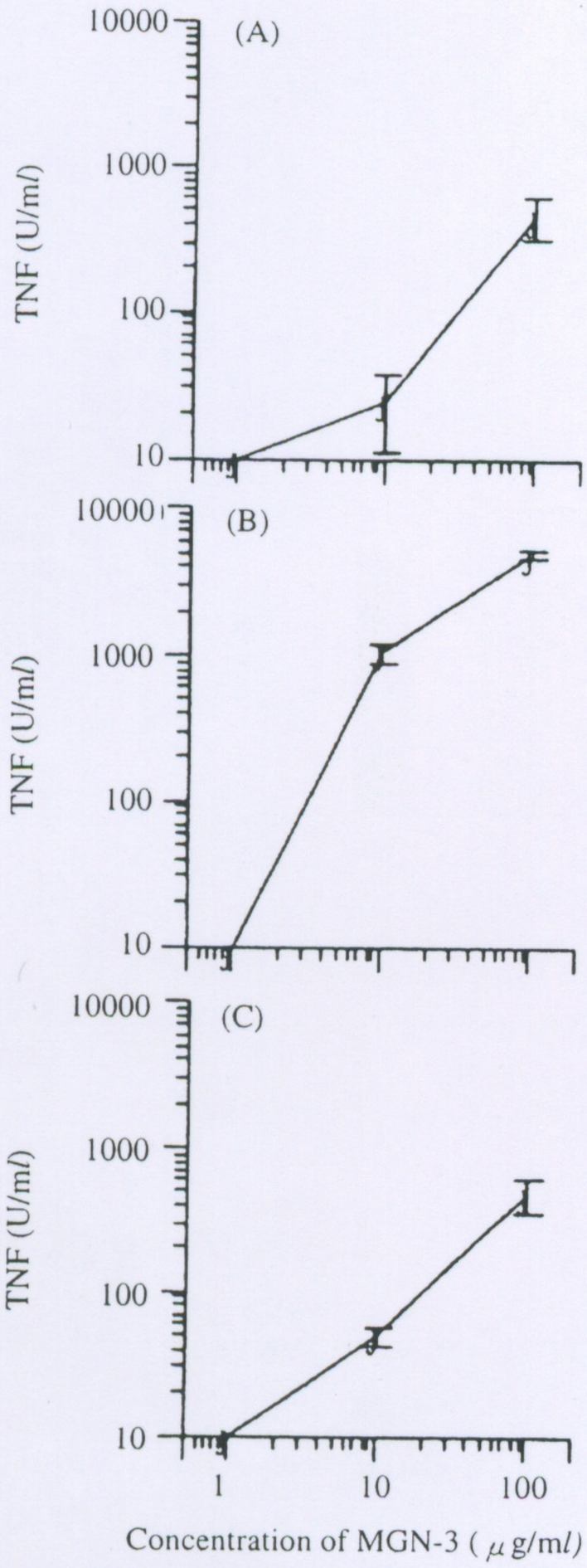

Fig. 4 Effect of MGN-3 on production of TNF- α by three macrophage models.

Murine P-M ϕ (A), murine RAW264.7 cells (B) and human U937 cells (C) were stimulated with MGN-3 (1, 10, 100 μg /ml).

The culture supernatant obtained at 4 h after the stimulation was subjected to determination of TNF- α by bioassay.

The data are the means±standard errors of the mean (SEM) of triplicate samples.

A representative result obtained from three independent experiments is shown.

