KIR3DL2 Binds to HLA-B27 Dimers and Promotes the Expansion of T Cells in Ankylosing Spondylitis

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*J Immunol* published online 25 February 2013
http://www.jimmunol.org/content/early/2013/02/24/jimmunol.1202926
KIR3DL2 Binds to HLA-B27 Dimers and Free H Chains More Strongly than Other HLA Class I and Promotes the Expansion of T Cells in Ankylosing Spondylitis

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The human leukocyte Ag HLA-B27 (B27) is strongly associated with the spondyloarthritides. B27 can be expressed at the cell surface of APC as both classical β2-microglobulin–associated B27 and B27 free H chain forms (FHC), including disulfide-bonded H chain homodimers (termed B272), B27 FHC forms, but not classical B27, bind to KIR3DL2. HLA-A3, which is not associated with spondyloarthritis (SpA), is also a ligand for KIR3DL2. In this study, we show that B272 and B27 FHC bind more strongly to KIR3DL2 than other HLA-class I, including HLA-A3. B272 tetramers bind KIR3DL2-transfected cells more strongly than HLA-A3. KIR3DL2Fc bound to HLA-B27–transfected cells more strongly than to cells transfected with other HLA-class I. KIR3DL2Fc pulled down multimeric, dimeric, and monomeric FHC from HLA-B27–expressing cell lines. Binding to B272 and B27 FHC stimulated greater KIR3DL2 phosphorylation than HLA-A3. B272 and B27 FHC stimulated KIR3DL2CD3ε-transduced T cell IL-2 production to a greater extent than control HLA-class I. KIR3DL2 binding to B27 inhibited NK IFN-γ secretion and promoted greater survival of KIR3DL2+ CD4 T and NK cells than binding to other HLA-class I. KIR3DL2+ T cells from B27+ SpA patients proliferated more in response to Ag presented by syngeneic APC than the same T cell subset from healthy and disease controls. Our results suggest that expansion of KIR3DL2-expressing leukocytes observed in B27+ SpA may be explained by the stronger interaction of KIR3DL2 with B272 FHC.

The Journal of Immunology, 2013, 190: 000–000.

The online version of this article contains supplemental material.

Abbreviations used in this article: AS, ankylosing spondylitis; B27, human leukocyte Ag HLA-B27; FHC, free H chain form; KIR, killer cell Ig-like receptor family; β2m, β2-microglobulin; MFI, mean fluorescence intensity; RA, rheumatoid arthritis; SEB, staphylococcal enterotoxin B; SpA, spondyloarthritis.
have proposed that KIR3DL2-B27 interactions promote the survival of proinflammatory leukocytes in SpA (17, 18).

By contrast with HLA-B27, HLA-A3 is not strongly associated with spondyloarthritis. We hypothesized that differences between the strength of binding of B27γ and B27 FHC and HLA-A3 to KIR3DL2 could explain the differential disease association of these different class I molecules.

We predicted that stronger interactions of B27 FHC with KIR3DL2 compared with HLA-A3 and other ligands would result in stronger effects on downstream functions modulated by KIR ligation.

In this study, we compare the strength of interaction of B27γ and B27 FHC and HLA-A3 and other HLA-class I antigens with KIR3DL2. We compare KIR3DL2 binding to HLA-B27 and other HLA-class I using KIR3DL2 reporter cells and class I tetramer and KIR3DL2Fc staining of transfected cells. We also study the effect of KIR3DL2 ligation by HLA-B27 and other ligands on receptor phosphorylation, cell proliferation and survival, and cytokine production. We show that cell surface B27 FHC (which include B27γ) are ligands for KIR3DL2. KIR3DL2 bind more strongly to B27 FHC than other characterized ligands. KIR3DL2 binding to B27 FHC inhibits IFN-γ production and promotes the survival of leukocytes to a greater extent than binding to other HLA-class I, including HLA-A3.

