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Markers of Atherogenesis in Patients with Chronic Rejection of Renal Allografts

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Patients with renal insufficiency are thought to be at higher risk for atherosclerotic complications, and pathogenesis of chronic kidney transplant rejection resembles, in some aspects, atherogenesis. In the present study, the authors evaluated risk factors for atherogenesis in patients with biopsy-proven chronic rejection and compared them with matched groups of transplant recipients without rejection and healthy Caucasians. Patients with chronic rejection had higher α-1-acid glycoprotein and fibrinogen, as well as soluble P-selectin concentration in the peripheral blood, as compared with patients with normal graft function. Surprisingly, the authors found no differences in E-selectin and ICAM-1. Moreover, they found patients with normal renal graft function to have higher acute phase proteins and P-selectin levels as compared with healthy persons. In conclusion, kidney transplant recipients are at increased risk for atherogenesis. Soluble P-selectin and acute phase proteins can be used for atherogenesis and/or chronic rejection monitoring in such patients.

Introduction

Chronic rejection of kidney transplants represents an important cause of kidney graft loss in the long term. In contradiction to alloantigen-dependent risk factors, the pathophysiological mechanisms caused by alloantigen-independent events in chronic rejection reveal some similarities with atherogenesis.[1] Recently, it has been observed that some markers of endothelial cell injury and dysfunction, such as soluble adhesion molecules and acute phase proteins, are increased in cardiovascular atherosclerotic diseases.[2-4] The aim of the present study was to evaluate the concentration of soluble adhesion molecules and acute phase proteins in peripheral blood of renal graft recipients with biopsy-proven chronic rejection. We hypothesized that patients with chronic rejection have higher atherogenesis markers levels.

Materials and Methods

Twenty-five kidney transplant recipients with biopsy-proven chronic rejection according to Banff classification; 26 age-, gender-; and follow-up-matched transplant recipients with normal (creatinine clearance 48 ml/min) kidney graft function; and 60 age- and gender-matched healthy blood donors were enrolled in the study. There were no significant differences in the prevalence of diabetes, use of lipid-lowering drugs, panel reactive antibodies, and HLA mismatches between groups. Patients with chronic rejection used more antihypertensive drugs than controls. All transplant recipients received cyclosporine-based immunosuppression. The Ethics Committee of the Institute for Clinical and Experimental Medicine approved the study protocol.

Soluble adhesion molecule plasma levels were evaluated using ELISA commercial kits (ELISA using human soluble ICAM-1™, human soluble P-selectin™, and human soluble E-selectin™ kits, R&D Systems Europe, Abington, UK), α-1-acid glycoprotein concentration was determined using immunoturbidimetry (SEVAC, Prague, Czech Re-

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Table 1 | **Experimental Groups and Atherogenesis Markers**

Group	Chronic Rejection	Transplant Controls	Blood Donors
N	25	26	60
Age (years)	49.2 ± 10.2	54.1 ± 9.7	50.6 ± 5.5
Follow-up (months)	52.0 ± 18.5	54.10 ± 3.3	NA
Creatinine clearance (mL/s)	0.6 ± 0.2[a]	1.2 ± 0.2	ND
Donor age (years)	42.9 ± 13.1[a]	27.7 ± 10.2	NA
PRA (%)	24.0 ± 29.6	14.9 ± 18.9	NA
BMI (kg/m^2)	28.3 ± 5.3	30.2 ± 5.0[c]	26.1 ± 4.1
Antihypertensive drugs (n)	2.3 ± 1.2[a]	1.4 ± 0.9	0
Proteinuria (g/day)	1.1 ± 1.5	0.23 ± 0.25	ND
$_S$P-selectin (μg/L)	216.0 ± 82.0[b]	155.9 ± 64.0	124.3 ± 37.0
$_S$E-selectin (μg/L)	63.4 ± 54.4	51.6 ± 26.2	42.8 ± 16.6
$_S$ICAM-1 (μg/L)	270.2 ± 89.5	269.1 ± 102.1	255.4 ± 50.7
Fibrinogen (g/l)	4.00 ± 0.91[b]	3.28 ± 0.70[b]	2.72 ± 0.66
α-1 acid glycoprotein (g/L)	1.51 ± 0.47[b]	1.05 ± 0.30[c]	0.62 ± 0.21
Cholesterol (mmol/L)	5.9 ± 1.3	6.2 ± 1.2[d]	5.4 ± 1.0
Triglycerides (mmol/L)	2.6 ± 1.3	2.3 ± 1.4	2.3 ± 0.5

