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Complement-fixing antibodies against denatured HLA and MICA antigens are associated with antibody mediated rejection☆

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ABSTRACT

Background: We have found antibodies against denatured HLA class I antigens in the serum of allograft recipients which were not significantly associated with graft failure. It is unknown whether transplant recipients also have denatured HLA class II and MICA antibodies. The effects of denatured HLA class I, class II, and MICA antibodies on long-term graft outcome were further investigated based on their ability to fix complement c1q.
Materials and methods: In this 4-year retrospective cohort study, post-transplant sera from 975 kidney transplant recipients were tested for antibodies against denatured HLA/MICA antigens and these antibodies were further classified based on their ability to fix c1q.
Results: Thirty percent of patients had antibodies against denatured HLA class I, II, or MICA antigens. Among them, 8.5% and 21.5% of all patients had c1q-fixing and non c1q-fixing antibodies respectively. There was no significant difference on graft survival between patients with or without antibodies against denatured HLA/MICA. However, when these antibodies were further classified according to their ability to fix c1q, patients with c1q-fixing antibodies had a significantly lower graft survival rate than patients without antibodies or patients with non c1q-fixing antibodies ($p = 0.008$). In 169 patients who lost renal grafts, 44% of them had c1q-fixing antibodies against denatured HLA/MICA antigens, which was significantly higher than that in patients with functioning renal transplants (25%, $p < 0.0001$). C1q-fixing antibodies were more significantly associated with graft failure caused by AMR (72.73%) or mixed AMR/CMR (61.9%) as compared to failure due to CMR (35.3%) or other causes (39.2%) ($p = 0.026$).
Conclusions: Transplant recipients had antibodies against denatured HLA class I, II, and MICA antigens. However, only c1q-fixing antibodies were associated with graft failure which was related to antibody mediated rejection.

1. Introduction

Accumulating evidence has shown that HLA and MICA antibodies are major causes of allograft rejection (Cai et al., 2013; Terasaki & Cai, 2008; Ozawa et al., 2007; Terasaki & Cai, 2005; Cai & Terasaki, 2005a; Terasaki, 2003; Lee et al., 2002). Sequence-based antibody-epitope mapping revealed that alloantibodies detected in transplant recipients recognized not only surface residues but also non-surface residues of target antigens (El-Awar et al., 2009; Cai et al., 2009; Cai & Terasaki, 2008; El-Awar et al., 2007; Cai et al., 2006a; Cai et al., 2006b). This finding suggested that there are two types of antibodies in transplant recipients, one targets intact antigen and the other targets denatured antigen. To investigate the clinical importance of both intact and denatured antigen specific antibodies, we did a cohort study to compare the long-term graft outcome of patients who developed either intact or denatured HLA class I antibodies. We demonstrated that only intact but not denatured HLA class I antibodies are predictive of graft failure (Cai et al., 2009).

Complement dependent cytotoxicity (CDC) assay was introduced into transplant clinic in 1964 to identify complement-fixing antibody which is cytotoxic against (potential) donor cell target (Terasaki & Rich, 1964; Terasaki & McClelland, 1964). Cytotoxicity of alloantibodies has been proved to be significantly associated with graft failure (Patel & Terasaki, 1969; Cook et al., 1987). As we discussed in our previous

Abbreviations: HLA, human leukocyte antigen; MICA, MHC class I chain-related gene A; AMR, antibody mediated rejection; CMR, cell mediated rejection.

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review, even though CDC assay was proved to be more significantly associated with graft failure, one of the major disadvantages of CDC assay is its low sensitivity (Cai & Terasaki, 2005b). In recent year, due to the development of solid phase HLA antibodies detection technology using luminex, a new technique to detect c1q-fixing antibodies based on Luminex technology has been introduced correspondingly and proved to be more effective to identify a subgroup of HLA/MICA antibodies which have significant association with graft outcome (Chin et al., 2011; Piazza et al., 2013; Yabu et al., 2011).

The questions we try to address in this study include: 1) what are the frequencies of denatured HLA class I, class II, and MICA specific antibodies in transplant recipients? 2) What are the frequencies of c1q-fixing and non c1q-fixing antibodies specific for denatured HLA and MICA antigens? 3) Do denatured HLA class II and MICA antibodies have a similar effect on graft outcome as denatured HLA class I antibodies as reported previously? 4) Will they have different effects on graft outcome if denatured HLA/MICA antibodies are further classified into c1q-fixing and non c1q-fixing antibodies based on their ability to bind complement fragment c1q? and 5) Is there any association between c1q-fixing antibodies and antibody mediated rejection in patients who lost allografts during 4-year follow-up post antibody testing?

