

Clinical Significance of Anti-endothelial Cell Antibody in Renal Transplant Recipients

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In order to evaluate the role of anti-endothelial cell antibody (AECA) in acute rejection in renal transplantation, serum AECA IgG titers were measured in 68 healthy controls, 111 chronic hemodialysis (HD) patients and 58 first renal transplant recipients. The AECA titer in hemodialysis patients was higher than in healthy controls (13.9 ± 5.0 vs. 4.8 ± 2.3 U/mL, $p < 0.01$). In transplant recipients, AECA titers were not affected by dialysis mode (HD vs. CAPD vs. non-dialysis; 9.6 ± 7.6 vs. 7.9 ± 3.9 vs. 11.9 ± 3.1 U/mL, $p > 0.05$). After renal transplantation, AECA titer was decreased significantly (vs. 4.7 ± 3.6 U/mL, $p < 0.01$). The serum AECA IgG titers increased significantly in recipients with acute rejection (6.9 ± 3.1 vs. 13.5 ± 9.9 U/mL, $p < 0.01$), but decreased to 5.6 ± 3.0 U/mL ($p < 0.01$) after formal rejection therapy. In the recipients with acute rejection ($n = 27$), the pre-renal transplant AECA titer was higher than in that without acute rejection (14.0 ± 8.6 vs. 7.7 ± 3.8 U/mL, $p < 0.01$). The results of this study lead us to conclude that pre- and post-renal transplant AECA titer might be a useful predictor for acute rejection and useful for monitoring acute rejection in renal transplant recipients.

Key Words : *Antiendothelial cell antibody, Kidney transplantation, Graft rejection, acute*

Introduction

Endothelial cells play a key role in the pathogenesis of vascular inflammation and immune processes¹. Antibodies against endothelial cells (antiendothelial cell antibody; AECA) have been reported in patients with Kawasaki syndrome², systemic lupus erythematosus³, rheumatoid arthritis⁴, hemolytic uremic syndrome⁵ and Berger's disease⁶. AECA can be detected in different unrelated autoimmune vasculitis and their presence generally correlates with disease activity. They also play a probable pathogenic role in *in vivo* vessel wall damage supported by different *in vitro* models of direct and complement- or cell-mediated cytotoxicity. In addition, AECA can be useful in diseases lacking other specific serological markers,

such as in Kawasaki syndrome or in idiopathic forms of vasculitis¹.

There have been several studies on molecular characterization of the endothelial cell target antigen against antibodies. In clinical transplant cases, a 90-100 kD kidney-specific antigen has been identified as the target for IgG antibodies eluted from rejecting kidneys⁷, and IgM antibodies associated with hyperacute rejection of a kidney transplant were directed against a 97-110 kD endothelial target antigen⁸. AECA was studied in cardiac and renal transplantation. AECA was detected in a proportion of renal transplant recipients who developed either accelerated, acute or chronic graft rejection, suggesting the role of AECA in graft rejection⁹⁻¹¹. In cardiac transplantation as well, AECA have been associated with hyperacute rejection¹² and humoral acute rejection^{13,14}.

Based on the above findings, serum AECA IgG titers were monitored before and after renal transplantation, and the association of AECA titers with acute rejection in

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renal transplantation was evaluated. Our data indicate that serum AECA titer is a useful predictor for acute rejection and immunologic monitoring in renal transplant recipients.

