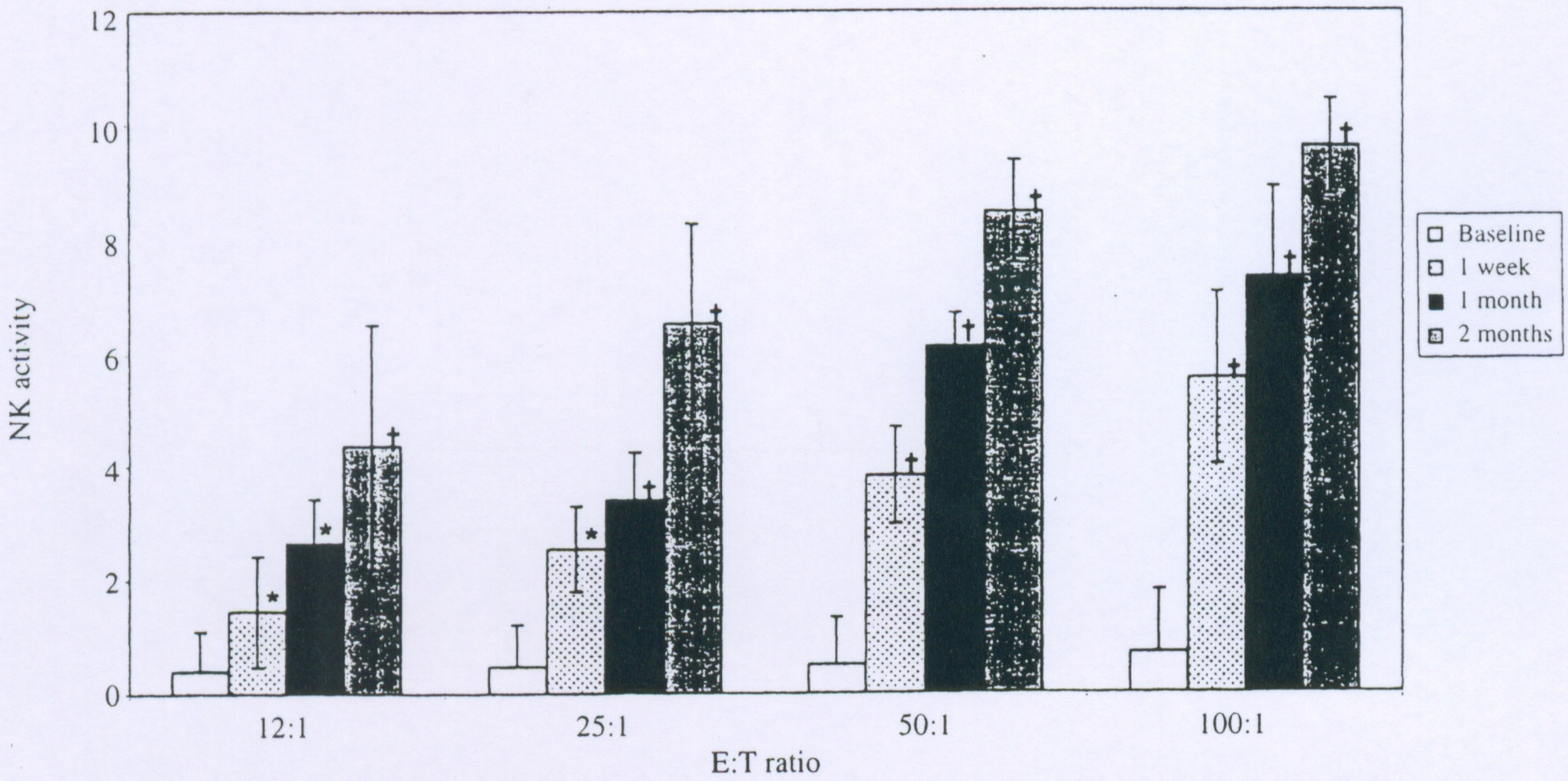

Fig. 5 Action of MGN-3 on natural killer (NK) activity against Raji cells at different effector : target ratios

Activity was examined at baseline, 1 week, 1 month and 2 months.
Mean±SD of eight different individuals. *$p<0.005$, †$p<0.001$

