ELSEVIER

Identification of MICA as a New Polymorphic Alloantigen Recognized by Antibodies in Sera of Organ Transplant Recipients

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ABSTRACT: MHC class I-related chain A (MICA) is an HLA-related, polymorphic gene the product of which may be recognized by a subpopulation of intestinal γδ T cells and may play a role in the activation of a subpopulation of natural killer cells. Using anti-MICA specific rabbit sera we previously demonstrated that freshly isolated monocytes, keratinocytes, fibroblasts, and endothelial cells express MICA. To analyze whether MICA may be a target for specific antibodies in sera of transplanted patients, we produced three recombinant MICA proteins consisting of the α1, α2, and α3 domains, and used them in an enzyme-linked immunosorbent assay. We found that several patients had specific antibodies against MICA. Most of them were detected in serum samples collected at different times after organ rejection. Although this finding raises the question of how these patients became immunized, the fact that the polymorphic, HLA-like MICA molecule, expressed at the cell surface of endothelial cells, is recognized by specific antibodies in sera of transplanted patients, suggests the MICA may be a target molecule in allograft rejection. *Human Immunology 61, 917–924 (2000).* © American Society for Histocompatibility and Immunogenetics, 2000. Published by Elsevier Science Inc.

KEYWORDS: transplantation; HLA; alloantigen; MICA

ABBREVIATIONS

Ags	antigens
ELISA	enzyme-linked immunosorbent assay
HLA	human leukocyte antigens
MHC	major histocompatibility complex
MICA	MHC class I-related chain A
NK	natural killer
PCR	polymerase chain reaction

INTRODUCTION

Recently, the HLA-related, polymorphic MICA gene was described [1]. It has been suggested that the 383 amino acid-encoded polypeptide has three extracellular domains (α1, α2, and α3), one transmembrane region, and a cytoplasmic tail. It has been reported that MICA may be recognized by a subpopulation of intestinal γδ T cells [2] and may play a role in the activation of a subpopulation of NK cells that express the NKG2D receptor [3].

Using anti-MICA-specific rabbit sera we previously demonstrated that freshly isolated monocytes, keratinocytes, fibroblasts, and endothelial cells express MICA, whereas peripheral blood $CD4^+$, $CD8^+$, or $CD19^+$ lymphocytes do not [4]. We also demonstrated by flow cytometry that MICA is expressed at the cell surface in endothelial cells and fibroblasts [5].

Classical HLA class I Ags are able to elicit the synthesis of alloantibodies in patients that have received many blood transfusions, multiparous women, or solid organ transplant recipients [6]. These antibodies can be detected by standard serological methods and flow cytometry [7], and their presence in serum of transplant recipients has been associated with poor outcome and

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TABLE 1 Primers used for amplification and sequencing MICA from genomic DNA

Name	Sequence	Notes
MICA6823	CGTTCTTGTCCCTTTGCCYGTGTGC	5′ primer for amplification of MICA from genomic DNA
MICA8999	GATGCTGCCCCCATTCCCTTCCCAA	3′ primer for amplification of MICA from genomic DNA
MICA6838	GCCYGTGTGCATTTCCTG	Sequencing primer for exon 2 of MICA; coding direction[a]
MICA7302	TGAGCCAGATCCAGTGGG	Sequencing primer for exon 2 of MICA; noncoding direction[a]
MICA7403	CCCTGGGCTGAGTTCCTC	Sequencing primer for exon 3 of MICA; noncoding direction[a]
MICA7843	ATAGCACAGGGAGGGTTT	Sequencing primer for exon 3 of MICA; noncoding direction[a]
MICA8263	CAGAGTGAGAACAGTGAA	Sequencing primer for exon 4 of MICA; coding direction[a]
MICA8705	AGGGACTTGTTATACACT	Sequencing primer for exon 4 of MICA; noncoding direction[a]
MICAINCBODY	CGCGCGCGCATATGGAGCCCCACAGTCTTCG	5′ primer for addition of restriction sites and amplification of MICA from cDNA
RECMICAEND	GCGCGGATCCTTACTAATGGTGATGGTGAT GGTGTACACGAGAGGGCAC	3′ primer for addition of restriction sites and His_6tag, and amplification of MICA from cDNA
T7PROMOTER	TAATACGACTCACTATAGGG	5′ primer for screening of MICA DNA in pET26b plasmid
T7TERMINATOR	GCTAGTTATTGCTCAGCGG	3′ primer for screening of MICA DNA in pET26b plasmid

[a] Cy5.5 Dye™-labeled sequencing primers were used.

rejection of HLA-mismatched kidney allografts [8], most likely because they recognize the HLA molecules and activate effector functions such as complement fixation.

