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Enhanced Macrophage Antitumor Effects of Protein A in Combination with IFN-γ

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In this study we examined the potential for the synergistic augmentation of the antitumor activity of inflammatory mouse peritoneal macrophages by stimulation with protein A combined with IFN-γ. The moderate augmentative effect induced by preincubation with protein A was demonstrated to be concentration-dependent, whereas IFN-γ had a very low activating effect. Following preincubation with both protein A and IFN-γ, a marked enhancement of macrophage activity was noted. In addition, based on the utilization of neutralizing antibody to TNF-α or the inhibition of NO production, TNF-α and NO were proven to be involved as mediators during the activation of tumoricidal macrophages by protein A in combination with IFN-γ. We also demonstrated that supernatants from macrophages treated with protein A plus IFN-γ contained both TNF-α and NO at markedly increased levels. Thus, tumor cell lysis in the combined system was mediated via TNF-α or NO. These results demonstrate the synergistic effects on mouse peritoneal macrophage function of protein A in combination with IFN-γ and suggest that combinations of such agents may serve as the basis for future in vivo immunotherapy.

Key words : Protein A, Interferon-γ, NO, TNF-α, Macrophage, Antitumor activity

INTRODUCTION

Since it was first found that the immune system was involved in the destruction of tumor cells, researchers have tried to find ways of enhancing the functions of effector cells like macrophages. Macrophages can be activated to a tumoricidal state by a variety of agents such as IFN-γ, lipopolysaccharide, or other microbial products (Dullens *et al.*, 1989; Gautam *et al.*, 1989; Paulnock *et al.*, 1990).

Activated macrophages play an important role in host defense against infections and cancer (Drysdale *et al.*, 1988). Protein A, a glycoprotein produced by *Staphylococcus aureus,* is a powerful immunostimulating agent (Catalona *et al.*, 1981; Sakane *et al.*, 1978). Protein A can bind plasma-blocking factors and modulate the reactivity of both cellular and humoral factors in a tumor-bearing host (Steel *et al.*, 1974). In addition, previous studies have shown that protein A stimulated antitumor activity in transplanted animals (Ray *et al.*, 1983; Ray *et al.*, 1984a; Ray *et al.*, 1984b) and in carcinogenic mice (Shukla *et al.*, 1996). This antitumor activity of protein A has been suggested to result from both the increase of macrophage number and the activation of macrophage (Prasad *et al.*, 1987). Good *et al.* (1990) suggested that injection of protein A corrected the immunosuppression caused by retroviral infection and it is well known that IFN-γ is amongst the most studied and characterized substance produced by mammalian cells, which primes phagocytic cells both in vitro and *in vivo* to enhance respiratory burst and microbicidal activity (Brenton *et al.*, 1986). Thus, both protein A and IFN-γ have shown immunomodulatory activities on macrophages as single agents. It might be expected that a combinations of two compounds would be beneficial. Recently, a combination of various biological response modifiers was credited with the marked regression of a murine tumor (Iigo *et al.*, 1986; Iigo *et al.*, 1989). Furthermore, cytokines such as TNF-α, IL-1, and IFN-γ have been shown to modulate macrophage functions (Hori *et al.*, 1987; Verstovsek *et al.*, 1992) by producing synergistic effects with other agents to induce the release of cytotoxic molecules from macrophages (Jiang *et al.*, 1992). With the recent use of cytokines or bacterial fractions as therapy for human diseases, an understanding of both the individual and the combined effects of these agents on macrophage function has become increasingly important. The purpose of the present study was to examine the capacity of protein A alone or in combination with IFN-γ to activate murine

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peritoneal macrophages to a tumoricidal level. The results demonstrate that preincu-bation with protein A alone caused moderate to marked enhancement of tumor cell lysis, whereas far greater augmentation of killing was achieved by a combination of protein A and IFN-γ.

