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In Vivo Tumor Inhibitory Effects of Nutritional Rice Bran Supplement MGN-3/Biobran on Ehrlich Carcinoma-Bearing Mice*

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This study was undertaken to investigate the in vivo anti-tumor activity of MGN-3/Biobran, a modified arabinoxylan rice bran. Swiss albino mice were inoculated intramuscularly in the right thigh with Ehrlich ascites carcinoma (EAC) cells. On Day 8, mice bearing a solid Ehrlich carcinoma (SEC) tumor were treated with MGN-3 via intraperitoneal injection. Tumor growth, cytokine production, and apoptotic effect of MGN-3 were examined. MGN-3 caused a highly significant delay in both tumor volume (63.27%) and tumor weight (45.2%) as compared to controls ($P < 0.01$). The mechanisms by which MGN-3 exerts its antitumor effect seem to involve its ability to induce apoptosis and immune modulation. MGN-3 induced a 1.8-fold increase in the percentage of apoptotic SEC cells as determined by flow cytometry and the histopathological examination. In addition, MGN-3 influenced plasma cytokine production by increasing the levels of tumor necrosis factor-α and interferon-γ, while downregulating levels of the immune suppressing cytokine interleukin-10. Data also showed that non-tumor-bearing mice intramuscularly injected with MGN-3 resulted in a twofold increase in natural killer activity. No adverse side effects due to MGN-3 treatment were observed; all animals displayed normal feeding/drinking and life activity patterns. These data may have clinical implications for the treatment of solid cancers.

INTRODUCTION

Cancer remains the largest cause of mortality in the world, claiming over 6 million lives each year (1). Anticancer drug therapies induce apoptosis in cancer cells but are mostly toxic, immune-suppressive, mutagenic, and carcinogenic (2–5). Previous studies have shown that several natural agents possess antitumor activity including ginger derivatives (6), *Allium sativum* (garlic) (7,8), vitamin E (9), Chinese herbal preparation (PC SPES) (10), protein-bound polysaccharide K (11), and yeast (12–14). This investigation serves to introduce MGN-3/Biobran into this group of anticancer agents. MGN-3 is an arabinoxylan extracted from rice bran that has been treated enzymatically with Shiitake mushroom extracts (15). Previous reports have shown MGN-3 to be a potent biological response modifier (BRM) that stimulates several different arms of the immune system including natural killer (NK) cells (16–19), T cells (15), B cells (15), and macrophages (20). In addition, MGN-3 is capable of sensitizing human leukemic cell surface CD95 receptors that are involved in the triggering of apoptosis (21). In this study, we investigated the in vivo antitumor activity of MGN-3/Biobran and its effects on cytokine production and NK cell activity. Data from this study revealed that both intraperitoneal (IP) and intratumoral (IT) injections of MGN-3 into tumor-bearing mice result in significantly reduced tumor volume. The mechanisms supporting this phenomenon may involve the immunomodulatory and apoptotic functions of this product. These data may suggest clinical applicability of MGN-3 in the treatment of solid tumors.

MATERIALS AND METHODS

Animals

Female Swiss albino mice (2 mo old) weighing 19 to 21 g were housed 5 per cage at constant temperature (24°C ± 2°C) with alternating 12-h light and dark cycles. Animals were provided with standard cube pellets and water ad libitum. The pellets consist of casein (12.5%), fats (1.0%), wheat flour (80%), bran (3.3%), olive oil (2.3%), dl-methionine (0.5%), vitamins and salt mixture (0.2%), and water (0.2%). The ratio of total

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calories was about 18% protein, 73% carbohydrate, and 9% fat. The pellets were purchased from Misr Oil & Soap Company (Cairo, Egypt). The actual food intake was monitored and found to be from 4 to 5 g/day/animal weighing 20 g ± 2 g.

Tumor Cell Lines

Two tumor cell lines were used in this study. Ehrlich ascites carcinoma (EAC) cells, kindly supplied by the National Cancer Institute, Cairo University, Egypt, were maintained by weekly IP transplantation of 2.5×10^6 cells. A yeast artificial chromosome-1 (YAC-1) cell line (a Moloney leukemia virus-induced mouse T-cell lymphoma of A/Sn mice origin) obtained from the American Type Culture Collection (ATCC, Rockville, MD) was acquired for use in NK activity assays.

Complete Medium (CM)

RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum, 2 mM glutamine, and 100 μg/ml streptomycin and penicillin was used to maintain cell cultures.