2. Patients and methods

2.1. Patients and characteristics

A total of 975 kidney recipients with follow-up information transplanted between 1984 and 2004 at the Charite-Universitätsmedizin in Berlin, Germany were enrolled in this study. Among them, 59% were female and 41% were male patients. Mean age of all recipients was 43.6 with a standard deviation of 13.2. Immunosuppressive drug therapy information was not available in 10% of patients. The other patients were on either double or triple therapy. The percentages of patients using PRD, CSA, FK, AZA, MMF, and RAPA were 81%, 46%, 44%, 25%, 39%, and 7% respectively. Eighty two percent of all patients received deceased donor transplants and 86.2% of all recipients were primary renal graft recipients. Patient sera were tested once at least 6 months post-transplant and graft function was monitored 4 year post antibody testing. The average time that the sera were tested was 6.8 $\pm$ 5.5 years post-transplant. Sixteen percent of all patients had pre-transplant antibodies detected by ELISA.

2.2. Detection of alloantibodies against denatured HLA and MICA antigens

LABScreen® Mixed antigen beads (One Lambda Inc., Canoga Park, CA) were spun down, washed once with PBS containing 0.1% BSA, then incubated in an appropriate volume of PBS containing 0.1% BSA at 90 °C for 5 min, then cooled down to 4 °C in a PCR machine (Gene Amp® PCR System 9700; Applied Biosystems, Foster City, CA). The beads were then washed with PBS containing 0.1% BSA 3 times in a 1.5 ml Eppendorf tube. The beads were then spun down and re-suspended in an appropriate volume of PBS with 0.1% BSA. Alloantibodies recognizing denatured HLA and MICA antigens were identified using heat-treated antigen beads using Luminex assay. An MFI of 1000 and test-to-control ratio of 2 was used as cutoff to define an antibody positive serum.

2.3. Detection of complement c1q binding HLA and MICA antibodies

Patient serum was heat inactivated at 56 °C for 90 min and then mixed with c1q (One Lambda Inc., Canoga Park). Mixed antigen beads which contain HLA class I, class II, and MICA antigens were mixed with patient serum with c1q and incubated for 1 h. Beads were washed three times and incubated with PE-conjugated anti-C1q antibodies. Then, beads were read with LabScan™ 100 after three time washes with wash buffer. A cutoff value of 200 MFI level was used to define a c1q binding antibody positive serum.

2.4. Statistical analysis

All study analyses were conducted using STATA version 9.0 (StataCorp LP, College Station, Texas). Survival rates were calculated using Kaplan-Meier methods. Statistical significances were determined by the log-rank test for comparison of survival curves. Hazard ratios and statistical significance of variables were calculated using Cox proportional hazard model.

3. Results

3.1. Frequencies of c1q-fixing and non c1q-fixing antibodies against denatured HLA class I, class II, and MICA antigens

Post-transplant sera from 975 renal transplant recipients enrolled in this study were tested for c1q-fixing antibodies against denatured HLA class I, class II, and MICA antigens. As shown in Fig. 1, 24%, 15%, and 17% of patients had detectable antibodies against denatured HLA class I, II, and MICA antigens. In combination, approximately 30% of all patients had antibodies specific for either HLA class I, II, or MICA antigens. Among all patients, 5.85%, 4.10%, and 4.21% of them had c1q-fixing antibodies, while 18.05%, 10.87%, and 12.62% had non c1q-fixing antibodies against denatured class I, II, and MICA antigens respectively. Overall, 8.51% and 21.54% of all patients had c1q-fixing and non c1q-fixing antibodies against denatured HLA and/or denatured MICA antigens.

3.2. Antibodies against denatured HLA/MICA are not predictive of graft failure

In this retrospective cohort study, one post-transplant serum from each patient was tested for antibodies against denatured HLA class I, II, and MICA antigens. Four-year graft survival rates post antibody testing were compared between patients with and without denatured HLA and/or MICA antibodies. Fig. 2 demonstrates that similar to denatured HLA class I specific antibodies (Fig. 2a, $p = 0.8594$), patients with antibodies specific for denatured HLA class II (Fig. 2b, $p = 0.8256$) and MICA (Fig. 2c, $p = 0.3492$) had comparable 4 year graft survival rates when compared to antibody negative patient groups. Graft survival difference remained statistically insignificant when all HLA class I, II, and MICA antibodies were considered ((Fig. 2d, $p = 0.4958$).

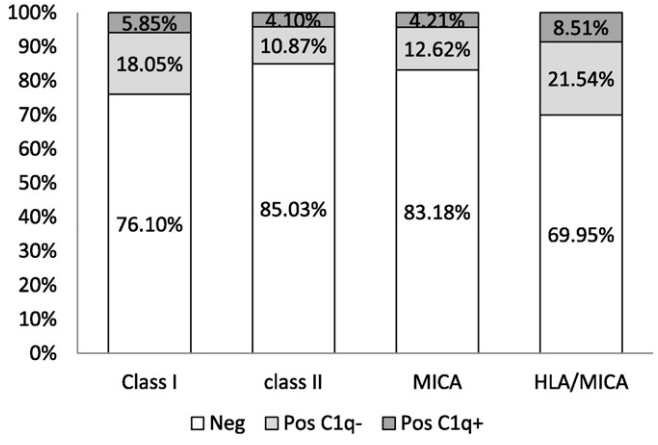

Fig. 1. Frequencies of c1q-fixing and non c1q-fixing antibodies against denatured HLA class I, class II, and MICA antigens.