Enhancement of Human Natural Killer Cell Activity by Modified Arabinoxylan from Rice Bran (BioBran/MGN-3)

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Summary

Modified Arabinoxylan from Rice Bran (BioBran/MGN-3) was examined for its augmentory effect on human NK (NK) cell activity *in vivo* and *in vitro*. Twenty-four individuals were given BioBran/MGN-3 orally at three different concentrations: 15, 30 and 45 mg/kg/day for 2 months. Peripheral blood lymphocyte-NK cell activity was tested by ^{51}Cr release assay against K562 and Raji tumor cells at 1 week, 1 month and 2 months post-treatment and results were compared with baseline NK activity. Treatment with BioBran/MGN-3 enhanced NK activity against K562 tumor cells at all concentrations used. In a dose-dependent manner, BioBran/MGN-3 at 15 mg/kg/day increased NK activity after 1 month posttreatment (twofold over control value), while significant induction of NK activity at 30 mg/kg/day was detected as early as 1 week posttreatment (three times control value). NK cell activity continued to increase with continuation of treatment and peaked (fivefold) at 2 months (end of treatment period). Increasing the concentration to 45 mg/kg/day showed similar trends in NK activity, however the magnitude in values was higher

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than for 30 mg/kg/day. After discontinuation of treatment, NK activity declined and returned to baseline value (14 lytic units) at 1 month. Enhanced NK activity was associated with an increase in the cytotoxic reactivity against the resistant Raji cell line. BioBran/MGN-3 at 45 mg/kg/day showed a significant increase in NK activity after 1 week (eightfold) and peaked at 2 months posttreatment (27 times that of baseline). Culture of peripheral blood lymphocytes (PBL) with BioBran/MGN-3 for 16 h demonstrated a 1.3 to 1.5 times increase in NK activity over control value. The mechanism by which BioBran/MGN-3 increases NK activity was examined and showed no change in cluster of differentiation (CD) 16^+ and $CD56^+$, $CD3^-$ of BioBran/MGN-3-activated NK cells as compared with baseline value; a four fold increase in the binding capacity of NK to tumor cell targets as compared with baseline value; and a significant increase in the production of interferon- γ (340-580 pg/m*l*) postculture of PBL with BioBran/MGN-3 at concentrations of 25-100 μg/m*l*. Thus, BioBran/MGN-3 seems to act as a potent immunomodulator causing augmentation of NK cell activity, and with the absence of notable side-effects, BioBran/MGN-3 could be used as a new biological response modifier (BRM) having possible therapeutic effects against cancer.

Introduction

There is an increasing body of evidence implicating the NK phenomenon as a discrete subpopulation of lymphocytes capable of mediating lysis of a variety of tumor target cells regardless of major histocompatibility components[1-5]. For example, it has been shown that patients with Chediak-Highashi syndrome who display selective natural killer (NK) deficiency are also prone to develop lymphoproliferative disorders[6]; defective NK cell activity predisposes patients to develop lymphomas[7]; genetic abnormalities associated with impaired NK activity may predispose animals to develop lymphoid malignancies[8, 9]; adoptive transfer of NK clone to beige mice provided resistance to radiation-induced thymic leukemia[10]; and data from our laboratory show that female mice that have lower NK activity compared with males are also more susceptible to tumor development than males[2]. These data taken together provide compelling evidence supporting the role of NK cells in host surveillance.

The various immunological functions of NK cells make them prime candidates as therapeutic agents. Interleukin-2 (IL-2) has been shown to boost NK activity in peripheral blood, both *in vitro* and *in vivo*. These activated NK cells have broader antitumor cytolytic capabilities, including lysis of fresh, uncultured, human tumor cells as well as a wide variety of tumor cell lines[11, 12]. Activated NK cells are defined as lymphokine activated killer (LAK) cells. Together, IL-2 and LAK cells have been used as adoptive immunotherapy against cancer[13]. However, although IL-2 has been reported to have promising results when administered to patients with advanced malignancies[14-16], the overall clinical success of IL-2 has been limited due to its severe side-effects.

