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The influence of vitamin B_{12} supplementation on the level of white blood cells and lymphocytes phenotype in rats fed a low-protein diet

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Abstract

Protein malnutrition has a negative effect on body composition and some blood parameters, especially in the young growing organism. One of nutritional factors which could protect against negative consequences of protein deficiency may be B group vitamins. The aim of the study was to investigate the effect of vitamin B_{12} supplementation on the immune system in rats fed a standard and a low-protein diet. Rats were fed a control (20% of energy from protein) or a protein-deficient diet (4.5% of energy from protein). Half of animals in each group were additionally supplemented with vitamin B_{12} (300% of the daily intake). The white blood cells analysis and lymphocytes immunophenotyping (number and percentage) were performed. Low-protein diets caused disturbances in WBC and lymphocyte subpopulations in both short- (30-day) as well as long-term periods (90-day). Vitamin B_{12} supplementation significantly reduced the negative impact of protein malnutrition after 30 days, however had no effect on long-term malnutrition. Furthermore, vitamin B_{12} addition in rats fed a control diet did not affect the studied parameters. This observation opens the promise of use of vitamin B_{12} supplementation to improve immune system parameters in protein malnourished organisms.

Key words: *protein deficiency, B_{12} supplementation, immune system, rats.*

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Introduction

The Food and Agriculture Organization of the United Nations estimated that nearly 870 million people (of about 7 billion people in the world) were suffering from chronic undernourishment in 2010-2012 [1]. Malnutrition is a serious problem mainly in developing countries, however approximately 20 million malnourished people originate from developed countries. Protein malnutrition is one of types of nutritional depletion. It occurs often in young or elderly people [2, 3]. Protein-depleted diet causes profound disturbances of body metabolic processes. Long-term consumption of a protein-deficient diet reduces body temperature in humans and animals, causing hypoalbuminemia, edema formation, damage of blood vessels and liver [3, 4]. It has been shown that a protein-deficient diet decreased the level of hemoglobin, hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) in rats [6]. It also attenuates the immune system function. Mice fed a low-protein diet had less resistance to pneumococcal infections, fewer mature and immature B cells in the lung and spleen, and a reduced production of specific antibodies compared with control mice [7]. Similarly, leopard frog tadpoles fed a protein-restricted diet had reduced phytohaemagglutinin and bacterial killing ability responses relative to tadpoles fed a high-protein diet [8].

Despite many side effects, a diet deficient in protein is recommended in the treatment of kidney diseases. In chronic renal disease, a low-protein diet delays the progression of kidney failure, especially in patients with proteinuria, and decreases the supply of phosphorus [9]. In addition, protein malnutrition could positively affect glucose metabolism and improves blood and urinary glucose levels and renal manifestations of diabetes in C57BLKS-db/db mice [10].

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It seems that B group vitamins, especially B_{12}, could minimize the effects of protein malnutrition in the hematological or immune system. It is known that vitamin B_{12} (cobalamin) is an essential coenzyme in the methylation reactions in the body. It also plays a very important role in the formation of red blood cells. Symptoms of B_{12} deficiency include hematological, neurological and cognitive disorders, including megaloblastic anemia, visual disturbances and memory loss [11, 12]. Therefore, we decided to investigate the effect of vitamin B_{12} on immunological parameters in rats fed a standard and low-protein diet. We used high doses of vitamin B_{12} (300% of the daily intake), as vitamin B_{12} toxicity has not been reported before. Moreover, some of the hematologist experts used them for the treatment of pernicious anemia in current clinical practice [13, 14].

Material and methods

Animals

The study was performed on 40 male Wistar rats with a body mass of 180 ±1.7 g (mean ± SEM). The experimental protocol was approved by the IV Local Ethic Committee for Animals Studies in Warsaw. Animals were cultured in metal cages (5 units per cage) in an air-conditioned room in standard conditions (12-hour light cycle, temperature 23°C). Rats were fed *ad libitum* a semisynthetic diet of energy value of 350 kcal/100 g (1466.5 kJ/100 g) for 90 days. Rats were divided into 4 groups: control (10 rats), control + vitamin B_{12} (10 rats), protein deficient (10 rats), and protein deficient + vitamin B_{12} (10 rats). In the control diet, 20% of energy was provided from protein and in the experimental diet, 4.5% only. Half of animals from the control and protein-restricted groups were supplemented with vitamin B_{12} to a level 3 times higher than in the control diet (300% of the daily intake).

