Objectives: The optimum induction and maintenance immunosuppressive therapy in renal transplantation is not yet established. In this study immunomonitorization of cytokine levels was performed to determine the immunologic status of kidney transplant patients to optimize the immunosuppressive therapy regimen.

Methods: We determined IL-1α, TNF-α, IFN-γ, IL-10 and TGF-β in 87 blood samples by ELISA (RayBiotech, Inc). Group 1 (n=35) included kidney transplant patients at postoperative 1st year whose preoperative blood samples were studied (n=35) and all were cross-match negative. Group 2 (n=10) were cross-match positive preoperative patients. Group 3 was the control group (7 healthy subjects). Those patients were immunosuppressed by Cyclosporine (CyA) based triple immunosuppressive regimen (CyA+Mycophenolate mophetyl+steroid).

Results: Cyclosporine (CyA) based triple immunosuppressive regimen was associated with acute rejection (p<0.001) and also with IL-10 (p<0.001), TGF-β (p<0.001), IL-1α (p<0.005), IFN-γ (p<0.001) positivity. In the healthy control group all of the cytokines were measured negative indicating an immunological balance. Hemodialysis for over 5 years significantly leads to TGF-β (p=0.021) positivity but it does not correlate with acute rejection after renal transplantation.

Conclusion: We found that immunomonitorization by cytokine secretion profile is a promising tool to determine immunological risk at renal transplantation patients and is necessary to optimize individual therapy decisions related to immunosuppression regimens, thereby improving transplant outcomes.

Key words: Renal transplantation, monitoring, cytokine, ELISA

Introduction

In renal transplantation, the continuing use of immunosuppressive drugs for long-term survival of the graft is also associated with nephrotoxicity and an increased susceptibility to infections, cardiovascular problems and malignancies. The optimum induction and maintenance of immunosuppressive therapy in renal transplantation is not established yet. Further, precise immunologic monitoring to determine the appropriate dosage of immunosuppressive drugs is not yet available.1

In vitro monitorization of the immune status of kidney transplant recipients may help to differentiate the patients in risk of rejection from patients prone to tolerance. Subsequently, medication can be adjusted to prevent rejection before it becomes clinically apparent or medication may be reduced or even withdrawn in patients considered to be tolerant.2 There are different methodologies which have been developed to monitor various organ transplants. Cytokine ELISA assays are the method of choice because they are sensitive, quantitative, can be performed with relatively little amount of sample and noninvasive. In this study immunomonitorization of cytokine levels was performed to determine the immunologic status of kidney transplant patients to optimize the immunosuppressive therapy regimen.

Patients and Methods

Group 1 (n=35) included kidney transplant patients at postoperative 1st year whose preoperative blood samples were also studied (n=35) and all were cross-match negative. Group 2 (n=10) were cross-match positive preoperative patients. Group 3 consisted control group (7 healthy subjects). Average patient age was 34.5±10.1 years (range 15-60 yrs). Average duration of renal replacement therapy before renal transplantation was 42.1±57.9 months (range 0-288 months). The type of renal replacement therapy were as follows; hemodialysis (n=27), CAPD (n=8). Those patients were immunosuppressed by Cyclosporine (CyA) based triple immunosuppressive regimen (CyA+Mycophenolate mophetyl+steroid).

All human serum samples from patients and healthy control sera were analyzed for IL-1α, TNF-α, IFN-γ, IL-10 and TGF-β concentrations by ELISA (Orgenium Laboratories, Finland).

ELISA technique was performed according to the following procedure. 50 ml standard or sample is added to each well. 50 ml ready for use biotin antibody is added and incubated 1 hour 30 minutes at room temperature. After adding 100 ml ready for use HRP-Streptavidin solution, it is incubated 30 minutes at room temperature, 50 ml TMB One-Step Substrate Reagent and 50 ml Stop Solution is added to each well. The concentrations in each well are determined by extrapolating the measured absorbance at 450 nm on an ELISA reader (ER 2005) to a standard curve.

