Original Article

Predominant Inflammatory and Th1 biased cytokine secretion pre- and post- kidney transplantation

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Abstract. A key goal in post-transplant monitoring is the diagnostic detection of harmful processes in the allograft early which can be easily and non-invasively assessed. Cytokines are crucial mediators involved in immune responses leading to rejection. It is known that episodes of viral infections and acute rejection can cause an increase in pro-inflammatory cytokines in transplant recipients. This study is significant since detailed analysis of cytokines was performed in kidney transplant patients pre- and post-transplant to assess the impact of graft implantation. Twenty patients with mean age of 35 years and comprising 8 females who underwent renal transplantation were included in the study. The mean follow-up time for the study cohort was 5 months. Using a multiplex microassay, twelve cytokines [IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, granulocyte–macrophage colony-stimulating factor, IFN-γ and tumour necrosis factor] were measured simultaneously before and after transplant. A strong pro-inflammatory response was seen as the levels of circulating IL-1β ($p<0.02$) and IL-6 ($p<0.01$) increased post-transplant. A Th1 bias was due to increased IFNγ ($p<0.05$) and absent IL-4 and IL-10 post-transplant. Levels of IL-1α, IL-2, IL-7, IL-12, GM-CSF and TNFα remained low and unchanged whilst IL-8 levels was reduced ($p<0.02$). These findings show a strong pro-inflammatory response with a Th1 cytokine bias and this immunological outcome places the patient at risk of graft rejection. We suggest that diagnostic parameters such as cytokines can be used to monitor allografts non-invasively and may have the potential to guide clinical decisions regarding immunosuppressive therapy which could improve outcomes post-transplantation.

Key words: Kidney transplant, allograft rejection, cytokines, Th1 bias

1. Introduction

It remains a challenge for clinicians to balance immunosuppressive therapy to allow graft maintenance whilst retaining an adequate immune response. The cytokine response to injury or trauma is of interest in terms of both its mediation of the acute phase response and its possible relation to the immunological depression after major surgery. Cytokines are crucial inflammatory mediators and the generation of Th1 cytokines has been associated with both antiviral responses and allograft rejection. Several studies examining renal transplant patients during acute rejection episodes, chronic rejection and long-term stable courses have shown a predominant Th2 cytokine response with decreased IFN-γ in the latter group suggesting a role for cytokines to monitor renal transplant injuries (1,2). In the current study, we aim to examine the levels of cytokines and chemokines in kidney transplant patients to investigate the influence of stress such as surgery on cytokine production. We report a strong inflammatory response and Th1 cytokine bias in renal transplant patients which increases the risk of graft rejection. This study highlights the need to monitor host immune responses as well as viral determinants in transplant patients. These findings suggest that diagnostic parameters to monitor allografts non-invasively may have the potential to guide clinical decisions regarding immunosuppressive therapy and could improve outcomes post-transplantation.

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2. Materials and methods

Patients and samples: Sera were collected retrospectively from the National Virus Reference Laboratory archive sample bank. Twenty renal transplant patients with mean age of 35 years (range 4 to 69 years) and comprising 8 females were included in the study. The follow-

up time for the study cohort ranged from 1 to 11.5 months with a mean time of 5 months. Pre- and post-transplant samples were retrieved. There were no episodes of acute rejection in the patients during the course of the study period. Standard immunosuppressive therapy included mycophenolate, tacrolimus and steroids. Depending on CMV serostatus prior to transplant, antiviral prophylaxis included ganciclovir. Ethical approval for this study was obtained from the Human Research Ethics Committee, University College Dublin.

Multiplex T cell cytokine microarray: Using the proteoplex microarray assay supplied by Novagen (MP Biomedicals, Geneva, Switzerland), 12 cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, granulocyte–macrophage colony-stimulating factor (GM-CSF), IFN-γ and tumour necrosis factor (TNF)-α) were measured simultaneously. Patients' sera were diluted according to the manufacturer's protocol and then incubated for 60 min on an orbital shaker at room temperature. After washing, incubation with human detection antibody cocktail was performed for a further 60 min. After rinsing, the detection system (sensilight PBLX fluorophore) was added for 90 min at room temperature in the dark. Slides were rinsed, dried and disassembled and returned to the manufacturer for scanning (633 nm Ex, 660 nm Em at 10 mm resolution) and analysis. Standard curves for all 12 cytokines analysed had R^2 values of between 0·94 and 1·00.

