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Clinically irrelevant circulating human leukocyte antigen antibodies in the presence of ventricular assist devices

Afzal Nikaein, PhD,[a] Nadim El-Awar, PhD,[b] Judson Hunt, MD,[c] Edward J. Rosenthal, MD,[c] Eric Eichhorn, MD,[c] Shelly Hall, MD,[c] James Boehrer, MD,[c] Mitchell Magee, MD,[c] Marc Pieniek, MD,[c] William Brinkman, MD,[c] and Todd Dewey, MD[c]

From the [a]Texas Medical Specialty, Dallas, Texas; [b]One Lambda, Inc, Los Angeles, California; and [c]Medical City Dallas Hospital, Dallas, Texas.

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INTRODUCTION: Identification of anti-human leukocyte antigen (HLA) antibodies by single-antigen beads (SAB) allows for prediction of donor-specific crossmatches (virtual crossmatches), thus facilitating the allocation of organs from deceased donors. However, the clinical relevance of HLA antibodies identified by SAB has been less than clear. This study demonstrates that sera from cardiac transplant candidates with a ventricular assist device (VAD) or infection may contain clinically irrelevant antibodies that bind to the beads but not to lymphocytes.

METHODS: Investigated were 5 cardiac transplant candidates (3 with VAD, all with infections, and 1 retransplant) with positive HLA antibodies detected by SAB, but negative by cytotoxicity. To determine clinical relevance of the antibodies, flow cytometric crossmatches (FCXM) were performed. Untreated beads and elution buffer–treated beads to dissociate the β-2 microglobulin and the peptide from the heavy chain were used.

RESULTS: The virtual crossmatch data were compared with data from actual FCXMs. Of 40 T-cell and B-cell FCXM, SAB-identified HLA antibodies were predictive for only 1 T-cell and 9 B-cell FCXM outcomes. Patients' sera contained a mixture of antibodies directed against cryptic epitopes on the heavy chain and exposed epitopes. The mean fluorescence intensity of antibodies varied from 1,040 to 11,000.

CONCLUSIONS: Sera from cardiac transplant candidates with or without VAD may contain natural antibodies that do not bind to intact antigens on the cell surface. Therefore, great care must be exercised before denying a life-saving transplant to these patients simply on the basis of SAB results.

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Sensitization to human leukocyte antigens (HLA) in heart transplant candidates prolongs waiting times for a compatible donor organ, forcing the increased use of ventricular assist devices as a bridge to transplantation.[1–4] These devices increase the level of sensitization due to infection or transfusion, which further complicates transplantation in these patients.

The advent of recent solid-phase immunoassays, in which purified HLAs are immobilized onto beads, has helped to better identify HLA antibody specificities.[5,6] As a result, transplantation of these patients has been facilitated by virtual crossmatching.[7–9] However, the clinical relevance of HLA antibodies detected by beads is questionable.

Recent studies of healthy donors and renal transplant recipients have shown that some of the antibodies detected by solid-phase immunoassay were against β2-microglobulin–free and peptide-free heavy chains (HC) of HLA.[10–12] These antibodies, referred to as natural antibodies, did not appear to have an effect on renal transplant outcome. Our study evaluated the presence of natural antibodies in heart transplant candidates with or without VAD.

Reprint requests: Afzal Nikaein, Texas Medical Specialty, Inc, 7777 Forest Lane, Dallas, TX 75230. Telephone: 972-566-5761. Fax: 972-566-7720.

E-mail address: nikaein@cs.com or afzal.nikaein@hcahealthcare.com

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Table 1 Comparison of Flow Crossmatch Outcome With Human Leukocyte Antigen Antibodies Detected by Untreated or Treated Beads[a]

Pt	AB binding (MFI range)		Donors tested (N)	HLA antigen tested in FCXM	FCXM HLA antigen result	
	To untreated bead	To treated bead			Positive	Negative
705	A1, 25, 26, 29, 31, 32, 33, 34 (1,600–2,800) R4, DQ6, 8 (1400–1600)	A1, 25, 26, 29, 31, 32, 33, 34 (1,700–2,500)	2	A31, A33, DQ8	A31, DQ8 (B+), A33, DQ8 (B+)	A31 (T–), 33 (T–)
30	A3, 23, 28, 30, 31, 32, B27, 37, 38, 49, 51, 52, 53, 57, 82 (1,062–4,365)	B82 (8,300)	8	A3, 23, B51, B27	A3 (T-/B+), B51 (T+/B+)	A23, B27 and 2 A3 cells
855	B76 (1,735)	None	1	B76	None	B76
879	A68, B18, Cw7 & week A25, 34, B35 (<1,000) DR103, 8, 12, 13, 16, 17, DQ4,5, 6 (1,040–5,360)	B27 (4,600)	4	A24, 25, 33, B18, 27, 35, 44, 57 DR13	DR13 (T–/B+)	A24, 25, B18, 27, 44, 35, 57
782	A23, 24, 32, B8, 18, 35, 49, 51, 52, 53, 57, 58, 75, 78 (4,800–11,000) DQ5, 9, 2 (3,040–3,846)	B8 (10,900)	6	A24, DQ5, B8, 35, DQ5	A24, B35 DQ5 (T–/B+)	3 A24, B8; 1 B35