MATERIALS AND METHODS

Mouse and tumor cell

The mouse strain used in this study was CD-1, which was obtained from Charles River Breeding Laboratories (Japan). For *in vitro* use, B16 tumor cell (ATCC, Rockville, MD) was grown in RPMI-1640 (Gibco, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), and streptomycin (100 μg/ml) (RPMI-FBS). The tumor cell line was passaged three times per week.

Chemicals and antibodies

Unless otherwise indicated, all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant murine IFN-γ, recombinant murine TNF-α and polyclonal anti-mouse TNF-α antibody (1×10^6 neutralizing units/ml) were purchased from Genzyme Corporation (Boston, MA). $Na_2{}^{51}CrO_4$ was purchased from Amersham Life Science LTD (England). Culture media and test reagents were assayed for endotoxin contamination by the limulus lysated test (E-Toxate; Sigma) and found to be less than 10 pg/ml.

Isolation of inflammatory peritoneal macrophages

Thioglycolate-elicited peritoneal exudate cells were obtained from 4~6 weeks old CD-1 male mice by the intraperitoneal injection of 1 ml of Brewer thioglycolate broth (4.05 g/100 ml)(Difco Labs, Detroit, MI) and by lavage of the peritoneal cavity with 5 ml of medium 3~4 days later. The cells were washed twice and resuspended in RPMI-FBS. Macrophages were isolated from the peritoneal exudate cells as described by Pyo *et al.* (1994). Briefly, peritoneal exudate cells were seeded at densities of $5\sim6\times10^5$ cells/cm^2 on teflon-coated petri dishes (100×15 mm) and the macrophages were allowed to adhere for 2~3 hours in a 5% CO_2 humidified atmosphere. Teflon-coated petri dishes were prepared by spraying with aerosolized teflon (Fisher Scientific, Pittsburgh, PA) and sterilizing with ultraviolet light for 3 hours. The nonadherent cells were removed by washing the dishes twice with 10 ml of prewarmed RPMI-1640 and the dishes incubated for 10 min at 40°C. The supernatants were then carefully removed and discarded and the plates were washed once with prewarmed Dulbecco's phosphate buffered saline (PBS) (Gibco, Grand Island, NY). Cold PBS (15 ml) containing 1.5% FBS (PBS-FBS) was added, followed by 0.3 ml of 0.1 M EDTA (pH 7.0). The plates were incubated for 15 min at room temperature and the macrophages were removed by rinsing 10 times using a 10 ml syringe. The detached cells were washed once with PBS-FBS and resuspended in RPMI-FBS. The viability of the detached cells was assessed by trypan blue exclusion and the proportion of macrophages was determined after cytoplasmic staining with acridine orange, followed by examination using fluorescence microscopy. Cell preparations were >95% viable and contained >95% macrophages.

TNF bioassay

TNF levels were determined in a cytotoxicity assay (Flick *et al.*, 1984) using TNF-sensitive L929 fibroblast (ATCC, Rockville, MD). 100 μl of L929 cells (4×10^5 cells/ml) in RPMI 1640 containing 5% FBS were added to 96 well mcrotiter plates (Nunc, Denmark). The plates were incubated overnight at 37°C in 5% CO_2 humidified incubator. After removal of the medium from each well, 50 μl of supplemented EMEM, 50 μl of sample and 50 μl of actinomycin D (2 μg/ml) were added to every well. After an 18-hour incubation in a humidified CO_2 incubator, the supernatants were discarded and the cells were stained for 10 min with 50 μl of 0.05% crystal violet in 20% ethanol. 100 μl of 100% methanol was added to each well to elute the stain from the cells. The optical density of each well at 595 nm was determined using a Molecular Device microplate reader (Menlo, CA). Murine recombinant TNF-α was used as a positive control to standardize the assay.