Materials and Methods
Recombinant protein expression

Recombinant class I proteins were expressed as inclusion bodies, refolded as either heterotrimeric or homodimer complexes and purified by gel exclusion chromatography, as previously described (5). Heterotrimers of HLA-class I comprise β2m, peptide class I H chains. HLA-A3 and HLA-B27 heterotrimer and homodimer tetramers were made as previously described (16).

FACS staining with tetramers and tetramer competition experiments

Tetrameric complexes were used to stain BaF3 cells transduced with KIR3DL1 and KIR3DL2, as previously described (16). For tetramer competition experiments, cells were first stained with a saturating concentration of PE-labeled tetramer before competition at room temperature with a 2-fold excess of tetramer without fluorescent label. Subsequently, FACS staining with fluorescent-conjugated tetramer was assessed at different times after adding unlabeled tetramer.

KIR3DL2Fc expression and purification, FACS staining, and pull-downs

A KIR3DL2Fc lentiviral expression cassette was constructed by overlapping PCR to generate cDNA encoding the D0, D1, and D2 domains of KIR3DL2*0101 fused to the Fc portion of human IgG1 cloned in PHR-SIN. Lentiviruses were used to transduce 293T cells and soluble KIR3DL2Fc purified from supernatants on protein A Sepharose.

A total of 200,000 LBL.721.221 (221) cells transfected with HLA-B27 or control HLA was stained for FACS analysis with 5 μg KIR3DL2 or control DR5Fc or parental 221 cells, EBV-immortalized B cell lines, or MACS-sorted (Miltenyi Biotec) CD14+ monocytes for 24 h with/without HCl10, W6/32, ME1, or isotype control IgG1/G2a mAbs (MOPC 123 and M514-1B12; BioLegend) at 50 μg/ml. Subsequently, supernatants were harvested for IL-2 ELISA, according to the manufacturer’s instructions (eBioscience).

The tetramer and KIR3DL2 constructs used in this study are summarized in a schematic in Supplemental Fig. 1.

Generation of T and NK cell lines and clones: coculture of T or NK cell lines with HLA-B27–expressing APC

T and NK cell lines and clones were generated from FACS-sorted CD4 T cells from B27+ SpA patients and maintained, as described previously (17, 18). LBL.721.220 and LBL.721.221 parental B lymphocyte–derived cell lines (abbreviated to 220 or 221) transfected with HLA-A3, HLA-B7, HLA-B8, HLA-B27, HLA-B27C67S, HLA-B35, HLA-B44, and HLA-B27 together with human tapasin have been described previously (21, 22). CD4 T or NK cell lines were labeled with CFSE, according to the manufacturer’s instructions (Invitrogen). T and NK cell cocultures with 221 APC were set up, as previously described for 220 cells (17, 23). A total of 1 × 10^6 T or NK cells was cocultured with 0.5 × 10^5 221 cells. On day 5, cells were stained for KIR3DL2 expression with the DX31 mAb, annexin V allophycocyanin (BD Biosciences) and Pacific blue Live Dead stain (InVitrogen) in annexin V buffer, according to the manufacturer’s instructions (BD Biosciences). Alternatively, in CFSE proliferation experiments, dead cells stained with Live Dead were excluded before FACS analysis. Total viable numbers of KIR3DL2+ CD4 T cells were enumerated with fluocount beads (Beckman Coulter).

Supernatants for IFN-γ ELISA (eBioscience) were harvested from NK cells after 0.24-h stimulation in R10 medium.

