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*J Immunol* 2010; 185:1935-1948; Prepublished online 7 July 2010; doi: 10.4049/jimmunol.1000424

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Antibodies to HLA-E in Nonalloimmunized Males: Pattern of HLA-Ia Reactivity of Anti–HLA-E–Positive Sera

Mepur H. Ravindranath,* Hugo Kaneku,* Nadim El-Awar,† Luis E. Morales-Buenrostro,‡ and Paul I. Terasaki*

Natural anti–HLA Abs found in sera of healthy nonalloimmunized males recognize HLA-Ia alleles parallel to those recognized by anti–HLA-E mAbs (MEM-E/02/06/07). Therefore, some of the HLA-Ia Abs seen in healthy males could be due to anti–HLA-E Abs cross-reacting with HLA-Ia. If anti–HLA-E Abs occur in healthy nonalloimmunized males, it can be assessed whether they evoke HLA-Ia reactivity as do mouse HLA-E mAbs. IgG and IgM Abs to HLA-E and HLA-Ia alleles are identified in sera of healthy males using microbeads coated with recombinant denatured HLA-E or a panel of rHLA-Ia alleles. The pattern of allelic recognition is comparable to that of anti–HLA-E mAbs. Sixty-six percent of the sera with HLA-E IgG have a high level of HLA-Ia IgG, whereas 70% of those with no anti–HLA-E Abs have no HLA-Ia Abs. HLA-E IgM/IgG ratios of sera are divided into four groups: IgM<sub>low</sub>/IgG<sub>Low</sub>, IgM<sub>High</sub>/IgG<sub>Low</sub>, IgM<sub>High</sub>/IgG<sub>High</sub>, and IgM<sub>Low</sub>/IgG<sub>High</sub>. These groups correspond to anti–HLA-Ia IgM/IgG ratio groups. When HLA-E IgM and IgG are absent or present in males, the IgM or IgG of HLA-Ia are similarly absent or present. The mean fluorescent intensity of HLA-Ia Abs correlates with that of anti–HLA-E Abs. Most importantly, HLA-E and HLA-Ia reactivities of the sera are inhibited by the shared, but cryptic, peptide sequences<sup>126</sup>LNEDLRSWTA<sup>135</sup>,<sup>137</sup>DAAQIS<sup>143</sup>. Therefore, Abs to the H chain of HLA-E may be responsible for some of the HLA-Ia allele reactivity of the natural HLA-Ia Ab in human sera. Absence of any anti–HLA-Ia Abs in 112 nonvegans and the presence of the same in vegans suggest that dietary meat proteins might not have induced the natural allo-HLA Abs. The Journal of Immunology, 2010, 185: 1935–1948.

Maj or histocompatibility complex class I molecules include highly polymorphic classical HLA class Ia (HLA-A alleles [n = 767], HLA-B alleles [n = 1178], and HLA-C alleles [n = 439]) and least polymorphic nonclassical HLA-Ib (HLA-E alleles [n = 9], HLA-F alleles [n = 21], and HLA-G alleles [n = 43]) (1–4). Abs to classical allo-HLA-Ia alleles, not directed to donor-mismatched HLA-Ia alleles, may occur in sera after transfusions, organ transplantation, and pregnancy. However, occurrence of Abs that react to polymorphic allo-HLA-Ia in the sera of normal, healthy, nonalloimmunized individuals (5–12) is perplexing, because the allo-HLA Ags do not occur in these individuals nor is the nature of the immunogens responsible for Ab production known. A pattern in the allelic specificity of the natural Abs is observed (12). The allelic specificities in healthy males are attributed to the exogenous Ags of microbes or pathogens (13–22) or to dietary meat proteins (12). Ag mimicry of several bacterial, fungal, or viral peptide sequences with HLA-Ia alleles (23) may explain single or a few allelic reactivities but not the entire pattern of the allelic specificities of the natural allo-HLA Abs. Speculation that the Abs are produced against meat proteins and peptides has not been verified.

Tongio et al. (7) suggested that natural allo-HLA Abs could be the result of spontaneous secretion of a B cell clone directed against a common autoantigen. Whether any self-Ags that mimic HLA-Ia alleles could elicit Abs that react to HLA-Ia alleles has never been considered, primarily due to Burnet’s hypothesis of clonal selection (24, 25), which forbids immunity against self-Ags. However, evidence has accrued for an emerging school of thought—termed the “immunological homunculus” (26–28) or “immunculus” (29)—that natural Abs could be due to autoimmune response against “a specific and limited set of self molecules” (26). Furthermore, Ag-microarray informatics documents that there are several natural autoantibodies against a battery of configurations or Ag determinants present in self-Ags (30). It is not known whether natural Abs against HLA-Ia alleles are due to any such self-Ags.

HLA-Ia (HLA-A, -B, and -C) molecules share several peptide sequences with a commonly expressed least polymorphic nonclassical HLA-Ib molecule, HLA-E (126:<sup>PRAPWMEQE</sup><sup>35</sup>, 137:<sup>EYWDRETR</sup><sup>65</sup>, 90:<sup>AGSHTLQW</sup><sup>97</sup>, 108:<sup>RFLRYGE</sup><sup>123</sup>, 115:<sup>QFAYDGKD</sup><sup>123</sup>, 117:<sup>AYD</sup> <sup>GKD</sup> <sup>D</sup> <sup>123</sup>, 128:<sup>LNE</sup><sup>LD</sup><sup>LSWTA</sup> <sup>135</sup>, 173:<sup>DAAQ</sup><sup>143</sup>, 153:<sup>RAYE</sup><sup>L</sup><sup>123</sup>, 163:<sup>TCVEWL</sup><sup>168</sup>, and 182:<sup>EPKHTHVT</sup> <sup>190</sup>) (31; 65:<sup>RSDA</sup><sup>2</sup> and 143:<sup>SEQN</sup><sup>125</sup>) are unique and specific to HLA-E. This finding led to the hypothesis that Abs produced against shared peptide sequences present in HLA-E may recognize the same peptide sequences present in HLA-Ia alleles. To test the hypothesis, the affinity of murine anti–HLA-Ia mAbs for HLA-Ia was recently examined using the synthetic shared peptide sequences to inhibit Ab binding to HLA-E and HLA-Ia (31). The binding of HLA-E mAbs to HLA-Ia Abs that were free of β<sub>2</sub>-microglobulin (β<sub>2</sub>m) confirmed the location of the epitopes on the H chain of the Abs. Strikingly, the nature of HLA-Ia alleles recognized by different HLA-E mAbs remained identical. The binding of HLA-E mAbs to HLA-Ia was inhibited dosimetrically by two adjacent peptides, 128:<sup>QFA</sup><sup>YDGKD</sup><sup>123</sup> and 137:<sup>DAAQ</sup><sup>143</sup>, but not by 126:<sup>LNE</sup><sup>LD</sup><sup>LSWTA</sup><sup>135</sup>, another more closely shared peptide sequence located between the two inhibitory peptides. The inhibitory peptide sequences in HLA-E are masked at the α2 helix terminal by β<sub>2</sub>m in intact HLA-E, but they are exposed in β<sub>2</sub>m-free soluble HLA-E (31).
HLA-Ia REACTIVITY OF HLA-E Abs IN HEALTHY MALES