MGN-3

MGN-3 is a processed hemicellulose that is obtained by reacting rice bran hemicelluloses with multiple carbohydrate hydrolyzing enzymes from shiitake mushrooms. The main chemical structure of MGN-3 is an arabinoxylan, with a xylose in its main chain and arabinose in its side chain (15). It contains polysaccharides (β1, 3-glucan, and activated hemicellulose). To guarantee the purity and consistency of MGN-3 production from batch to batch, 9 parameters are measured, each by a specific method and specification (Table 1). In addition, the effective fraction of MGN-3/Biobran raw material is measured by the standard anthrone test (22). In the current study, MGN-3 dissolved in 0.9% saline was given to mice bearing solid Ehrlich carcinoma (SEC) via IP or IT injection [40 mg/kg body weight (BW)]. MGN-3 was provided by Daiwa Pharmaceuticals Co. Ltd. (Tokyo, Japan).

TABLE 1
Analysis of MGN-3 from batch to batch

Parameter	Test method	Specification
Appearance	Organoleptic test	Light brown powder
Moisture	Loss on drying test	<8.0
Protein	Lowry method	8.0~15.0%
Ash	Dry ash method	5.0~10.0%
Carbohydrates	Anthrone method	65.0~80.0%
Arsenic (as As_2O_3)	DDTC-Ag method	<5.0 ppm
Heavy metals (as Pb)	Heavy metals limit test	<20.0 ppm
Aerobic plate count	Standard plate-count method	$< 3.0 \times 10^3$ CFU/g
Coliform bacteria	Confirmation of gas production-BGLB broth	Negative

Abbreviations are as follows: DDTC, Diethyldithiocarbamate; CFU, colony forming units; BGLB, Brilliant Green Lactose Bile.

Experimental Design

Tumor transplantation and IP and IT injections of MGN-3. On Day 0, female Swiss albino mice were inoculated intramuscularly with 0.2mL EAC cells (2.5×10^6 cells) in the right thigh of the lower limb. Posttumor cell inoculation, mice bearing a solid Ehrlich tumor mass of ~100 mm^3 were randomly divided into 2 groups for IP treatment: 1) mice bearing solid tumors receiving IP injections of phosphate-buffered solution (PBS; n = 13) and 2) mice bearing solid tumors receiving IP injections of MGN-3 (n = 13). Similarly, IT-treated mice were randomly divided into 2 groups: 1) mice receiving IT injections of PBS (n = 10) and 2) mice receiving IT injections of MGN-3 (n = 10). A control group of tumor-free mice (n = 8) was included in each experiment to establish a baseline for all parameters under investigation. MGN-3 dosage was 40 mg/kg BW provided in a single 0.1 ml shot. Animals receiving IP injections commenced treatment on Day 8 post-EAC cells inoculation with continued treatment 3 times a week for 3 wk. Animals receiving IT injections commenced treatment on Day 11 post-EAC cell inoculation and continued treatment twice a week thereafter for 5 wk.

Tumor volume (TV) and tumor weight (TW) analysis. Time interval measurements of TV using digital Vernier calipers were conducted from Day 8 to Day 35 or Day 11 to Day 45 post-EAC cells inoculation. Data collected were plugged into the following formula to obtain tumor volume: TV (mm^3) = $0.52\,AB^2$, where A is the minor axis and B is the major axis. On Day 35, after receiving IP injections of MGN-3, mice were euthanized, and solid tumors were excised for TW determination, photographed for tumor regression, and processed for histopathological studies.

BW Changes

Animals bearing SEC treated IP with MGN-3 or PBS were examined for BW changes: (initial BW on Day 0, last and net final BWs on Day 35). Net final BW = (final BW − tumor weight). BW gain was determined as the difference between initial and net final BW.

Apoptosis as Determined by Flow Cytometry

Flow cytometry analysis was used to measure the percentage of apoptotic cancer cells in tumor bearing mice that were either IP treated with PBS or MGN-3. Dead cells were detected by fluorescein-conjugated Annexin V and propidium iodide technique (Annexin V-FITC apoptosis detection kit, BioVision Research Products, Mountain View, CA). Cells in suspension were prepared as described by Tribukait et al. (23). Cells were acquired by FACScan (Becton Dickinson, San Jose, CA) and analyzed by Cell-Quest software.

Histopathological studies. Histopathological studies were carried out to evaluate the apoptotic figures in the tumor-bearing mice treated IP with MGN-3 or PBS groups. The tumor tissues were fixed in 10% neutral buffered formalin for at least 24 h, processed for paraffin sections (4 μm thick), and stained with hematoxylin and eosin (H&E). Sections were examined for apoptotic changes under a light microscope.

Cytokine Analysis

On Day 35 posttumor cells inoculation plasma was collected from 3 animal groups: tumor-free mice (control), tumor-bearing mice PBS treated, and tumor-bearing mice MGN-3 treated IP. Animals fasted for 16 h before sampling, and plasma was used to examine the levels of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-10 (IL-10). Cytokine concentrations were measured via mouse cytokine specific enzyme-linked immunoabsorbent assay kits provided by CytImmune Sciences Inc. (Rockville, Maryland).

