Lymphocytic HLA-A mRNA Is a Reliable Indicator of Acute Rejection in Renal Transplantation

S.M. Zhou, J. Tian, R. Sun, W.F. Shi , Z.G. Peng, and X. Zou

ABSTRACT

Background. Rejection in renal transplantation is the most frequent event causing transplant failure. It is important to identify parameters to predict rejection, which are helpful in a timely fashion.

Methods. Fifty-nine renal transplant recipients were divided into two groups: group 1 (stable renal function) and group 2 (acute rejection episodes). The levels of HLA-A mRNA in peripheral blood lymphocytes (both pre- and posttransplantation) were measured by reverse transcriptase-polymerase chain reaction (RT-PCR) with glucose-6-phosphate dehydrogenase (G6PDH) as an internal reference. The TEST software was used to analyze the relative expressions of HLA-A mRNA.

Results. There was no statistical significance between features of the two groups pretransplant versus normal controls. Posttransplant, the HLA-A mRNA levels decreased significantly compared to those of pretransplant and normal control individuals. The levels of HLA-A mRNA among the 10 patients with acute rejection episodes were significantly increased. There was no significant change in the lymphocyte populations in the early stage of an acute rejection episode compared with the prerejection value.

Conclusion. HLA-A mRNA expression was strongly correlated with immune status. The HLA-A mRNA levels may provide an effective and reliable indicator to predict acute rejection episodes in renal transplantation.

KIDNEY transplantation is the best approach to combat end-stage renal disease (ESRD). During the early phase of kidney transplantation, rejection is the most frequent event causing graft failure. With the development of various strategies to deal with rejection, the rate of acute rejection episodes has decreased dramatically. However, acute rejection is still a major obstacle in kidney transplantation. Recently, graft biopsy with histopathology has proven to be the gold standard to evaluate acute rejection episodes (ARE).[1] Needle biopsy, a well-established procedure, is tedious with a low (but considerable) complication rate. In addition, needle biopsy is invasive; it can cause kidney injury.[2] With the development of immunosuppressants, the clinical manifestations of acute rejection are usually not obvious. When rejection prediction is based on histological criteria alone, the episode may be over- or underestimated.[3] Sometimes a graft with normal renal function also manifests lymphocyte infiltration. Furthermore, the procedure cannot be repeated many times during the life of the transplant. For these reasons, great efforts have been made to develop rapid, noninvasive, and sensitive peripheral blood or urinary indicators for early detection of ARE.

The major histocompatibility antigen complex (MHC) is a group of closely related genes encoding MHC molecules that determine the immune response.[4,5] The human MHC encodes human leukocyte antigens (HLA), which play important roles in the development of transplant rejection.[6,7] Major histocompatibility complex-I (MHC-I) antigens are constitutively expressed on all nucleated cells (HLA class I); HLA-A is particularly important because of

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Table 1. The Primes for HLA-A mRNA and Intrareference (G6PDH)

	HLA-A mRNA	G6PDH
Downstream primer	5′-AGCTCTTCCTCCTCCACAT-3′	5′-GCACGAAGTGCATCTGGCTCC-3′
Upper primer	5′-TCACCCTGAGATGGGAGC-3′	5′-ACCTGACCTACGGCAACAGA-3′
Probe	5′FAM-CGGTCGTTACTACGGGTGCT-TAMRA3′	5′FAM-TGAAGCTCCCTGACGCCTACG-TAI
Length	129bp	99bp

its role in graft rejection. Le Morvan et al[8] reported that the immune response correlated with the levels of MHC-I mRNA. Minguela et al[9] found that the expression of this gene was significantly elevated during the development of rejection.

In previous studies, we observed that the levels of HLA-A mRNA correlated with rejection of liver transplantations.[1,9] To establish an early noninvasive method to diagnose acute renal transplant rejection, we examined the levels of HLA-A mRNA in peripheral blood lymphocytes. We adopted the method of fluorescence-quantitative–PCR (FQ–RT-PCR) using glucose-6-phosphate dehydrogenase (G6PDH) as an internal reference to determine the levels of HLA-A mRNA, seeking to determine whether HLA-A mRNA was an accurate parameter to predict acute rejection of kidney transplantations.