In this study, we tested the ability of a new immunomodulator called BioBran/MGN-3, an enzymatically modified arabinoxylan from rice bran with no notable side-effects, to enhance human NK cell activity both *in vivo* and in *vitro*.

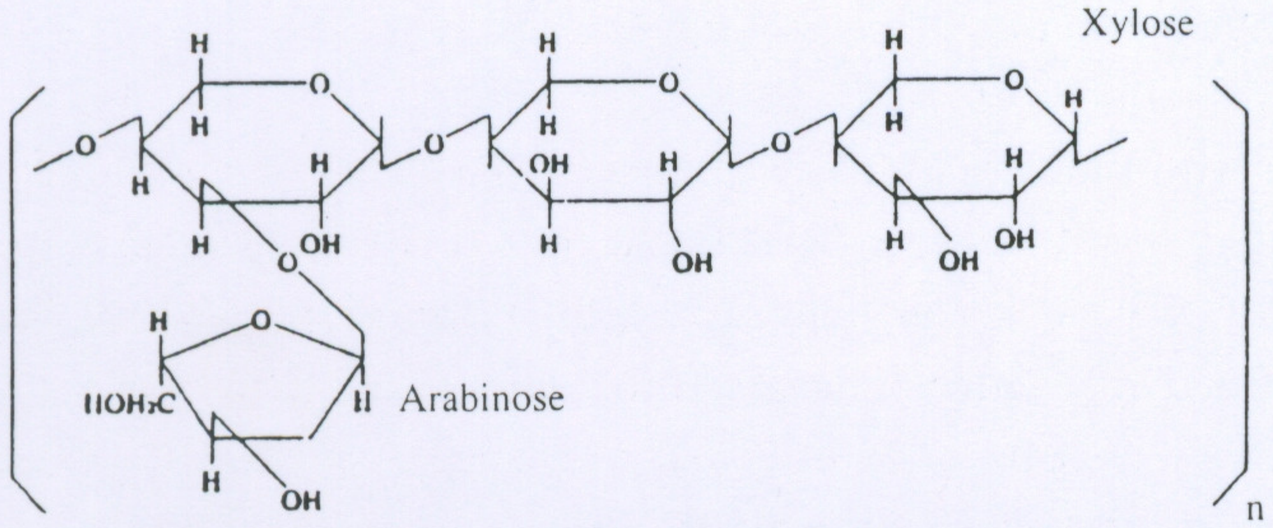

Fig.1 Main chemical structure of MGN-3.
It is an arabinoxylan with a xylose in its main chain and an arabinose polymer in its side chain

Materials and methods

1. Human subjects and treatment with BioBran/MGN-3

Twenty four healthy control subjects (15 females and nine males) participated in this study. Subjects ranged in age from 20-46 years, with a mean age of 34 years. They had not ingested any medications or vitamins for at least 2 weeks prior to their participation. Moreover, the subjects did not have a history of chronic diseases. During the course of the experiment that extended for 2 months, the women were not menstruating nor taking oral contraceptives—both of which can affect the level of NK cell activity.

Subjects were divided into three groups of eight individuals and were given BioBran/MGN-3 orally for 2 months. Group 1, group 2 and group 3 received BioBran/MGN-3 in doses of 15 mg/kg/day, 30 mg/kg/day and 45 mg/kg/day, respectively. Twenty m*l* of blood was drawn from each individual before treatment (day zero) and at different intervals posttreatment—1week, 1month and 2 months.

2. BioBran/MGN-3

BioBran/MGN-3 is an arabinoxylan from rice bran, a polysaccharide that contains β-1,4-xylopyranose hemicellulose, that has been enzymatically treated with an extract from hyphomycetes mycelia and was prepared in 500 mg tablets (Daiwa Pharmaceutical Co., Ltd., Tokyo, Japan). **Fig.1** shows the main chemical structure of BioBran/MGN-3.