Methods

1. Patients

In all, 68 healthy subjects, 111 hemodialysis (HD) patients and 58 first renal transplant recipients were studied. In the control group, mean age was 38 years (range 22-60) and sex ratio (MF) was 48:20. In the HD patients, mean age was 50 years (range 27-63), sex ratio (MF) was 57:54 and mean duration of HD was 57 months (range 19-96). In the renal transplant recipients, mean age was 38 years (range 26-50), sex ratio (MF) was 32:26, method of dialysis (HD/CAPD/none) was 44: 10: 4 and mean duration of dialysis was 29 months (range 8-50). All recipients received 12 mg/kg/day of cyclosporine A starting 2 days prior to the transplantation and the dosage was subsequently adjusted to maintain a trough cyclosporine A plasma concentration within the desired range. Intravenous methylprednisolone (125 mg) was administered intraoperatively, just prior to restoring blood flow to the allograft. Postoperatively, intravenous methylprednisolone (125 mg/day in two divided doses) was administered for 48 h. Beginning on the 3rd post-transplant day, 60 mg prednisolone per day was administered until day 7, at which time the steroid dosage was tapered to 15-20 mg/week for 1 month. Acute rejection was observed in 27 of the 58 renal allograft recipients and diagnosed by graft biopsy findings based on the Banff schema¹⁵. Of the 27 graft biopsies, 10 were very mild AR, 4 were grade I AR, 3 were grade II AR, 3 were normal, 3 were others and 4 were inadequate specimens. Rejection episodes were treated with a 6-day course of intravenous methylprednisolone (250 mg every 12 h for 3 days, and then 125 mg every 12 h for 3 days), followed by gradual tapering to maintenance doses.

2. Serum Sample Collection

Serum samples of renal transplant recipients were serially obtained before and after renal transplantation with 3-5 day interval for 1 or 2 months after transplantation. Serum samples were collected and stored at -20 until used.

3. Endothelial cell culture

Endothelial cells were harvested from human umbilical cord veins by collagenase using established methods¹⁶. Endothelial cells were grown onto 0.1% gelatin coated

tissue culture flasks (Costar, Cambridge, MA, USA), in medium M-199 (Gibco BRL, Gaithersburg, MD, USA) supplemented with 20% heat-inactivated newborn calf serum, 200 U/mL penicillin, 200 g/mL streptomycin, 2 mM L-glutamine, 25 g/mL endothelial cell growth factor (Boehringer Mannheim, Germany) and 5 U/mL heparin. Cells were fed every three days and, when confluent, subcultured by exposure to 0.05% trypsin-0.01% EDTA (Gibco BRL, Gaithersburg, MD, USA). The cells were used between passage 2 to 5.

4. Anti-endothelial cell antibody assay

The ACEA were detected using the method of Calos *et al.*⁹ with an ELISA. Cultured endothelial cells placed onto gelatin coated 96-well microtiter plates (Costar, Cambridge, MA, USA) at a concentration of 2×10^4 cells/well in complete medium. Cells reached a confluent monolayer in about 48 hr. Plates were washed twice with Hank's Balanced Salt Solution (HBSS, Flow Labs., Irvine, Scotland, UK). After two times, washes with HBSS, 100ug of the test serum (diluted 1:100 with HBSS), were added to each well triplicate. The plates were incubated for 2 hr at room temperature and then washed with HBSS three times. One hundred microliters of peroxidase conjugated rabbit anti-human IgG (diluted 1:1000 with HBSS) were added to each well and incubated for 1 h at room temperature. Four time washes were then performed with HBSS. One hundred microliters of o-phenylenediamine dihydrochloride were added to each well and incubated for 15 min at room temperature. And then 100 μ l of 3M NaOH were added to each well. The plates were read at 405 nm in an ELISA reader.

The amount (U/mL) bound of anti-human IgG of the positive control sera at the standard dilution of 1/50 was arbitrarily chosen as 100 U/mL endothelial cell activity. Positive control sera were run on each plate and test sample binding activity was expressed as a percentage of the positive reference sera. Values greater than three standard deviations above the mean value of 48 normal subjects were considered increased.

5. Statistical analysis

Mean values are reported \pm SD. Nonparametric tests were used for the statistical analyses. The Wilcoxon rank sum test was used for an unpaired comparison between

the two groups, and the Wilcoxon Sign Rank Test for comparison within the group. $P < 0.05$ was considered significant.