Materials and Methods

Serum samples

Healthy, nonalloimmunized blood donors. The samples included the sera of 208 Mexican males (mean age: 33.1 ± 10.3 y) and 9 females collected at the National Institute of Medical Sciences and Nutrition Salvador Zubirán, after approval by that institution’s ethics board (12). In addition, fresh sera from two healthy Indian male donors were also obtained and used to identify specificities. The sera were restricted to HLA-Ia reactivity of the sera not comparable to those recognized by mAb-E/02; they are presented in the figures with boldface ID numbers.

Healthy, nonalloimmunized Indian vegetarian male blood donors. Eleven Indian male volunteers (ages 19–45 y) were interviewed to ascertain their vegetarian diet since birth. They were strict lactovegans (no meat or eggs). After signing consent, these vegans were paid to donate 5 ml blood for the study; after HLA-Ia typing, the sera were aliquoted and stored in a freezer. These individuals had no history of transfusions or transplants and had no recent infections.

Immunofluorescence with SABs. Multiplex Luminex-based immunofluorescence was used, as described earlier, to detect the presence of Abs that react to HLA-E and HLA-Ia alleles in the sera (31). In this investigation, we used sera diluted 1:10 with PBS (pH 7.2). Using dual-laser flow-cytometric principles of Luminex xMAP multiplex technology, the single-Ag (allele) assays were carried out for data acquisition and analysis of HLA-Ia and HLA-E Abs. The Luminex assays use microbeads on which individual HLA Ags have been covalently bonded (xMap assays). xMap microbeads including two reporter fluorochromes that are proportionately varied to identify them as one of 100 possible bead identifiers. The LABScreen (One Lambda, Canoga Park, CA) consists of a panel of color-coded microspheres (SABs coated with single Ag HLA alleles) to identify Ab specificities. The array of HLA Ags representing various alleles on the beads are listed at the One Lambda Web site (www.onelambda.com) under Ab-detection products/LABScreen Single Ag Product sheet/LABScreen Single Ag Product sheet/HLA-Ia Ags. Products in LS1A04 include 31 HLA-A, 50 HLA-B, and 16 HLA-C alleles, but not all existing HLA-Ia alleles are represented in the beads analyzed. HLA-E fold H chain (10 mg/ml in MES buffer) was obtained from Dr. Jianhong Cao (Immune Monitoring Laboratory, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA). The HLA-E H chain is attached to 5.6-μm polystyrene microspheres by a process of simple chemical coupling at One Lambda. Data generated with Luminex Multiplex Flow Cytometry (LABScan 100) were analyzed using computer software. The protocol was identical to that reported earlier (31, 36). PE-conjugated anti-human IgG or IgM Abs were used for immunolocalization of the Ab bound to Ags coated onto the microbeads. The reporter fluorochrome intensity was measured in a specialized flow cytometer together with the microbead identifiers, and the fluorescence measurement was classified by bead identifier. Fluorescence intensity was collected from a sample ≥90 beads. The trimmed mean is obtained by trimming a percentage off the high and low ends of a distribution and finding the mean of the remaining distribution. Trimmed mean fluorescence intensity (MFI) for the SAB reactions are obtained from the output (.csv) file generated by the flow analyzer was adjusted for background signal using the formula (sample #N bead – sample negative control bead). The MFI was compared with that of negative control

Table 1. Level (MFI) and incidence of anti–HLA-E IgG Abs in the healthy sera from nonalloimmunized males with (positive group) or without (negative group) anti–HLA-Ia natural Abs

<table>
<thead>
<tr>
<th>Group</th>
<th>Anti–HLA-E IgG (MFI)</th>
<th>n</th>
<th>Incidence (%)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive group (I)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Anti–HLA-Ia Ab positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[MFI &gt; 1000]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;2000</td>
<td>22/96</td>
<td>22.9</td>
<td>2605 ± 601</td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>18/96</td>
<td>18.8</td>
<td>815 ± 157</td>
<td></td>
</tr>
<tr>
<td>&gt;1250</td>
<td>63/96</td>
<td>66</td>
<td>(Group IA)</td>
<td></td>
</tr>
<tr>
<td>&lt;1250</td>
<td>33/96</td>
<td>34</td>
<td>(Group IB)</td>
<td></td>
</tr>
<tr>
<td><strong>Negative group (II)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Anti–HLA-Ia Ab negative)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[MFI &lt; 500]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;2000</td>
<td>11/112</td>
<td>9.8</td>
<td>2908 ± 637</td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>50/112</td>
<td>44.6</td>
<td>663 ± 227</td>
<td></td>
</tr>
<tr>
<td>&gt;1250</td>
<td>34/112</td>
<td>30</td>
<td>(Group IIA)</td>
<td></td>
</tr>
<tr>
<td>&lt;1250</td>
<td>78/112</td>
<td>70</td>
<td>(Group IIB)</td>
<td></td>
</tr>
</tbody>
</table>

Absence of anti–HLA-Ia IgG in 112 males (meat eaters); 70% of them do not have anti–HLA-E IgG Abs. *79.4% had MFI < 1500; 12/51 had MFI 1501–1922.

FIGURE 1. Incidence (%) of the presence (MFI > 1250) and absence (MFI < 1250) of anti–HLA-E IgG Abs in the sera of positive (with HLA class Ia Abs) and negative (with no HLA class Ia Abs) groups of healthy nonalloimmunized Mexican males.

In addition, identical similarities noted between the kinds of HLA-Ia alleles recognized by different murine anti–HLA-Ia mAbs and by natural anti–HLA IgG in the sera of healthy males (12, 31) led to the hypothesis that HLA-E Abs may be responsible for HLA-Ia reactivity of natural HLA-Ia Abs in healthy human males.

HLA-E, one of the nonclassical HLA-Ib molecules discovered in 1987 (1–3), is uniquely distributed on several cells, predominantly endothelial cells, immune cells (B, T lymphocytes, NK cells, monocyte, and macrophages), trophoblasts, and some tumor cells, including melanoma (32–34). Increased cellular expression of HLA-I results in the release of HLA-E in the circulation (32). Soluble HLA-E (sHLA-E) is observed in sera or plasma of patients with immune-mediated vascular diseases or Kawasaki disease (a systemic pediatric vasculitis), as well as in normal individuals (32, 35). Abs may be generated by sHLA-E consequent to removal of β2m, which may expose cryptic epitopes masked by β2m. These Abs may bind to the shared peptide sequences of HLA-Ia alleles.