The fact that MICA is expressed at the cell surface of endothelial cells, and this HLA-related Ag has a polymorphic nature, suggests that MICA may also elicit the formation of specific antibodies in sensitized recipients of organ transplants.

In this study, we addressed the question of whether MICA allele products might be recognized by antibodies in serum of transplanted patients. For this experiment, we produced three recombinant MICA proteins, and developed an ELISA assay to detect the alloantibodies. We also analyzed whether such antibodies are allele specific and whether antibodies against MICA are distinct from antibodies against other HLA class I molecules.

MATERIALS AND METHODS

Anti-MICA Rabbit Sera

The production of rabbit antibodies against MICA has been described previously [4]. Serum #620 was raised against a peptide corresponding to amino acid residues 42–60 (MA42–60, RAKPQGQWAEDVLGNKTWD) of the translated sequence of MICA, whereas sera #621 and #622 were raised against a peptide corresponding to the amino acid residues 140–160 (MA140–160, MNVRNFLKEDAMKTKTHYHAM) of the translated sequence of MICA [1].

Patients

A panel of sera from 73 transplant recipients were used in these experiments. The patients included 56 kidney allograft recipients and 17 recipients of transplants of other organs. The sera selected were from patients with early rejection, high panel reactive antibodies, or presence of antibodies against keratinocytes determined by immunofluorescence on sections of human skin. CU is an adult African American man who received two unrelated kidney transplants, one in 1986 and the second in 1995. The first kidney was rejected 5 years after the transplant, while the second kidney was rejected 6 months after the transplant. CJ is an adult Hispanic man who in 1986 received an unrelated kidney transplant, which was rejected 9 years later. Also, a panel of 20 human sera from healthy volunteers was used.

Sequence-Based Typing of MICA

Genomic DNA was extracted from a panel of cell lines, using a standard phenol/chloroform method. Genomic DNA was also extracted from whole blood from different human patients, but using a commercial kit (QIA amp DNA Blood mini kit, Qiagen, Valencia, CA). MICA exons 2–5 were amplified by PCR using the primers MICA6823 and MICA8999 (Table 1). PCR reactions were performed in a GeneAmp PCR 9600 system (Perkin Elmer, Norwalk, CT), with 1.5 mM $MgC1_2$ and 1.9 U of Taq polymerase (Life Technologies, Gaithersburg, MD) per tube, as follows: one denaturation step at 94°C for 2 min, 40 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 50 sec, and extension at 68°C for 2 min, and a final extension at 72°C for 10 min.

PCR-amplified genomic MICA DNA was typed by sequencing using a VGI OpenGene DNA sequencer (Visible Genetics, Toronto, Ontario, Canada), using the Cy5.5-labeled sequencing primers MICA6838, MICA7302, MICA7403, MICA7843, MICA8263, and MICA8705 (Table 1). Sequence reactions were performed with a Thermo Sequenase dye terminator cycle

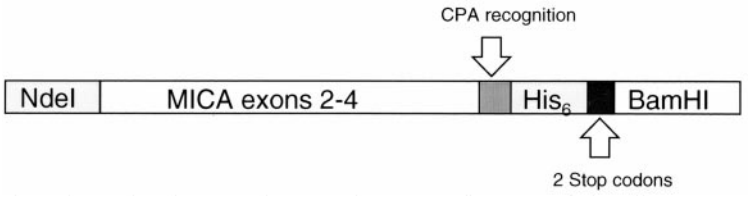

FIGURE 1 Diagrammatic representation of the DNA construct generated to produce recombinant MICA. MICA cDNA was produced from RNA isolated from HeLa and HCT116 cells. Amplification of exons 2–4, and addition of cloning sites for NdeI and BamHI, a carboxipeptidase A (CPA) recognition sequence, the histidine tag (His_6), and two stop codons was performed by PCR using the primers MICAINCBODY and RECMICAEND (Table 1). The amplified DNA products were cloned into pET26b, ligated, and used for transformation of NovaBlue *E. coli* cloning hosts. Plasmids without mutations were purified, and used to transform BL21 (DE3) *E. coli* expression hosts. Expression was induced with IPTG, and rMICA proteins were purified by affinity chromatography with a Ni^{2+} column.

kit (Amersham, Arlington Heights, IL). Conditions for sequence reactions were: one denaturation step at 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 70°C for 1 min, and a final extension at 72°C for 2 min.

