Antibodies to HLA-E may account for the non-donor-specific anti-HLA class-Ia antibodies in renal and liver transplant recipients

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Abstract

The non-donor-specific anti-HLA-Ia antibodies correlate significantly with lower graft survival in organ transplant patients. Based on our earlier findings that anti-HLA-E murine monoclonal antibodies (MEM-E/02 and 3D12) reacted with different HLA-Ia alleles and the peptides shared by HLA-E and HLA class Ia alleles inhibited the HLA-Ia reactivity of the anti-HLA-E antibodies in normal non-alloimmunized males, the possibility of that anti-HLA-E IgG may account for the non-donor-specific anti-HLA-Ia antibodies in the allograft recipients was examined by multiplex-Luminex/C226-immunoassay. About 73% of renal and 59% of liver transplant patients’ sera with high level of anti-HLA-E IgG showed reactivity to different non-donor HLA-Ia alleles. About 50% renal and 52% liver allograft recipients’ sera with low level of anti-HLA-E IgG had no reactivity to any HLA-Ia alleles; however, the IgG isolated from the same sera with protein-G columns showed the presence of anti-HLA-E IgG with HLA-Ia reactivity. Furthermore, both recombinant HLA-E and the IgG-free serum containing soluble HLA-E (sHLA-E) inhibited HLA-Ia reactivity of anti-HLA-E murine monoclonal IgG significantly. The data suggest that the HLA-Ia reactivity of the anti-HLA-E antibody accounts for the non-donor-specific anti-HLA-Ia antibodies. It is proposed that the sHLA-E heavy chain, shed in circulation after organ transplantation, may expose cryptic epitopes of HLA-E to elicit anti-HLA-E IgG antibodies, which may cross react with HLA-Ia alleles due to the peptide sequences shared between them. This study provides a new explanation for the presence of non-donor-specific antibodies for non-existing HLA-Ia alleles, frequently observed and correlated with survival in organ transplant recipients.

Keywords: HLA-E, HLA-Ia, kidney transplantation, liver transplantation

Introduction

MHC class I [both classical Ia (HLA-A, -B and -C) (1) and non-classical Ib (HLA-E, -F and -G) (2)] is expressed on the cell surfaces with or without β2M (3, 4). While HLA-Ia is polymorphic, HLA-E is oligomorphic (5), with two well-defined alleles HLA-E107R and HLA-E107G, the latter expressed at higher levels than HLA-E107R (6). HLA-E is ubiquitously distributed in tissues (HLA-E is found in kidney, skin, liver, salivary gland, urinary bladder, thyroid, stomach and endometrium, in endothelial cells from all types of vessels, including arteries, veins, capillaries, lymphatics and kidney glomerali, spleen, lymph nodes and high endothelial venules, with a strong expression in B and T lymphocytes, monocytes, macrophages and megakaryocytes (but not in erythrocytes) (7) and cell lines (7–9) and is over expressed in malignant cells after HLA-Ia expression is down-regulated or lost (9–19). Expression of HLA-E among mesenchymal, epithelial and hematopoietic cells increases upon inflammation or tumorogenesis, releasing soluble HLA-E (sHLA-E) antigen into circulation (7, 19–21). Because HLA-E heavy chain (HC) shares several peptide sequences with HLA-Ia alleles [See Table in ref. (22), the mAbs (MEM-E/02, E/06, E/07, 3D12) produced against recombinant HLA-E (rHLA-E) HC recognized an array of HLA-Ia alleles that shared one or more peptide sequences with HLA-E (22–25). Most commonly shared sequences117AYDGKDY123 and137DTAAQIS143 inhibited not only anti-HLA-E mAbs but also their HLA-Ia reactivity (22–25). Both anti-HLA-E antibodies and HLA-Ia reactivity of the sera of normal, healthy non-alloimmunized males were also inhibited by the above-shared sequences (25), thereby supporting the contention that HLA-Ia reactivity in normal healthy human sera could be due to anti-HLA-E antibodies.

In the present investigation, we hypothesize that the reactivity of non-donor-specific antibodies (NDSA), to non-existing HLA-Ia alleles, observed in organ transplant recipients could be a consequence of HLA-Ia allelic reactivity of anti-HLA-E
antibodies, resulting due to the shared peptide sequences in the HCs of HLA-E and HLA-Ia. The postulate is examined by (i) comparing the incidences of anti-HLA-E antibodies as well as the reactivity with HLA-Ia alleles in the sera of kidney and liver allograft recipients, (ii) isolating IgG from the sera with protein-G columns to test the presence of anti-HLA-E IgG and HLA-Ia reactivity, (iii) comparing the pattern of HLA-Ia reactivity of the patient’s sera with that of the murine anti-HLA-E mAbs, (iv) detecting the presence of sHLA-E antigen in the sera with no detectable levels of anti-HLA-E antibodies or HLA-Ia reactivity and (v) assessing the inhibition HLA-Ia reactivity of murine anti-HLA-E mAbs with IgG-free serum (IFS) containing sHLA-E and rHLA-E.

Methods

Sera from transplant recipients

This study utilizes a fraction of the archived sera sent to the Terasaki Foundation Laboratory (TFL) from different transplant centers around the world. Renal transplant (RT) sera were collected for research from allograft recipients between 1984 and 2005 at Charité-Universitätsmedizin, Berlin, after approval by the Institutional Ethics Committee and informed consent from the participating patients (26)—some of whom had received more than one allograft. (Supplementary Table 1 is available at International Immunology Online). HLA typing of donors and recipients was performed at Campus Virchow-Klinikum, Berlin. The Supplementary Table 1, available at International Immunology Online, provides the patients and sera’s general characteristics. Sera of liver transplant (LT) recipients participating in the North Italy Transplant Program were obtained after approval by the Institutional Ethics Committee and informed consent from the participants (27), all of whom had received re-transplant after failure of a first allograft (Supplementary Table 2 is available at International Immunology Online).

