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ORIGINAL ARTICLE

IL-10 Gene polymorphism and graft outcome in live-donor kidney transplantation

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Abstract

Background: The description of polymorphisms in many of the key immunoregulatory molecules involved in the rejection process has offered a possible explanation for the individual variation in susceptibility to rejection and differences in allograft survival independent of the many known contributory factors. The aim of this work is to study the impact of IL-10 cytokine gene polymorphism on renal transplant clinical course and outcome.

Methods: This work studied 50 transplant recipients maintained on sirolimus based immunosuppression for IL-10 cytokine gene polymorphisms. After transplantation patients were divided into two groups. Group (A) patients (12 patients) received sirolimus, tacrolimus and steroid, while Group (B) patients (38 patients) received sirolimus, mycophenolate mofetil and steroid. Results were correlated with acute and chronic rejection episodes as well as graft and patient outcome.

Results: In our study, we found no impact of IL-10 on incidence and degree of acute rejection episodes, incidence of chronic allograft nephropathy, pathological changes in protocol biopsies, graft function and graft and patient survivals.

Conclusion: From this work, we can conclude that the potential impact of IL-10 cytokine gene polymorphisms on renal transplant clinical course and outcome have shown no influence, and probably other genes rather than IL-10 could be involved as key molecules for graft function.

Key words: IL-10, gene polymorphism, graft outcome, kidney transplantation, live-donor.

INTRODUCTION

The increasing success at preventing acute renal allograft rejection has resulted in rejection rates of less than 20% and one-year graft survivals of more than 90%.This success rate has led to focus on the improvement in long-term allograft survival, and the adjustment of immunosuppression to the individual need. Currently, all patients are treated with broad-spectrum immunosuppression with their myriad side effects. However, we do know that some patients may experience either discontinuation or substantially lower immunosuppression without suffering any ill effects (1).

A possible explanation for the individual variation in rejection susceptibility and differences in allograft survival has been offered by the polymorphisms description of immunoregulatory molecules involved in the rejection process. Several interesting observations have been made that would suggest that genetic vari-

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ability influencing allograft survival reaches beyond that of the major histocompatibility complex (MHC) molecules**.** The influences of polymorphisms in cytokines, cytokine receptors, chemokines, adhesion molecules, and co-stimulatory molecules are among some of those (2).

The combination of a high production of TNF-α with low IL-10 production was associated with acute rejection in heart transplant patients, whereas high producers of both TNF-α and IL-10 were associated with acute rejection in renal transplant patients (1).

A functional role of polymorphisms has also been implicated in the chronic rejection process (2). Polymorphisms in both interferon-γ (INF-γ) and transforming growth factor-β (TGF-β) have been associated with allograft fibrosis in lung transplant recipients.

Two polymorphisms in the adhesion molecule intercellular adhesion molecule-1(ICAM-1) have been associated with increased chronic renal allograft dysfunction (1), while one polymorphism in ICAM-1 was found to have a protective effect with regard to chronic rejection in cardiac transplant recipients.

These data are just some of the increasing evidence that there is a degree of validity to the hypothesis that polymorphisms in immunoregulatory molecules contribute to the heterogeneity in outcomes after transplantation (2).

We aimed to examine the relationship between recipient cytokine genotype and clinical outcome in patients with a surviving allograft of at least five years and to assess its sensitivity and specificity in predicting acute allograft rejection and chronic allograft rejection.

Materials and methods

The material for this work comprised 50 recipients of first renal transplants who were transplanted in the Mansoura Urology and Nephrology Center. Exclusion criteria consisted of prior transplantation, recipients younger than 18 years, pre-transplant chemistries demonstrating a total cholesterol greater than 300mg/dl, triglycerides greater than 400mg/dl, white blood cell count less than 4,000/mm^3 or platelets less than 150,000/mm^3 or change of the basal immunosuppressive protocol for whatever reason.