Table 1 Total NK cells posttreatment with MGN-3

Dose (mg/kg/day)	Baseline		1month after treatment	
	$CD56^+/CD3^-$	$CD16^+$	$CD56^+/CD3^-$	$CD16^+$
15	4.7±3.1	not tested	4.3±2.4	not tested
30	6.4±1.8	12.2±1.7	8.2±3.6	12±5.3
45	4.2±2.6	10±2	6.4±2.4	9±2.4

1 week (7.4 lytic units as compared with 0.9 lytic units for baseline). Activity continued to increase and peaked at 2 months after treatment (24.6 lytic units). After discontinuation of treatment, NK activity gradually declined to 10.9 lytic units at 2 weeks and 6.3 lytic units after 1 month. Enhancement of NK activity was detected at all E:T ratios (**Fig. 5**).

4. Quantification of total NK cells

Flow cytometry was used to analyze changes in the total NK cells cultured with BioBran/MGN-3 (at all doses). **Table 1** demonstrates that treatment with BioBran/MGN-3 at different concentrations had no significant effect on the percentages of total NK cell population as identified by $CD56^+$ $CD3^-$ and $CD16^{+-}$ monoclonal antibodies, respectively.

5. Percent conjugates

The binding capacity of NK cells to K562 tumor targets was examined after 1-month treatment (45 mg

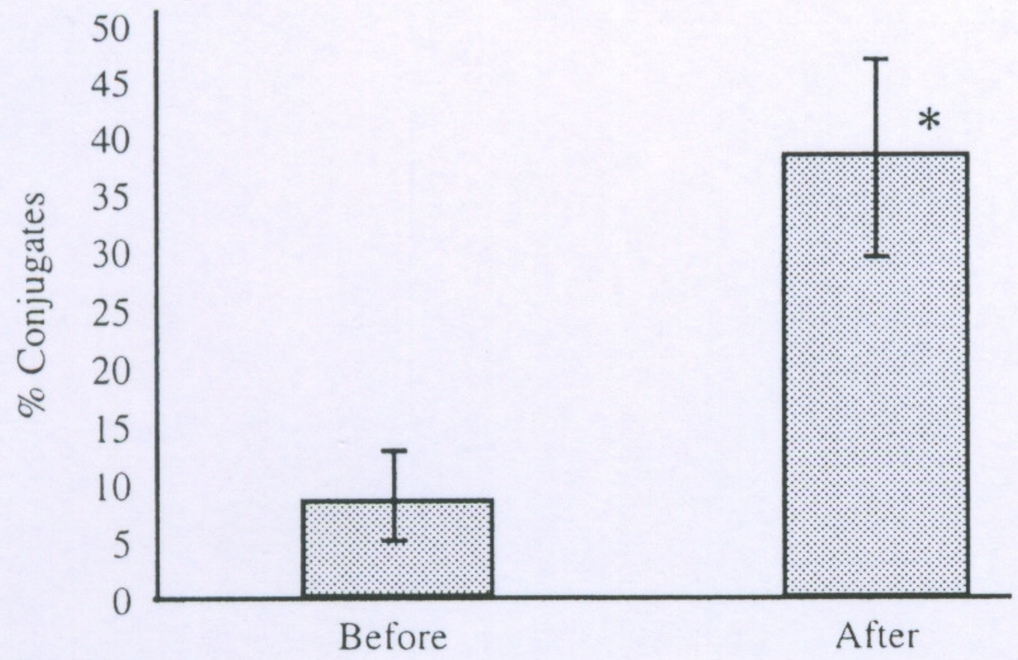

Fig. 6 Percentage of conjugate formation between natural killer (NK) cells and K562 target cells

Conjugate formation was examined at baseline (zero day) and at 1 month, posttreatment.
Mean ±SD of five different individuals. *$p<0.005$