Production and Purification of His_6-Tag Recombinant MICA001, MICA008, and MICA009v Proteins

Total RNA was extracted from HeLa (MICA*008 homozygous) and from HCT116 (MICA*001/MICA*009v, which is a novel MICA allele that has a silent mutation in codon 336 when compared with MICA*009) cell lines by standard phenol/chloroform extraction and isopropanol precipitation. cDNA retrotranscription was performed with a commercial kit (Advantage RT-forPCR kit, Clontech, Palo Alto, CA), using oligo$(dT)_{18}$ primer and Moloney murine leukemia virus (MMLV) retrotranscriptase.

Double-stranded MICA cDNA encoding for exons 2–4 (α1 to α3 domains), as well as addition of the His_6-tag sequence and the cloning sites, was performed by PCR using the primers MICAINCBODY and REC MICAEND (Table 1). The schematic representation of the constructs is shown in Figure 1. To minimize the introduction of mutations during the PCR, the reaction was performed with an Expand High Fidelity PCR System (Boehringer Mannheim, Indianapolis, IN) in a GeneAmp PCR 9600 system (Perkin Elmer), using buffer with 1.5 mM $MgC1_2$ provided with the kit by the manufacturer. PCR reaction conditions were as follows: one denaturation step at 94°C for 2 min, 10 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 2 min, and extension at 72°C for 1 min, 20 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 2 min, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were gel purified using a QIAquick Gel extraction kit (Qiagen). Gel-purified DNA and plasmid pET26b (Novagen, Madison, WI) were digested simultaneously with NdeI (New England Biolabs, Beverly, MA) and BamHI (New England Biolabs), in BamHI buffer (New England Biolabs) for 3 h at 37°C. Digested DNA fragments were gel purified using a QIAquick Gel extraction kit (Qiagen). Ligation of the amplified MICA DNA and the plasmid was performed with T4 DNA ligase (Novagen). *Escherichia coli* cloning hosts (NovaBlue, Novagen) were transformed with 1 ng of the ligation reaction products, and transformed bacteria were selected in 30 μg/ml kanamycin (Gibco, Gaithersburg, MD). Screening for the presence of MICA DNA inserts was performed by PCR with T7 promoter and T7 terminator primers (Novagen, Table 1) and Taq polymerase. PCR conditions were as follows: denaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 1 min, and extension at 68°C for 1 min; and final extension at 72°C for 10 min.

Colonies with MICA DNA inserts were grown, and plasmids were purified using a QIAwell 8 Plus plasmid kit (Qiagen) and sequenced using a VGI OpenGene DNA sequencer (Visible Genetics) to check for the absence of mutations. Plasmids with no mutations were used to transform BL21(DE3) *E. coli* expression hosts (Novagen). Kanamycin-resistant colonies were selected and grown in liquid LB medium. Expression of recombinant MICA proteins (rMICA) was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG, Calbiochem, San Diego, CA) for 3 h. Induced bacteria were washed with 50 mM Tris HC1 pH = 8.0, resuspended in SDS-PAGE sample buffer and analyzed by SDS-PAGE or used for the purification of the recombinant proteins.

Purification of the His_6-tag rMICA proteins was performed with His-Bind purification kit (Novagen) in the presence of 6 M urea (Sigma, St. Louis, MO). Briefly, induced BL21 (DE3) hosts expressing the proteins were washed with binding buffer (5 mM imidazole, 0.5 M NaC1, 20 mM Tris HC1, pH = 7.9), DNA was sheared by sonication, and centrifuged at 20,000 *g* for 15 min. The pellet was resuspended in binding buffer with 6 M

urea and incubated on ice for 1 h to dissolve the inclusion bodies, centrifuged at 39,000 *g* for 20 min, and the supernatant was applied to a resin with immobilized Ni^{2+} cations after filtration using a 0.45 μm filter. His_6-tag recombinant proteins were eluted with elution buffer (1 M imidazole, 0.5 M NaC1, 20 mM Tris HC1, pH = 7.9) with 6 M urea. Fractions were analyzed by SDS-PAGE and Coomassie blue staining. Fractions containing the recombinant proteins were pooled and stored at −70°C. Protein concentration of rMICA proteins was determined by the Micro BSA Assay (Pierce, Rockford, IL) using bovine serum albumin as standard.