Immunoassay with single antigen beads

To detect the presence of antibodies in the sera that react to HLA-E and HLA-Ia alleles, multiplex LumineX®-based immunoassay (One Lambda, Canoga Park, CA, USA) was used, as described earlier (22–25). The sera were diluted 1/10 with PBS (pH 7.2). Using dual-laser flow cytometry of LumineX® xMAP® multiplex technology, the single antigen assays were carried out for data acquisition and analysis of anti-HLA-Ia and anti-HLA-E antibodies (22). The LABScreen® Single Antigen (One Lambda) assay consists of a panel of color-coded microspheres (single antigen beads, SAB) coated with HLA antigens to identify antibody specificities. The array of HLA antigens representing various alleles on the beads is listed at one lambda website under Antibody detection products/Lab-screen® Single Antigen Product sheet HLA-Ia combi-LS1A04-Lot 003 Worksheet Rev-1. The single HLA-Ia antigens in LS1A04-Lot 0G3 contain 31 HLA-A, 50 HLA-B and 16 HLA-C molecules. rHLA-E folded HC [10 mg ml⁻¹ in morpholine ethane sulfonic acid (MES) buffer] obtained from the Immune Monitoring Lab, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA. Data generated with Luminex Multiplex Flow Cytometry (LABScan® 100) were analyzed using computer software, the protocol the same as that reported earlier (22, 25). Trimmed mean fluorescence values for the SAB reactions are obtained from the output (csv file generated by the flow analyzer and are adjusted for blank and background signal using the formula described below. To express the values of anti-HLA antibodies at specified dilution, the sample-specific fluorescence value [trimmed mean fluorescence intensity (MFI)] for each beads are taken into consideration. Four different kinds of S # N are obtained: [1] trimmed MFI for serum antibodies, [2] trimmed MFI for the negative control beads used for serum antibody; [3] trimmed MFI for HLA-coated beads (PE-conjugated second antibody only) and [4] Trimmed MFI for the negative control beads (with PE-Conjugated second antibody only). Normalized trimmed MFI is calculated as follows: (S # N value of [1] – S # N value of [2]) – (S # N value [3] – S # N value of [4]). The HLA-Ia microbeads have inbuilt control beads, which included those coated with human IgG (considered positive control but not applicable as positive control for when we used murine mAb in this study) and serum albumin (HSA/BSA) (negative control), respectively. For HLA-E, we have separately included identical control beads (both positive and negative controls). Each experiment is done in duplicate. For each analysis, at least 100 beads are counted. Mean and standard deviation of MFI for each alleles are recorded. All the data are stored and archived at TFL. Origin Graphics Software® is used to plot the data. Basic statistical analyses are carried out with Excel software.

HLA-E: electrophoresis and western blot

Electrophoresis of sera was accomplished with 12% Bis–Tris Mini Gels in Mini-Cell (Invitrogen, Carlsbad, CA, USA). Both reduced and non-reduced samples were prepared following Invitrogen’s protocol. Eight microliter of serum were used to prepare a 15 μl volume of sample for application in each well. Electrophoresis was conducted for 35 min (MES buffer) or 50 min (MOPS buffer); Voltage: 200 V constant at 100–125 mA/gel at start and 60–80 mA/gel toward the end. The gels were transferred to Invitrogen’s iBlot™ Dry Blotting System (IB1001), with polyvinylidene difluoride (PVDF) membrane used to transfer the proteins from gel membrane in 7 min (as recommended in Invitrogen User Manual for iBlot™ Dry Blotting System [for details, see Version C, at 7 February 2007, 25-0911, pages 10–12], and murine anti-HLA-E mAb MEM-E/02 and/or MEM-E/06 were used to immunostain the PVDF membrane. Using a scanner, brightness and color contrast of the fractions were adjusted with Adobe photoshop. Subjecting diluted sera (8 μl made into 15 μl) to electrophoresis may not render sharp bands, as would immunoprecipitated HLA-E.

Inhibition of HLA-Ia reactivity of murine mAb with sHLA

Different dilutions of murine anti-HLA-E mAb MEM-E/02 (MA1-19309; Affinity Bioreagents, Golden, CO, USA) were prepared with PBS, pH 7.2, and incubated for 1 h with rHLA-E or the IFS from patient Mi92. The IFS was obtained after eluting the serum in a protein-G column and used after establishing the presence of sHLA-E. Inhibition of HLA-Ia reactivity of the mAb MEM-E/02 was assessed. IFS (sera after passing through protein G) was used as experiment and
PBS (value presented) was control (PBS value refers to MFI values obtained with mAb MEM-E/02). HLA-E also serves as a positive control for IFS for it contains sHLA-E. The differences in the values between IFS and rHLA-E points out to the differences in the concentration. No additional controls were used since treated-murine mAb was tested at different dilutions in a dose-dependent manner. The inhibition was assessed at every dilution of mAb MEM-E/02 by comparing the treated values against untreated (PBS only). The differences obtained (after treating with serum IgG or rHLA-E) for each allele were compared pairwise by paired sample t-test (two tailed). Both the raw values obtained for PBS only or IFS or rHLA-E as well as the P² values for each dilution of mAb are presented in the final table. The dosimetric differences in the raw values and two tailed P (P²) values were used as proof of significance.

Elution of IgG from sera using protein-G columns

The following protocol was used for isolating IgG from the sera of allograft recipients. The objective is to find out whether anti-HLA-E IgG present in sera is not reactive for HLA-E antigen, due various interfering factors. If anti-HLA-E IgG is present, we wanted to test the HLA-Ia reactivity of the purified anti-HLA-E IgG. For this purpose, the sera representing different antibody groups [antibody positive or non-reactive (negative) for HLA-E] were passed through protein-G columns obtained from Thermo Fisher Scientific (Rockford, IL, USA). The recombinant protein-G contains two Fe-binding domains and devoid of albumin and cell surface-binding sites. This was coupled to beaded agarose and ultralink resins. Protein-G agarose resin binds to all human IgG subclasses but not to IgM, IgD, IgA or serum albumin. The serum (250 µl) was applied to the protein-G agarose resin after washing the resin twice with PBS, pH 7.2. The serum was mixed well, incubated for 10 min and allowed to pass through the resin. The serum-treated resin was washed three times with wash buffer (PBS, pH 7.2). The agarose-bound IgG was eluted in three vials using acidic buffer (pH 2.8; 400 µl) and recovered in alkaline buffer (pH 8.5; 10 × 40 µl) to neutralize the eluates. sHLA-E, if bound to IgG, will be dissociated by the acid pH of the buffer used for eluting IgG. Three eluates (each 400 µl) were collected and the concentrations of the fractions were determined using the calculations provided in the manual of Eppendorf BioPhotometer (Copyright by 1998 by Eppendorf—Netheler—Hinz GmbH, Hamburg, page 87). The calculation of the protein-G fractions was carried out using the calculated factor: \[ C = 1.55 \times \text{absorbancy at } A_{280} - 0.76 \times \text{absorbancy at } A_{260} \] to obtain the concentration of the protein (milligrams per milliliter). Then, the eluates were diluted 1/10 and tested for anti-HLA-Ia and anti-HLA-E reactivity. In the process of protein-G elution, the IgGs were diluted 4.8-fold and then further diluted 1/10 to test the presence of anti-HLA-E IgG and HLA-Ia reactivity. The final dilution of IgG was 48-fold.

Statistical analyses

Inhibition studies required statistical analyses. Therefore, paired sample t-test was carried out on MFI of different HLA-Ia alleles, using Microsoft Excel and only two-tailed P values are considered for the level of significance, as presented in a table.