NK Cell Studies

Preparation of splenic cells. Non-tumor-bearing mice were injected daily with MGN-3 (100 μg/ml) in the thigh. A total of 14 days posttreatment, mice were sacrificed by cervical dislocation. Spleens were removed, teased in CM, and contaminating erythrocytes were lysed with distilled water for 20 s at room temperature (27°C). Single cell suspensions were washed once with Hanks balanced salt solution (HBSS), and cells were resuspended to a concentration of 1×10^7 cells/ml CM. Cells were counted using a hemocytometer and a light microscope.

Measuring NK activity by ^{51}Cr-release assay. A standard Cr-release assay for measuring NK cell activity was employed as follows: YAC-1 tumor target cells (5×10^6) were labeled with 100 μCi of sodium chromate solution for 1h in 0.1 ml CM. Tumor cells were washed 4 times in 5 ml HBSS, and 1×10^4 cells were pipetted into 96-well, round-bottomed Linbro plates (Linbro Chemical Co., Hamden, CT). Effector cells were pipetted into quadruplicate wells to give effector:target (E:T) ratios of 100:1, 50:1, 25:1, and 12.5:1. Following a 4-h incubation at 37°C, 0.1 ml of supernatant from each well was collected and counted in a gamma counter. The percentage of isotope released was calculated using the following formula:

$$\%\text{lysis} = \frac{\text{Exp. Rel.} - \text{Sp. Rel.} \times 100}{\text{Total Rel.} - \text{Sp. Rel.}}$$

where Sp. = spontaneous and exp =/experimental. In order to address variability in cell yield or recovery from different cell populations, the units of lytic capacity were used. Dose response curves for the populations were obtained by plotting the percentage of target cells killed on the y-axis and the E:T ratio on the x-axis. Lytic units (LUs) were then defined as the number of effector cells extrapolated from the dose response curve, with 1 LU corresponding to the number of effector cells required to achieve 15% lysis (16). LU/1 $\times 10^7$ is the number of LUs in 10 million effector cells.

Effector-target cell conjugate assay. The capacity of splenic NK effector cells to form conjugates with YAC-1 target cells was measured in both the MGN-3-treated group and the control group as previously described (24). Splenic cells (1×10^5) were incubated with 5×10^5 target cells in 1 ml of CM. The cell mixture was pelleted at 130 *g* for 10 min and incubated for 1 h at 4°C. Pellets were gently resuspended, and cytocentrifuged smears were prepared and stained with Giemsa. The percentage of conjugates was determined by counting 200 lymphocytes (bound and free) in triplicate samples.

Adverse Effects of MGN-3 (Toxicity)

Mice were examined daily for adverse side effects by MGN-3 as assessed by changes in the normal feeding/drinking cycles and life activity patterns for the entire treatment period. Changes in BW were also recorded weekly.

Statistical Analysis

Cytokine values are reported as the mean ± SE, and data were analyzed using one-way analysis of variance measures followed by post hoc tests for multiple comparisons. Statistical significance of other data was determined by Student's *t*-test. Differences were considered significant at the $P < 0.05$ level.

RESULTS

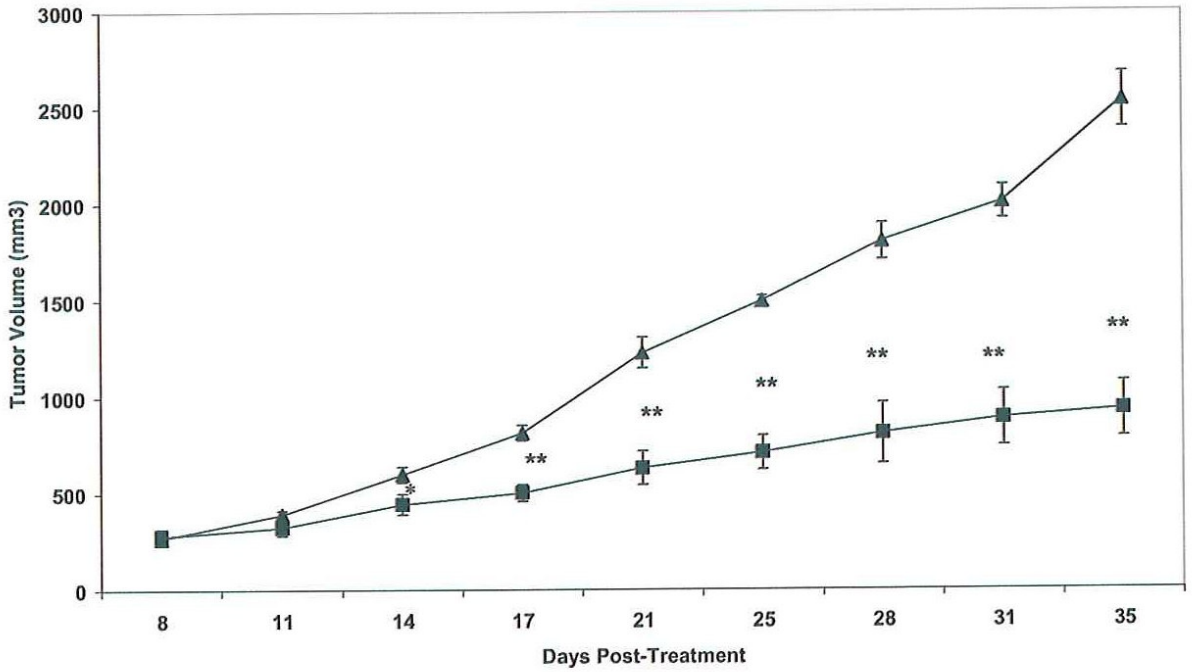
Adverse Effects of MGN-3 (Toxicity)