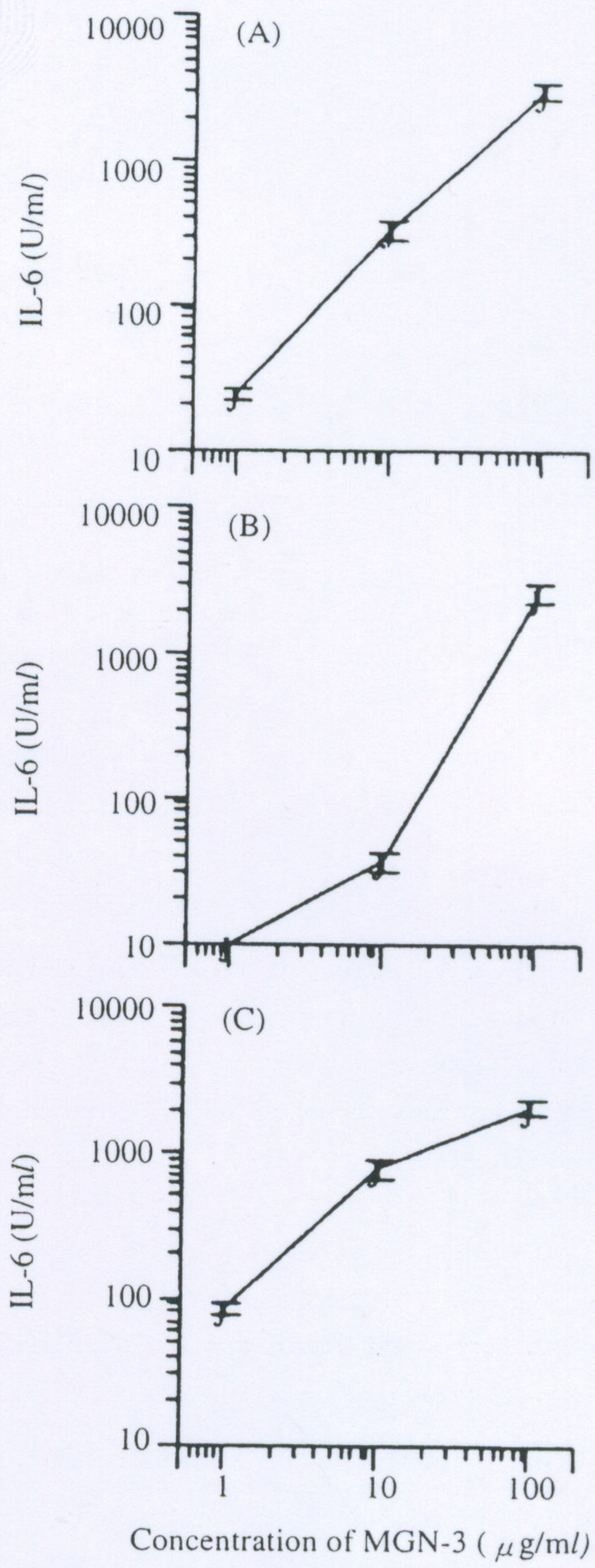

Fig. 5 Effect of MGN-3 on production of IL-6 by three macrophage models.

Murine P-M ϕ (A), murine RAW264.7 cells (B) and human U937 cells (C) were stimulated with MGN-3 (1, 10, 100 μg /ml).

The culture supernatant obtained at 48 hr after the stimulation was subjected to determination of IL-6 by bioassay.

The data are the means±standard errors of the mean (SEM) of triplicate samples.

A representative result obtained from three independent experiments is shown.

(10-80 U/ml) was detected post treatment of the three cell types with 1 μg/ml of BioBran/MGN-3, and augmented when increasing the concentration of BioBran/MGN-3 at 10 and 100 μg/ml. Response of human U937 cells to BioBran/MGN-3 was the highest among the three models.