Results

B27 dimer tetramers bind more strongly to KIR3DL2 than HLA-A3

HLA-class I molecules are expressed as heterotrimeric complexes with H chain, β2m, and peptide. In addition to β2m-associated heterotrimer, HLA-B27 is also expressed as β2m-FHC dimers (termed B27γ). We first compared staining of KIR3DL2-transfected BaF3 cells with HLA-A3 heterotrimers and B27γ tetramers. B27γ tetramer consistently stained KIR3DL2 transfectants more strongly than HLA-A3 heterotrimer tetramers (Fig. 1A, 1B). B27γ tetramers did not stain parental BaF3 cells (Fig. 1A). B27γ tetramers may have an enhanced avidity for KIR3DL2 because each dimer tetramer incorporates eight molecules of HLA-B27 compared with HLA-A3, which incorporates four molecules. Because of this, we stained KIR3DL2–expressing cells with nontetramerized recombinant B27 dimer, HLA-A3, or control HLA-class I protein. Subsequently, bound protein was detected by staining with extravidin PE. Recombinant B27 dimer protein stained KIR3DL2 transfectants more strongly than HLA-A3 and other HLA-class I proteins (Fig. 1C).

Next, we studied the ability of B27 dimer and HLA-A3 heterotrimer to compete for binding to KIR3DL2. We stained KIR3DL2–expressing cell lines with fluorescent-conjugated HLA-A3 heterotrimer or B27 dimer tetramers and then measured remaining bound tetramer at 30 and 60 min after adding unlabeled tetramer.

Analysis of KIR3DL2 phosphorylation

Transduced T cells were starved overnight in DMEM without supplements (D0). A total of 5 × 10^6 Jurkat cells transduced with C-terminal hemagglutinin-tagged KIR3DL2 was stimulated with 10 ng/ml staphylococcal enterotoxin E and 5 × 10^6 parental or transfected 221 cells in DMEM without supplements for 20 min prior to staining with anti-KIR3DL2 mAb for 10 min on ice (clone 1; Innate Pharma, Marseille, France). After washing in ice-cold TBS, cells were lysed in lysis buffer with phosphatase inhibitors (Thermo Scientific). KIR3DL2 was subsequently immunoprecipitated by tumbling with anti-mouse IgG Dynabeads (Dynal). Immunoprecipitates were washed and resolved by reducing SDS-PAGE. Resolved lysates were Western blotted with a mix of anti-phosphotyrosine mAbs (PY20, Invitrogen; P-Tyr 100, Cell Signaling) and were subsequently reprobed with anti-hemagglutinin mAb (HA-7; Sigma-Aldrich).

KIR3DL2CD3ε reporter cell assay

A KIR3DL2CD3ε PHR-SIN lentiviral expression cassette was generated by overlapping PCR with primers designed to amplify the extracellular and transmembrane portions of KIR3DL2*0101 and the cytoplasmic region of CD3ε. KIR3DL2CD3ε PHR-SIN has been previously described (19). Subsequently, KIR3DL2CD3ε and KIR3DL2CD3ε lentiviruses were made as described previously for LILRB2 (20).

All experiments for cytokine assay were set up in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% FCS and antibiotics (R10) (16). A total of 200,000 transduced T cells was incubated with 200,000 221B27 or parental 221 cells, EBV-immortalized B cell lines, or MACS-sorted (Miltenyi Biotec) CD14+ monocytes for 24 h with/without HCl10, W6/32, ME1, or isotype control IgG1/G2a mAbs (MOPC 123 and M514-1B12; Bio-Legend) at 50 μg/ml. Subsequently, supernatants were harvested for IL-2 ELISA, according to the manufacturer’s instructions (eBioscience).

