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**REVIEW ARTICLE**

# HLA-C: An Accomplice in Rheumatic Diseases

Ruby J. Siegel,[1] S. Louis Bridges Jr,[2] and Salahuddin Ahmed[1,3]

Human leukocyte antigen c (HLA-C) is a polymorphic membrane protein encoded by the HLA-C gene in the class I major histocompatibility complex. HLA-C plays an essential role in protection against cancer and viruses but has also been implicated in allograft rejection, preeclampsia, and autoimmune disease. This review summarizes reports and proposed mechanisms for the accessory role of HLA-C in rheumatic diseases. Historically, contributions of HLA-C to rheumatic diseases were eclipsed by the stronger association with HLA-DRB1 alleles containing the "shared epitope" with rheumatoid arthritis. Larger genetic association studies and more powerful analytical approaches have revealed independent associations of HLA-C with rheumatic disease–associated phenotypes, including development of anticitrullinated peptide antibodies. HLA-C functions by presenting antigens to T cells and by binding activatory and inhibitory receptors on natural killer (NK) cells, but the exact mechanisms by which the HLA-C locus contributes to autoimmunity are largely undefined. Studies have suggested that HLA-C and NK cell receptor polymorphisms may predict responsiveness to pharmacotherapy. Understanding the mechanisms of the role of HLA-C in rheumatic disease could uncover therapeutic targets or guide precision pharmacologic treatments.

## INTRODUCTION

By definition, rheumatic diseases are those that cause chronic and progressive damage to joints and connective tissue (1). According to a 2010 World Health Organization (WHO) Global Burden of disease study, rheumatic and musculoskeletal diseases are the second leading cause of disability worldwide, affecting two billion people (2). In 2013, arthritis-attributable medical expenses and loss of earnings reached 303 billion dollars annually in the United States alone (3).

In many rheumatic diseases, genetic and environmental risk factors interact to incite and perpetuate the development of autoimmunity. The strong association between the major histocompatibility complex (MHC) genomic region and autoimmune diseases has been established for over 50 years (4). Located within the MHC, human leukocyte antigen (HLA)-C gene has shown independent association with specific subsets of rheumatoid arthritis (RA) as well as psoriatic arthritis (PsA) (5), ankylosing spondylitis (6), systemic lupus erythematosus (SLE) (7) and Crohn's disease (8). HLA-C molecules leverage both the adaptive and innate arms of the immune system in contrast with HLA-DRB1, which has no innate immune functions. HLA-C and other class I molecules contribute to adaptive immunity by binding intracellularly derived peptides and presenting them to $CD8^+$ cytotoxic T cells. HLA-C also drives innate immunity by activating or inhibiting natural killer (NK) cells through interactions with killer immunoglobulin-like receptors (KIR) (9).

Numerous genome-wide association studies (GWAS) have advanced our understanding on the pathogenesis of rheumatic diseases. For example, a GWAS meta-analysis with autoantibody-positive RA patients identified the top-ranked gene module including (in order of significance): TAP2, HLA-C, HLA-A, TAPBP, and LILRB1 genes (10).

## HLA-C

The HLA-C gene is located within the MHC on chromosome 6p21.33 (https://www.ncbi.nlm.nih.gov/gene/3107). The MHC is a gene-rich region containing at least 269 loci and includes genes encoding HLA class I (HLA-A, -B, and -C), nonclassical class I (HLA-E, -F, and -G), class I–like molecules (MICA and MICB), and HLA class II (HLA-DR, -DQ, and -DP) molecules (11). HLA-C is a heterodimeric class I molecule consisting of a heavy chain and a light chain (beta-2 microglobulin). The heavy chain is encoded by

[1]Ruby J. Siegel, MS: Washington State University College of Pharmacy, Spokane; [2]S. Louis Bridges, Jr, MD, PhD: University of Alabama at Birmingham; [3]Salahuddin Ahmed, PhD: Washington State University College of Pharmacy and Pharmaceutical Sciences, Spokane, Washington; and University of Washington School of Medicine, Seattle, Washington.

