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Chapter 30

NK Immunorestoration of Cancer Patients by MGN-3, A Modified Arabinoxylan Rice Bran (Study of 32 Patients Followed for up to 4 Years)

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NK cells have been characterized as non-B cells or non-T cells lacking the characteristics of mature macrophages which develop from the bone marrow independently of thymic influence.[1,2] NK cells play a crucial role in tumor rejection, immune surveillance, resistance to infections, and immune regulation.[3-5] NK cell destruction of cancer cells involves a sequence of events.[6] First, the NK cell recognizes and binds to the cancer cell. This process requires receptor-to-receptor interaction. Next, the NK cell releases granules which penetrate the cancer cell and ultimately kill it. The NK cell is then free to bind to another cancer cell and repeat the same process

However, cancer cells know how to fight back in a sort of cell war. We found for the first time in our laboratory that cancer cells can destroy WBCs through the phenomenon of phagocytosis.[7-10] We have observed three ways in which this is done. The cancer cell can extend two arms around the WBC or it can develop a cup-shaped opening where the WBC is drawn inside. A third way is for the cancer cell to extend a long arm to capture the WBC and finally draw it inside the cancer cell where it is digested. In addition, extensive work by others has shown that cancer cells secrete immune-suppressive substances which inhibit the function of the immune system.[11-13]

Many attempts have been made in the last 25 years to strengthen the power of the immune system using different biological response modifiers (BRMs). These are substances originating from bacteria and fungi which possess immunoaugmentory properties.[4,14,15] In addition, some kinds of cytokines serve as BRMs such as interferons, interleukin-2 and interleukin-12.[16,17] There are two problems associated with these BRMs: 1st) toxicity and 2nd) the development of hyporesponsiveness in which a single

administration of the BRM can significantly enhance NK cell activity, but that repeated administration of the same BRM results in depression of NK cell activity.[18-20] It is interesting to note that MGN-3 has advantages over other BRMs. It is nontoxic and has not shown hyporesponsiveness in the four years that the patients have been followed. This work was undertaken in order to investigate the augmentory effect of a new BRM known as MGN-3 on NK cell function and T and B cell proliferation in 32 patients. Tumor-associated antigens were reported for selected patients.

Patients, Materials and Methods

PATIENTS

The present study was carried out on 32 cancer patients. Patients had different types of malignancies: prostate[10], breast[12], multiple myeloma[5], and leukemia. The majority of the patients first went through a debulking done using conventional therapies such as surgery, radiation, or chemotherapy.

MATERIALS

MGN-3. MGN-3 is an arabinoxylan extracted from rice bran that is treated enzymatically with an extract from shiitake mushrooms. It is a polysaccharide that contains (-1,4 xylopyronase hemicellulose. MGN-3 is commercially known as Biobran (Daiwa Pharm., Co., Ltd., Tokyo, Japan).

METHODS

Treatment Protocol. Patients were given MGN-3 (3 g/day) daily by mouth.

Tumor-Associated Antigens (TAA) .TAA for each type of malignancy was measured prior to MGN-3 treatment and one month posttreatment.

Tumor Cell Line. K562, a human erythroleukemic cell line, was used as the target. Tumor cells were cultured in complete medium (CM) that consisted of RPMI-1640 supplemented with 10% fetal calf serum and 1% antibiotic (100 U penicillin and 100 g/ml streptomycin).

Preparation of Peripheral Blood Lymphocytes (PBL). PBLs were prepared from fresh heparinized peripheral venous blood by Ficoll-Hypaque density gradient centrifugation. Cells were washed two times with Hanks balanced salt solution (HBSS) and resuspended to 10 x 106 cells/ml in CM.

51CR-Release Assay for Measuring NK Activity. NK activity was measured by a standard 4 hr 51Cr-release assay. Briefly, 1 x 104 51Cr-labelled tumor target cells in 0.1 ml CM were added to different wells of a 96-well microtiter plate. Effector cells were then pipetted into quadruplicate wells to give E:T ratios of 12:1, 25:1, 50:1, and 100:1. After a 4 hr incubation at 37°C, the plates were centrifuged (1,400 rpm for 5 min) and 0.1 ml of supernatant from each well was collected and counted in a gamma counter (Beckmann G50, Beckmann Instruments).

