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Effects of Cyanocobalamin on Immunity in Patients with Pernicious Anemia

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Key Words
Pernicious anemia · Vitamin B_{12} · Immunity · Natural killer cells · CD4/CD8 ratio

Abstract
Objective: The aim of the study was to evaluate the role of vitamin B_{12} in patients with pernicious anemia. ***Materials and Methods:*** This study was conducted prospectively at the Turgut Özal Medical Center, Department of Hematology, between April and November 2002. Absolute numbers and ratio of the surface antigens of T and B lymphocyte subgroups, CD4/CD8 ratio were calculated in order to evaluate changes in leukocyte and lymphocyte numbers; natural killer (NK) cell count, serum C3, C4, and levels of immunoglobulins G, A, and M were also measured to evaluate vitamin B_{12} effect on immunity. Values obtained before treatment with cyanocobalamin were compared with those found during peak reticulocyte count. ***Results:*** In vitamin B_{12}-deficient patients, absolute numbers of CD4+ and especially CD8+ lymphocytes were found to be decreased; CD4/CD8 ratio increased, and NK cell activity was depressed. After cyanocobalamin treatment, absolute numbers and percentage of lymphocyte subgroups were elevated. Increased CD4/CD8 ratio and depressed NK cell activity were restored and levels of C3, C4, and immunoglobulins were elevated. ***Conclusion:*** These findings suggest that vitamin B_{12} has important immunomodulatory effects on cellular immunity, and abnormalities in the immune system in pernicious anemia are restored by vitamin B_{12} replacement therapy.

Introduction

The role of vitamin B_{12} in human immunity is still obscure. There are few studies reporting changes observed in immune parameters after vitamin B_{12} administration in patients with pernicious anemia [1–5]. However, some of these studies were case reports or inadequately designed clinical trials.

The most important function of vitamin B_{12} is in DNA synthesis where it is necessary for cell replication. In this process vitamin B_{12} acts with folic acid. It is believed that vitamin B_{12}, which has a role in cell division, also acts as a modulator of human immunity; it facilitates the production of T lymphocytes recruited in cellular immunity, restores abnormally increased CD4/CD8 ratio and maintains the count of lymphocyte subgroups in the normal range [6]. An in vitro experimental study demonstrated that vitamin B_{12} was implicated in concanavalin A-dependent T cell production and pokeweed mitogen-dependent immunoglobulin synthesis in B cells [7]. In animal models, immune defense against viruses and

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Table 1. Some blood parameters of patients before treatment

Parameters	Min.	Max.	Mean	SD	Normal range
Hemoglobin, g/dl	3.1	10.30	7.1	1.86	11.5–16
Hematocrit, %	8.6	30.50	20.6	5.89	34–45
Mean corpuscular volume, fl	97	139.00	113.7	12.44	80–95
Platelet count, $\times 10^3$/ml	6	528.00	142.9	104.62	150–450
Leukocyte count, $\times 10^3$/μl	1.1	11.70	4.4	2.13	4–11
LDH, U/l	530	13,429	4,960.9	3,129.44	200–350
Potassium, mmol/l	3.6	4.90	4.2	0.27	3.5–5.5
Uric acid, μmol/l	130.8	547.4	326.5	95.7	154–422
Unconjugated bilirubin, μmol/l	0.4	16.7	5.2	2.3	0–10
Conjugated bilirubin, μmol/l	0.22	6.4	1.4	1.2	0–4
Vitamin B_{12}, pg/ml	50	180	85.2	73.4	200–900
Folic acid, ng/ml	0.92	22.3	6.7	5.07	2.7–16.1

bacteria was found to be depressed in vitamin B_{12} deficiency [8].

In this study we evaluated the role of vitamin B_{12} in patients with pernicious anemia. The measurements included the activity of subgroups of T and B lymphocytes and natural killer (NK) cells, levels of serum C3, C4 and immunoglobulins before and after vitamin B_{12} administration.