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**FIGURE 1.** B27₂ protein and tetramers bind to KIR3DL2-transfected cells more strongly than HLA-A3. (A) Representative FACS stain of KIR3DL2-transfected Baf3 cells with saturating concentrations of B27 dimer and HLA-A3 tetramers. Geometric MFIs for staining were 206 and 52 for B27 dimer and HLA-A3 tetramer staining, respectively. Representative of one of four independent experiments. (B) Titration of PE-labeled B27₂ and HLA-A3 tetramer in FACS staining of KIR3DL2-transfected Baf3 cells. Representative stain from one of four independent experiments. (C) FACS staining of KIR3DL2 Baf3 cells with B27 dimer (B27₂), HLA-A3, HLA-B8, and HLA-B27 protein. Representative staining of one of three independent experiments. (D) B27₂ tetramers compete for binding to KIR3DL2 more strongly than HLA-A3. Left-hand top panel. B27₂ tetramer competition with PE-labeled HLA-A3 tetramer bound to KIR3DL2 Baf3 cells. Staining with HLA-A3 tetramer at 0, 30, and 60 min after addition of HLA-A3 or B27₂ tetramer. Left-hand bottom panel. HLA-A3 tetramer competition with PE-labeled B27₂ tetramer bound to KIR3DL2 Baf3 cells. Staining with B27₂ tetramer at 0, 30, and 60 min after addition of HLA-A3 or B27₂ tetramer. Results are presented as the mean reduction in the geometric MFI of tetramer staining from three independent experiments ± 1 SD.

B27₂ tetramers effectively competed HLA-A3 heterotrimer tetramer binding to KIR3DL2 transfectants, whereas HLA-A3 tetramers had little effect on B27₂ binding (Fig. 1D, upper and lower panels). B27 dimer tetramers competed for bound B27 dimer tetramer weakly. HLA-A3 tetramers also competed for bound HLA-A3 tetramer weakly (Fig. 1D). B27₂ tetramer staining of KIR3DL2-expressing cell lines was strongly inhibited by H chain mAb, which recognizes B27 dimers compared with HLA-A3
tetrimer staining, which was only weakly inhibited (HC10 or HCA2; Supplemental Fig. 1). KIR3DL2 staining by both tetramers was inhibited by anti-KIR3DL2 mAb (DX31) (Supplemental Fig. 1).

KIR3DL2 binds more strongly to cell surface B27 FHC than other HLA-class I

We next compared binding of KIR3DL2Fc fusion protein to HLA-B27 and other HLA-class I molecules. KIR3DL2 Fc consistently stained HLA-B27–transfected LBL.721.221 cells (hereafter referred to as 221B27 cells) more strongly than parental 221 cells or 221A3 and 221B35 cells (Fig. 2A, 2B). Fig. 2B shows KIR3DL2Fc staining of transfected cells expressed as a ratio with the mean fluorescence intensity (MFI) for W632 staining after subtracting the MFIs for background KIR3DL2 staining of parental 221 cells. The 221B35 cells did not stain with KIR3DL2Fc significantly above background staining of parental 221 cells. We hypothesized that the increased staining of B27-transfected cells with KIR3DL2Fc could result from KIR3DL2 binding to B27 FHC expressed by these cells (22). Thus, we stained 221B27 with KIR3DL2Fc and analyzed bound protein precipitated with protein G from cell lysates by SDS-PAGE and Western blot with HC10 mAb. Bands corresponding to B27 dimers, monomers, and multimers were detected by Western blot (Fig. 2B). No bands were detected when Western blots were reprobed with the anti-β2m Ab BBM-1 (results not shown).

We reasoned that stronger binding of B27 FHC would stimulate greater KIR3DL2 ITIM phosphorylation compared with other class I ligands. Thus, we compared KIR3DL2 tyrosine phosphorylation in superantigen-activated KIR3DL2-expressing Jurkat cells with transfected 221 cells. HLA-B27 ligation consistently stimulated greater phosphorylation of KIR3DL2 compared with HLA-A3, HLA-B35, or parental 221 cells (Fig. 2C).

We then determined whether the stronger binding of B27 dimers to KIR3DL2 in vitro could be translated into differences in functional interactions with this receptor. We transduced Jurkat T cells with KIR3DL2Cd3e and measured functional interactions with plate-immobilized recombinant B27 dimers and HLA-class I heterotrimers by measuring IL-2 production by ELISA. B27 dimers, but not control HLA-B27 heterotrimers, consistently stimulated IL-2 production above background levels in the presence of PMA and anti-CD28 (Fig. 3A). Equivalent quantities of immobilized B27 dimer consistently stimulated greater production of IL-2 than HLA-A3 heterotrimers.