The specific objective of this study was to evaluate the hypothesis that HLA-Ia reactivity of HLA-E Abs may account for HLA-Ia allelic specificities in healthy human sera. The presence or absence of Abs to HLA-E in the sera of 220 nonalloimmunized healthy males was correlated with the presence or absence of natural Abs reacting to HLA-Ia alleles on LABScreen single-Ag beads (SABs) using dual-laser flow-cytometric principles of Luminex xMAP multiplex technology. The HLA-Ia reactivity of IgM and IgG in human sera was compared with the HLA-Ia allelic recognition of murine mAb-E/02.

![Graph showing incidence of anti–HLA-E IgG Abs in healthy males](http://www.jimmunol.org/)

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showing that natural allo-HLA Abs are not restricted to meat eaters.

Table II.

<table>
<thead>
<tr>
<th>ID</th>
<th>HLA</th>
<th>Anti–HLA Class Ia Ab Levels (MFI)</th>
<th>Anti–HLA-E IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;500</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>1GB020508</td>
<td>A26, A32</td>
<td>A*</td>
<td>A<em>1102/B</em>2403/A*6602M</td>
</tr>
<tr>
<td></td>
<td>B8, B51</td>
<td>B*</td>
<td>B<em>0702/B</em>4801/B*5501</td>
</tr>
<tr>
<td></td>
<td>Cw07,Cw14</td>
<td>Cw*</td>
<td>Cw*0403</td>
</tr>
<tr>
<td>2SV020608</td>
<td>A*</td>
<td>A</td>
<td>A<em>0101/A</em>0201/A*0203</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A*3601</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>B<em>3701M/B</em>4101M/B<em>5401M/B</em>8201M/Cw*0801M</td>
</tr>
<tr>
<td>3GN020708</td>
<td>A31, A33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B44, B62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4JA020708</td>
<td>A*</td>
<td></td>
<td>A*0101M</td>
</tr>
<tr>
<td></td>
<td>Cw01, Cw08</td>
<td></td>
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<tr>
<td>5JM021308</td>
<td>A2, A26</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>B8, B51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cw07, Cw07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6JgM021308</td>
<td>A*</td>
<td></td>
<td>A<em>3001/A</em>3101</td>
</tr>
<tr>
<td></td>
<td>B8, B51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cw07, Cw07</td>
<td></td>
<td></td>
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<tr>
<td>7VM021308</td>
<td>A*</td>
<td></td>
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<td></td>
<td>B8, B51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cw07, Cw07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B7, B52</td>
<td></td>
<td>B<em>4501/B</em>4801</td>
</tr>
<tr>
<td></td>
<td>Cw07, Cw12</td>
<td></td>
<td>Cw<em>1502/Cw</em>0102</td>
</tr>
<tr>
<td>9MJ021208</td>
<td>A*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10RP021808</td>
<td>A*</td>
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<tr>
<td></td>
<td>B35, B40</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Cw03, Cw04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13RG022508</td>
<td>A*</td>
<td></td>
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</tbody>
</table>

HLA typing is also provided to show that none of the anti–HLA-Ia Abs correspond with the HLA alleles found in the individuals.

Letter M after allele refers to IgM.

Bold type denotes alleles also recognized by anti–HLA-E mAb E/02.
HLA-E IgG > 1250 (30% of 112 individuals); and 4) group IIB: anti–HLA-Ia IgG < 1250 (70% of 112 individuals).

Fig. 1 summarizes the relative incidence of anti–HLA-E and anti–HLA-Ia Abs in Mexican male donors. Among those positive for HLA-Ia IgG, there was a greater number of individuals (group IA; 63/96) who had a high level of anti–HLA-E IgG. Strikingly, among those negative for HLA-Ia IgG, a higher incidence of individuals (Group IIB; 78/112) had no or low levels of anti–HLA-E IgG. Table II shows the MFI of HLA-E IgG and HLA-Ia IgM and IgG Abs found in 11 Indian vegans. HLA typing of these individuals is also provided to show that neither of the anti–HLA-Ia Abs, IgM or IgG, corresponds with the HLA alleles found in the individuals; this finding is in accordance with our earlier article (12) on Mexican males reporting that the natural anti–HLA-Ia Abs found in healthy individuals do not possess matching HLA alleles.

Anti–HLA-E IgM/IgG ratio groups: prevalence of HLA-Ia IgG Abs in individuals with increased levels of anti–HLA-E IgG

Sera from all groups of healthy, nonalloimmunized males were examined for anti–HLA-E IgM/IgG ratios; the following groups were observed (Fig. 2). Group I (IgMLow/IgLowlow; n = 12): IgM and IgG Abs were absent or undetectable (MFI < 1250). Seven of them had HLA-Ia IgG, with two reacting only to HLA-A*alleles. Group II (IgMHigh/IgLowlow; n = 23): MFI of IgM Abs was significantly greater (two-tailed p value < 0.0001) than that of IgG. Ten (44%) of them showed HLA-Ia reactivity, with one reacting only to HLA-A*. Group III (IgMHigh/IgGHigh; n = 14) had equal MFI levels for IgM and IgG. Twelve of them (86%) showed HLA-Ia reactivity. Group IV (IgMLow/IgGHigh; n = 11): MFI of IgM Abs was significantly lower (two-tailed p value < 0.0001) than that of IgG. In this group, MFI of IgG was >2000. Nine of the 11 sera (82%) showed HLA-Ia reactivity.

Comparing the different groups for anti–HLA-E IgM/IgG ratio, it is evident that the percentage of individual sera reacting to anti–HLA-Ia IgG increased from 29% (I) < 44% (II) < 86% (III) = 82% (IV). HLA-Ia IgG with a high level of anti–HLA-E IgG was found in 84% (21/25) of the individuals in groups III and IV.

Meat proteins or peptides do not account for natural anti–HLA-Ia Abs

The Mexican males in our study consumed meat products. Nevertheless, 112 sera of the male donors did not have Abs to allo-HLA class Ia molecules, suggesting that consumption of meat protein is unrelated to the presence of natural anti–HLA-Ia Abs. Analysis of anti–HLA-E Abs of 112 males revealed that 78 (70%) had no anti–HLA-E IgG Abs (Fig. 1). The absence of anti–HLA-E Abs in 70% of those with no natural HLA-Ia Abs is striking. In addition, Table II shows the presence of HLA-E and anti–HLA-Ia Abs in the vegan sera, which supports the irrelevance of meat consumption to the occurrence of natural anti–HLA-Ia Abs.

