Human Immunology 72 (2011) 1045-1048

Contents lists available at SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Heart transplantation with donor-specific antibodies directed toward denatured HLA-A*02:01: a case report

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ARTICLE INFO

Article history:
Received 10 June 2011
Accepted 4 August 2011
Available online 10 August 2011

Keywords:
Natural HLA antibodies
HLA antibody in males

ABSTRACT

The development of solid-phase assays for antibody detection has aided in the frequent detection of human leukocyte antigen (HLA) antibodies in nonalloimmunized males. Some scientists have reported that these HLA antibodies are produced to pathogens or allergens and the reactivity with HLA coated beads is the result of cross-reactive epitopes. These antibodies may also be directed toward cryptic epitopes exposed on the denatured beads. In this report, we describe the case of a heart transplanted patient who exhibited anti-HLA-A*02:01 donor-specific antibodies detected with a bead-based assay (Luminex) and undetected with the complement-dependent cytotoxicity (CDC) test. Posttransplant monitoring, carried out with CDC and with Luminex on sera from this patient collected at the 2nd, 4th, 8th, and 12th posttransplant weeks and at 1 year confirmed the presence of anti-HLA-A*02:01 in all serum samples. Additional tests carried out with denatured and intact HLA molecules using single antigen beads demonstrated that the antibody was directed toward a cryptic epitope. One year after transplantation the patient is doing well. No sign of antibody-mediated rejection was observed throughout the follow-up. A comprehensive evaluation of the anamnesis and of antibodies is critical to avoid needless exclusion of organ donors.

1. Introduction

Human leukocyte antigen (HLA) antibodies are usually produced by alloimmunization resulting from transfusions, pregnancies, or transplants. Nevertheless, articles published in the past century reported the existence of "natural antibodies" to HLA, detectable with complement-dependent cytotoxicity (CDC) directed mostly against HLA-B8 antigen [1,2]. The introduction of solid-phase assays [3] for antibody detection made it possible to search more precisely for such natural antibodies. Antibodies reacting in bead-based assays are frequently reported in unsensitized healthy males [4,5].

The most likely explanation for this phenomenon is that these antibodies are produced to pathogens or allergens and the reactivity with HLA coated beads is the result of cross-reactive epitopes, as described by some scientists [6,7]. However, it has also been postulated that the process of attaching the HLA molecules to the beads causes denaturation of the molecules. As a consequence, epitopes that would not be present in naturally expressed HLA molecules are exposed on the cell surface. El-Awar et al. [4] discovered that treatment of the HLA antigens coupled to single antigen (SA) beads with an acid buffer dissociates the β2-microglobulin and the peptide from the HLA class I heavy chain and that the reaction strength of certain weak antibodies in 1 serum sample increased many fold when tested with dissociated forms of HLA class I antigens. Finally, it has also been suggested that some of the natural HLA-Ia antibodies seen in healthy males could be anti-HLA-E antibodies cross-reacting with HLA-Ia alleles [8].

Here we describe the case of a heart transplanted patient who exhibited natural anti-HLA donor-specific antibodies that were detected with a bead-based assay [3] but were not detected with the CDC test [9].

2. Subjects and methods

2.1. Patient and heart donor characteristics

A 48-year-old patient (RSA) was diagnosed with dilative cardiomyopathy at the age of 4 years. At 7 years he was involved in a severe car accident and suffered multiple fractures; no data are available about possible blood transfusions during that period. Except for this episode, the recent immunologic history did not reveal any immunologic stimulus. At 40 years, his cardiac function began to deteriorate and he was placed on the waiting list for heart transplantation in the North Italy Transplant program [10]. His blood group was A^+ and HLA typing was HLA-A*11,*24; B*07,*40;

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0198-8859/11/$32.00 - see front matter © 2011 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2011.08.001

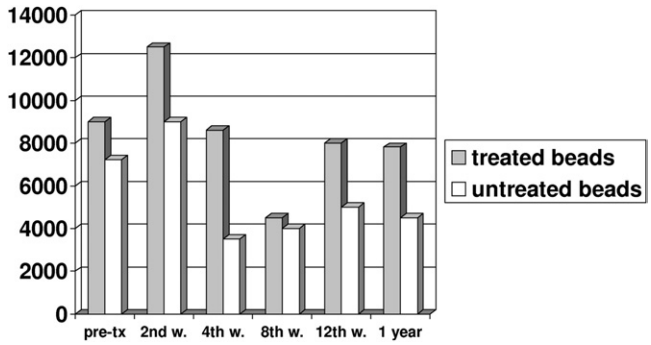

Fig. 1. Normalized mean fluorescence intensity values of serum samples collected at different intervals and tested with denatured and untreated SA beads (gray and white columns, respectively).