Before kidney transplantation all patients were subjected to extensive clinical, laboratory, radiological and immunological evaluation. After transplantation patients were prospectively randomised into two groups. Group (A) patients (12 patients) received sirolimus within 24 hours after completion of surgery in a dose of 10mg/day orally for three days, and then maintained on 5mg/day. Further doses were concentration controlled to keep 24-hour whole blood trough level between 6-12ng/ml. Tacrolimus was also administered to this group of patients on the third day post-operative, provided that creatinine clearance was above 50ml/min. Tacrolimus was started at 0.03mg/kg/day in two divided doses. Further doses were subsequently adjusted to maintain 12-hour whole blood trough level of 3-7ng/ml (3).

Group (B) patients (38 patients) received sirolimus and were maintained on a single oral morning dose of 10mg/day. Further doses were concentration controlled to keep 24-hour whole blood trough level between 10-15ng/ml. Mycophenolate mofetil 1gm twice a day was begun the morning after surgery. Patients remained on this dose unless side effects such as gastrointestinal toxicity or leucopenia necessitated dose reduction.

All patients in both groups were given basiliximab 20mg intravenously at surgery and on day four post-operative. Patients in both groups received intravenous methyl prednisolone 500mg one day before and on day of surgery. Oral prednisolone was then given at a dose of 1mg/kg/day which was then gradually tapered down to 0.1mg/kg by the 10^{th} month post-transplantation.

Following kidney transplantation, all recipients were evaluated regarding graft function, tolerance to drugs, drug monitoring, radiological evaluations, histopathological examination of the graft biopsy, and immunological screening.

In this work, renal allograft tissue histopathologic examination was carried out in cases of: delayed graft function**,** nephrotic range proteinuria**,** episodes of renal dysfunction (25% increase in creatinine from base line) unexplained by pre-renal, post-renal causes or high tacrolimus trough level in Group A patients, in addition to routine protocol core biopsy at one year post-transplantation that was carried out in 43 patients in the study group.

Immunological screening

All the patients were investigated for gene polymorphism in IL-10 (at position -1082) as follows:

I. RNA isolation:

RNA isolation was performed using automated MagNA pure Lightcycler (LC) System and MagNA pure LC RNA Isolation Kit (Roche Applied Science,

Mannheim, Germany). Isolated RNAs were reverse transcribed and amplified using LC RNA Master Hybridization Probes Kit (Roche Applied Science, Mannheim, Germany) and PCR Master SYBR Green Kit (GeneCraft, Germany) according to the manufacturer's instructions.

II. Detection of cytokine polymorphisms of IL-10 (at position -1082):

Lightcycler RNA Master Hybridization Probes Kit (Roche molecular biochemicals, Mannheim, Germany) was used to determine the level of IL-10. Real-time PCR reaction using 20µL was performed with 7.0µL RNA and 7.5µL enzyme mix in addition to 0.5µM of each primers and 0.25µM of each probes. The forward primer was 5'-AGCTGAGAACCAAGACCCAGA-3' and the reverse primer was 5'-GGGCTGGGTCAGCTATCC-3'. Two hybridization probes (Metabion, Germany) were designed to recognise adjacent internal sequences within the amplified fragment. One was labelled at the 5` end with LC Red 640, and phosphorylated at the 3` end to prevent probe elongation by the Taq DNA polymerase. The other was labelled at the 3` end with fluorescine (Flu). The sequences of the two probes were 5'- CGGCGCTGTCATCGATTTCTTCCCT-3' Flu and LC Red 640-5'-TGAAAACAAGAGCAAGGCCGTGGAGC-3'. After 25 minutes incubation at 60°C for reverse transcription, a first denaturation step of one minute at 95°C was started. Amplification was then performed for 45 cycles of denaturation at 95°C for 10s, annealing at 58°C for 15s, and extension at 72°C for 20s. Continuous fluorescence was monitored at the annealing step for each sample (4). Results were interpreted as follows: AA (Low producer), GA (Intermediate producer) and GG (High producer) (4, 5 and 6).