Subsequently, we studied whether cell surface HLA-B27 bound to KIR3DL2Cd3e–Jurkat T cells. The 221B27 cells stimulated greater IL-2 secretion than 221A2, 221A3, 221B7, 221B35, 221B27C67S, and parental 221 cells (Fig. 3B). HLA-class I transfectants expressed similar quantities of class I, as assessed by FACS staining with W632 mAb (Supplemental Fig. 2). Parental Jurkat T cells and KIR3DL2Cd3e–transduced Jurkat T cells produced negligible quantities of IL-2 in response to these stimuli (Supplemental Fig. 3). IL-2 production by KIR3DL2Cd3e–transduced Jurkat T stimulated with 221B27 cells was inhibited by KIR3DL2-specific mAb (DX31), H chain (HC10), and W632 Abs (Fig. 3B). By contrast, the B27-specific Ab ME1 had no significant effect (Fig. 3B).

LBL.721.220 cells transfected with HLA-B27 (hereafter referred to as 220B27) also express cell surface B27 H chain dimers and multimers (22). These cells lack functional human tapasin and as a consequence form more unstable B27 heterotrimers on their cell surface. B27 dimers and multimers form from unstable cell surface B27 heterotrimers (22). Supertransfection of 220B27 cells with human tapasin (220B27huTPN), which optimizes peptide cargo and stabilizes B27 heterotrimers, reduces the level of cell surface B27 dimer expression (16). As a consequence, 220B27huTPN cells...
express higher levels of ME1 and W632-reactive B27 and lower levels of HC10-reactive B27 FHC. As expected, 220B27 cells stimulated production of IL-2 by KIR3DL2CD3ε-transduced T cells to a greater extent than parental 220 cells or 220 cells transfected with control HLA-A1, HLA-B8, or HLA-A3. Results presented as mean values from triplicate samples (pg/ml) ± 1 SD. (C) IL-2 production by KIR3DL2CD3ε-transduced T cells stimulated with 221B27 cells with HC10, W632, ME1, and IgG2a and IgG1 isotype control mAbs or the anti-KIR3DL2 mAb (DX31). Results presented as mean values from triplicate samples (pg/ml) ± 1 SD are representative of four independent experiments. (D) IL-2 production by KIR3DL2CD3ε-transduced T cells stimulated with parental LBL.221.220 cells, 220B8, 220B27 cells with HC10, W632, ME1, and IgG2a and IgG1 isotype control mAbs and 220B27 cells transfected with human tapasin (HuTPN). Results presented as mean values from triplicate samples (pg/ml) ± 1 SD are representative of four independent experiments.

We have shown that B27 dimers and H chains are expressed by APC in B27⁺ SpA patient peripheral blood and synovial fluid (5, 7). We sought to determine whether HLA-class I on the surface of APC from B27⁺ SpA patients and B27⁺ healthy controls could interact with KIR3DL2 on KIR3DL2CD3ε Jurkat T cells. The representative FACS stains in Fig. 4A and 4B show the relative levels of expression of HC10 and W632-reactive forms of HLA-class I by EBV-transformed B cells and purified monocytes from B27⁺ individuals. B cell lines and monocyte lines from B27⁺ healthy controls and B27⁺ SpA patients both stimulated IL-2 production by KIR3DL2CD3ε-transduced T cells (Fig. 4C, 4D). Reporter cell IL-2 production was inhibited by HC10 and W632 mAbs, but not isotype control mAb (Fig. 4A, 4B). B cell lines from healthy controls also weakly stimulated IL-2 production by KIR3DL2CD3ε-transduced T cells (data not shown).