HLA-Ia molecules recognized by the sera of normal males parallel the HLA-Ia molecules recognized by the murine anti–HLA-E mAb

Murine mAb-E/02 raised against human rHLA-E (MFI: 17,000) reacted strongly with many of the HLA-B and HLA-Cw alleles coated onto Lot 2 and Lot 5 of LABScreen beads but minimally with HLA-A. The number of HLA-Ia alleles reacting to mAb-E/02 decreased with dilution of the mAb (48 at 1:300; 14 at 1:600; and 7 at 1:1200) (31). Very few HLA-A alleles reacted with mAb-E/02; their MFI can be ranked as follows: A*2402 (with Lot 2: 1159; with Lot 5: 797), A*3301 (with Lot 2: 852; with Lot 5: 1944), A*1101 (with Lot 2: 683; with Lot 5: 1382), and A*2403 (with Lot 2: 531; with Lot 5: <500).

Fig. 3 shows that the specificities of HLA-Ia alleles recognized by natural Abs in sera of normal subjects (at dilution 1:10) are similar to HLA-Ia alleles recognized by the anti–HLA-E mAb MEM-E/02 (at dilution 1:300) reported earlier (31). The upper x-axis in Fig. 3 lists the HLA-Ia alleles reacting to mAb-E/02. Although the MFI of the reactivity differed between Lot 2 and Lot 5, the mAb reacted with the same set of HLA-Ia molecules in both of the lots. The figure shows the results obtained with IgG Abs in healthy male sera tested against SABs of Lot 2. Only one patient’s (T-338) serum Ab reacted with HLA-A alleles, whereas none of the other sera Abs reacted to any of the HLA-A* alleles coated onto the microbeads (Lot 2). The total number of HLA-Ia alleles recognized by the sera of these subjects ranged from 3 (T-249) to 21 (T-354). It was considered a 100% match when all of the HLA-Ia-allele specificities of a serum were matched with HLA-Ia-allele specificities of mAb-E/02. For example, T-86 and T-364 showed 100% match. Individuals showing >50% match are shaded gray (Fig. 3). A 50–100% match between the HLA-Ia reactivity of human sera and that of anti–HLA-E mAb-E/02 suggests a positive relationship between the HLA-Ia reactivity and HLA-E of human sera. The number of individuals recognizing each allele is listed in the bottom x-axis and ranges from 1–13. The most prevalent alleles recognized by the healthy male sera are B*8201 (n = 13), Cw*0302 (n = 13), Cw*0102 (n = 11), B*0702 (n = 10), Cw*0303 (n = 9), B*5601 (n = 9), B*4402 (n = 8), Cw*1701 (n = 8), B*1502 (n = 7), B*1512 (n = 7), Cw*1502 (n = 7), Cw*1203 (n = 7), Cw*0202 (n = 7), B*3701 (n = 6), Cw*0501 (n = 6), and Cw*1802 (n = 6). Most importantly, the sera of these individuals recognized HLA-Ia alleles B and C, but they rarely reacted with HLA-A alleles, very similar to murine mAb-E/02. Possibly, the human anti–HLA-E Abs, very
much like murine mAb-E/02, are recognizing the same or similar discontinuous peptide sequences among HLA-E, -B, and -C (Fig. 4). Evidently, anti–HLA-E Abs in normal human sera and murine mAbs are inaccessible to the epitope constituting the inhibitory peptides in HLA-A, possibly because of substitution of the amino acid residues in the epitope (DTAAQI). Fig. 5 summarizes the salient difference between alleles that bind and do not bind to human sera or murine mAb-E/02. The figure shows that the failure of anti–HLA-E Ab to recognize HLA-A could be due to stearic hindrance (from recognizing the epitope) caused by substitution of amino acids near the epitopes. In the epitope DTAAQI, threonine (T) is replaced by methionine (M) at position 138 in all HLA-A alleles; in some, isoleucine (I) is replaced by threonine at position 142. In addition, in HLA-A alleles, glutamic acid (E) at position 114 is replaced by basic amino acids, such as histidine (H), arginine (R), or glutamine (Q). These acid–base shifts may alter the orientation of the epitopes and affect Ab binding.

**FIGURE 3.** The HLA-Ia molecules recognized by IgG Abs in sera of normal males follow the pattern of HLA-Ia alleles recognized by the murine anti–HLA-E mAb. Upper x-axis, Data above the box represent MFI obtained with the mouse mAb MEM-E/02 (at 1:300 dilution) with beads coated with HLA-E (MFI 17,000) and a series of HLA class Ia molecules that include 1 A*allele, 22 B* alleles, and 14 Cw* alleles. Shaded alleles imply that they are recognized more frequently (6–13 sera) by the Abs in the sera of normal healthy nonalloimmunized subjects. The two numbers separated by a slash (/) above each allele refer to MFI obtained with Lot 2 (LS1A04-Lot 002) and/or Lot 5 (LS1A04-Lot 005) beads. The differences in MFI signify differences in the Ag coating between the two lots of beads. B* and Cw* alleles are ranked based on MFI observed with Lot 2 beads. Left y-axis columns, The first column refers to the serial numbers of the 38 individuals whose sera were tested. The second column refers to the identification of each individual. These individuals belong to the positive group presented in Fig. 1 and Table I. The third column refers to the MFI of anti–HLA-E IgG Abs. Right y-axis columns, The left columns show the incidence and percentage of alleles recognized by serum Abs of each patient that match HLA-Ia alleles recognized by mAb-E. In some individuals, the HLA-Ia Ag recognized by the sera is 100% comparable to that recognized by mouse mAb MEM-E/02. The lowest incidence was 22% (ID T-239). Note that 29 of 38 individuals had >50% match, with HLA class Ia beads recognized by mouse mAb E/02, indicating that the natural anti–HLA-E Ab could be due to anti–HLA-E Abs found in them, because the allele specificities of HLA-Ia recognized by the sera of normal males is identical to those recognized by the murine anti–HLA-E mAb. Central box, For every individual, the natural anti–HLA class Ia Ab similar to those recognized by the mouse mAb E/02 is present in the small shadowed box. Although the MFI cutoff for anti–HLA-Ia Ab is set at 1250, the MFI cutoff for anti–HLA-Ia Abs is 1000. Values plotted in the central box are from Lot 5 values of anti–HLA-Ia Abs. MFI 500 < 1000, MFI 1000–2000, MFI > 2000, MFI > 5000. Lower row of x-axis, The total number of Abs recognizing each allele is indicated by the number of boxes in each column.
Anti–HLA-Ia IgM Abs in healthy males: parallel trends between IgM/IgG profiles of HLA-Ia and HLA-E Abs

Because HLA-E Abs also occur as IgM, the presence of natural IgM Abs to HLA-Ia alleles was screened to determine whether HLA-Ia reactivity of healthy male IgM parallels that of anti–HLA-E IgM. Because HLA-E IgM in healthy males mimics the pattern of HLA-Ia reactivity of murine mAbs, HLA-Ia reactivity of human IgM is also comparable to that of murine mAbs.

