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HLA Class I Allelic Sequence and Conformation Regulate Leukocyte Ig-Like Receptor Binding

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Leukocyte Ig-like receptors (LILRs) are a family of innate immune receptors predominantly expressed by myeloid cells that can alter the Ag presentation properties of macrophages and dendritic cells. Several LILRs bind HLA class I. Altered LILR recognition due to HLA allelic variation could be a contributing factor in disease. We comprehensively assessed LILR binding to >90 HLA class I alleles. The inhibitory receptors LILRB1 and LILRB2 varied in their level of binding to different HLA alleles, correlating in some cases with specific amino acid motifs. LILRB2 displayed the weakest binding to HLA-B*2705, an allele genetically associated with several autoimmune conditions and delayed progression of HIV infection. We also assessed the effect of HLA class I conformation on LILR binding. LILRB1 exclusively bound folded β2-microglobulin–associated class I, whereas LILRB2 bound both folded and free H chain forms. In contrast, the activating receptor LILRA1 and the soluble LILRA3 protein displayed a preference for binding to HLA-C free H chain. To our knowledge, this is the first study to identify the ligand of LILRA3. These findings support the hypothesis that LILR-mediated detection of unfolded versus folded MHC modulates immune responses during infection or inflammation. The Journal of Immunology, 2011, 186: 000–000.

Human leukocyte Ag class I proteins direct the functions of both adaptive and innate immunity through their recognition by the TCR and leukocyte receptor complex-encoded receptors, which include members of the killer Ig-like receptor (KIR) and leukocyte Ig-like receptor (LILR) families. LILRs expressed on professional APCs can influence adaptive immune responses (1) by modulating cytokine release and co-stimulatory receptor expression (2–6).

LILRs are termed activating (LILRA) or inhibitory (LILRB) on the basis of their cytoplasmic domains. Inhibitory LILRs possess a long cytoplasmic tail containing ITIMs (7–9), whereas activating LILRs possess a short cytoplasmic tail and associate with the adaptor molecule FceRγ (10, 11). The putative soluble protein LILRA3 has no known signaling ability (12, 13). The best characterized members of the LILR family are the inhibitory receptors LILRB1 (ILT2/LIR1/CD85j) and LILRB2 (ILT4/LIR2/CD85d), which recognize a wide range of classical and nonclassical HLA class I proteins (8, 9, 14–22). The activating receptor LILRA1 has been shown to bind HLA-B27, the product of which is associated with diseases such as ankylosing spondylitis (23).

Classical HLA class I proteins are highly polymorphic (24). KIRs, expressed on NK cells and some T cell subsets, recognize subsets of HLA class I alleles. Their binding may be influenced also by peptide bound to class I (25–30). KIR variation, in conjunction with that in HLA class I, is associated with diseases, including viral infections (31, 32), autoimmunity (33, 34), and complications of pregnancy (35). The broad specificity of LILRB1 and LILRB2 results from their interaction with the conserved β2-microglobulin (β2m) subunit (LILRB1) and/or the α3 domain of HLA class I (20, 36). Despite this, a study of four different HLA class I alleles indicated a range of affinities for LILRB1 and LILRB2 (21). Such differences in the affinity and avidity of ligand binding can influence signaling through LILR and the activation of APCs bearing them (5). Consequently, variations in affinity for individual HLA class I alleles might be detected by genetic association of LILR with disease (37).

Although HLA class I molecules usually require association with β2m and antigenic peptide before they can progress to the cell surface (38), they can subsequently dissociate to generate open conformers that lack peptide and/or β2m (39, 40). Unfolded HLA class I molecules are a feature of activated lymphocytes (41–47) and their recognition and functions are of growing interest (45, 48). The inhibitory receptor LILRB2 can bind to β2m-free forms of both the HLA-B27 allele as well as the nonclassical HLA-G molecule (20, 23). The activating receptor LILRA1 has also been shown to engage β2m-free forms of HLA-B27 (23).

