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Clinical evaluation of the endothelial tie-2 crossmatch in ABO compatible and ABO incompatible renal transplants

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ABSTRACT

The necessity of detection of other than the classical major histocompatibility complex (MHC) and MHC class I-related chain A (MICA) directed antibodies prior to organ transplantation has already been repeatedly reported. A commercial flow cytometric endothelial crossmatch (CM) using isolated peripheral blood tie-2 positive cells provides a tool to detect non-MHC antibodies in addition to antibodies directed to MHC class I and II. The vast majority of circulating tie-2 positive cells expresses HLA–DR but not the A, B blood group antigens. Tie-2 cells are circulating surrogate endothelial cells. In this retrospective study we evaluated the endothelial CM in 51 renal transplantations, 30 with ABO compatible grafts and 21 with ABO incompatible grafts. Fifteen of the ABO compatible recipients (group A) developed unexplained rejection episodes (RE) while the remaining 15 had no RE (group B). Five cases of group A and none of group B had a positive tie-2 CM before transplantation ($p = 0.042$). A positive tie-2 CM was also correlated with graft failure in ABO compatible transplants ($p = 0.02$). No significant correlation was found between a positive pre-transplant tie-2 CM and RE in the ABO incompatible group. This study strongly suggest that a positive tie-2 CM may predict post-transplantation complications in ABO compatible grafts while negative reactions are not predictive. The test is not significantly correlated with RE in ABO incompatible grafts possibly due to applied desensitization.

1. Introduction

The endothelium of a transplanted organ is the first target for the humoral and/or cellular alloimmune response. Preformed antibodies attack the cells either directly by activating complement, indirectly via antibody dependent cell cytotoxicity (ADCC) or both [1]. The A and B blood groups antigens which are expressed on the endothelium are the "first" main barrier for successful organ transplantation followed by the products of the major histocompatibility complex (MHC). These molecules are differentially expressed on resting endothelial cells (EC) but activated cells as it happens in a transplanted organ have increased expression of these molecules [2]. Both the classical human leucocytes antigens HLA-A, B, C and non-classical, e.g. HLA-E or other molecules as the MHC class I-related chain A (MICA) antigens [3] are expressed on their cell surfaces.

Besides these antigens the endothelium expresses other molecules, some of which are shared by monocytes. The EC expresses tissue specific autoantigens, as the type 1- receptor of angiotensin II [4] or proteins characterized by their molecular weight such as the one of 97–110 kDa protein [5]. Antibodies against these molecules have been associated with acute rejection of renal transplants [5,6]. Other proteins like the 38–40 kDa, 54 kDa, 60 kDa, 90–100 kDa and 97–110 kDa are expressed on both EC and monocytes [5,7–10]. Among these molecules is the tie-2 tyrosine kinase receptor of angiopoietin [11,12], expressed on mononuclear monocytes, macrophages, lymphocytes and polymorphonuclear cells [13]. Culture of tie-2 positive cells on fibronectin-coated culture wells induces their differentiation without proliferation to

Abbreviations: ADCC, antibody dependent cell cytotoxicity; EC, endothelial cell; PEC, precursor endothelial cell; RTx, renal transplantation; IS, immunosuppression; MPA, mycophenolate acid; mTORi, mammalian target of rapamycin; PP, plasmapharesis; IVIg, intravenous immunoglobulin; PRA, panel reactive antibody(ies); Cr–Cl, creatinine clearance; APAAP, Alkaline Phosphatase-Anti-Alkaline Phosphatase; AMR, antibody mediated rejection; CMR, cellular mediated rejection; AR, acute rejection.

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phenotypically and morphologically entities resembling EC [13]. Taking this into account, tie-2 positive cells might be considered as precursor endothelial cells (PEC) [14]. Antibodies against these cells have been associated with cellular and antibody mediated rejections [15–21].

Several assays have been developed over time trying to allow the reliable detection of antibodies towards the endothelium not being directed against MHC class I and/or class II [22]. All methods were hampered by the fact that the targets' cells are only available after an invasive approach. So, to perform an endothelial crossmatch using as target biopsy material was not easy. In a first instance monocytes were used for testing/crossmatching because they were thought to express similar products as EC on their cell surface. Results, however, showed that this is the case for some but not all of the expressed molecules. Recently, a new assay was introduced for the detection in the serum of antibodies against this type of "precursor" endothelial, tie-2 positive cells [17–21]. This procedure is based on their isolation from the peripheral blood of the prospective organ donor and the use of them as targets for the antibodies' serum of the prospective recipient. Because of the procedure only living donor peripheral blood can be currently used. Earlier, we presented a case of an accelerated rejection and graft loss in a renal transplant recipient with preformed IgM antibodies against donor tie-2 positive cells [20]. In the present study, we retrospectively investigate the clinical relevance of such antibodies on a cohort of 51 renal transplants performed in our unit. The predictive value regarding transplantation outcome and rejection episodes are evaluated.

