Effect of Modified Arabinoxylan from Rice Bran (BioBran/MGN-3) on NK Cell Activity of Human Peripheral Blood Lymphocytes

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Introduction

Every day, genetic damage is caused by a variety of environmental factors, such as air pollution due to exhaust gases, chemical carcinogens including pesticides, and lifestyle habits such as unbalanced meals and cigarette smoking. Cells with damaged genes cannot behave normally and some become proliferative tumour cells. These abnormal cells are eliminated by white blood cells and natural killer (NK) cells, which carry out immune functions at an early stage before the tumour cells become fully malignant and cause widespread disease. Tumour cells that fail to be eliminated by early immunity can be removed by a more powerful immune mechanism. The cells involved include macrophages, dendritic cells, and lymphocytes such as helper T cells and killer T cells. These carry out acquired immunity, and the anti-cancer immunomodulatory substances (cytokines) produced play important roles in this process. Typical cytokines involved include interleukin 2 (IL-2), interferon γ (IFN-γ), interleukin 12 (IL-12), interleukin 18 (IL-18), and tumour necrosis factor (TNF-α). The reactions mediated by cytotoxic cells such as NK cells and killer T cells are referred to as cellular immunity. C ancer patients are known to show a decrease in cellular

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immune activity across a range of functions.[1)]

Plant polysaccharides derived from mushrooms and herbs counteract the decline in immune function by enhancing lymphocyte cytotoxicity and their ability to produce cytokines, and thus exert an anti-cancer effect. Mushrooms which have been shown to exert anti-tumour or immunomodulatory effects include agaricus, shiitake (lentinan), reishi, enokidake, maitake (maitake D-fraction), kawaratake (krestin), and suehirotake (sonifilan). Some of these are used in dietary supplements. β-1,3-glucan polysaccharides have been shown to be responsible for the lymphocyte stimulating effect of these mushrooms.[2)] However, β-1,3-glucans are a polymeric dietary fibre, which passes through the intestinal tract without being absorbed. To exert an immunomodulatory action, the active ingredient must be absorbed into the bloodstream and make contact with effector cells such as lymphocytes and macrophages. It is thought that some of the polysaccharides degraded by enteric bacteria are absorbed and exert such an effect, while most pass unused through the intestine.

In this study, we investigated the physiologically active ingredient that activates cellular immunity in BioBran/MGN-3 with a relatively low molecular weight. BioBran/MGN-3 mainly contains modified hemicellulose, produced by partially degrading a soluble hemicellulose fraction from rice bran with a carbohydrate-hydrolyzing enzyme complex from shiitake mushroom culture filtrate. This product is already on the market and has been reported to be effective orally against various diseases including cancer[3)] and HIV infection.[4)] Studies have also been made into its ability to scavenge reactive oxygen species and to activate macrophages, and its therapeutic effect in the treatment of diabetes mellitus.

The main physiological effect of this product is its action on biological defences in which NK cells play a major role. Thus, it has been demonstrated that culture of peripheral blood lymphocytes with this product increases cytotoxicity of NK cells.[5)]

NK cells are lymphocytes that are able to kill certain target cells without becoming sensitised to antigen; they are neither T cells nor B cells and account for about 5%-10% of human peripheral blood lymphocytes.[6,7)] When killer T cells, which differentiate from T cells after stimulation by virally infected cells or tumour antigens, find and kill target cells, receptors on the T cell surface recognise antigen-derived peptides and MHC (major histocompatibility complex) proteins at the same time and attack the cells which carry them. However, in order for NK cells to mount an attack, an MHC antigen should not exist, and the antigen need not to be presented by an MHC molecule. In other words, the cytotoxicity of NK cells is not restricted by the MHC, meaning that they can attack different cell types including syngeneic, homogeneous, and heterogeneous cells.[7-9)] NK cells have attracted attention for their particular properties, including immunosurveillance of tumours and an ability to prevent viral infection. It has also been suggested that NK cells are closely involved with the differentiation and growth of haematopoietic stem cells, organ transplantation, and the development of certain haematological diseases, collagen disease, diabetes melli-tus, and gastrointestinal disorders, through their production of cytokines and killer activity.[10)] As mentioned above, NK cells play an important role at the forefront of biological defence. Enhanced cytotoxicity resulting from activation of NK cells may therefore promote elimination of abnormal cells and activation of the immune system overall by increased cytokine production.