ELISA

Polystyrene plates (Maxisorb, Nunc, Carrollton, TX) were coated with 500 ng of rMICA protein per well. After blocking with 5% non-fat dry milk (Bio Rad, Hercules, CA) in phosphate-buffered saline (PBS) for 1 h at 37°C, plates were incubated with either rabbit anti-mica peptide sera diluted 1/2,000 or human sera diluted 1/5 or 1/10 for 1 h at 37°C and washed three times with PBS-Tween 20, 0.05%. Thereafter, the plates were incubated with either peroxidase-conjugated goat anti-rabbit or alkaline phosphatase-conjugated anti-human IgG (Bio Rad) for 1 h at 37°C, washed three times with PBS-Tween, and developed with appropriate substrates (H_2O_2/*o*-phenylenediamine for peroxidase, and *p*-nitrophenylphosphate for alkaline phosphatase). Optical density of the resulting color reactions was determined with a Titertek Multiscan MKII ELISA reader at 492 nm for peroxidase and at 405 nm for the alkaline phosphatase-conjugated antibodies. All tests were performed in duplicate. Controls in which wells were coated with PBS instead of antigen were used to determine presence of nonspecific binding of the sera. ELISA reactions were considered positive when the optical densities exceeded the mean absorbance of the normal human control sera, plus three standard deviations. For determination of antibodies against HLA class I the GTI ELISA kit (Genetic Testing Institute, Brookfield, WI) ELISA kit was used and was performed as recommended by the manufacturer.

RESULTS

To select cells for the production of soluble recombinant MICA proteins, we performed sequence-based typing of MICA of a panel of cell lines, and selected two of them. HeLa was found to be MICA*008 homozygous, and HCT116 was MICA*001 and MICA*009v. We called MICA*009v a novel MICA allele that is a variant of MICA*009 that has a silent mutation (C→T) at position 336 of the nucleotide sequence that encodes for the mature polypeptide (C. Y. Marcos, unpublished results).

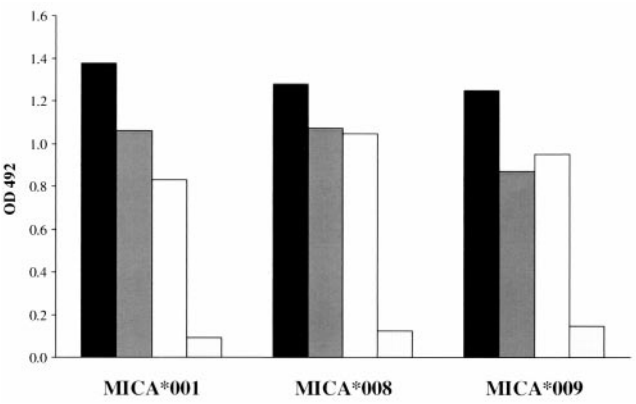

FIGURE 2 Reactivity of rabbit anti-MICA peptide sera with purified rMICA*001, rMICA*008, and rMICA*009v proteins by ELISA. ELISA with rMICA proteins, and rabbit sera #620 (black bars), #621 (gray bars), and #622 (white bars) was analyzed on plates coated with each recombinant protein. All three recombinant proteins were recognized by the anti-MICA peptide sera. NRS: normal rabbit serum (light gray bars).

Thereafter, we isolated the RNA from these two cell lines, retrotranscribed it into cDNA, and used this cDNA to clone exons 2–4 of MICA (α1 to α3 domains). The His_6-tag recombinant proteins were expressed as inclusion bodies, solubilized in urea, and purified by affinity chromatography using a resin with immobilized Ni^{2+} cations.

The purified rMICA proteins were used in an ELISA, and their binding to the solid phase was assessed by the reactivity of the rabbit anti-MICA peptide sera #620, #621, and #622 (Fig. 2). The three rabbit sera reacted with all the recombinant MICA proteins tested.

