

Detection of Antibodies Against Major Histocompatibility Complex Class I-Related Chain A in Long-term Renal Graft Recipients

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Abstract

Objectives: To determine the prevalence, allele specificity, and intensity of anti-MICA antibodies in long-term renal graft recipients and to investigate their association with impaired renal function.

Materials and Methods: Sixty-eight long-term (> 10 y) renal graft recipients were divided into 2 groups: (1) patients with impaired renal function (serum creatinine ≥ 2 mg/dL, n=6); (2) patients with normal renal function (serum creatinine < 176.8 μ mol/L, n=62). Anti-MICA antibodies were tested using Luminex single antigen beads assays and the frequency, specificity, and intensity of these antibodies were compared between 2 patient groups.

Results: MICA antibodies were detected in 33% of impaired renal function patients and 15% of normal renal function patients ($P > .05$). Anti-MICA*027 antibodies were found in 11.76% of patients, whereas antibody to MICA*012 was found in 2.94% of patients. Interestingly, among all antibody specificities, MICA*001, *004, *007, *009, *012, and *018 were found more frequently in impaired renal function patients than in normal renal function patients. The peak mean fluorescence intensity

levels of MICA antibodies in impaired renal function patients were significantly higher than those in normal renal function patients ($P < .05$).

Conclusions: Our data suggest that increased prevalence and intensity of anti-MICA antibodies are associated with impaired renal graft function in long-term renal graft recipients and some MICA antibodies might be more important than others in mediating graft rejection.

Key words: Major histocompatibility complex class I-related chain A, Alloantibody, Antibody specificity, Kidney transplant, Graft function

Introduction

MICA, an endothelial cell surface antigen, is a target for both humoral and cellular immune responses during graft rejection.¹ It is reported that presensitization of kidney transplant recipients against MICA antigens is associated with an increased frequency of graft loss and might contribute to allograft loss among recipients who are well matched for HLA.² In 2 prospective trials, Terasaki provided strong evidence to show that, similar to HLA antibodies, MICA antibodies also were associated with graft failures.³ However, it is not clear whether allele specificity and intensity of MICA antibodies are associated with impaired renal function in renal graft recipients.

By using Luminex single antigen beads assays (One Lambda, Inc., Canoga Park, CA, USA), we determined the prevalence, allele specificity, and intensity of anti-MICA antibodies in patients with either normal or impaired renal graft function. We investigated the association between MICA

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antibodies and impaired renal function in long-term kidney transplant recipients.

Materials and Methods

A total of 68 renal graft recipients (42 men, 26 women) who underwent deceased-donor transplant between 1984 and 2000 were included in this study. The patients were divided into 2 groups based on the levels of serum creatinine: (1) normal renal function (NRF), serum creatinine < 176.8 $\mu\text{mol/L}$, $n=62$) and (2) impaired renal function, serum creatinine $\geq 176.8 \text{ nmol/L}$, $n=6$) (Table 1). Mean posttransplant follow-up times were 187.97 ± 52.44 months for patients with normal renal function (NRF) and 159.33 ± 61.85 months for patients with impaired renal function (IRF). This study was performed strictly in accordance with the Declaration of Istanbul on organ transplantation. The study was also approved by the Ethics Committees of our hospital, and all patients gave written informed consent.

Table 1. Characteristics of the patients.

Variables	NRF (n=62) I	IRF (n=6) I	P value
Patient age (y)	56.89 $\pm$ 9.12	56.00 $\pm$ 4.60	$P = .973$
Months posttransplant	187.97 $\pm$ 52.44	159.33 $\pm$ 61.85	$P = .457$
Male/female (n)	39/23	3/3	$P = .866$
ABO blood group (A/B/AB/O)	18/15/7/22	3/2/1/0	$P = .688$
Immunosuppressive treatment CsA/FK506/Rapa + MMF + Pred	55/4/3	3/3/0	$P = .076$

Abbreviations: CsA, cyclosporine; FK506, tacrolimus; IRF, impaired renal function; MMF, mycophenolate mofetil; NRF, normal renal function; Pred, prednisone; Rapa, sirolimus

Sera and MICA antibody tests

The most recent serum sample from each patient was obtained and tested for MICA antibodies using Luminex single antigen beads based upon the manufacturer's instructions (Luminex PRA single antigen MICA antibody testing was performed) as described previously.⁴ MICA antibodies were tested with Luminex single antigen beads, which include MICA *001, *002, *004, *007, *009, *012, *017, *018, *019, and *027 antigens. Each Luminex single antigen beads group (5 μL) was incubated with 25 μL of undiluted serum for 30 minutes. After washing, 100 μL of fluorescein isothiocyanate-conjugated antihuman IgG solution (diluted 1:100) was added and incubated for 30 minutes. After washing and fixing, the sample was analyzed by Luminex. Data

were analyzed by Fusion software (Fusion Software, Cleveland, OH, USA), and Ration ≥ 6 was considered to be positive.