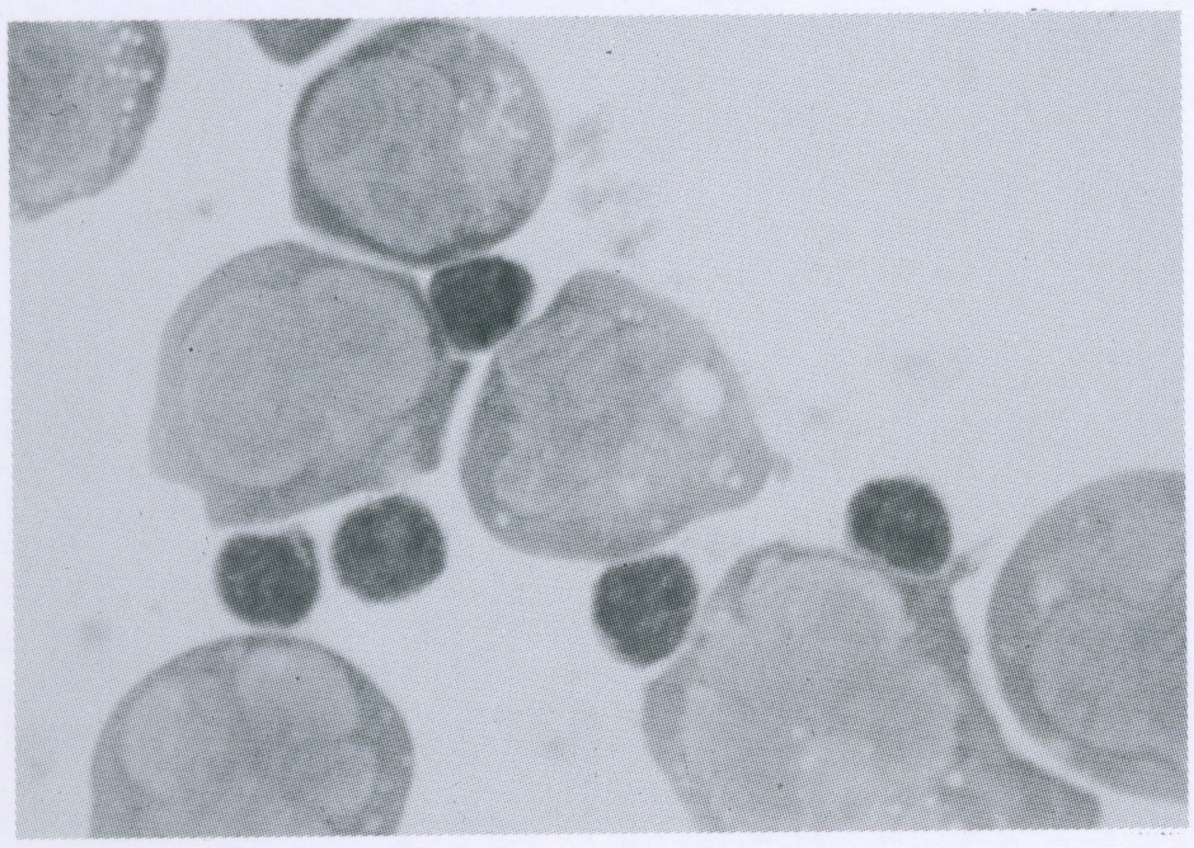

Fig. 7 Natural killer (NK) effector-tumor targets conjugate formation

Peripheral blood lymphocyte from an individual treated with MGN-3 (45 mg/kg/day) for 1 month and cultured with K562.
Note increased binding of NK cells to tumor cells and 1 NK cell binding to 3 tumor cells.
Giemsa stained.

/kg/day) with BioBran/MGN-3. **Figs. 6** and **7** show that the percentage of conjugate formation increased significantly posttreatment (38.5%), compared with baseline (9.4%).

In vitro studies

1. NK activity post culture with BioBran/MGN-3

Culture of PBL with BioBran/MGN-3 for 16 h resulted in an enhancement of NK cell activity that was dose-dependent. BioBran/MGN-3 at a concentration of 25 μg/m*l* increased NK activity by 130%. NK