Statistical analysis

Qualitative data were displayed in cross tabulations, and quantitative data were described in terms of arithmatic mean ± SD. Bivariate techniques were used for initial evaluation of contrasts. Thus, the chi-square and Fisher's exact tests were used for comparisons of frequencies of qualitative variables; the Mann-Whitney test and the unpaired t-test were used for comparisons of means of two quantitative variables. A p-value <0.05 was considered significant. All analyses were carried out using the computer package SPSS for Windows, version 16.

Results

This work involved 38 male and 12 female patients with a mean age of 33.86 ± 9.79 years. These patients received their allografts from relatively young donors (mean = 34.38±10.42 years) who were related in 82% of the cases. The duration of pre-transplant hemodialysis ranged from 0-48 months during which period they received a mean of 3.11±2.89 blood units. The mean duration of post-transplant follow up was 72.48±6.28 months (range= 62-85) (Table 1).

Table 1: Demographic and clinical characteristics of the 50 renal allograft recipients at commencement of the study

1. Recipient age (19-57) M ± SD (range), years	33.86 ± 9.79
2. Recipient gender (76%) M/F[1] (M %)	38/12
3. Donor age, M ± SD (21-65) (range), years	34.38 ± 10.42
4. Donor gender (48%) M/F[1] (M %)	24/26
5. Donor relation (% of related) (82%) R/U[2]	41/9
6. Duration of HD[3] (0-48) M ± SD (range), months	15.88 ± 15.74
7. Number of pre-transplant blood (1-10) M ± SD (range), units	3.11 ± 2.89
8. Post-transplant follow-up (62-85) M ± SD (range), months	72.48 ± 6.28

1: Male/Female
2: Related/Unrelated
3: Hemodialysis

Our data showed that there was no statistically significant difference between HLA and DR matching, and sum of HLA and DR in relation to incidence of acute rejection (P. 0.613, 0.317 and 0.783 respectively) or chronic allograft nephropathy (P. 0.565, P. 0.268 and P. 0.129 respectively).

Analysis of the data in relation to IL-10 production The 50 patients included in this study were divided into three groups according to the level of IL-10 pro-duction {low (n=22), intermediate (n=15) and high (n=13)}.

The three groups of renal allograft recipients were comparable with respect to donor age, donor gender, recipient age, recipient gender, original kidney disease, donor relation and duration of hemodialysis (Table 2).

Table 2: Demographic and clinical characteristics of the 50 renal allograft recipients in relation to IL-10 production

	IL-10 production			P value
	Low (n=22)	Inter-mediate (n=15)	High (n=13)	
Age of donor M ± SD (range), years	32.41 ± 9.16	37.47 ± 12.92	34.15 ± 9.09	0.356
Sex of donor				
-Male	12	7	5	
-Female	10	8	8	0.650
Age of recipient M ± SD (range), years	32.86 ± 10.53	35.27 ± 8.18	33.92 ± 10.78	0.772
Sex of recipient				
-Male	18	10	10	
-Female	4	5	3	0.568
Original kidney disease				
-Membranous GN[1]	0	1	0	
-FSGS[2]	1	0	1	
-Chronic pyelonephritis	4	3	3	
-Nephrosclerosis	1	0	0	
-ESRD[3]	2	3	2	0.773
-Hereditary	1	0	0	
-Amyloidosis	0	2	0	
-Stone kidney disease	3	2	2	
-APCKD[4]	1	0	0	
-Unknown	9	4	5	
Consanguinity				
-Parents	4	3	2	
-Sibling	14	8	8	
-Offspring	0	0	1	0.465
-Other relative	0	1	0	
-Unrelated	4	3	2	
Duration of HD[5] (median), months	16.5	5	7	0.223

1: Glomerulonephritis
2: Focal Segmental Glomerulosclerosis
3: End-stage Renal Disease
4: Adult Polycystic Kidney Disease
5: Hemodialysis

In our study, there was no statistically significant difference between the three groups regarding the number of acute rejection episodes, degree of the first attack of acute rejection, and incidence of chronic allograft nephropathy. Only one recipient from the first group (low producers) suffered a second attack of acute cellular rejection (Table 3).

