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Association of Kidney Transplant Failure and Antibodies Against MICA

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ABSTRACT: Despite the progress in renal transplantation, acute rejection and graft failure still occur and chronic rejection continues to be the main problem in long-term allograft survival. Although kidney transplant rejection has been linked to anti-HLA antibodies, not all patients with failed kidney transplants have anti-HLA antibodies, indicating that other loci may be involved. Sera of 63 patients who experienced kidney rejection were compared against sera of 82 patients with functioning transplants. Sera were examined for IgG and IgM anti-HLA Class I and II antibodies. They were also tested by cytotoxicity against panels of 26 endothelial cell lines, 8 MHC class I chain-related gene A (MICA) recombinant cell lines, and 28 B lymphoblast cell lines. Among patients whose transplants failed, 65% had anti-HLA antibodies compared with 45% of those with functioning kidneys ($p < 0.05$). Similarly, among those whose transplants failed, 41% had anti–endothelial cell antibodies in contrast to 22% in functioning patients ($p < 0.05$). Among patients whose grafts failed, 52% had anti-MICA antibodies versus 21% of those with functioning grafts ($p < 0.001$). Eleven patients with failed grafts and 32 with functioning grafts were negative for all of the above. However, 6 of the former and 7 of the latter showed positive cytotoxicity against B lymphoblasts ($p < 0.05$). Taking all antibodies together, 92% of patients with graft failure had antibodies as opposed to 70% of patients with functioning grafts ($p < 0.001$). We postulate that antibodies against HLA, MICA, endothelial cells, and B lymphoblasts could be independently involved in the slow process of chronic graft failure. *Human Immunology 67, 683–691 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: Kidney-transplant rejection; MICA; endothelial cell; B lymphoblast

ABBREVIATIONS

HLA human leukocyte antigen
MICA MHC class I chain-related gene A
PBS phosphate-buffered saline

INTRODUCTION

We previously summarized the evidence indicating that anti–human leukocyte antigen (HLA) antibodies are the likely cause of chronic immunologic rejection [1, 2]. Although rejection can often be attributed to HLA, research has also shown that graft failure can occur in HLA identical transplants. Previous research has established graft-versus-host reactions and graft failure among HLA-identical sibling donor kidney transplants as well as a 38% 10-year kidney graft failure in HLA-identical sibling transplants [3]. Further evidence that HLA alone is not responsible for graft failure is provided by a comparison of kidney transplant survival in HLA-identical sibling donors and unrelated living donors in which calculations suggested that as many as 38% kidney graft failures in deceased donor transplants are the result of other histocompatibility loci [3]. Finally, a recent study by Opelz indicated that anti–non-HLA antibodies contribute substantially to long-term kidney-transplant failure in identical sibling donor transplants, suggesting the need for characterizing non-HLA antigens that can lead to graft failure [4].

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To test for antibodies against non-HLA, we used recombinant MICA cell lines and a panel of endothelial cells, as endothelial cells are likely targets of alloresponse, and antibodies against endothelial cells have been found in renal transplant recipients before and after transplantation [5–10]. Antibodies to MHC class I chain-related gene A (MICA) antigens [11] are interesting given that the MICA locus is highly polymorphic and closely linked to the HLA-B locus [11]. Zwirner *et al.* identified MICA antigens which are expressed by endothelial cells and monocytes but not by lymphocytes [12]. In other research, MICA antibody was found in patients whose kidney transplants had been rejected [13, 14].

Our aim here was to conduct a systematic study of antibodies ageinst HLA, MICA, and endothelial cells in patients who had failed grafts and in those with functioning grafts. Finally, for those patients with none of the above antibodies, antibodies reactive against B-lymphoblast cells were sought by cytotoxicity.

PATIENTS AND METHODS

Patients

We retrospectively examined the sera of 173 patients who had undergone either a first-kidney or combined first-kidney and pancreas transplantation performed in Nantes, France, between January 1998 and March 2003. The sera were taken until December 2003 as part of an annual check-up to test for immunologic response, which is the accepted procedure at this institution to monitor for rejection. One serum sample was available for each patient was available in this study and tested in December 2003. Patients were followed in Nantes until May 2005. A total of 28 patients who had graft failure before serum collection were used as references for the study (After-Failure group). The average number of days between the sample collection date and the failure date in the After-Failure group was 173 days (5–1087 days).

Unfortunately, the exact cause of each failure could not be accurately determined. We assumed that not all failure were the result of chronic rejection. Because the sera had been collected over a long period of time, biopsy data, especially those from C4d staining, were not uniformly available.

After testing was completed in December 2003, a total of 146 patients (excluding the After-Failure group) were followed; of those, 63 patients had experienced kidney rejection or had died before the end of the study (the Before-Failure group). The average number of days between the sample collection date and failure date in the Before-Failure group was 164 days (0–1193 days). A total of 82 patients with functioning first transplants from the same center were used as control subjects. Most of the study and control patients had initial triple therapy (cyclosporine [CsA], steroids, and azathioprine or mycophenolate mofetil [MMF]), with an anti-IL2 receptor monoclonal antibody, Basiliximab, induction. Some recipients were also participating in experimental protocols and were given other monoclonal antibodies or related molecules (anti-LFA1, anti-CD4 or CTLA4-Ig), Sirolimus, Everolimus, or FTY720 instead of the induction therapy described above. All patients with acute rejection episodes were treated with steroid boluses as first line therapy and with anti-lymphocyte globulins in case of cortico-resistance. The immunosuppressive treatment was modified according to clinical events [15]. "Return to dialysis" was considered to indicate graft loss.

Detection of Anti-HLA Antibodies

Testing for anti-HLA antibodies was performed by flow cytometry or Luminex methods, using FlowPRA®, or LABScreen® assays, respectively, according to the manufacturer's specifications (One Lambda, Inc., Canoga Park, CA). IgG anti-HLA antibody screening was done using the antihuman reagent provided by the kits. IgM anti-HLA antibody screening was performed using R-Phycoerythrin-conjugated AffiniPure $F(ab')_2$ Fragment Donkey Anti-Human IgM, Fc5μ Fragment Specific (Jackson ImmunoResearch Laboratories, West Grove, PA). Antibodies to HLA DP (0101, 0201, 0301, 0401, 0501, and 1101) were tested with FlowPRA® single antigen HLA-DP beads.

Antibodies were detected by the fluorescent signal which were measured on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) or LABScan 100™(One Lambda Inc, Canoga Park, CA). The bead was considered positive when it exceeded the cut-off value set by the manufacturer. We confirmed negative levels of these tests with 20 normal healthy men.

Cytotoxicity Tests With Recombinant MICA Cell Lines

MICA-expressing cell lines were produced from the m-HMY2.CIR cell line, which expressed no HLA Class I and II antigens. We electrotransformed these host cells with vector plasmid, MICA cDNA (*001,*002,*004,*007,*008,*012,*017, and *018). cDNA's of MICA were modified with the coding sequences (exon 2 to 4), signal peptides (exon 1), transmembrane regions (exon 5), and cytosolic tails (exon 6 and 7) with G418 resistance. All cell lines were cultured in RPMI Medium 1640 with 15% fetal bovine serum, 1% of L-glutamine penicillin/streptomycin solution, membrane filtered, and 600 μg/ml G418 sulfate, and incubated at 37°C in a 5% CO_2 humidified