Animals were monitored to observe potential toxic side effects of MGN-3 treatment. No adverse side effects or unintentional deaths were observed during the treatment period (35 days). In addition, MGN-3 IP-treated mice showed a significant final BW gain.

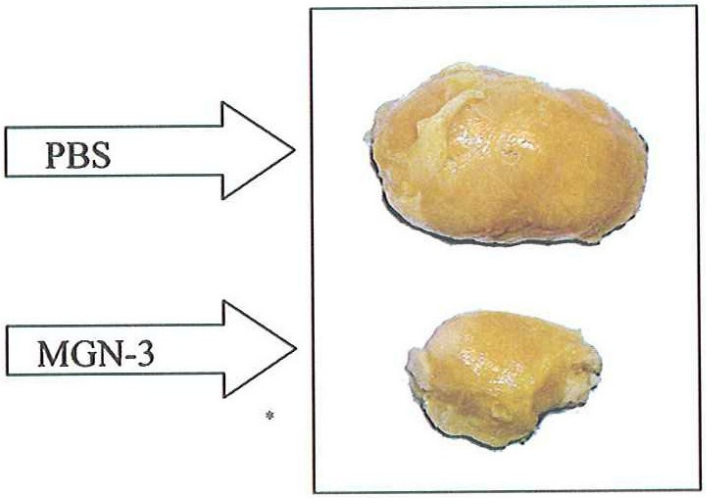
TV

IP injection of MGN-3. Figure 1A shows that IP-administration of MGN-3 in tumor-bearing mice resulted in retardation of solid tumor development that became significant as early as Day 14 posttreatment. By Day 35, the percent TV differences between MGN-3 treated (931.98 mm^3 ± 144.76) and PBS-treated mice (2537.11 mm^3 ± 143.52) reached –63.27% ($P < 0.01$). Photographs of the tumor isolated from MGN-3-treated mice clearly demonstrate significant tumor regression as compared to PBS-treated mice. Similar levels of tumor regression were observed in all groups tested (Fig. 1B).

IT injection of MGN-3. IT injection was performed to examine an alternative route by which MGN-3 may exert an antitumor effect in vivo. Figure 2 shows that IT administration of MGN-3 suppressed tumor growth that began on Day 28 posttreatment. TV differences became significant on Day 36 when tumor delay reached –32.24%, $P < 0.05$. TV differences became highly significant when percent divergence between



(a)



(b)

FIG. 1. A: In vivo effect of intraperitoneal injection of MGN-3 on tumor volume. Mice were inoculated in the right thigh with Ehrlich ascites carcinoma cells. At Day 8, after tumor cells inoculation, mice bearing solid Ehrlich carcinoma were injected with MGN-3 (■) and change of tumor volume (TV) mm^3 was examined at Day 8 until Day 35. The figure represents the change of TV mm^3 during the time course; results were compared with control phosphate-buffered solution (PBS)-treated mice-bearing tumor (▲). Each value represents the mean ± SE. $^*P < 0.05$, $^{**}P < 0.01$ as compared to PBS group at the corresponding time point. B: Photograph of tumor regression, PBS-treated and MGN-3-treated mice bearing tumor. Data is representative of all groups examined.