Fig. 6 provides four kinds of profiles of 25 male sera. Group A shows that when HLA-E IgM and IgG Abs are absent in healthy males, the natural IgM and IgG Abs to HLA class Ia alleles are negligible or absent. In Group B, when HLA-E IgM is present and IgG is absent, the natural IgM to HLA class Ia alleles occurs without IgG. In Group C, when HLA-E IgM and IgG Abs are present, IgM and IgG Abs to HLA class Ia alleles also occur. Most importantly, HLA-Ia reactivity of IgM differs with HLA-Ia reactivity of IgG. Rarely, parallel HLA-Ia IgM/IgG reactivity was observed between IgM and IgG. An HLA-Ia allele (B*1512) in one individual (T131) reacted to IgM and IgG. The independent HLA-Ia reactivity in this group of individuals (with anti–HLA-E IgM and IgG) underscores that HLA-E IgM and IgG react differently to HLA-Ia alleles. In group D, when HLA-E IgM is absent and IgG is present, the natural IgG Abs to HLA class Ia alleles occur without IgM Abs.

Anti–HLA-Ia IgM/IgG ratio groups parallel anti–HLA-E IgM/ IgG ratio groups. IgM Abs reacting to HLA-Ia alleles in healthy males mimic the HLA-Ia reactivity of mouse mAbs. All of these observations support the hypothesis that HLA-Ia reactivity of HLA-E Abs may account for some of the HLA-Ia allelic specificities of IgM and IgG natural Abs.

Normal male sera can also recognize HLA-Ia alleles not recognized by mAb-E/02

Not all sera in Fig. 3 showed 100% match with the HLA-Ia alleles recognized by mAb-E/02. Comparing peptide sequences of HLA-E with those of HLA-Ia, one can observe that HLA-E shares several peptide sequences with HLA-Ia alleles. The mAb-E/02 recognized one of the most common discontinuous epitopes, peptide sequences 117AYDGKDY and 117DTAAQIS (Fig. 4), as evidenced by peptide-inhibition studies (31). However, several shared peptide sequences are not recognized by mAb-E/02 or other murine MEM-mAbs, but they seem to be recognized by normal human sera. These are discussed below.

FIGURE 4. Crypticity of the inhibitory discontinuous peptides (DTAAQIS and QFAYDGKDY). The position of the inhibitory discontinuous peptides (DTAAQIS and QFAYDGKDY) is shown in yellow. A, The stearic hindrance (*) caused by $\beta_{1m}$ is shown. B, Exposed peptide sequences in $\beta_{1m}$-free HLA-E. C, The exposed amino acids constituting the inhibitory discontinuous peptide sequences. The position of each amino acid is indicated by an asterisk (*) and the number. Based on peptide-inhibition studies, the possible exposed amino acid residues (in bold letters) recognized by the Abs are inferred; they essentially include two tyrosines and two adjacent aspartic acids.

58EYWDRETR$^{36}$. The location of the specific epitope is presented in Fig. 7. This epitope is uniquely expressed by A*6813, B*1516, B*1517, and B*1567 (lower right panel). B*1516 is recognized by several human sera (left panel). Because of the nonavailability of some beads in the multiplex assay (A*6813, B*1516, and B*1567), only those Abs bound with B*1516 could be detected in the sera of several individuals. Such specific reactivity is possible only if the anti–HLA-E Abs were produced against the epitope 58EYWDRETR$^{36}$.

157RAYLED162. This epitope is located in the $\alpha_3$ domain of the H chain of HLA-E, as shown in Fig. 8. The HLA-C alleles with the epitope are listed in the bottom panel; they include Cw*0701, Cw*0702, Cw*0703, Cw*0704, Cw*0706, Cw*0711, Cw*0717, Cw*0718, Cw*0719, Cw*0720, Cw*0721, Cw*0726, Cw*0729, Cw*0730, Cw*0736, and Cw*0742. Because beads are available only for Cw*0702, reactivity to Cw*0702 alone could be assessed. If anti–HLA-E Ab is raised against this epitope, the sera may react selectively to these alleles, including Cw*0702. Such a Cw*0702-specific Ab occurred in the sera of one female and five normal males, suggesting that they may have had anti–HLA-E Ab reacting to the specific epitope found on Cw*0702.

182EPPKTHVT190. This epitope is located in the $\alpha_2$ domain of the H chain of HLA-E, as shown in Fig. 9. This unique epitope is restricted to B*8201 and is shared only by HLA-E but not by any other HLA-Ia or Ib (F and G) alleles. Among sera of healthy, nonalloimmunized subjects, two categories of sera recognized this epitope: sera with Abs reacting to B*8201, as well as with other HLA-Ia molecules (Fig. 10 shows that 53 males, as well as 5 females who showed reactivity, reacted to B*8201 and to other HLA-Ia molecules [Fig. 3]) and sera with Abs specifically reacting to B*8201. One such nonspecific serum (MR022108) reacting only to B*8201 was observed and reassessed using single-single beads containing only B*8201.

The binding of the nonspecific sera to single-single beads of B*8201 was inhibited strongly (75%) by the synthetic peptide AYLED but not by the nonspecific peptide QISQR (Fig. 10A). However, such strong inhibition was not observed with the poly-specific sera, although they reacted with B*8201 (Fig. 10B). The AYLED inhibition of binding of the polyspecific sera was mild (range, 13–46% with different individuals). The observations indicate that Abs in monospecific sera may be specifically recognizing B*8201, whereas Abs in the polyspecific sera may still be recognizing the discontinuous epitope recognized by mAb-E/02. B*8201 is also recognized by mAb-E/02 in Lots 2 and 5. HLA-E has the B*8201-specific epitope RAYLED and the shared discontinuous epitopes DTAQQIS and AYDGKDY commonly found in other HLA-B and HLA-C alleles (Table I). To determine whether Abs in the polyspecific sera recognize DTAQQIS and/or AYDGKDY, we used these peptides to inhibit binding of the sera to B*8201. As expected, the synthetic peptides DTAQQIS and QFAYDGKDY inhibited B*8201 binding of the polyspecific serum of T-244 (in group 1), but they failed to inhibit the B*8201 monospecific serum MR-1 (MR022108) (group 2) (Fig. 10C). The results confirmed that different shared peptide sequences in HLA-E can elicit Abs to HLA-Ia alleles. The epitope responsible for immunogenicity can be assessed by specific peptide-inhibition studies, as documented above.
Discussion

Since 1973, there have been reports of Abs reacting to a group of polymorphic allo-HLA-Ia in the sera of normal and healthy nonalloimmunized individuals (5–12), although such reactivity was dismissed in normal sera as “nonspecific binding of normal sera to the solid support used with the conjugated Ag” (37). The current study was designed to examine the HLA-Ia specificities of normal sera using the LABScreen Single Ag assay (One Lambda). This assay consists of a panel of color-coded microspheres (SABs) coated with HLA alleles to identify Ab specificities; the alleles may include intact and b2m-free H chains. Although this is a sensitive tool, it is logical to anticipate nonspecific binding. However, appropriate correction of the controls permitted reproducible recognition of specific HLA-Ia molecules and ruled out nonspecific binding to microbeads; the necessary correction consisted of microbeads coated with human serum albumin as negative controls for sera tested at 1:10 dilution.