Nitrite determination

Macrophage cultures were treated with protein A, IFN-γ or the combination of the two, at with various concentrations and the accumulation of Nitrite in the culture supernatants was measured using the assay system described by Ding *et al.* (1988). Briefly, 100 μl of supernatant was removed from each well into an empty 96 well microtiter plate. After the addition of 100 μl Griess reagent to each well, absorbance at 550 nm was measured using a Molecular Device microplate reader. Nitrite concentration was calculated from a $NaNO_2$ standard curve. The levels of nitrite are indicative of NO production. Griess reagent was prepared by mixing 1 part of 0.1% naphthylethylenediamine dihydrochloride in distilled water with 1 part of 1% sulfanilamide in 5% phosphoric acid.

Macrophage-mediated cytotoxicity

The assay for macrophage cytotoxicity is based on an assay described Hori *et al.* (1987). Briefly, Macrophages (1×10^5 cells/well) were plated into 96-well microtiter

plates and incubated with protein A, IFN-γ or the combination of the two at various concentrations for 18 hours at 37°C in a 5% CO_2 incubator. In some experiments, cytokine antibody or metabolic pathway inhibitor were added with the activating agents. After two washings with warm media, ^{51}Cr-labeled tumor target cells were added (1×10^4 cells/well : an initial effector : target cell ratio of 10 : 1), to the macrophages for an additional 18 hours incubation. The plates were then centrifuged at 300×g for 5 min and the aliquots (0.1 ml) of the cell-free supernatant solutions were harvested for counting in a Packard Cobra series gamma counter (Meriden, CT).

Evaluation of ^{51}Cr release

The percentage of specific ^{51}Cr release was calculated as

$$\%\ \text{of specific}\ ^{51}\text{Cr release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Experimental release is the radioactivity released in wells containing activated effector cells and target cells; spontaneous release is the radioactivity released from target cells incubated in the medium alone; maximum release is the radioactivity from target cells lysed with 1% (v/v) Triton X-100. All experiments were repeated at least three times.

Statistical analysis

Statistical differences between groups were determined by the Student's t-test.

RESULTS

In initial studies, tumor cell lysis by freshly isolated inflammatory macrophages was assessed after preincubation with various concentrations of protein A. At the concentration used, protein A did not exert any significant effect on the cells (data not shown). As shown in Table I, protein A alone can activate macrophages sufficiently to kill tumor cells. The relationship was concentration-dependent. The augmentative effects of IFN-γ on macrophage function seem to be modest. Although the data shown represent an initial effector : target cell ratio of 10 : 1, qualitatively similar results with more specific ^{51}Cr release were obtained at higher effector : target cell ratios (data not shown).

It has been known that IFN-γ can accelerate the biological activities of other cytokines or bacterial products and is thought likely to act by immunomodulation rather than by direct action on tumor cells (Trinchieri *et al.*, 1986). To test for possible additive or synergistic effects, macrophage function after preincubation with the combination of protein A and IFN-γ was examined. The incubation of macrophages with protein A at a concentration of 100 μg/ml and IFN-γ at 10 U/ml produced significant levels of tumor cell lysis. Thus, the combinations of IFN-γ with protein A at different concentrations were shown to be synergistic in terms of inducing the activation of peritoneal macrophages (Table I).

Table I. Enhancement of macrophage function By IFN-γ in combination with protein A

Addition during preincubation	Percentage specific lysis
	10:1 (Effector:Target)
Nil	2.3±0.8
Protein A (1 μg/ml)	11.7±2.5†
Protein A (10 μg/ml)	17.6±3.1†
Protein A (50 μg/ml)	36.6±2.0†
Protein A (100 μg/ml)	40.7±1.7†
IFN-γ (10 U/ml)	6.3±1.3†
IFN-γ+Protein A (1 g/ml)	16.7±1.9†‡
IFN-γ+Protein A (10 g/ml)	29.4±2.2†‡
IFN-γ+Protein A (50 g/ml)	67.9±5.8†‡
IFN-γ+Protein A (100 g/ml)	73.2±3.8†‡

Macrophages were incubated for 18 hours with medium alone or IFN-γ, protein A alone or in combination. Macrophages were co-cultured for 18 hours with radiolabeled B16 target cells at an initial effector:target ratio of 10:1. The results are mean±S.D. of quintuplicates from a representative experiment.
†: Significantly different from control (no treatment); $P<0.001$.
‡: Significantly different from the group treated with protein A or IFN-γ alone; $P<0.001$.