The mechanism of NK cell activation by BioBran/MGN-3 and the identity of the active constituent are unknown. In this study, we performed an investigation with the aim of defining the active ingredient and elucidate the mechanism by which it activates NK cells.

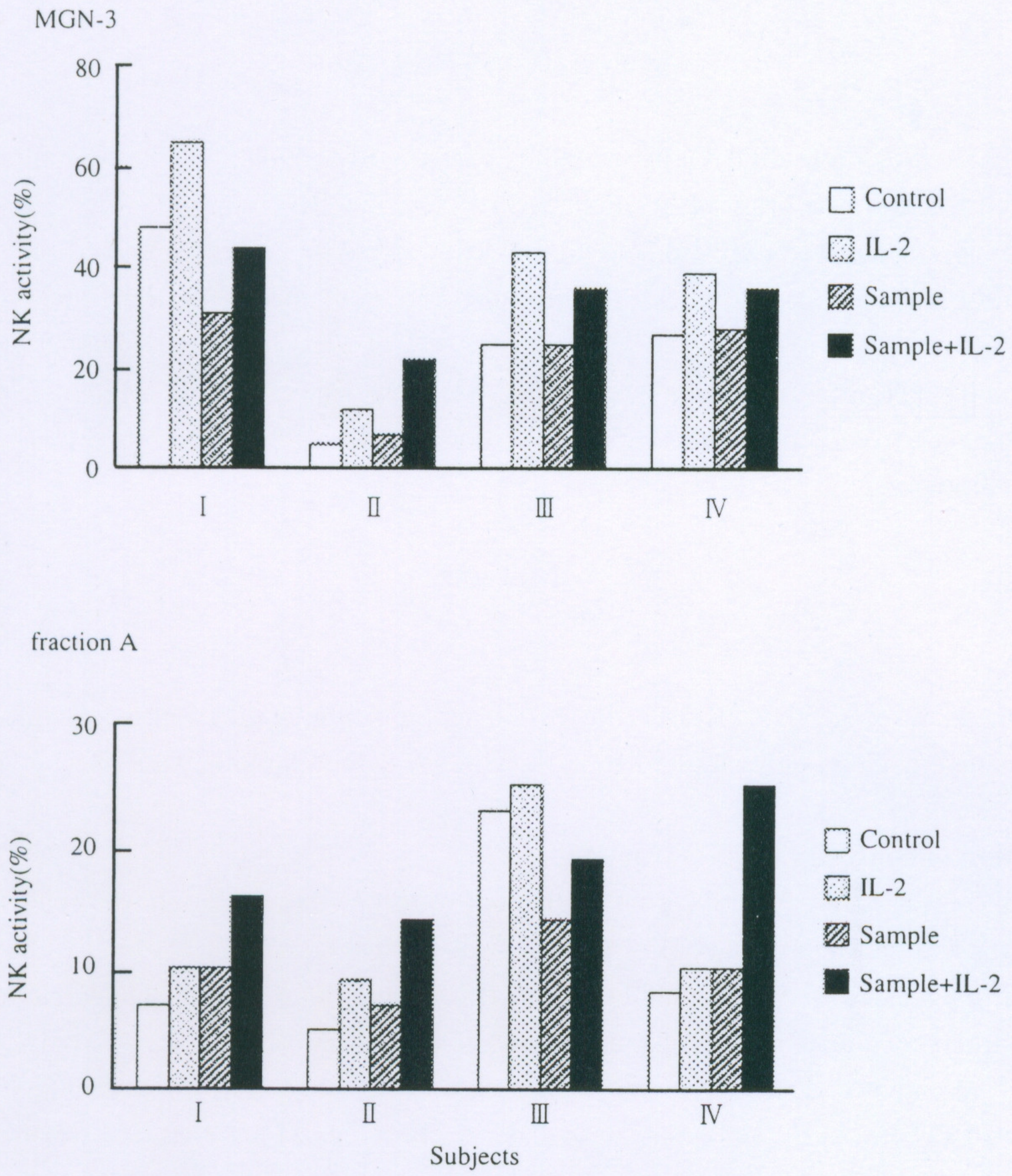

Fig.1 NK activity of MGN-3 or fraction A

FCS-RPMI 1640 was added one day before measurement, and K-562 cells (2-5×10^5 cells/m*l*) in logarithmic phase were used in experiments.

