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## MIC EXPRESSION IN RENAL AND PANCREATIC ALLOGRAFTS

Hankey, Kim G.<sup>1</sup>; Drachenberg, Cinthia B.<sup>2</sup>; Papadimitriou, John C.<sup>2</sup>; Klassen, David K.<sup>3</sup>; Philosophe, Benjamin<sup>4</sup>; Bartlett, Steven T.<sup>4</sup>; Groh, Veronika<sup>5</sup>; Spies, Thomas<sup>5</sup>; Mann, Dean L.<sup>1,6</sup>

Department of Pathology and Divisions of Nephrology and Transplant Surgery, University of Maryland Medical System, Baltimore, Maryland 21201, and Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109

<sup>1</sup> Department of Pathology, University of Maryland, MSTF Room 700.

<sup>2</sup> Department of Pathology, University of Maryland Medical System.

<sup>3</sup> Division of Nephrology, University of Maryland Medical System.

<sup>4</sup> Division of Transplant Surgery, University of Maryland Medical System.

<sup>5</sup> Clinical Research Division, Fred Hutchinson Cancer Research Center.

<sup>6</sup> Address correspondence to: Dean L. Mann, University of Maryland, Department of Pathology, MSTF Room 700, 10 South Pine Street, Baltimore, Maryland 21201.

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### Abstract [TOP](#)

Background. MHC class I chain-related antigen A (MICA) and MHC class I chain-related antigen B (MICB) are HLA class I related products of polymorphic MHC genes. Constitutive expression in normal tissue is limited to gut epithelium but can be induced in other epithelial cells by stress. Specific antibodies against MICA have been reported in the serum of patients who had rejected kidney allografts, suggesting a potential role for these molecules in transplant immunopathology. However, expression of MICA and MICB in transplanted organs has not been demonstrated. In this study, we report the expression of MICA and MICB in renal and pancreatic allograft biopsies, which were obtained due to clinical signs of rejection.

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**Methods.** A monoclonal antibody directed against MICA and MICB was used to perform indirect immunohistochemistry on formalin fixed, paraffin embedded needle biopsies of kidney and pancreas allografts. The results of staining were then compared to the standard light microscopic evaluation of the biopsies for rejection.

**Results.** A total of 53 individual renal transplant biopsies and 19 pancreas transplant biopsies were assayed for expression of MIC. Histologically, renal biopsies were diagnosed as no rejection, acute tubular necrosis (ATN), acute rejection (AR), chronic rejection (CR), and acute and chronic rejection (ACR). No staining was observed in 7 of 10 kidneys showing no rejection. All 11 of the kidney biopsies with AR were positive, as were the 11 ATN cases, 9 of the 11 kidney biopsies with CR, and 7 of the 10 with ACR. The acini of normal, nontransplanted, pancreas, control specimen were consistently negative; however, islets were positive in all specimens. The acini and islets of five histologically normal pancreas biopsies were positive, as were the four biopsies with AR, seven biopsies with CR, and two with ACR.

**Conclusions.** MICA and MICB are expressed in epithelial cells in allografted kidney and pancreas that show histologic evidence of rejection and/or cellular injury. In addition to previous findings of alloantibodies against MICA, expression of these gene products may play a role in allograft rejection.

MHC Class I Chain-related antigen A (MICA) and MHC Class I Chain-related antigen B (MICB) are two members of a recently described family of divergent HLA class I genes that map centromeric to the HLA-B locus in the human MHC. MIC genes encode products that are class I-like in structure but with several major differences. They are not associated with  $\beta$ 2-microglobulin or peptide ligands. Using monoclonal antibodies against these molecules, constitutive surface expression of at least MICA on some cells in the normal gut epithelium, but not in brain, lung, thyroid, liver, kidney, skin, spleen, adrenal glands, placenta, and tonsil, has been found. In addition, and unlike classical HLA class I molecules, expression was not found on lymphocytes. With permissive cell types (fibroblasts, epithelial, and endothelial cells) MICA and MICB mRNA can be induced by conditions that increase expression of heat shock proteins, HSP70 (HSPA1 genes), strongly suggesting that expression of these molecules is related to cellular stress (1). This may be reflected by the frequent expression of MIC in epithelial tumors and by their induction on CMV-infected cells (2,3).