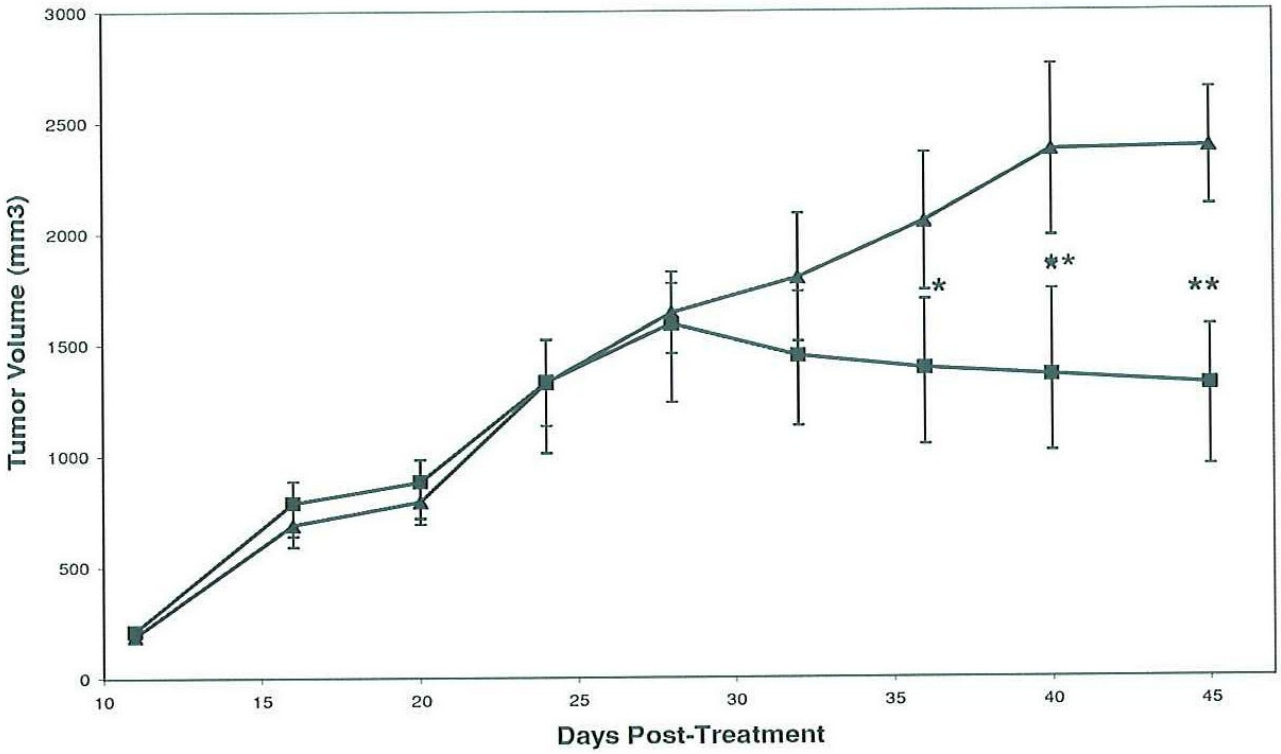
MGN-3-treated and PBS-treated tumor-bearing mice reached –44.83%, $P < 0.01$ on Day 45 of tumor cells inoculation.

TW

Table 2 shows the effect of IP treatment with MGN-3 on TW examined at the end of the experiment on Day 35. The TW of PBS-treated mice was found to be 6.62 ± 0.38 g; on the other hand, the TW in MGN-3-treated group was 3.63 ± 0.45 g, representing a highly significant change ($P < .01$) in TW, a difference of 45.2%.

BW

Changes in BW were examined during the 35 days of the experiment. As shown in Table 2, IP administration of MGN-3 to SEC-bearing mice caused a significant BW gain of +7.32%, $P < 0.025$.



ı vivo effect of intratumoral injection of MGN-3 on tumor volume. Mice were inoculated in the right thigh with Ehrlich ascites carcinoma cells. At :r tumor cells inoculation, mice bearing solid Ehrlich carcinoma were injected with MGN-3 twice a week for 5 wk at concentration of 40 mg/kg body The change on total volume mm^3 was examined at Day 11 until Day 45, and results were compared with the phosphate-buffered solution (PPS)-treated ng mice (▲). Each value represents the mean ± SE. $^*P < 0.05$, $^{**}P < 0.01$ as compared to PBS group at the corresponding time point.

is of SEC

:ytometry. The data in Table 3 show the flow cytome- sis of the percentage of apoptotic cells in SEC-bearing Day 35 posttumor cell transplantation. PBS-treated aring mice show 42.61 ± 5.56% apoptotic cells. IP with MGN-3 significantly increased the percentage tic SEC cells (74.68 ± 4.22%). This represents a 1.8- < 0.0001) increase in the percentage of apoptotic SEC

athological examination. Histopathological sec- e examined for the presence of apoptotic cells in tumor- nice posttreatment IP with MGN-3. Figure 3A shows a ıcrease in the number of apoptotic tumor cells in MGN- mice as compared with only a few scattered, individual cells in the tumors of PBS-treated, SEC-bearing mice

e Levels

? effects of MGN-3 treatment on the levels of plasma FN-γ, and IL-10 measured on Day 35 posttumor trans- n are shown in Table 4.

x plasma level. Data in Table 4 show no significant es in the levels of TNF-α between the control tumor- (1,100 pg/ml ± 10.4) and PBS-treated, tumor-bearing 146 pg/ml ± 16). However, the treatment of MGN-3 in significantly elevated levels of TNF-α production (1,271.9 pg/ml ± 13.5, $P < 0.01$), approximately 11% higher than mice bearing SEC, and 15% higher than control.

