GENE EXPRESSION PROFILES OF PATIENTS WITH ANTIBODY-MEDIATED REJECTION AFTER CARDIAC TRANSPLANTATION

To the Editor:

Antibody-mediated rejection (AMR) is characterized by interstitial edema, prominent endothelial cell damage, occasional inflammatory cells, donor-specific antibodies and C4d deposition, and may cause acute graft loss after heart transplantation.[1–3] Unfortunately, there is no non-invasive method to accurately predict or diagnose AMR.

Peripheral blood mononuclear cell (PBMC) gene signatures allow for identification of patients at risk of rejection.[4] We conducted a pilot study to test the hypothesis that patients with AMR show specific PBMC gene expression profiles.

We included all patients at our center who were part of the Cardiac Allograft Rejection Gene expression Observational (CARGO) study[4] and evaluated with gene microarrays. Gene probes with expression values present in <70% of the samples were filtered retaining 4,688 probes of the original 7,370. AMR was defined as new-onset graft dysfunction in the absence of cellular rejection, with light-microscopic criteria of endothelial swelling, requiring specific treatment according to our institutional practice. Repeat samples from the same patients were averaged. Candidate genes were identified by Significance Analysis of Microarrays (SAM).[5] Functional analysis was performed with High Throughput GOminer[6] (HTGM) and Gene Set Enrichment Analysis (GSEA).[7] Clinical variables were compared using a *t*-test or chi-square test when appropriate.

Of the 121 center patients participating in the CARGO study, 45 provided 105 PBMC microarrays. Five patients, providing 17 samples, met the clinical criteria for AMR (Table 1). SAM identified 388 gene transcripts differentially expressed between the two phenotypic conditions with a false-discovery rate (FDR) of 10% (Figure 1). HTGM identified more than 30 gene ontology categories enriched by differentially expressed genes, including genes related to cell metabolism, protein biosynthesis, immune response, apoptosis, humoral response and cell proliferation (Table 2). Gene set enrichment analysis identified 5 gene sets enriched with FDR of <25%, 16 at a nominal *p*-value <1% and 29 at <5%. Enriched gene sets at FDR <25% included the apoptosis/TNFR1 pathway, genes identified in the proliferation of stem cells, genes involved in DNA damage and DNA repair, and genes from the NKT pathway.

In summary, patients meeting the clinical criteria for AMR have specific gene signatures that correlate with AMR and suggest a cross-linked pathophysiology between different forms of rejection as described in renal transplantation.[8] The relationship between T- and B-cell–mediated rejection is not well understood. T-cell mediation may be required for all phases of the alloimmune response, whereas B-cell–mediated antibodies may play a role as the alloimmune response progresses,[9] reflecting a complementary interaction between the innate and adaptive immune system.

This first report correlating the clinical entity of antibody-mediated cardiac allograft rejection gene expression profiling may help to identify the high-risk patient and develop surveillance methods for this prognostically dismal clinical phenotype.

Table 1. Clinical Characteristics

	AMR^+ ($n = 5$)	AMR^- ($n = 40$)	*p*-value
Age, mean ± SD (range)	56.6 ± 6.7 (66–50)	58.4 ± 12.6 (22–76)	0.76
Male	2 (40%)	30 (75%)	0.14
Race			
Black	2 (40%)	2 (5%)	
White	2 (40%)	32 (80%)	
Hispanic	1 (20%)	5 (12.5%)	0.16
Etiology			
Ischemic cardiomyopathy	3 (60%)	22 (55%)	
Dilated cardiomyopathy	2 (40%)	11 (27.5%)	
Other	0 (0%)	7 (17.5%)	0.57
Pre-transplant LVAD	3 (60%)	8 (20%)	0.085
Cross-match	1 (20%)	0 (0%)	0.11
Re-transplant (heart)	2 (40%)	2 (5%)	0.01
1-year mortality	3 (60%)	6 (15%)	0.047

LVAD, left ventricular assist device.