DRB1*04,*13. Antibody screening with CDC was negative; anti HLA-A*02:01 antibodies were identified with SA beads (One Lambda, Inc., Canoga Park, CA). In March 2010 he underwent a cardiac transplant. The extended incubation CDC crossmatch against autologous and donor T and B lymphocytes was negative. The donor HLA typing was A*02:01,*24; B*35,*51; DRB1*07,*11.

Immunosuppressive treatment included basiliximab, cyclosporine, and azathioprine; no maintenance steroids were used. Posttransplant monitoring was carried out with CDC and with Luminex methodology on sera from this patient collected at the 2nd, 4th, 8th, and 12th posttransplant weeks and at 1 year. Endomyocardial biopsies were performed in accordance with the established protocol schedule and graded according to the International Society Heart & Lung Transplantation biopsy grading scale, as indicated elsewhere [11,12]: weekly during the first month, biweekly during the next 2 months, monthly until the 6th month, and bimonthly throughout the first year. No episode of acute myocardial rejection or sign of antibody-mediated rejection was observed throughout the follow-up.

2.2. Antibody screening and crossmatching with the CDC test

In the North Italy Transplant program, sera from patients on the waiting list are regularly screened with a CDC test. Screening involved the use of a whole lymphocyte population consisting of a panel of 50 cell types from Caucasian blood donors that incorporated most of the HLA-A, HLA-B, and HLA-DR antigens normally detected in our population. The CDC protocol used was the standard method previously described [9]. Briefly, the patient's serum and positive and negative controls were dispensed onto Terasaki trays. The negative control comprised pooled sera from male, nontransfused, group AB blood donors who tested negative for anti-HLA antibodies. The positive control comprised pooled sera from patients with more than 80% panel-reactive antibodies and a titer >1:4. The trays were either used immediately or stored at −40°C. Fresh panel cells were added to each well, and trays were incubated at 22°C for 60 minutes. Rabbit complement was added to each well, and the trays were incubated for another 60 minutes at 22°C. Finally, a cell staining solution composed of acridine orange, ethidium bromide, and quenching ink (Fluoroquench, One Lambda) was added to each well of the tray. The percentage of panel-reactive antibodies was calculated and the specificity was evaluated with the support of Lambda Scan Analysis software.

Sera that gave a positive result with CDC but a negative result with Luminex were typically retested with and without dithiothreitol to differentiate immunoglobulin G from immunoglobulin M isotype antibodies.

A CDC crossmatch was carried out with a pretransplant serum against T and B lymphocytes following the same procedure as that described for screening.

2.3. Sera adsorption with HLA-A*02:01-positive cells

The pretransplant and the most recent posttransplant sera of RSA were incubated with A*02:01-positive packed total lymphocytes at 24°C for 60 minutes. The adsorption was repeated 4 times.

2.4. Luminex methodology

IgG anti-HLA reactivity in the sera was tested with a bead-based screening assay [3,13]. Briefly, we used the LABScreen mixed kit (One Lambda), which simultaneously detects class I and class II antibodies with microbeads coated with purified class I and class II HLA antigens. Results above a cutoff value of 3.0 were considered positive. The SA kit (One Lambda) was also used to identify HLA specificities. The tests were carried out according to the manufacturer's instructions and analysis was performed with One Lambda software (HLA Fusion, version 2.0).

For the denaturation assay, SA beads were treated with a denaturing buffer (0.3 M glycine–HCl, pH 2.7, with 1% bovine serum albumin). Briefly, beads were added to denaturing buffer at a 1:10 ratio. After 1 hour of incubation at 24°C, beads were washed with LabScreen wash buffer and resuspended with LabScreen storage solution 2 at the original bead volume. After this treatment the beads were ready to use.

