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Donor non-specific MICA antibodies in renal transplant recipients

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ABSTRACT

Despite recent advances in solid organ transplantations, an antibody mediated rejection caused by donor specific antibodies is still a major problem in kidney graft survival. Besides HLA-induced humoral response, antibodies against MICA antigens have recently attracted attention because of their possible role in graft rejection.

The aim of our study was to establish whether renal recipients produce antibodies against MICA molecules due to the transplantation and if they are specific for MICA antigens of the donors. MICA antibody screening was performed in 124 kidney recipient sera. 22 sera, that were found to be MICA antibody positive, were further examined for MICA antibody profiles and compared with donor MICA alleles.

The analysis of MICA antibody positive sera showed mostly more complex reactivity patterns. A significant fraction of patient sera (59%) reacted not only with the donor MICA antigens, but also with other MICA patterns. A match between antibody specificities and MICA antigens was observed in 41% of renal recipients only. On the other hand, as much as in 36% of recipient sera were detected antibodies against their own MICA molecules.

We did not prove a complete correlation between the recipient MICA antibody specificities and MICA antigens of the donor. We assume that MICA antibody induction occurs not only due to the allogeneic stimulation itself but also due to other factors that need to be elucidated.

Introduction

The human major histocompatibility complex class I chain-related gene A (MICA) is a member of the non-classical HLA class I family showing the greatest degree of polymorphism (Fodil et al. 1996). Unlike classical HLA molecules, MICA expression is limited to dendritic cells, monocytes, activated T cells, keratinocytes, fibroblasts, endothelial and epithelial cells (Zwirner et al. 1998, 1999; Molinero et al. 2006). MICA can be also upregulated on tumors and infected cells as a marker of stress (Bauer et al. 1999; Groh et al. 1998). Products of the gene, MICA molecules, do not present any antigens but they act as a ligand for several immune cells including natural killer (NK) cells and gamma/delta T cells expressing lectin-like NKG2D receptors (Bauer et al. 1999). Activation mediated through NKG2D leads to their cytolytic responses.

Despite recent advances in solid organ transplantations, antibody mediated rejection (AMR) caused by donor specific antibodies is still a major problem in a kidney graft survival. Besides HLA-induced humoral response, antibodies against MICA antigens have recently attracted interest because of their possible role in graft rejection. Several studies have already described the presence of antibodies against MICA in kidney allograft recipients and immunohistochemical techniques enabled to identify localized expression in podocytes within the glomeruli of renal transplant recipients with acute rejection, together with infiltrating mononuclear cells, B cells, CD8+ T, and NK cells (Zwirner et al. 2000; Mizutani et al. 2005; Zou et al. 2006; Li et al. 2010). The presence of specific antibodies against MICA has been reported in patients with decreased kidney graft survival, even if antibodies against HLA were absent (Zou et al. 2007). Anti-MICA antibodies are supposed to react with epitopes defined by amino acid polymorphisms of MICA molecules in a similar way as anti-HLA antibodies react with structurally defined epitopes on the HLA molecular surface. The binding of antibodies with MICA may initiate complement independent mechanisms

Abbreviations: AMR, antibody mediated rejection; HLA, human leukocyte antigen; MICA, major histocompatibility complex class I chain-related gene A; NK, natural killer.

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Table 1
Characteristics of kidney graft recipients.

Gender (M/F)	78/46
Age	21–64 (mean 48)
Rejection (absent/present)	70/54
AMR/TMR + AMR	49/33
Post-transplant HLA antibodies (positive/negative)	84/40
Post-transplant MICA antibodies (positive/negative)	35/89

AMR, antibody mediated rejection; TMR, T cell mediated rejection; HLA antibodies, antibodies against HLA; MICA antibodies, antibodies against MICA.

of graft damage resulting in vascular thrombosis and loss of graft function (Sumitran-Holgersson et al. 2002).

The aim of our work was to compare recipient MICA antibody profiles with donor MICA alleles and to find out whether recipient antibodies against MICA are donor-specific, i.e. whether the transplant itself is the cause of MICA antibody production in kidney transplant recipients.

Materials and methods

Subjects

Serum samples from 124 renal graft recipients were obtained during a routine follow-up at the *National Transplantation Organisation in Bratislava, Slovakia*. Between the years 2007 and 2012, sera were collected from each patient: First sample before the transplant procedure and the second collection was done between one to six months after the transplantation. Clinical as well as laboratory parameters were investigated in the patient group with focus on the occurrence of the antibody mediated rejection. Antibodies against MICA, HLA-I, and HLA-II molecules were determined. Detailed characteristic of the study group is given in Table 1. The control group comprised 38 healthy volunteer subjects with no reported history of kidney disease.

A written informed consent for enrolling in the study and for personal data management was obtained from all subjects. All investigations were carried out in accordance with the principles of the Declaration of Helsinki and the study was approved by the Independent Ethics committee of the Comenius University Faculty of Medicine in Bratislava (Project approval No. 206/2009).