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Ligation of KIR3DL2 by HLA-B27 inhibits NK IFN-γ production and promotes NK cell survival to a greater extent than binding to HLA-A3, HLA-B7, and HLA-B35

We next investigated the effect of KIR3DL2 binding to HLA-B27 on cytokine production and survival of primary NK cells. The 221B27 cells inhibited the production of IFN-γ by KIR3DL2-expressing NK cells to a greater extent than cells expressing control HLA-class I (Fig. 5A). Inhibition of NK cytokine production by 221B27 cells was reduced by anti-KIR3DL2 and HC10 mAbs, but not isotype control mAb (Fig. 5B). Stimulation with 221B27 cells promoted the survival of KIR3DL2-expressing NK cells to a greater degree than parental 221 cells and cells expressing control HLA-class I (Fig. 5C). The effect of 221B27 cells in promoting NK cell survival could be inhibited by anti-class I (HC10 and W632) and anti-KIR3DL2 (DX31) mAbs (Fig. 5D).

Stimulation with HLA-B27 enhances the survival of KIR3DL2+ CD4 T cells compared with stimulation with HLA-A3 and other HLA-class I: increased expansion of Ag-stimulated KIR3DL2+ T cells in B27+ SpA patients

We next studied proliferation and survival of primary KIR3DL2+ CD4 T cells, stimulated with 221B27 cells by CFSE dilution. The 221B27 cells promoted the survival of staphylococcal enterotoxin B (SEB)-activated KIR3DL2+ CD4 T cells to a greater degree than parental 221 and 221A3 (Fig. 6A). Superantigen-driven proliferation and survival of KIR3DL2+ CD4 T cells with 221B27-transfected cells were inhibited with anti-KIR3DL2 (DX31), anti-class I H chain (HC10), and W632 mAbs, but not isotype control IgG2a mAb (Fig. 6B).

We have previously shown increased proportions of CD4 T cells expressing KIR3DL2 in B27+ SpA patients (17, 23). We hypothesized HLA-B27 on APC could promote the survival of Ag-stimulated KIR3DL2+expressing leukocytes in ankylosing spondylitis (AS).

Thus, we compared the survival of CFSE-labeled KIR3DL2+ CD4 T cells stimulated with superantigen presented by APCs in PBMC samples from B27+ SpA patients and healthy B27+ and B27− controls and patients with rheumatoid arthritis (RA). PBMC were labeled with CFSE and stimulated with SEB for 5 d. Viable proliferating CD4 T cells expressing KIR3DL2 or other KIR were determined by FACS staining.
We observed increased numbers of viable proliferating KIR3DL2+ CD4 T cells from SpA patients after stimulation with superantigen compared with KIR3DL2 CD4 T cells from healthy and RA disease controls (Fig. 6C). By contrast, we did not see similar expansions of CD4 T cells expressing other KIR (Supplemental Fig. 4A). Because of variability in the percentages of T cells expressing KIR and responding to superantigen between individuals, we counted numbers of KIR3DL2+ CD4 T cells before and after stimulation using fluocount beads. We then compared the fold increase in KIR3DL2+ CD4 T cells between AS patients and controls when cells were stimulated with superantigen. Fig. 6D shows that there was a greater increase in numbers of KIR3DL2 CD4 T cells in patients compared with B27+ healthy controls and RA disease controls. Expansion of KIR3DL2+ CD4 T cells in AS patients was not due to Vβ bias toward SEB-binding Vβ in this population in patients as FACS staining showed no enrichment for SEB-reactive Vβ in patients (Supplemental Fig. 4B). We also observed a greater fold increase in the number of CD4-negative T cells between patients and controls (Supplemental Fig. 4C). SEB-driven expansion of KIR3DL2+ CD4 T cells in AS patients was inhibited by anti-KIR3DL2 mAb (DX31) and B27 FHC-specific mAb (HD6) (Supplemental Fig. 4D). By contrast, B27 FHC-specific mAb had little effect on expansion of KIR3DL2+ CD4 T cells in B27+ healthy controls (Supplemental Fig. 4D).