Subjects and Methods

This study was conducted prospectively in Turgut Özal Medical Center, Department of Hematology, between April and November 2002. Thirty patients (16 male and 14 female, age 17–75, average 55 years) with pernicious anemia were enrolled in the study. Diagnostic criteria of the patients are summarized in table 1. All patients showed low serum levels of vitamin B_{12} (the average value and the normal range were 85 and 200–900 pg/ml, respectively). Diagnosis was based on the medical history, macro-ovalocytosis in peripheral blood, megaloblastic changes in bone marrow, low serum levels of vitamin B_{12}, increased serum LDH and indirect bilirubin levels, and grade 4 atrophic gastritis in endoscopic biopsy.

In order to evaluate changes in leukocyte and lymphocyte numbers, absolute numbers and the ratio of the surface antigens of T and B lymphocyte subgroups, CD4/CD8 ratio, NK cell activity, serum C3, C4, and immunoglobulin G, A, and M (IgG, IgA, IgM) levels were measured before treatment and compared with the values obtained during peak reticulocyte count.

Cyanocobalamin (Dodex) was administered (1,000 μg/day intramuscularly) until the reticulocyte crisis occurred and serum vitamin B_{12} levels returned to the normal range.

Surface Marker Analysis

Peripheral blood was obtained in tubes containing EDTA K3. Ten microliters of monoclonal antibody solution was added to 100 μl of peripheral blood and incubated at room temperature for 30 min. The mononuclear cell fraction was collected by centrifugation on lymphocyte separation medium (Coulter Q-PREP, Epics Immunology Workstation, USA). Mononuclear cells were analyzed by flow cytometry (Beckman Coulter Epics Altra) before and after treatment. Surface marker analysis was performed within 2 h of blood collection. Commercial monoclonal antibodies used for immunophenotyping were as follows: CD3 T cell FITC clone UCHT1, CD4 T cell FITC clone MT310, CD7 T cell FITC clone DK24, CD8 T cell FITC clone DK25, CD10 CALLA RPR clone SS2/36, CD16 FC gamma receptor III FITC clone DJ130C, CD19 B cell FITC clone HD37, CD20 B cell FITC clone B-Lyl, CD22 B cell FITC clone 4KB128, CD56 NK cell RPE clone-MOC-1 (DAKO Denmark).

Complement and Immunoglobulin Analysis

Serum C3, C4, IgA, IgM, and IgG were measured by the nephelometric method (Behring 100 Analyzer, Newark, N.J., USA), using the Dade Behring (Newark, N.J., USA) commercial kit.

Statistical Analysis

Statistical analysis was done by SPSS (Statistical Program for Social Sciences, version 10.0, Chicago, Ill., USA). Significance of differences was evaluated with paired t test; $p < 0.05$ was regarded as statistically significant.

Results

Mean leukocyte count before and after treatment was 4,432.3 ± 2,131.7 and 5,416.1 ± 1,697.5/mm³, respectively. Increase in leukocyte count with treatment was statistically significant ($p = 0.009$). Lymphocyte counts before and after treatment were 2,850.1 ± 781.7 and 2,514 ± 830/mm³, respectively, and the difference was statistically significant ($p = 0.008$; table 2, fig. 1).