The percentages of isotopes released were calculated by the following formula:

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

Spontaneous release (SP) from target cells was no more than 8–10% of total release. Total release was measured by adding 0.1 ml Triton X-100 (Sigma Chemical Co.) to designated wells. Lytic units (LU) were calculated from effector titration curves with one LU defined as the number of effector cells required to achieve 20% lysis for K562.

NK Granularity. Percall frationated PBL were adjusted to 2.5 x 10.6 /ml and centrifuged on slides at 1,000 rotations /minute for 5 minutes using a cytospin cytocentrifuge (Shandon Southern Institute, Sewickley, PA). Slides were air dried, fixed in 100% Meoh, stained with 5% Giemsa solution for 10 minutes. Stained preparations were examined for the granularity of NK cells (21).

In Vivo T and B Lymphocyte Proliferation. We investigated the in vivo effects of MGN-3 on T and B cell proliferation using 3H-thymidine uptake. MNCs were prepared from peripheral blood of five cancer patients before and at one month after treatment with MGN-3. Cells were incubated at 2 x 105 cells/ml in CM. Cells were treated with 10 g/ml of phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM) for three days. One (Ci of 3H-thymidine (New England Nuclear) was added to the cell cultures for the last 18 hrs. DNA was harvested and 3H-thymidine uptake was determined by scintillation counter. All experiments were done in triplicate and data expressed as counts per minute (cpm).

Statistical Analysis. Student T test was used to examine the significance of difference between NK activities and T and B cell response to mitogens before and after treatment with MGN-3.

RESULTS

1—NK Cell Activity

Fig. 1 demonstrates the baseline values of cytotoxic responses of NK cells in 32 cancer patients. Patients demonstrated overall significant low level in NK function. Depression in NK activity was observed in patients with different types of malignancies as follows: prostate, 11.1 LUs; breast, 11.4 LUs; MM, 7.3 LUs; and leukemia, 4.3 LUs. Studies performed on peripheral blood lymphocytes from 12 participants one to two weeks after the primary studies revealed no statistically significant differences in NK cell activity in comparison with the initial results. Treatment with MGN-3 resulted in significant increase in NK activity up to tenfold. MGN-3 augmentory effect was detected in all types of malignancies: prostate, 41.9 LUs; breast, 33 LUs; MM, 31.9 LUs; and leukemia, 51.4 LUs. Individuals varied in response to augmentory effect of MGN-3.