In all the diets, 15% of energy came from fat, including approx. 2% from essential fatty acids (EFA). Diets were enriched with mineral salts and vitamins in accordance with the guidelines for rats. The energy value of the low-protein diet was supplemented with carbohydrates (Table 1). Animals and food containers were weighed twice a week, and then an average mass and consumption of individual diet per rat was calculated.

Blood samples were collected on EDTA after day 0, 30 and 90 of the experiment from tail vein of rats. On the 90th day after blood collection rats were sacrificed by cervical dislocation while unconscious (morbital anesthesia).

Cytometric analysis

The total white blood cells number was measured in the hematological analyzer in accordance with the manufacturer's protocol (MEK-6450, Nihon Kohden). The percentage of white blood cells populations was determined using FSC/SSC/FL-3 parameters (FACS Calibur, BD). Subpopulations of lymphocytes were labeled by surface staining of blood cells with fluorochrome-conjugated rat antibodies, according to the manufacturer's protocol (BD Biosciences). Two panels were established:

1. CD45a-FITC, CD161-PE, CD3-APC,
2. CD8-FITC, CD4-PE, CD3-APC.

Before the flow cytometry analysis, red blood cells were lysed (10 min, Lysing Solution BD Biosciences).

Table 1. Diet composition

Diet component	20% of energy from protein		4.5% of energy from protein	
	g/100 g	kcal	g/100 g	kcal
Sunflower oil	0.41	3.69	0.55	4.95
Lard	5.45	48.91	5.31	47.57
Casein	18.97	60.70	4.56	14.59
Egg powder	1.61	9.33	0.20	1.16
Wheat flour	19.43	67.61	19.43	67.61
Wheat starch	30.00	120.00	30.00	120.00
Potato starch	9.14	–	11.36	–
Sugar	10.00	39.90	23.59	94.12
Mineral mix [1)]	4.00	–	4.00	–
Vitamin mix [2)]	1.00	–	1.00	–

[1)] 1000 g of the mineral mixture contains: 322 g of $KHPO_4$, 300 g of $CaCO_3$, 167 g of NaCl, 102 g of $MgSO_4$, 75 g of $CaHPO_4$, 27.5 g of $FeC_6P_5O_7$, 5.1 g of $MnSO_4$, 0.8 g of KJ, 0.3 g of $CuSO_4$, 0.25 g of $ZnCl_2$, 0.05 g of $CoCl_2$

[2)] 1000 g of the vitamin mixture contains: 545000 IU of vitamin D_3, 1 g of vitamin K, 30 µg of vitamin B_{12}, 10 g of choline chloride, 1.01 g of folic acid, 0.03 g of biotin, 10 g of inositol, 10 g of PABA, 1250000 IU of vitamin A, 1.5 g of vitamin B_6, 2.5 g of vitamin E, 5 g of vitamin B_1, 25 g of vitamin C, 5 g of vitamin PP, 2.5 g of vitamin B_2, 25 g of calcium pantothenate

Table 2. Mean ± SEM of daily feed intake and final body mass of rats. Rats were fed for 90 days with a control (20% of energy from protein) or protein-deficient diet (4.5% of energy from protein) supplemented or not with 300% of the daily intake of vitamin B_{12}

	20% of protein *n* = 10 (control group)	**20% of protein + + vitamin B_{12} *n* = 10**	**4.5% of protein *n* = 10**	**4.5% of protein + + vitamin B_{12} *n* = 10**
Body mass (g)	451.7 ±9.4	448.7 ±8.2	349.2 ±10.8[a]	333.7 ±7.2[a]
Daily feed intake (g)	16.8 ±0.2	17.3 ±0.2	15.8 ±0.3[a]	15.3 ±0.2[a]

Significance ($p < 0.05$): [a]comparison to the control group, [b]comparison to 4.5% of protein group

Table 3. White blood cells (number and percentage) in blood of rats fed for 30 days a control (20% of energy from protein) or protein-deficient diet (4.5% of energy from protein) supplemented or not with 300% of the daily intake of vitamin B_{12}. White blood cells numbers were measured in hematological analyzer (MEK-6450, Nihon Kohden) and lymphocytes immunophenotyping were performed by flow cytometry (FACS Calibur, BD). Results are shown as mean ± SEM. Significance ($p < 0.05$)