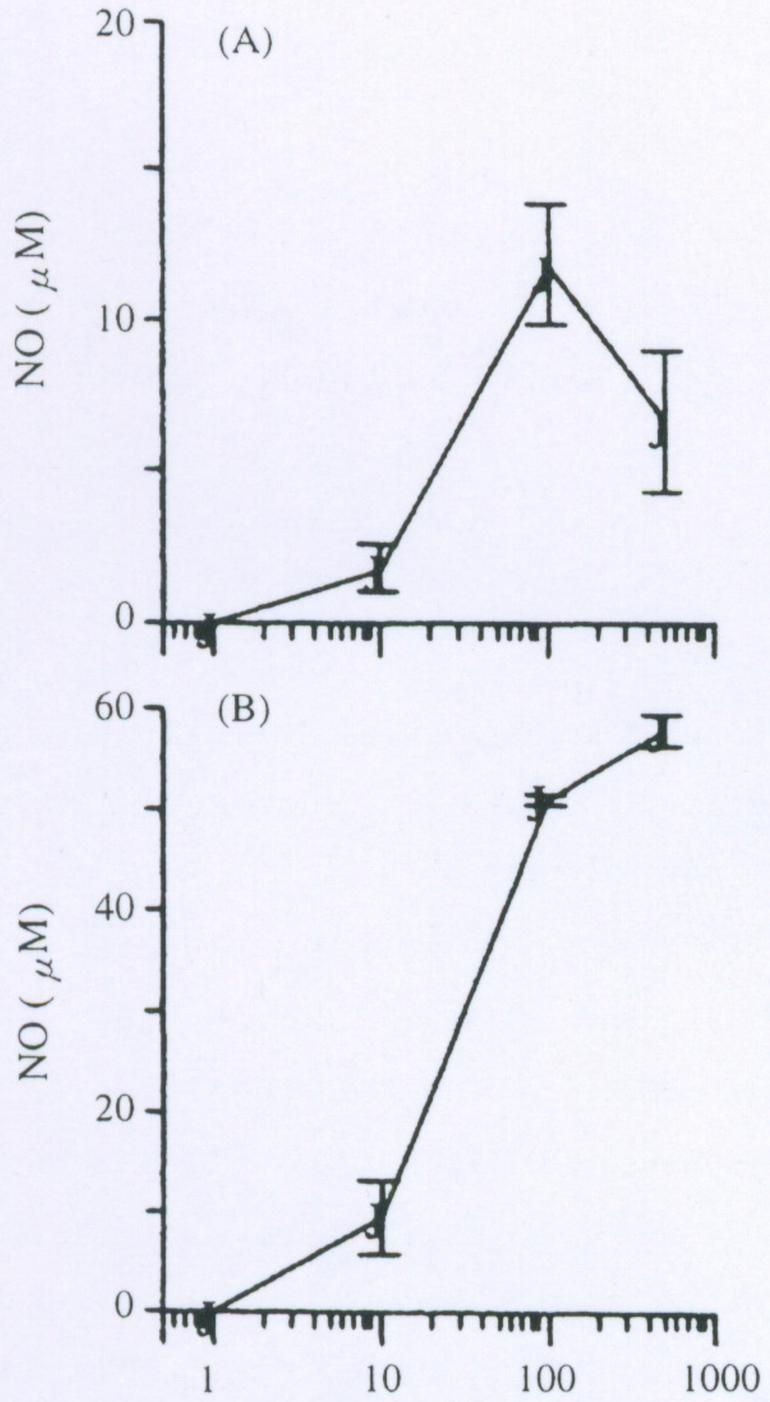

Fig. 6 Effect of MGN-3 on production of NO in two macrophage models.

Murine P-M ϕ (A) and murine RAW264.7 cells (B) were stimulated with MGN-3 (1, 10, 100, 500 μg/m*l*).
The culture supernatant obtained at 48 hr after the stimulation was subjected to determination of NO by Griess reagent.
The data are the means ±standard errors of the mean (SEM) of triplicate samples.
A representative result obtained from three independent experiments is shown.

8. Production of NO

Production of NO in response to BioBran/MGN-3 was examined in both murine macrophage models; murine P-M ϕ and RAW264.7 cells (**Fig.6**). Production of NO was detected post treatment of both cells with 10 μg/m*l* of BioBran/MGN-3, however, increasing the concentration to 100 μg/m*l* resulted in a significant production of NO in both types of cells, but RAW264.7 cells were more responsive than P-M ϕ and produced about 5 fold of NO at 100 μg/m*l* of BioBran/MGN-3. Production of NO by human U937 cells was examined. Neither BioBran/MGN-3 nor LPS induced NO production by U937 cells. It is generally known that human cells are slowly responsive in the production of NO, following exposure to various immuno-stimulants.

9. Action of high dose of BioBran/MGN-3 (500 μg/m*l*) cell apoptosis

U937 cells were cultured with BioBran/MGN-3 at concentration of 500 μg/m*l* for 2 days and cell survival was determined. The results show that the background of dead cells was 2%. Co-culture of cells with BioBran/MGN-3 resulted in an insignificant change in cell survival as compared with the background of

cells.

10. NO production

Action of high dose of BioBran/MGN-3(500 μg/m*l*) on NO production was examined using murine P-M ϕ and RAW264.7 cells. Data in **Fig.6** show that treatment with BioBran/MGN-3 caused only a slight fluctuation in the level of NO by both types of cells as compared with 100 μg/m*l*.

Discussion

BioBran/MGN-3 is an arabinoxylan extracted from rice bran that is treated enzymatically with an extract from shiitake mushroom[14]. The various biological functions of BioBran/MGN-3, such as anti-HIV activity, increased T and B cell proliferation[14], and NK immunomodulatory function[15, 28] have been previously demonstrated. In this paper, we examined the ability of BioBran/MGN-3 to activate another arm of the immune system, the macrophages. We tested many important properties associated with macrophages activation, such as production of cytokines (TNF-α and IL-6) and NO from 3 different macrophage models. Data of the present study demonstrated that BioBran/MGN-3 significantly enhances macrophage phagocytic activity. The effect depends on the type of macrophages and the BioBran/MGN-3 dose applied. In this study, we used 100 and 500 μg/m*l* of BioBran/MGN-3 for studying the effects of this agent on phagocytosis and spreading, while we used a much smaller concentration (1-100 μg/m*l*) for studying its effects on cytokine and NO production. Earlier studies demonstrated that concentrations $\geqq$100 μg/m*l* of BioBran/MGN-3 are necessary to cause significant activation of cells, such as NK cells[15, 16] and sensitization of leukemia HUT 78 cells to anti-CD95 antibody-induced apoptosis[20], while induction of cytokines such as IFN-γ secretion by human PBL was noticeable at 1-10 μg/m*l* of BioBran/MGN-3[16]. Data from this study showed that treatment with BioBran/MGN-3 at a higher concentration of 500 μg/m*l* resulted in an insignificant change in NO production, as compared with 100 μg/m*l* (**Fig.6**). In addition, this concentration did not cause apoptosis of U937 cells. Several investigators examined the enhancement of macrophage phagocytosis by natural agents; these include ginsan[2], Panax ginseng[29], shi-ka-ron and Chinese herbs[30], Perilla frutescens var. crispa[31], Platycodon grandiflorum[32, 33], and fermented papaya preparation[34].

