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Detection of antibodies in eluates of immunoadsorption causing humoural rejection in patients after solid organ transplantation

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Abstract

The influence of antibodies (AB) against human leukocyte antigen (HLA) on antibody mediated rejection (AMR) is still discussed controversially. Here we demonstrate to what extent post transplant detected HLA-AB and non-HLA-AB against Angiotensin II type 1 receptor (AT1 R-AB), endothelin-1 type A receptor (ETA R-AB) and glycoprotein (GP) IIb/IIIa, Ia/IIa, Ib/IX affect the graft outcome.

A total of 13 transplant recipients (9 kidneys and 4 hearts) suffering from AMR were analysed. Before immunoadsorption (IA) treatment HLA-AB (CDC) in sera were detected in 27% versus 39% in eluates and 46% versus 87% by using ELISA. We could not find any AB against GP in sera. In eluates, however, we could detect AB against GP: GP IIb/IIIa in 86% of all samples with titres from 1:1 to 1:32, GP Ib/IX (up to 1:32) in 76% and GP Ia/IIa with titres from 1:1 to 1:16 in 82%. Further we detected anti-endothelial cell antibodies (AECA) against receptors AT1 and ETA in sera before IA in 22%, after IA in 10% and in eluates in 42% of all samples. The antibody titres vary from 1:1 to 1:256.

Our investigation pointed out, that AMR is still possible without detectable AB in serum and consolidates the hypothesis that clinical relevant non-HLA-AB and HLA-AB are partly fixed on the graft. IA is qualified to detach these fixed AB.

Keywords: Donor-specific antibody; HLA-antibody; Non-HLA-antibody; Antibody-mediated rejection; Immunoadsorption; Graft survival; Eluat

1. Introduction

Vascular rejection is the B-cell-mediated production of immunoglobulin G (IgG) AB against the transplanted organ. Donor specific HLA-AB (HLA-DSA) represent a major risk factor for AMR and are strongly associated with reduced graft survival [1−3].

However current biomarkers of AMR, such as HLA-DSA, do not detect all forms of AMR. The influence of non-HLA-AB on graft functionality is not yet clear. In the literature several investigators [4−7] reported about AECA causing higher rates of acute rejection episodes and shortening the heart or kidney transplant survival. While HLA-AB have an alloimmune origin, AECA like AT1 R-AB and ETA R-AB are autoantibodies. In addition to MHC molecules and blood group antigens, non MHC encoded antigens may also be targets for AMR.

Extracorporeal procedures are pathophysiologically based on removing circulating pathogenic substances. The effect of IA on organ-bound AB and immunocomplexes is uncertain and may be indirectly measured by improvement of the affected organ.

The quick removal of AB and other plasma factors via IA remains an effective and supportive method for treating AMR, but there is no satisfactory answer when, how often and for how long treatment should be administered. Is it possible that the behaviour of the AB concentration in the medium after elution from protein A sepharose column (eluate) can give an answer?

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2. Patients

At our institution, basic rejection prevention therapy consists of Mycophenolate Mofetil (target level: 1.5–3 μg/ml), FK 506 (target level: 8–10 μg/ml) and Prednisolone.

When patients presented with graft failure a few hours after heart TX, extracorporeal treatment was immediately initiated. In acute rejection episodes or suspicion of impending rejection (decline in ejection fraction, arrhythmias) the first step was to administer methylprednisolone pulse therapy (3 × 1 g). Extracorporeal therapy was only initiated when this method failed regardless if patients were tested positive for antibodies. Heart biopsy was routinely done in all patients according to the transplantation protocol.

In cases of renal TX extracorporeal therapy was initiated only when acute rejection was biopsy-proven according to Banff classification, methylprednisolone pulse therapy failed and the patients serum was tested positive for DSA.

9 patients after kidney TX and 4 patients after HTX were treated and the non-HLA-AB and HLA-AB were examined. All patients intended for treatment were assigned by the clinic and gave their written informed consent.

3. Methods

In total patients received 73 high-volume Protein A immunoadsorptions (Immunosorba®, Fresenius Medical, Bad Homburg, Germany). In order to overcome AMR 3 to 25 treatments per patient were performed.

HLA- and non-HLA-AB were tested in sera before and after IA as well as in eluates. Eluates are defined as solution resulting from elution of protein A sepharose columns and neutralised with TRIS-buffer.

Following methods were used for the detection of HLA- and non-HLA-AB:

1. Complement dependent lymphocytotoxicity test (CDC) according to standards of the National Institute of Health (NIH). CDC identifies the most important AB in crossmatch and screening tests – those responsible for hyper acute or accelerated AMR.
2. Solid-phase enzyme-linked immunosorbent assay (ELISA): QUIKSCREEN® identifies IgG-AB HLA-Class I and B-SCREEN® IgG-AB HLA-Class II (GTH DIAGNOSTICS, USA)
3. Luminex® technology (BMT-Onlamda) – this assay uses purified HLA glycoprotein bound to latex beads. The binding of alloantibodies is detected by fluorochrom-conjungated secondary antibodies in a dual laser flowcytometer. Luminex is more specific and sensitive than CDC and ELISA in detecting HLA-AB. This technology is not influenced by the presence of non-HLA-AB, auto reactive or therapeutic cytotoxic AB [11,12].
4. Non-HLA-AB against AECA such as AT1 R-AB- and ETA R-AB-IgG-antibodies (ELISA, CellTrend GmbH, Luckenwalde, Germany)
5. Non-HLA IgG-AB against GP (GP IIb/IIIa, GP Ib/IX, GP Ia, IIa) (ELISA, PAKPLUS®, GTH DIAGNOSTICS, USA)

4. Results

The number of detected HLA-AB in sera before IA and in eluates using the CDC and ELISA test are shown in Table 1. The HLA-AB titres differ from 1:4 to 1:512.

The results of AB detection against AT1 and ETA are shown in Table 2. The AB titres vary from 1:1 to 1:256.

The results of all tested non-HLA-AB are presented in Table 3.

The AB titres against GP IIb/IIIa differ from 1:1 to 1:32; against GP Ib/IX from negative to 1:32 and against GP Ia/IIa from 1:1 to 1:32. More than 80% of all tested eluates are positive for non-HLA-AB. Overview of four patients (Table 4).

4.1. Patient (1), acute rejection, Grade 3a (ISHLT 1990)

The performance of 4 PA-IA treatments resulted in complete elimination of all HLA-AB (without statement of donor specificity). Serum was tested positive for HLA-AB DQ6 and HLA-AB DQ9. These HLA-AB were detected in Luminex® technology only. No non-HLA-AB were detected. However in the eluates of IA (Fig. 1) AB against HLA- and non-HLA-AB could be detected.

4.2. Patient (2), no biopsy during acute rejection episode

Using CDC and Luminex® only HLA-DSA of the specificity anti-HLA-DQ2 and HLA-DQ3 were detected in sera (Fig. 3). Non-HLA-AB were only detected in eluates of IA (Fig. 2). After insufficient TPE regarding graft-functionality, 5 PA-IA treatments were needed to improve graft-functionality successfully, measured by the rise of EF.

4.3. Patient (3), acute rejection Grade 3b-4 (ISHLT 1990)

This patient developed a hyper acute rejection without detection of panel reactive AB and despite of a negative

Table 1
Number of detected HLA-AB in serum before IA and in eluate of 67 performed IAs.

Methods	Serum before IA		Eluate	
	Negative	Positive	Negative	Positive
CDC	49 (73%)	18 (27%)	41 (61%)	26 (39%)
ELISA	36 (54%)	31 (46%)	9 (13%)	58 (87%)