(D) Measurement of NK cell activity

NK cell activity was measured using an established fluorescence method.[12]

Results and Discussion

As blood two days after collection was used in this study, the effectiveness of the separated PBMC for assay of NK activity and toxicity of BioBran/MGN-3 for PBMC were studied first. Viable and dead cells were counted using the trypan-blue elimination method[11] after culture for 1,3, and 5 days. Viability was 92% immediately after separation and 80% after 5 days in culture. No significant differences in viability were observed in PBMC treated with BioBran/MGN-3. From this result, it was judged that BioBran is non-toxic for PBMC.

After partial purification of BioBran/MGN-3, the fraction obtained by Sephadex G-25 chromatography

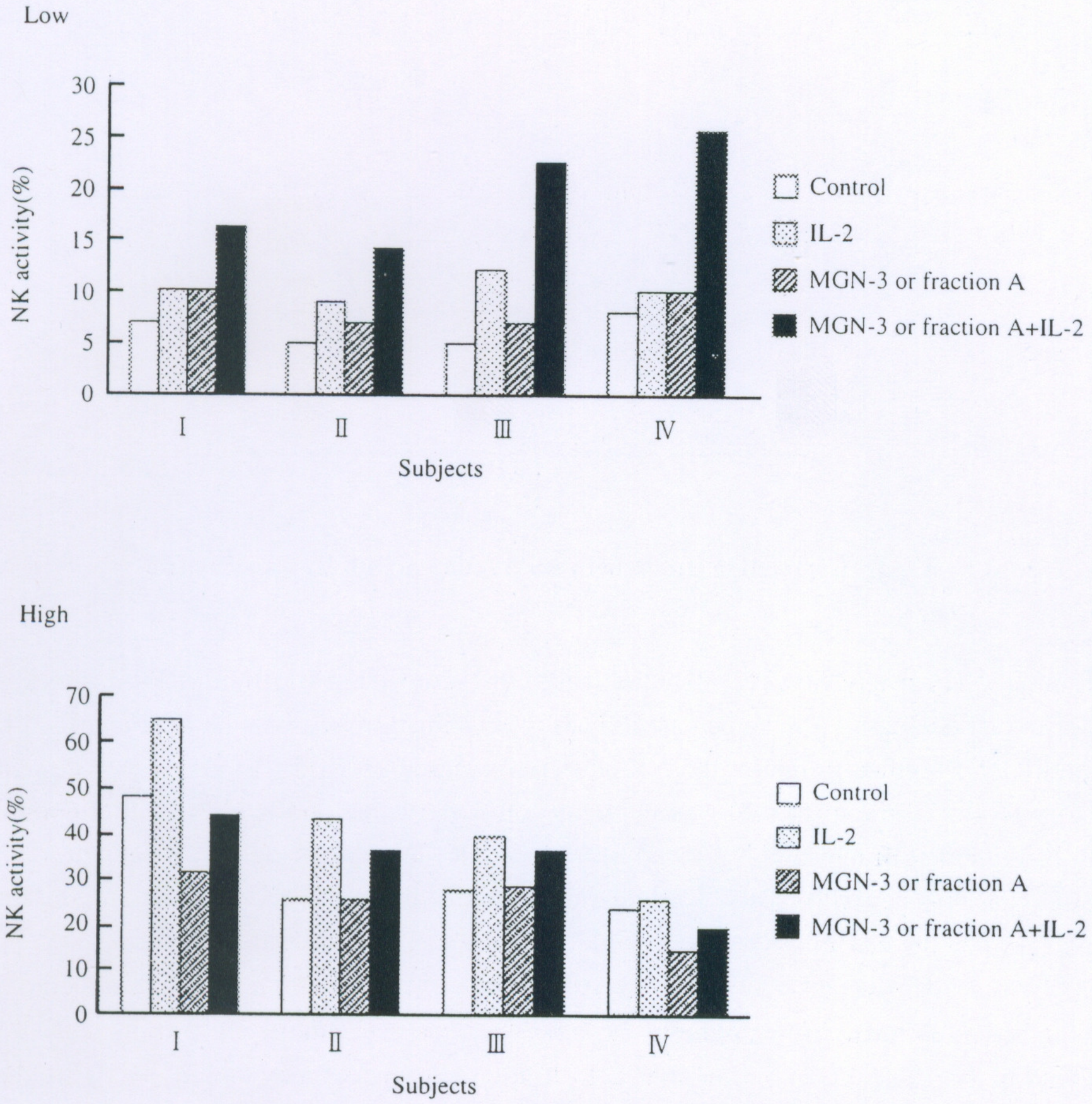

Fig.2 Effects for the low or high NK activity subjects

was designated A. Of the fractions obtained by Sephadex G-75 chromatography, that corresponding in molecular weight to cytochrome C was designated B, and that between blue dextran and cytochrome C was designated C. NK activity increased in the presence of all three fractions, but the effect depended on the cells used. Concomitant treatment with IL-2 tended to increase NK activity more than treatment with any of the fractions alone. However, the effect varied greatly depending on the cells used, and the activity did not increase in a similar way. As shown in **Fig. 1**, the degree of increase in NK activity also varied in the positive control containing IL-2. Treatment with BioBran/MGN-3 and fraction A increased NK activity in some cases but left the activity unchanged or reduced in others. Depending on the cells used, combined treatment with BioBran/MGN-3 and IL-2 produced conflicting results, increasing activity in some cases but decreasing it in other cases relative to treatment with IL-2 alone.

Analysis of the results from all blood cells showed that the effect on samples with a high control NK activity was different from that on samples with low activity. The results are summarised in **Fig. 2**. In blood cells with low initial NK activity, treatment with either BioBran/MGN-3 or IL-2 led to increased activity, which was higher after combined treatment with both than after either one alone. In blood cells