IFN-γ plasma level. PBS-treated, tumor-bearing mice showed insignificant differences in plasma IFN-γ (142.1 pg/ml ± 10.6) when compared to the control tumor-free mice (158.75 pg/ml ± 19.82). Treatment with MGN-3 showed an enhancement in plasma IFN-γ levels (404.1 pg/ml ± 58.3, $P < 0.01$) of 184.4% over PBS-treated, tumor-bearing mice, and 154.54% over control mice (Table 4).

IL-10 plasma levels. Data depicted in Table 4 illustrate that plasma concentrations of the immune-suppressing cytokine IL-10 dramatically increased by over twofold in PBS-treated, tumor-bearing mice (858.5 pg/ml ± 140.9, $P < 0.01$) as compared to the control tumor-free group (405.5 pg/ml ± 25.0, 111.71%). Administration of MGN-3 resulted in IL-10 plasma levels of 465.3 pg/ml ± 30.0, similar levels to that of the tumor-free control group.

NK Cell Studies

NK activity. NK cell activity was examined 2 wk after daily intramuscular treatment with MGN-3. Using YAC-1 cells as targets, MGN-3-treated mice showed elevated NK cell activity as compared to control untreated mice at various E:T ratios (Fig. 4). A significant increase (twofold) between controls and MGN-3-treated mice was detected at each comparison ratio:

TABLE 2
Effect of IP injection of MGN-3 on the body weight (g) and tumor weight (g)[a]

Group	Tumor-Bearing Mice	
Parameters	PBS	MGN-3
Initial Body weight (g) (at Day 0)		
Mean ± SE	20.21 ± 0.47	20.64 ± 0.51
No. of mice	13/13	13/13
Final Body weight (g) (at day 35)		
Mean ± SE	26.67 ± 0.58	25.78 ± 0.54
No.of mice	11/13	13/13
Tumor weight (g) (at day 35)		
Mean ± SE	6.62 ± 0.38	3.63 ± 0.45[b]
Net final Body weight (g)		
Mean ± SE	20.05 ± 0.48	22.15 ± 0.63[c]
Body weight gain (g)	−0.15 g	+1.51 g
% of change from the initial body weight	(−0.79%)	(+7.32%)

[a]Abbreviations are as follows: IP, intraperitoneal; PBS, phosphate-buffered solution. Each value represents the mean ± SE of the corresponding number of animals/group. Net final body weight = (Final body weight at Day 35 − Tumor weight at Day 35). Body weight gain (Net final body weight – Initial body weight).

[b]Significant at $P < 0.01$ as compared to the PBS group.

[c]Significant at $P < 0.025$ as compared to the initial body weight.

TABLE 3
Effect of IP injection of MGN-3 on the percentage of apoptotic tumor cells in mice bearing solid ehrlich carcinoma as determined by flow cytometry at day 35 posttumor cells inoculation[a]

Group	Tumor- Bearing Mice	
Parameters	PBS	MGN-3
% Apoptotic tumor cells		
Mean ± SE	42.61 ± 5.56%	74.68 ± 4.22%
% of Change from controls	—%	* 75.26%
Significance	—%	$P < 0.0001$
No. of mice	8	8

[a]Abbreviations are as follows: IP, intraperitoneal; PBS, phosphate-buffered solution.

from 2.7% to 5.5% at 12.5:1, 5.1% to 13.6% at 25:1, 8.4% to 17.4% at 50:1, and 10.6% to 22.1% at 100:1. When calculated in terms of LUs, statistical analysis showed a significant increase in NK cell activity in MGN-3 treated mice (27.1 LUs) as compared to controls (8.3 LUs, $P < 0.01$).

Percent conjugate formation. To examine whether MGN-3 treatment affects NK cell binding capacity to tumor cell targets, we compared the percentage of conjugate formation between NK cells obtained from either MGN-3-treated or control mice against YAC-1 tumor cells. As shown in Fig. 5, the proportion of conjugate-forming cells in MGN-3-treated mice was significantly higher (27.5%) as compared to control mice (14%, $P < 0.01$), representing a twofold increase.