Statistical analysis: The Mann Whitney U test was used to compare pre- and post-transplant values.

3. Results

Twelve cytokines and chemokines were measured pre-and post-transplant. Proinflammatory cytokines; IL-1β (pre: 9.3 ± 9.9 vs post: 148.1 ± 24.3, $p<0.02$) and IL-6 (pre: 16.9 ± 4.3 vs post: 393.7 ± 33.5, $p<0.01$) were increased in the renal transplant patients when serum samples were examined (Fig. 1A & B). The anti-viral cytokine, IFN-γ (Fig. 1C) was also increased significantly post-transplant (11.7 ± 4.2 pg/ml vs 91.3 ± 51.1 pg/ml, $p<0.05$). However, IL-8, a neutrophil chemoattractant, was significantly reduced post-transplant (489.0 ± 81.2 pg/ml vs 82.7 ± 19.6 pg/ml, $p<0.02$, Fig.1D). Low levels of several other cytokines including IL-1α, IL-2, IL-4, IL-7, IL-10, IL-12, GM-CSF and TNFα were measured (<10pg/ml) and these levels remained unchanged post transplant. Viral immune status analysis revealed the patient cohort to be 100% negative for hepatitis B and 92% negative for hepatitis C. Seropositivity to herpes simplex, varicella zoster, EBV and CMV were 64%, 93%, 88% and 58% respectively. Three patients seroconverted as a result of CMV infection during the course of the study, however no differences were observed in circulating cytokine levels when compared to non-infected patients.

4. Discussion

We conducted this study to profile cytokine and chemokine response patterns after renal transplantation and demonstrated an immune deviation towards a robust inflammatory response observed by the significant increase in IL-1 and IL-6 levels post-transplant. IL-6 is essential for the regulation of immune processes that induce IL-2 dependent antigen-specific helper and cytotoxic T cell proliferation and differentiation (3). IL-6 has also been shown to resolve innate responses and switch the immune system towards an adaptive response (4). Recently, a novel role for IL-6 in the development and differentiation of the Th17 cell subset has been reported (5). In contrast to a recent report which showed that levels of TNF-α of more than 45 pg/ml correlated with histology in both acute and chronic kidney transplant rejection (6), the current study cohort showed low levels and no significant changes in TNF-α levels.

Currently, serum creatinine is widely used as the main strategy to monitor renal allografts. When it deteriorates by more than 10-20%, and rejection is a concern, most centres perform a biopsy to determine the cause of allograft dysfunction (7). It is now accepted that serum creatinine lacks specificity and sensitivity and the need for more robust non-invasive monitoring tools which are capable of detecting subclinical allograft inflammation, interstitial fibrosis, tubular atrophy and transplant glomerulopathy remains to be determined. Research to evaluate biomarkers in urine and the circulation have focussed on candidates identified in tissue biopsies of acute and chronic inflammation. One such candidate is IFNγ and its downstream targets eg. CXCL9, CXCL10 and CXCL11, all of which have been identified in tissue biopsies (8). In the current study, circulating cytokines were measured in a similar time frame used for assessing biopsies (4-12 months) (7).