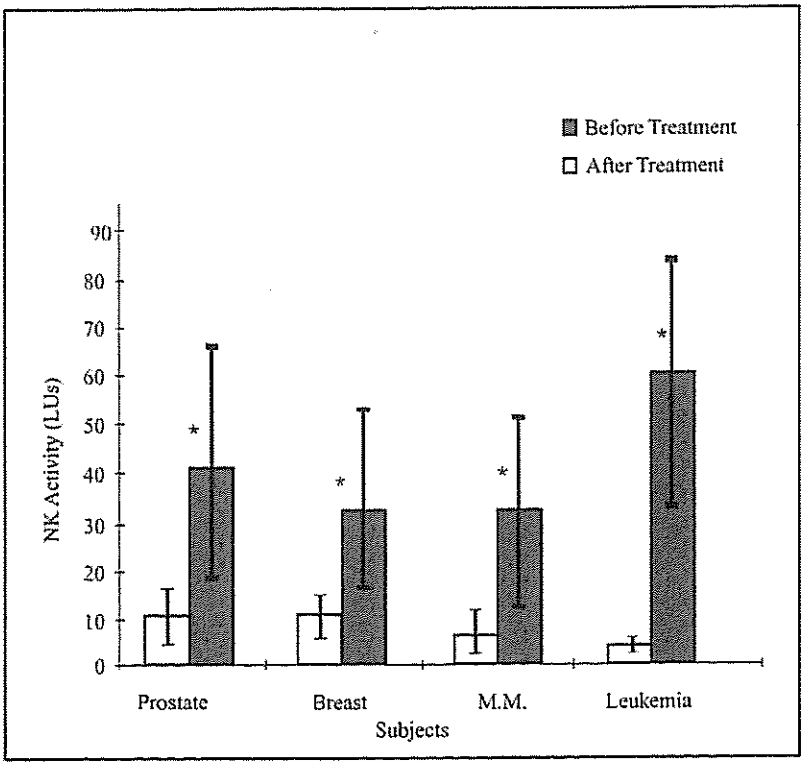

Fig. 1. Effects of MGN-3 on NK cell activity of 32 patients at one to two weeks after treatment. Malignancies were: prostate (10), breast (12), multiple myeloma—MM (5), and leukemia (5). LUs at 20% *P< 0.001.

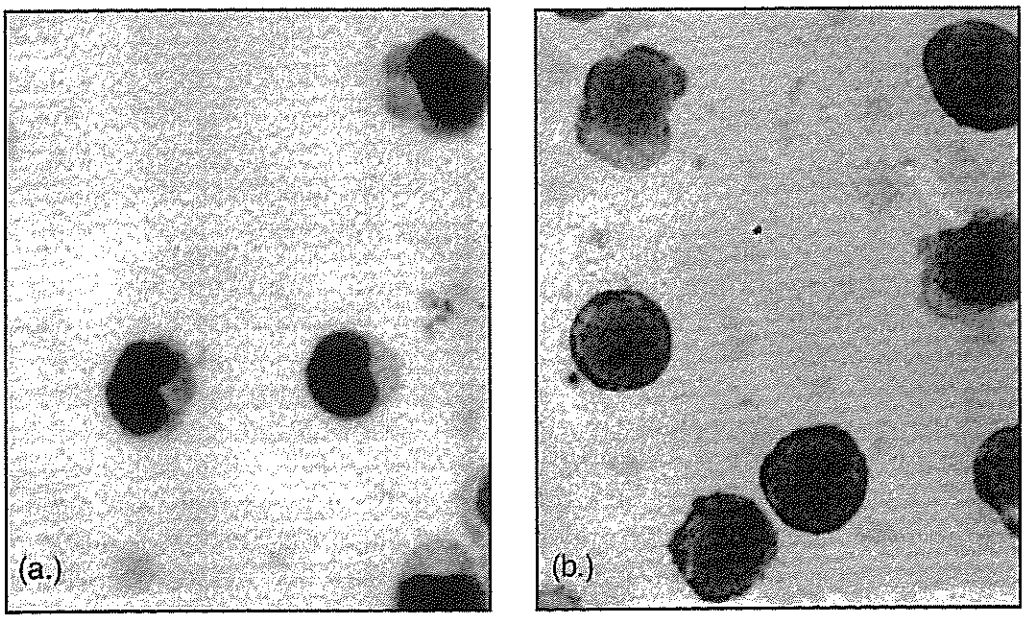

Fig 2. (a). Cytocentrifuge preparation of PBL-NK cells isolated from cancer patient before treatment with MGN-3. (Giema, X740). Notice high nuclear cytoplasmic ratio and absence of granules. (b). Cytocentrifuge preparation of PBL-NK cells of the same patient at one week post-treatment with MGN-3. Cells demonstrated high granular content.

2—NK Granularity

Cytospin cytocentrifuge preparation of PBL-NK cells before treatment showed low or absent granularity (Fig. 2a). On the other hand, treatment with MGN-3 resulted in significant increase in the granular content at one week post-treatment (Fig. 2b). The MGN-3-activated NK cells demonstrated an increase in the binding capacity and killing of cancer cells (Fig. 3).