Thereafter, we performed an analysis of the reactivity of different human sera with these rMICA proteins. For this analysis, we performed a standard amplification ELISA, coating the plates with these Ags, and incubating them with the human sera, diluted 1/5 and 1/10. In the first screening, we found seven patients with reactivity above the cut-off against rMICA*001, six patients with reactivity above the cut-off against rMICA*008, and nine patients with reactivity above the cut-off against rMICA*009 (Fig. 3). To further analyze the reactivity of these human sera, we performed a time-course analysis of the presence of anti-MICA antibody in the serum samples from patient CJ (Fig. 4). Patient CJ showed no anti-MICA antibody in serum sample collected before the transplant. However, at different periods of time after the kidney transplant (when he developed rejection and failure of the transplant), these antibodies were apparent in the serum samples, and the intensity of binding fluctuated during the time covered in this study (Fig. 4A). A similar pattern of reactivity was observed with MICA*001, MICA*008, and MICA*009.

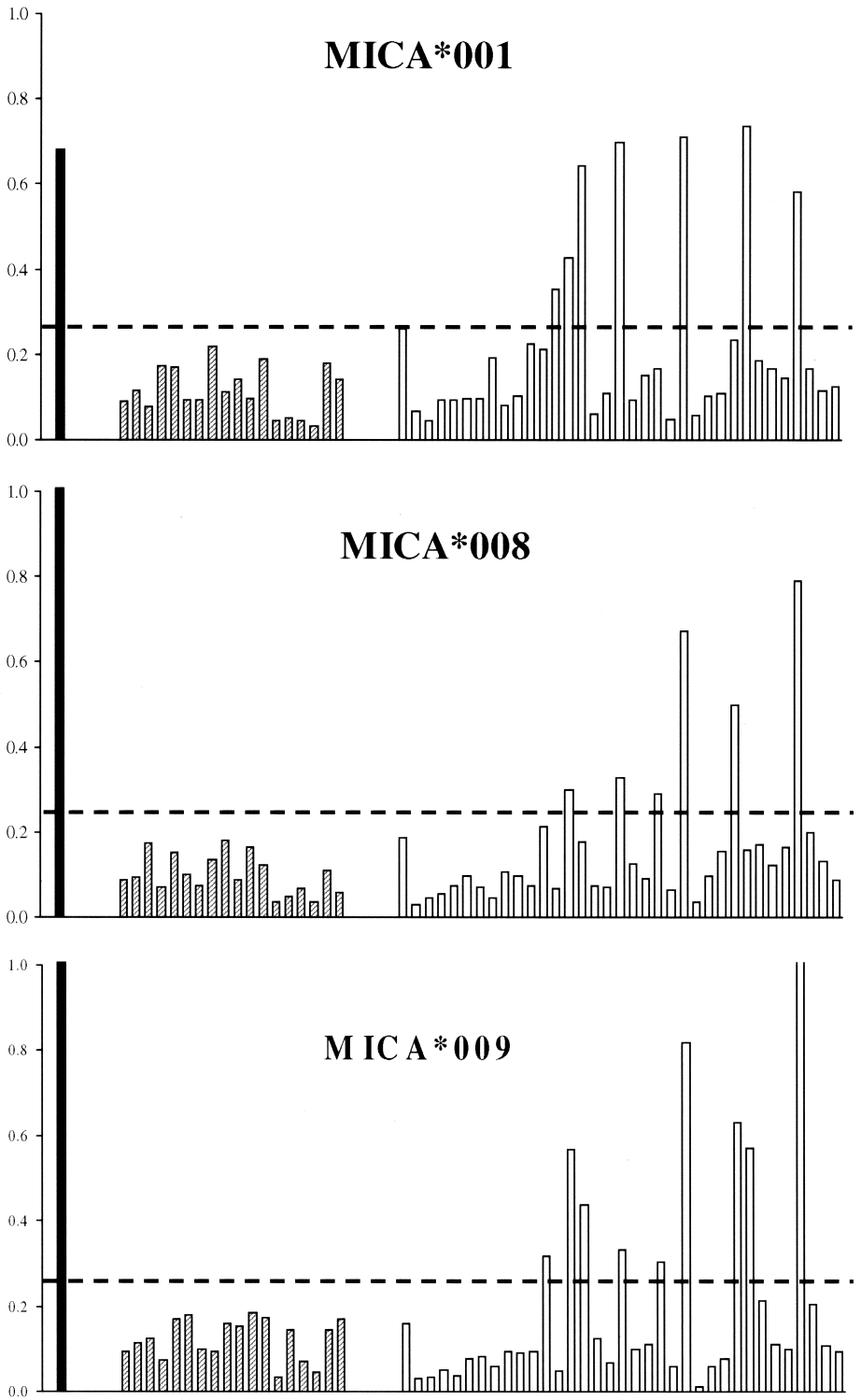

FIGURE 3 ELISA with recombinant MICA proteins and sera from normal subjects and kidney transplant recipients. The three panels show reaction with rMICA*001 (top), MICA*008 (middle), and MICA*009v (bottom). The positive control was a hyperimmune human alloantiserum (black bars); 18 sera from healthy normal human donors (gray bars); and 35 sera from kidney transplant recipients (white bars). In each panel the threshold level above which reactions were considered positive is shown by a thick black dashed line. Among the transplant patients 7 were positive with MICA*001, 5 with MICA*008, and 6 for MICA*009v. The results indicate that antibodies to MICA alleles developed in kidney transplant recipients.

