as mPMs. In the preliminary experiment using a kit, CytoTox 96® Non-radioactive Cytotoxicity Assay (Promega, Woods, Hollow Road

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Abbreviations used: 3H-TdR – tritium thymidine; BB – bovine bone; ET ratio – effector : target ratio; IFN-γ – interferon-gamma; i. p. – intraperitoneally; LPS – lipopolysaccharide; MCP-1 – monocyte chemoattractant protein-1; mPMs – murine peritoneal macrophages; PBS – physiological buffered saline; PECs – peritoneal exudate cells; PS – porcine skin; TLR4 – Toll-like receptor 4.

Madison, WI, USA), the amount of LDH released from 1 x 10^5 of lysed PECs was similar to that of the lysed adherent population of 1 x 10^5 of PECs. It showed that the number of adherent PECs approximated that of PECs. In order to easily wash non-adherent target cells by centrifugation, round-bottomed microtiter plates were used. Non-adherent target cells equally settled on the round bottoms when the plate was not centrifuged. mPMs (approximately 1 x 10^5/well) were incubated with reagents in the wells, each containing 0.2 ml of 5% FCS modified MEM at 37 °C for 24 h. After washing the wells with PBS twice, target cells (5 x 10^3/well) were added to the wells, each containing 0.2 ml of 5% FCS modified MEM followed by an additional 48 h incubation. Tritium thymidine (3H-TdR) was added to all wells (final concentration: 1 μCi/ml) approximately 7 h before the termination of the incubation. Thereafter they were washed with 0.2 ml of PBS twice and mixed with 0.2 ml of 1% sodium lauryl sulfate, and 0.1 ml of the 0.2 ml was mixed with 3 ml of a scintillator (Ready Flow III, Beckman). Scintillation of the mixture was counted with a scintillation counter (Beckman LS3801). When target cells were incubated with effector cells, 3H-TdR uptake by the target cells was calculated by subtracting 3H-TdR uptake by effector cells from that by both effector and target cells.

Culture supernatants of mPMs incubated with a stimulant. mPMs (1 x 10^6/well) were incubated with or without 15 mg/ml of PS gelatin or 500 ng/ml of LPS in wells of a 24-well culture plate, each containing 2 ml of 5% FCS modified MEM at 37 °C for 24 h. After washing the cells, they were incubated in 2 ml of 5% FCS modified MEM at 37 °C for 24 h. Then the supernatants were collected and stored at –20 °C until used for anti-tumor assay or cytokine detection assay. Target cells (5 x 10^3/well) were incubated in wells of a 96-well round-bottomed microtiter plate, each containing 50 μl of 5% FCS modified MEM and the supernatant (150 μl) at 37 °C for 48 h. The manner of pulse by 3H-TdR was described above.

Ultrafiltration. The supernatant was filtered with centrifugal filter devices, models YM-10 at 14 000 g for 30 min (Millipore Corporation, Bedford, MA, USA) and filtrates and retentates were reconstituted with the original medium. The membrane nominal molecular weight limit of YM-10 is 10 000 in Daltons.

Fluorescent immunohistochemistry. The cell samples were fixed with 4% formaldehyde solution in phosphate buffered saline (PBS) for 15 min at room temperature. Then, they were washed three times with PBS, after which incubated with 0.1% Triton X-100 in PBS for 5 min at room temperature. Then they were incubated 10% normal goat serum (NGS) in PBS at room temperature for 30 min to block non-specific binding. Further they were incubated with optimum concentrations of primary antibody diluted with 10% NGS in PBS for 60 min at room temperature. As primary antibody, rabbit polyclonal antibody against asialo GM1 (natural killer cell marker) and rat anti-mouse F4/80 (IgG2b) (macrophage marker) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and AbD Serotec (Oxford, UK), respectively. All antibodies were labeled using Zenon Labeling Kits (Molecular Probes, OR, USA). The cells and polyclonal antibodies were observed using an immunofluorescence microscope (Axio Observer. Z1; Zeiss, Oberkochen, Germany) with a magnification of x100 and a halogen lamp of 100 W. Images were photographed using a digital camera (AioCam MRm, HRc; Zeiss) and AxioVision 4.6 software (Zeiss). The photographs made with 4 different filters were later merged with the AxioVision 4.6 software to get the quadruple-stained pictures.

