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ORONIGAL ARTICLE

Reconstruction of NK Cells During Complex Cancer Treatment

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ABSTRACT

AIM: The role of glucan in potentiation of immune system is well established, including effects on cancer development. In this study, we focused on effects of short term glucan supplementation in patients during complex cancer treatment.

MATERIALS AND METHODS: We measured the levels of leptin and changes in NK cell numbers after oral application of 200 mg/day of glucan.

RESULTS: We found significant improvement in total numbers of NK cells. Levels of leptin remained unchanged.

CONCLUSIONS: Our findings show the significant effects of glucan supplementation on induction of NK cell, suggesting that addition of glucan to the diet could be beneficial for prevention of cancer remission. The glucan-supplemented group exhibited some improvements in psychic conditions, nutritional state and overall feeling of well-being. Longer supplementation seems to be necessary.

Key Words: Glucan; Cancer; Leptin; obesity; NK cells

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INTRODUCTION

The obesity trend is, at least in developed countries,continuous and alarming[1]. Besides a significant reduction in lifespan, obesity also increases the risk of additional diseases[2-4]. The most commonly obesity-related diseases are cardiovascular diseases, type II diabetes and some malignant diseases[2,5,6]. Recently, the focus of intensive studies is on the relation between increased weight and a higher risk of cancer development[7,8]. Numerous research studies evaluated several components of the immune response for possible relations to the cancer development. In addition, various inductors of some mediators (such as insulin growth factor), increased release of steroid hormones, adipokines and other cytokines were subjects of recent experiments[2,5,6,9].

One of the most important molecules influencing immune mechanisms is leptin produced by adipose cells[7,10]. This hormone is a product of a gene Ob and its levels correlate with an individual's weight[5]. Additional studies confirmed that leptin has significant effects on both natural and adaptive immunity[5,7]. Leptin strongly affects the population of natural killer (NK) cells which can have Ob receptor. Binding with this receptor results in higher cytotoxicity[5,6]. *In vitro* studies revealed that leptin is necessary not only for formation of NK cells, but also for their activation[6,11]. Leptin is involved in all aspects of NK cell biology - from proliferation to differentiation, activation and cytotoxicity[11]. Low cytotoxic activity of peripheral blood cells is related to a higher risk of development of malignant diseases[6]. A long-term study showed that low activity of NK cells directly correlates with the risk of malignancies[12].

In cancer patients, activity of their immune system is directly endangered by environment induced by cancer cells[13,14]. Expression of some inhibitory molecules results in inhibition of activity of cytotoxic T lymphocytes. Effector cells of natural immunity, such as NK cells, cytokine-induced killer cells $CD3^+$ $CD56^+$ CIK cells and γδ T lymphocytes are extremely important, as they are not subject of MHC restriction and form the first line of anti-tumor surveillance[15]. These cells act as a bridge between natural and adaptive immunity and they share some common mechanisms including perforin-mediated toxicity and cytokine

secretion[16]. *In vitro* studies and clinical trials revealed that each of these components of nonspecific immunity has some unique characteristics. NK cells, $CD56^-$ and $CD56^+$ lymphocytes are able of fast activation[14] and a speedy attack of abnormal cells (mostly tumor and virus-infected cells). Their absolute numbers and function can be changed by microbiome alterations.

Stress and aging are important factors affectingimmune systemquality[17]. Cancer patients usually suffer from a cascade of stressors, starting with the original diagnosis, followed up with confirmation or diagnosis, surgery, radiation, chemotherapy and subsequent endless control evaluations. Many patients, particularly women after breast ablation, are significantly influenced by a quality of family environment and by health care quality. Stress levels are strongly influenced by quality of ambulatory and hospital care. Acute stressis slowly changed into chronic stress which is a non-adaptive phenomenon[17]. Connection between central nervous system and immune system was repeatedly confirmed with the main effects persisting stress signals. Persisting stress results in slower healing, slower reparation of physical conditions and higher sensitivity of infections[17]. In addition, stress reactions can negatively influence the control over some infections. Activation of EB infection leading to subsequent decrease in NK and Th cell numbers can serve as an example[18]. Stress can lead to significant changes in DNA reparation processes and in homeostatic processes. Cancer disease combined with stress can result in a serious imbalance of regulation. Anxiety, worries about the future and the results of clinical treatment can significantly influence the quality of immune reactions, as documented in a study of patients with Gulf War Syndrome[18]. In numerous patients were found conditions such as increase of depression with subsequent higher riskof persisting chronic inflammatory problems and activation of compensatory anti-inflammatory reflex system (CIRS), which can be characterized as negative immunoregulatory process[19]. Permanent stress is often accompanied with inflammation is directly connected to the nutrition status, mainly to obesity.