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Address correspondence to Salahuddin Ahmed, PhD, Department of Pharmaceutical Sciences, Washington State University College of Pharmacy, SPBS Room 411, 412 East Spokane Falls Boulevard, Spokane, WA 99210. E-mail: salah.ahmed@wsu.edu.

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eight exons; polymorphisms within exon 2 and exon 3 are responsible for the peptide binding specificity of each class I molecule.

Most genes in the MHC are highly polymorphic, especially in the sequences encoding the peptide-binding groove of the classical HLA. Tens of millions of individuals have been HLA genotyped for enrollment in bone marrow donor registries or for solid organ transplantation. Such a high volume of molecular genotyping, combined with advances in DNA sequencing technology, continues to reveal an exponentially growing number of unique alleles at each locus. There is evidence that pathogen-mediated selection has driven the evolution of polymorphisms in HLA-A, -B, and -C (12). Genetic diversity in the classical and nonclassical MHC ensures that human immune systems have nearly infinite potential to bind and recognize any foreign agents, pathogenic or altered-self peptides, and carbohydrates or lipids.

## DIFFERENCES BETWEEN HLA CLASS I AND II IN RHEUMATIC DISEASES

The potential mechanisms by which HLA-C contributes to rheumatic disease pathogenesis have not been elucidated. The best-known MHC associations with rheumatic disease include HLA-B27 with ankylosing spondylitis and a group of HLA-DRB1 alleles that share a five amino acid motif known as the "shared epitope," which strongly correlates with RA (4).

The role of HLA-C is likely to resemble that of its fellow class I HLA-B27 and diverge from that of HLA-DRB1 because of several inherent differences between MHC class I and II molecules.

**Type of peptides presented.** Both HLA-C and HLA-DRB1 are cell-surface glycoproteins whose role includes initiating the adaptive immune response by binding and presenting peptide antigens to T lymphocytes. The peptides bound by HLA class I represent the array of protein products currently being synthesized in a cell, derived from protein degradation in the ubiquitin proteasome system (13). Like other class II molecules, HLA-DRB1 presents extracellularly derived peptides to $CD4^+$ helper T cells. These complementary routes of antigen presentation offer protection from intracellular threats, like viruses or mutation, and extracellular threats, such as toxins or pathogenic microbes.

**Expression.** HLA class I molecules are constitutively expressed on nearly all nucleated cells in the body, whereas class II molecules are only constitutively expressed on thymic epithelial cells and professional antigen-presenting cells, such as macrophages, dendritic cells, and B cells (14). Expression levels of HLA-C have effects that demonstrate its pivotal role in immunity: a lone single nucleotide polymorphism (SNP) that correlates with increased HLA-C expression is associated with better control of human immunodeficiency virus (HIV) levels but greater risk of Crohn's disease (15).

**Protein structure.** Class I and class II HLA differ in protein conformations, with functional implications. HLA-C and the other class I molecules are heterodimers consisting of one transmembrane HLA heavy chain noncovalently bound to the nonpolymorphic β2-microglobulin (β2m) for stability (11). HLA-DRB1 and the other class II molecules consist of two polypeptides, the α and β chains, both of which are anchored in the cell membrane (11). Mature HLA class I, but not class II, exhibit the ability to dissociate from bound peptide and β2m to become an "open conformer" also known as a "free heavy chain" at the cell surface (16). Although no studies have directly investigated the role of these alternate conformations of HLA-C in rheumatic diseases, there are parallels with putative mechanisms for HLA-B27 in ankylosing spondylitis.