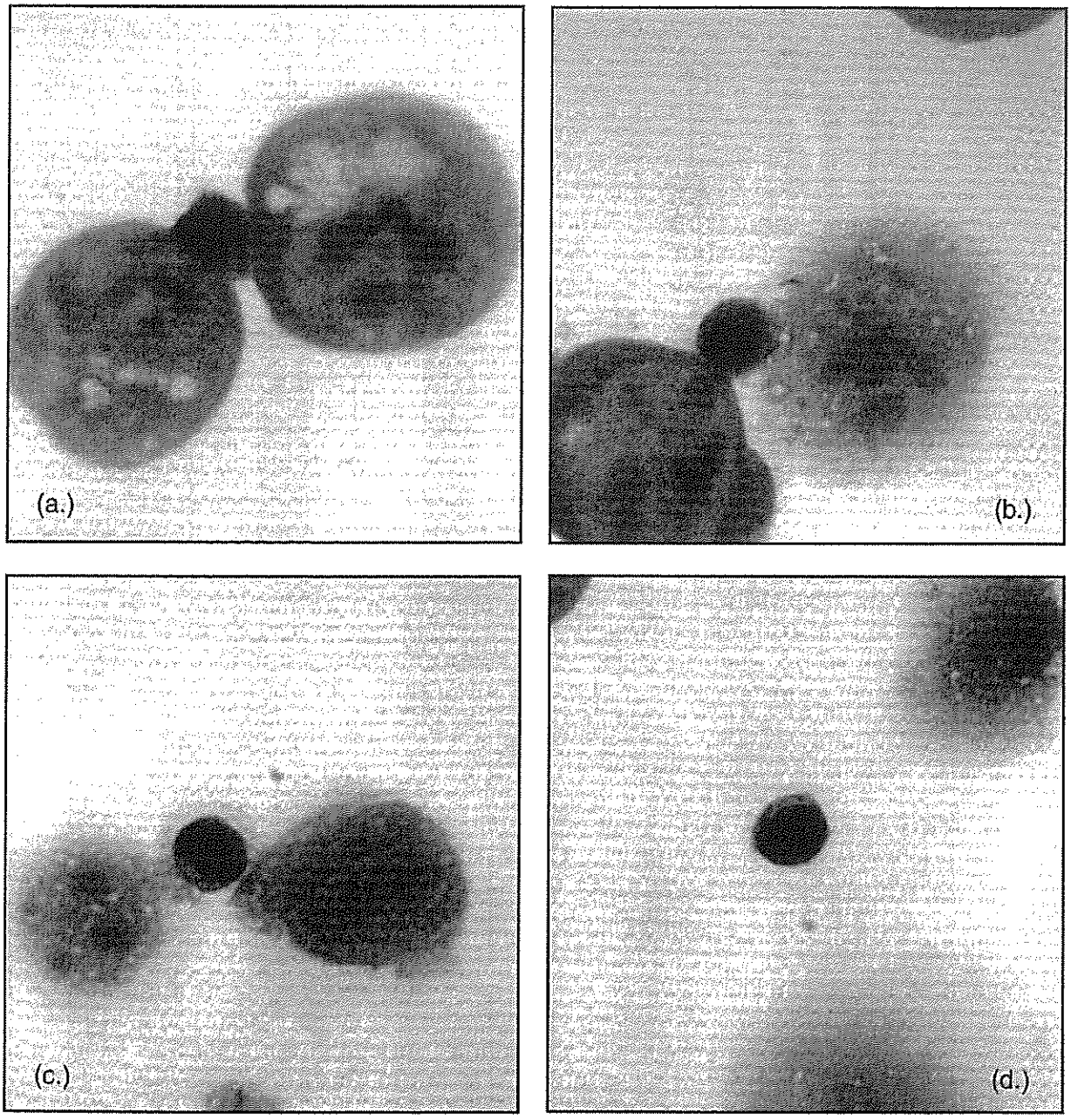

Fig. 3. Cytocentrifuge preparation of two K562 tumor cell destruction by one NK cell. NK cells were activated by MGN-3. (a) First step in the process represented by binding of NK cell to tumor cells. (Giema, X740). (b) Preparation showing one tumor cell is dead. (Giema, X740). (c) Preparation showing both tumor cells are dead while NK cell in between is still alive. (Giema, X740). (d) Cytocentrifuge preparation showing NK cell detach itself from the dead tumor cells. (Giema, X740).

3—In Vivo T and B Lymphocyte Proliferation

Fig. 4 shows that treatment with MGN-3 significantly increased T cell proliferation as indicated by their response to PHA and Con A mitogens. B cell proliferation also increased post-treatment with MGN-3 as indicated by their response to PWN, a B cell mitogen, as compared to baseline value.