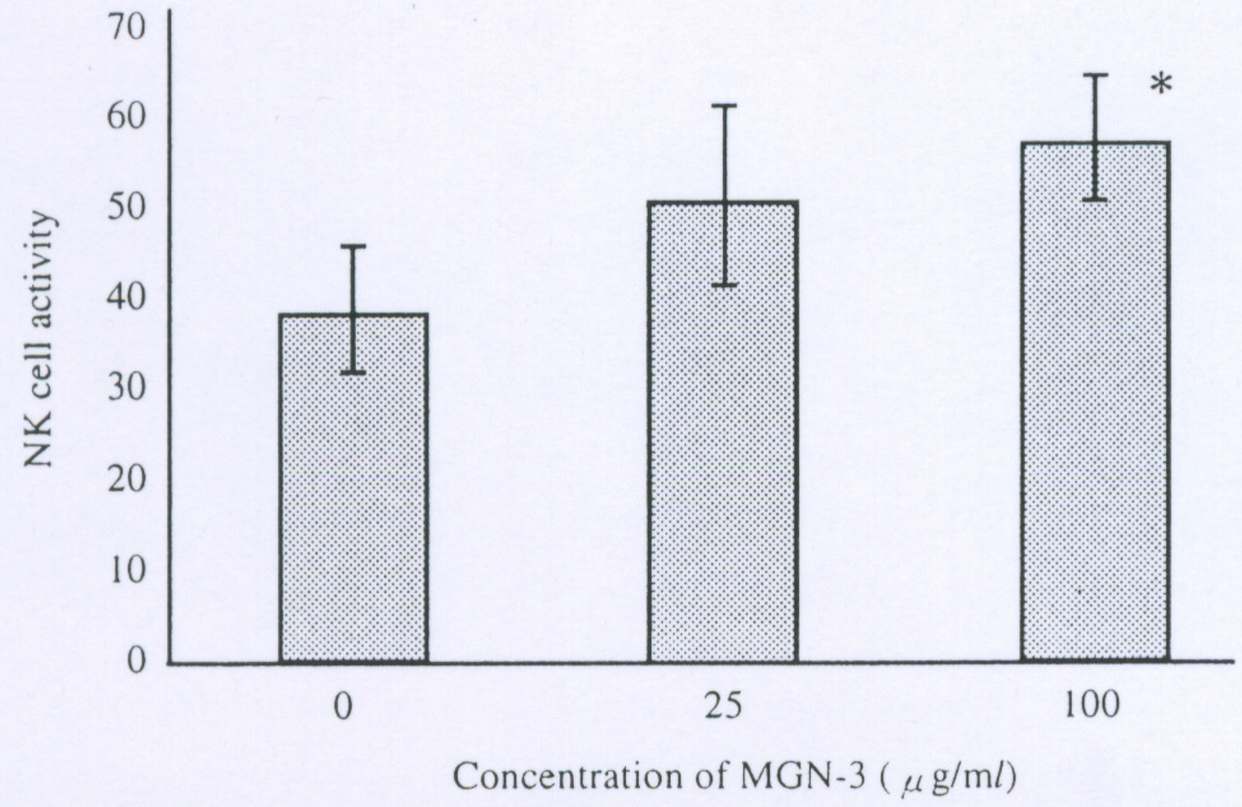

Fig. 8 *In vitro* effect of MGN-3 on natural killer (NK) activity

PBL were cultured for 16 h with MGN-3 at two different concentrations; 25 and 100 μg/m*l*.
NK activity examined at 100:1.
Mean±SD of five different individuals in each cencentration. *$p<0.005$