3. Complete medium

Complete medium consisted of RPMI-1640 supplemented with 10% fetal calf serum and 1% antibiotic (100 U penicillin and 100 μg/ml streptomycin).

4. Tumor cell lines

Two human tumor cell lines were used as targets: sensitive K562, an erythroleukemic cell line, and resistant Raji, a Burkett cell lymphoma.

5. Preparation of peripheral blood lymphocytes (PBLs)

PBLs were prepared from fresh heparinized peripheral venous blood by Ficoll-Hypaque density gradient centrifugation. Cells were washed three times with Hanks balanced salt solution and resuspended to 10×

10^6 cells/ml in complete medium.

6. Culture of PBLs with BioBran/MGN-3

PBLs from six healthy control subjects were adjusted to 1×10^6 cells/m*l* in complete medium and cultured with BioBran/MGN-3 at concentrations of 25 and 100 μg/m*l* for 16 h. PBLs were then washed twice and examined for NK activity at effector: target (E:T) ratios of 100: 1.

7. ^{51}Cr-release assay for measuring NK activity

NK activity was measured by a standard 4-h ^{51}Cr-release assay. Briefly, 1×10^4 ^{51}Cr-labeled tumor target cells in 0.1 m*l* complete medium 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-h incubation (37℃), the plates were centrifuged (1,400 rpm for 5 min) and 0.1 m*l* of supernatant from each well was collected and counted in a gamma counter (Beckmann G50, Beckmann Instruments).

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

$$\%\ \text{lysis} = \frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Total release} - \text{Spontaneous release}} \times 100$$

Spontaneous release from target cells was no more than 8-10% of total release. Total release was measured by adding 0.1 m*l* Triton X-100 (Sigma Chemical Co.) to designated wells. Lytic units were calculated from effector titration curves with one lytic unit defined as the number of effector cells required to achieve 30% lysis for K562 and 8% lysis for Raji cells. This serial performance of NK cytotoxic assay was based on criteria for a reproducible NK test and was intended to help minimize associated errors[17].

8. NK subpopulations

NK cell subset enumeration was carried out with PBLs from individuals for baseline and after 1 month treatment with BioBran/MGN-3. A single laser flow cytometer (Epics Profile, Coulter Epics, Inc., Hialeah, FL), which discriminates forward and right-angle light scatter, as well as two colors, was used with a software package (Quad Stat, Coulter). Mononuclear cell populations were determined by two-color direct immunofluorescence, by using a whole-blood staining technique with the appropriate monclonal antibody and flow cytometry[18]. Fluorescein isothiocyanate-(FITC, CD3-FITC), or phycerythrin (PE, CD56-PE)-conjugated monclonal antibodies (Coulter Immunology) were selected for the determination of NK cell subsets. To monitor lymphocyte markers, bitmaps were set on the lymphocyte population of the forward-angle light scatter versus a 90° light scatter histogram. The percentage of positively stained cells for each marker, as well as the percentage of double-stained lymphocyte positive for the respective surface markers, were determined.

9. Effector: target cell conjugate assay

The capacity of NK effector cells from different treatment conditions to form conjugates with K562 targets was measured as previously described[19]. Briefly, 1×10^5 lymphocytes were incubated with 1×10^6 K562 target cells in 1.0 m*l* complete medium in 12×75 mm glass tubes, pelleted at 130 G for 5 min and incubated for 1 h at 4℃. The pellets were gently resuspended and cytocentrifuged. Smears were prepared