Results

Outline of the results

The results are presented in three sections: Sections 1 and 2 pertain to allograft recipients. Section 1 includes documentation of (1) parallel incidence of anti-HLA-E antibodies and HLA-Ia reactivity of the sera and (2) the pattern of HLA-Ia reactivity of the patients’ sera was compared with the HLA-Ia reactivity of murine mAb MEM-E/02. The upper row of Figs. 1 and 3) denotes HLA-Ia allelic reactivities observed with anti-HLA-E mAb for comparison with that recognized by the anti-HLA-E antibodies of patients. Similarly, the HLA-Ia alleles reactive to anti-HLA-E mAb are indicated in the Tables 2 and 4 (3). Sera that had no or low levels of anti-HLA-E antibodies with poor HLA-Ia reactivity were examined for (i) the presence of sHLA-E and (ii) anti-HLA-E IgG with HLA-Ia reactivity, after eluting IgG with protein-G. Section 2 describes inhibition of HLA-B and HLA-Cw reactivity of murine mAb MEM-E/02—at different dilutions—by rHLA-E and sHLA-E in IFS of LT recipient, Mi92. The significant binding of both sources of sHLA-E with anti-HLA-E mAb indicates that sHLA-E can quench the reactivity of the serum antibodies to both HLA-E and HLA-Ia.

Kidney transplantation: defining the sera based on anti-HLA-E antibodies and HLA-Ia reactivity

Table 1 shows HLA types of allograft recipients and donors with the presence and absence of HLA-Ia reactive donor-specific antibodies (DSA) and NDSA and anti-HLA-E antibodies (MFI expressed in thousands). Allograft recipients’ sera were categorized into two major groups as (i) those with a detectable level (MFI ≥ 500) of IgG antibodies to HLA-Ia alleles (Group A, n = 42) and (ii) those with no or low detectable level (MFI < 500) of antibodies to HLA-Ia alleles (Group B, n = 22). These groups were further categorized by the cutoff level (for anti-HLA-E antibodies) determined earlier in normal, healthy non-alloimmunized males (25): (i) Group A-1 (anti-HLA-Ia+) with detectable level (≥1000) of anti-HLA-E IgG (n = 32); (ii) Group A-2 (anti-HLA-Ia+) without detectable level (<1000) of anti-HLA-E IgG (n = 10); (iii) Group B-1 (anti-HLA-Ia-) with detectable level of anti-HLA-E IgG (n = 12) and (iv) Group B-2 (anti-HLA-Ia-) without detectable level of anti-HLA-E IgG (n = 10).

Of 64 patients’ sera, 44 (A-1 and B-1) had anti-HLA-E antibodies; 73% of the 44 reacted with HLA-Ia alleles. No anti-HLA-E antibodies could be detected in the sera of 20 patients, and 50% of the 20 showed no HLA-Ia reactivity. Table 2 shows MFI levels (values expressed in thousands) of allo-HLA-Ia reactivity of NDSA and DSA (in bold and italic in shaded box) of different groups. Most of the HLA-B and Cw alleles and very few HLA-A alleles recognized by the Group A-1 sera of allograft recipients (alleles in bold) parallel with the HLA-Ia alleles recognized by mAb MEM-E/02 (22, 25) (for details, see the Table legends). In Group A-1, 20 of 32 sera recognized three or more of HLA-B and Cw alleles, while 11 of 32 sera reacted with three or more of HLA-A alleles—as observed earlier with the sera of normal non-alloimmunized
In general, there is a tendency for decreased HLA-B and HLA-Cw (based on the number and MFI) with the decrease in MFI of anti-HLA-E antibodies. The table also shows MFI levels (values expressed in thousands) of allo-HLA-Ia reactivity of NDSA and DSA (underlined) of both Groups A-1 and A-2. In Group A-2, sera of 5 of 10 patients recognized three or more of HLA-B and HLA-Cw alleles, very similar to murine anti-HLA-E mAbs (for details, see table legends).

It is important to note from Tables 1 and 2 that sera of 29 of 42 RT patients (Group A) had no DSA and only NDSA reacting from 2 to 23 HLA-Ia alleles. One patient in these groups had 4 alleles for DSA and 36 alleles for NDSA. Presence of high numbers of NDSA in spite of the low level of anti-HLA-E antibody in addition to Groups B-1 and B-2 suggested the crypticity of anti-HLA antibodies (reactive against both HLA-E and HLA-Ia alleles) in the allograft recipients and also possibly the factors, such as sHLA-E or anti-idiotypic antibodies formed against anti-HLA-E antibody, affecting the detectability of both the anti-HLA antibodies.

Evidence for bound anti-HLA antibodies in renal allograft recipients

The possibility of sHLA-E in the sera to affect the detectability of anti-HLA-E antibodies and, consequently, the
HLA-Ia reactivity were tested by eluting IgG with protein-G columns from the sera of Group A-1 and more importantly in Group B-2 sera and then testing the 1/48-diluted eluates for anti-HLA-E IgG and HLA-Ia reactivity. Figure 1 shows that the Group B-2 eluates (patients # 1497/#1645/#2009) had high levels of anti-HLA-E-IgG (MFI > 5000). The intensity increased with elution (eluate 1 > 2 > 3), confirming the presence of bound or cryptic anti-HLA-E IgG in the sera of Group B-2 patients. The pattern of HLA-Ia reactivity is parallel to that of mAb MEM-E/02. A-1 eluates (positive control) also showed an increase in MFI (>5000) of anti-HLA-E IgG plus increased HLA-Ia reactivity whose number and intensity corresponded with the increased MFI of anti-HLA-E IgG.

Appearance of anti-HLA-E IgG and HLA-Ia reactivity in the sera after isolating the IgG suggests the presence of sHLA-E that may block IgG antibodies from binding to HLA-E and HLA-Ia. Possibly anti-idiotypic antibodies may not be the interfering factors since they would have been eluted by protein-G column along with anti-HLA-E IgG, such that anti-HLA-E IgG would not react to the antigen in the immunosassay.

Since sHLA-E is suspected to interfere with anti-HLA-E binding, efforts were made to examine sHLA-E in the sera. The presence of sHLA-E was assessed by electrophoresis, western blotting and then immunostaining with murine mAbs MEM-E/02 and MEM-E/06. The profile of the western blots of untreated sera (Fig. 2) shows the presence of diffused sHLA-E; the staining intensity observed in the group B-2 sera is very intense. Possibly, the excess sHLA-E in the serum may be blocking the antibodies from reacting to HLA-E and HLA-Ia.
Table 1. HLA type of recipients and donors, presence of DSA and NDSA, antibodies to HLA-E (MFI \times 1000) and HLA-Ia (number of alleles recognized) in renal allograft recipients

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Recipient's HLA</th>
<th>Current donor's HLA</th>
<th>Previous donor's HLA</th>
<th>HLA class Ia reactivity and anti-HLA-E antibodies</th>
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<td>A, A/B, B/Cw, Cw/</td>
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<td>4719</td>
<td>3, 30/13, 35/4, 6</td>
<td>3, 30/13, 35/4, 6</td>
<td>n/a</td>
<td>NDSA, (+), 7/5/0, 0.2</td>
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<td>Group B-1 (n = 12)</td>
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Anti-HLA-E antibodies account for NDSA in Transplant patients

Downloaded from https://academic.oup.com/intimm/article-abstract/24/1/43/695703 by guest on 25 December 2018
**Sera of LT recipients: defined by anti-HLA-E antibodies and HLA-Ia reactivity**

Table 3 shows HLA types of liver donors with the presence and absence of HLA-Ia reactive DSA and NDSA. The anti-HLA-Ia antibodies were measured at two different time intervals A1, B1, and C1 refers to sera tested at 1/3 dilution and A2, B2, and C2 refers to sera tested at 1/10 dilution. The number in parenthesis refers to DSA. Sera of the LT recipients were also classified into four groups. MFI cutoff for presence or absence of anti-HLA-E IgG was kept higher (2000) for LT patients than for healthy non-alloimmunized males (1250) because all the recipients were re-transplants, and several had vascular inflammatory diseases, which are known to promote both expression of HLA-E on the cell surface and the shedding of HLA-E, a factor that may induce antibody production against HLA-E.