We sought to perform what we believe to be the first comprehensive study of LILR binding to the products of different HLA class I alleles using a panel of >90 single Ag beads (SABs) (49). We also determined the binding of LILR to “unfolded” or β2m-free forms of HLA class I.

Abbreviations used in this article: FHC, free H chain; KIR, killer Ig-like receptor; LD, linkage disequilibrium; LILR, leukocyte Ig-like receptor; β2m, β2-microglobulin; MFI, mean fluorescence intensity; SAB, single Ag bead.

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Materials and Methods
Cloning of LILR sequences and production of LILR-Fc DNA constructs

Full-length LILRB1, -B2, and -A1 were amplified from macrophase cDNA using the primers listed in Supplemental Table I. RNA extraction and subsequent cDNA synthesis were performed as previously described (50). All PCRs were performed using Phusion polymerase (Finzymes) with the following cycling parameters: 2 min at 98˚C followed by 11 cycles of 98˚C for 10 s, 68˚C for 30 s, and 72˚C for 60 s, followed by 21 cycles of 98˚C for 10 s, 65˚C for 30 s, and 72˚C for 60 s, followed by 10 cycles of 98˚C for 10 s, 60˚C for 30 s, and 72˚C for 60 s. PCRs were carried out on either MJ Research (Reno, NV) Dyad DNA engines or MJ research PTC-200 thermal cyclers. PCR products were HindIII and XbaI (New England Biolabs) digested and then ligated into the p3'SFLAG-CMV-9 vector (Sigma-Alrich) using a Rapid DNA ligation kit (Roche). The full protein coding sequence of LILR A3 was amplified from dendritic cells using primers NK1076 and NK645 (Supplemental Table I) and cloned into TOPO vector (Invitrogen) following the manufacturer’s recommended protocol.

The extracellular coding region of each LILR was subsequently amplified from the cloned sequences by PCR using the appropriate primer pairs listed in Supplemental Table I. The extracellular sequence of LILRB3 was amplified from a clone supplied by Professor Marco Colonna. PCR products were ligated into the Signal plgplus vector containing the human IgG1-Fc domain (R&D Systems) following a HindIII and XbaI restriction digest. Sequences were verified by cycle sequencing using BigDye Terminator version 3.1 methodology (Applied Biosystems) and an Applied Biosystems 3730xl DNA analyzer.

Transfections and production of LILR-Fc fusion proteins

Prior to transfection, HEK293T cells were maintained in RPMI 1640 supplemented with 10% FBS and penicillin-streptomycin (50 U/ml) and incubated at 37˚C with 5% atmospheric CO2. HEK293T cells were transfected with Signal plgplus vector containing LILR-Fc coding sequence or an empty Signal plgplus vector using jetPEI transfection reagent (Polyplus Transfection), following the manufacturer’s recommended protocol. Culture media were replaced with fresh Pro293a serum-free medium (Lonza) 24 h posttransfection. Supernatants were harvested 5 d posttransfection and concentrated using an Amicon Ultra 15 centrifugal filter column (Millipore) with a nominal molecular mass limit of 30 kDa. The retentate was then washed twice with 10 ml PBS while on the filter column.

The concentration of Fc protein was assessed by ELISA.

MHC SAB binding assay

Fc-fusion proteins at a concentration of 1 μM were screened against LAMBScreen HLA class I SAB (LS1A04 lot no. 005; One Lambda, Canoga Park, CA) and HLA class II-coated beads (LSM12 lot no. 008; One Lambda), according to the manufacturer’s standardized protocol. The HLA class I SAB panel contained HLA-A (n = 31), -B (n = 50), and -C (n = 16) alleles, the protein sequences of which are provided in the Supplemental Materials section (Supplemental Fig. 12). Binding was assessed on a Luminex LABCScan 100 (One Lambda), and the median fluorescence intensity (MFI) value was obtained. For each bead, the MFI values of each LILR-Fc were normalized for background binding by subtracting the MFI values of the Fc-negative control. Results for B*3701 and B*4701 were normalized for background binding by subtracting the MFI values of the Fc-negative control.