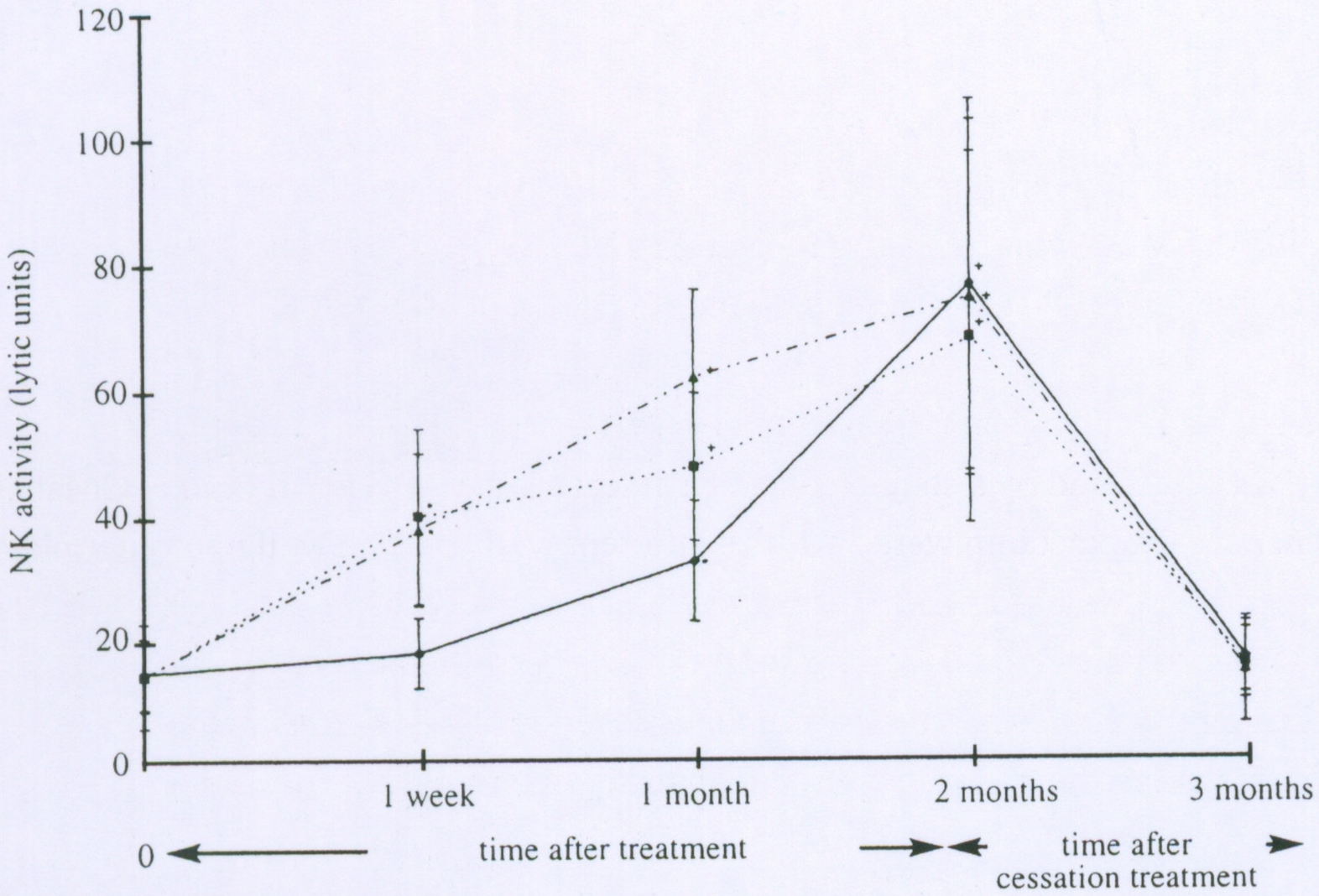

Fig. 2 Dose range and time course of natural killer (NK) cell activation by MGN-3 against K562 tumor cells

Activity is expressed as number of lytic units at 30%.
MGN-3 at 15 mg/kg/day (●), 30 mg/kg/day (◆) and 45 mg/kg/day (▲).
NK activity was examined at baseline zero day, 1 week, 1 month, 2 months and 3months.
Means±SD of eight different individuals in each dose. †$p<0.001$

using a cytospin cytocentrifuge (Shandon Instruments) and stained with Giemsa. The percentage of conjugates was determined by counting 200 lymphocytes (bound and free) in triplicate samples.