Allo–HLA-Ia recognition of normal sera was confirmed earlier (12) by testing the IgG in the sera of 424 healthy nonalloimmunized Mexican male blood donors over a 6-mo period and using SAB manufactured at different sources (One Lambda; Gen-Probe Transplant Diagnostics, Stamford, CT). We also investigated previously (12) whether individuals carrying natural Abs to HLA class I Ag have the specific HLA Ags by obtaining high-resolution HLA typing for these individuals. After sample-size calculations, it was noted that a sample size of 20 is sufficient to examine the HLA profiles. Thirty individuals were randomly selected for DNA typing using LABType SSO (One Lambda). None of the subjects had matching HLA class Ia Ags for anti-HLA Abs, suggesting that healthy individuals who carry natural anti-HLA IgG Abs do not possess matching HLA alleles and that HLA-Ia Ags expressed by healthy males may not be the causative agent for HLA-Ia reactivity of sera (12). Observations in Table II on natural anti–HLA-Ia IgG Abs and HLA typing in vegans also support the above suggestion. These findings support the basic content of this study that some, if not all, naturally occurring allo-HLA class Ia Abs observed in healthy individuals could be due to cross-reactivity of anti–HLA-E Abs with HLA-B and HLA-Cw alleles.

The relevance of naturally occurring anti–HLA-Ia Abs in nonalloimmunized individuals, particularly with respect to transplant patients, is emerging. It was shown that transfused patients have Abs directed against many infrequent HLA-Ia alleles, including B*1512 (B76), A*8001, and A*3002 (38). The specificities of these alloantibodies match specificities found in sera of normal subjects. Whether these Abs result in unnecessary exclusion of donors depends on whether the Abs have the same clinical effect as alloantibodies. As will be reported in a separate study, many specificities of the nondonor-specific Abs produced after transplantation are also found in normal males and with greater frequency (39–42). Even if they are irrelevant to graft rejection, specificities of these Abs must still be identified to prevent unnecessary exclusion of potential transplant donors.

The prevalence of IgM that recognizes allo–HLA-Ia in the healthy sera without prior alloimmunization was documented previously (7). The present investigation further confirms the presence of IgM and IgG in healthy male sera reactive to HLA-Ia alleles coated onto different lots of SABs. The nonavailability of single beads for some HLA-Ia alleles imposes a limit on screening natural allo-HLA Abs. The progress made so far on the discovery...
FIGURE 6. The HLA-Ia alleles and HLA-E recognized by the IgM and IgG Abs in the sera of healthy males compared with those recognized by the murine anti–HLA-E mAb. Upper row 1, top rows above the box, The HLA-Ia alleles recognized by the mouse mAb MEM E/02 are indicated. Upper row 2, Refers to IgM/IgG profiles of HLA-Ia and HLA-E. Left column, The first column is the identification of individual serum that was tested. The second column refers to Abs: upper row, IgM; lower row, IgG. Right column, HLA-E IgM/IgG status. The left-most column refers to anti–HLA-Ia observed in the individual sera: upper row, IgM; lower row, IgG. The IgM or IgG row boxes denote the MFI of the alleles as follows: □ MFI < 500, □ MFI 500–1,000, □ MFI 1,000–2,000, □ MFI 2,000–5,000, □ MFI > 5,000, □ MFI > 10,000. Note that the allele specificities of HLA-Ia recognized by IgM and IgG Abs in the sera of healthy males are identical to those recognized by the murine anti–HLA-E mAb. A, HLA-Ia alleles IgM+/Ig− group versus HLA-E IgM+/Ig− group. Three individuals are represented (T-363, T-106 & T-325). Empty rows signify no reactivity for HLA-Ia IgM and IgG Abs for A*, B*, and Cw* alleles, as well as for
of natural HLA Abs in humans projects that, in the future, a greater number and incidence of natural allo-HLA IgG Abs will be recorded in healthy nonalloimmunized males.

A number of investigators provided different explanations for the presence of natural HLA Abs (7, 11, 12, 43). Some contended that they are the result of a polyclonal, continuous immune response against exogenous Ags (5, 6, 8–12). Tongio et al. (7) thought that natural HLA-Ia reactivity of sera could be due to spontaneous secretion of Ab by one or more B cell clones developed against common autoantigens (7). The number of HLA-Ia specificities of natural Abs in Mexican healthy nonalloimmunized males, as assessed in neat sera or sera diluted 1:3, range from 1 to 64, with MFI cutoff at 500. Investigating the same cohort of male sera at 1:10 dilution, HLA-Ia reactivity ranged from 1 to 13, also with MFI cutoff at 500. Although studies of vegans’ sera are few, these observations, taken with those made on 112 healthy meat eaters, do not support the contention that meat proteins can be the immunogens for multivarious natural anti–HLA-Ia Abs. There is certainly scope for future investigations on natural anti–HLA-Ia Abs in vegans.

Natural Abs against self-Ags (commonly called autoantibodies) are present in normal childhood (30, 44) and adult nonalloimmunized (45–47) human sera. Ag-microarray informatics revealed the presence of a battery of several natural autoantibodies against several configurations or Ag determinants present in self-Ags (30). It is in this context that our investigation documents that human nonclassical HLA-E shares several peptide sequences with HLA-Ia alleles, as well as that a number of healthy nonalloimmunized males may have Abs against HLA-E.

The expression of HLA-E in its soluble state in circulation without β2m and shedding β2m-free sHLA-E under the influence of cytokines (IFN-γ), tissue necrosis, and injury (32–35) suggests that sHLA-E might have elicited Abs against exposed epitopes. Peptide-inhibition experiments with synthetic, but shared, peptide sequences identify the epitope of the murine mAb MEM-E/02 as 117AYDGKDY123 and 137DTAAQIS143, the sequences located on or near the α2 helix of the HLA class I Ags (31). In the native state (when associated with β2m), the epitope sequences 117AYDGKDY123 and 137DTAAQIS143 may be masked by β2m (Fig. 4A). However, when free from β2m, the epitope may be exposed (Fig. 4B) without any interference. Possibly, the sequences 117AYDGKDY123 and 137DTAAQIS143 (Fig. 4C) may become immunogenic to elicit Ab response or exposure for Ab recognition.

