

EFFECTS OF CELL WALL EXTRACTS OF GRAM POSITIVE BACTERIA (MPGC) ON HUMAN IMMUNITY AND TUMOR GROWTH IN ANIMALS

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ABSTRACT

Muramyl polysaccharide glycan complex (MPGC) was tested for its immunostimulatory effects on human mononuclear cells and lymphocytes and for its anti-tumor effects in the S-180 mouse sarcoma model. MPGC is a non-toxic purified extract of the bacterial cell walls of Gram positive bacteria. *In vitro* MPGC (0.1 mg/mL) stimulated the production of Interleukins 1, 6, and 12, and stimulated human lymphocyte proliferation. A mixture of cytokines produced by MPGC (0.1 mg/mL)-stimulated human monocytes resulted in the maturation of immature human dendritic cells as evidenced by flow cytometric quantitation of CD83. Tumors were established in Kun Ming mice (3-4 weeks old, 19-21 grams each, mixed male/female, 10 animals per group) after subcutaneous injection of S-180 sarcoma cells in the flank. Intraperitoneal MPGC (250 mcg/dose, daily for 14 days, first injection 2 days after tumor establishment) resulted in 75% inhibition of tumor growth. Using the same model and conditions, intravenous MPGC (250 mcg/dose, daily for 14 days, first injection 2 days after tumor establishment) resulted in 77% inhibition of tumor growth compared to controls. We conclude that MPGC has immunostimulatory and anti-tumor qualities and should be studied further as an immunotherapeutic agent for cancer.

BACKGROUND

Bacterial and fungal cell wall extracts have been used as immune stimulants and anti-tumor agents. Examples are Bacillus Calmette-Guerin (BCG),¹ Polysaccharide K,² beta 1,3, glucan,³⁻⁶ the Maruyama vaccine,⁷ and extracts of *Bifidobacterium*, *L. lactis*, *L. fermentum*, *L. acidophilus*, *S. lactis*.⁸

Muramic acid is a component of bacterial cell walls with immunostimulatory qualities that may be partially responsible for the anti-tumor effects of Gram positive bacterial extracts.⁹ Muramyl peptides (comprised of two muramic acids bound together) sensitize macrophages to phosphatidylserine and muramic acid, both of which are found preferentially on tumor cells. Muramyl peptides up-regulate monocyte cytokine genes (IL-1 beta, IL-6, IL-8, IL-12, macrophage chemotactic and activating factor, and tumor necrosis factor alpha but not IL-2 or IL-10) and activate monocyte-mediated tumoricidal activity.¹⁰ Muramyl peptides increase the ability of macrophages to recognize virally infected cells, including cells infected with oncogenic viruses.¹¹ Muramyl peptides and muramic acid are not selectively internalized by monocytes, and therefore have been associated with toxicity. Monocytes/macrophages have mannose receptors that allow them to readily internalize polysaccharides that contain mannose.

Muramyl polysaccharide-glycan complex (MPGC), is a non-toxic bacterial cell wall extract of *Lactobacillus fermentum* that contains muramic acid moieties attached to variable-length mannose-rich polysaccharides. The mannose-rich polysaccharides promote internalization of the entire muramic acid-containing complex.

In this study MPGC was tested for immunostimulatory activity and anti-tumor activity in a mouse fibrosarcoma model.

MATERIALS AND METHODS

PRODUCTION OF EXTRACT

Lactobacillus fermentum was grown at 37°C in 1500-ml Lactobacillus MRS Broth (Fisher Scientific) for 24 hours. The broth was then centrifuged at 10,000xg for 30 min. The pellet was washed with 1000 mL of 0.15 NaCl, resuspended in 400 mL deionized water (2 mL/gram of pellet) and 8 mL glacial acetic acid, final pH 2.0 (2% acetic acid concentration). This mixture was heated to 100°C for 2 hours with continuous stirring. Additional water was added to maintain a volume of 400 mL. The mixture was cooled and centrifuged at 10,000xg for 20 minutes. The supernatant was saved and the pH adjusted to 7.0 using NaOH. The pellet was retained and extracted again as above. The resulting supernatant was pooled with the first. The pooled supernatant was ultrafiltered using a 3-KD membrane equipped stirred cell (Amicon/Millipore Corp) until the volume was reduced to 20-ml. The filter retentate was retained and mixed with 20-ml chloroform to remove lipids. This was centrifuged at 5000xg for 10 minutes, saving the upper layer. The upper portion was then heated to 100°C with nitrogen purging to remove traces of chloroform. To the resulting solution 2.3-ml trichloroacetic acid (TCA) was added and incubated at 4°C overnight. The solution was centrifuged at 25,000xg for 15 minutes. The supernatant was retained and pH adjusted to 7.0 using NaOH. The resulting solution was ultrafiltered using a 3KD equipped Amicon Stir Cell until volume and was reduced to 0.5-ml. To the solution 100-ml deionized water was added and ultrafiltration continued until the volume was reduced to 5-ml. The resulting retentate was collected and lyophilized to produce the dry extract herein referred to as MPGC.