DISCUSSION

In this study, we examined the effect of MGN-3 against tumor growth using SEC-bearing Swiss albino mice as a model. MGN-3 exhibited a potent antitumor effect in vivo as indicated by significant tumor regression post-IP and IT injection with MGN-3. The mechanisms by which MGN-3 exerts its antitumor effect seems to involve both immune-modulatory and apoptotic effects. With respect to tumor immunology, tumors

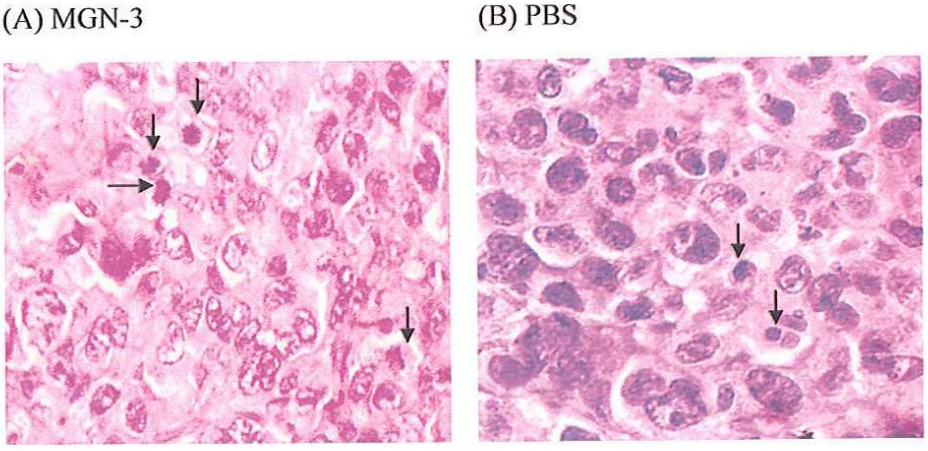


FIG. 3. Cross sections of solid Ehrlich carcinoma-bearing mice. A: Section of MGN-3-treated tumor demonstrates extensive apoptosis. B: Section of control tumor shows most of tumor cells are viable except for a few spots of apoptosis. (× 400 magnification with hematoxylin and eosin stain; arrows point to apoptotic cells). PBS, phosphate-buffered solution.

TABLE 4
Effect of IP injection of MGN-3 on plasma cytokine levels in mice bearing solid ehrlich carcinoma on day 35 of tumor cells inoculation[a]

Groups / Parameters	Control mice (without tumor)	Tumor-Bearing Mice	
		PBS	MGN-3
TNF-α (pg/ml)			
Mean±SE	1,100 ± 10.4	1,146 ± 16	1,271.9 ± 13.5[c,d]
% Change[b]	—%	(4.17%)	(15.63%)
No. of mice	8	11	10
IFN-γ (pg/ml)			
Mean±SE	158.75 ± 19.8	142.1 ± 10.6	404.1 ± 58.3[c,d]
% change[b]	—%	−10.46%	154.54%
No. of mice	8	11	10
IL-10 (pg/ml)			
Mean±SE	405.5 ± 25.0	858.5 ± 140.9[c]	465.3 ± 30.0[d]
% change[b]	—%	111.71%	14.75%
No. of mice	8	11	10

[a]Abbreviations are as follows: IP, intraperitoneal ; PBS, phosphate-buffered solution; TNF-α, tumor necrosis factor-alpha; IFN- γ, interferon-gamma; IL-10, interleukin-10. Each value represents the mean ± SE from the indicated number of mice.

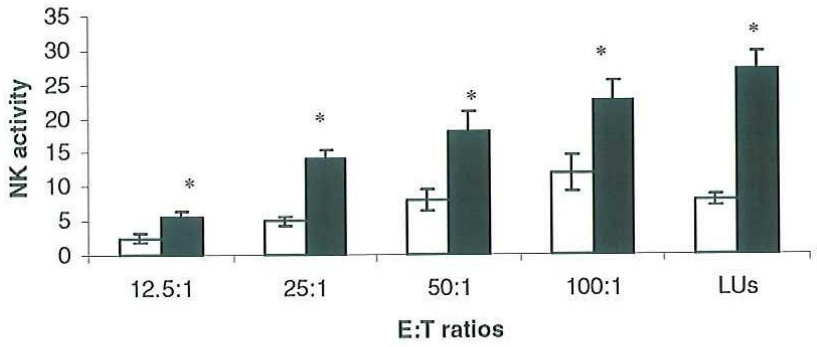
[b]Percent change as compared to control group.

[c]Significantly different from the control group at 0.01 level.