	20% of protein *n* = 10 (control group)	**20% of protein + + vitamin B_{12} *n* = 10**	**4.5% of protein *n* = 10**	**4.5% of protein + + vitamin B_{12} *n* = 10**
Monocytes (× 10^3/µl)	0.51 ±0.07	0.4 ±0.06	0.29 ±0.04[a]	0.55 ±0.08[b]
Monocytes (%)	5.0 ±0.8	4.1 ±0.4	3.9 ±0.4	4.5 ±0.4
Neutrophils (× 10^3/µl)	0.9 ±0.1	0.9 ±0.1	1.3 ±0.1[a]	1.80.3[a]
Neutrophils (%)	8.5 ±0.9	9.8 ±1.0	19 ±1.5[a]	14.7 ±1.2[a]
Eosinophils (× 10^3/µl)	0.13 ±0.01	0.14 ±0.01	0.06 ±0.01[a]	0.10 ±0.01[a]
Eosinophils (%)	1.3 ±0.2	1.4 ±0.1	0.9 ±0.1[a]	0.8 ±0.1[a]
Lymphocytes (× 10^3/µl)	8.9 ±0.6	8.6 ±0.6	5.43 ±0.6[a]	9.4 ±0.8[b]
Lymphocytes (%)	85.2 ±1.5	84.6 ±1.3	76.2 ±1.6[a]	80.0 ±1.3
(B cells) CD45a+ (× 10^3/µl)	2.4 ±0.2	2.3 ±0.2	1.0 ±0.1[a]	2.2 ±0.2[b]
(B cells) CD45a+ (%)	26.8 ±1.3	26.6 ±0.8	18.3 ±1.1[a]	23.6 ±1.2[b]
(T cells) CD3+ (× 10^3/µl)	5.3 ±0.5	4.9 ±0.5	3.7 ±0.4[a]	5.9 ±0.5
(T cells) CD3+ (%)	58.7 ±1.9	56.2 ±2.1	67.8 ±1.2[a]	62.2 ±1.7
CD4+ (× 10^3/µl)	3.7 ±0.3	3.4 ±0.4	2.7 ±0.2[a]	4.3 ±0.3[b]
CD4+ (%)	41.2 ±0.2	39.4 ±2.0	49.6 ±1.3[a]	45.4 ±1.1
CD8+ (× 10^3/µl)	1.9 ±0.2	1.6 ±0.2	1.1 ±0.1[a]	1.7 ±0.2[b]
CD8+ (%)	20.5 ±1.0	18.4 ±1.0	19.4 ±0.7	18.3 ±0.7
CD4/CD8 ratio	2.0 ±0.1	2.1 ±0.1	2.6 ±0.1[a]	2.5 ±0.1[a]
NK cells (CD161+) (× 10^3/µl)	0.37 ±0.05	0.33 ±0.04	0.25 ±0.04[a]	0.37 ±0.04[b]
NK cells (CD161+) (%)	4.3 ±0.5	4.0 ±0.6	4.9 ±0.6	4.0 ±0.3
NKT cells (CD161+, CD3+) (× 10^3/µl)	0.13 ±0.01	0.13 ±0.01	0.07 ±0.01[a]	0.12 ±0.01[b]
NKT cells (CD161+, CD3+) (%)	1.4 ±0.1	1.5 ±0.1	1.3 ±0.1	1.2 ±0.1

[a]comparison to the control group, [b]comparison to 4.5% of protein group

Statistical analysis

Statistical evaluation of the results was performed by one-way ANOVA, and the significance of differences between the groups was verified with a Bonferroni multiple comparison post test (Graph Pad Prism software package).

Results

Body mass and diet consumption

Results of final body mass and daily feed intake are presented in Table 2. Rats fed a protein-deficient diet (4.5% of energy from protein) had a decreased body mass (about 23%, $p < 0.001$) in comparison to the control group. A lowered daily feed intake has also been noted in rats fed a diet restricted in proteins (about 6%, $p < 0.05$). Vitamin B_{12} supplementation did not affect the body mass and daily feed intake in both control and protein-malnourished groups.

Cytometric analysis

Blood analysis performed on day 1, after randomization of rats groups, did not reveal any significant differences between groups. Mean ± SEM number of white blood cells identified by cytometric analysis, was: lymphocytes 7.3 ±0.2 × 10^3 (82.2 ±0.6%); monocytes, 0.6 ±0.1 × 10^3 (7.1 ±0.3%); neutrophils 0.8 ±0.1 × 10^3 (9.4 ±0.4%) and eosinophils 0.1 ±0.01 × 10^3 (1.4 ±0.1), respectively. Average (± SEM) number of lymphocytes subpopulations

