Cent. Eur. J. Med. • 3(2) • 2008 • 199-202
DOI: 10.2478/s11536-008-0011-4

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Central European Journal of **Medicine**

IL-2, IL-6 and IL-8 levels remain unaltered in the course of immunosuppressive therapy after renal transplantation

Research Article

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Received 4 January 2008; Accepted 2 February 2008

Abstract: Cytokines regulate the immune reactions elicited by renal transplantation (RT). This study was designed to investigate the blood serum levels of IL-2, IL-6, IL-8 in 25 RT patients (10 female and 15 male, mean 5.4±2.7 yrs after RT) three times over a six-month period during standard immunosuppressive therapy with cyclosporine A, azathioprine and prednisolone. The levels of IL-2, IL-6 and IL-8 were tested with ELISA Quantikine Human Interleukin Immunoassay (R&D Systems, detection level 7,0.7 and 10 pg/cm^3, respectively). There was no significant alternation of blood serum levels of IL-2, IL-6 and IL-8 in the study patients.

Keywords: *Cytokines • Lymphocytes • Transplantation • Immune system*

1. Introduction

Cytokines regulate immune response and the inflammatory reactions in parallel with numerous biological reactions. A number of cytokines are released in response to the variety of antigens, bacterial lipopolysaccharides, lectins [1] and mediate information processing among inflammatory cells; this is particularly relevant in the setting of renal transplantation (RT) [2]. Immune disorders are attributed to complex alternations of the reactivity of immune competent cells, phenotype alternations of immune receptors and cell surface adhesion antigens in the milieu of abnormal levels of immune mediators. The causative role of immune dysfunction after RT is driven by immune incompatibility and rejection of graft organ [3]. The transplantation drives immune reactions involving the recognition and active damage of the transplanted organ. It further involves specific immune response of immune competent cells. Immune abnormalities in patients with chronic renal failure is likely to be secondary and only in some patients it may be determined by a given HLA configuration. Relatively fast and appropriate normalization of immune function after RT demonstrates the lack of permanent immune abnormalities in chronic renal failure [4-6]. The assessment of cytokine mediators and their receptors is useful in the research of graft rejection mechanisms. Molecular techniques are able to provide the insight at transcriptional and translational levels of inflammatory cell processing of cytokines and cytokine receptors and respective biopsy materials. Another available option is the assessment of cytokine levels in tissues and peripheral blood serum. The assessment of the soluble receptors for IL-2 and IL-6 in systemic circulation

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and graft-derived fluids may be complicated with their increase due to both the infection and rejection reactions. Moreover, gene activation of IL-2 and IL-4 and the cytokine expression may occur before the first clinical symptoms of the graft rejection. Therefore, the assessment of cytokine levels in RT patients as means of early detection of rejection may be a clinically useful diagnostic tool. This study was therefore designed to investigate the levels of IL-2, IL-6 and IL-8 in patients after renal transplantation within three consecutive six month periods.

2. Material and Methods

2.1. Experimental Design

The experimental construct of the study included blood serum levels of IL-2, IL-6, IL-8 in 25 RT patients (10 female and 15 male, mean 5.4±2.7 yrs after RT), which were routinely monitored three times within a period of six-months. All subjects taking part in the experiment had gone under objective and projective tests. Laboratory tests were administered to the subjects for instance, C reactive protein, urea, and creatine in the serum. Conditions that determined whether or not the test was reliable enough to be used in the experiment included, but were not limited to, a lack of clinical and biochemical marks of transplant rejection. Additional criteria that pertained to all patients was the lack of smoking as a habitual routine. Patients who satisfied the requirements for being included in the study were consulted by a nephrologist, an ophthalmologist and a vascular surgeon in order to exclude micro and macroangiopathy (Table 1). Among the healthy individuals taking part in the study, no control group was created. The internal threat of mortality was not an issue. All patients who committed themselves to the experiment had participated fully throughout the entire study. Patients underwent immunosuppressive therapy with cyclosporine A (100-225 mg daily), azathioprine (50-100 mg daily) and prednisolone (5-10 mg per day).

2.2. The blood serum levels of IL-2, IL-6, IL-8

The levels of IL-2, IL-6 and IL-8 were tested with ELISA Quantikine Human Interleukin Immunoassay (R&D Systems). The lowest levels detection levels of IL-2, IL-6 and IL-8 were 7.0, 0.7 and 10 pg/cm^3, respectively. Blood samples were drawn from antecubital veins on a fasting basis.

Table 1. The clinical characteristics of patients.

	Assay I	Assay II	Assay III
Number of patients	25	25	25
Mean age (x ± SD)	54,3±9,8	54,3±9,8	54,3±9,8
Mean age after renal transplant. (x ± SD)	5,4±2,7	5,4±2,7	5,4±2,7
Urea (mmol/l)	7,0±1,3	6,5±1,7	7,2±1,4
Creatynine (μmol /l)	98,5±20,3	100±23,4	102,7±14
CRP (mg/dl)	4,2± 0,5	5,6± 0,7	4,1± 0,4

2.3. Statistical analysis

The results are expressed as arithmetic mean ± standard deviation in MFI (mean fluorescence intensity) units. The differences were analyzed with Mann-Whitney U test. The significance levels in all cases was at $p<0.05$.