Table 2. Immune parameters before and after cyanocobalamin treatment

Parameters	Treatment		p value	Normal range
	before	after		
Leukocytes/mm^3	4,432 ± 2,131	5,416 ± 1,697	0.009	4,000–11,000
Lymphocytes/mm^3	2,850 ± 781	2,514 ± 830	0.008	1,500–3,500
CD4, %	44.22 ± 7.64	44.33 ± 7.65	NS	26–58
CD4/mm^3	1,048 ± 337	1,056 ± 373	NS	1,000–2,000
CD8, %	26.69 ± 10.23	29.42 ± 10.9	0.002	20–42
CD8/mm^3	606 ± 239	689 ± 318	0.035	240–1,000
CD4/CD8 ratio	2.00 ± 1.05	1.80 ± 0.83	0.06	1.5–2.0
CD3, %	69.06 ± 11.71	73.01 ± 8.02	0.042	57–81
CD3/mm^3	1,573 ± 512	1,752 ± 542	0.015	1,200–2,500
CD7, %	44.29 ± 7.64	46.85 ± 12.94	NS	34–69
CD7/mm^3	1,002 ± 353	1,044 ± 376	NS	1,000–2,000
CD10, %	7.60 ± 7.76	7.86 ± 7.23	NS	4–12
CD10/mm^3	181 ± 185	190 ± 222	NS	100–300
CD16 + CD56, %	17 ± 7.28	19 ± 7.45	NS	8–28
CD16 + CD56/mm^3	264 ± 112	287 ± 126	NS	212–318
CD19, %	12.50 ± 6.93	18.76 ± 6.98	0.016	10–27
CD19/mm^3	254 ± 149	348 ± 146	0.002	200–400
CD20, %	18.50 ± 6.95	17.37 ± 7.68	NS	11–25
CD20/mm^3	412 ± 142	355 ± 144	0.035	300–500
CD22, %	14.43 ± 6.09	12.98 ± 6.39	NS	10–20
CD22/mm^3	322 ± 134	271 ± 147	NS	200–400
C3, g/l	0.75 ± 0.21	0.92 ± 0.27	0.001	0.8–1.5
C4, g/l	0.21 ± 0.15	0.21 ± 0.14	NS	0.15–0.35
IgG, g/l	10.07 ± 2.77	11.28 ± 3.44	0.04	7–15
IgA, g/l	2.41 ± 1.63	3.81 ± 2.76	0.01	0.8–4
IgM, g/l	0.93 ± 0.45	1.16 ± 0.57	0.03	0.4–2.6

NS = Not significant.

Effects of Cyanocobalamin Administration on T Lymphocyte Subsets

Mean absolute number and CD4 (%) before and after treatment were 1,048.6 ± 337.3/mm^3, 44.2 ± 7.6 (%) and 1,056.4 ± 373.4/mm^3, 44.3 ± 7.6 (%), respectively. The difference was not statistically significant ($p = 0.894$). Mean absolute number and the CD8 (%) before and after treatment were 606.95 ± 239.26/mm^3, 26.6 ± 10.2 (%) and 689.09 ± 308.22/mm^3, 29.4 ± 10.9 (%), respectively. The increases in the absolute number and CD8 (%) were statistically significant ($p = 0.035$ and $p = 0.002$). The mean CD4/CD8 ratio before and after treatment was 2.00 ± 1.05 and 1.80 ± 0.83, respectively ($p < 0.06$). The abnormally high CD4/CD8 ratio before treatment declined after 1 week of cyanocobalamin administration.

The absolute number and the CD3 (%) and CD7 (%) cells were elevated with treatment. However, the increment was statistically significant for CD3 cells but not for CD7 (table 2, fig. 1).

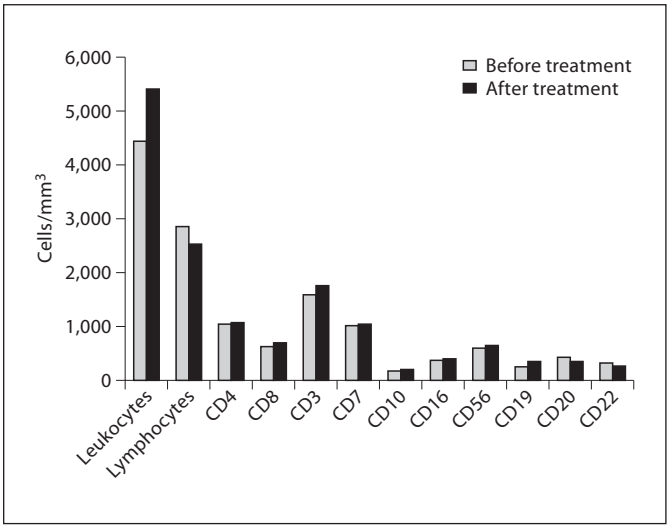

Fig. 1. Changes in immune parameters by cyanocobalamin treatment.