Discussion

In this study, we show that KIR3DL2 binds more strongly to HLA-B27 FHC species (which include B27 dimers) than to HLA-A3 and other HLA-class I. B27 dimer tetramers bound more strongly to KIR3DL2 and competed more effectively for binding to KIR3DL2 than HLA-A3 tetramers, suggesting that B27 dimers are stronger ligands. The fact that nontetramerized B27 dimer protein also bound more strongly to KIR3DL2-expressing cell lines than HLA-A3 suggests that this was not simply due to differences in tetramer stoichiometry. Moreover, B27 dimer tetramer binding to KIR3DL2 was strongly inhibited by the H chain–specific monoclonal HC10. The HLA-A H chain mAbs HCA2 and HC10 mAb only slightly reduced HLA-A3 binding to KIR3DL2, suggesting that binding of B27 FHC and HLA-A3 to this receptor is distinct. HCA2 binds within and HC10 binds close to a region of HLA-class 1, which binds to the D0 domain of KIR3DL1 (23-25). KIR3DL1 has a related structure to KIR3DL2. KIR3DL2Fc stained 221B7 cells more strongly than HLA-A3 and control HLA-class I–transfected cells.

We also observed greater phosphorylation of KIR3DL2 when T cells were stimulated with 221B7 cells compared with stimulation with parental 221, 221A3, and 221B35 cells. This increased phosphorylation of KIR3DL2 ITIMs by B27 also suggests stronger binding compared with other HLA-class I.

Recombinant B27 dimers stimulated greater production of IL-2 by KIR3DL2 reporter cells compared with HLA-A3 and HLA-B27 heterotrimers. Moreover, 221B7 cells stimulated greater production of IL-2 by KIR3DL2CDε reporter cells compared with 221A3, parental 221 cells, and control HLA-class I–transfected cells. The 221A3 cells stimulated production of more IL-2 by KIR3DL2CDε-reporter cells compared with stimulation with 221 cells expressing control HLA-class I, but not HLA-B27. These results suggest that peptide MHC complexes and possibly other forms of HLA-A3 are weaker ligands for KIR3DL2 than HLA-B27 H chain forms.

The 220B27 cells also stimulated greater production of IL-2 by KIR3DL2CDε-transduced T cells compared with cells expressing control HLA-class I. The 220B27 cells lack functional tapasin, which optimizes peptides bound to HLA-class I. We have shown that B27 FHC form from β₂m-associated HLA-B27 with suboptimal peptides (22). Thus, 220B27 cells express increased levels of B27 dimers and other FHC species because of the presence of more B27 peptide MHC complexes suboptimally loaded with peptide. Consistent with this hypothesis, 220B27 cells transfected with human tapasin stimulated less IL-2 production by reporter cells compared with 220B27 cells.

The 221B7 cells inhibited IFN-γ production by KIR3DL2-expressing NK cells to a greater extent than cells expressing other HLA-class I. The 221B7 inhibition of IFN-γ production was reduced by H chain and anti-KIR3DL2 Abs, suggesting that at least part of this effect was due to KIR3DL2 binding to B27 FHC species. The 221B7 cells also promoted the survival of KIR3DL2-expressing NK and T cells more strongly than HLA-A3 and other HLA-class I–transfected cells. We have shown that KIR3DL2-expressing leukocytes are expanded in B27+ spondyloarthropathy patients (17, 18). KIR ligation inhibits activation-induced cell death of leukocytes (17, 26). The increased proportions of KIR3DL2+ expressing leukocytes in SpA patients could thus be due to stronger KIR3DL2 binding to B27 promoting immune cell survival.