Abbreviations: PRA = panel reactive antibody; BMI = body mass index; NA = not applicable; ND = not done.
a. $P < 0.05$
b. $P < 0.01$ chronic rejection vs. transplant controls.
c. $P < 0.01$
d. $P < 0.05$ transplant controls vs. blood donors.

public), fibrinogen was measured using a Fibrinogen Reagent™ kit with bovine thrombin (Immuno AG, Vienna, Austria), and a Sysmex CA 6000™ coagulation analyzer (Toa Medical Electronics Co, Kobe, Japan).

The results are given as mean ± standard deviation. Data were compared by ANOVA test with Scheffé comparison. Values of $P < 0.05$ were defined as statistically significant.

Results

We observed a significant increase of $_S$P-selectin, fibrinogen, and a-1-acid glycoprotein levels in patients with chronic rejection as compared with transplant controls. However, we found no differences in $_S$E-selectin and $_S$ICAM-1 concentrations. There were no differences in total cholesterol level, triglycerides, and body mass index between patients with chronic rejection and transplant controls. Moreover, we found patients with normal renal graft function to have higher acute phase protein concentrations when compared with healthy subjects (Table 1).

Discussion

Soluble adhesion molecule levels have been suggested to be a clinical readout of ongoing atherosclerosis.[4] Surprisingly, in our study we have demonstrated a significant increase only in $_S$P-selectin level; the $_S$E-selectin and $_S$ICAM-1 plasma levels did not differ among groups. This observation is not explained by impaired kidney elimination in the chronic rejection group since soluble adhesion molecules have similar molecular weight. The fact that $_S$E-selectin and $_S$ICAM-1 levels are not raised in kidney transplant recipients during rejection or in stable state with good graft function was also pointed out by Alcalde and others.[5] Discrepancies between the grade of expression of E-selectin, as well as ICAM-1 within graft tissue and its serum levels, have been described in heart transplantation.[6] These facts explain why soluble adhesion molecules, as estimated from peripheral blood, have not been suggested to date as a tool for detecting chronic rejection.

Kidney transplant recipients have received immunosuppressants such as cyclosporine A and

steroids. Both drugs decrease E-selectin and ICAM-1 synthesis in the endothelium[7] without blocking of constitutive or cytokine-inducible expression of P-selectin in human endothelial cells.[8] But how should the raised serum levels of $_s$P-selectin in the kidney transplant recipients with chronic rejection be interpreted? While P-selectin, like E-selectin, is produced by inflammatory endothelium,[9] the greater proportion of $_s$P-selectin in the blood originates in activated platelets after release from their alpha granules.[10] Recent experimental data suggest P-selectin plays an important role in the pathophysiology of ischemia/reperfusion injury and in the development of chronic rejection as well as in atherogenesis.[11,12]

Kidney transplant recipients had increased systemic inflammatory response. This impression is based on the finding of increased levels of the "positive" acute phase proteins α-1 acid glycoprotein, and fibrinogen in patients with chronic rejection as well as in patients with normal renal graft function.

In conclusion, kidney transplant recipients are at increased risk for atherogenesis. Patients with chronic rejection seem to be at higher risk when compared with those with normal graft function. The increase of soluble P-selectin and of acute phase proteins fibrinogen and α-1-acid glycoprotein could be used for detection of both atherosclerosis and chronic rejection in kidney transplant recipients treated by cyclosporine-based immunosuppression.

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