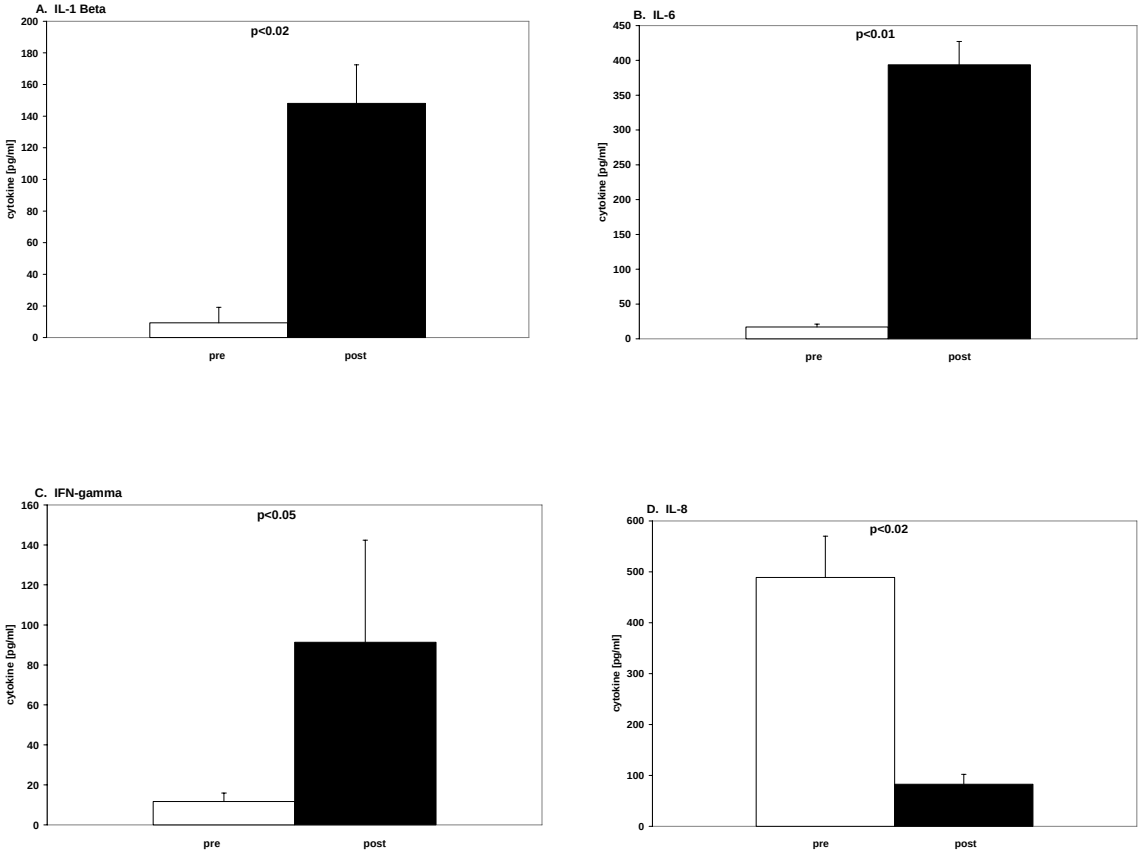

Fig. 1. Serum cytokine and chemokine levels measured in pg/ml are shown as the mean ± SD for 20 patients at pre- and post- renal transplantation. A. IL-1β levels, B. IL-6 levels, C. IFN-γ levels and D. IL-8 levels. Statistical significance is shown as *p* values

In agreement with studies examining transcript in biopsies (8) or urine (9), a significant increase in IFNγ levels post transplant was observed.

The importance of cytokines and chemokines in the innate response is evident as they play a key role in controlling leukocyte mobility and migration, components which mediate the efficiency of the induced immune response.

IL-8 is an inflammatory chemokine which is known to induce recruitment of neutrophils to sites of infection and in the current study levels of IL-8 were significantly reduced post-transplant. Previous studies investigating epigenetic regulation have shown that methylation of the IFNγ promoter at CpG sites is an important mechanism which explains the low IFN-γ production by T cells (10,11). In contradiction to the common epigenetic paradigm, in which methylation of the promoter CpG sites silences gene expression, methylation of the IL-8 gene has been shown to enhance IL-8 production in a breast carcinoma cell line (12). Taken together, the increased IFN-γ and reduced IL-8 in patients post-transplant in the present study may be due to common epigenetic modifications and further studies are currently underway.

5.Conclusion

Emerging results indicate that T-cell monitoring by cytokine ELISpot evaluation, as part of a comprehensive risk assessment platform, has the potential to guide decision making and improve outcomes after transplantation (13). Our findings suggest that monitoring circulating cytokines and chemokines may provide a less expensive and less time consuming tool for close follow-up of patients.

Acknowledgments

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