The observation that these genes are constitutively expressed in gut epithelium together with the knowledge that the gut contains an abundance of a subset of  $\gamma\delta$  T cells led to the demonstration that gut epithelial cells expressing these molecules could be recognized and killed by these  $V\delta 1$ - $\gamma\delta$  T cells (4). In addition, MIC molecules function as ligands for NKG2D, an activating receptor that triggers natural killer (NK) cells and provides costimulation to  $\gamma\delta$  T cells and CD8 T cells (3,5, 6).

Both MICA and MICB genes are polymorphic, however, the extent of allelic diversity is considerably less than that found in other class I genes. The distribution of alleles in the population has been reported, and linkage disequilibrium with alleles of the HLA class I B locus has been identified (7,8). A potential role for MIC gene products in graft rejection was suggested by the results of studies where antibodies to several MICA alleles were found in sera from patients who had rejected renal allografts (9). However, expression of these gene products on cells in allografted organs has not been documented.

In this study, MIC expression was examined in paraffin-embedded blocks of archival renal and pancreatic allograft needle biopsies from different individuals who received a single-organ transplant. The monoclonal antibody, mAb 6D4, which reacts with an epitope common to the products of both genes, was used (4). Specimens were selected for study based on histologic criteria, which classified the rejection status of the organ. The diagnosis of acute and chronic rejection in the kidney was made following the 1997 Banff grading scheme (10), whereas the diagnosis of acute and chronic pancreas allograft rejection was made using previously described grading systems (11,12). The paraffin embedded biopsies were sectioned at 5  $\mu$ m onto poly-L-lysine-coated glass slides. After deparaffinization and rehydration, the sections were subjected to target retrieval using Dako Target Retrieval Solution (Dako, Carpinteria, CA), rinsed in phosphate-buffered saline, quenched with peroxide, and treated with MaxiTags Protein Blocking Agent (Shandon, Pittsburgh, PA). The sections were subsequently incubated for 30 min with a dilution of monoclonal antibody 6D4 (anti-MICA/B) or isotype-matched control immunoglobulin. The Peroxidase EnVision System (Dako) was used to complete staining followed by counterstain with hematoxylin, dehydration in graded alcohols, and xylene mounting.

Photomicrographs depicting MIC expression found in allografted kidneys and pancreas are shown in [Figure 1, A and B](#). The patterns shown here are representative of the reactivity of the monoclonal antibody that was observed in allografted organs that were classified by histologic criteria of rejection.

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Figure. 1. Immunohistochemical detection of MIC in kidney (A) and pancreas (B) allografts. (1) Normal, nontransplanted kidney. (2) Acute tubular necrosis. (3) Acute rejection. (4) Chronic rejection. (5) Normal, nontransplanted pancreas. (6) Acute rejection. (7) Chronic rejection.

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In contrast to normal kidney, which shows no immunochemical evidence of MIC expression, the majority of biopsies with histologic evidence of rejection or acute tubular necrosis (ATN) showed positive staining of the tubular epithelium in the proximal and distal tubules. Weak and diffuse staining of the tubules or moderate staining of individual cells or cell clusters in the tubules was noted in biopsies with ATN. This pattern varied from that found in biopsies with acute rejection, where the staining of the tubules was consistently more intense and diffuse. Finally, specimens diagnosed with chronic rejection stained weakly positive with a diffuse pattern punctuated by occasional sites of focal intensity.

[Figure 1B](#) shows a normal nontransplanted pancreas with positive expression of MIC in the islets only. This was a consistent observation wherever an islet was seen in the pancreas biopsies studied. The acinar cells in pancreas allografts diagnosed with acute rejection showed intense and diffuse pattern of positive staining. Although pancreas specimens with chronic rejection showed reduction in acini due to fibrosis, the remaining cells were positive with a pattern similar to that seen in acute rejection pancreas.