Table 4. White blood cells (number and percentage) in blood of rats fed for 90 days a control (20% of energy from protein) or protein-deficient diet (4.5% of energy from protein) supplemented or not with 300% of the daily intake of vitamin B_{12}. White blood cells numbers were measured in the hematological analyzer (MEK-6450, Nihon Kohden) and lymphocytes immunophenotyping were performed by flow cytometry (FACS Calibur, BD). Results are shown as mean ± SEM. Significance ($p < 0.05$)

	20% of protein *n* **= 10 (control group)**	**20% of protein + + vitamin B_{12}** *n* **= 10**	**4.5% of protein** *n* **= 10**	**4.5% of protein + + vitamin B_{12}** *n* **= 10**
Monocytes (× 10^3/µl)	0.59 ±0.04	0.61 ±0.09	0.35 ±0.04[a]	0.40 ±0.06[a]
Monocytes (%)	7.4 ±0.6	8.1 ±0.8	5.5 ±0.4	6.4 ±0.4
Neutrophils (× 10^3/µl)	0.9 ±0.1	1.18 ±0.2	1.8 ±0.2[a]	1.8 ±0.3[a]
Neutrophils (%)	10.7 ±0.9	15.6 ±1.3	28.9 ±2.6[a]	29.0 ±1.9[a]
Eosinophils (× 10^3/µl)	0.06 ±0.01	0.08 ±0.02	0.03 ±0.01[a]	0.03 ±0.01[a]
Eosinophils (%)	0.8 ±0.1	0.9 ±0.2	0.4 ±0.1	0.5 ±0.1
Lymphocytes (× 10^3/µl)	6.7 ±0.6	5.9 ±0.9	4.13 ±0.4[a]	4.09 ±0.6[a]
Lymphocytes (%)	81.1 ±1.4	75.4 ±1.6	65.1 ±2.5[a]	64.2 ±2.1[a]
(B cells) CD45a+ (× 10^3/µl)	1.3 ±0.1	1.2 ±0.2	0.8 ±0.1[a]	0.8 ±0.1[a]
(B cells) CD45a+ (%)	19.0 ±1.0	15.6 ±1.2	18.5 ±1.0	17.4 ±1.0
(T cells) CD3+ (× 10^3/µl)	4.6 ±0.5	5.2 ±0.7	2.7 ±0.3[a]	2.8 ±0.3[a]
(T cells) CD3+ (%)	68.0 ±1.7	68.1 ±1.9	62.6 ±1.9	68.5 ±2.1
CD4+ (× 10^3/µl)	2.9 ±0.3	3.3 ±0.4	1.8 ±0.2[a]	2.0 ±0.2[a]
CD4+ (%)	42.5 ±1.4	44.1 ±1.5	43.8 ±1.0	48.4 ±1.6
CD8+ (× 10^3/µl)	1.8 ±0.2	2.0 ±0.3	0.9 ±0.1[a]	0.9 ±0.1[a]
CD8+ (%)	26.7 ±1.0	24.9 ±1.0	20.6 ±0.9[a]	22.3 ±0.7[a]
CD4/CD8 ratio	1.6 ±0.1	1.8 ±0.1	2.1 ±0.1[a]	2.2 ±0.1[a]
NK cells (CD161+) (× 10^3/µl)	0.34 ±0.05	0.49 ±0.09	0.12 ±0.02[a]	0.14 ±0.02[a]
NK cells (CD161+) (%)	5.2 ±0.5	6.3 ±0.5	2.8 ±0.2[a]	3.4 ±0.4[a]
NKT cells (CD161+, CD3+) (× 10^3/µl)	0.14 ±0.01	0.19 ±0.04	0.08 ±0.01[a]	0.09 ±0.01[a]
NKT cells (CD161+, CD3+) (%)	2.2 ±0.2	2.3 ±0.3	1.9 ±0.2	2.0 ±0.1

[a]comparison to the control group, [b]comparison to 4.5% of protein group

amounted to: B cells (CD45a+) – 1.8 ±0.1 × 10^3, T cells (CD3+) – 4.4 ±0.2 × 10^3, CD4+ – 3.1 ±0.1, CD8+ – 1.6 ±0.1 × 10^3, CD4/CD8 ratio – 1.9 ±0.1, NK cells (CD161+) – 0.2 ±0.1 × 10^3, NKT cells (CD161+, CD3+) – 0.2 ±0.01 × 10^3. This represented 25.5 ±0.6% (CD45a+), 61.1 ±0.8% (CD3+), 42.4 ±0.6% (CD4+), 21.7 ±0.4% (CD8+), 2.6 ±0.1% (CD161+), 1.8 ±0.1% (CD3+, CD161+) of lymphocytes, respectively.