MOUSE SARCOMA MODEL

INTRAPERITONEAL

Mixed gender Kun Ming mice 3-4 wk of age, weighing 20-22 grams were bred and housed at Beijing Hepatitis Institute, Beijing, China. 0.2 mL of phosphate buffered saline (PBS) solution containing 8×10^5 S-180 murine sarcoma cells were injected subcutaneously in the left groin of each animal. The mice were randomly assigned to groups of 10. After 24 hours, either 0.1 mL of normal saline as a control, or 0.1 mL containing 250 ug of MPGC dissolved in normal saline was injected intraperitoneally into each animal. The animals received treatment daily for 14 days, at which point the experiment was terminated. On days 9 and 14 two diameters of the subcutaneous tumors were measured by calipers. On day 14, the animals were sacrificed and tumors were resected and weighed.

INTRAVENOUS

The study was conducted as above except for route of administration.

LYMPHOCYTE PROLIFERATION

Human lymphocytes were collected by density gradient centrifugation from whole blood collected in EDTA anticoagulant tubes. The number of lymphocytes was determined and the cells were suspended in AIM-V culture medium with Interleukin-2 and 2-mercaptoethanol (2-ME). The cells were then equally divided into three sets and transferred to tissue culture flasks. To one third of the flasks, 32 micrograms/ml of MPGC was added. To one third of the flasks 160 micrograms/ml of MPGC was added. Nothing was added to the final third of the flasks which

served as controls. After a 3 day incubation at 37° in 95% air, 5% CO₂ the number of lymphocytes were compared using a Coulter Epics flow cytometer.

CTYOKINE PRODUCTION

Human mononuclear cells were collected by density gradient centrifugation from whole blood collected in EDTA anticoagulant tubes and resuspended in RPMI growth medium. The monocytes were allowed to attach to tissue culture flasks (25 cm² Falcon) for 1 hour after which time the RPMI containing contaminating platelets and lymphocytes was remove. RPMI growth medium supplemented with 10% fetal calf serum was added to each flask. To one third of the flasks, 0.1 mg/ml of MPGC was added. To one third of the flasks 1.0 mg/ml of MPGC was added. Nothing was added to the final third of the flasks which served as controls. After a 2 day incubation at 37° in 95% air, 5% CO₂, the growth media from the flasks were removed and analyzed for concentrations of interleukins-6, and -12 using ELISA assays.

RESULTS

MOUSE SARCOMA MODEL

A summary of the inhibitory effects of MPGC on sarcoma tumor growth in mice is in table 1.

Table 1
IP=Intraperitoneal injection;
IV=Intravenous injection

S-180 Mouse Tumor Model		
Tx (250ug/dose)	Tumor Weight (grams)	Tumor Growth Inhibition
Control	2.40	0%
MPGC IP	0.60	75% p<.001
MPGC IV	0.54	77% p<.001

LYMPHOCYTE PROLIFERATION

There was a dose dependent increase in lymphocyte proliferation induced by MPGC. The results are summarized in Figure 1.

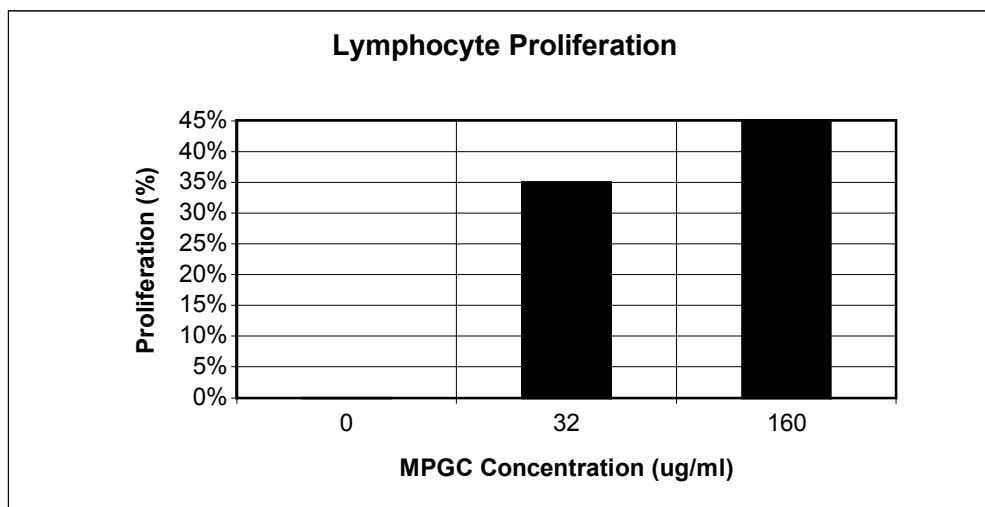


FIGURE 1.

CYTOKINE PRODUCTION

It was found that MPGC significantly induced the production of Interleukin 6 and Interleukin 12. Results are summarized in Table 2.

Table 2

FA Extract Effect on MCM Cytokines		
FA (mg/ml)	IL-6 (ng/ml)	IL-12 (pg/ml)
0	0	0
0.1	499	1880
1	700	690

DISCUSSION

Data were presented demonstrating that MPGC has immune-stimulating and anti-tumor qualities. MPGC should be studied further to elucidate its anti-tumor effects and mechanisms of action.

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This research was supported by and performed at the BioCommunications Research Institute, 3100 N. Hillside Ave. Wichita, KS 67219.