Statistics. Difference between 2 groups was evaluated using Student's t-test. Difference between 2 groups in tumor sizes was evaluated using Mann-Whitney test because each population of tumor size did not show statistic normal distribution. Difference between 2 groups in survival was evaluated using generalized Wilcoxon test. *p* values of 5% or less were considered statistically significant.

RESULTS

Comparison of PS gelatin with LPS in inducing anti-tumor activities of mPMs. Fig. 1 shows that mPMs incubated with PS gelatin exerted anti-tumor activity at effector : target ratio (ET ratio) 2, which was statistically significant ($p < 0.05$). On the other hand, mPMs intact or incubated with LPS enhanced proliferation of target cells at ET ratio 1~2. Intact mPMs exerted anti-tumor activity at ET ratio 16. mPMs incubated with LPS exerted anti-tumor activity at ET ratio 8. LPS of 500 ng/ml was optimal in stimulating cytokine secretion and 15 mg/ml of PS gelatin also optimal in suppressing tumor cell proliferation (data not shown).

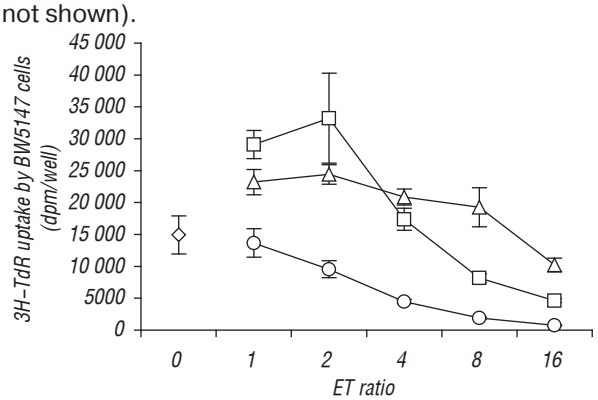

Fig. 1. Comparison between LPS and PS gelatin in induction of anti-tumor activities in mPMs. mPMs were incubated with 500 ng/ml of LPS (□) or 15 mg/ml of PS gelatin (○) or without both (△) in 5% FCS modified MEM at 37 °C for 24 h. Those mPMs were collected with a scraper. Then they or no mPMs (◇) were incubated with BW5147 cells at the indicated ET ratio at 37 °C for additional 24 h

Induction of anti-tumor activity of mPMs by synergistic action of LPS and IFN-γ. Fig. 2 shows that mPMs stimulated with 1 ng/ml of IFN-γ to exert anti-tumor activity, that as low as 0.1 ng/ml of IFN-γ synergistically enhanced anti-tumor activity of mPMs with 500 ng/ml of LPS and that 1 ng/ml of IFN-γ additionally augmented anti-tumor activity of mPMs with 10 mg/ml of PS gelatin.

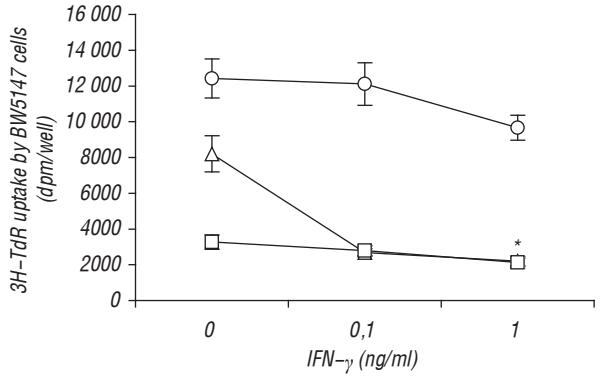

Fig. 2. Synergistic action of LPS and IFN-γ inducing anti-tumor activity in mPMs. mPMs were incubated with the indicated concentration of IFN-γ in the presence of 500 ng/ml of LPS (△), 10 mg/ml of PS gelatin (□) or absence of both (○) at 37 °C for 24 h, and those reagents were washed out. Then they were incubated with BW5147 cells at 37 °C for additional 24 h. *Statistically different from control (□, 0) at $p < 0.05$.