To date we have tested 230 sera obtained from 72 patients (Table 2). Among 56 kidney recipients that we tested, 12 were found to react with rMICA by ELISA. We also observed such reactivity in 4 of 11 heart recipients and in 2 of 5 recipients of lung transplants. Overall, 25% of the patients and 31.3% of their sera were found to contain antibodies against MICA.

To determine the allospecificity of the immune response in these patients we performed sequence-based typing of the MICA antigens in three of the recipients

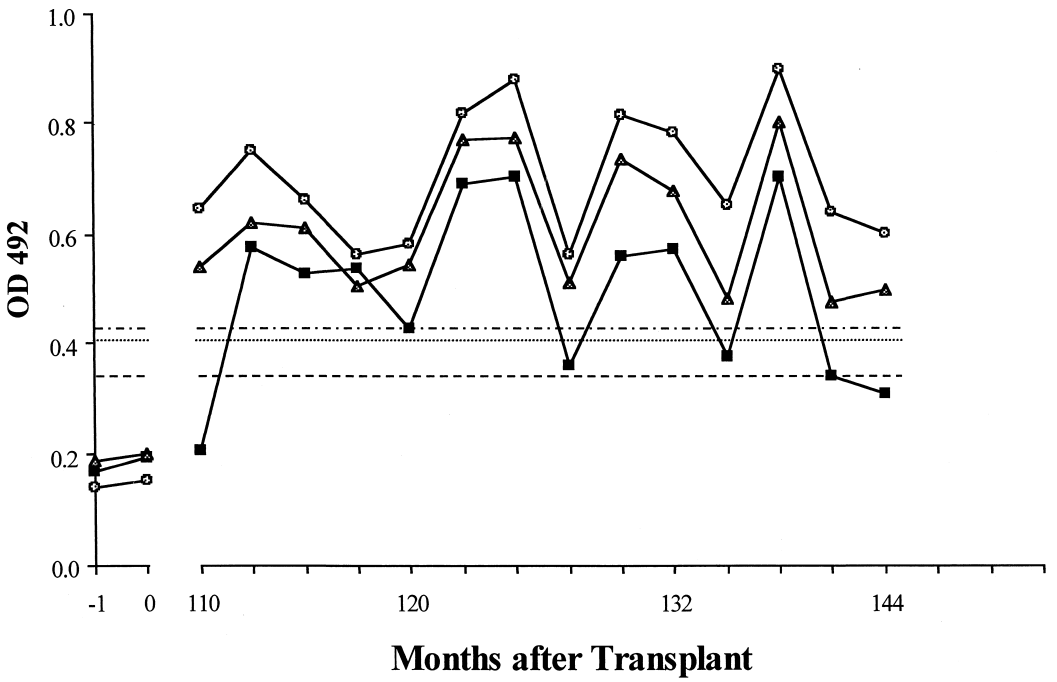

FIGURE 4 Time-course analysis of the presence of anti-MICA antibody in sera from kidney recipient CJ, by ELISA with rMICA*001 (■), rMICA*008 (○), and rMICA*009v (△). The cut-off for each rMICA allele is indicated by a horizontal dashed line as follows, rMICA*002 (· - · - ·), rMICA*008 (· · · · ·), and rMICA*009 (——). The time in months after the transplant is shown, rejection occurs after no break at 110 months.

known to have antibodies against different rMICA proteins (Table 3). Patient UC was typed as MICA*008/*015 and his serum contained antibodies against MICA*001 but not against MICA*008 or MICA*009v. In the case of patients JC and LL, they made antibodies against the three alleles that we tested (Table 3) and the results of sequencing showed that they did not possess any of those alleles.

