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Interleukin-21 in hemodialyzed patients: Association with the etiology of chronic kidney disease and the seropositivity against hepatitis C virus infection

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ABSTRACT

Objectives: IL-21 is a new pleiotropic cytokine involved in immune system regulation.
Design and methods: We determined IL-21 in the plasma of hemodialyzed (HD) patients and healthy controls, and we tried to identify the factors which could affect its levels.
Results: Detectable levels of IL-21 were observed in the similar percent of HD patients and controls, but its concentration was twice lower in HD patients. The patients with detectable IL-21 had lower inflammatory state, reflected by IL-6 and TNF-α, compared to those with undetectable IL-21. The association was between IL-21 and the presence of glomerulonephritis ($p<0.05$), anti-HCV seropositivity ($p<0.001$) and the markers of liver function.
Conclusions: IL-21 is decreased in HD patients and is not affected by gender, age, inflammation, the vintage of HD, type of medication and type of used dialysis membrane. The etiology of chronic kidney disease and anti-HCV seropositivity independently affect its plasma levels in these patients.

Introduction

Hemodialysis (HD) patients show clinical signs of an immune defect characterized by an increased susceptibility for infection and a decreased immune response to T-cell dependent antigens. The precise mechanisms responsible for this immune defect are poorly understood [1]. Although acquired immunity (via antibody production) is impaired in HD patients [2, 3], the current data suggest that T-lymphocyte-dependent immune response is deficient, predisposing to infections and inadequate response to vaccination [4, 5]. In fact, in HD patients vaccinated with hepatitis B surface antigen, the delayed development of antigen-specific cells in the central memory T cells, defective or almost absent generation of antigen-specific effector memory T cells, decreased hepatitis B surface antigen-specific T cell proliferation and anti-hepatitis B antibodies production were observed [6].

Interleukine 21 (IL-21) is a pleiotropic cytokine secreted almost exclusively by CD4(+) T cells [7] that profoundly affects the growth, survival, and functional activation of B, T, and natural killer lymphocytes in concert with other cytokines or activating stimuli [8]. As a consequence of its ability to act on multiple cells of the immune system, IL-21 has the potential to impact both innate and adaptive immune responses. It is also known to play a major role in development of B cells and to promote the antibodies production [9, 10]. Consistent with these broad actions, IL-21 has been shown to affect autoimmunity, tumor-specific responses and immunity to infection [11–13].

To our knowledge, there are no data concerning IL-21 in uremia. In this study we compared plasma IL-21 concentration between HD patients and healthy volunteers. The results were statistically analyzed to evaluate the influence of etiology of renal disease, residual renal function, gender, HD process, type of dialysis membrane and medication on this cytokine level. Moreover, the inflammatory markers: interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were also evaluated in the plasma because inflammation usually accompanies HD, and IL-21 has been perceived as the new mediator of inflammation [14].

Materials and methods

Subjects

Fifty-seven patients on chronic HD were enrolled in the study. Twenty-two had some residual renal function (RRF), daily urine volume ranged from 300 to 3000 mL, median 1500 mL/d; and thirty-five were anurics. All patients were clinically stable and free of active infections and autoimmune diseases. None of the patients received immunosuppressive treatment, lipid-lowering agents, nonsteroidal anti-inflammatory drugs or antioxidants such as vitamins E, C or allopurinol at the time of the study. Body mass index (BMI)

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was calculated by dividing the dry weight in kilograms by the square of the height in meters. Dialysis adequacy was assessed by measuring the weekly fractional urea clearance (Kt/V). The presence of cardiovascular disease (CVD) was based on one or more of typical clinical symptoms or prior ECG or exercise test. The patients received conventional 4-h HD, three times weekly, with a bicarbonate dialysate and low molecular weight heparin–enoxaparin (n = 50) or unfractionated heparin (n = 7) as anticoagulation. One of the following membranes was assigned to the patients randomly when they were admitted to the clinic: modified cellulose membrane, n = 20; polysulfone membrane, n = 15; hemophan membrane, n = 16 and cuprophane membrane, n = 6. None of the patients had serum detection of hepatitis B virus (HBV) surface antigen, and 15 were seropositive for the antibodies against hepatitis C virus (anti-HCV).

End-stage renal disease was attributed to glomerulonephritis in 16 cases, diabetes mellitus type 2 in 10, interstitial nephritis in 10, polycystic kidney disease in 5, secondary amyloidosis in 6, hypertensive nephropathy in 4 and was undetermined in 6 cases.

Twenty-eight sex- and age-matched healthy subjects who were receiving no drugs or vitamin supplements at the time of the study volunteered as controls for determinations of IL-21, IL-6 and TNF-α in the plasma. All were on a regular diet and did not have any history of hypertension, diabetes mellitus or renal disease.

The study was conducted in accordance with the Declaration of Helsinki (1985 amendment) and was approved by the ethical guidelines of Medical University in Bialystok. The informed consent was obtained from each participant.

Blood sampling and laboratory measurements

Investigations were performed in the morning under fasting conditions. Blood samples were taken directly from the arteriovenous fistula immediately before the beginning of a routine 4-h HD session. In the controls blood was drawn from the antecubital vein using EDTA as anticoagulant. Platelet poor plasma samples were prepared conventionally, aliquoted and stored at −30 °C until the assay.