Anti-Tumor Activity of the Culture Supernatant of mPMs Incubated with LPS or PS Gelatin. Fig. 3 shows that both supernatants of mPMs incubated with LPS and PS gelatin suppressed 3H-TdR uptake by MH134 cells and that the activity of the supernatant of mPMs incubated with PS gelatin was higher than that with LPS. The supernatant of mPMs alone enhanced 3H-TdR uptake by MH134 cells.

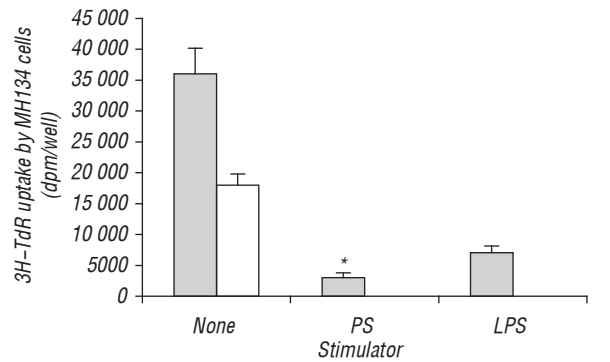

Fig. 3. Anti-tumor activity of culture supernatant of mPMs stimulated by LPS or PS gelatin. mPMs were incubated with or without (None) 15 mg/ml of PS gelatin (PS) or 500 ng/ml of LPS (LPS) in 5% FCS modified MEM at 37 °C for 24 h. After discarding stimulators, they were incubated in the new medium at 37 °C for additional 24 h. The supernatants were collected. MH134 cells were incubated in 150 μl of the supernatant and 50 μl of 5% FCS modified MEM (closed) or in 200 μl of the medium alone (opened) at 37 °C for 48 h. *Statistically different from LPS at $p < 0.01$.

Molecular size of the factor having the anti-tumor activity in the culture supernatant. The filter which does not pass molecules of more than 10 kDa suggested that the molecular weight of the anti-tumor activity was over 10 kDa (Fig. 4).

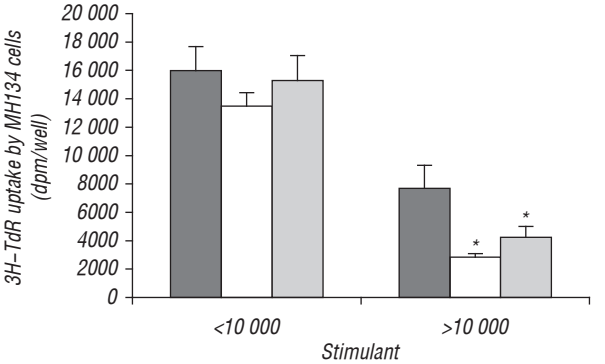

Fig. 4. Molecular size of the factor having the anti-tumor activity in the culture supernatant. The culture supernatant of mPMs with or without (black) 15 g/ml of PS gelatin (white) or 500 ng/ml of LPS (grey) was ultrafiltered with microcon YM-10 (10 kDa). *Statistically different from control (black) at $p < 0.01$.

IL-1β, IL-6, TNF-α and IFN-γ in the culture supernatant of mPMs incubated with LPS or PS gelatin. In the experiment shown in Fig. 5, the anti-tumor activity of the supernatant of mPMs incubated with PS gelatin or LPS was examined. In this experiment, concentrations of anti-tumor cytokines, i. e., IL-1β, IL-6, TNF-α and IFN-γ in those supernatants were measured. Fig. 5 shows that LPS was more excellent than PS gelatin in stimulating mPMs to secrete cytokines (IL-1β, IL-6, TNF-α and IFN-γ).

Effect of anti-TNF-α antibody on the anti-tumor activity of the supernatant. Fig. 6 shows that the anti-tumor activity of the supernatant of mPMs incubated with PS gelatin was not affected by anti-TNF-α antibody, but that with LPS was partially inhibited by the antibody.

Involvement of Toll-like receptor 4 in induction of anti-tumor activity of mPMs by PS gelatin. Macrophages of C3H/HeJ mice lack Toll-like receptor 4 (TLR4). Fig. 7 shows that PS gelatin stimulated mPMs of both C3H/HeN and /HeJ mice to exert anti-tumor activity whereas LPS did mPMs of C3H/HeN mice only.