[d]Significantly different from the PBS tumor-bearing group at 0.01 level.

re characterized by their ability to avoid host immune system 25–27). An Ehrlich tumor is a rapidly growing carcinoma with ery aggressive behavior. It can avoid host immune response by ecreting a variety of immunosuppressive cytokines, resulting ı the dysfunction of cytotoxic cell immuno-surveillance (25), hich plays a role in controlling tumor growth (28,29). Indeed, bservations demonstrating that an imbalance in the cytokine etwork is associated with tumor evolution established the ba- is for therapeutic approaches to modify cytokine production to avor tumor rejection (30).

Results presented here show that MGN-3 is a potent in vivo ıducer of both TNF-α and IFN-γ, a finding that has been previously reported in vitro using human peripheral blood lymphocytes (19), macrophages, and macrophage cell lines (20). Both TNF-α and IFN-γ are known to present strong antitumor activities (31–33). Type I interferons (IFN-α and IFN-β) are used for the treatment of hepatocellular carcinoma (34–36). Type I IFNs activate NK cells (37) and cytolytic T lymphocytes (38,39) via increased production of IL-1, IL-6, and TNF-α (40–42). Additionally, MGN-3 downregulates the tumor-induced increase in plasma levels of the immunosuppressive cytokine IL-10, which has been shown to inhibit the synthesis of several cytokines including IL-2 and IL-3 in Th1 cells (43,44), IL-4 and IL-5 in Th2 cells (30), and TNF-α and IFN-γ in NK cells (45,46).



IG. 4. In vivo effect of MGN-3 on natural killer (NK) activity. Non-tumor-bearing mice were treated with MGN-3 for 2 wk, and splenic NK activity was ıeasured by means of a 4-h Cr-release assay and is expressed at different effector:target (E:T) ratios and as number of lytic units (LUs). Data represent the mean : SD of 3 mice/group. * $P < 0.01$ as compared to control untreated mice.

TABLE 4
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No. of mice	8	11	10
IL-10 (pg/ml)			
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% change[b]	—%	111.71%	14.75%
No. of mice	8	11	10

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re characterized by their ability to avoid host immune system 25–27). An Ehrlich tumor is a rapidly growing carcinoma with ery aggressive behavior. It can avoid host immune response by ecreting a variety of immunosuppressive cytokines, resulting n the dysfunction of cytotoxic cell immuno-surveillance (25), /hich plays a role in controlling tumor growth (28,29). Indeed, bservations demonstrating that an imbalance in the cytokine etwork is associated with tumor evolution established the ba- is for therapeutic approaches to modify cytokine production to avor tumor rejection (30).

Results presented here show that MGN-3 is a potent in vivo nducer of both TNF-α and IFN-γ, a finding that has been previously reported in vitro using human peripheral blood lymphocytes (19), macrophages, and macrophage cell lines (20). Both TNF-α and IFN-γ are known to present strong antitumor activities (31–33). Type I interferons (IFN-α and IFN-β) are used for the treatment of hepatocellular carcinoma (34–36). Type I IFNs activate NK cells (37) and cytolytic T lymphocytes (38,39) via increased production of IL-1, IL-6, and TNF-α (40–42). Additionally, MGN-3 downregulates the tumor-induced increase in plasma levels of the immunosuppressive cytokine IL-10, which has been shown to inhibit the synthesis of several cytokines including IL-2 and IL-3 in Th1 cells (43,44), IL-4 and IL-5 in Th2 cells (30), and TNF-α and IFN-γ in NK cells (45,46).

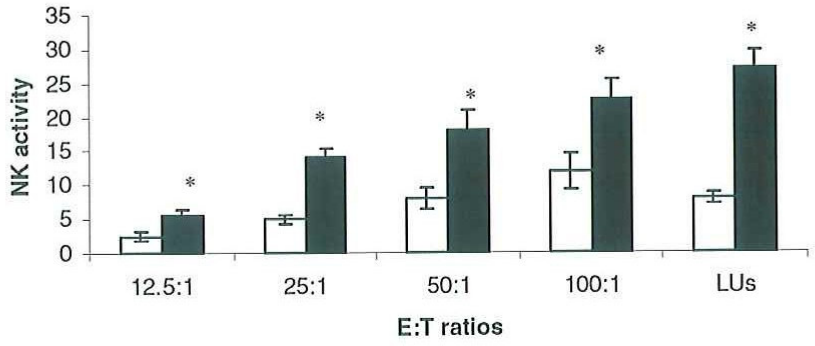

FIG. 4. In vivo effect of MGN-3 on natural killer (NK) activity. Non-tumor-bearing mice were treated with MGN-3 for 2 wk, and splenic NK activity was measured by means of a 4-h Cr-release assay and is expressed at different effector:target (E:T) ratios and as number of lytic units (LUs). Data represent the mean ± SD of 3 mice/group. * $P < 0.01$ as compared to control untreated mice.

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