[Table 1](#) summarizes the frequency of MIC expression in kidney biopsies grouped by histologic findings. The majority (7/10) of the specimens that had no evidence of rejection were completely negative. All biopsies diagnosed as ATN (11/11) and acute rejection (11/11) were positive, as were the majority of the specimens with histologic findings of acute/chronic rejection (7/10) or chronic rejection (9/11). Overall, there was good agreement between the cellular expression of these gene products and histologic criteria, indicating rejection and/or conditions that are associated with cellular injury.

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Table 1. Results of immunohistochemical staining of kidney allograft biopsies using mAb 6D4 in relation to histological findings

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We considered the possibility that the clinical indications for a biopsy correlated with the observed expression of MIC in samples selected for study. All 15 samples that were obtained to evaluate delayed graft function were positive, as were 24 of the 33 evaluated for elevated creatinine. Of particular interest was the expression of MIC in the three biopsy specimens, where other evidence of cellular rejection/injury was lacking. Subsequent biopsies in two of these patients (within 2 months) showed histologic evidence for ATN and chronic allograft nephropathy. One might speculate that the MIC expression that was observed was an early indication of cellular stress that was subsequently manifested.

In the needle biopsies from pancreas, all samples except one showed some evidence of MIC antigen expression ([Table 2](#)). Most notably, this included five specimens considered to be histologically negative for rejection; however, one of these biopsies was performed due to infection at the site of the graft. Because MIC expression is most probably related to stress, it is conceivable that an infectious process may induce their expression.

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Table 2. Results of immunohistochemical staining of pancreas allograft biopsies using mAb 6D4 in relation to histological findings

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Diffuse patterns of antibody reactivity ranged from intense to weak staining involving the majority of the acinar tissue. Weak and diffuse reactivity was observed in all five specimens where no rejection was seen by histologic criteria. In contrast, staining was the most intense and diffuse in specimens that met the histologic criteria of acute rejection. Prior studies have established the lack of MIC expression in normal kidneys but had not examined the pancreas. Reactivity of the monoclonal antibody with cells in pancreatic islets was observed in the allograft biopsies as well as in nontransplanted, postmortem organs. The expression of MIC in the islets may be constitutive, or in the case of the allografts, it may be associated with islet injury due to treatment with immunosuppressive agents, such as FK506 and cyclosporine. To state definitively that one or more of the cell types that make up this structure constitutively expresses these genes, it is necessary to examine additional normal, nontransplanted pancreas sections using multiple antibodies that recognize MIC.

The observations made in this study raise a number of questions as to the relevance of MIC expression in allografted organs. For instance, the expression may precede the cellular infiltration that characterizes the histologic criteria for rejection. The relationship of expression to rejection may be established by prospective studies in renal allografts where biopsies are obtained at the time or before the surgical procedure and at intervals subsequent to engraftment. Such a study may reveal if the expression of these genes is an early marker for tissue rejection.

There is strong evidence that the NKG2D molecule expressed on NK,  $\gamma\delta$ , and some CD8+  $\alpha\beta$  T cells is a costimulatory receptor that interacts with MICA and MICB (6). Identification of these receptors on cellular infiltrates in allografted organs may be informative in regard to their role in rejection. Immunosuppressive regimens are directed at abrogating the development of acquired cellular and humoral immunity to the grafted tissue by the host. Unlike other MHC products that incorporate peptides that are recognized and targeted by  $\alpha\beta$  T cells, there is no evidence that peptides are presented by MICA molecules. Rather their cell surface expression alone triggers T cell recognition. It is conceivable that immunosuppressive regimens do not affect cellular cytotoxicity mediated through MICA recognition by cytotoxic T cells expressing the NKG2D receptor. One might hypothesize that the graft rejection that occurs despite adequate immunosuppression is a consequence of this innate immune response rather than acquired immunity.

To our knowledge, this is the first report that documents MIC expression in allografted kidneys and pancreas. These observations raise a host of questions, which will only be addressed by additional investigations.