1) Group A-1 (anti-HLA-Ia+): anti-HLA-E IgG+ (60% or 12/20 patients);
2) Group A-2 (anti-HLA-Ia+): anti-HLA-E IgG− (40% or 8/20 patients);
3) Group B-1 (anti-HLA-Ia-): anti-HLA-E IgG+ (45% or 9/20 patients); and
4) Group B-2 (anti-HLA-Ia-): anti-HLA-E IgG− (55% of 11/20 patients).

Sera of 21/40 patients (53%) with anti-HLA-E antibodies showed reactivity to HLA-Ia alleles; sera of 11/21 patients (52%) with no detectable anti-HLA-E antibodies showed no HLA-Ia reactivity. HLA-Ia alleles recognized by antibodies of LT recipients (Table 4) are identical to those recognized by anti-HLA-E mAb MEM-E02 (shown in bold and see Table legend for details) and those not recognized by the mAb MEM-E/02 are not in bold. As with mAb-MEM-E/02, the sera of several patients reacted with few HLA-A alleles. It is important to recall from Tables 3 and 4 that sera of 10 of 20 LT patients (Group A) had no DSA and only NDSA reacting from two or more HLA-Ia alleles.

**Cryptic or bound anti-HLA antibodies in LT patients**

Nine B-1 patients had anti-HLA-E IgG and 13 B-2 patients did not. Non-detectability of anti-HLA-E or anti-HLA-Ia antibodies could be due to antibodies being bound with sHLA or other protein factors (e.g. anti-idiotypic antibodies). The results (Fig. 3) obtained with sera IgG with protein-G columns revealed the bound nature of both anti-HLA-E antibodies. The isolated IgG in eluates 2 and 3 had remarkably higher reactivity to HLA-E and HLA-Ia alleles. B-2 sera (Mi33/Mi37/Mi79/Mi98) showed a unique pattern upon isolation of IgG. Neither the sera nor the eluate # 1 showed reactivity to HLA-E or HLA-Ia. In eluate 1—during elution with acid buffer—the bound HLA-E might have been dissociated from the IgG, rebound after neutralization with sHLA, which would have been eluted in the first collection tube. Eluates 2 and 3 showed that the isolated IgG reacted with both HLA-E and HLA-Ia alleles at varying number and intensities, the pattern of HLA-Ia recognition similar to that of anti-HLA-E mAb MEM-E/02.

**sHLA-E in sera of LT recipients**

To detect sHLA-E in the sera of Group A-2 LT recipients Mi92/Mi59/Mi24 and group B-2 Mi39/Mi45/Mi63, the western blots of their electropherograms were immunostained with mAbs MEM-E/02 and MEM-E/06. Figure 4 shows the high level of sHLA-E in group B-2 Ags; no sHLA-E could be detected in patient Mi59.

**Can sHLA-E in sera inhibit HLA-Ia reactivity of anti-HLA-E mAb MEM-E/02?**

The binding of mAb MEM-E/02 to beads coated with HLA-Ia alleles was measured before or after incubating different dilutions of the mAb with rHLA-E or with IFS (sHLA-E).
Table 2. MFI values of anti-allo-HLA-Ia (NDSA) and anti-HLA-E antibodies in different Groups of the renal allograft recipients (n = 64)