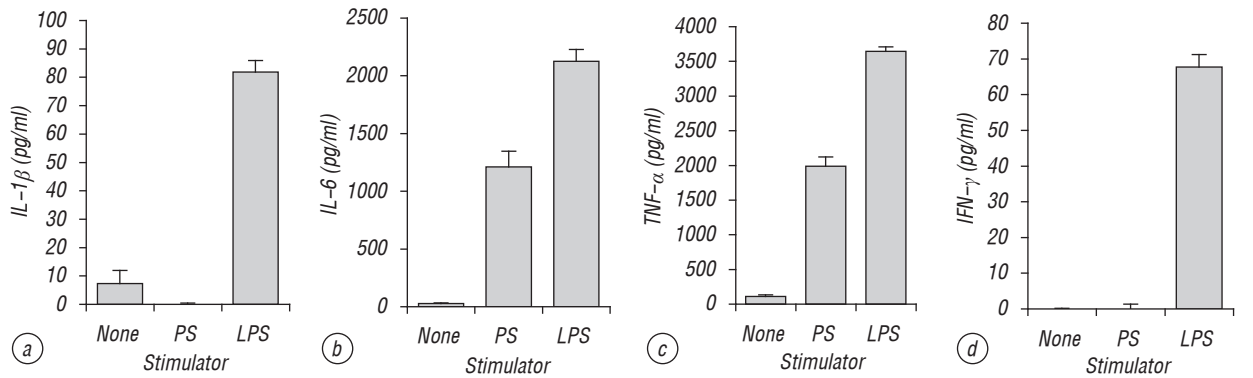

Fig. 5. IL-1β, IL-6, TNF-α and IFN-γ in culture supernatants of mPMs incubated with LPS or PS gelatin. mPMs were incubated with or without (None) 15 mg/ml of PS gelatin (PS) or 500 ng/ml of LPS (LPS) in 5% FCS modified MEM at 37 °C for 24 h. After discarding stimulators, they were incubated in the new medium at 37 °C for additional 24 h. Supernatants were collected and the amounts of IL-1β (*a*), IL-6 (*b*), TNF-α (*c*) and IFN-γ (*d*) were measured

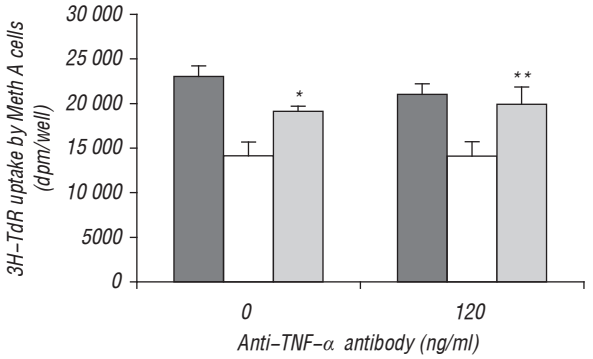

Fig. 6. Effect of anti-TNF-α antibody on the anti-tumor activity of the culture supernatant. The supernatant (150 µl/well) of mPMs incubated with or without (black) 15 mg/ml of PS gelatin (white) or 500 ng/ml of LPS (grey) was incubated with or without 120 ng/ml of anti-mouse TNF-α antibody (25 µl/well) at 37 °C for 1 h, and Meth A cells (5 x 10^3/25 µl/well) were added to the wells followed by an additional 24 h incubation. *Statistically different from control (black) at $p < 0.01$, **not different.

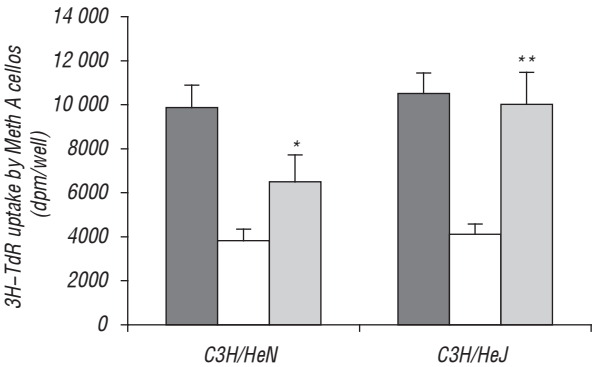

Fig. 7. Involvement of TLR 4 in inducing anti-tumor mPMs by PS gelatin. mPMs of C3H/HeN or /HeJ were incubated with or without (black) 10 mg/ml of PS gelatin (white) or 500 ng/ml of LPS (grey) at 37 °C for 24 h. After removing those reagents, MH134 cells were incubated with the mPMs for additional 24 h. *Statistically different from control (black) at $p < 0.05$, **not different.