To assess the level of β2m-associated HLA class I on each bead population, the HLA class I-specific mouse IgG1 mAb W6/32 was used and Ab binding was detected using PE-conjugated rabbit F(ab’)2 anti-mouse IgG (both from AbD Serotec, Oxford, U.K.). The level of β2m was measured using the mouse mAb BBM1 (Santa Cruz Biotechnology). The presence of HLA class I free H chain (FHC) was assessed using the mouse mAbs HC10 (51), HCA2 (52) (provided by Prof. Hidde Ploegh, Cambridge, MA), and L31 (53, 54) (provided by Dr. Patrizio Giacomini, Rome, Italy). These FHC-specific Abs vary in their recognition of allelic subsets; alleles that carry Thr or Asn at residue 80 and Arg at the mature protein are recognized by HCA2 (55). L31 recognizes alleles with Phe or Tyr (56) (prominently HLA-C alleles) (54), whereas HC10 binds strongly to alleles carrying Arg (57) (mainly HLA-B and -C alleles) (55).

To assess binding of LILR-Fc molecules to HLA class I FHC, β2m was removed from SABs by incubating the beads in pH 3.0 citrate buffer (0.131 M sodium citrate, 0.066 M sodium phosphate, and 2% BSA) for 2 min, then washing twice in PBS supplemented with 1% BSA (56).

Ab blocking assay

The HLA class I Abs W6/32 and HC10 were assessed for their ability to block LILRB1, -B2, and -A1 binding to β2m-associated and FHC forms of C*0602 expressed on stably transfected 721.221 cells. Concentrated supernatants containing 0.5 μM LILR-Fc fusion protein were incubated for 1 h at 4˚C with PE-labeled F(ab’)2 coat anti-human Fc Ab (109-116-170; Jackson ImmunoResearch Laboratories) at a concentration of 5 μg/ml. Meanwhile, 1 × 107 cells were incubated for 30 min at 4˚C with either 1 μg W6/32 (Sigma-Aldrich), 10 μl HC10 supernatant, 1 μg IgG2a isotype Ab (Sigma-Aldrich), or W6/32 and HC10 in combination and brought to a volume of 25 μl with PBS/2% BSA. Twenty-five microliters of the LILR-Fc/secondary Ab solution was then added to the cell/Ab mixture and incubated for a further 1 h at 4˚C. Cells were washed twice with PBS/2% BSA followed by a final wash in PBS. The level of LILR-Fc binding was measured using a FACSscan flow cytometer (BD Biosciences). The same procedure was performed on cells previously treated with pH 3.5 citrate buffer (0.131 M sodium citrate, 0.066M sodium phosphate, and 2% BSA) for 30 s at 4˚C followed by two washes with PBS/2% BSA to increase the level of HLA class I FHC.

Statistical analysis

The relationship between LILR-Fc binding and level of β2m-associated HLA class I (as determined by W6/32) was assessed by non-linear regression (GraphPad Prism 5) generating a curve of best fit and R2 values.

Differences in LILR-Fc binding between groups of HLA class I alleles were statistically assessed using a two-tailed Mann–Whitney U test (Graph pad 5). LILR MFI values were normalized against level of HLA class I by division using W6/32 (for β2m-associated HLA) or HC10 (for FHC) MFIs prior to statistical analysis.

Results

Differential binding of LILRA1, LILRA3, LILRB1, and LILRB2 to HLA class I locus and allele products

On the basis of sequence similarities with LILRB1, the activating receptor LILRA1 and the soluble receptor LILRA3 are predicted to engage with HLA-class I (36). LILRA1 has been shown to bind both HLA B27 (23), but as yet no other alleles of HLA-class I have been investigated and no binding studies have been reported for LILRA3. Using Fc fusion proteins, we assessed the binding of LILRA1, LILRA3, LILRB1, LILRB2, and LILRB3 to beads coated with allomorphs of either HLA class I or HLA class II. LILRA1, LILRA3, LILRB1, and LILRB2 bound HLA class I but not HLA class II. No binding of LILRB3 to either HLA class I or HLA class II was detected (data not shown). The inability of LILRB3 to bind HLA class I is in accordance with previous studies and predictions (22, 23, 36).