<table>
<thead>
<tr>
<th>Group</th>
<th>N = 32 [anti-HLA-E + (MFI &gt; 1000)/anti-HLA-Ia + (MFI &gt;= 500)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>[HLA-E (8.2)] - A<em>0101 (1.5), A</em>0201 (1.0), A<em>0203 (0.7), B</em>1512 (0.7) - (NDSA = 4, DSA = 0)</td>
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<td>[HLA-E (7.9)] - B<em>0702 (6.9), B</em>1301 (12.2), B<em>1302 (10.2), B</em>2708 (8.0), B<em>4001 (5.7), B</em>4002 (8.0), B<em>4006 (6.8), B</em>4402 (0.8), B<em>4403 (1.3), B</em>4501 (1.0), B<em>4701 (3.5), B</em>4801 (2.6), B<em>7301 (2.7), B</em>8101 (8.0)/Cw*0202 (1.0) - (NDSA = 17, DSA = 0)</td>
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<td>[HLA-E (4.9)] - A<em>0101 (0.7), B</em>0702 (1.3), B<em>1301 (0.7), B</em>3701 (0.7), B<em>4002 (0.6), B</em>4006 (0.5), B<em>4201 (1.1), B</em>5101 (0.5), B<em>5102 (0.7), B</em>5201 (0.7), B<em>5501 (1.0), B</em>6701 (1.0), B*6101 (1.4) - (NDSA = 13, DSA = 0)</td>
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<td>[HLA-E (3.0)] - A<em>0201 (12.1), A</em>0203 (10.3), A<em>0206 (5.3), A</em>6901 (3.6)/B<em>0702 (12.1), B</em>1301 (0.7), B<em>1503 (0.9), B</em>2705 (2.0), B<em>2708 (2.6), B</em>4001 (1.9), B<em>4002 (2.7), B</em>4006 (2.2), B<em>4201 (3.4), B</em>4701 (0.9), B<em>4801 (1.0), B</em>5501 (2.0), B<em>5601 (0.7), B</em>5701 (1.6), B<em>5703 (2.4), B</em>5801 (1.7), B<em>6101 (10.6), B</em>6201 (1.2)/Cw*0702 (0.7) - (NDSA = 19, DSA = 4)</td>
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<td>[HLA-E (2.9)] - B<em>1502 (1.9), B</em>1510 (1.6), B<em>1511 (0.9), B</em>3601 (2.0), B<em>5101 (2.0), B</em>5102 (2.6), B<em>5201 (0.8), B</em>5301 (1.9), B*7801 (2.0) - (NDSA = 9, DSA = 0)</td>
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<tr>
<td>A2</td>
<td>[HLA-E (2.8)] - B<em>1501 (0.6), B</em>1502 (0.6), B*1512 (6.8) - (NDSA = 3, DSA = 0)</td>
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<td>[HLA-E (2.6)] - A<em>0301 (0.5)/B</em>5101 (3.3), B<em>5102 (2.8), B</em>5201 (4.1)/B*7801 (1.6) - (NDSA = 2, DSA = 3)</td>
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<td>[HLA-E (2.4)] - A<em>0101 (2.8), A</em>3601 (0.8), A<em>6801 (0.6)/B</em>5101 (0.7), B*5102 (1.1), - (NDSA = 5, DSA = 0)</td>
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<td>[HLA-E (2.2)] - B<em>4801 (0.6), B</em>8101 (0.8), - (NDSA = 2, DSA = 0)</td>
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<td>[HLA-E (2.1)] - B<em>1501 (0.8), B</em>1503 (0.7), B<em>1510 (0.6), B</em>4901 (0.7), B<em>5001 (0.6), B</em>5601 (0.6) - (NDSA = 6, DSA = 0)</td>
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<td>[HLA-E (1.9)] - A<em>0201 (0.8), A</em>0203 (0.6), A*0206 (0.5) - (NDSA = 3, DSA = 0)</td>
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<td>[HLA-E (1.9)] - A<em>0206 (0.5)/B</em>4402 (3.4) - (NDSA = 2, DSA = 0)</td>
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<td>[HLA-E (1.8)] - A<em>3001 (4.5), A</em>3002 (6.1)/B*5101 (0.7) - (NDSA = 3, DSA = 0)</td>
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<td>[HLA-E (1.4)] - B<em>1511 (1.4), B</em>4402 (0.5), B<em>4403 (0.8), B</em>4501 (0.68), B*8201 (0.9) - (NDSA = 5, DSA = 0)</td>
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<td>[HLA-E (1.3)] - A<em>2301 (8.3), A</em>2402 (6.2)/B*2403 (5.9) - (NDSA = 1, DSA = 2)</td>
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<td>[HLA-E (1.3)] - B*1502 (0.8) - (NDSA = 1, DSA = 0)</td>
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<td>[HLA-E (1.3)] - B<em>0702 (0.5), B</em>0801 (0.7), B<em>1401 (0.5), B</em>1510 (0.9), B<em>4201 (0.6), B</em>5501 (0.6), B*6701 (0.5) - (NDSA = 7, DSA = 0)</td>
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<td>[HLA-E (1.3)] - A<em>2301 (2.6), A</em>2402 (2.2)/B<em>2403 (1.5)/B</em>0801 (0.7) - (NDSA = 3, DSA = 1)</td>
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<td>[HLA-E (1.3)] - B<em>1512 (1.2), B</em>5701 (6.2), B<em>5703 (8.3), B</em>5801 (7.1) - (NDSA = 3, DSA = 1)</td>
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<td>[HLA-E (1.3)] - A<em>2501 (0.8), A</em>3303 (1.7), A<em>3402 (1.2), A</em>6601 (0.6), A<em>6602 (2.6), A</em>6801 (1.5), A*6802 (0.8) - (NDSA = 7, DSA = 0)</td>
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<td>[HLA-E (1.3)] - A<em>2501 (0.5), A</em>2601 (1.1), A<em>2901 (0.8), A</em>2902 (0.7), A<em>3002 (0.6), A</em>3402 (0.6), A<em>4301 (0.8), A</em>6601 (1.0) - (NDSA = 7, DSA = 1)</td>
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<td>[HLA-E (1.2)] - B<em>1502 (0.6), B</em>1516 (0.6), B<em>3501 (0.9), B</em>5101 (0.9), B*5301 (0.7) - (NDSA = 5, DSA = 0)</td>
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<td>[HLA-E (1.1)] - A<em>0101 (19.1), A</em>0201 (1.8), A<em>0203 (1.4), A</em>0206 (0.8), A<em>0301 (5.3), A</em>1101 (7.2), A<em>2301 (5.5), A</em>2402 (6.1), A<em>2403 (5.3), A</em>2501 (0.8), A<em>2601 (1.8), A</em>3402 (0.9), A<em>3601 (12.1), A</em>4301 (1.2), A<em>6601 (1.7), A</em>6801 (1.7), A<em>6802 (1.0), A</em>6901 (0.7), A<em>8801 (5.9), B</em>0801 (0.7), B<em>1512 (1.9), B</em>2101 (21.1), - (NDSA = 21, DSA = 1)</td>
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<td>[HLA-E (1.1)] - A<em>3001 (4.1), A</em>3002 (4.5)/B<em>3101 (3.3), B</em>0702 (0.7), B<em>4201 (0.6), B</em>6701 (0.5), B<em>8101 (0.9), B</em>8201 (0.6)/Cw*1402 (2.0) - (NDSA = 9, DSA = 0)</td>
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<td>[HLA-E (1.0)] - A<em>0201 (8.4), A</em>0203 (7.5), A<em>0206 (4.3), A</em>2301 (0.8), A<em>2402 (0.6), A</em>2403 (0.7), A<em>6801 (4.9), A</em>6802 (3.2), A<em>6901 (2.8)/B</em>0702 (0.7), B<em>1512 (1.9), B</em>5703 (0.6), B*8101 (0.6) - (NDSA = 10, DSA = 3)</td>
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### Table 2. Continued