To confirm that macrophage activation to a tumoricidal state by protein A, with or without IFN-γ, was not due to lipopolysaccharide contamination of the activating agent solutions, an experiment using polymyxin B was performed. The addition of polymyxin B (10 μg/ml) to activation cultures had no effect on the tumoricidal activity of macrophage induced by protein A alone or by protein A in combination with IFN-γ, indicating that activation was not due to lipopolysaccharide contamination (data not shown). The amount of polymyxin B used had been shown to be effective at neutralizing lipopolysaccharide (Verstovsek *et al.*, 1993).

A number of compounds have been implicated in macrophage cytotoxicity including TNF and NO, which are currently believed to be the primary species involved killing cells (Decker *et al.*, 1987; Hibbs *et al.*, 1987a). To determine whether these compounds were involved in the IFN-γ/protein A-mediated cytolysis, we attempted to halt cytolysis by neutralizing their activity or by inhibiting their production. At the concentrations employed neither antibody nor inhibitor exerted any gross adverse effects on the cells. The concentrations used in this study have been shown to both neutralize TNF-α and inhibit the production of NO (Pyo *et al.*, 1993). The inclusion of anti-TNF-α antiserum in the

Table II. Inhibition of tumoricidal activity of protein A, IFN-γ or protein A+IFN-γ-activated macrophages by antibody or inhibitor

Addition during preincubation	Percentage specific lysis 10:1 (Effector:Target)
Nil	4.3±0.9
Protein A (100 μg/ml)	37.4±2.4
Protein A+anti-TNF-α (500 U/ml)	26.6±1.9
Protein A+NMMA[a] (0.5 mM)	39.9±4.6
IFN-γ(10 U/ml)	8.2±2.7
IFN-γ+anti-TNF-α(500 U/ml)	8.0±1.6
IFN-γ+NMMA (0.5 mM)	6.4±0.8
IFN-γ (10 U/ml)+Protein A(100 g/ml)	62.7±5.2[†]
IFN-γ+Protein A+anti-TNF-α (500 U/ml)	40.1±3.1[‡]
IFN-γ+Protein A+NMMA (0.5 mM)	35.9±4.4[§]

Macrophages were cultured for 18 hours in 96-well plates with the indicated activating agents in the presence or absence of antibody or inhibitor. Macrophages were co-cultured for 18 hours with radiolabeled B16 target cells at an initial effector: target ratio of 10:1. The results are mean±S.D. of quintuplicates from a representative experiment.
a: NG-monomethyl-L-arginin
[†]: Significantly different from control (no treatment); P<0.001.
[‡]: Significantly different from protein A-treated; P<0.005.
[§]: Significantly different from IFN-γ+protein A-treated; P<0.005.

medium during cytotoxicity assays partially inhibited both protein A-mediated and IFN-γ/protein A-mediated killing of tumor cells. Alternatively, the inclusion of NMMA, an analog of L-arginine known to inhibit the production of NO, a in medium during cytotoxicity assays halted, in part, IFN-γ/protein A-mediated cytolysis of tumor cells. However, it did not affect the protein A mediated system (Table II).