SABs were used next to assess the influence of isotypic and alleloypic variation of HLA class I on LILR binding. The level of HLA class I on each SAB was determined using the mAbs BBM1 (anti-β2m) and W6/32 (a pan-HLA class I Ab). Their binding profiles correlated strongly (Supplemental Fig. 1). The binding of LILRA1, LILRA3, LILRB1, and LILRB2 to the product of each HLA class I allele was compared with that of W6/32 (Supplemental Fig. 2). Of all the receptors tested, LILRB1 binding correlated best with level of HLA class I, as indicated by W6/32 reactivity (Fig. 1). However, LILRB1 binding to HLA-A alleles displayed considerable variability (Fig. 1). HLA-A alleles with Ala193 and Val194 (numbers correspond to the mature protein) were significantly associated with a lower level of LILRB1 binding (Fig. 1, Supplemental Fig. 6B). These two variable positions correspond to an established binding site of LILRB1 (36). Two further variations, serine at position 207 and glutamine at 253, are in almost complete linkage disequilibrium (LD) with Ala193 and Val194 and were also strongly associated with weaker binding (Fig. 1, Supplemental Fig. 6B). Alleles that carried a serine at position 246 also displayed lower binding, although this was less significant (Fig. 1, Supplemental Fig. 6B) and is most likely due to linkage with the Ala193 and Val194 polymorphisms.
The presence of Val194, in the absence of the other polymorphisms, as found in several HLA-B alleles (such as B*35, B*51, and B*58) and most HLA-C alleles, did not influence LILRB1 recognition. None of the HLA-A polymorphisms correlating with altered LILRB1 binding appeared to influence LILRB2 recognition (Supplemental Fig. 6C). No other HLA variation correlated with altered LILRB1 binding.

In contrast to LILRB1, there was a greater degree of variability in LILRB2 binding to SABs. This protein displayed strongest binding to HLA-A and weakest binding to a subset of HLA-B alleles, including B*2705 and B5701 (Fig. 2, Supplemental Fig. 6A). We were unable to identify any HLA-A or -B residue positions that correlated with the overall binding pattern of LILRB2. However, the presence of a cysteine at residue 1 (Cys1) and/or an aspartic acid at position 9 (Asp9) of HLA-C correlated with significantly stronger LILRB2 binding (Supplemental Fig. 7). Residue 1 is located on an exposed surface distal from the β2m and LILR binding sites, whereas position 9 is located on the β2m-pleated sheet of the α1 domain. This residue influences peptide loading within the groove. Variation at positions 1 and 9 of class I did not influence LILRB1 binding (Supplemental Fig. 7).

LILRA1-Fc and LILRA3-Fc binding profiles did not correlate with levels of HLA class I on SABs as determined by W6/32 reactivity. Both of these LILRs displayed an overall preference for HLA-C (Fig. 3A, B, Supplemental Fig. 6A), binding strongest to alleles that carry Asp9 (Supplemental Fig. 7B). Direct comparison of the HLA class I binding profiles for LILRA1 and LILRA3 revealed a strong correlation (Fig. 3C), suggesting that these two receptors share highly similar HLA recognition sites.