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<tr>
<th>Group</th>
<th>N = 32 [anti-HLA-E + (MFI &gt; 1000)/anti-HLA-Ia + (MFI &gt; 500)]</th>
<th>Group B-2</th>
<th>N = 10 [anti-HLA-E – (MFI &lt; 1000)/anti-HLA-Ia – (MFI &lt; 500)]</th>
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<td>735</td>
<td>[HLA-E (1.0)] – B<em>4702 (1.8), B</em>2705 (1.1), B<em>2708 (1.3), B</em>4201 (3.1), B<em>5401 (2.3), B</em>5501 (1.0), B<em>5601 (0.9), B</em>6701 (1.6), B<em>7301 (0.9), B</em>8101 (1.2), B*6201 (1.0) – (NDSA – 11, DSA – 0)</td>
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<td>[HLA-E (1.0)] – B<em>1512 (0.5), Cw</em>0302 (12.0), Cw<em>0303 (0.9), Cw</em>0304 (2.1) – (NDSA – 4, DSA – 0)</td>
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<td>[HLA-E (1.0)] – A<em>3303 (0.8), A</em>3402 (0.5), A<em>6801 (0.7), A</em>6802 (0.6), B<em>0702 (1.0), B</em>0801 (2.4), B<em>1401 (1.5), B</em>1402 (1.2), B<em>1510 (0.6), B</em>1601 (1.2), B<em>2708 (1.0), B</em>5701 (2.2), B<em>3801 (13.0), B</em>3901 (5.0), B<em>4101 (1.3), B</em>4201 (3.1), B<em>5401 (2.2), B</em>5501 (4.8), B<em>5601 (1.1), B</em>5901 (1.0), B<em>6701 (5.4), B</em>8201 (0.5) – (NDSA – 23, DSA – 0)</td>
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<td>1339</td>
<td>[HLA-E (1.0)] – B<em>0702 (0.6), B</em>2705 (0.7), B<em>4801 (0.5), B</em>8101 (0.5) – (NDSA – 4, DSA – 0)</td>
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<td>[HLA-E (1.0)] – Cw<em>0202 (0.9), Cw</em>0401 (1.2), Cw<em>0602 (1.8), Cw</em>1701 (0.5) – (NDSA – 4, DSA – 0)</td>
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<td>[HLA-E (1.0)] – A<em>0201 (0.6), A</em>0203 (0.5), A<em>1102 (1.2), B</em>5701 (1.1), B<em>5801 (1.4), B</em>5801 (1.3) – (NDSA – 6, DSA – 0)</td>
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<tr>
<td>Group A-2</td>
<td>N = 10, [anti-HLA-E – (MFI &gt; 1000)/anti-HLA-Ia + (MFI &gt; 500)]</td>
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<td>9607</td>
<td>[HLA-E (0.8)] – A<em>0201 (1.2), A</em>0203 (0.6)/B<em>1301 (4.9), B</em>1302 (4.2) – (NDSA – 2, DSA – 2)</td>
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<td>1800</td>
<td>[HLA-E (0.8)] – A<em>0201 (10.5), A</em>0203 (10.4), A<em>0206 (6.1), A</em>6901 (1.9), B<em>5101 (0.8), B</em>5301 (0.8), B<em>5701 (6.7), B</em>5703 (8.2), B*5801 (2.7) – (NDSA – 9, DSA – 0)</td>
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<td>9778</td>
<td>[HLA-E (0.8)] – B<em>7301 (1.7), Cw</em>0702 (9.5), Cw<em>1601 (1.4), Cw</em>1701 (0.5) – (NDSA – 4, DSA – 0)</td>
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<td>[HLA-E (0.6)] – A<em>0101 (1.6), A</em>0301 (2.4), A<em>1102 (3.9), A</em>1103 (5.5), A<em>2501 (1.1), B</em>2601 (0.9), B<em>3601 (0.9), B</em>4301 (1.1), A<em>6601 (1.7), A</em>8001 (2.7), B<em>1002 (6.0), B</em>1003 (1.9), B<em>1004 (1.9), B</em>1402 (0.2), B<em>1402 (1.2), B</em>1501 (0.8), B<em>1502 (1.4), B</em>1503 (1.4), B<em>1510 (2.3), B</em>1511 (0.7), B<em>1512 (1.7), B</em>1801 (0.0), B<em>2708 (3.2), B</em>3501 (1.5), B<em>3901 (1.4), B</em>4101 (1.7), B<em>4002 (1.5), B</em>4006 (1.2), B<em>4101 (1.2), B</em>4201 (4.5), B<em>4401 (1.2), B</em>4801 (1.4), B<em>5001 (1.3), B</em>5401 (1.7), B<em>5501 (2.5), B</em>5601 (1.9), B<em>6701 (3.5), B</em>7301 (0.8), B<em>7801 (0.7), B</em>9101 (4.5), B*6201 (2.0) – (NDSA – 36, DSA – 4)</td>
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<td>412</td>
<td>[HLA-E (0.6)] – A<em>0101 (1.1), A</em>3601 (0.6), B*3701 (1.6) – (NDSA – 3, DSA – 0)</td>
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<td>10271</td>
<td>[HLA-E (0.6)] – A<em>0101 (17.0), A</em>0201 (2.9), A<em>0203 (2.5)/A</em>0206 (1.3), A<em>2301 (1.2), B</em>5701 (0.8), B<em>5703 (1.3), B</em>5801 (1.2) – (NDSA – 4, DSA – 4)</td>
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<td>[HLA-E (0.5)] – B*3701 (0.9) – (NDSA – 1, DSA – 0)</td>
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<td>1323</td>
<td>[HLA-E (0.4)] – A<em>2501 (3.5), A</em>2601 (3.2), A*6601 (3.2) – (NDSA – 3, DSA – 0)</td>
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<td>633</td>
<td>[HLA-E (0.3)] – A<em>3301 (0.7), A</em>6602 (2.3), B<em>4002 (0.5), B</em>4401 (0.7) – (NDSA – 6, DSA – 0)</td>
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<tr>
<td>4719</td>
<td>[HLA-E (0.2)] – A<em>0101 (0.6), A</em>0201 (3.2), A<em>0203 (2.6)/A</em>0206 (1.5), A<em>6901 (2.0), A</em>6902 (1.7), A<em>6901 (1.2), B</em>1512 (2.2), B<em>4402 (1.8), B</em>4501 (2.1), B<em>4801 (2.1), B</em>8201 (1.7) – (NDSA – 12, DSA – 0)</td>
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</tbody>
</table>