To investigate whether immunization by pregnancy could induce development of MICA antibodies, we screened several sera from multiparous women known to contain anti-HLA antibodies (Table 4). Six such sera were found to react by ELISA with MICA*008 or MICA*009v. None of them reacted with MICA*001. Five additional multiparous sera containing anti-HLA antibodies did not react with the MICA alleles tested (Table 4).

Absorptions were performed to investigate whether antibodies against MICA and those specific for other HLA class I antigens, present in the same serum, were independent of each other (Table 5). Serum CA, a human strong alloimmune serum known to react with HLA class I antigens from most donors, also showed strong binding in the ELISA test with rMICA*008. When this serum was absorbed with rMICA*008, reactivity against rMICA*008 was reduced by 93%. Absorption with

TABLE 2 Development of anti-MICA antibodies in transplant recipients

Type of transplant	Patients tested	Patients positive	Sera tested	Sera positive
Kidney	56	12	205	62
Heart	11	4	18	6
Lung	5	2	7	4
Total	72	18 (25%)	230	72 (31.3%)

TABLE 3 MICA alleles of patients that made anti-MICA antibodies

Subject ID	MICA sequence typing	Antibodies detected		
		MICA*001	MICA*008	MICA*009
UC[a]	008,015	+	−	−
JC[b]	002,002	+	+	+
LL[c]	002,012	+	+	+

[a] UC was typed as HLA-A1, A33, B8, B57 by standard serology methods and his serum contained also antibodies against HLA-A2, A9 and Bw4.

[b] JC was typed as HLA-A2, A3, B39. During his early course, when anti-MICA antibodies were present, reactivity against HLA was not detected, later HLA panel reactivity rose to 100%.

[c] LL was typed as HLA-A29, A30, B18, B53, and HLA panel reactivity was 71%.

TABLE 4 ELISA with rMICA of pregnancy sera with anti-HLA class I antibodies

Serum ID[b]	Specificity of the anti-HLA class I Ab	OD_{405}[a]		
		MICA*001	MICA*008	MICA*009v
P30910	A1	−[c]	0.438	0.373
P2194	A2	−	0.258	−
Whitley	A11	−	0.332	0.243
Applin	B8	−	0.289	0.296
P2210	B8	−	−	−0.340
CT209	B13	−	−	0.280

[a] Mean of normal sera + 3 SD = 0.216 − 0.310 in different experiments.

[b] Five other pregnancy sera with anti-HLA antibodies did not react with these MICA alleles.

[c] "−" means that the OD produced by that serum was below the cut-off.

HeLa cells, which are known to express MICA*008, reduced the reactivity against the same antigen by 87%. In contrast, absorption of serum CA with pooled human platelets resulted in a reduction of the reactivity against rMICA*008 of only 40.8% (Table 5). ELISA testing for anti-HLA class I antibodies showed the opposite effect. Reduction of HLA activity after absorption with rMICA*008 or HeLa cells resulted in only 7.1% and 21.7% reduction, respectively, whereas absorption with platelets produced a loss of 94.4% of the anti-HLA reactivity, as determined by ELISA using affinity chromatography-purified HLA class I antigens.

DISCUSSION

To investigate the presence of MICA-specific antibodies in sera of transplanted patients, we isolated RNA from the HeLa and HCT116 cell lines, and used them to produce three recombinant MICA proteins. These proteins consisted of the three putative extracellular domains (Fig. 1), and were the products encoded by the alleles MICA*001, MICA*008, and MICA*009v. The purified recombinant proteins were recognized by the rabbit anti-MICA peptide sera #620, #621, and #622 previously produced [4] in an ELISA assay (Fig. 2). This finding suggests that MICA*001, MICA*008, and MICA*009v bind effectively to the solid phase and the sera from rabbits immunized with MICA peptides do indeed recognize MICA-recombinant proteins. In contrast, serum from a rabbit immunized with MICB peptide did not bind to the MICA alleles, indicating specificity of the MICA ELISA assay.

TABLE 5 Absorption of MICA and HLA antibodies from serum CA a strong human alloimmune serum

Absorbed with	rMICA*008		Pool of HLA class I antigens	
	OD_{405}	% decrease	OD_{405}	% decrease
Unabsorbed	0.620	N/A	1.82	N/A
rMICA*008[a]	0.042	93.2	1.69	7.1
Unabsorbed	0.825	N/A	2.322	N/A
HeLa[b]	0.105	87.3	1.817	21.7
Unabsorbed	0.901	N/A	2.322	N/A
Platelets[c]	0.533	40.8	0.129	94.4

[a] Recombinant MICA*008 was added to a dilution of serum CA (1 ng r-MICA for 1 μl serum and incubated 1 hr at room temperature with mixing at 15-min intervals.