3. Results

The initial six-month follow-up demonstrated a tendency of blood serum increase of IL-2 if compared to the baseline levels but this increase was not significant. After another six- month time period there was also an insignificant decrease in these levels. The initial blood serum levels of IL-6 were decreased during the first and the consecutive follow-up period and it also appeared statistically insignificant. Furthermore, there was only a tendency of IL-8 to decrease, accompanied by insignificant increases after a twelve month time period (Table 2).

Table 2. Serum IL-2, IL-6 and IL-8 concentrations in renal transplantation patients.

Assay I-III		IL-2 (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)
I	6 mths (n=25)	67,1 ± 135,4	81,6 ± 61,5	50,1 ± 34,2
II	12 mths (n=25)	78,2 ± 59,0 *	74,7 ± 63,7*	48,6 ± 31,2*
III	18 mths (n=25)	69,5 ± 41,4**	82,7 ±77,07**	52,9 ± 46,1**

** p>0,05 II assay vs. I assay; ** p > 0,05 III assay vs. I assay.*

4. Discussion

Beside dialysis is renal transplantation, the possible renal replacement therapy available in the end stage of chronic renal failure. This option is however related to immune complications elicited by the alien antigens and is accompanied by self-propelling immune responses of the rejection of transplanted tissues; this involves cellular

interactions regulated predominantly by cytokine network, cell surface receptors of immune competent cells and various adhesive molecules [7]. Immune tolerance is still beyond the scope of therapeutic intervention despite the remarkable progress of research on transplantation immunology. The immune response to the transplanted tissue involves induction and an effector phase. The induction phase includes antigen presentation, their recognition and the proliferation of antigen-specific host lymphocytes. It may be both direct when donor MHC antigens are presented to host T lymphocytes by antigen presenting cells (APC), and indirect, when donor antigens are presented by host APC to the host T lymphocytes [2,8]. The presentation reaction is mediated by T cell receptor (TCR). The response of helper T lymphocytes to the antigen presentation is supported by co-receptors facilitating an adhesion reaction between T lymphocytes and APC as well as by signaling that accelerates the activation cascade. The effector phase involves the activation of specific immune mechanisms. After the antigen recognition, the macrophage release IL-1, leads to the proliferation of T lymphocyte helper cells. Activated helper T lymphocytes release IL-2, accelerating proliferation of cytotoxic lymphocytes. Moreover, activated T lymphocytes produce interferon γ and other chemotactic factors. Beside the activation of cytotoxic T lymphocytes, helper T lymphocytes may also initiate delayed hypersensitivity reactions, which involve non-specific, cytokine-mediated activation of macrophages and polymorphonuclear leukocytes. Helper lymphocytes mediating the induction of transplantation responses are present in host lymphatic organs and are activated by contact with donor or host antigen presenting cells. Activated T lymphocytes helpers proliferate and differentiate into cells capable of facilitating activation of resting cytotoxic T lymphocytes and transformation of lymphocytes B into effector cells by the release of cytokines and the direct contact. IL-6 and IL-1 regulate the process of antigen presentation leading to the activation of T lymphocyte helpers capable of activating cytotoxic T lymphocytes, principally by the release of IL-2, lymphocyte maturation factor (IL-12 and IL-4, IL-5, IL-6, IFN-γ), activation of natural killer lymphocytes (IL-2), proliferation of lymphocytes B (IL-2, IL-4, IL-5), differentiation of lymphocytes B (IL-2, IL-4, IL-5, IL-6). T lymphocyte helpers activate macrophages and recruit the remaining inflammatory cells within graft tissue including polymorphonuclear leukocytes, mast cells and plasmocytes. Moreover, certain cytokines may either suppress or accelerate the immune reaction to transplanted tissue at different stages. Cytokines not only control the proliferation and the differentiation of immune cells but also regulate leukocyte migration due to their effect on the expression of adhesion molecules including IL-1 and TNFα. Under normal conditions, cytokines increase expression of MHC antigens or induce their expression on the cell lacking their expression. IFN-γ is the most potent inducer of MHC expression, whose action is supported by TNF ability to increase the expression of class I and II MHC antigens on B lymphocytes, monocytes and macrophages [9,10]. The multiple sites of biological action of cytokines in the course of transplantation reaction appeared a useful tool for graft rejection monitoring and immunosuppressive therapy which was specifically aimed to cytokines and the cytokine receptors [12]. The reliability of circulating IL-8 levels was demonstrated in monitoring RT patients [13], whereas other data indicated a superior index value of urine levels of IL-8 if compared with the peripheral blood serum for the purpose of rejection monitoring [14]. IL-6 was reportedly the most sensitive index of graft rejection in RT patients [15-19].

The current study demonstrated constant blood serum levels of IL-2, IL-6 and IL-8 within an eighteen month follow-up time period in RT patients that may be related to appropriate dose of immune suppressive therapy and adequate tolerance of the graft tissue (Table 2). Although the mechanisms underlying cellular and immune interactions in RT patients remain elucidated, the available data might reflect the chronic inflammatory reactions associated with graft rejection.

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