We further investigated the expression of effector molecules elicited by macrophages in response to IFN-γ/protein A. In these experiments, after macrophages were cultured in the presence of activating agents, TNF-α and nitrite concentrations were measured in the culture supernatants. Table III shows that protein A stimulated TNF-α production by macrophages, in a concentration-dependent fashion. However, macrophages subjected to activation with IFN-γ/protein A exhibited a greater increase in TNF-α release. It can be seen in Table IV that nitrite concentrations were also significantly increased during the combined treatment. Thus, the combination of IFN-γ and protein A induces dose-dependently a release of TNF-α or NO. Moreover, anti-TNF-α antiserum or NMMA was able to inhibit, in part, TNF-α or nitrite production, respectively (Table III and IV). Interestingly, protein A had no effect on levels of nitrite produced by macrophages. Taken together, these results suggest that TNF-α and NO are involved in the antitumor activity of IFN-γ/protein A-stimulated macrophages.

Table III. Release of TNF-γ by macrophages induced by protein A, IFN-γ alone or combination as determined by L929 fibroblast bioassay

Treatment	TNF-α (pg/ml)[a]
Nil	0.1
Protein A 1 μg/ml	0.2
10 μg/ml	18±3
50 μg/ml	122±21
100 μg/ml	582±69
IFN-γ (10 U/ml)	22±7
IFN-γ+Protein A 1 μg/ml	25±10
IFN-γ+Protein A 10 μg/ml	40±5
IFN-γ+Protein A 50 μg/ml	233±18
IFN-γ+Protein A 100 μg/ml	1377±143
IFN-γ (10 U/ml)+Protein A (100 μg/ml) +anti-TNF-α (500 U/ml)	454±23[†]

[a]Macrophages were incubated for 18 hours with protein A, IFN-γ alone or protein A plus IFN-γ. TNF-α activity was assayed by techniques described in Materials and Methods. Results are the means±SD of 3 experiments.
[†]: Significantly different from IFN-γ+protein A-treated; P<0.005.

Table IV. Effect of protein A, IFN-γ alone or combination on the production of NO_2^-

Treatment	NO_2^- production (M)[a]
Nil	1.7±0.7
Protein A 1 μg/ml	1.8±0.3
10 μg/ml	1.7±0.5
50 μg/ml	2.1±0.2
100 μg/ml	1.9±0.3
IFN-γ (10 U/ml)	3.3±1.2
IFN-γ+Protein A 1 μg/ml	28.3±0.9
IFN-γ+Protein A 10 μg/ml	35.9±4.2
IFN-γ+Protein A 50 μg/ml	40.1±2.8
IFN-γ+Protein A 100 μg/ml	50.3±5.5
IFN-γ (10 U/ml)+Protein A (100 μg/ml) +NMMA (0.5 mM)	21.2±3.5[†]

[a]Macrophages were incubated for 18 hours with protein A, IFN-γ alone or protein A plus IFN-γ. NO_2^- production was measured by techniques described in Materials and Methods. Results are the means±SD of 3 experiments.
[†]: Significantly different from IFN-γ+protein A-treated; P<0.005.

DISCUSSION

Macrophages can be activated by a variety of stimuli, to kill tumor cells. A number of reports indicate that protein A can direct natural killer cells to act against tumor cells and increase both the number of macrophages and macrophage phagocytosis (Ray and Bandyopadhyay, 1983; Ray et al., 1984a; Ray et al., 1984b; Singh et al., 1987). In addition, Prasad et al. (1987) have showed that tumor regression by protein A treatment might be related to increased macrophage activity. Macrophages

may also be activated for tumor cytotoxicity by IFN-γ in combination with microbial and nonspecific agents (Herriott *et al.*, 1987). In the study presented herein, the effects of protein A in combination with IFN-γ on the generation of macrophage tumoricidal activity were measured using inflammatory macrophages from the peritoneal cavity of CD-1 mice. It was found that purified peritoneal macrophage could be activated to a tumoricidal state by protein A or by combinations of protein A and IFN-γ. Furthermore, by using TNF-α antibody or a metabolic pathway inhibitor, it was shown that TNF-α and NO are involved in the increased macrophage tumoricidal activity induced by protein A plus IFN-γ.