Descriptive data are presented as the mean $\pm$ standard deviation. Significance of differences between frequencies was determined by the Fisher exact test. The probability factor < .05 was considered significant.

Results

As shown in Table 2, the prevalence of MICA antibodies was higher among those with IRF than those with NRF (2/6, 33% vs 9/62, 15%) ($P > .05$). Table 3 lists the allele specificities of MICA antibodies in all patients with MICA antibodies. Figure 1 shows the frequency of specificities in descending order. Note that anti-MICA*027 antibodies were found in 11.76% of patients, whereas antibody to MICA*012 was found in 2.94% of patients, demonstrating the selectivity of anti-MICA antibodies in renal graft recipients. Interestingly, among all these specificities, MICA*001, *004, *007, *009, *012, and *018 were significantly higher in patients with IRF than in patients with functioning renal grafts ($P < .05$, .01, .05, .01, .05, .05 (Figure 2).

Table 2. MICA antibodies in patients with normal or impaired renal function.

MICA antibody	NRF (n=62)	IRF (n=6)	P value
Positive (n=11)	15% (9/62)	33% (2/6)	$P > .05$
Negative (n=57)	85% (53/62)	66% (4/6)	

Abbreviations: IRF, impaired renal function; NRF, normal renal function

Table 3. Specificities of MICA antibodies in renal graft recipients.

Subject (n)	Graft function	Allele specificity of MICA antibody
6	NRF	027
11	NRF	019
12	NRF	001, 002, 007, 012, 017, 018, 019, 027
15	NRF	007
16	IRF	001, 007, 012, 018, 027, 004, 009; 002
19	NRF	019
28	IRF	001, 002, 007, 017, 018, 019, 027, 004, 009
49	NRF	027
62	NRF	002, 007, 018, 027, 001
65	NRF	002, 007, 017, 027, 004, 009, 019
68	NRF	001, 018

Abbreviations: IRF, impaired renal function; NRF, normal renal function

With proper negative controls, mean fluorescence intensity (MFI) in Luminex antigen bead assay measures the relative amount of antigen-specific antibodies in serum samples. Figure 3 shows that the peak MFI levels of MICA antibodies in patients with

IRF are significantly higher than those in patients with NRF (17064 ± 2121 vs 4111 ± 2172 ; $P < .05$).

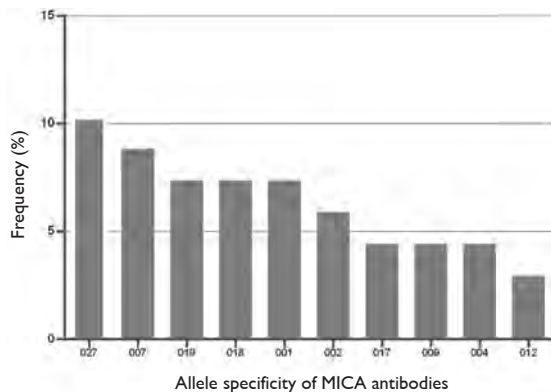


Figure 1. Frequency of anti-MICA antibody specificities in long-term kidney recipients.

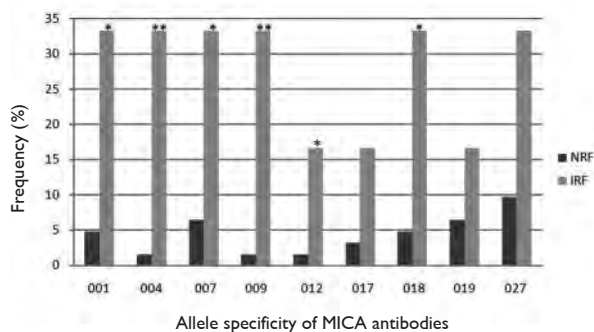


Figure 2. Comparison of frequencies of MICA antibody specificities in patients with normal renal function and impaired renal function (* $P < .05$, ** $P < .01$).

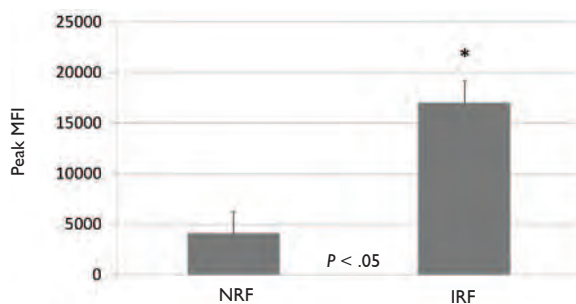


Figure 3. Peak mean fluorescence intensity (MFI) of MICA antibodies in patients with normal renal function and impaired renal function.