MATERIALS AND METHODS

Subjects

The study comprised 59 patients (range, 13–53 years) who had undergone renal transplantations from October 2005 to May 2007. Their primary diseases were glomerulonephropathies. The follow-up periods ranged from 4 weeks to 2 months. We obtained informed consent from all patients prior to initiation of the study. Participants were categorized into the following three subgroups: group 1 consisted of those who developed an acute rejection episode after renal transplantation (10 cases; 7 males and 3 females), 6 of which were diagnosed by renal biopsy and the other 4 by biochemical analysis. The diagnosis of an acute rejection episode with a renal biopsy used the Banff 2003 system.[10] The second group contained 49 patients (27 males, and 22 females) who had normal renal function following transplantation. All 49 patients displayed normal serum creatinine (Scr) levels (less than 1.14 mg/dL). The last group of 60 healthy individuals (range, 37–57 years) served as normal controls. This study design was approved by our Ethical Committee.

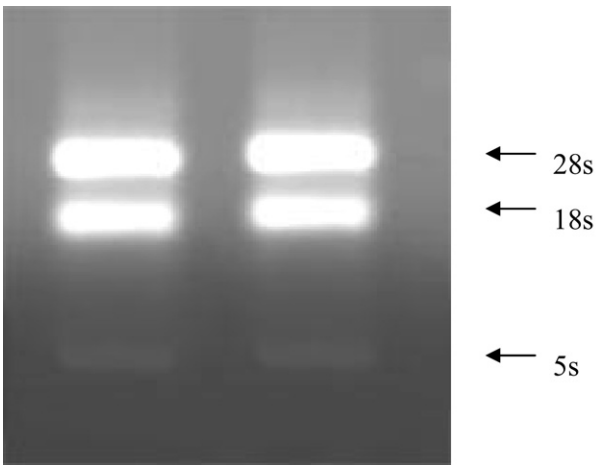

Fig 1. RNA metamorphic electrophoresis. The brightness of the 28S band is about twice that of the 18S band. The diffuse of two trip is not present, indicating the quality of the RNA is efficient for RT-PCR.

Collection of Peripheral Blood Samples

The blood samples from the first two groups of patients were obtained during their hospitalization. Blood samples (2 mL of each) were first taken before the operation and administration of an immunosuppressant, and then every day during the first week posttransplantation. During the following 3 months, blood samples were obtained weekly. All patients were instructed to return to the hospital immediately in case of fever, lumbodynia, or oliguria.

Extraction of Lymphocytic Cells and Total RNA Preparation

Blood samples were centrifuged at 3000 rpm (4 °C/5 min) immediately or no more than 4 hours after collection. We aspirated cells into two tubes that contained 2 mL lymphocyte-separation medium (Huajing Biology-Technology Limited, Shanghai, China); the ratio between blood and medium was 3:2. After centrifugation (20 min/2000 rpm), the lymphocyte layer was aspirated and total RNA extracted using the Trizol method.[11] The purity of isolated RNA was determined by measurement of the ratio of absorbance at 260 and 280 nm (A260/280 ratios), and concentrations evaluated by absorbance at 260 nm using an ultraviolet-visible spectrophotometer (Beckman, Germany).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Information about primers and probes for HLA-A mRNA and G6PDH (Fuxing Medical Technology Limited, Shanghai, China) is

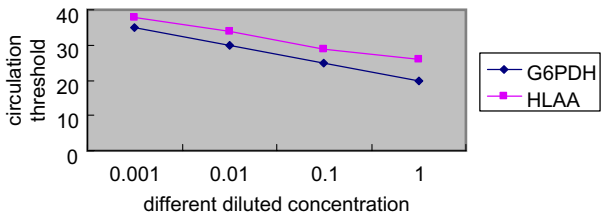

Fig 2. Detection of amplification efficiency analyzed by REST software. Calculated by REST software, the coefficients R^2 are 0.94 and 0.99, respectively; the slopes are −3.78 and −4.91, respectively. This indicates that the amplification efficiency of G6PDH was at the same speed as that of target gene (HLA-A mRNA). We can use the value of Ct to calculate the relative expression of HLA-A mRNA.