Table 3: Relation between number, degree, management and response to therapy in acute rejection episodes; incidence of chronic allograft nephropathy and IL-10 production

	IL-10 production			P value
	Low (n=22)	Inter-mediate (n=15)	High (n=13)	
Number of acute rejection episodes				
- Zero	17	14	12	
- One	4	1	1	0.578
- Two	1	0	0	
Degree of 1st acute rejection				
-ACR[1]grade 1	1	0	0	
-BLR[2]	4	1	1	0.792
Management of 1st acute rejection				
-Steroids	5	1	1	
-Other therapy	0	0	0	
Response of 1st acute rejection to therapy				
-Complete response	5	1	1	
Degree of 2nd acute rejection				
-ACR[1]grade 1	1	0	0	
-BLR[2]	0	0	0	
Management of 2nd acute rejection				
-Steroids	1	0	0	
-Other therapy	0	0	0	
Response of 2nd acute rejection to therapy				
-Complete response	1	0	0	
Chronic allograft nephropathy				
-No	20	14	12	
-Yes	2	1	1	0.964

1: Acute Cellular Rejection
2: Border Line Rejection

Regarding the results of the first biopsy, it showed no statistically significant difference between the three groups and according to Banff classification, all first biopsies showed Border Line Rejection {N.B. Those rejecters were all from the first group (low producers)}. Regarding the results of the second biopsy and the Banff classification of these biopsies, there was no statistically significant difference between the three groups. Only two recipients were

subjected to a third graft biopsy (one from the first group {low producers} with its result as normal and the second one from the third group {high producers} who had chronic rejection). Forty-three recipients were subjected to protocol biopsy one year after transplantation with no statistically significant difference between the three groups regarding the results of these protocol biopsies (Table 4).

Table 4: Relation between results, Banff classification of the graft biopsies; results of protocol biopsies and IL-10 production

	IL-10 production			P value
	Low (n=22)	Inter-mediate (n=15)	High (n=13)	
Result of 1st biopsy				
-Insufficient	1	0	0	
-Normal	2	1	2	0.385
-ACR[1]	4	0	0	
-Acute FK toxicity	1	1	0	
Banff classification of 1st biopsy				
- BLR[2]	4	0	0	
-ACR[1] grade 1	0	0	0	
Result of 2nd biopsy				
-ACR[1]	2	1	1	
-Acute FK toxicity	2	0	0	0.595
Banff classification of 2nd biopsy				
-BLR[2]	0	1	1	
-ACR[1]grade 1	2	0	0	0.135
Result of 3rd biopsy				
-Normal	1		0	
-Chronic rejection	0		1	0.384
Protocol biopsy				
-Yes	20	13	10	
-No	2	2	3	0.513
Result of protocol biopsy	(n=20)	(n=13)	(n=10)	
-Normal	5	5	1	
-Chronic tubulointerstitial fibrosis (mild degree)	14	7	9	0.465
-Chronic tubulointerstitial fibrosis (moderate degree)	1	1	0	

1: Acute Cellular Rejection
2: Border Line Rejection

Analysis of MMF group (n=38) in relation to IL-10 production showed no statistically significant difference between the three groups regarding the number of acute rejection episodes, incidence of chronic allograft nephropathy, incidence, degree and onset of proteinuria, or results of protocol biopsies (Table 5).

Table 5: Analysis of MMF group in relation to IL-10 production

	IL-10 production			P value
	Low (n=18)	**Inter-mediate (n=8)**	**High (n=12)**	
Number of acute rejections				
-Zero	15	7	11	
-One	2	1	1	0.868
-Two	1	0	0	
Chronic allograft nephropathy				
-Yes	0	0	1	
-No	18	8	11	0.329
Proteinuria				
-No	13	7	9	
->1gm/day for >6 months	5	1	3	0.693
Degree of proteinuria	(n=5)	(n=1)	(n=3)	
-1-3gm/day	3	1	3	
->3gm/day	2	0	0	0.358
Onset of proteinuria (median), months	8	2	48	0.142
Protocol biopsy				
-Yes	16	6	9	
-No	2	2	3	0.544
Result of protocol biopsy	(n=16)	(n=6)	(n=9)	
-Normal	5	3	1	
-Chronic tubulointerstitial fibrosis (mild degree)	11	3	8	0.256
-Chronic tubulointerstitial fibrosis (moderate degree)	0	0	0	

Analysis of FK group (n=12) in relation to IL-2 production also showed no statistically significant difference between the three groups regarding the number of acute rejection episodes, incidence of chronic allograft nephropathy, incidence of proteinuria, or results of protocol biopsies (Table 6).