Results

1. Anti-endothelial cell antibody titers in normal controls and dialysis patients

The AECA titer in patients with HD was higher than that in healthy controls (13.9 ± 5.0 vs. 4.8 ± 2.3 U/mL, $p < 0.01$) (figure 1). In transplant recipients, AECA titers were 9.6 ± 7.6 , 7.9 ± 3.9 and 11.9 ± 3.1 U/ml in recipients with HD, CAPD, and non-dialysis, respectively. This suggests that AECA titers were not affected by the dialysis modes (figure 2).

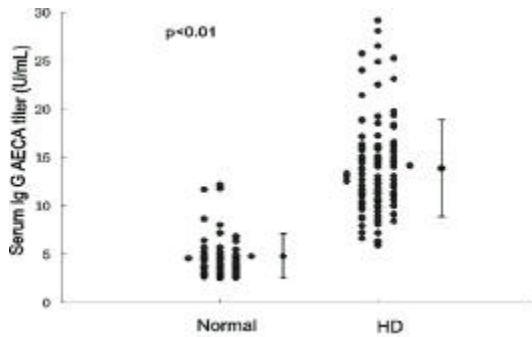


Figure 1. AECA titers in sera of healthy controls and hemodialysis (HD) patients.



Figure 2. AECA titers in sera of recipients before kidney transplantation (KT) according to dialysis modality

2. The AECA titers after kidney transplantation and recipients with acute rejection

The AECA titers at 1 week after the transplantation were significantly decreased as compared with pre-transplant titers (4.7 ± 3.6 U/mL vs. 10.6 ± 7.1 , $p < 0.05$) (Figure 3). In recipients without acute rejection episode, significant

changes of AECA titers were not reported for following 2 months. Thus AECA titers at 2 months after the transplantation were similar to those at 1 week (5.0 ± 2.1 vs. 4.7 ± 3.6 U/mL) (figure 3).

In acute rejection episodes ($n=27$), AECA titers increased significantly compared to prerejection titer (6.9 ± 3.1 to 13.5 ± 9.9 U/mL, $p < 0.01$) (figure 4), but decreased with successful anti-rejection treatment (13.5 ± 9.9 U/mL vs.

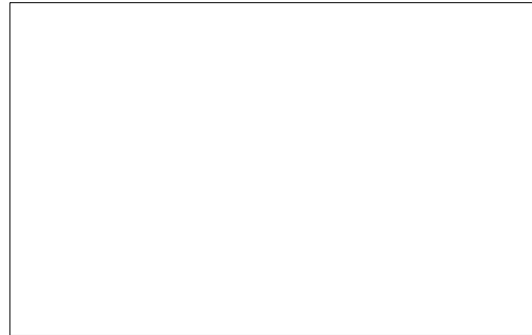


Figure 3. Serial changes of AECA titers in patients without acute rejection episodes



Figure 4. Serial changes of AECA titers in patients who showed acute rejection episodes

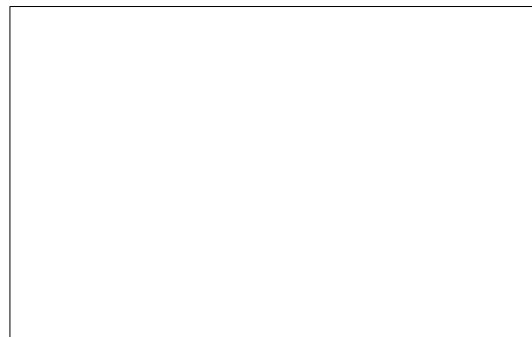


Figure 5. The relationship between the pre-transplant AECA titers and acute rejection (AR)

5.6 ± 3.0 U/mL, $p < 0.01$) (figure 4). In the recipients who experienced acute rejection ($n=27$), the pre-transplant AECA titer was higher than that without acute rejection ($n=31$) (14.0 ± 8.6 vs. 7.7 ± 3.8 U/mL, $p < 0.01$) (figure 5).

Discussion

Our study demonstrates an association of AECA titer with acute rejection in renal transplant recipients. The AECA IgG titers significantly increased in recipients with acute rejection, and decreased with successful anti-rejection treatment. The pre-transplant IgG AECA titers in recipients with acute rejection were higher than in those without acute rejection episode. This finding suggests that AECA itself might be responsible for acute rejection processes and elevated AECA titer in pre-transplant recipients may be a predictor of acute rejection.