Chronic inflammatory responses in an obese population are often connected to induction of various diseases[6]. It is speculated that quality of diet and most of all regular psychical problems, depression and fatigue can help to negatively influence immune mechanisms[19]. Changes in adipose function, leptin levels, dysfunction of hypothalamic pituitary adenocorticoidal axes and oxidative stress play an important part in an inflammatory reaction of obese individuals. Homeostasis of energetic metabolism affecting leptin can influence a homeostasis of thymus. Leptin, together with additional anti-inflammatory cytokines, is involved in a support of Th1 cell differentiation and cytokine production. Leptin plays an important role not only in regulation of energetic homeostasis, but also in regulation of neuroendocrine and immune functions. Inhibition or at least changes on cytokine-leptin cooperation can serve as preventive marker of cancer risk in obese women[5]. Leptin OBR and interleukin (IL)-1 system are important in breast cancer, Numerous papers described expression of leptin, CB-Ra IL-1, IL-1α, IL-1β, IL-1 receptors ($IL\text{-}1R^{+I}$ and $IL\text{-}1^{+II}$) and their antagonist IL-1Ra. These molecules are involved in inflammatory reaction. IL-1 and leptin are secreted by cancer cells. A direct proof of the role of leptin in breast cancer induction was demonstrated in a mouse study where leptin-deficient mice (ob/oba or ob/ob) did not suffer from experimental breast tumors[5]. In NK cells leptin is involved in cell development, differentiation, proliferation, activation and toxicity[7,20]. These actions are mediated partly via activation of signal transducers and activators of transcription (STAT)-3 and expression of perforins and IL-2 genes[10]. NKT present in fat tissue can regulate and reduce obesity and obesity-related diseases including diabetes Type II[21]. Functional deficit of NK cells in obese individuals is well established and numbers of $CD3^+$ $CD56^+$ cells are lower comparing to non-obese population. In addition, lower levels of a marker of NK cell function, TRAIL were described[6]. On the other hand, weight increase leads to chronic hyperinsulinemia, insulin resistance and subsequently to a higher risk of cancer[2]. The statement,reduction of BMI is now considered to be an important factor for cancer risk reduction[9]. It is important to remember that only one criterion will not solve the problem. Induction of colorectal carcinoma and other malignant diseases is connected to numerous risks including smoking, nutrition, obesity and systemic inflammation generated by leptin. Genetic definition of leptin receptors might help to establish some prevention[2]. In developed countries, obesity is reaching epidemic proportions and it is expected that over 1 billion adults are currently obese[1].

During the last two decades significant attention was devoted to studies of functions and bioactivities of β-glucans. The most common activities were found to be immunostimulatory effects in both infectious and cancer diseases[1,22-25]. Their ability to stimulate both cellular and humoral immunity as well as both nonspecific and specific immune response is well established. Some studies even suggest that glucan can be effective in an obese population, as they can regulate food intake and appetite and help to reduce weight. Soluble fibers in the form of glucomannans can influence weight already at a daily dose 1.24 g. Lower appetite after glucan supplementation might be connected with fermentation of glucan in the gut and with the formation of short chain fatty acids which are rapidly absorbed. Glucan might serve as crucial regulators of energy[1]. Induction of some hormones in gastrointestinal tract caused by glucan treatment is currently subject of intensive studies. Nutrition in general is important not only for prevention of diseases, but also in subsequent effects on physiological functions[1,23]. Our study is focused on the possible effects of glucan supplementation of an obese population after initial treatment of cancer.

MATERIALS AND METHODS

Glucan

Yeast-derived insoluble Glucan #300 (> 85% dry w/w basis) was purchased from Transfer Point (Columbia, SC, USA). This glucan contains 96% carbohydrates and 2.1% proteins. Neutral sugar analysis confirmed 91.3% glucose and 8% mannose.