[b] HeLa cells are known to be homozygous for MICA*008.

[c] Pooled human platelets from 750–1000 donors.

When we tested human sera by ELISA with these rMICA proteins, we were able to detect anti-MICA antibodies in some of the samples from different patients that received an organ allograft (Fig. 3). A time-course analysis of the presence of these antibodies showed that they were absent in pretransplant samples (patient CJ), but these anti-MICA antibodies were consistently detected in the serum samples collected at different times after the transplant (Fig. 4). In another case (patient CU), we were able to demonstrate that the reactivity was allele specific because this patient had antibodies against MICA*001, but not against MICA*008 or MICA*009v (Table 3). This finding indicates that MICA is an alloantigen.

We chose MICA*008 for this study because it appears to be the most frequent MICA allele. It was found in 55% of 484 Caucasian subjects in a recent study [9]. Together with MICA*001 and MICA*009v, the three recombinant proteins used in the present study would represent about 60% of a Caucasian donor population. The frequency of antibodies in transplant recipients observed in this study should therefore be interpreted with caution. Nevertheless, with our limited panel, we detected antibodies against MICA in 25% of organ transplant recipients and in 31% of the sera tested (Table 2). One can assume that the actual frequencies are somewhat higher. Additional rMICA proteins are being prepared, which will cover about 90% of the population to gener-

ate more confident data about the prevalence of anti-MICA alloantibodies in the population.

To investigate whether patients with anti-MICA alloantibodies do or do not express the MICA alleles against which they show reactivity, we performed sequence-based typing using genomic DNA from patients CU, CJ, and LL (Table 3). We found that CU (the patient who had antibodies against MICA*001 but not against MICA*008 or MICA*009v) is MICA*008/MICA*015, CJ (the patient who had antibodies against MICA*001, MICA*008 and MICA*009v) is MICA*002 homozygous, and LL, who also reacted with the three alleles, is typed as MICA*002/MICA*012. These results clearly demonstrate that some patients develop antibodies against different MICA alleles, but those antibodies are not directed against the alleles these patients express. How these patients become immunized is a question for which we still do not have a definitive answer. We could speculate that during the course of multiple blood transfusions these patients receive during the waiting time until they receive a transplant, they might receive an immunizing dose of cells expressing MICA. In the case of a regrafted patient, there is the additional possibility that during the first allograft, the patient mounted an alloimmune response against MICA expressed by cells in the allograft, and that these alloantibodies may play some role in the outcome of a second allograft. This hypothesis is supported by the findings with patient CJ, who developed anti-MICA antibodies only after the onset of the rejection of the transplanted kidney. Transfusions would be the most likely cause of immunization against MICA in recipients waiting for their first transplant of kidney, heart, or lung (Table 2).

In addition, it appears that pregnancy can induce some antibodies against MICA. We found anti-MICA reactivity in 6 of 11 pregnancy sera used for HLA typing. These sera were clearly positive but the OD readings were not high (Table 4).

Because the pregnancy sera chosen for these experiments also had strong activity against class I HLA antigen (Table 4), we performed absorption experiments to demonstrate that anti-MICA antibodies and anti-HLA antibodies were independent. Serum CA, a strong alloimmune serum, was known to contain high titer of antibodies against HLA class I antigens and showed strong reactions with MICA-recombinant proteins. Absorption of this serum with pooled human platelets removed virtually all the detectable anti-HLA class I antibodies but produced only a modest reduction in the binding to MICA*008 (Table 5). In contrast, absorption with rMICA*008 or with HeLa cells, known to express this antigen [3], had little or no effect on anti-HLA reactivity of this serum, but removed the anti-MICA antibodies almost completely. These results confirm that anti-HLA and anti-MICA antibodies are specific and can be separated by absorption. It is therefore very unlikely that binding to rMICA could be due to antibodies against HLA present in some of the sera tested.

The possible role of preexisting antibodies against MICA on the outcome of transplants is currently under investigation. We can speculate that these anti-MICA antibodies may bind to the endothelial cells of the transplanted solid organ and induce cell destruction of the grafted tissue. Regardless of the role of the alloantibodies against MICA on the outcome of allografts, we definitively have demonstrated that MICA is an alloantigen capable of inducing synthesis of specific alloantibodies in transplanted patients.

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