Biochemical and hematological parameters were determined by standard laboratory methods. LDL cholesterol was calculated using the Friedewald's formula. Serum HBV surface antigen and anti-HCV antibodies were determined by third generation microparticle ELISA kits manufactured by Abbott, using an AxSYM analyzer (Abbot Laboratories, Abbot Park, IL).

The concentrations of IL-21 and the proinflammatory cytokines: IL-6 and TNF-α in the plasma were determined by Human IL-21 ELISA, Human IL-6 HS ELISA and Human TNF-α ELISA kits, Bender MedSystems GmbH, Vienna, Austria.

Statistical analysis

The normally distributed data provided by Shapiro–Wilk's W test were expressed as mean ± SD. The non-Gaussian data were presented as median (range), and were log-transformed prior to statistical analysis.

Multiple group comparisons were performed by one-way analysis of variance, and significant differences between groups were assessed by Kruskal–Wallis Test (Nonparametric ANOVA). For comparison of parameters between healthy volunteers or between 2 groups of HD patients, unpaired *t*-test or Mann–Whitney *U*-test was used as appropriate. We used the χ^2 test for categorical variables. Correlations were determined using Pearson's linear and quasi-Newton and Rosenbrock's regression analysis when appropriate. Multiple regression analysis was used to assess the combined influence of variables on IL-21. A two-tailed p value <0.05 was considered statistically significant.

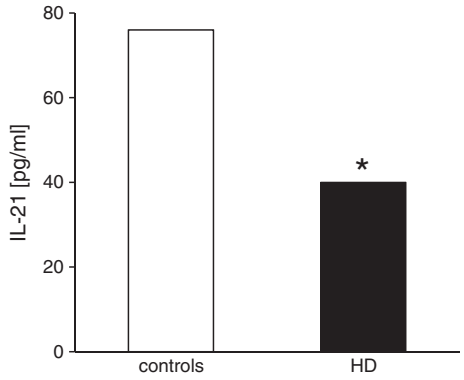

Fig. 1. The plasma IL-21 levels in healthy controls and hemodialyzed (HD) patients.

Results

At the time of the study, 57% from healthy controls and 49% from HD patients had detectable IL-21 levels (the limit of detection was determined to be 20 pg/mL). Plasma IL-21 concentrations were significantly higher in healthy controls compared to HD patients with detectable IL-21 levels; p = 0.0274 (Table 2 and Fig. 1). The clinical and biochemical characteristics, concomitant diseases and medication for two groups of HD patients: with detectable IL-21 levels (group A) and with IL-21 below detection limit (group B) are listed in Table 1. Fibrinogen was lower and prothrombin time was

Table 1
Clinical and biochemical characteristics of hemodialyzed patients with detectable IL-21 levels (group A) and with IL-21 below detection limit (group B).

	A	B	p
	n = 28	n = 29	
Sex, M/F	14/14	19/10	0.5995
Age, years	61.79 ± 13.52	59.86 ± 13.98	0.5995
BMI, kg/m^2	25.86 ± 4.93	24.55 ± 3.41	0.2779
Hemodialysis vintage, months	33.0 (8.132)	26.5 (4–241)	0.5335
Kt/V	1.26 ± 0.26	1.18 ± 0.29	0.4457
Hemoglobin, g/L	112.3 ± 12.4	109.0 ± 13.2	0.3226
White blood cells, ×10^9/L	5.70 ± 1.38	6.00 ± 1.79	0.7739
Lymphocytes, %	24.61 ± 6.47	24.25 ± 7.17	0.8440
Total cholesterol, mmol/L	5.43 ± 1.14	5.11 ± 1.31	0.3343
HDL-cholesterol, mmol/L	1.15 ± 0.34	1.18 ± 0.33	0.8188
LDL-cholesterol, mmol/L	3.62 ± 0.95	3.22 ± 1.34	0.2191
Triglycerides, mmol/L	1.46 (0.35–4.93)	1.43 (0.54–5.43)	0.9731
Total protein, g/L	68.0 ± 5.7	66.7 ± 0.61	0.4727
Albumin, g/L	38.4 ± 4.6	38.2 ± 3.7	0.8882
ALT, IU/L	17.0 (7.0–90.0)	15.0 (5.0–104.0)	0.6781
AST, IU/L	18.0 (8.0–92.0)	17.0 (9.0–49.0)	0.8371
Bilirubin, μmol/L	7.52 ± 3.76	7.87 ± 5.30	0.7123
Fibrinogen, μmol/L	9.61 ± 2.28	11.05 ± 2.24	0.0233
Prothrombin time, s	16.42 ± 4.03	14.29 ± 2.00	0.0169
aPTT, s	36.34 ± 6.60	36.80 ± 4.40	0.7622
Systolic blood pressure, mm Hg	137.41 ± 25.92	148.79 ± 22.98	0.0888
Diastolic blood pressure, mm Hg	82.96 ± 13.25	84.48 ± 10.89	0.6424
Erythropoietin treated, %	79	83	0.9463
Erythropoietin dose, U/kg/week	91.60 (20.81–161.62)	82.22 (18.73–155.84)	0.8465
Anti-HCV seropositivity, %	32	14	0.0357
Cardiovascular disease, %	68	72	0.6725
ACE inhibitor, %	64	66	0.8680
Calcium channel antagonist, %	50	69	0.6043
B-blocker, %	50	48	0.8770
Alpha-blocker, %	18	14	0.7219

ACE = angiotensin I-converting enzyme; BMI = body mass index, ALAT = alanine aminotransferase, AST = aspartate aminotransferase, aPTT = activated partial thromboplastin time.