Antibody of the reactivity of the alleles due to DSA is underlined. Values in parenthesis refer to the MFI of the respective alleles (x1000). Most of the B and Cw allelic reactivities match with the HLA-Ia reactivity of the murine anti-HLA-E mAb for HLA-E (22, 24). Data obtained after 1/10 dilution of sera are presented. Allelic reactivity due to DSA is underlined. HLA-Ia allelic reactivities that match with the HLA-Ia reactivity of the anti-HLA-E murine mAb MEM-E02 (lot #2, alleles are recognized as positive if the show MFI > 1000 at dilution 1/600 for anti-HLA-E antibody (22) are presented in bold. Also, note that some of the HLA-Ia alleles not presented in bold, match with the HLA-Ia reactivity of the anti-HLA-E murine mAb 3D12, due to differences in the recognition of the shared peptides (24). The number of HLA-Ia alleles reacting to sera Group A-1 show a tendency to increase as the level of anti-HLA-E IgG increases.
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Donor’s HLA</th>
<th>HLA class Ia reactive antibodies (antibody numbers)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A, A/ B, B/</td>
</tr>
<tr>
<td>A, A/ B, B/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A-1 (n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mi92</td>
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<td>NDSA (DSA?)</td>
</tr>
<tr>
<td>Mi59</td>
<td>2, 3/ 5, 35/</td>
<td>NDSA</td>
</tr>
<tr>
<td>Mi127</td>
<td>11, 24/ 62, 46/</td>
<td>NDSA and DSA</td>
</tr>
<tr>
<td>Mi114</td>
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<td>NDSA</td>
</tr>
<tr>
<td>Mi184</td>
<td>3, 33/ 18, 65/</td>
<td>NDSA</td>
</tr>
<tr>
<td>Mi50</td>
<td>26, 68/ 38, 44/</td>
<td>NDSA and DSA</td>
</tr>
<tr>
<td>Mi19</td>
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<tr>
<td>Mi11</td>
<td>Not available</td>
<td>NDSA or DSA</td>
</tr>
<tr>
<td>Mi117</td>
<td>Not available</td>
<td>NDSA and DSA</td>
</tr>
<tr>
<td>Mi24</td>
<td>2, 3/ 44, 35/</td>
<td>NDSA</td>
</tr>
<tr>
<td>Mi119</td>
<td>Not available</td>
<td>NDSA or DSA</td>
</tr>
<tr>
<td>Mi123</td>
<td>Not available</td>
<td>NDSA</td>
</tr>
<tr>
<td>Group A-2 (n= 8)</td>
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<td></td>
</tr>
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<td>NDSA and DSA</td>
</tr>
<tr>
<td>Mi18</td>
<td>2, 25/ 7, 35/</td>
<td>NDSA and DSA</td>
</tr>
<tr>
<td>Mi122</td>
<td>Not available</td>
<td>NDSA</td>
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<tr>
<td>Mi60</td>
<td>3, 24/ 7, 40/</td>
<td>NDSA and DSA</td>
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<td>Mi85</td>
<td>3, —/ 18, —/</td>
<td>NDSA</td>
</tr>
<tr>
<td>Mi110</td>
<td>24, 68/ 7, 40/</td>
<td>NDSA and DSA</td>
</tr>
<tr>
<td>Mi103</td>
<td>2, —/ 44, 62/</td>
<td>NDSA</td>
</tr>
<tr>
<td>Group B-1 (n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mi12</td>
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<td>None</td>
</tr>
<tr>
<td>Mi113</td>
<td>Not available</td>
<td>None</td>
</tr>
<tr>
<td>Mi14</td>
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<tr>
<td>Mi21</td>
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<tr>
<td>Mi95</td>
<td>9, 30/ 18, 49/</td>
<td>NDSA (at 1/3 dilution)</td>
</tr>
<tr>
<td>Mi42</td>
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<td>None</td>
</tr>
<tr>
<td>Mi61</td>
<td>Not available</td>
<td>None</td>
</tr>
<tr>
<td>Mi88</td>
<td>Not available</td>
<td>None</td>
</tr>
<tr>
<td>Mi41</td>
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<td>None</td>
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<tr>
<td>Group B-2 (n = 11)</td>
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<td></td>
</tr>
<tr>
<td>Mi110</td>
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<td>Mi44</td>
<td>Not available</td>
<td>None</td>
</tr>
<tr>
<td>Mi45</td>
<td>Not available</td>
<td>None</td>
</tr>
<tr>
<td>Mi39</td>
<td>2, 3/ 51, —/</td>
<td>NDSA</td>
</tr>
<tr>
<td>Mi79</td>
<td>Not available</td>
<td>None</td>
</tr>
<tr>
<td>Mi180</td>
<td>Not available</td>
<td>None</td>
</tr>
<tr>
<td>Mi37</td>
<td>Not available</td>
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</tr>
<tr>
<td>Mi63</td>
<td>11, 24/ 35, 44/</td>
<td>NDSA and DSA (at 1/3 dilution)</td>
</tr>
<tr>
<td>Mi33</td>
<td>Not available</td>
<td>None</td>
</tr>
</tbody>
</table>

HLA-Ia reactivity was carried out at different dilutions of sera as indicated in the table. The bold number under the column of donor’s HLA refers to the mismatch HLA types. Cw alleles were not examined during typing. (Ø) = sera were tested at 1/3 dilution early during the analysis. However, when sera were tested at 1/10 dilution, some antibodies were prominent and others could not be detected. For further analysis and interpretation, only the data obtained from 1/10 dilution will utilized.
Table 4. MFI values of anti-allo-HLA-Ia (NDSA) and anti-HLA-E antibodies in different groups of the liver allograft recipients (n = 40)

<table>
<thead>
<tr>
<th>Group A-1 N = 12 [anti-HLA-E + (MFI &gt; 2000)/anti-HLA-IIa + (MFI &gt; 500)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mi92</td>
</tr>
<tr>
<td>Mi59</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group A-2 N = 8 [anti-HLA-E + (MFI &lt;2000)/anti-HLA-IIa + (MFI &gt; 500)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mi18</td>
</tr>
<tr>
<td>Mi7</td>
</tr>
</tbody>
</table>

| Group B-1 N = 9 [anti-HLA-E + (MFI >2000)/anti-HLA-IIa + (MFI < 500)] | Group B-2 N = 11 [anti-HLA-E + (MFI <2000)/anti-HLA-IIa + (MFI < 500)] |
|---------------------------------------------|
| Mi12 | [HLA-E (2.1)] [NDSA – 0; DSA – 0] | Mi110 | [HLA-E (1.8)] [NDSA – 0; DSA – 0] |
| Mi113 | [HLA-E (2.4)] [NDSA – 0; DSA – 0] | Mi44 | [HLA-E (1.2)] [NDSA – 0; DSA – 0] |
| Mi14 | [HLA-E (2.1)] [NDSA – 0; DSA – 0] | Mi45 | [HLA-E (0.7)] [NDSA – 0; DSA – 0] |
| Mi21 | [HLA-E (2.7)] [NDSA – 0; DSA – 0] | Mi39 | [HLA-E (0.7)] [NDSA – 0; DSA – 0] |
| Mi95 | [HLA-E (2.6)] [NDSA – 0; DSA – 0] | Mi79 | [HLA-E (1.0)] [NDSA – 0; DSA – 0] |
| Mi42 | [HLA-E (3.1)] [NDSA – 0; DSA – 0] | Mi90 | [HLA-E (0.8)] [NDSA – 0; DSA – 0] |
| Mi61 | [HLA-E (2.3)] [NDSA – 0; DSA – 0] | Mi9 | [HLA-E (1.2)] [NDSA – 0; DSA – 0] |
of A-1 LT recipient Mi92. The MFI obtained for different HLA-Ia alleles with PBS versus rHLA-E or PBS versus IFS (containing sHLA-E) were subjected paired sample t-test and two-tailed P values were obtained. Table 5 shows that rHLA-E significantly inhibited HLA-Ia reactivity of the mAb (P < 0.0001). Inhibition of the mAb's HLA-Ia reactivity by the IFS (but containing sHLA-E) varied at different dilutions of the mAb (1/100: NS; 1/200 and 1/400: P = 0.04; 1/800 and 1/1600: P < 0.0001). The dosimetric variation points out that the sHLA-E in the IFS is capable of interfering with HLA-E and HLA-Ia alleleic reactivity of the serum antibodies.

Discussion

Anti-HLA-E antibodies may account for NDSA in allograft recipients: enumeration of evidences

Antibodies against an array of allo-HLA class-Ia alleles are present in the sera of allograft recipients (26, 28) in whom these antibodies are unrelated to donor HLA—i.e., NDSA, which are correlated significantly (P < 0.0001) with lower graft survival (26, 28). Since NDSA co-exists with DSA in the sera of several transplant recipients, previous investigators have postulated that NDSA are antibodies reacting to HLA-Ia alleles that share a specific amino acid (aa) at a particular position of the HC in donor-specific HLA-Ia alleles, and several such aa-variants were listed (29–31). In the present report, we have documented (Tables 1–4) that Group A sera of 29 of 42 RT patients and 10 of 20 LT patients had no DSA but only NDSA reacting to 2–23 HLA-Ia alleles. NDSA of these patients cannot simply be attributed to shared ‘aa’ of DSA and NDSA, for there is no DSA in them. Therefore, the postulate NDSA may be sharing a specific amino acid at a particular position of HC is untenable (29–31) Moreover, the postulate fails since ‘anti-allo-HLA-Ia antibodies’ occur in both pre- and post-transplantation sera devoid of DSA. It should be noted that anti-allo-HLA-Ia antibodies are frequently found in the sera of healthy people (25) and evidences were presented to show that the HLA-Ia reactivity of the normal sera could be due to the presence of anti-HLA-E antibodies in the sera (25).