Table 6: Analysis of FK group in relation to IL-10 production

	IL-10 production			P value
	Low (n=4)	Intermediate (n=7)	High (n=1)	
Number of acute rejection				
-Zero	2	7	1	
-One	2	0	0	0.091
Chronic allograft nephropathy				
-Yes	2	1	0	
-No	2	6	1	0.351
Proteinuria				
-No	4	6	1	
->1gm/day for >6 months	0	1	0	0.677
Degree of proteinuria		(n=1)		
-1-3gm/day		1		
->3gm/day	0	0	0	
Onset of proteinuria (median), months		4		
Protocol biopsy				
-Yes	4	7	1	
-No	0	0	0	
Result of protocol biopsy				
-Normal	0	2	0	
-Chronic tubulointerstitial fibrosis (mild degree)	3	4	1	0.719
-Chronic tubulointerstitial fibrosis (moderate degree)	1	1	0	

All rejection episodes were managed and responded completely to pulse steroid therapy.

Lastly, the three groups were comparable regarding the graft survival (P. 0.496) and the 50 patients who were included in this study were alive with functioning grafts at the last follow-up.

Discussion

Genetic variations including single nucleotide polymorphisms (SNPs), dinucleotide repeats and microsatellites have been identified in a number of genes encoding cytokines, cytokine receptors, chemokines and their receptors, adhesion molecules. Several of the polymorphisms were located in the promoter region of the gene, affect transcription and translation, and not infrequently determine the level of expression of the protein product. A wide variety of disease states ranging from infection susceptibility and autoimmunity to hypertension have been associated with known sequence variations (7).

IL-10 gene polymorphism and acute rejection

IL-10 is known to inhibit antigen-dependent proliferation of T cells in vitro and to suppress responses in the primary mixed lymphocyte reaction (8), but appears to have paradoxical effects in organ transplantation. Although elevated IL-10 gene expression has been associated with reduced rejection of heart and liver transplants (9 and 10), in kidney transplant recipients high IL-10 status has been associated with increased acute rejection (2 and 11).

While IL-10 polymorphisms have been reported to affect the risk of rejection in several reports (12 and 2), there are a number of reports that have been unable to detect the influence of IL-10 (13 and 14).

In our study, we did not find associations between IL-10 gene polymorphism and incidence or degree of acute graft rejection. In contrast to our findings, many reports found an association between IL-10 gene polymorphism and the risk of rejection, although some of these reports have conflicting results regarding the association between the level of IL-10 production and the risk of rejection.

The first group found that high producers of IL-10 have higher incidence of rejection, like the study conducted by Sankaran and co-workers who studied 115 renal allograft recipients, where all patients with primary function were given cyclosporine A (CsA) monotherapy. Transplant patients with delayed graft function were commenced on a triple therapy regimen of CsA, prednisolone and azathioprine. When all patients were analysed independently of HLA-DR matching, no association was found between cytokine gene polymorphism and rejection episodes. However, the recipient IL-10 high producer genotype showed an association with increased rejection episodes only when the HLA-DR-mismatched transplants were analysed separately (2).

The explanation was that IL-10 may enhance antibody responses against the graft as reported by Merville and co-workers in 1995 (15) and low IL-10 responses can be used to predict a low risk for acute graft rejection (16). Furthermore, IL-10 is a potent stimulator, inducing differentiation and proliferation of B cells, thus driving the immune response towards the humoral pathway (17).