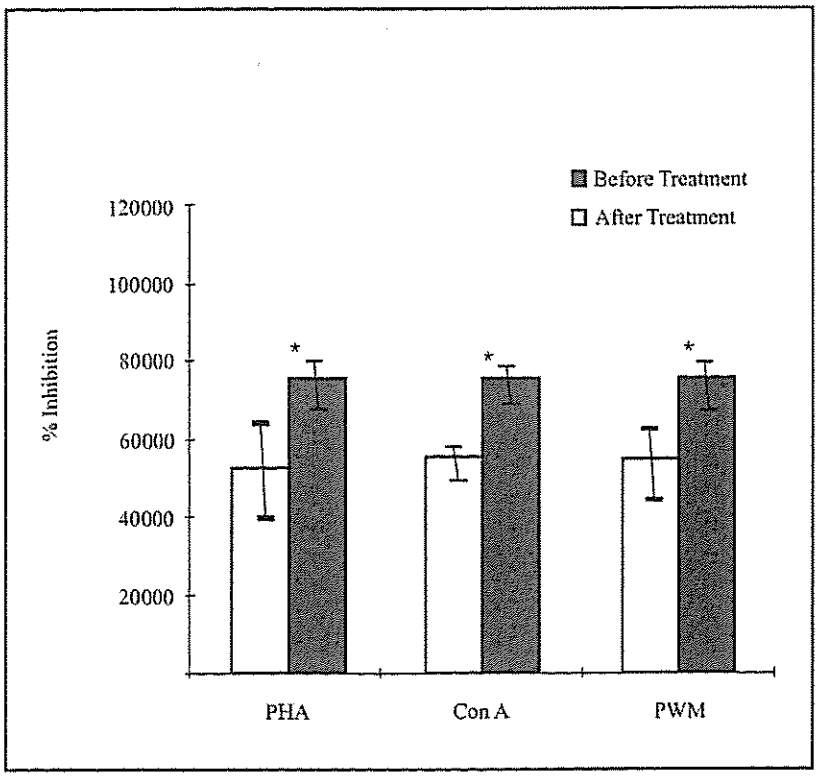

Fig. 4. In vivo action of MGN-3 on T and B cell mitogen response at one month after treatment. MNC were cultured for three days in the presence of PHA, Con A and PWM. Data represent mean (s.d. of five individuals. *P<0.001).

4—TAA and NK Activity in a Selection of Patients

Patients were monitored for tumor-associated antigens: prostate, PSA; and multiple myeloma, BJP or IGg; while breast cancer was monitored by CEA and CT Scan one to two times per year. A selection of patients having different types of malignancies were analyzed.

A 39-year-old patient, Mr. K., diagnosed with acute myelogenous leukemia (AML), was treated with chemotherapy which brought the WBCs count to 5.6, the normal range being between 4.5 and 10.5. He was off chemo and began taking MGN-3 in January of 1995. His WBC count has been within the normal range from that time. The patient's baseline NK activity was 7.9 LUs and increased to 113 LUs within one week after treatment with MGN-3. His NK activity level has remained high for about four years now.

Mr. Y., a 52-year-old Japanese store manager, was also diagnosed with AML. He did not follow conventional therapy. His WBC was 18,700 per milliliter on March 31, 1998. He began taking MGN-3 and on April 30th his WBC dropped to 11,000. His condition has been kept quite stable since that time.

In 1994, Mr. R. came to us suffering from prostate cancer. Hormonal therapy had brought his PSA level to 0.1 but it was known that the marker would increase again with

time. The patient was given MGN-3 and his PSA level has remained within the normal level for the last four years.

Ms. M. had a recurrence of breast cancer in April of 1995 and was treated with surgery followed by chemotherapy. She started MGN-3 after completion of her chemotherapy and since then all CAT scans have been negative. There has been no evidence of recurrence seen in CAT scans or biopsies. The patient's baseline NK cell activity was 16.4 LUs, which increased twofold one week post-treatment with MGN-3. Her activity increased further to 128 LUs and has remained at a high level over the years.

