Ege Tıp Dergisi 43(2) : 83 - 85, 2004

THE INTERLEUKIN – 6 – 174 C ALLEL IS ASSOCIATED WITH EARLY ACUTE REJECTION IN PEDIATRIC KIDNEY ALLOGRAFTS

ÇOCUK BÖBREK ALLOGRAFTLARINDA ERKEN AKUT REJEKSİYON İLE İNTERLÖKİN – 6 – 174 C ALLELİ İLİŞKİLİDİR

Caner KABASAKAL Afig BERDELİ Sevgi MİR

Department of Pediatrics, Faculty of Medicine, Ege University, Bornova, İzmir

Anahtar Sözcükler : Interleukin-6, kidney allograft, gene polymorphism, acute rejection, children
Key Words : Working adolescent, Psycho-Social Problems

SUMMARY

Interleukin-6 (IL-6) is a pleiotropic cytokine with a central role in inflammation, host defense and tissue injury. Although it is considered as predominantly proinflammatory cytokine; recently, IL-6 has been recognized as having additional immunosuppressive properties. In this study, we investigated the impact of IL-6 -174 G/C promoter genotype on acute rejection in 14 patients out of 55 kidney recipients. In 14 acute rejection presenting patients, early acute rejection (within first 3 months) was found significantly frequent in -174 C allel carriers, compared to non-carriers (33.3% vs 10.3% p<0.05). Our results imply a higher risk of early acute rejection in carriers of -174 C allel compared to non-carriers, and the relative risk of early acute rejection is 4.3 fold higher in IL-6 -174 C allel carrriers.

ÖZET

Interlökin-6 (IL-6) inflamasyon, konakçı savunması ve doku hasarında önemli rol oynayan çok işlevli bir sitokindir. Daha çok proinflamatuar sitokin olarak kabul edilmekle birlikte, son zamanlarda IL-6'nın immunsupressif rolü de saptanmıştır. Bu çalışmada, IL-6 -174 G/C promoter genotipinin, 55 böbrek nakli hastasından 14'ünde görülen akut rejeksiyona etkisi incelenmiştir. Akut rejeksiyon gözlenen 14 hastada, erken (ilk 3 ay içinde) akut rejeksiyon görülmesinin -174 C allel taşıyanlarda, taşımayanlara oranla anlamlı şekilde daha fazla olduğu bulunmuştur (%33,3 ile %10,3 p<0.05). Bu çalışmanın sonuçları, -174 C allel taşıyanlarda erken akut rejeksiyon riskinin daha fazla olduğu ve taşımayanlara oranla 4,3 kat daha fazla rölatif risk altında olduğunu düşündürmektedir.

INTRODUCTION

Advances in transplanation management including HLA monitoring, new immunosupression modalities and doses, have beeen occured in recent years. However, rejection and early graft loss are still important clinical problems. Acute (18%) and chronic rejection (28%) are considered responsible for almost the half of graft losses (1,2).

Interleukin-6 is a pleiotropic cytokine with a central role in inflammation, host defense and tissue injury. IL-6, produced by many different cell types, is considered as predominantly proinflammatory cytokine, and has recently been recognized as having additional anti-inflammatory and immunosupressive properties (3,4,5).

Yazışma adresi: Caner KABASAKAL, Department of Pediatrics, Faculty of Medicine, Ege University, Bornova, İzmir
Makalenin geliş tarihi : 31.05.2004 ; kabul tarihi : 09.06.2004

In transplantation, the relevance of the recipients IL-6 genotype for acute rejection is still controversial (6). No association was shown between acute rejection and recipient IL-6 polymorphism (7). However, the same authors showed an association between the donor IL-6 genotype and both severity and incidence of acute rejection (8). Müller-Steinhardt M et al. suggested C allel carriers had an inferior three year graft survival (9). We investigated the impact of kidney recipients' IL-6 -174 promoter genotype on acute rejection.