Protocol

The same protocol described previously[25,26] was used throughout this study. Briefly, a randomized, double-blind, placebo-controlled trial compared β-glucan #300 and a placebo in people after complex surgical, radiation and/or chemotherapy treatment of cancer. Out of a total of 32 patients, the placebo group consisted of 14 patients (8 females and 6 males with an average age of 70.0 ± 7.0 years). Glucan-supplemented group involved 18 patients (14 females and 4 males with an average age 65.3 ± 10.4 years). The cancer types in females were 20x breast cancer and 2x ovarian cancer, in males it was 8x colorectal cancer, 1x breast cancer and 1x bronchial cancer. All patients underwent basic immunologic screening with evaluation of full spectrum of inflammatory reaction, cancer markers and cellular immunity. This screening was followed up with evaluation of indicators of iron metabolism, nutritional state (leptin, prealbumin) and additional methods, based on the clinical

Table 1 Leptin levels in supplemented and control patients(A-Start, B-End).

	Glucan		Placebo	
	A	B	A	B
n	14	14	8	8
Mean	16 534	16 471	20 888	14 924
SD	9 373	8 978	10 348	8 087
t-test	$P = 0.471$		$P = 0.071$	

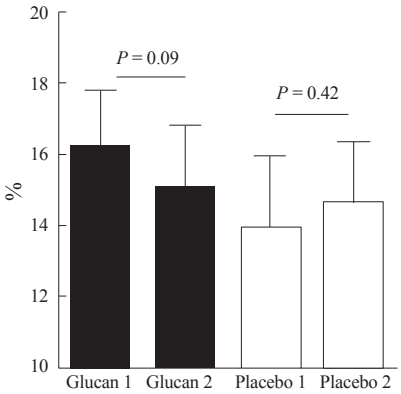

Figure 1 NK cell percentage in the groups of patients supplemented with β-glucan and placebo (1 = day 0, 2 = day 60).

syndromes of additional health problems.

During the intervention period, the subjects consumed 200 mg/d of β-glucan or placebo for 60 days. The glucan and placebo capsules looked identical. Subjects were routinely evaluated by the medical staff. This study was performed in agreement with Helsinki declaration (revised version 2000.09.01) and was in full compliance with the rulesfor clinical testing in the Czech Republic.

Tests

NK cells were measured using flow cytometer FACSCalibur (BD Biosciences, USA) with 4 color test. We used anti-CD16-FITC, CD56-FITC, CD56-PE, CD45-PerCP, CD3-APC, CD11b/PE and HLA-DR/PE monoclonal antibodies (BD Biosciences, USA). NK cells were identified as $CD45^+$, $CD16^+$, $CD56^+$ and $CD3^-$ cells. Absolute numbers were calculated from numbers of leukocytes and lymphocytes (hematological analyser XS 800) and relative percentage of NK cells.

Leptin levels were determined using an ELISA kit Human Leptin (Orgenium, Gentaur Molecular Products, San Jose, CA, USA).

Statistical analysis

Statistical significance was evaluated by a pair *t*-test using GraphPad Prism 5.04 software (GraphPad Software, USA). An average and standard deviation was evaluated after determination of composition of standard values (D'Agostino, Pearson). In case of non-standard composition, we converted the values into logarithms.

RESULTS

In the glucan supplemented group, the percentage of NK cells at the beginning of the study was 16.22 ± 1.53. After 60 days, we found a small decrease to 15.05 ± 1.73 %. In the placebo group, the change from 13.91 ± 2.0 to 14.62 ± 1.70 % was also statistically not significant (Figure 1). The reference range in the immunological laboratory is 6 to 19%.

When we measured the total numbers of NK cells, the glucan-supplemented group showed significant increase from 241 ± 17 to 295 ± 14 cells/mL ($P \leqslant 0.02$), whereas in the placebo group there was nonsignificant decrease from 239 ± 17 to 200 ± 15 cells/mL (Figure 2). The laboratory norm is 250 to 400 cells/mL.

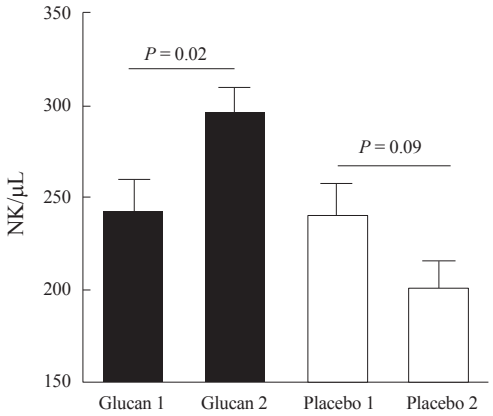

Figure 2 Mean (SD) values of NK cells in patients supplemented with β-glucan and placebo (1 = day 0, 2 = day 60).