Winn's test to prove generation of anti-tumor mPMs by PS gelatin in vitro. When mPMs incubated with PS gelatin in vitro were injected with MH134 cells i.p. or subcutaneously (s. c.), tumor-bearers of each group survived for longer periods than those injected with mPMs incubated in the absence of PS gelatin (Fig. 8, *a*, *c*) and the subcutaneous tumors grew more slowly than those of the control mice (Fig. 8, *b*).

Effects of PECs, PS gelatin and both on tumor growth in vivo. When PECs, PS gelatin or both were injecetd with MH134 cells i. p, the survival of each group did not differ statistically (Fig. 9, *a*). On the other hand, PS gelatin plus PECs or PS gelatin alone suppressed the tumor growth as compared with PECs alone when they were injected with MH134 cells s. c. (Fig. 9, *b*) and moreover PS gelatin plus PECs but not PS gelatin alone had the tumor-bearers survive for a longer period than PECs alone (Fig. 9, *c*).

Identification of Adherent PECs. Fluorescent immunohistochemistry showed that more than 90% of adherent PECs was F4/80-positive and at most 10% of them was asialo GM1-positive (data not shown).

DISCUSSION

PS gelatin was observed to suppress proliferation of tumor cells *in vitro* [2, 3]. However, when PS gelatin administered *in vivo*, it was expected to be easily degraded by various proteases, e. g., lysosomal enzymes in mPMs. Therefore, the effect of mPMs on degradation of PS gelatin was investigated by means of *in vitro* anti-tumor activity of PS gelatin. In this experiment, adherent PECs were regarded as mPMs since more than 90% of them had F4/80 antigen. mPMs were added to the incubation of tumor cells with PS gelatin. If mPMs degrade PS gelatin, the anti-tumor activity must be attenuated. Unexpectedly mPMs

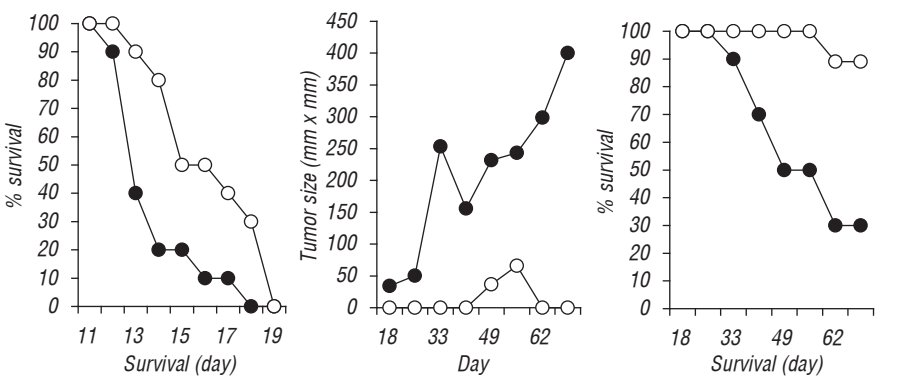

Fig. 8. Winn's test to prove the stimulation of mPMs by PS gelatin *in vitro*. mPMs (4×10^6/dish) were incubated with (○) or without (●) 15 mg/ml of PS gelatin in dishes at 37 °C for 48 h. Then they were collected with a scraper, and mPMs/dish were mixed with 1×10^5 of MH134 cells and the mixture was inoculated i. p. (*a*) or s. c. (*b, c*). Both are statistically different at $p < 0.05$ (*a*), at $p < 0.05$ on day 18, 26, 33, 38 and 64 (*b*) and at $p < 0.01$ (*c*)