AB, antibody; HLA, human leukocyte antigen; FCXM, flow cytometry crossmatch; MFI, mean fluorescence intensity.
[a]Some of the crossmatches were performed with more than 1 serum from each patient.

Materials and methods

The study included 52 patients listed for heart transplantation. Of these, 14 (27%) had received the Heartmate XVE[2] or Heartmate II[12] (Thoratec Corp, Pleasanton, CA) VAD. Complement-dependent cytotoxicity (CDC) enhanced with anti-human globulin (AHG) showed 5 of these 14 patients (35.7%) had no HLA antibodies, but the beads technologies showed that they were sensitized. These 5 patients had no or very low levels of HLA antibodies before VAD support, which elevated after VAD placement in 3, after persistent wound infection in 3, or after other infections requiring a clinic visit or hospitalization. Only 4 (10%) of the remaining 38 listed patients with no VAD were sensitized by the CDC-AHG and beads methods.

Sensitization was detected by solid-phase immunoassay using single HLA antigen-coated beads.[6] Sera were studied further to determine if their HLA antibodies bound to intact HLA antigens in flow cytometric crossmatches (FCXMs) using pronase-treated lymphocytes from actual or surrogate donors. Lymphocytes were treated with pronase to breakdown or modify the FC receptors to reduce non-specific binding of immunoglobulin (Ig) G to the cell surface. A total of 40 flow crossmatches were performed with T or B lymphocytes from actual or surrogate donors using different serum samples from each patient. All serum specimens tested in crossmatch were also tested for antibody reactivity by beads.

HLA antibodies were recharacterized at One Lambda, Inc (Los Angeles, CA) for their allospecificity in addition to natural antibody reactivity using untreated or treated beads. Treated beads were HLA-coated beads that were treated with ImmunoPure IgG Elution Buffer (Pierce, Rockford, IL), then blocked with 2% bovine serum albumin.[13] W6/32 monoclonal antibody was included as positive control, and sera from non-transfused males were used as a negative control.[10] Sensitized patients who had positive CDC-panel reactive antibody (PRA), positive alloantibodies detected by treated beads, no reactivity to untreated beads, and a positive flow crossmatch were excluded.

Results

We took 2 approaches to determine the nature of HLA antibodies in 5 patients with negative CDC-AHG PRA, but positive bead reactivity. One was to identify whether the antibodies were alloantibodies and would bind to intact antigens on the surface of lymphocytes. To this approach, FCXMs were performed with actual or surrogate donors who were positive for 1 or more unacceptable antigens. The second approach was to use treated beads to investigate if the antibodies were natural antibodies against the HC of the HLA antigens and would not bind to lymphocytes in FCXM. By definition, natural antibodies do not bind to the intact HLA molecules on the cell membrane.[11]

Untreated beads express intact HLAs as well as β2-microglobulin (β2m)–free, peptide-free HC–only molecules; therefore, these beads detect antibodies against intact and HC antigens. However, the treated beads contain only β2m and peptide–free HC molecules; therefore, antibodies to intact antigens do not bind to these beads. Antibodies binding to treated beads only or to untreated and treated beads, but not to lymphocytes (CDC-PRA or FCXM), indicate the presence of antibodies to HC. These antibodies, referred to as natural antibodies, may bind to hidden or cryptic epitopes (normally masked by β2m and peptide of the groove) or exposed epitopes of intact antigens on the beads. Weak alloantibodies may not be detected in cellular assays. However, these antibodies do not bind to treated beads.

Crossmatch results in relation to unacceptable antigens and strength of antibodies detected by mean fluorescence intensity (MFI) are reported in Table 1. Multiple serum samples from these patients were tested by FCXM. At least 1 natural antibody, with or without alloantibodies, was detected in all 5 patients. Only 1 of 36 T-cell and 9 of 36 B-cell crossmatches with different donors were weakly positive.