Also, Yang and Liu found that patients with acute

rejection have a higher IL-10 level than those without rejection. When HLA-DR mismatched, the recipient with IL-10 GG genotype (high producer) showed a higher occurrence of acute rejection than those with low producer genotype in the first three months after renal transplantation (18).

On the other hand, the second group found that low producers of IL-10 have higher incidence of rejection. In a study conducted by George and others, they found that the frequency of IL-10 (-1082) AA, a genotype associated with low production of IL-10, was high in the rejection group (34 patients) when compared to a rejection-free group (71 patients), even in recipients with HLA-DR matched transplants (P=.01) (19). These results were consistent with the experimental work done, suggesting the dominant role of IL-10 as an anti-inflammatory cytokine (20 and 21).

In 2007, Amirzargar and co-workers studied 100 kidney transplant recipients who had at least one year of post-transplantation follow-up. All recipients were prescribed cyclosporine or tacrolimus, azathiopurine or MMF, and methylprednisone treatment after transplantation. They found that the low-producer IL-10 genotype was higher in acute rejection episodes (22).

On the contrary, our results are in agreement with the findings reported by Marshall and co-workers who studied 22 polymorphisms in 11 cytokine and cytokine receptor genes (including IL-10) and found no association between any polymorphism and the incidence or severity of acute rejection (23).

Lastly, Chen and co-workers in their study in 2014, which included 325 renal transplant recipients, found no association between IL-10 genotype and incidence of rejection or graft survival (24).

IL-10 gene polymorphism and chronic rejection A functional role of polymorphisms has also been implicated in the chronic rejection process (2).

In our study, there was no statistically significant difference between IL-10 production and the incidence of chronic rejection.

Similar results were published by Canossi and co-workers in 2007 when they studied 86 renal transplant recipients. The average follow-up was 78 months after transplantation and all patients received cyclosporine/prednisolone/azathioprine or mycophenolate mofetil. Only nine patients had chronic rejection. Canossi and co-workers failed to prove a significant relationship between IL-10 gene polymorphism and incidence of chronic rejection (25).

On the contrary, Uboldi de Capei and his team studied 416 first cadaveric renal allograft recipients, and after 10 years post-transplantation, the graft was still functional in 171 patients (41%) – with 102 of these 171 patients also typed for cytokine polymorphisms by PCR-SSP. They found that patients who were high IL-10 producers and HLA Class I mismatched (but matched for Class II) were protected from chronic rejection {P=0.0008} (26). The explanation suggested by them was that interferon-γ, which induces the expression of MHC Class II molecules, is not inhibited by IL-10 (27).

An association between IL-10 (-1082) SNP genotypes and chronic rejection after kidney transplantation was investigated in another study that reported an association (which was not statistically significant) between the high IL-10 producer genotype (GG) and a better graft function five years post-transplant, whereas patient survival after one and five years was not associated with any IL-10 genotype (28).

IL-10 gene polymorphism, and graft and patient survival

Analysis of our data showed that there was no impact of IL-10 production on either patient or graft survival. This can be explained by the comparable results in all groups regarding the incidence of both acute and chronic rejections.

In agreement with our findings, a very large study conducted by Mytilineos and co-workers who studied 4,199 renal transplant recipients (of whom 2,298 were first transplants and 1,901 were re-transplanted) from 73 transplant centres participating in the Collaborative Transplant Study. Caucasian patients who were transplanted between 1987 and 2000 were studied. They did not find an effect of IL-10 gene polymorphisms on kidney graft survival (29).

On the other hand, Uboldi de Capei and colleagues who studied 102 renal allograft recipients found that in patients with grafts that maintain function for more than 10 years, survival of patients with a functioning organ can be associated with the high IL-10 producer genotype (26).

Conclusion

Our study showed that there was no role for IL-10 gene polymorphism on the incidence of acute rejection, chronic rejection and patient and graft survival. However, the genetic polymorphisms examined in this study addressed only a fraction of all cytokine genes involved in the immune response, and addi-

tional studies are needed to be conducted to clearly explore the role of cytokine gene polymorphism in renal transplantation outcome.

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