LILRA1, LILRA3, and LILRB2 bind HLA class I FHC

Two members of the LILR family, LILRA1 and LILRB2, have previously been shown to bind β2m-free forms of the HLA-B27 allele and the nonclassical HLA-G allele (20, 23, 57). It is possible that FHCs of other HLA class I alleles that are present on activated lymphocytes (41–47) may also act as LILR ligands. Levels of unfolded HLA-class I on SABs were determined using the Abs HCA2, HC10, and L31, all of which bind unfolded H chains of allelic subsets of HLA-class I, as described in Materials and Methods (Supplemental Fig. 3). Binding of LILRA1-Fc, LILRA3-Fc and LILRB2-Fc positively correlated with HC10 Ab staining, unlike LILRB1-Fc (Fig. 4A). To further assess LILR binding to FHCs of HLA class I, β2m was liberated from HLA class I trimeric complexes using acid treatment. Removal of β2m was confirmed by the almost complete loss of W6/32 binding (Supplemental Fig. 4). The presence of the remaining HLA class I FHC was confirmed by Ab staining (Supplemental Fig. 4). Of the
receptors tested, LILRA1, LILRA3, and LILRB2 all bound FHCs, whereas LILRB1 binding was abrogated by the loss of β2m (Supplemental Fig. 5). Interestingly, LILRA1 and -A3 displayed increased binding to SABs following the removal of β2m, whereas LILRB2 binding was slightly reduced (Fig. 4B).

Blocking with mAbs W6/32 and HC10 confirms differential LILR recognition of alternative forms of HLA class I

The Abs W6/32 and HC10 were used to block the interaction of LILR-Fc proteins to a 721.221-HLA-C*0602 transfectant, both before and after the partial removal of β2m (using a mild acid treatment) to assess further the influence of HLA class I conformation on LILR binding. W6/32 dramatically reduced the binding of LILRB1 and LILRB2-Fc to untreated cells (Fig. 5A,5C), in accordance with previous reports (12, 58), whereas HC10 had a weak effect on LILRB2 and no effect on LILRB1-Fc binding. LILRA1-Fc (and the negative control LILRB3-Fc) failed to bind cells prior to acid treatment.

The level of LILRB1-Fc binding diminished following acid treatment (reflecting the reduction in the level of β2m-associated HLA class I; Fig. 5B) and was completely abrogated by W6/32, whereas HC10 had little influence (Fig. 5A). In contrast, W6/32 had only a moderate effect on LILRB2-Fc binding to acid-treated cells, whereas HC10 displayed a greater level of blocking (Fig. 5A, 5C). The blocking of LILRB2 binding by either W6/32 or HC10 is consistent with binding of this receptor to both β2m-associated and FHC forms of HLA class I. The increased level of HC10-mediated blocking, following acid treatment, suggested that FHC was the dominant LILRB2 ligand on these cells. LILRA1 binding, which was predominantly to acid-treated cells, was greatly reduced by HC10 but was unaffected by W6/32 (Fig. 5A, 5C), suggesting that this receptor interacts with FHC.

**FIGURE 5.** Differential blocking of LILR binding by W6/32 and HC10 mAbs. W6/32, HC10, and an IgG2a isotype control were assessed for their ability to block the interaction of LILR-Fc proteins to a 721.221-HLA-C*0602 transfectant both before and after mild acid treatment. W6/32 and HC10 were used both singly and in combination. Representative results are shown in A. The effect of acid treatment on the binding of W6/32 (red), HC10 (blue), and IgG2a isotype mAb (black) are shown in B. Combined results of the blocking experiment are displayed in C. LILRA3 binding was not determined as it gave a high level of nonspecific binding to cells.
The ability of HC10 to block LILRB2 and -A1 binding to FHC suggests that some LILR contact sites are centered on the peptide-binding groove, as the HC10 binding site is located on the edge of the empty peptide-binding groove within the α1 helix.

**HLA class I variation influences LILR binding of FHC**

Using acid-treated SABs, LILRB2-Fc binding correlated well with overall level of FHC (as assessed with HC10 binding) (Fig. 6A), with a preference for the FHC of HLA-A compared with that of HLA-B and -C (Supplemental Fig. 8). Significantly, LILRA1 and -A3 displayed marked preferential binding to HLA-C FHC (Fig. 6A, Supplemental Fig. 8). These locus-specific preferences were consistent with those found prior to the acid treatment of SABs (Supplemental Fig. 6A). The binding patterns of LILRA1 and LILRA3 to FHC of all HLA class I alleles tested were highly comparable (Fig. 6B), providing further evidence that these receptors share highly similar binding sites.