To demonstrate that the HLA antigens on the beads were denatured by mild acid treatment, we used the monoclonal antibody G46–2.6 (BD Biosciences Pharmingen, San Josè, CA), which recognizes a monomorphic epitope of HLA class I antigens. All sera were also tested following the manufacturer's instructions with C1QScreen (One Lambda) for identification of complement-binding antibodies. Finally, the pretransplant and the most recent posttransplant sera neat and adsorbed with A*02:01-positive cells, as well as an anti A*02:01 serum from a kidney patient, were retrospectively tested with class I clean beads, which do not have denatured antigens on them (kindly provided by One Lambda).

3. Results

The first RSA serum sample collected in November 2009 tested negative with CDC and positive with Luminex assay. An SA test revealed the presence of antibodies directed against HLA-A*02:01, −02:03, −02:05. The same results were obtained with sera collected after transplantation at the 2nd, 4th, 8th, and 12th weeks and at 1 year. Sera retested with denatured SA beads were positive. The normalized mean fluorescence intensity values of sera tested with beads untreated and treated with the denaturing buffer are reported in Fig. 1.

Using the C1qScreen tool, all RSA sera tested negative, indicating that the antibodies were noncomplement binding. Table 1 summarizes the results of the retrospective analysis on RSA pretransplant and the most recent posttransplant sera, neat and adsorbed with A*0201-positive cells, carried out with the different types of beads. The antibody G46−2.6 (BD Biosciences Pharmimgen) reacted positively with regular beads and negatively with denatured beads (Fig. 2).

Table 1
Normalized mean fluorescence intensity (MFI) values of RSA's serum samples and of an A*02:01-positive serum tested under different conditions

Serum	Single antigen (SA) beads	SA denatured beads	Class I clean beads	C1qScreen
Pretreatment neat	7,200	9,000	500	50
Pretreatment adsorbed	5,330	8,500	300	45
Posttreatment neat	4,500	7,800	81	55
Posttreatment adsorbed	4,200	7,500	75	60
Anti-A02:01 typing serum	8,516	120	13.375	Not tested