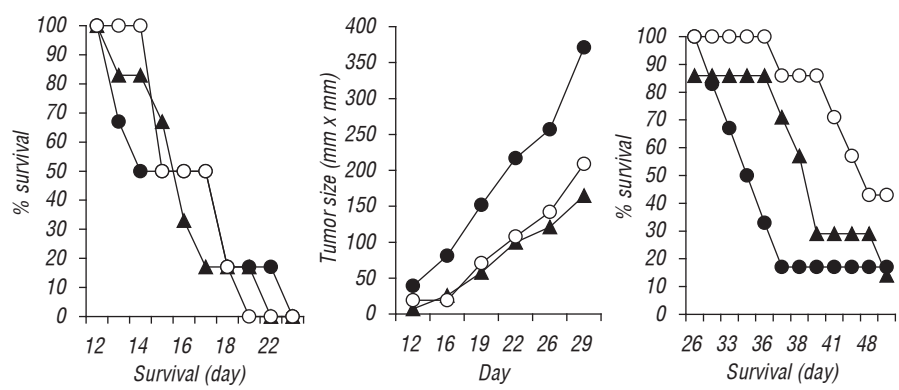

Fig. 9. Effects of PECs, PS gelatin and both on tumor growth *in vivo*. PECs (1×10^7) (●), 25 mg of PS gelatin (▲) or both (○) were inoculated into the peritoneal cavity with 5×10^5 of MH134 cells (*a*). PECs (2×10^6) (●), 1.75 mg of PS gelatin (▲) or both (○) were inoculated s. c. with 1×10^5 of MH134 cells (*b, c*). Both (○, ▲) are not different from control (●) statistically (*a*) and statistically different from control (●) at $p < 0.05$ on day 16, 19, 22 and 26 (*b*). Both (○, ●) are statistically different at $p < 0.05$, but both (▲, ●) are not different statistically (*c*)

augmented the anti-tumor activity of PS gelatin (data not shown), and so it was examined whether or not PS gelatin stimulated mPMs to be cytostatic or cytocidal in the present study. In addition, BB gelatin also became to obtain anti-tumor activity in the same experimental system whereas it did not exert anti-tumor activity by itself (data not shown). This may be related with its activity to stimulate mPMs to secrete various cytokines, which was greater than that of PS gelatin [2].

mPMs incubated with PS gelatin exerted anti-tumor activity *in vitro*. The experiment using double chamber culture indicated that the anti-tumor activity of mPMs incubated with PS gelatin did not require the contact with target cells (data not shown). However, a possibility that the contact of target cells with activated mPMs exerts an additional anti-tumor effect is not deniable. The culture supernatant of mPMs incubated with PS gelatin exerted higher anti-tumor activity than that with LPS (Fig. 3).

LPS stimulated macrophages [2] or cells of a macrophage cell line [12] to secrete a variety of cytokines or an anti-tumor factor other than IL-1β, IL-6 and TNF-α, respectively. Previously we reported that PS gelatin stimulated mPMs to secrete IL-6, IL-12 and TNF-α and other cytokines but its activity was lower than that of BB gelatin and by far lower than that of LPS [2]. Clarke *et al.* [13] described that inhibition of macrophage-mediated killing was associated with decreases in superoxide, nitric oxide and TNF-α production in response to provocative stimulus. In this study, LPS induced larger amounts of cytokines, i. e., IL-1β, IL-6, TNF-α and IFN-γ in mPMs than PS gelatin. However, mPMs stimulated by LPS exerted lower anti-tumor activity than those stimulated by PS gelatin. The anti-tumor activity of the supernatant of mPMs stimulated by LPS but not PS gelatin partly depends on TNF-α. The ultrafiltration study showed that the active molecule did not pass through 10 kDa filter. Therefore superoxide or nitric oxide is suggested not to be the candidate for the anti-tumor activity of the culture supernatants generated by PS gelatin and LPS.

Lorsbach *et al.* [10] reported that synthesis of NO was increased when cells of a mouse macrophage cell line, RAW 264.7 were costimulated with LPS and IFN-γ. This increase paralleled increase in cytotoxicity. In our experiment, the synergism between LPS and IFN-γ also was shown in exerting anti-tumor activity of mPMs. However, the synergism between IFN-γ and PS gelatin was not shown (Fig. 2).

It is well known that LPS stimulates macrophages via TLR-4 [13, 14]. Previously we reported that PS gelatin as well as LPS stimulated mPMs to secrete TNF-α via TLR-4 [2]. In the present study, however, mPMs of C3H/HeJ mice (TLR-4 deficient) incubated with PS gelatin exerted anti-tumor activity in vitro whereas those incubated with LPS did not. This finding suggests that induction of anti-tumor activity in mPMs by PS gelatin depends on another pathway than cytokine signaling pathway via TLR 4.