The level of LILRB2 binding to HLA-A correlated with variation at positions 9, 144, and 145 of the mature protein, as normalized to a histidine at position 9, 144, and 145 of the mature protein, as normalized to the empty peptide-binding groove within the α1 helix.

**FIGURE 6.** The binding of LILRB2, -A1, and -A3 to acid treated HLA class I SABs. A, The level of LILR binding was compared with the overall level of acid-treated FHC on each bead as assessed by HC10. LILRB2 binding correlated with levels of FHC, whereas LILRA1 and -A3 displayed a preference for HLA-C. Only alleles that bound well to HC10 in addition to either L31 or HCA2 were analyzed. B, The binding patterns of LILRA1 and -A3 correlated strongly with each other but not with LILRB2. All SABs were used in this analysis.

Variation at residue 9 also appeared to influence the binding of LILRA1, -A3, and -B2 to HLA-B and HLA-C FHC. All three LILRs displayed strongest binding to HLA-B and -C alleles carrying Asp3 (namely HLA-B*0801, HLA-C*0602, C*0702, and C*1802) (Fig. 7B, Supplemental Fig. 8). However, the influence of HLA-C Asp3 was not dependent on the removal of β2m by acid treatment (Supplemental Fig. 7B).

The presence of a free cysteine at position 1 of HLA-C was also significantly associated with higher binding of LILRA1, -A3, and -B2 (Fig. 7C), as it was for LILRB2 binding prior to acid treatment (Supplemental Fig. 7A). Free cysteines enable homodimer formation for HLA-G and HLA-B27 (23, 57) and the free cysteine at position 1 of some HLA-C alleles may be acting in a similar manner. Dimerization of HLA-C could be important in vivo interactions by encouraging the clustering of HLA-C and, in doing so, maximise LILR binding, thereby increasing the avidity of the interaction with the dimeric LILR-Fc molecules.

Interestingly, most alleles carrying Cys1 are in strong LD with a polymorphism known as −35C, which is associated with delayed onset to AIDS (59). Conversely, most alleles carrying Gly3 display strong LD with the variant associated with rapid onset (termed −35T). Consequently, LILRA1 bound significantly higher to the FHC products of −35C-linked alleles (p = 0.038, Mann–Whitney U test, GraphPad Prism 5) (Supplemental Fig. 11). LILRB2 and LILRA3 also displayed preference for −35C-linked alleles (p = 0.053) (Supplemental Fig. 11).

None of the polymorphic sites that altered LILR binding of FHC occurred within the recognition sites of the mAbs used for the purpose of normalization, namely HC10, L31, and HCA2, which all bind within the α1 helix. Consequently, it is unlikely that these findings are due to altered mAb binding.

**Discussion**

We assessed the binding of four LILR proteins to a panel of classical HLA class I allelic products. LILR binding was clearly influenced by HLA allelic variation: LILRB1 exhibited a lower affinity for a subset of HLA-A alleles, LILRB2 displayed a lower affinity for several HLA-B alleles, including HLA*B2705, whereas LILRA1 and LILRA3 generally displayed a greater preference for HLA-C alleles, particularly following the removal of the free cysteine at position 1 of some HLA-C alleles by acid treatment. Additionally, LILRA1 binding was reduced for HLA-C alleles following the removal of β2m by acid treatment. Variation in HLA-B alleles, particularly following the removal of β2m by acid treatment.