The following observations favor the contention that presence of NDSA in the sera of liver and renal allograft recipients could also be due to anti-HLA-E antibodies. The number of HLA-Ia alleles reacting to sera group A-1 show a tendency to increase as the level of anti-HLA-E IgG increases.
Table 5. Inhibition of HLA-B and HLA-Cw allelic reactivity of different dilutions of mAb MEM-E/02 (lot # 1) by rHLA-E and IFS of a liver second allograft recipient (MI92, Group A-1)

<table>
<thead>
<tr>
<th>MEM-E/02 positive HLA-la alleles</th>
<th>MFI of mAb MEM-E/02</th>
<th>MFI of mAb MEM-E/02</th>
<th>MFI of mAb MEM-E/02</th>
<th>MFI of mAb MEM-E/02</th>
<th>MFI of mAb MEM-E/02</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Incubated with</td>
<td>Untreated</td>
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<tr>
<td></td>
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<td>IFS</td>
<td>PBS only</td>
<td>IFS</td>
<td>PBS only</td>
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<td>2035</td>
<td>1440</td>
<td>219</td>
<td>1051</td>
<td>869</td>
</tr>
<tr>
<td>B*1401</td>
<td>7341</td>
<td>7080</td>
<td>230</td>
<td>5326</td>
<td>5372</td>
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<tr>
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<td>2386</td>
<td>115</td>
<td>1758</td>
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<td>2913</td>
<td>169</td>
<td>1855</td>
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<td>754</td>
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<td>1749</td>
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<td>2877</td>
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<td>2362</td>
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<tr>
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<td>181</td>
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<td>8806</td>
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</tbody>
</table>

*p (2-tail) 0.0913 < 0.0001 0.0413 < 0.0001 0.0385 0.0011 0.0001

IFS is obtained after passing the serum through protein-G column; IgG anti-idiotypic antibodies, present if any, are removed.
Seventy-three percent of renal patients with anti-HLA-E antibodies showed HLA-Ia reactivity; 50% with no detectable anti-HLA-E antibodies showed no HLA-Ia reactivity. Fifty-three percent of LT patients with anti-HLA-E antibodies showed HLA-Ia reactivity; 52% with no anti-HLA-E antibodies showed no HLA-Ia reactivity.

The pattern of HLA-Ia reactivity of the sera and the IgG eluted from the sera of allograft recipients is comparable with that of the array of HLA-Ia alleles recognized by the mAbs (22–24). Like anti-HLA-E mAbs, the sera recognized HLA-B and Cw but only few HLA-A alleles.

IgG isolated (with protein-G columns) from the sera that were negative for both anti-HLA-E and anti-HLA-Ia antibodies showed both HLA-E and HLA-Ia reactivity. However, reappearance of HLA-Ia reactivity may not have been due to allo-HLA-Ia antibodies because the patients did not have allo-HLA-Ia alleles as immunogen. HLA-E occurs in kidney tissues and endothelial venules in these patients. As pointed out earlier (7), they may over-express upon inflammation and shed into circulation as sHLA-E. In support of this contention, this investigation documents sHLA-E in sera of allograft recipients.

The IgG-free LT sera (that contained sHLA-E) as well as the rHLA-E significantly inhibited the HLA-Ia reactivity of various dilutions of anti-HLA-E mAb.

Support for the hypothesis from IgG eluted from the sera groups

Group A-2 had IgG antibodies to HLA-Ia but not HLA-E. Such antibodies might have been generated by other non-classical HLA-Ib alleles (F* and G*) or by other auto-antigens or anti-HLA-E IgG may be bound to HLA-E-specific peptides. Group B-1 included sera positive for anti-HLA-E without HLA-Ia reactivity—possibly because anti-HLA-E antibodies were reacting to a peptide epitope that was not a shared sequence (146SARDA71 and 143EQKSNDA152) (25). The anti-HLA-E antibodies may recognize HLA-Ia alleles not available on the LABScreen beads. Sera and eluate 1 of LT recipients (B-2) did not react with HLA-E or HLA-Ia but HLA-E antibody and HLA-Ia reactivity appeared in eluates 2 and 3. Since IgG was eluted with acidic buffer and recovered in neutralizing buffer, sHLA-E and anti-HLA-E IgG recovered by acid buffer may have rebound upon neutralization of the eluate, which may be why eluate #1 failed to detect either free HLA-E antibody or HLA-Ia reactivity.

This is a significant finding for clinical approach to transplant patients who do not show apparent NDSA reactivity. If IgG are isolated from their sera using protein-G column, not only anti-HLA-E IgG will be revealed but also concomitantly, the HLA-Ia reactivity of NDSA.

This observation supports the contention that non-detectability of anti-HLA-E antibodies in some sera (A-2, B-2) is indeed due to the antibody bound to sHLA-E, and this study provides evidence for sHLA-E being in the sera of liver and renal allograft recipients. The molecular sizes of sHLA-E match those described earlier (19, 32). Puppo et al. (32) also reported that individual serum levels of sHLA had a wide distribution and were not related to HLA-Ia phenotype. A significant correlation was observed between cell surface expression of β2M-free HC and soluble-free HC (33). The increase in serum free HLA HC was greater than that of β2M–HC complex during ischemia after LT, acute graft rejection and acute graft-versus-host-disease following allogeneic bone marrow transplantation (34). Our observations support the contention that sHLA-E in sera can be a biomarker to monitor the clinical course of liver and renal allograft.

In conclusion, it may be stated that the inflammation is a major event that occurs soon after allograft transplantation, which is capable of causing over-expression of HLA-E (7) and consequent shedding of sHLA-E, free (as HC) or bound to β2M (19–21). As envisaged and postulated by the late Professor Sercarz (35), the shed HC of sHLA-E may expose epitopes that are cryptic when complexed with β2M, resulting in autoantibody production against HLA-E in sera of allograft-recipients. Since HLA-E shares several peptide sequences with HLA-Ia (22–25), the antibodies produced against sHLA-E may recognize HLA-Ia alleles in the beads and account for HLA-Ia reactivity of NDSA. In view of the above observations, it is concluded that NDSA is, indeed, the result of HLA-Ia reactivity of one or more of anti-HLA-Ib antibodies produced as a consequence of inflammation, cell death and consequent shedding of sHLA-Ib molecules in circulation, after organ transplantation.

Supplementary data

Supplementary data are available at International Immunology Online

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Declaration of Interest

Professor Paul I. Terasaki is a major shareholder in One Lambda Inc. (Canoga Park, Los Angeles, CA).

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