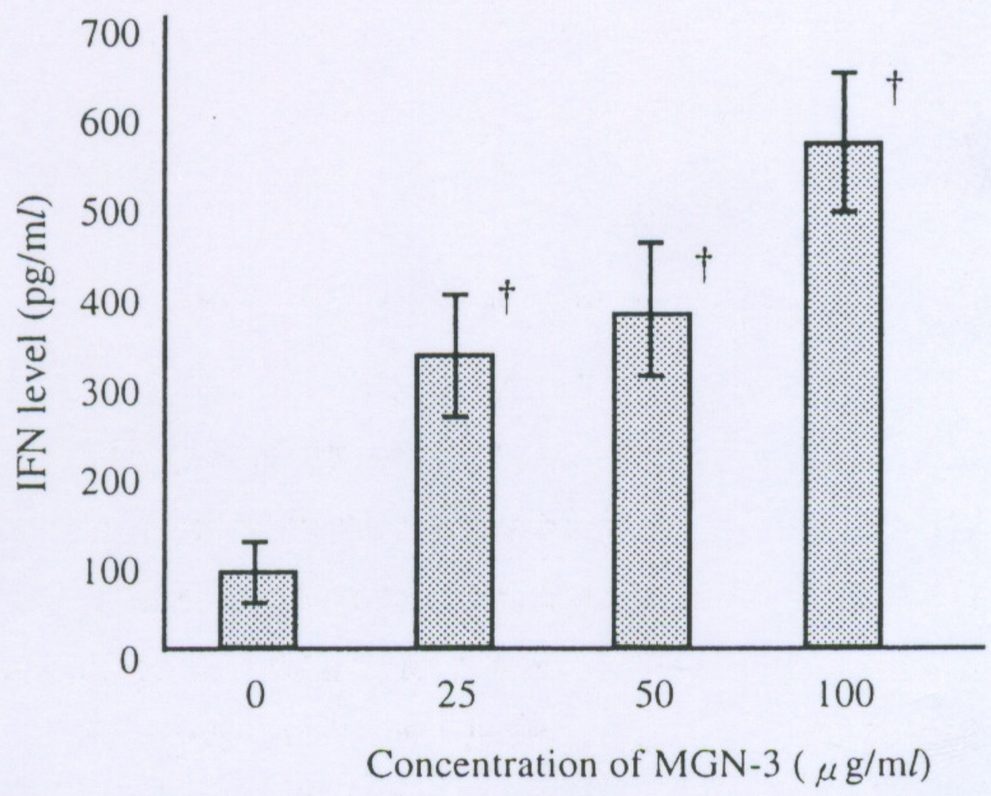

Fig. 9 *In vitro* action of MGN-3 on interferon (IFN)-γ production

PBL were cultured with MGN-3 at different concentrations: 25, 50 and 100 μg/m*l*
Mean±SD of five different individuals in each concentration. † $p<0.001$

activity was further enhanced (150%) when the BioBran/MGN-3 concentration was increased to 100 μg /m*l* (**Fig. 8**).

2. Production of IFN-γ

Fig. 9 summarizes results of the effect of BioBran/MGN-3 on the production of IFN-γ. PBL cultured without BioBran/MGN-3 showed little IFN-γ. BioBran/MGN-3 treatment resulted in a significant increase in IFN-γ production that was dose-dependent. Treated PBL at BioBran/MGN-3 concentrations of 25, 50 and 100 μg/m*l* demonstrated 340, 390 and 580 pg/m*l* of IFN-γ production, respectively.

Discussion

In this study a new biological response modifier, BioBran/MGN-3, was examined for its ability to enhance human NK cell activity *in vivo* and *in vitro*. The results showed that BioBran/MGN-3 is a potent biological response modifier (BRM) as manifested by significant induction of NK cytotoxicity upon BioBran/MGN-3 ingestion—the augmentory action was detected not only against sensitive K562 targets, but also against cell lines known to be highly resistant to NK activity, such as Raji, a Burkitt lymphoma, and Daudi another Burkitt cell line (data not shown). That BioBran/MGN-3 potent biological response modification is also shown by the decline of NK activity after discontinuation of treatment, as well as an increase in NK activity postculture with BioBran/MGN-3 for 16 h. The immunomodulatory function of BioBran/MGN-3 depended on two factors. Firstly, the concentration. As shown in **Figs. 2** and **3**, BioBran /MGN-3 increases NK activity in a dose-dependent manner. BioBran/MGN-3 at 15 mg/kg/day demonstrated an increase in NK cytotoxicity against K562 tumor cells after 1-month treatment, while higher concentrations of 30 and 45 mg/kg/day resulted in a significant induction of NK activity after 1-week treatment. Activity continued to increase and peaked at 2 months. Secondly the augmentory function of BioBran /MGN-3 is differential among subjects.

The type of BioBran/MGN-3-activated killer cells is not fully defined, but it may be similar to the IL-2-induced LAK cell phenomenon with respect to heterogeneity. Both types of cells are associated with increased conjugate formation between peripheral blood mononuclear cells and tumor targets[12]. Consequently, various effector cells may be involved. While NK cells may represent a major component of BioBran/MGN-3-activated cells, as seen in increased lysis of the classical NK-sensitive K562, lysis of resistant tumor cell lines (Raji and Daudi) may involve either NK cells and/or other effector cells having anti-cancer activity such as cytotoxic T-lymphocytes. Another similarity between the two induced cell types (BioBran/MGN-3 and IL-2) is the upregulation of $CD25^+$ and $CD69^+$ receptors[20].