Levels of leptin changed only slightly in glucan group from 16 534 ng/mL to 16 141 ng/mL, whereas in the placebo group we observed nonsignificant decrease from 20 882 ± 10 348 to 14 924 ± 8 087 (Table 1). The laboratory norms for men is 8 000 to 15 000 ng/mL, for women 12 000 to 22 000 ng/mL. Finding of changes of leptin levels were accompanied by findings of changes in weight. In glucan supplemented group we found slight increase from 75.62 to 75.81 kg, in the control group we found significant decrease from 81.89 to 78.6 kg ($P \leqslant 0.0284$).

DISCUSSION

Starting points of cancer disease are closely associated with obesity[2,8]. These processes are manifested by induction of inflammatory proteins with characteristic long-term inflammation and are mostly induced *via* adipocytes producing various proteins involved in inflammation[13,14].

Successful treatment of malignant disease by surgery and subsequent irradiation and/or chemotherapy is not the final phase of treatment. It is crucial to keep following the patients and to make sure that the quality of all their physiological functions is wellmaintained. It is well known that the quality of complex treatment affects the possibility for longer remission. It is imperative to not only maintain high quality nutrition and to eliminate stress, but also tofulfill the feeling of necessity of taking care of its own health. It will result in reaching the optimal level of immune reactions necessary for reparation of physiological functions[1,8,23-25].

This area is currently under intensive attention from both scientists and physicians. Supplementation with vitamins, minerals and other supporting materials is often used in many malignant diseases[8,22,23]. With more than 14 000 scientific publications, it is not surprising that glucan is the most commonly used natural immunomodulatory[23,26]. The ability of glucans to regulate and significantly improve various physiological processes from defense reaction to stress regulation[27,28], cholesterol level or regulation of blood sugar is well documented (for review see[26]). In this study, we evaluated the effects of glucan in patients after primary therapeutic solution of malignant disease and in the period after surgery and after irradiation or chemotherapy. The aim of this glucan supplementation was to keep or even improve the quality

of the NK system, which is necessary for post-treatment therapy of this disease.

Support of anticancer immunity is connected with NKT type I, whereas type II is involved in the suppression of anticancer defense[20]. NK cells type I induce lysis of cancer cells both directly (via perforin/granzyme mechanisms) and indirectly (*via* induction of secretion of Th1 cytokines and NK cell activation). In comparison to CIK cells and γδ T lymphocytes, NK cells from cancer patients are more active in both cytokine secretion and cytotoxic activities[14] and are an ideal candidate for adaptive cell immunotherapy. In our study we found a decrease in NK cell numbers in the control group and a significant increase of NK cell numbers in the glucan-supplemented group. When calculated as percentage of NK cells, the trend is opposite, but not significant.

Our findings might be influenced by the weight of patients. In the glucan-supplemented group, only small change was found, but in the placebo group we found significant weight reduction. In a short term of evaluation, we do not consider nutritional changes as significant[8], despite the desperate need for a weight decrease in patients with high BMI[17]. Our findings showed the same dynamic of changes in levels of leptin - nonsignificant changes in the glucan-supplemented group, stronger (but still statistically nonsignificant) decrease in the placebo group. Leptin is known to stimulate breast cancer growth via cooperation with additional adipokinins such as hepatocyte growth factor. However, leptin can not only support the invasion of cancer cells, but also support the cancer growth indirectly by stimulation of abiogenesis[15].

The importance of how cancer cells escape immune mechanisms has been studied intensively for decades[11,29]. It is well established that strong deficit in iNKT cells can be a precondition to insufficient clinical effects of cancer treatment[11,13,15]. The percentage of these cells in total lymphocyte population is clearly negative prognostic factor[30]. NK cells isolated from cancer patients produce more cytokines and have higher anticancer toxicity[14]. Experiments demonstrated that higher cytotoxic activity of peripheral blood lymphocytes correlates with a lower risk of cancer growth[12].

CONCLUSIONS

Our findings show the significant effects of glucan supplementation on induction of NK cell in patients treated for cancer. We hypothesize that addition of glucan to the diet could be beneficial for prevention of cancer remission. The glucan-supplemented group exhibited some improvements in psychic conditions, nutritional state and overall feeling of well-being. However, our observation used only a short-term application of glucan and clearly the evaluation of a longer time will be necessary.

CONFLICT OF INTERESTS

There are no conflicts of interest with regards to the present study.

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