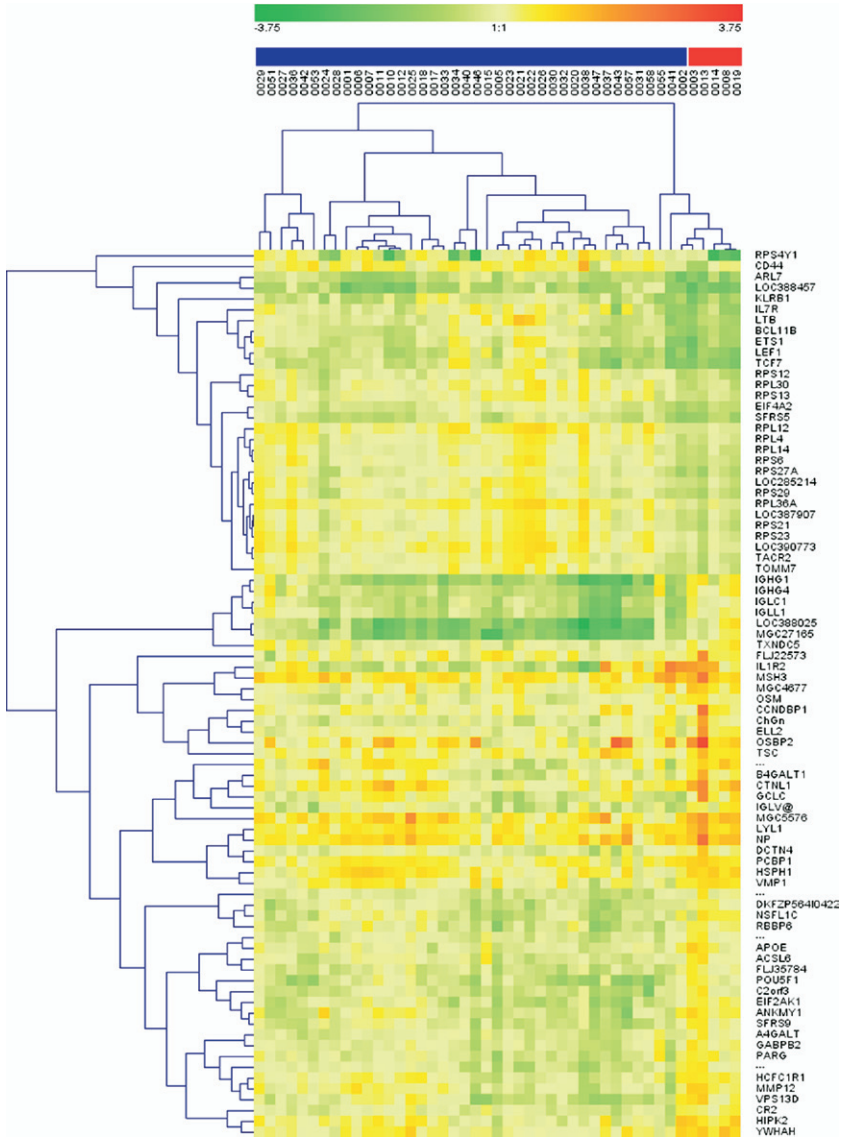

Figure 1. Gene expression profiles of patients with antibody-mediated rejection. Expression Heat Map performed on the average gene expression per patient of 105 samples obtained from 5 patients with antibody-mediated rejection (red) and 40 patients without antibody-mediated rejection (blue), clustered according to the expression of 80 top-ranking genes with statistically significant differences in gene expression based on Significance Analysis of Microarrays (FDR $<5\%$) using average distance and Pearson correlation metrics. Gene expression profiles of patients meeting the criteria for antibody-mediated rejection cluster together.[10]

Table 2. Selected Gene Ontology Categories Enriched by Differentially Expressed Genes

Gene Ontology	Category	Total Genes	Genes	Enrichment	FDR
GO:0050852	T cell receptor signaling pathway	6	4	32.4	0
GO:0050851	Antigen receptor-mediated signaling	9	4	21.6	0.002
GO:0042981	Regulation of apoptosis	301	18	2.9	0.0028
GO:0030098	Lymphocyte differentiation	37	6	7.8	0.0033
GO:0050863	Regulation of T cell activation	32	5	7.6	0.0159
GO:0030217	T cell differentiation	20	4	9.7	0.0166
GO:0030097	Hemopoiesis	82	7	4.2	0.0288
GO:0006959	Humoral immune response	111	8	3.5	0.0284
GO:0016064	Humoral defense mechanism	74	6	3.9	0.0431

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