Whereas HLA-A3 binding to KIR3DL2 is dependent on the sequence of complexed peptide, B27 dimers bind to KIR3DL2 in a peptide-independent fashion (14, 16). This could also result in higher densities of B27 ligand at the surface of APC available for binding to KIR3DL2 compared with HLA-A3. Higher densities of ligand could also result in stronger interactions with KIR3DL2. HLA-A3–expressing individuals have NK cells with poor effector function, suggesting that HLA-A3 may only interact weakly with KIR3DL2 in vivo in “licensing” NK cells (27). Our findings suggest that studying how HLA-B27 expression could “license” better KIR3DL2 NK cell effector function merits further investigation.

We hypothesize that KIR3DL2-B27 interactions could promote the expansion of T cells and other leukocytes in vivo. In support of this, superantigen-activated KIR3DL2–expressing T cells in PBMC samples from SpA patients proliferated more than the same immune subset in control samples. We observed greater proliferation of both CD4+ and CD4− T cells expressing KIR3DL2 in SpA patients. Although we have previously shown enrichment for IL-23R expression on KIR3DL2-expressing CD4 T cells, we did not observe similar enrichment on CD4+ T (18). This suggests that the increased expansion of KIR3DL2 T cells in these assays is not simply because of increased levels of IL-23 in SpA patients promoting the survival of these cells. We did not observe significantly increased proliferation of KIR3DL2+ expressing T cells from B27+ healthy controls compared with other control samples. We have previously reported intermediate proportions of KIR3DL2+ CD4 T cells in healthy B27+ controls compared with B27− controls (18). The difference in proliferation of KIR3DL2 CD4 T cells between B27+ SpA patients and B27− controls might be explained by higher levels of expression of B27 FHC ligands for KIR3DL2 expressed by APC from B27+ SpA. Indeed, in support of this, we and others have reported higher levels of expression of B27 and other FHC on the surface of peripheral blood monocytes from B27+ SpA patients (5, 7, 28, 29).

KIR3DL2CDε T cell interactions with B27 were inhibited by HC10, W632, and HD6, but not by ME1 Abs (this study) (7). W632 and HD6 Abs inhibited KIR3DL2 binding more weakly than HC10 in these assays. HC10 was most effective at inhibiting KIR3DL2-expressing T cell interactions, suggesting that the different inhibitory Abs recognize different B27 species that share the HC10 epitope. By contrast, ME1 recognizes a population of β₂m-associated HLA-B27 molecules and did not inhibit KIR3DL2 interactions. W632 and ME1 recognize distinct epitopes of β₂m-associated HLA-class I. W632 recognizes a conformational-dependent epi-
tope in the α2 and 3 domains of HLA-class I close to residues 86 and 121 of class I H chains (30). By contrast, ME1 binds to a conformational βm-dependent epitope in the α1 domain of HLA-B27 that is critically dependent on interactions between residues 67 and 71 (31). W632 has also been reported to recognize a population of B27 dimers on transfected cells and βm-free HLA class I on transfected and Daudi cells, but does not inhibit B27 dimer tetramer binding to KIR3DL2 (32, 33).

Our results suggest that B27 FHC ligands for KIR3DL2 are heterogeneous. KIR3DL2Fc pulled down monomeric, dimeric, and multimeric H chain forms from B27-expressing cells. Dimerization and multimerization of B27 H chains could increase the binding avidity for KIR3DL2. Alternatively, monomeric B27 H chains could bind more strongly to this receptor than other HLA-class I H chains.

Monocytes and B cells from B27+ SpA patients and B27+ healthy controls stimulated production of IL-2 by KIR3DL2-CD3ε-transduced T cells, which was inhibited by HC10 and W632 mAbs. Thus, reduced T-cell responses against viral and self-epitopes and HLA-B27 subtypes differentially associated with ankylosing spondylitis.

One possibility is that KIR3DL2 ligation could divert the pro-inflammatory cell signaling pathways mediating spondylarthropathies and are ligands for paired Ig-like receptors. Immunol. Lett. 16: 1699–1710.


Disclosures

The authors have no financial conflicts of interest.

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