The mechanism by which BioBran/MGN-3 boosts NK activity appears to be via its ability to induce IFN production. Most agents that can activate NK cells appear to be acting by their ability to induce, either *in vivo* or *in vitro*, IFN production[21]. Several bacterial immunomodulators, such as *bacilli* Calmette-Guérin and *Corynebacterium parvum*, have been found to induce IFN and augment NK activity[22]. Among the IFN stimulators studied so far, bacteria appear to have specificity for induction in large granular lymphocytes (LGLs) alone[23]. Other biological agents can induce rapid IFN production from LGLs and it is the production of IFN which produces the self-activation of NK activity in LGLs. Our work shows that BioBran /MGN-3 (25-100 μg/m*l*) induces PBL IFN- γ production (340-580 pg/m*l*) in culture. This suggests that BioBran/MGN-3 enhances IFN production, which in turn may augment NK activity. Our work also shows the ability of BioBran/MGN-3 to stimulate the production of other cytokines such as TNF- α [20].

It is possible that the increase in NK cell activity from ingestion of BioBran/MGN-3 may be due to an increase in the activity per cell and not due to an increase in the actual NK cell number. This was confirmed by noting that lower E:T ratios (12:1 and 25:1) achieved a maximum induction in NK activity (in comparison with 100:1), while flow cytometry analysis showed no significant changes in total NK cells after treatment (compared with baseline values). The results also showed an increase in the binding capacity of BioBran/MGN-3-cultured effector cells to tumor targets (four fold). The increased number of NK cells in conjugates with no increase in NK cell numbers in PBLs after treatment suggests that BioBran

/MGN-3 increases the binding capacity of NK cells as well as other cell populations to tumor targets. It is well established that the NK-tumor cell interaction proceeds through several discrete stages[24] including E: T cell recognition and binding; triggering and activation of the NK cells; release of the granules from NK cells and binding to receptor sites on the tumor cell surface; and tumor target cell death. This binding process is a key event in the activation of NK cells.

The anticancer activity of natural BRMs may involve activation of different arms of the immune system. For example, killed bacteria (*Corynebacterium parvum* and *Bacilli* Calmette-Guérin) may involve NK activation[25-28]; lectins from plants such as *griffonia simplicflial-β4* isolectin may involve activation of macrophage[29]; bitter melon protein may involve neutrophil activation[30]; and lentinan isolated from edible mushroom, *Lentius edodes* (Berk) Sing. possesses augmentory effects on NK and killer T-cell activity[31-35]. With respect to BioBran/MGN-3, activated cells are mainly NK cells although cytotoxic T-lymphocytes may be involved in the activation process. Extracted hemicellulose from rice bran fiber has known unique biological effects; for example, α-glucan from rice bran shows potent antitumor activity in mice[36], and arabinose and xylose from rice bran fiber shows defensive effects against bis(tri-*n*-tributyltin)oxide-induced thymic atrophy in rats[37]. Unprocessed rice bran fiber and cholestyramine have been observed to increase peripheral blood leukocytes in humans[38]. The product used in this study is a modified arabinoxylan from rice bran. Modification occurs by enzymatic treatment with an extract from *Hyphomycetes mycelia*, which shows a high augmentory effect on human NK activity *in vivo* and *in vitro*, as well as in mice and rats (data not shown). BioBran/MGN-3 was examined for toxicity using blood chemistry analysis utilizing Panel 20 which includes liver enzymes (SGOT and SGPT). After 1-month treatment, no abnormalities were detected for these parameters as compared with baseline. We conclude that the high augmentory effect of BioBran/MGN-3 and the absence of notable side-effects make this material a promising immunotherapeutic agent for treating cancer patients. Preliminary studies in this regard are very encouraging. NK immunomodulatory function by BioBran/MGN-3 was detected in 27 patients afflicted with different types of malignancies[39], additionally, the relationship between the immunomodulatory and anticancer properties of BioBran/MGN-3 was assayed in five patients with breast cancer[40].

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