Vascular endothelial cells are strategically positioned within a transplanted organ and are considered possible targets in most forms of rejection^{1,17}. Recently, AECA was described in sera from patients affected by autoimmune diseases, and it plays a probable pathogenetic role in *in vivo* vessel wall damage supported by different *in vitro* models of direct and complement- or cell-mediated cytotoxicity. It is generally accepted that AECA activity does not involve HLA class I and II or ABO blood group antigens¹. In transplantation, AECAs directed against non-HLA molecules on graft endothelium have been detected in a proportion of allograft recipients who developed accelerated, acute or chronic graft rejections, suggesting that antibodies reactive with the endothelium may take part in graft rejection^{8-11,18-21}. Karuppan *et al.*⁸ reported that IgM antibodies reactive against endothelial cells are associated with hyperacute and accelerated renal transplant rejection. Perrey *et al.*¹⁸ demonstrated that the presence of IgG antibodies directed against endothelial cells determinants in renal transplant recipients is associated with graft failure. Al-Hussein *et al.*¹⁹ also showed that the presence of AECA was correlated with acute and chronic rejection. Based on previous findings and our data, AECA seems to be associated with rejection process.

Several studies investigated the pathogenetic role of these antibodies in inducing the cell wall damage. AECA in Kawasaki syndrome display a complement-dependent endothelial cytotoxicity on cytokine-activated (interferon (INF)- γ , interleukin (IL)-1 or β , and tumor necrosis factor (TNF)- α) but not resting endothelial cells².

Karuppan *et al.*⁸ demonstrated that non-activated endothelial cells were not killed by the antibodies, but cytotoxic effect of AECA can be observed when activated endothelial cells bind with IL-1. The activation of endothelial cells may occur *in vivo* during transplantation. After renal transplantation, endothelial cells can be activated by ischemia-reperfusion injury and inflammatory mediators (IL-1, TNF- α , and INF- γ)²²⁻²⁴. The activated endothelial cells seem to be another target of acute rejection.

In this study, we found that AECA titers in end stage renal disease patients were higher than in healthy controls. The nature of the stimulus that increases production of AECA in uremic condition is not determined but two possibilities can be speculated on. First, active role of kidney in the metabolism of AECA, considering reduction of AECA IgG titer in the post-transplant period. Second, preactivated immune state in uremic condition may stimulate AECA production²⁵⁻²⁷. In contrast, decreased AECA titers after successful renal transplantation suggested the increased clearance of AECA via kidney and influence of immunosuppressive drug.

Acute rejections are considered to be due to the activation of a cell-mediated immune response and the role of humoral rejection is not well documented. Classical categories of cellular (lymphocyte) and humoral (antibody) mechanisms of rejection are known to be inter-related²⁸. Antibody production by B cells is regulated by T cells through both cell to cell contact and secretion of regulatory lymphokines including IL-2, IL-4, and INF- γ . In this study, AECA titers were increased during acute rejection but subsequently decreased with successful anti-rejection therapy, suggesting the possibility of involvement of humoral rejection in acute rejection process. Although humoral immune responses throughout the course of the transplantation have not been measured, it would appear that both types of immune responses may occur during the course of transplantation and may contribute to the acute rejection. Increased AECA IgG titer after transplantation was associated with acute rejection. High level of pre-transplant AECA IgG titer is related with acute rejection, also. This finding suggests the role of AECA in pathogenesis of acute rejection.

The clinical importance of AECA remains to be established, but it is important to note that AECA assay frequently detects the presence of a clinically significant antibody which is not detected by the lymphocytotoxicity test and which correlates with rejection. This correlation of the AECA detection with clinical course appears to be well established.

In conclusion, monitoring for the presence of AECA may provide a valuable prognostic indicator of graft rejection and immunologic status in renal transplant patients.

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