Many previous observations (31–35, 48) support the contention that HLA-E without β2m would have a conformation essentially similar to HLA-E with β2m. Particularly, in our previous reports (31, 48), we showed the dosimetric inhibition of the binding of mAbs (E/02, E/06, and E/07) to HLA-E H chains, on one hand, and HLA-Ia alleles, on the other, by the two adjacent peptides 117AYDGKDY123 and 137DTAAQ143 but not by 128LNEDLRSWTA135, another shared peptide sequence located between the two peptides. Such unique inhibition of the binding of mAbs to β2m-free HLA-E is possible only when the sequences occur in the conformation shown in Fig. 4C. Importantly, this observation points out that the mAbs do not recognize a linear sequence but recognize only a discontinuous sequence (48). The precise location of the binding epitopes is on the terminal zone of α2 helix. It is known that these helical configurations are highly stable with or without β2m. Moreover, Fig. 4A shows that β2m inhibres on the territory of the peptide sequence (115QFAYDGKDY123), which is an extension of the α2 helix, a stable entity. We hypothesized that β2m would interfere with accessibility of the Ab to the binding domain. To test the hypothesis, we completed a series of experiments using another anti–HLA-E–specific mAb (3D12). The results document that the mAb binds to microbeads coated with β2m-free HLA-Ia but not to

![Figure 7](https://example.com/figure7.png)

**FIGURE 7.** Location of the peptide sequence shared between HLA-E and B*1516. A unique HLA-E peptide (**EYWDRET**R) shared with B*1516 (lower right panel) is located in the α1 helix and may be involved in recognition by the normal sera Abs. The sera of individuals (body type denotes females) reacting to the beads containing B*1516 are indicated in the left panel. MFI < 1000, MFI 1000–2000, MFI > 2000, MFI > 5000. The position of the most common inhibitory peptide (DTAAQIS) is in yellow and is located on the α2 helix.
the beads coated only with intact HLA-Ia. The beads coated only with intact HLA are under patent submission by One Lambda. The details of the observations will be reported elsewhere. Furthermore, ElliPro web tool (49) analyses for discontinuous epitopes confirmed that the sequences 115QFAYDGKDY123 and 137DTAAQIS142 are immuno-
genic, as is, in β2m-free HLA-E.

Not all of the alleles recognized by murine mAb MEM-E/02 are recognized by the sera of healthy males. Purity and concentration of the mAb, on one hand, and the presence of other proteins, other serum components, and dilution of sera, on the other hand, may account for differences between mAb and human sera (or among different human sera). The most striking findings are the total

FIGURE 8. Location of the peptide sequence shared among HLA-E, Cw*0702, and other closely related alleles. A unique HLA-E peptide (182EPKTHV190) shared by Cw*0702 and other related alleles (lower panel) is located in the α3 domain and may be involved in recognition by the normal sera Abs. Top panel, The sera (bold type denotes a female) reacting to the beads containing Cw*0702 are shown. MFI < 1000, MFI 1000–2000, MFI > 2000, MFI > 5000. Middle panel, The inhibitory peptides (DTAAQIS and QFAYDGKDY, shown in yellow) are located on the α2 helix.

FIGURE 9. Location of the peptide sequence shared between HLA-E and B*8201. Lower right panel, A unique HLA-E peptide (167RAYLED172) shared with B*8201 is located at the α2 helix and may be involved in recognition by some of the normal sera Abs. Left panel, The sera of individuals (bold type denotes females) reacting to the beads containing B*8201. Two types of sera reacting to B*8201 were observed in normal individuals. Type 1 reacts only to B*8201; type 2 reacts to B*8201, as well as to other B* and Cw* alleles. MFI < 1000, MFI 1000–2000, MFI > 2000, MFI > 5000. Upper right panel, The position of the inhibitory peptides (DTAAQIS and QFAYDGKDY shown in yellow) is also located on the α2 helix.
FIGURE 10. Synthetic peptide AYLED, shared between B*8201 and HLA-E, inhibited sera with Abs monospecific for B*8201 (A, Group 2, MR022108 and MR060408) but failed to inhibit sera with Abs polyreactive to several HLA-Ia molecules (B, Group 1, T-75, T-96, and T-244). The nonspecific control peptide (QISQR) did not inhibit either sera type. C. The HLA-E common epitope DTAAQI inhibited a representative serum from group 1 (T-244, \(n = 3\)), but it failed to inhibit group 2 (MR-1:MR022108, \(n = 3\)), which specifically recognizes B*8201. Although peptide QFAYDGKDY inhibited 24% of the serum of T-244, the inhibition was not significant. Both peptides failed to inhibit binding of MR sera to B*8201. These observations show that anti–HLA-E Abs in sera of healthy individuals are polyclonal and not monoclonal. Therefore, some individuals may have Abs that may not bind to specific epitopes recognized by the murine monoclonal anti–HLA-E Ab MEM-E/02. In addition, sera may recognize another epitope shared between HLA-E and class Ia molecules, such as \(157\)RAYLED\(162\) (an epitope shared between HLA-E and B*8201). The figure provides proof that sera of individuals may contain Abs that specifically recognize \(157\)RAYLED\(162\) and not the other alleles recognized by MEM-E/02. This is well illustrated by sera MR022108 and MR060408, which recognized only B*8201 and none of the other HLA-B or C alleles. The figure also shows that several sera (T75, T96, and T244) recognized B*8201 and several other HLA-B and -C alleles similar to MEM-E/2.
absence of natural HLA-Ia IgG Abs in 112 healthy meat eaters and the presence of natural HLA-Ia Abs in vegans. Furthermore, neither murine mAb-E/02 nor anti–HLA-E Abs in normal human sera can recognize the epitope constituting the inhibitory peptides in HLA-A, possibly because of substitution of the amino acid residues in the epitope (DTAAQI). Fig. 5 documents that the failure of anti–HLA-E Ab to recognize HLA-A could be due to stearic hindrance caused by substitution of amino acids near the epitopes. In the epitope DTAAQI, T is replaced by M at position 138 in all HLA-A alleles, and at position 142 in some alleles, I is replaced by T. In addition, in HLA-A alleles, glutamic acid (E) at position 114 is replaced by basic amino acids, such as H, R, or Q. These acid–base shifts may alter the orientation of epitopes and affect Ab binding.