Results

There was no graft loss at postoperative 1st year after renal transplantation. TNF-α was negative in all cases. Figure 1 shows number of positive cytokines among postoperative 1st year renal transplantation patients. IL-1α production may be up to infection, injury, or antigenic challenge. IL-10 positivity may be considered as over dosage of steroid. Figure 2 shows number of positive cytokines among preoperative cross-match negative renal transplantation recipients. Figure 3 shows number of positive cytokines among preoperative cross-match positive renal transplantation patients which does not indicate to very abnormal levels of cytokines.

Among the involved patients five acute rejection episodes was detected (5/35). Cyclosporine (CyA) based triple immunosuppressive regimen was associated with acute rejection (p<0.001) and also with IL-10 (p<0.001), TGF-β (p<0.001), IL-1α (p<0.005), IFN-γ (p<0.001) positivity. In the healthy control group all of the cytokines were measured negative indicating an immunological balance. Hemodialysis for over 5 years significantly leads to TGF-β (p=0.021) positivity but it does not correlate with acute rejection after renal transplantation.

Discussion

The capability of differentiation of renal transplant recipients with the risk of acute rejection from
recipients prone to tolerance by in vitro immunomonitorization methods would make the immunosuppressive regimen adjustable to prevent rejection before it becomes clinically apparent or, dosage reduction in case of tolerance.

A study which was conducted at the Leiden University Medical Centre, included patients who were transplanted between 2004 and 2005 and none of the patients had active cytomegalovirus infection. All patients received immunosuppressive therapy consisting of a combination of prednisone, cyclosporine, mycophenolate mofetil and/or rapamycine. This study revealed that the ratio of IFN-γ and IL-10 producing donor-specific cells is a potential in vitro parameter for the immune status of transplant recipients in relation to their donor graft. If confirmed, this in vitro tool may help to optimize the immunosuppressive dose and regimen in transplant recipients.2

Zheng X et al. has investigated the serum levels of cytokines in hand transplant recipients.3 They have measured the serum levels of IL-2, IL-10, TNF-alpha, and IFN-gamma in 2 hand transplant recipients by enzyme-linked immunosorbent assays (ELISA). The serum levels of IL-2, TNF-alpha and IFN-gamma were decreased significantly in both patients during the first post transplant week, then increased gradually to the pretransplant levels, and finally decreased and maintained the low level. The serum level of IL-10 in both patients increased significantly during the first post transplant week, and decreased gradually to the low level. At posttransplant fifth month, the serum level of IL-10 re-increased moderately. They concluded Han FH et al. investigated the relationship between the changes of Th1/Th2 cytokine expression in peripheral blood. They performed flow cytometry on the day before transplantation and 3rd, 5th, and 7th day of operation in pancreas transplant recipients. They concluded that change of expression of Th1 and Th2 cytokines may be an important parameter in determining whether acute rejection occurs after pancreatic transplantation.4

Fujii N et al. determined serum cytokine concentrations of allogenic stem cell recipients. Serum concentrations of IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-alpha and IFN-gamma were concur-

Figure 1

Cytokine positive cases among postoperative 1st year renal transplant patients. (n=35)

Figure 2

Cytokine positive cases among preoperative cross-match negative renal transplant patients. (n=35)

Figure 3

Cytokine positive cases among preoperative cross-match positive renal transplant patients. (n=10)
rently measured by the cytometric bead array (CBA) technique. Their results indicate that the ratio of a particular cytokine/cytokine could be a potential diagnostic marker for aGVHD, more sensitive than the serum level of a given cytokine.5

Asmis R et al. determined the contribution of blood monocytes in relation with the production of inflammatory cytokines in whole blood from hemodialysis patients. TNFalpha, IL-6 and IL-10 serum levels in the whole blood were measured with ELISA. Intracellular levels of TNFalpha, IL-6 and IL-10 in monocytes are indistinguishable between hemodialysis patients and healthy controls.6

According to our results, healthy group is indicating an immunological balance; however hemodialysis for over 5 years significantly leads to TGF-β positivity. Cyclosporin (CyA) based triple immunosuppressive therapy regimen was related with acute rejection. We suggest that immunomonitorization by cytokine secretion profile is a parameter to determine the immunological risk at renal transplantation and is necessary to optimize the individual immunosuppressive therapy decision, thereby improving transplant outcome.

References