MHC compatibility between effector macrophages and target cells may be important in the recognition process leading to macrophage cytotoxicity (Jiang *et al.* 1992). Our results show that protein A / IFN-γ-activated macrophages from allogeneic CD-1 mice may be cytotoxic for B16 (Table I) or P815 target cells (data not shown), suggesting that macrophage-mediated cytotoxicity might be MHC unrestricted and not directly dependent on the recognition of cell-associated tumor-specific antigen, expressed by the tumor cells. However, we do not eliminate the possibility that tumor-specific transplantation Ag expressed on tumor cells, could synergize with protein A / IFN-γ-activated macrophages to maximize the activation for tumor cell killing.

Macrophage-elicited cytotoxicity is mediated partly via the secretion of a number of soluble mediators including cytokines, NO, and oxygen radicals (Decker *et al.*, 1987; Hibbs *et al.*, 1987; Carlsen and Prydz, 1988). We could show that protein A / IFN-γ strongly induced the release of TNF-α or NO in a dose-dependent manner. Protein A was also able to induce the release of TNF-α but not the generation of NO. In addition, protein A-induced cytotoxicity was partially blocked by anti-TNF-α serum, but not by NMMA, suggesting that TNF-α may play an important role in the tumoricidal activity of macrophages induced by protein A. If the protein A effect is specific to TNF-α production, it is reasonable to speculate that molecules like receptors, which mediate the protein A effect, may exist on macrophage surfaces and are important in the production of TNF-α. Signaling through surface molecules would induce functional changes that may contribute to the induction of TNF-α gene expression.

Protein A / IFN-γ induced markedly higher release of TNF-α and NO, compared with protein A alone, which would be necessary for killing tumor cells. Since a lymphokine such as IFN-γ primes macrophages to respond to a second signal that triggers the macrophages to lyse tumor cells (Uhing *et al.*, 1989; Hart *et al.*, 1992), it is possible that IFN-γ in synergism with protein A may provide a secondary signal to macrophages to increased NO production. These findings additionally support our conclusion that TNF-α and NO are involved in the antitumor activity of IFN-γ / protein A-stimulated macrophages. However, we can not eliminate the possibility that other toxic molecules released by activated macrophages are involved in IFN-γ / protein A-treated macrophages. Recently, Mishra *et al.* (1992) have suggested that respiratory burst of macrophages was triggered by protein A. Furthermore, an important aspect of macrophage-mediated tumor cell killing is the binding of effector macrophages to tumor cells through Gal/GalNAc macrophage lectin (Oda *et al.* 1989).

In summary, the data presented in this report demonstrates that the treatment with IFN-γ / protein A results in a synergistic augmentation of macrophage function and that the combination of protein A with IFN-γ is a potent stimulator of both the synthesis and the release of cytotoxic mediators. These results suggest that the combination of protein A with IFN-γ might achieve *in vivo* enhance-ment of macrophage function. However, *in vivo* use may be limited by the side effects caused by the systemic administration of this combination. Further studies with animal models are necessary to provide a preclinical basis for clinical applications and the usefulness of IFN-γ / protein A against malignancy.

REFERENCES CITED

Brenton, G., Zeni, L., Cassatella, M. A. and Rossi, F., Gamma interferon is able to enhance the oxidative metabolism of human neutrophils. *Biochem. Biophys. Res. Commun.*, 138, 1276-1282 (1986).

Carlsen, E. and Prydz, H., Activation of monocytes more than one process. *Scand. J., Immunol.*, 27, 401-404 (1988).

Catalona, W. J., Ratliff, T. L and Mccool, R. E., Interferon induced by S. aureus protein A augments natural killing and ADCC. *Nature*, 291, 77-79 (1981).