MICA antibody determination and epitope analysis

Screening for MICA antibodies in patient sera was performed by multiplexing, using LABScreen® MICA mixed Class I & II, *OneLambda Inc*. The procedure was performed according to manufacturer's instructions. Patient sera were first analyzed using beads coated with either mixed MICA or HLA molecules. Subsequently antibodies reactive to beads were detected with an anti-IgG PE-conjugated secondary antibody on Luminex AtheNA 100IS. Evaluation was performed by the *HLA Fusion*™ software, *OneLambda*.

MICA antibody positive sera were further analyzed for MICA antibody profiles using the Luminex single antigen bead assay (LABScreen MICA Single Antigen – Group 1, *OneLambda Inc.*), which enables to detect the following MICA epitope specificities: MICA*001, *002, *004, *007, *009, *012, *015, *017, *018, and *027. Evaluation was performed by the *HLA Fusion*™ software, *OneLambda*. Confirmation analysis of MICA epitope specificities was performed using LIFECODES LSA™ MIC, *Gen-Probe* which enables to identify the following MICA specificities: MICA*001, *002, *004, *005, *006, *007, *008, *009, *011, *012, *015, *016, *017, *018, *019, *024, *028, *029, *030, *033, *036, *037, *041, *042, *043, *046, *050, and *051. Detection was performed by Luminex AtheNA 100IS and the software *Match it!*, *Gen-Probe*.

Details of the procedures are available elsewhere (Seyhun et al. 2012; Lemy et al. 2010).

Sequence analysis of the MICA gene

Sequence analysis of the MICA genes was performed as previously described (Durmanova et al. 2011). Briefly, genomic DNA isolated from peripheral blood samples by the phenol–chloroform extraction was used as a template for MICA gene amplification (2201 bp DNA fragment including exon 2, 3, 4 and 5) (Fodil et al. 1996). Purified PCR products (*EXO SAP-IT kit*, *USB*) were then sequenced using *ABI PRISM BigDye Terminator v3.1 ready reaction-cycle sequencing kit* (*Applied Biosystems*). Each exon (i.e. 2, 3 and 4) was sequenced individually on both strands of the purified MICA fragments using six primers as reported by Katsuyama et al. (1999). The sequencing reaction was run on the 3130 ABI PRISM Genetic analyser (*Applied Biosystems*). To distinguish alleles with the same polymorphism in the extracellular domains (ambiguities), fragment analysis based on GCT repeat recognition in the exon 5 was performed (Tosh et al. 2006). The PCR products of the exon 5 were separated by capillary electrophoresis using the 3130 Genetic analyzer and their size was confirmed using the gene scan-500 LIZ size standard and GeneMapper software (*Applied Biosystems*). The MICA alleles were determined by SeqScape software (*Applied Biosystems*) using a MICA allelic sequence library, including the reference sequence reported on http://www.ebi.ac.uk/imgt/hla/align.html. Finally allele and genotype frequencies were calculated using the *Arlequin* statistical software.

Results

124 renal recipient sera were screened for HLA and MICA antibodies. Out of these, 35 sera were found to be positive for antibodies against MICA. Further, the MICA antibody specificities were assessed. However, the sensitive determination of MICA antibody specificities confirmed MICA antibody positivity only in a 22 sample cohort and the remaining 13 sera were excluded from the study. Out of the 38 control subject, 2 (5.3%) were found to be MICA antibody positive.

Antibody profiles from 22 MICA antibody positive patients showed mostly more complex reactivity patterns (Table 2). The most frequent MICA antibodies were directed against MICA*018 and MICA*001. MICA antibody positive sera were also more frequently HLA sensitized, either for HLA class I or class II HLA antigens. Only five kidney recipients were found to be HLA antibody negative.

Analysis of MICA gene distribution among our donors and anti-MICA positive recipients showed that MICA*008 was the most frequent one with the occurrence rate of 31%, followed by MICA*002 with the frequency of 14%.

By matching MICA allele profiles of donors and MICA antibody epitopes of their respective recipients, we were able to find a correlation only in 9 donor–recipient pairs (41%). In 13 recipient sera no antibodies against MICA molecules of the graft were detected, only antibodies against other MICA antigens were present. In conclusion, the majority (59%) of MICA antibodies in patients were not donor-specific as they did not react with the donor MICA molecules (Table 2). The majority of renal recipients (16 of 20 patients, i.e. 80%) developed donor-specific MICA antibodies de novo in the post-transplantation period, only three recipients had detectable MICA antibodies prior to the transplantation and sera from other two individuals were not available for testing.

Comparison of the MICA allele polymorphism in the recipients with their respective MICA antibody profiles disclosed post-transplantation production of antibodies directed against their own MICA antigens in 8 individuals (36%). In addition, donor specific