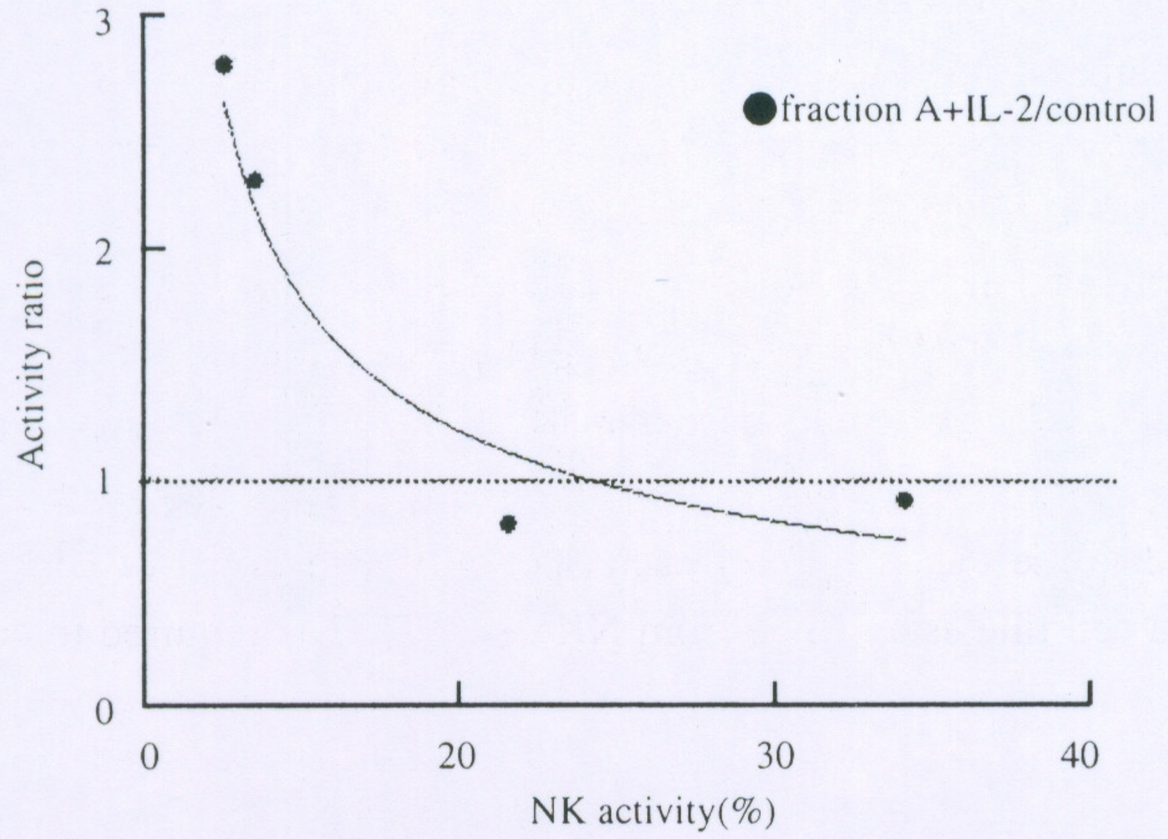

Fig.3 Correlation curves between fraction A with IL-2 and control

with high initial NK activity, however, IL-2 increased the activity but BioBran/MGN-3 caused almost no increase, or in fact decreased it in some cases. During combined treatment with IL-2 and BioBran/MGN-3, BioBran/MGN-3 seemed to inhibit the NK activity increased by IL-2. To investigate these seemingly contradictory results, a graph (**Fig. 3**) was plotted to show the value of NK activity after combined treatment with IL-2 and BioBran/MGN-3 divided by control NK activity (the degree of activation) against the control value (baseline activity). This clearly indicates that BioBran/MGN-3 tended to inhibit the increase in NK activity when the baseline NK activity of blood cells was above a certain value.

In this study, molecular-weight fractionation was also performed using an ultrafiltration membrane on the fraction obtained by affinity chromatography. A molecular species with the effect of increasing NK activity was found in the 12-15 kDa fraction after gel filtration chromatography and in the 10-30 kDa fraction after ultrafiltration. The two separation procedures produced similar results, suggesting that the size of this entity is within these ranges. However, as the estimates for molecular weight for both methods are based on the assumption that the molecule is spherical, the actual size of this sugar compound may be lower.

The results for the active fraction separated by ultrafiltration showed that the degree of activation of NK cells was dose-dependent. The fraction was shown to activate NK cells even at the low concentration of 10 mg/m*l*.

The compound produced results which appeared at first sight to be inconsistent. However, it is unlikely that two different substances are responsible independently for the increase and decrease of NK activity. It is unknown how BioBran/MGN-3 regulates NK activity at an appropriate physiological level *in vivo*.