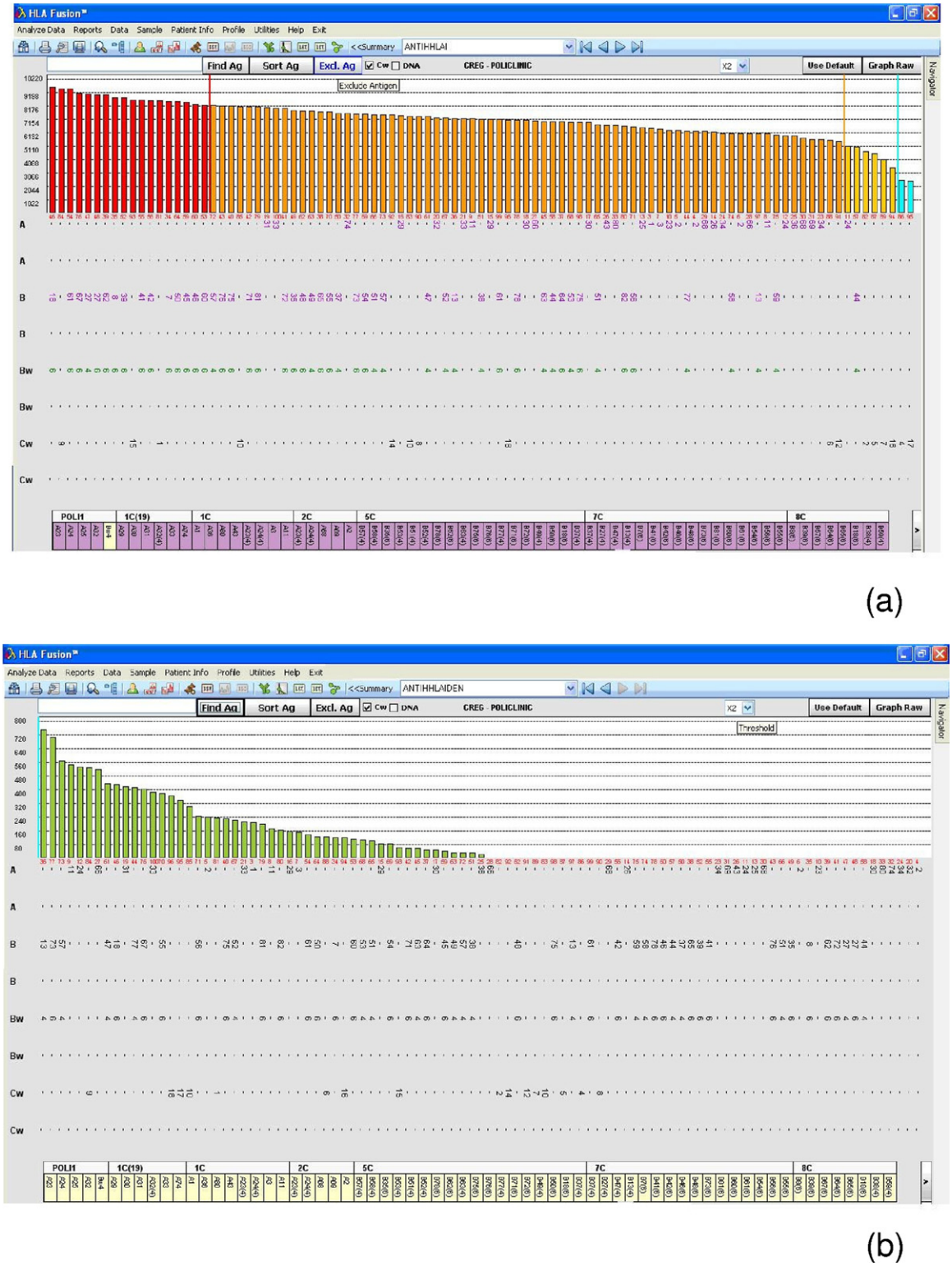

Fig. 2. LabScreen single-antigen assay with an R-PE-conjugated antihuman HLA-A, -B, -C monoclonal antibody (G46-2.6), tested with regular beads (a) and with denatured beads (b). The *y* axis indicates the mean fluorescence intensity values and the *x* axis denotes single-antigen beads.

4. Discussion

We have reported the case of a patient with a negative immunologic history and anti-HLA-A*02:01 antibodies detectable with solid-phase assay before and after heart transplantation from an A*02:01-positive donor. The possibility that our finding was the result of a mix-up of samples was excluded because the same reactivity pattern was observed on a new serum sample and on 5 posttransplant sera over a 7-month period. The strength of these antibodies was enhanced using beads denatured by mild acid treatment. On the basis of the results we postulated that these antibodies were directed to cryptic epitopes exposed on the denatured beads or to microbial agents cross-reacting with HLA molecules.

The clinical urgency of the patient, the absence of immunologic stimuli, and the negativity of CDC crossmatch led us to allow heart transplantation despite donor-specific anti HLA-A*02:01 reactivity. Additional testing carried out retrospectively using class I clean beads indicated that the anti A*02:01 antibody detected in the serum was targeting a cryptic epitope more accessible on the denatured beads.

This donor-specific antibody seems not to adversely affect transplant outcome. Currently, at 1 year posttransplant, the patient is doing well. Only 3 of 14 endomyocardial biopsies were mildly positive for cellular rejection at the 4th and 10th weeks and at the 6th month after transplantation, but no specific treatment for rejection was necessary.

Other cases of renal patients transplanted despite the presence of HLA donor-specific antibodies reactive with beads but negative with native antigen have been reported [14] but, to the best of our knowledge, this is the first case described in the heart transplant setting.

One might rightly say that this is an anectodal case and may thus not be extrapolated to all circumstances. In fact, some open questions remain regarding whether natural antibodies resulting from microbial cross-reactivity have the same potential irrelevance. Again, we are not able to assess whether complement-fixing antibodies or those with stronger MFIs have an impact on mid- and long-term heart transplant outcome.

Nevertheless, our case demonstrates the importance of defining whether HLA-specific antibodies evidenced by Luminex alone are directed versus true allogeneic epitopes able to activate complement and possibly T cell receptor or antibodies directed against cryptic epitopes. In addition, this case underscores the relevance of obtaining a complete clinical history and the importance of the combined use of CDC and solid-phase assays for pretransplant assessment and posttransplant antibody monitoring. The inconsistency between the results from the bead array technique and the clinical data prompted us to investigate the true nature of the antibodies detected only with the solid phase test with the ultimate aim of avoiding to inappropriately deny an individual access to a transplant.

Acknowledgments

The authors are grateful to One Lambda, Inc., for technical assistance in testing complement-binding antibody with C1q Screen and for providing class I clean beads.

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