This study strongly suggests that a positive tie-2 CM may predict post-transplantation complications in ABO compatible grafts. The test is not correlated with RE in ABO incompatible grafts possibly due to applied desensitization.

2. Material and Methods

2.1. Study design

In the Laikon Hospital, Athens, 171 consecutive ABO compatible and 27 consecutive ABO incompatible renal transplants from living related donor (RTx) were performed between June 2005 and September 2011. A negative current Complement Dependent Cytotoxicity (CDC-AHG, anti-human globulin) crossmatch and T/B flow cytometry crossmatch (FCM) was required for the inclusion in the study. To identify patients who might have pre-formed anti-endothelial IgG or IgM antibodies we retrospectively crossmatched by FC tie-2 crossmatch (CM) the following categories of recipients and donors: (a) 30 ABO compatible and (b) 21 ABO incompatible RTx recipients. We used the serum at the day of transplantation (day 0). The ABO compatible RTx recipients were divided in two groups: (a) the group A included 15 patients who had experienced unexplained RE 3.0 ± 4.7 months after the transplantation and (b) the group B consisted of 15 patients who had no rejection episodes the same post transplantation period and were chosen as control group. Accordingly, in the recipients of an ABO incompatible graft, 6 patients had experienced rejection episodes. Patients who received ABO incompatible grafts had different induction therapy compared to recipients of an ABO compatible graft and were analyzed separately. All 51 patients were selected for the absence of anti-HLA and anti-MICA donor specific antibodies (DSA), following the operating procedures of our laboratory [23]. The mean follow up period was 36.1 ± 18.9 months (range 3.6–75.7) for the group A patients, 32.8 ± 17.6 (range 5.0–52.8) for the group B patients and 40.4 ± 20.1 months (range 3.6–75.7) for the ABO incompatible transplants recipients.

Table 1
Demographics of the patients analyzed in the present study.

	ABO compatible graft (n = 30)	ABO in compatible graft (n = 21)	p[a]
Age (years)	40 ± 12	38 ± 11	NS
Gender			NS
Males	22(73.3%)	17(80.9%)	
Females	8(26.6%)	4(19.0%)	
HLA mismatch			
Class I (A,B,Cw)	2.4 ± 1.0(0–5)	2.5 ± 1.3 (1–5)	NS
Class II (DR,DQ)	1.5 ± 0.8(0–3)	1.4 ± 1.0(0–4)	
Prior transplants	0	0	NS
Pregnancy	3	1	NS
Transfusion	11	7	NS
HLA sensitization			
PRAs(0–5)	28(93.3%)	19(90.4%)NS	
PRAs (5–15)[b]	2 (6.6%)	2 (9.5%)	
IS			
Scheme A[c]	30	0	0.0001
Scheme B[d]	0	21	
Follow up	5 days–52.8 months	3.5–69.4 months	NS

[a] Chi-square test.
[b] No patient had PRA > 15%.
[c] Scheme A: corticosteroids, mycophenolate acid (MPA) and a calcineurin inhibitor either CsA or tacrolimus.
[d] Scheme B: corticosteroids, a mammalian target of rapamycin inhibitor (mTORi) and a calcineurin inhibitor either CsA or tacrolimus.

We investigated the presence of IgG and IgM anti-endothelial cell antibodies in the pre renal transplantation (RTx) sera and analyzed the association to rejection episodes. Demographics including recipient's age, gender, HLA sensitization, factors of sensitization, HLA mismatch and immunosuppression (IS) therapy are shown in Table 1.

Institutional Review Board approval was obtained from Laikon Hospital for the retrospective tie-2 crossmatch. Informed consent was obtained from recipient and donor prior bleeding as well.

2.2. Clinical protocols

Renal transplant recipients of ABO compatible graft received corticosteroids, mycophenolate acid (MPA) and a calcineurin inhibitor (CsA or tacrolimus; Scheme A; Table 1). Renal transplant recipients of ABO incompatible grafts received a mammalian target of rapamycin inhibitor (mTORi) instead of MPA (Scheme B; Table 1). An anti-interleukin-2R inhibitor was administered routinely as induction therapy in both groups.

Desensitization protocol: All renal transplant candidates of an ABO incompatible graft underwent desensitization before transplantation. The desensitization protocol was a combination of a single dose of rituximab, antigen-specific immunoadsorptions and a single dose of IVIg. More specific, 30 days prior to scheduled transplantation patients received a single dose (375 mg/m^2) of the anti-CD20 antibody rituximab. Removal of the anti-A and anti-B antibodies (ABab) was achieved by four repeated Glycosorb-ABO immunoadsorptions preoperatively on days -6,-5,-2,-1 in order to attain an ABab titre of $\leqslant$1:32 at the day of transplantation. One day before transplantation a single dose of IVIg 0.5 g/kg body weight was administrated. Three additional immunoadsorptions were performed postoperatively on days 2, 5 and 7.

2.3. Methods

Patients and donors were typed for HLA-A, B, C, DR and DQ serologically (CDC) and by DNA (One Lambda, Canossa Park, USA)