Discussion

It has been reported previously that both preexisting and de novo MICA antibodies were associated with transplant rejection or graft loss,^{2, 3, 5-7} though some cohorts could not confirm the adverse effect of MICA antibodies on renal graft outcomes.⁸ Zou and associates² reported that antibodies against MICA alleles were detected in 217 of 1910 patients (11.4%)

before transplant. The presence of MICA antibodies has been associated with renal allograft rejection.² Ozawa's data demonstrated that MICA antibodies were found in 12% of 266 patients posttransplant, and these antibodies were found to be more frequent in patients with graft failure (21%) than in patients with successful grafts (7%).⁹

We know that rapid progress of antibody-mediated hyperacute rejection is related to a large amount of preexisting alloantibodies and it usually occurs in ABO-incompatible or highly sensitized patients. However, in current transplant clinics, transplants are performed primarily in ABO-compatible, low-sensitized patients. Moreover, highly effective immunosuppressive drug therapies are used widely in transplant recipients. Therefore, unlike hyperacute rejection, antibody-mediated acute or chronic graft function loss might not result primarily from thrombosis-related rapid vascular occlusion, but from a progressive damage-repair-damage pathological process. The pathological injury follows the law of "quantitative change to qualitative change." The process speed of antibody-mediated rejection is associated with many factors, which include intensity of antibody, the capability of transplanted organ tissue repair, and the efficacy of immunosuppressive treatments.¹⁰ A previous study demonstrated that de novo antibodies developed on average approximately 1 year after transplant, and led to an increase in serum creatinine > 2 mg/dL in an average of 29 months and graft failure in 44 months.¹¹ In some cases, it may take more than 10 years for an antibody to cause graft failure.¹¹ Subjects of our study subjects are all renal graft recipients who survived more than 10 years after the transplant. We showed that in these long-term allograft recipients, 33% of patients with IRF had MICA antibodies, which is higher than in patients with functioning renal grafts (15%) ($P > .05$). Statistical significance was not found between the 2 groups, most likely because MICA antibodies were rarely detected in patients with IRF and NRF. In addition, the number of patients in the study was too small to reach statistical significance. However, if combined with the MICA antibody specificity differences in Figure 2, our findings seemed to be consistent with the previous reports that suggested a significant association between graft failure and MICA antibodies.

In Zou's study, 5 MICA allele antigens (MICA*001, *002, *004, *008, and *009) were used as

antibody targets.² In Ozawa's study, MICA antibodies were tested using a group of 10 MICA antigens (MICA*001, *002, *004, *007, *009, *012, *017, *018, *019, and *027).⁹ Possibly because almost all transplant recipients are not typed routinely for MICA antigens when they receive organ transplants, frequencies of different allele-specific MICA antibodies were not analyzed further and reported in these previous studies.^{2, 9} In addition, the association between intensity of MICA antibody and graft outcome was not investigated in these 2 studies.

In the present study, MICA antibodies were tested using a group of 10 MICA antigens, which included MICA *001, *002, *004, *007, *009, *012, *017, *018, *019, and *027. We not only demonstrated that MICA antibodies were detected more frequently in IRF patients than in patients with successful grafts; we also found that MICA *001, *004, *007, *009, *012, and *018 seemed to be more substantially associated with IRF than others. Interestingly, the peak MFI levels of MICA antibodies in patients with IRF are significantly higher than in patients with NRF ($P < .05$), which further confirms the effect of MICA antibodies in IRF. However, to avoid any premature conclusions, these findings from our current study need to be verified in a larger patient population. Epitope analysis of positive MICA antibodies also may be necessary to further investigate the relation between positive specificities in the same patient. We previously reported that owing to the shared epitopes within different antigens, 1 antibody may cause multiple antigen reactions.^{12,13} For instance, MICA*001, *004, *007, *009, *012, and *018, which were found more frequently in IRF patients, share an amino acid of Glutamine at the position of 91 of their peptide sequences.

In conclusion, our data suggest that increased prevalence and intensity of anti-MICA antibodies are associated with impaired renal graft function in long-term renal graft recipients and that some MICA antibodies might be more important than others in mediating graft rejection. The major limitation of this study is the lack of donor and recipient MICA typing information. Therefore, we could not define whether these MICA antibodies are donor specific or nondonor specific. In addition, because of the

unavailability of pretransplant sera, we could not determine whether these antibodies were preexisting or de novo. Further investigations are warranted to address these remaining questions and to verify reported findings, namely, whether MICA antibodies are donor specific,¹⁴ preexisting, or de novo, and to elucidate the relation between HLA antibodies and MICA antibodies.^{4, 15}

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