MATERIAL AND METHODS

Subjects

Fifty three of pediatric kidney recipients, on follow up in pediatric transplant clinics, were included in this study. Out

of 53 patients, twenty-nine were females and twenty-four males, with mean transplantation age of 12.2±4.2 years, and were on follow up for 0.5 to 13 years (median:2 years). The primary diseases of the patients were given in Table 1. All patients received primary kidney transplantation; 30 were from cadaveric donors and 23 from living related donors (LRD). Immunosupressive treatment included azathioprine, prednisolone and cyclosporine in patients transplanted from LRD until August 2001 (n:14) and basiliximab was added thereafter (n:9). Immunosupressive treatment in patients with cadeveric allografts included azathioprine, prednisolone, antithymositglobuline (ATG) and cyclosporine was added (when creatinine decreased below 3mg/dl) until April 2002 (n:11), and thereafter changed with FK506 (n:19). Rejection episodes were defined by clinical diagnosis (elevated serum creatinine in the absence of other pathology, including infection, urinary tract obstruction, allograft artery stenosis or cyclosporine A toxicity) and confirmed by biopsies, evaluated according to Banff criteria (10). Informed parental concent was obtained for genetic analysis.

Table 1. The etiology of chronic renal failure

Primary disease	Patients	%
Chronic pyelonephritis and Reflux nephropathy	26	49
Chronic glomerulonephritis	13	24
Focal segmental lomerulosclerosis	9	17
Chronic tubulointerstitiel nephritis	2	4
Congenital malphormation	1	2
Amyloidosis	1	2
Congenital nephrotic syndrome	1	2

DNA extraction and IL-6 Genotyping

Genomic DNA was prepared from whole blood using Qiagen mini Blood DNA purification kits (QIAGEN, Ontaria Canada) according to manufacturer's.

Polimeraz chain reaction (PCR) was used to determine -174 C/G polymorphism on the promoter region of IL-6 gene. For this aim; forward primer (F)- 5'-TGACTTCAGCTTTACTCTTGT- 5 pmol/µl, revers primer (R) -5' CTGATTGGAACCCTTATTAAG 5 pmol/µl (MWG,Germany), 200 µM concentration of mixture of dinucleotide trifosfate (dNTP) (dATP;dCTP;dGTP;dTTP) (Sigma), 0.5 U AmpliTaq Gold DNA Polimeraz (Applied Biosystems), 1× PCR buffer from, Gene Amp 10× PCR buffer of the same enzyme, 2 mM concentration of MgCl2 from 25 mM MgCl2 which has been prepared for the same enzyme and to complete the total volum to 25µL, deionized water were used. After 23 µl of this mixture was filled a tube, 2 µl DNA of patient was added on.

PCR procedures were performed by GeneAmp 9700 ThermalCycler (PE Applied Biosystems; Foster City; USA). For IL-6 gene, thermal cycling was as follows: 1 cycle of 95o C for 5 min, followed by 32 cycles of 95oC for 30 seconds, 55oC for 30 seconds and 72 oC for 90 seconds, and a final extention at 72 oC for 7 min. 5 µl of PCR product, that was 198 bp, were mixed 1 µl loading buffer and were controlled on a 2% agarose gel by comparing 100-bp DNA marker 5 µl of positive PCR product was digested with SfaNI restriction enzyme (New England, Biolabs; UK) at 37 oC overnight.

The identified genotypes were named according to the presence or absence of the enzyme restriction sites, so SfaNI (G/G) (G/C) and (C/C) are homozygotes for the presence of the site (140/58bp), heterozygote for the presence and absence of the site (1498/140/58bp), and homozygote for the absence of the site (198bp), respectively.

Statistical analysis

Genotype frequencies were measured for IL-6 -174 G/C promoter polymorphism; and the occurance of biopsy proven acute rejection episodes was investigated. We looked for the associations of IL-6 promoter polymorfism with acute rejection episodes and early acute rejection episod (within the first 3 months). Associans were assessed using 2x2 and 2x3 contingency analysis tables and the x2 test with Fisher's exact tests where appropriate. Odds ratio (OR) and the OR 95% confidence interval (CI) were also calculated for significant associations.