Decker, T., Lohmann-Mathes, M. L. and Gifford, G., Cell associated tumor necrosis factor (TNF) as a killing mechanism of activated cytotoxic macrophages. *J. Immunol.*, 138, 957-962 (1987).

Ding, A. H., Nathan, C. F. and Stuehr, D. J., Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. *J. Immunol.*, 141, 2407-2412 (1988).

Drysdale, B. E., Agarwal, S and Shin, H. S., Macrophage-mediated tumoricidal activity: mechanisms of activation and cytotoxicity. *Prog. Allergy*, 40, 111-161 (1988).

Dullens H. F. J., De Weger., R. A., Van Der Maas, M., Den Besten, P. J., Vandebriel, R. J. and Den Otter, W., Production of specific macrophage-arming factor precedes cytotoxic T lymphocyte activity *in vivo* during tumor rejection. *Cancer Immunol. Immunother.*, 30, 28-33 (1989).

Flick, D. A. and Gifford, G. E., Comparison of *in vitro* cell cytotoxicity assays for tumor necrosis factor. *J. Immunol.,* 68, 167-175 (1984).

Gautam, S. and Deodhar, S., Generation of tumoricidal effector cells by human C-reactive protein and muramyl tripeptide: a comparative study. *J. Biol. Response Modif.,* 8, 560-569 (1989).

Good, R. A., Ocasawara., M., Liu, W. T., Lorenz, E. and Day, N. K., Immunosuppressive actions of retroviruses. *Lymphology,* 23, 56-59 (1990).

Hart, P. G., Whity, G. A., Picoli, D. S. and Hamilton, T. A., Synergistic activation of human monocyte by granulocyte macrophage colony stimulating factor (GM-CSF) and interferon gamma (IFN-γ): increased tumor necrosis factor but not interleukin-1 activity. *J. Immunol.,* 141, 1516-1521 (1992).

Herriott, M. J. and Leu, R. W., Activation of mouse macrophages for migration inhibition and for tumor cytotoxicity is mediated by interferon-priming and triggering by various agents. *J. Interferon Res,.* 7, 165-172 (1987).

Hibbs, J. B., Tanitor, R. R. and Vavrin, Z., Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science,* 235, 473-476 (1987).

Hori, K., Ehrke, M. J. and Mihich, E., Effect of recombinant tumor necrosis factor on tumoricidal activation of murine macrophages: synergism between tumor necrosis factor and interferon gamma. *Cancer Res.,* 47, 5068-5073 (1987).

Iigo, M., Nakajima, Y., Nishikata, K. and Hoshi, A., Tumor target derived soluble factors synergize with IFN-γ and IL-2 to activate macrophages for tumor necrosis factor and nitric oxide production to mediate cytotoxicity of the same target. *J. Biol. Response Modif,.* 8, 147-154 (1989).

Iigo, M., Sakurai, M., Tamura, T., Saijo, N. and Hoshi, A., Synergistic inhibition of the growth of adenocarcinoma 755 by the combination of interleukin-2 and interferon-beta. *Proc. JPN. Acad. Ser B,.* 62, 275-278 (1986).

Jiang, H., Stewart, C. A., Fast, D. J. and Leu, R. W., Tumor target derived soluble fators synergize with IFN-γ and IL-2 to activate macrophages for tumor necrosis factor and nitric oxide production to mediate cytotoxicity of the same target. *J. Immunol.,* 149, 2137-2146 (1992).

Mishra, A., Dwivedi., P. D., Verma, A. S. and Ray, P. K., Mechanism of enhanced phagocytic response in protein A treated rat macrophages. *Immunol. Lett.,* 34, 289-295 (1992).

Morrison, C. J., Brummer, E. and Stevens, D. A., Effect of recombinant tumor necrosis factor on tumoricidal activation of murine macrophages:synergism between tumor necrosis factor and interferon gamma. *Infect. Immunol.,* 57, 2953-2958 (1989).