BioBran/MGN-3-induce macrophage phagocytic activity was paralleled with an increase in cytokine secretion. Significant levels of TNF-α secretion were triggered by the presence of BioBran/MGN-3 in a dose dependent manner. TNF-α is a multifunctional cytokine. It is produced by macrophage in order to destroy microorganisms[35-37] and is involved in the early phase of the cytokine cascade; it induces NO and IL-6 production[38]. In addition, TNF-α promotes the generation of T cell-mediated antitumor cytotoxicity[39], the generation of lymphokine-activated killer cells (LAK)[40], and also regulates interleukin-2-mediated activation of immature human NK cells[41-43]. Previously we have reported that BioBran/MGN-3 is a potent inducer of TNF-α from human peripheral blood lymphocytes (PBL) and also increases levels of TNF-α from NK cells[16]; this may suggest a possible involvement of this cytokine in the enhancement of NK activity in healthy subjects[15] and in cancer patients[28, 44, 45] post treatment with BioBran/MGN-3.

Interleukin-6 (IL-6) is another cytokine produced by macrophage in order to destroy microorganisms. Data in the present study reveals a consistent increase in the level of IL-6, post treatment with BioBran /MGN-3. The effect, in a dose dependent manner, was noted in the three macrophage models

being studied. IL-6 has multiple biological activities in various cell types: IL-6 synergizes with IL-1 and promotes T cell proliferation, T helper cells differentiation, and the development of T cell-mediated cytotoxicity by $CD8^+$ cells[46, 47].

It has been shown also that nitric oxide (NO) is produced by activated macrophages. NO is synthesized endogenously by the enzyme nitric oxide synthase (NOS) in activated macrophages. It contributes to immune function, and in particular to 'non-specific host defense'. In addition, NO plays an important role in the killing of intracellular microbial pathogens, and possesses tumoricidal activity[48]. Treatment with BioBran/MGN-3 increased NO levels in the two murine types of macrophages used. The effect was dose dependent. Increased production of NO was detected at a low concentration of BioBran/MGN-3 (10 μg/m*l*), and further increased at 100 μg/m*l*. However, augmenting the concentration to 500 μg/m*l* caused only a slight fluctuation in the level of NO production by both types of cells, as compared with 100 μg/m*l*. Production of NO by human U937 cells post treatment with BioBran/MGN-3 was also examined. Neither BioBran/MGN-3 nor LPS induced NO production by U937 cells. It is known that human cells have a slow response in the production of NO, following exposure to various immuno-stimulants such as bacterial lipopolysaccharide (LPS) and interferon- γ [49]. It was reported that interferon is the key cytokine for the induction of NOS2 in macrophages[50, 51]. The cytotoxic actions of NO against tumor cells appear to be related to inhibition of several heme-containing enzymes which are found in the mitochondrial electron transport complex and the citric acid cycle[52].

Data in the present study reveal a differential response among M ϕ models towards the augmentory effect of BioBran/MGN-3 on enhancing phagocytosis and secretion of cytokines and NO. The reason for this phenomenon is not known, but it could be attributed to the difference in the type of macrophage model: human versus murine, cell line versus murine P-Mϕ, or due to the mechanisms of activation of macrophage models (i.e difference in the pathways of activation and possible interference of different cytokines of the activation process)[53, 54]. In conclusion, we have presented evidence for the role of BioBran/MGN-3 in the enhancement of phagocytic activity, and in the production of cytokine and NO within the three macrophage models. This data suggests BioBran/MGN-3 may be a useful agent for fighting against microbial infection; it also indicates that BioBran/MGN-3 is a potent BRM that is not restricted to specific immune cells, but may cause an overall activation of the immune system.

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