There are many reports of molecules with immunomodulatory effects, mainly enhancement of NK activity, but BioBran/MGN-3 is particularly interesting as it increased NK activity in PBMC with low initial NK activity but decreased activity in PBMC with high initial NK activity. NK cells play an important role, particularly in cellular immunity, where they are activated by cytokines. However, when the immune response is expressed as humoral immunity, it leads to the production of cytokines involved in antibody production and allergic reactions. Some of these cytokines inhibit tumour suppression by cellular immunity, resulting in an inhibition of NK activity.[9,13,14] Given this, it is thought that BioBran/MGN-3 may stimulate the production of cytokines determining cellular immunity or humoral immunity, resulting in an

increase or decrease of NK activity.

The immunomodulatory effect of mushrooms is mainly attributable to β-glucan, which activates macrophages through cell surface receptors.[15] This β-glucan receptor is less specific and also reacts with mannose and N-acetyl-D-glucosamine.[16] Macrophages recognise sugar chains on cell surfaces and exert a phagocytic action. This enables them to recognise lectins and many types of sugars. Activation of the immune system by BioBran/MGN-3 may be mediated by macrophages.

As the blood cell fraction used in this study contained all the lymphocytes from the original blood and not just NK cells, in order to verify the above hypothesis it will be necessary to separate different lymphocyte populations and analyse the activity of each one. No clear effect of BioBran/MGN-3 was demonstrated on KHYG-1, a cell line established from NK cells. IL-2 is required to culture KHYG-1, and thus cells of KHYG-1 always have a high NK activity. This is a possible reason why BioBran/MGN-3 had no clear effect on KHYG-1.

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