RESULTS

In this study group we studied IL-6 -174 promoter polymorphism in transplant recipients and found C allel frequency 0,26 and G allel frequency 0,74. Overall patients developed acute rejections and number of acute rejection episodes showed no association with IL-6 -174 promoter genotypes GG,GC,CC and -174 C allel carriers (GC/CC) (Table 1). Fourteen patients developed acute rejection episodes. First episode of acute rejection after transplantation were diagnosed in 11 (78%) of patients within the first 3 months, in one (7%) patient between 3-6 months and in two (14%) patients later than 6 months. Genotype frequencies were shown in Table 3, while the significant association between -174 C allel carriers and early acute rejection episodes, within the first 3 months following kidney transplantation (Tables 2,3). Early acute rejection was found significantly frequent in -174 C allel carriers, compared to non-carriers (33.3% vs 10.3% $p<0.05$). Carrying IL-6 -174 C allel, increases the relative risk of early acute rejection (<3 months) 4.3 times (Odd's ratio 4.33; 95 Confidence Interval 1.001-18.767).

Table 2. The association between acute rejection and IL-6 polymorphism

	GG	GC/CC	GC	CC	p
Acute rejection (+)	5/29	9/24	7/20	2/4	
Acute rejection (-)	24/29	15/24	13/20	2/4	NS
Number of AR <2	26/29	5/24	4/20	1/4	
≥2	3/29	19/24	16/20	3/4	NS
Early AR 0-3months	3/29	8/24	7/20	1/4	
> 3 months	26/29*	16/24*	13/20	3/4	P<0.05*

AR: Acute rejection

Table 3. Genotype frequencies (n:53) and associations with early acute rejection (0-3 months)

Genotype	Frequencies(n:53)		Early acute rejections (0-3 months)			
	Total	%	negative	%	positive	%
CC	4	7.5	3	75.0	1	25.0
GC	20	37.7	13	65.0	7	35.0
GC/CC	24	45.2	16	66.7	8	33.3*
GG	29	54.7	26	89.7	3	10.3*

* Fisher's exact test $p<0.05$; Odd's ratio 4.33, 95% CI (1.001-18.767)

DISCUSSION

It has been reported that many cytokines and chemokines took part both in acute and chronic rejection in clinical and experimental studies. It is widely accepted that among cytokines and chemokines, especially IL-6, which is the main mediator of the acute phase reaction, has an important role allograft rejection. Increased IL-6 expression during renal allograft rejection has been shown by in situ hybridization (11,12,13), immunohistochemistry (14,15).

The influence of cytokine gene polymorphism in kidney transplantation has been investigated by a few study groups. Unfortunately little consensus has been achieved between different studies. In kidney transplantation a study suggested no association between acute rejection and recipients polymorphism, including IL-6 -174 promoter polymorphism (7). However the same group claimed the -174 CC genotype of the kidney donors was identified as a major risk factor for the occurence of acute rejection episodes (8).

Several published studies reported no association between acute rejection and recipients IL-6 -174 promoter polymorphism (7,12,14). These authors looked for an association with total acute rejection episodes detected during graft survival (16,17) or only in first 30 days (7). Recent two studies reported definite association between -174 C allel carriers and inferior graft survival in three years follow-up (18,9). However, no data exists regarding early acute rejection, longer than first 30 days.

This study reports, to our knowledge, for the first time a significant association of IL-6 -174 promoter polymorphism with early acute rejections detected within the first three months of transplantation. Carriers of -174 C allel were identified as having an association with ($p<0.05$) and higher risk of (Odd's ratio 4.33) early acute rejection in the first three months. One possible explanation could be that the higher dosage of immunsupressive drugs might disquise the impact of the IL-6 -174 genotype in the first 30 days of transplantation. However, we believe additional studies will be required to understand more clearly.

In conclusion, we report a significant association of the IL-6 -174 C promoter polymorphism with early acute rejection within the first three months. Our results imply a higher risk of early acute rejection in carriers of -174 C allel versus non-carriers.