It should be noted that anti–HLA-E Abs in the sera of healthy individuals are polyclonal and not monoclonal. Therefore, one Ab may target the same epitope recognized by the murine mAb MEM-E/02. The binding of this Ab can be inhibited by the peptides constituting the epitope, whereas in another serum an anti–HLA-E Ab can target a different epitope but is also shared by HLA-E and HLA-Ia Ags. Examples are 58EYWDRER65 (B*1516) (Fig. 7), 182EPPKTHVT190 (Cw*0702) (Fig. 8), and 157RAYLED162 (B*8201) (Fig. 9). The sera of some individuals may specifically recognize one of these epitopes and not those recognized by MEM-E/02. In such instances, the sera will bind only to B*1516, Cw*0702, or B*8201. For example, sera MR022108 and MR060408 recognize only B*8201 and none of the other HLA-B or -C alleles, whereas several other sera (T75, T96, and T244) recognize B*8201 in addition to several other HLA-B and -C alleles that are similar to MEM-E/2. In this instance, the binding may not be specific to B*8201 (157RAYLED162) but may bind the discontinuous epitopes recognized by MEM-E/02 Ab.

This study categorizes the healthy sera into four groups (Fig. 11), based on polyclonality of the anti–HLA-E Abs. Group 1 (n = 74/220) has both IgG Abs to HLA-E and several HLA-Ia alleles. Group 2 contrasts with group 1 in that IgG Abs to HLA-E and HLA-Ia are absent (n = 78/220). These two groups support the contention that HLA-E Abs may account for some, if not all, of the natural HLA-Ia Abs. Group 3 (n = 34/220) has IgG Abs to HLA-E, but those to HLA-Ia are absent. There are two possibilities: the Abs to HLA-E may be reacting to a peptide that is not a sequence shared between HLA-E and any other HLA-Ia Ags (two such sequences are identified 121RSARDTA71 and 143SEQKSNDASE152) or the Abs to HLA-E may recognize HLA-Ia alleles not available on the LABScreen beads, so that HLA-Ia reactivity could not be observed. Group 4 (n = 34/220) has IgG Abs to HLA-Ia but not to HLA-E. Again, there are two possibilities: the HLA-Ia reactivity may be due to exogenous or environmental Ags (such as those of bacteria, virus and parasites) or if HLA-E Abs are not detectable in the sera of individuals who carry Abs to HLA-B or -C alleles, the Abs may be generated by sources other than HLA-E, such as microbial Ags. There is no doubt that some viral and bacterial Ags may mimic one or more HLA-Ia alleles. In such cases, epitopes are specifically shared between microbial Ags and HLA. Attributing Abs to HLA-Ia to the presence of such microbial Ags requires three important prerequisites: identifying specific peptide sequences shared by the microbial Ags and HLA-Ia molecules, distinguishing HLA class Ia alleles that express those sequences from those that do not, and using such shared sequences, once identified and synthesized, in inhibition assays similar to those reported in this article and in earlier studies (31). The percentage of inhibition would confirm whether the Abs are specifically generated in response to microbial Ags.

Innumerable studies in the literature identify such specific peptide sequences of microbial Ags in HLA class I molecules, and several investigators identified, in humans, the Abs that specifically recognize such sequences (12); however, dosimetric-inhibition studies are lacking in the literature. One cannot entirely rule out the possible existence of microbial Ags that carry the discontinuous epitopes recognized by MEM-E/02. An extensive basic local alignment search tool search may reveal such shared epitopes. Our efforts to look for the discontinuous epitome of MEM-E/02 on known microbial Ags were futile. Very few microbial Ags carry partial epitopes; therefore, there is a need to expand the basic local alignment search tool search. However, this study provides background for further investigations related to possible immune responses due to Ag mimicry by microbial peptide sequences with MHC class I and other cell-surface molecules. Alternatively, the reactivity could be due to other self-Ags, including other HLA-Ib alleles.

Finally, additional experiments are required to establish that IgG induced specifically after immunization with HLA-E in humans can react with HLA-Ia alleles. These experiments would shed light on whether the anti–HLA-E Abs in human sera are a consequence of autoimmune response to HLA-E. Although this study does not rule out the possibility that HLA-Ia reactivity observed in the sera of healthy individuals can be caused by exogenous Ags, such as microorganisms, it remains to be documented whether such Abs are capable of reacting to HLA-E. A sequential correlation between the

![Figure 11](http://www.jimmunol.org/Downloadedfrom)
formation of allo–HLA-Ia IgM and IgG Abs following pathogen infection with the appearance of HLA-E Abs would elucidate this question. This study leads to another important question: whether the nonodonor-specific Abs observed in allograft recipients (50–52) are also due to Abs produced against nonclassical HLA-Ib molecules. Experiments in progress would shed light on these issues.

In conclusion, the results of this investigation reconfirm earlier findings (12) on the occurrence of natural IgG Abs against HLA-Ia in healthy humans. The healthy individuals who carry natural anti–HLA-Ia IgG or IgM Abs do not possess matching HLA-Ia alleles; therefore, the HLA-Ia Ags expressed by healthy males may not be the causative agent for the HLA reactivity of sera. The observation that 60% of males with high levels of HLA-E IgG had IgG Abs against HLA-B and HLA-Cw, as well as that 70% of individuals who did not have anti–HLA-E IgG were devoid of IgG Abs for HLA-B and HLA-Cw alleles, favors the contention that HLA-Ia reactivity in healthy individuals could be due to HLA-E IgG Abs present in those individuals. The prevalence of HLA-Ia IgG is 84% in individuals with increased levels of HLA-E IgG. IgG and IgM isotypes specific to HLA-E and HLA-Ia alleles were identified in the sera. The different IgM/IgG ratio groups of anti–HLA-E Abs observed in normal males suggest that there may be a constant stimulation of specific B cell clones in nonalloimmunized males due to release and shedding of HLA-E. The HLA-Ia reactivity of HLA-E Abs mimics, to a major extent, the HLA-Ia reactivity of muriere–HLA-E mAbs. Because anti–HLA-E Abs in human sera are polyclonal, some of the serum Abs may bind to HLA-Ia epitopes recognized by the murine anti–HLA-E mAbs, whereas others may bind to HLA-Ia epitopes not recognized by the murine mAbs. All of these findings support the hypothesis that HLA-E Abs may account for the HLA-Ia reactivity in human sera.

Acknowledgments
We thank Dr. Jar-How Lee and Thoa Nong at One Lambda for conjugating the HLA-E with beads. Most of the technical analyses reported in this investigation were done with the help of Chien-wei Chen, former research associate. HLA-Ia IgM/IgG analyses with Lot 5 were carried out by Tho Xuan Pham, a research associate at the Terasaki Foundation Laboratory. We also thank Robert L. Schoenborg for editorial assistance with the resubmitted manuscript.

Disclosures
P.I.T. is a major shareholder of One Lambda, Inc. The other authors have no financial conflicts of interest.

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