Unusual recognition of individual HLA class I alleles by LILR may alter the overall balance between activating and inhibitory signals within immune cells and subsequently contribute to the development of disease. LILRB2 displayed considerably weaker binding to HLA-B*2705 on untreated SABs. B*2705 is genetically associated with several autoimmune conditions, particularly ankylosing spondylitis (60). Thus, it is possible that the comparatively weak interaction of HLA B*2705 with the inhibitory receptor LILRB2 may influence immune activity during disease. For example, as LILRs have been shown to regulate cytokine secretion, reduced LILRB2-mediated inhibition of APCs could encourage the production of inflammatory cytokines such as TNF-α that strongly contribute to the immunopathology of ankylosing spondylitis. Conversely, an increased tendency for activation could be of benefit in controlling infection: HLA-B27 is strongly associated with delayed onset of AIDS in HIV patients, as is HLA-B*5701 (61), another allele that displayed weak binding to LILRB2. These weaker LILRB2–ligand interactions may promote more effective immune responses against HIV-infected cells. This is in contrast to
HLA-B*3503, an allele associated with rapid onset of AIDS that has previously been found to bind LILRB2 more strongly than the neutral HLA-B*3501 allele. The presence of HLA-B*3503 results in greater inhibition and reduced dendritic cell responsiveness (37). Further work will explore the link between the strength of LILRB2 interaction with HLA-B27/57 and APC responses.

Variations in LILRB2 binding to HLA-B did not correlate significantly with HLA polymorphism, which is in contrast to LILRB1 binding patterns of HLA-A. It is possible that this variation resulted from other factors, such as the nature of the peptides presented by HLA class I and the levels of their stability as FHCs. The influence of bound peptide on LILRB2 binding has been previously shown for a soluble form of LILRB1 (50). LILRA3 is predicted to encode a soluble protein (64, 65) and may therefore compete with LILRA1 to provide a negative regulatory effect, as previously shown for a soluble form of LILRB1 (50). LILRA3 is not present on all leukocyte receptor complex haplotypes (64). LILRA3 deficiency has been associated with the development of Sjögren’s syndrome and multiple sclerosis (66–68).

Our results are consistent with differential LILR recognition of alternative forms of HLA class I: LILR1 binding is solely restricted to β₂m-associated HLA class I, LILRB2 is able to bind both β₂m-associated and FHC forms, whereas LILRA1 only recognizes FHC HLA class I. The LILRB1 and -B2 profiles are in accordance with their crystal structures, which revealed a β₂m requirement for LILR1, but not LILRB2, binding (20, 36, 69). The ability of LILRA1 and LILRB2 to bind FHCs of HLA-B*2705 has been reported previously (57, 70). LILRB2 also bound FHC forms of HLA-G and HLA-Cw4 (20). In this study, we show that recognition of HLA class I FHC is a feature of LILRA1, LILRA3, and LILRB2.

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class I complex (20, 36, 69). Additional interaction of the peptide-binding groove with the third and fourth Ig domains of LILR2, -A1, and -A3 cannot be ruled out, as the crystal structures of these domains have yet to be determined.

LILR activity on professional APCs can strongly influence adaptive immune responses (2, 3, 6, 71, 72). Consequently, LILR-mediated recognition of FHC could represent a novel mechanism of immune regulation by detection of altered conformation of HLA class I complexes. Increased cell surface expression of FHC is associated with the activation of lymphocytes (41–47) and potentially APCs (73). HLA-C is particularly prone to forming FHC due to its unusual stability in the absence of β2m (54, 77), and this form of HLA-C is specifically upregulated during macrophage differentiation (78). Increased levels of FHC influence HLA class I clustering on activated T cells (44, 79, 80). This clustering could enhance receptor recognition and consequent cellular activity (81).

The notion that increased FHC levels are symptomatic of cellular activation is complemented by the association of inflammatory disease activity with serum β2m levels (e.g., see Ref. 82), presumably arising from the dissociation of the HLA class I complex.

LILR-mediated detection of FHC may not be limited to direct cell–cell interactions: FHC is the dominant form of HLA class I on exosomes (83), and it constitutes the major form of soluble HLA in serum (shed from cells by the action of metalloproteases) (46, 84–86).

Our findings demonstrate an influence of allelic variation and conformation of HLA class I on LILR binding. Further work is required to assess the functional implications in immune responses.

Disclosures
The authors have no financial conflicts of interest.

References


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