Discussion

MGN-3 is considered to be a potent BRM as manifested by inducing increased activity of NK cells in animals and humans. Mice injected IP with MGN-3 showed a several-fold increase in NK cell activity at two days post-treatment. Other studies where MGN-3 was mixed with food and fed to rats also demonstrated increased NK cell activity in a dose-dependent manner. Studies were also carried out on healthy subjects who received MGN-3 orally. A 2.3-fold increase in NK activity was seen at one week post-treatment with MGN-3 at 30 and 45 mg/kg/d while lower doses of 15 mg/kg/d took one month to increase NK activity by twofold.[22]

We thought it would be of particular interest to investigate the augmentory effect of MGN-3 on NK activity in cancer patients. Patients went through chemotherapy or radiation therapy as debulking was necessary. However, NK activity becomes depressed as a result of these therapies. Given a need for natural immunity in tumor control, we felt it may be clinically important to enhance NK activity in order to destroy the remaining cells that escape from chemo and radiation. It is possible to enhance NK cell activity with the usage of different BRMs. However, toxicity and hyporesponsiveness associated with many BRMs limit their usage. MGN-3 is a safe product and patients did not develop hyporesponsiveness during the four years of the study. NK cells are sensitive indicators of activation by BRMs.Their monitoring has been used to document alterations in the activity of circulating immune cells during therapy with these agents. Enhancement of NK activity by MGN-3 was detected as early as one to two weeks post-treatment and it was maintained at a high level with continuation of MGN-3 treatment.

The therapy of debulking followed by MGN-3 immunotherapy had a practical application in the 32 cancer patients. Parallel to the increase in NK activity, patients demonstrated a gradual decrease in the level of TAAs with no signs of recurrence for the four years of the study.

The mechanism(s) by which MGN-3 increases NK cell activity has been examined. Based on our studies, it appears that two mechanisms are involved in the activation of NK cells by MGN-3. First, by the increase in NK cell granularity and second, by the elevation of cytokine production. Regarding granularity, NK cells in our patients have low granularity or granules might be absent altogether. It is interesting to note that MGN-3 treatment significantly increases the granular content of NK cells (Fig. 2). The granules are not only situated in the cytoplasmic portion but also between the nuclear and cellular membranes. Exocytosis of NK granules and secretion of pore-forming molecules (perforins) stored as cytoplasmic granules may represent one of the most important mechanisms to kill cancer cells by the NK cell system.[23-25] The important role of granules in NK cells' destruction of their tumor targets has been indicated by the observation that isolated and purified granules are lytic for a variety of tumor cell types.[24] Therefore, we

believe that the increased level of granularity of NK cells is an important factor in the enhancement of these effector cells by MGN-3.

With respect to cytokines, several cytokines have been shown to affect NK cell proliferation or cytolytic activity. Of these, the interferons (IFN) and IL-2 have been the most extensively studied.[16, 17, 26, 27] Suppression in NK activity in cancer patients was related to defective lymphokine production. It appears that augmentation of NK cell cytotoxic function by MGN-3 is parallel to a significant increase in the levels of different cytokines. The heavy granulation of LGL may indicate secretory function. It is not known whether production of various lymphokines is a multipotential property of a single subset of LGL. It is more likely that different subsets of LGL are responsible for different lymphokines. In vitro studies showed that PBLs treated with MGN-3 have significantly increased production of TNF-(and IFN-(.[28] In addition, patients with different types of malignancies showed an increase in levels of IL-2, IL-12, TNF-(and IFN-(post-treatment with MGN-3 (data not shown), suggesting that the apparent enhancement in NK cytotoxicity by MGN-3 could be cytokine mediated.

Our work has shown primarily the dramatic effect of MGN-3 on NK cells; however, there is evidence obtained from healthy control subjects that other immune cells, T cells and B cells, have shown increased function post-treatment.[29] In this study, we have found that patients also demonstrated elevation of T and B cell function as evidenced by their proliferation response to different mitogens. This suggests that MGN-3 causes overall immune stimulation.

The preliminary results of the present studies are encouraging enough to warrant continued investigation in multiple clinical trials. ∞

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