REFERENCES

1. Feld LG, Stablein D, Fivush B, et al: Renal transplatation in children from 1987-1996: The annual report of the North American Pediatric Renal Transplant Cooperative Study. Pediatr Transplant 1997;1:146-162.
2. Opelz G, Mytilineos J, Scherer S, Schwarz V: Clinical implications of DNA typing in organ transplantation. The collaborative transplant study. Transplant Proc 1997; 29:1524-1527.
3. La Flamme AC, Pearce EJ: The absence of the IL-6 does not affect Th2 cell development in vivo, but does lead to impaired proliferation, IL-2 reseptör expression, and B cell responses. J Immunol 1999; 162:5289-5837.
4. Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. Immunol Today 1997;18:428-432.
5. Xing Z, Gauldie J, Cox G et al:IL-6 is antiimflamatory cytokine required for controlling local or systemic acute inflmmatory responses. J Clin Invest 1998; 101:311-320.
6. Hahn AB, Kasten-Jolly JC, Constantino DM, Graffunder E, Singh TP, Shen GK, Conti DJ: TNF-α,IL-6, IFN-γ and IL-4 receptors (alpha)-chain variant Q576R: effects on renal allograft outcome. Transplantation 2001;72:660-665.
7. Marshall SE, McLaren AJ, Haldar NA, Bunce M, Morris PJ and Welsh KI: The impact of recipient cytokine genotype on acute rejection after renal transplantation.Transplantation 2000;70:1485-1491.
8. Marshall SE, McLaren AJ,Mckinney EF, Bird TG, Haldar NA, Bunce M, Morris PJ and Welsh KI: Donor cytokine genotype influences the development of acute rejection after renal transplantation. Transplantation 2001;71:469-476.
9. Müller-Steinhardt M, Hartel C, Müller B, Kirchner H, Fricke L: The ınterleukin 6 -174 promoter is associated with long-term kidney allograft survival. Kidney International 2002;62:1824-1827.
10. Solez K, Axelsen RA, Benediktsson H et al. International standardization of criteria for hystologic diagnosis of renal allograft rejection: The Banff working classification of kidney transplant pathology. Kidney Int 1993; 44:411-422.
11. Hancock WH,Whitley WD, Tullius SG et al. Cytokines, adhesion molecules, and the pathogenesis of chronic rejection of rat renal allografts. Transplantation 1993;56:643-650.
12. Tovey MG, Deglise-Favre A, Schoevaert D. Differential in situ expression of cytokine genes in human renal transplantation. Kidney Int 1993;43 [Suppl 39]: S129-S132.
13. Vandenbroecke C, Caillat-Zucman S, Legendre C, et al. Differential in situ expression of cytokines in renal allograft rejection. Transplantation. 1991; 51:602-609.
14. Fukatsu A, Matsuo S, Tamai H, Sakamoto N, Matsuda T, Hirano T. Distribution of interleukin-6 in normal and diseased human kidney. Lab Invest 1991;65:61-66.
15. Raasveld MH, Weening JJ, Kerst JM, Surachno S, ten Berge RJM. Local production of interleukin-6 during acute rejection in human renal allografts. Nephrol Dial Transplant 1993;8:75-78.
16. Poli F, Boschiero L, Giannoni F,Toninin M, Ancona G, Scalamogna M, Berra S and Sirchia G: TNF-α, IFN-γ, IL-6, IL-10 AND TGF-β1 gene polymorphisms in renal allografts. Transplant Proc 2001;33:348-349.
17. Reviron D, Dussol B, Andre M, Brunet P, Mercier P and Berland Y: TNF-α and IL-6 gene polymorphisms and rejection in kidney transplantation recipients. Transplant Proc 2001;33:350-351.
18. Müller-Steinhardt M, Fricke L, Müller B, Ebel B, Kirchner H and Hartel C: Cooperative influence of the IL-6 promoter polymorfisms -597,-572 and -174 on long-term kidney allograft survival. Am J Transplant 2004;4:402-408.