A protein-deficient diet caused significant changes in white blood cells number and percentage as well as lymphocytes subpopulations after 30 days of experience (Table 3). The following has been observed: a decrease in total lymphocytes (~40%, $p < 0.001$) monocytes (~45%, $p < 0.05$) and eosinophils (about 55%, $p < 0.001$) as well as an increase in neutrophils (about 50%, $p < 0.01$). This caused also significant changes in WBC (white blood cells) percentage distribution in blood. A reduced number of total lymphocytes in rats fed a low-protein diet resulted in a significant decrease in most of lymphocytes subpopulations number ($p < 0.01$), only NKT cells were insignificantly decreased ($p = 0.064$). Percentage analysis of lymphocytes subpopulations revealed a significant increase in CD3+, associated mainly with an elevation of CD4+, and a decrease in lymphocytes CD45a+. The elevation of CD4+ resulted in a significant increase in CD4/CD8 ratio (approximately 30%, $p < 0.05$).

Vitamin B_{12} supplementation did not affect the number and percentage of white blood cells populations in blood of rats fed a control diet for 30 days. However, 30-day addition of vitamin B_{12} to a low-protein diet almost completely reduced the negative impact of protein malnutrition. White blood cells and lymphocytes subpopulations numbers and percentage were similar to results obtained in the control group, except neutrophils (increase both in the number and percentage, $p < 0.001$) and eosinophils (decrease both in the number and percentage, $p < 0.05$). Rats fed a protein-deficient diet supplemented with vitamin B_{12} presented also an increased CD4/CD8 ratio.

Mean (± SEM) number and percentage of WBC and lymphocytes subpopulations of blood collected from rats fed for 90 days a control (20% of energy from protein) or protein-deficient (4.5% of energy from protein) diets additionally supplemented with vitamin B_{12} are presented in Table 4. Protein-malnourished rats presented a lower number of total white blood cells (approximately 25%, $p < 0.01$, data not shown), what resulted in diminution in the number of most WBC populations. Interestingly, a protein-deficient diet caused an increase in neutrophils number (about 45%, $p < 0.01$) in comparison to the control diet. Additionally, some other changes were observed in WBC percentage distributions. Rats fed a protein-deficient diet had a decreased percentage of total lymphocytes and eosinophils (approximately 20% and 50%, $p < 0.01$) while the percentage of neutrophils was increased (~170%, $p < 0.001$). Percentages of CD3, CD4+ and CD45a+ lymphocytes were not changed, however the percentage of CD8+ was significantly decreased in rats fed a low-protein diet. A lower level of CD8+ lymphocytes in protein-malnourished rats resulted in a significant increase in the CD4/CD8 ratio (30%, $p < 0.05$). Additionally, rats fed a protein-deficient diet had a reduced level of NK cells (approximately 50%, $p < 0.01$).

Ninety days of vitamin B_{12} supplementation did not affect the number and percentage of white blood cells as well as lymphocyte subpopulations in rats fed a control diet, however did not protect rats from a negative effect of a low-protein diet consumption.

Discussion

Nutrition is one of the basic needs required for correct functioning of live organisms. Early malnutrition may have harmful effects not only on the immune function, but also on the development of the mental system, gastrointestinal tract, and can cause metabolic syndrome [15]. Protein malnutrition reduces body mass in both humans and animals and decreases antioxidant levels [16, 17]. It elicits an early elevation of blood glucocorticoid levels to magnitude reminiscent of critical illness in weanling murine systems fed a low-protein diet [18]. Both short and long time of protein deprivation disrupt some blood parameters (e.g. decreased hemoglobin level, MCV, MCH), impair immune functions and increase sensitivity to infections [6, 19, 20].

Protein-deficient diets significantly decreased body and organ mass of rats in comparison to animals fed a control diet [15]. In the present work we also noted that relationship. Rats fed a protein-deficient diet (4.5% of energy from protein) had a lower body mass by approximately 23% than rats fed a control diet (20% of energy from protein). It was caused by a lower food intake observed in the protein-malnourished rats (Table 2) and decreased feed conversion ratio (about 35%). As expected, tissue weight of liver, kidney, spleen, and muscle was decreased in rats fed a low-protein diet. However, when the weight of organs was expressed relative to body mass, we did not observe any significant differences (data not shown).