Oda, S., Sato, M., Toyoshima, S. and Osawa, T., Binding of activated macrophages to tumor cells through a macrophage lectin and its role in macrophage tumouricidal activity. *J. Biochem.,* 105, 1040-1043 (1989).

Paulnock, D. M. and Lambert, L. E., Identification and characterization of monoclonal antibodies specific for macrophages at intermediate stages in the tumoricidal activation pathway. *J. Immunol.,* 144, 765-773 (1990).

Prasad, A. K., Singh, K. P., Saxena, A. K., Mathur, N. K. and Ray, P. K., Increased macrophage activity in protein A treated tumor regressed animals. *Immunopharmac. Immunotoxicol.,* 9, 541-561 (1987).

Pyo, S., Gangemi, J. D., Ghaffar, A. and Mayer, E. P., PolyI:C-induced antiviral and cytotoxic activities are mediated by different mechanisms. *Int. J. Immunopharmac.,* 15, 477-486 (1993).

Pyo, S., The mechanism of poly I:C-induced antiviral activity in peritoneal macrophage. *Arch. Pharm. Res.,* 17, 93-99 (1994).

Ray, P. K. and Bandyopadhyay, S., Inhibition of rat mammary tumor growth by purified protein A-a potential anti-tumor agent. *Immunol. Commun.,* 12, 453-464 (1983).

Ray, P. K., Bandyopadhyay. S., Dohadwala, M., Canchanapan, P. and Mobin, J., Antitumor activity with nontoxic doses of protein A. *Cancer Immunol. Immunother.,* 18, 29-34 (1984a).

Ray, P. K., Mohammed, J., Allen, P., Raychaudhurl, P., Dohadwala, M., Bandyopadhyay, S. and Mark, R., Effect of frequency of plasma adsorption over purified protein A containing *Staphylococcus aureus* on regression of rat mammary adenocarcinomas: Modification of antitumor immune response and tumor histopathology. *J. Biol. Resp. Modif.,* 3, 39-59 (1984b).

Sakane, T. and Green, I., Protein A from *Staphylococcus aureus*-a mitogen for human T lymphocytes and B lymphocytes but not L lymphocytes. *J. Immunol.,* 120, 302-311 (1978).

Singh, K. P., Saxena, A. K., Prasad, A. K., Dwivedi, P. D., Zaidi, S. I. A. and Ray, P. K., Effect of protein A on mast cell numbers and macrophage phagocytic activity. *Immunopharmac. Immunotoxicol.,* 9, 281-297 (1987).

Shukla, Y., Verma, A. S., Mehrotra, N. K. and Ray, P. K., Antitumour activity of protein A in a mouse skin model of two-stage carcinogenesis. *Cancer Lett.,* 103, 41-47 (1996).

Steele, G., Ankerest, J. and Sjogren, J. O., Alteration in *in vitro* antitumor activity of tumor-bearer sera by absorption with *Staphylococcus aureus* Cowan I. *Int. J. Cancer,* 14, 83-92 (1974).

Trinchieri, G., Kobayashi, M., Rosen, M., Loudon, R., Murphy, M. and Perussia, B., Tumor necrosis factor and lymphotoxin induced differentiation of human myeloid cell lines in synergy with immune interferon.

J. Exp. Med. 164, 1206-1211 (1986).

Uhing, R. J. and Adams, D. O., Molecular events in the activation of murine macrophages. *Agents and Actions,* 26, 9-14 (1989).

Verstovsek, S., Maccubbin, D. L., Ehrke, M. J. and Mihich. E.. Tumoricidal activation of murine resident peritoneal macrophages by interleukin-2 and tumor necrosis factor. *Cancer Res.*, 52, 3880-3885 (1992).

Verstovsek, S., Maccubbin, D. L., Ehrke, M. J. and Mihich., E., Polymyxin B-mediated lysis of tumor cells. *Int. Arch. Allergy Immunol.*, 100, 47-53 (1993).