10. Interferon-γ production

Peripheral blood mononuclear cells from five individuals were collected, adjusted to 10×10^6 cells/m*l*, and cultured with BioBran/MGN-3 at different concentrations (0, 5, 50 and 100 μg/m*l*) for 16 h. Culture supernatants were then collected and analyzed for interferon (IFN)-γ using enzyme-linked immunoabsorbent assay.

11. Blood chemistry

Five individuals were given BioBran/MGN-3 (45 mg/kg/day) for 1 month. At day zero and the end of treatment, 5 m*l* of blood was drawn from each subject for blood chemistry analysis using Panel 20 which includes liver enzymes (serum glutamic-oxaloacetic transaminase[SGOT], and serum pyruvic-oxaloacetic transaminase [SGPT]).

12. Statistical analysis

The statistical analysis software (SAS) procedure of analysis of variance was used to examine the effects before and after treatment with BioBran/MGN-3 in different determinations; the effect of changing the ratios between E:T cells; and the interaction of the two effects.

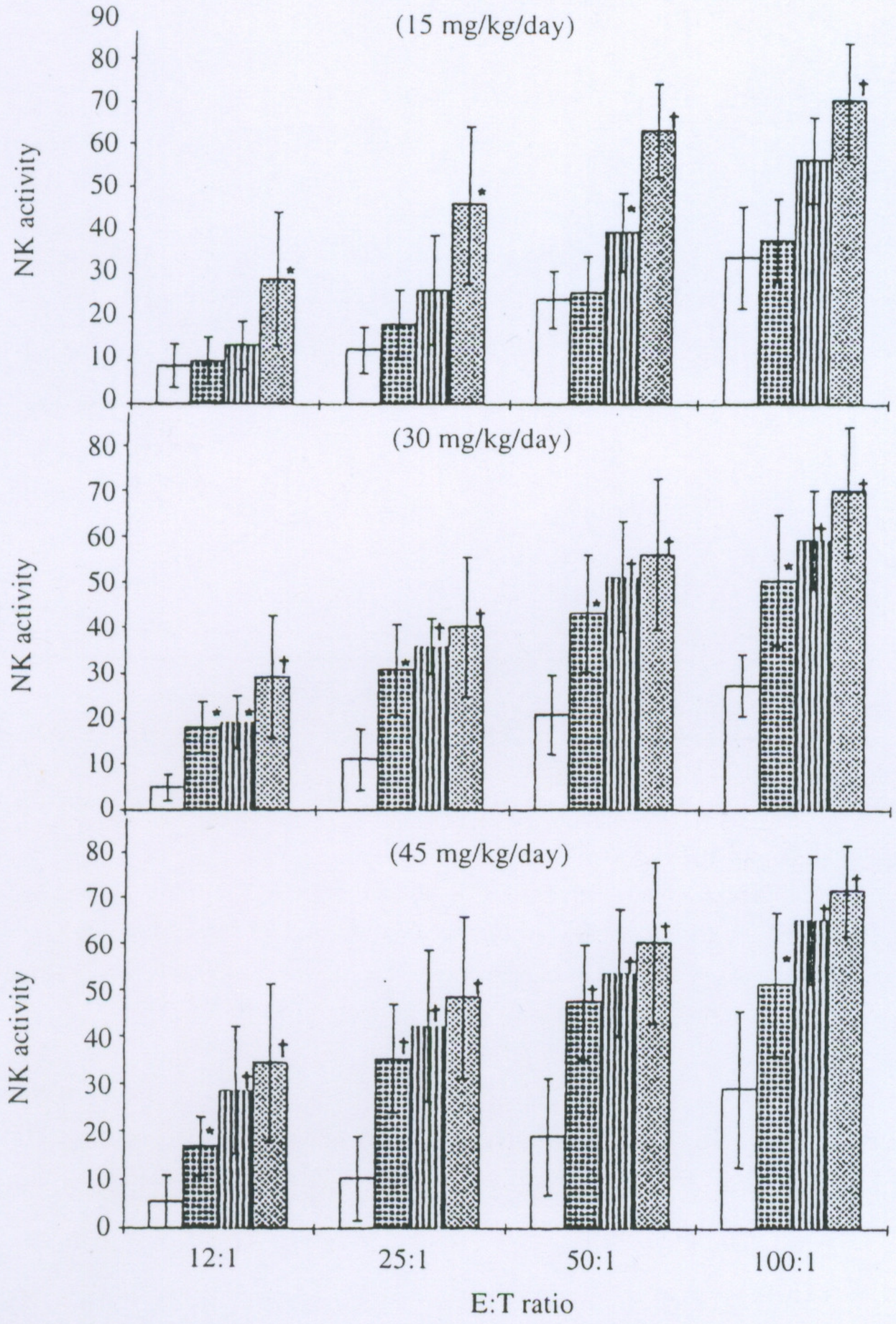

Fig. 3 Natural killer (NK) cell activation by MGN-3 against K562 at different effector: target (E:T) ratios
MGN-3 at 15, 30 and 45 mg/kg/day.
NK activity was examined at baseline, 1 week, 1 month, 2 months and 3 months posttreatment.
Mean±SD of eight different individuals in each dose. *$p<0.005$, †$p<0.001$

Results

1. Augmentation of NK activity by ingestion of BioBran/MGN-3

Results of NK activity enhancement by ingestion of BioBran/MGN-3 were expressed with respect to dose-response and time-course of changes in NK activity; E:T ratios; quantification of NK cells; and percentage of conjugate formation.

To test the reproduceability of the results, the present study was repeated with the same individuals after 4-6 month intervals, and by assaying NK function at different E: T ratios (12:1, 25:1, 50:1 and 100:1). These results were similar with regard to enhancement of NK function by BioBran/MGN-3 (second trial