It is considered that vitamin B_{12} could participate in regulation of body mass. Roman-Garcia *et al.* had shown that body mass of mice fed a B_{12}-deficient diet was decreased in comparison to control mice [21]. On the other hand, Abu-Samak *et al.* suggested that Jordanian overweight male youth were a risk group of vitamin B_{12} deficiency [22]. In the present study, vitamin B_{12} supplementation did not affect body mass and feed consumption in both control and protein-deficient groups. Comparison of vitamin B_{12} and vitamin B_6 supplementation in rats fed a low-protein diet (results obtained in our previous work, [6]) suggested that vitamin B_6 was a better factor to correct the negative influence of protein malnutrition on body mass.

Malnutrition inhibits the immune function and results in higher susceptibility to infectious diseases. Nutritional deprivation induces atrophy of lymphoid tissues such as the spleen and thymus, and reduces the number of circulating B and T cells [23]. Feeding sows with a low-protein diet during gestation affects immune competence in piglets that may have serious consequences for host defense against bacterial pathogens [24]. Moreover, protein malnutrition caused a significant reduction in bone marrow cell compartments, which results in B-cell depletion [25]. In the present study, we have observed disorders in the number and percentage of total WBC in blood of rats fed a low-protein diet. Significant changes were already noted on day 30 of the experiment and were increased on day 90. Unsurprisingly, a protein-deficient diet lowered the number of lymphocytes (in all studied subpopulations), monocytes, eosinophils, however raised the number of neutrophils. Additionally, after 30 days of the protein malnutrition diet, a decrease in B-cell percentage was also noted, which returned to control values on day 90 of the experiment.

Protein malnutrition is prevalent in cancer patients and occurs as a consequence of an imbalance between nutritional needs of the patient, demands of the tumor and availability of nutrients in the body [26]. Main immunological anti-cancer defenses in the organism include lymphocytes CD8+ and NK cells. A decreased NK cells number has been shown in malnourished cancer patients, which was increased when appropriate arginine-enriched nutrition was applied [27]. Moreover, Reynolds *et al.* observed impaired poly (I:C)-induced natural killer cell function in CBA/J mice fed for 2 weeks a protein-calorie malnutrition diet [28]. In the same experiment, NK cell activity in Swiss mice receiving a 2.5% protein diet (for two or three weeks) was not significantly impaired. In the present work, a short time of a protein-restricted diet (30 days, 4.5% of protein) did not affect cellular immunity of rats, however long time protein deprivation (90 days) resulted in a significant decrease in CD8+ lymphocytes and NK cells percentage. This may suggest one of mechanisms by which protein malnutrition secondarily promotes the development of the cancer.

The most important function of vitamin B_{12} is DNA synthesis, necessary for cell division, whereby it could modulate human immunity. Deficiency of the vitamin causes anemia and modifies NK cytotoxicity, lymphocytes B and lymphoproliferation in aged rats [29]. Addition of vitamin B_{12} in B_{12}-deficient patients facilitates the production of T lymphocytes recruited in cellular immunity, restores an abnormally increased CD4/CD8 ratio and maintains the count of lymphocyte subgroups in the normal range [30]. Similarly, Erkurt *et al.* demonstrated that the intake of vitamin B_{12} in patients with anemia causes an increase in the absolute number of CD8+ as well as a slight increase in CD 4+ lymphocytes [31]. In the present study, B_{12} supplementation did not affect white blood cells as well as lymphocytes subpopulations (number and percentage) in the control group (both after 30 and 90 days). A 30-day period of B_{12} addition in the protein-deficient group reduced the negative effect of malnutrition. This applies to the total number of white blood cells, number of lymphocytes (in all subpopulations) and monocytes, and percentage of CD3+, CD45A+ and CD4+ cell populations. These results suggest a protective role of vitamin B_{12} supplementation in the blood immunological system in the short-time protein malnourished organism. Unfortunately, long-time vitamin B_{12} administration did not reduce the adverse effect of protein deprivation. Absence of positive B_{12} impact after 90 days of the experiment may result from the extreme exhaustion of the body caused by the protein-restricted diet.

Conclusions

A high level of vitamin B_{12} supplementation (300% of the daily intake) did not affect the immune system in rats fed for 30 and 90 days a standard diet. At the same time, B_{12} enrichment had a beneficial impact in rats fed for 30 days a protein-deficient diet. These results suggest a positive effect of vitamin B_{12} supplementation on the immune system in the protein-deficient organism, but to fully understand the mechanism by which vitamin B_{12} affects the immune system in protein-malnourished organisms this needs to be precisely investigated.

The authors declare no conflict of interest.

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