Effects of Cyanocobalamin on B Lymphocyte Subsets

The absolute number and CD10 (%) were slightly elevated with treatment. However, the increment was not statistically significant. The absolute number and CD19 (%) were significantly elevated when compared with pretreatment values. The absolute number and CD20 (%) declined after treatment, but only the former was statistically significant. CD22 (%) declined too, but not significantly. The absolute numbers of B lymphocyte subsets before and after cyanocobalamin treatment are shown in table 2 and figure 1.

Effects of Cyanocobalamin Administration on NK Lymphocyte Subsets, Serum Immunoglobulin and Complement Levels

Both absolute number and CD16 (%) and CD56 (%) were found to be elevated after treatment although the increase was not significant. These changes are also listed in table 2 and figure 1.

Serum levels of immunoglobulins and complement before and after treatment are given in table 2. While a significant elevation was observed in C3 level after treatment, C4 level showed only a slight elevation (table 2). When compared with pretreatment values, IgG, IgA and IgM levels were elevated after treatment. Increment in immunoglobulin levels with treatment was statistically significant (table 2).

Discussion

Vitamin B_{12} deficiency results in megaloblastic anemia, peripheral nervous system disorder, and in animal models, depression of immune defense against viruses and bacteria [8]. It has been reported that vitamin B_{12} treatment restores the immune parameters in patients with megaloblastic anemia [1–5].

Concanavalin A-dependent T cell proliferation and pokeweed mitogen-dependent immunoglobulin synthesis in B cells were also shown to be increased by vitamin B_{12} [7]. In contrast to these observations, Soler et al. [9] and Carmel et al. [10] reported that in patients with pernicious anemia there was no increase in CD4+/CD8+ ratio or a significant decrease in CD8+ absolute number. Cyanocobalamin has been reported to restore both abnormal CD4+/CD8+ ratio, and decrease in absolute number of CD8+, but no change in absolute number of CD4+ cells was observed [6]. We observed that cyanocobalamin administration had restored the decrease in absolute number of CD8+ cells which was the main abnormality, but no significant increase in absolute number of CD4+ cells was seen.

Absolute numbers of CD3 and CD19 significantly increased after cyanocobalamin administration, but CD10 and CD7 were slightly elevated. The levels of serum IgG, IgA, and IgM were significantly elevated. These observations are consistent with the proposition that vitamin B_{12} favors humoral and cellular immunity. Lymphocyte apoptosis can be prevented by vitamin B_{12} administration and may thus improve immunologic abnormalities observed in pernicious anemia. Previous studies suggested that ineffective hematopoiesis caused by vitamin B_{12} deficiency concerned primarily CD8+ cells [11, 12]. In our patients with pernicious anemia, decline in absolute numbers of lymphocytes and CD8+ cells was partially restored by vitamin B_{12} administration. However, the role of CD8+ in this regard warrants further studies.

The cyanocobalamin-mediated recovery from depressed NK cell function in our vitamin B_{12} patients are in accordance with another study where lymphocyte and NK cell functions were completely restored and serum levels of vitamin B_{12} returned to normal after 1 year of vitamin B_{12} treatment [6]. Hsing et al. [13] reported that increase in absolute numbers of CD3–, CD16+, and CD57+ cells (these cells have strong NK cell activity) after vitamin B_{12} treatment had augmented the antitumor activity.

Two studies reported that serum C3, IgG, and IgM levels declined in vitamin B_{12}-deficient rats and this decline was restored after vitamin B_{12} administration [14, 15]. Our patients also showed a significant increase in serum levels of C3, IgG, IgA, and IgM, and a slight elevation in C4 level after vitamin B_{12} treatment. These effects of vitamin B_{12} may be responsible for the decrease observed in the incidence of infections in vitamin B_{12}-deficient patients after treatment.

Conclusion

The replacement of vitamin B_{12} in patients with pernicious anemia restored, at least in the early phase of treatment, the significant decrease in the number of CD8+ cells and the depression of NK cell activity and serum immunoglobulin levels. Assessment after a prolonged treatment or recovery from anemia might have shown a more substantial improvement of the immunological parameters. These observations may contribute to our understanding of some of the potential protective effects of vitamin B_{12}.

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