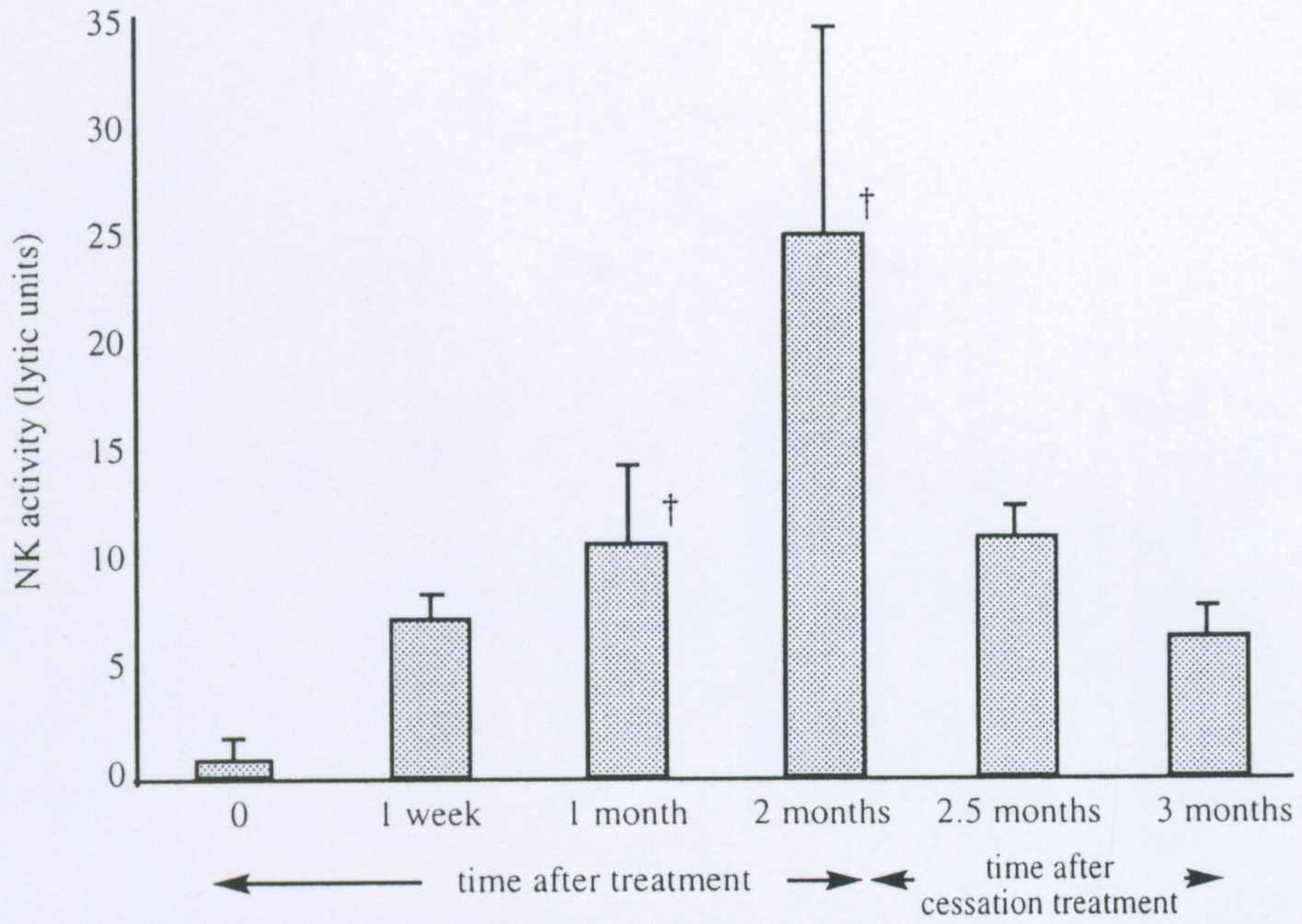

Fig. 4 Time course of natural killer (NK) activation by MGN-3 (45 mg/kg/day) against Raji cells

Activity was expressed as number of lytic units at 8% NK activity at baseline (zero day), 1week, 1month, 2 months, 2.5 months and 3 months.

Mean ±SD of eight different individuals. † $p<0.001$

data not shown).

2. Effect against K562

Dose-range and time-course of NK activation by BioBran/MGN-3 was examined. **Fig. 2** shows the augmenting effect of BioBran/MGN-3 on NK cell activity against K562 tumor cells (activity expressed as number of lytic units). BioBran/MGN-3 at a dose of 15 mg/kg/day showed no changes at 1 week as compared with baseline values, however a twofold increase in NK cytotoxicity was detected after 1 month of treatment. Increasing the dose to 30 mg/kg/day resulted in a significant enhancement of NK activity (310% over baseline) that was detected as early as 1 week. The activity of NK cells continued to increase with continuation of treatment. The peak response was observed at the end of the treatment period (2 months) where NK activity increased fivefold (68.2 lytic units in comparison with 13.6 lytic units for baseline). Increasing the dose to 45 mg/kg/day demonstrated a similar increasing trend in NK activity but the values were higher in magnitude than those for 30 mg/kg/day. Discontinuation of treatment resulted in a decline of NK activity and at 1 month, NK activity returned to baseline. **Fig. 3** shows NK cell activity against K562 at different E:T ratios. All subjects demonstrated enhancement in their NK activity, however, there was a differential response among different individuals toward the augmentory function by BioBran/MGN-3. An increse in activity was detected for all E:T ratios, but the level of enhancement was higher at lower E:T ratios (12:1, 25:1) than at higher ratios (50:1, 100:1).

3. Effect against Raji cells

Fig. 4 summarizes NK activation against Raji cells. The augmentory effect of BioBran/MGN-3 was examined at 45 mg/kg/day taken for 2 months. Activated NK cells were able to kill Raji cells as early as