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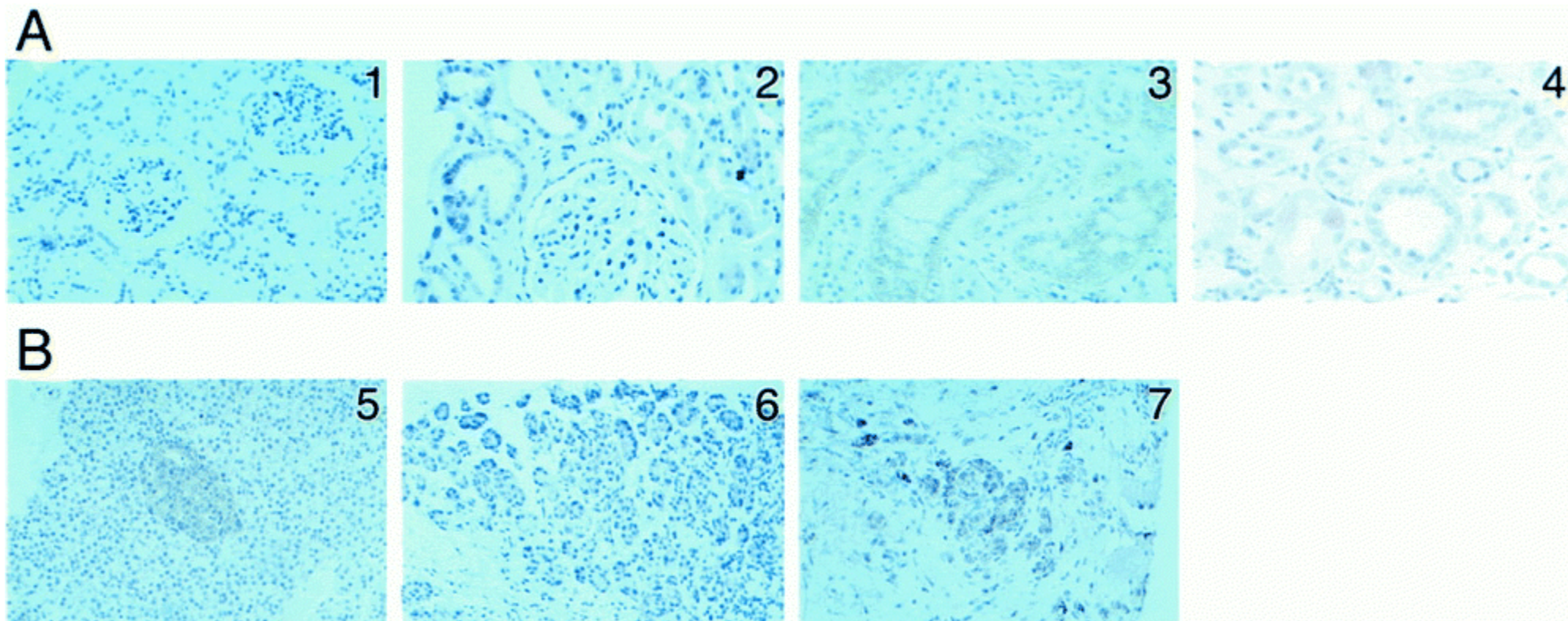


Figure. 1. Immunohistochemical detection of MIC in kidney (A) and pancreas (B) allografts. (1) Normal, nontransplanted kidney. (2) Acute tubular necrosis. (3) Acute rejection. (4) Chronic rejection. (5) Normal, nontransplanted pancreas. (6) Acute rejection. (7) Chronic rejection.

**TABLE 2. Results of immunohistochemical staining of pancreas allograft biopsies using mAb 6D4 in relation to histological findings**

Results with mAb 6D4	Histological findings			
	No rejection	Acute rejection	Chronic rejection	Acute/chronic rejection
Negative	0	0	1	0
Positive	5	4	7	2

Table 2. Results of immunohistochemical staining of pancreas allograft biopsies using mAb 6D4 in relation to histological findings

**TABLE 1. Results of immunohistochemical staining of kidney allograft biopsies using mAb 6D4 in relation to histological findings**

Results with mAb 6D4	Histological findings				
	No rejection	ATN	Acute rejection	Chronic rejection	Acute/chronic rejection
Negative	7	0	0	2	3
Positive	3	11	11	9	7

Table 1. Results of immunohistochemical staining of kidney allograft biopsies using mAb 6D4 in relation to histological findings