The anti-tumor activity of mPMs incubated with PS gelatin was exerted in vivo as well as in vitro regardless of inoculation site. Miyasaka *et al.* [15] reported that PECs elicited by i.p. inoculation of 3% thioglycollate medium exerted anti-tumor activity *in vitro* but not *in vivo*. The PECs rather increased the number of lung metastases when injected with tumor cells intravenously. When the mixture of PS gelatin, PECs and tumor cells or that of PS gelatin and tumor cells was inoculated s. c., the tumor growth was suppressed to the same extent in each group as compared with the mixture of PECs and tumor cells as control, and the survival of the former but not the latter was significantly prolonged as compared with the control. These findings suggest that some immunological mechanism in which PECs, especially PECs activated by PS gelatin, participated induced the prolonged survival. When the mixture of PS gelatin, PECs and tumor cells or that of PS gelatin and tumor cells was inoculated i.p., the survival of tumor bearers was not prolonged. These *in vivo* experiments suggest that the contact of PECs with PS gelatin for an appropriate duration *in vivo* is required for their activation and that once they are activated, e.g., incubation *in vitro*, they exert anti-tumor activity also in such a large space as peritoneal cavity. PS gelatin might disappear rapidly from peritoneal cavity when injected i. p., and might remain *in situ* when injected s. c. However, results of this experiment using PECs but not adherent PECs cannot exclude involvement of natural killer cells since PECs are expected to contain more natural killer cells than adherent PECs.

This study using female mice cannot discuss the effect of sex.

In conclusion, PS gelatin is a new macrophage stimulant and its ability to induce anti-tumor macrophages is higher than that of LPS or IFN-γ. The mechanism remains to be clarified. One study is under investigation whether or not PS gelatin administered repeatedly i. p. would prolong the survival of mice bearing MH134 tumor, and another is identifying the anti-tumor molecule in the culture supernatant of mPMs stimulated by PS gelatin.

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ПРОТИВООПУХОЛЕВАЯ АКТИВНОСТЬ МЫШИНЫХ ПЕРИТОНЕАЛЬНЫХ МАКРОФАГОВ, ИНДУЦИРОВАННАЯ ЖЕЛАТИНОМ КОЖИ СВИНЬИ

***Цель*: изучить *in vitro* противоопухолевую активность мышиных перитонеальных макрофагов, индуцированную желатином кожи свиньи. *Методы:* противоопухолевую активность макрофагов оценивали по включению меченного тимидина опухолевыми клетками-мишенями. Уровень цитокинов, секретируемых в культуральную среду, определяли с помощью ELISA. *Результаты:* способность желатина индуцировать противоопухолевую активность макрофагов была сильнее, чем у липополисахарида *E. coli*. Комбинация липополисахарида и интерферона-γ (IFN-γ) синергично стимулировала макрофаги, что показано и для комбинации желатина с IFN-γ. Противоопухолевая активность культурального супернатанта макрофагов, инкубированных с желатином, была выше, чем в случае применения липополисахарида, хотя липополисахарид индуцировал более сильную секрецию противоопухолевых цитокинов (IL-1, IL-6, TNF-α, IFN-γ) макрофагами. Антитела против TNF-α частично угнетали противоопухолевую активность культурального супернатанта макрофагов, инкубированных с липополисахаридом, но не с желатином. Желатин индуцировал противоопухолевую активность макрофагов как C3H/HeJ мышей, так и мышей C3H/HeN, в то время как липополисахарид влиял только на макрофаги C3H/HeN мышей. Макрофаги, стимулированные *in vitro,* показывали противоопухолевую активность *in vivo*. Более того, желатин стимулировал клетки перитонеального экссудата *in vivo* при одновременном подкожном введении. *Выводы:* желатин свиной кожи индуцирует противоопухолевую активность макрофагов у мышей, причем более эффективно, чем липополисахарид *E. coli*. Механизм действия желатина отличается от механизма действия липополисахарида и остается пока невыясненным до конца.**

***Ключевые слова:* желатин свиной кожи, макрофаги, цитотоксичность.**