## GWAS AND RELATED APPROACHES

Most rheumatic diseases are complex phenotypes that make the search for high effect-size genetic determinants difficult. These diseases often result from the cumulative weak contributions of a constellation of gene variants, so that the identification of any individual risk allele requires a sizable study cohort and sophisticated analysis algorithms (17). After the association between HLA and RA was first described in the late 1970s (18–20), the vast majority of studies focused on the HLA-DRB1 locus, whereas other loci with potentially smaller contributions remain underinvestigated. Strong linkage disequilibrium across the human MHC has made it a challenge to determine the individual contribution of other HLA loci, including HLA-C (21). Jawaheer et al published one of the first studies that identified, in addition to HLA-DRB1, an independent significant genetic association between rheumatism and markers present in the genomic region extending from HLA-C to the C4 gene (22). Although these markers were identified on a segment of a highly conserved ancestral A1-B8-DR3 haplotype, other disease-associated haplotypes lacking DRB1*03 tended to include these non-DRB1 markers.

Advances in DNA sequencing technology and bioinformatics have made GWAS popular, thereby advancing our understanding of conditions with complex heritability (23). In GWAS, hundreds of thousands of SNPs are tested simultaneously in a large number of cases and controls in association with disease phenotype to determine the individual contribution of each genetic variant (24). Univariate regression is commonly used to analyze individual SNP associations in GWAS. Such an approach, which determines the marginal (or independent) association of each SNP with the disease, works best for conditions with a single causal variant or multiple unlinked variants (25). The heritability of complex diseases such as RA is still largely undefined. Explanations for the observed "missing heritability" (26) include epigenetic effects, gene-environment, gene-gene, and protein-protein

interactions (PPIs), plus structural variants not identified on SNP genotyping platforms.

Gene association networks may provide insight into gene-gene interactions, which are important in the development of RA. To this end, Huang et al applied an analytical method called backward genotype trait association (BGTA) to a large data set from the North American Rheumatoid Arthritis Consortium (NARAC) (27). The investigators focused on RA candidate gene regions based on a literature search rather than scanning the whole genome. The BGTA method captures interactions between markers associated with a disease when used to evaluate unphased multilocus genotypes from case-control data (28). The authors analyzed two partially overlapping sets of candidate genes. In gene set 1, the authors studied 531 SNPs from 27 candidate genes identified from the RA literature to discover a strong independent association of RA with HLA-DRB1. Analysis of gene set 2 focused on gene-gene interactions and consisted of 24 candidate genes (336 SNPs), including 17 genes from gene set 1 and 7 additional genes from the literature. HLA-C, -B, and -A were found within a node of the RA gene association network, with significant interactions among themselves and with macrophage migration inhibitory factor, vascular endothelial growth factor, and NF-kappa-B inhibitor-like protein 1 (NFKBIL1).

A recent investigation by Xiao et al incorporated information from gene expression microarray and PPI networks generated by the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database into the analysis of GWAS data to successfully identify several interacting gene modules associated with RA (10). Subjects in the GWAS meta-analysis included 5539 autoantibody-positive RA patients along with 20 169 healthy controls. For RA synovium, the top-ranked gene module included TAP2, HLA-C, HLA-A, TAPBP, and LILRB1 genes, in order of significance. Functional enrichment analysis of the highest ranked gene modules emphasized the role of antigen processing and presentation by HLA class I in disease pathogenesis at the RA synovium.

Another study utilized a related approach to identify significantly RA-associated SNPs in the Wellcome Trust Case Control Consortium RA data set (25 176 SNPs). The analytical method, as described elsewhere (29,30), combines functional genomic information of a SNP with the *P* value of a SNP from a GWAS study. Results of the study identified five gene subnetworks associated with RA in the human PPI network. In the top-scoring subnetwork associated with RA, HLA-C was found in overrepresented Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. HLA-C–related SNPs were identified in relationship with cell adhesion molecules and antigen processing and presentation pathways. Interestingly, HLA-C was among the experimentally verified RA-associated genes and pathways.

More than one genetic association study has implicated SNPs clustered within and around the HLA-C locus with anticitrullinated peptide antibody (ACPA) positivity. Lee et al conducted a study including subjects of self-reported European-American ancestry from multiple clinical consortia, analyzing 1769 SNPs across the MHC region in 869 $ACPA^+$ cases and 1193 controls (21). Of all significant associations with RA in $ACPA^+$ cases, the analysis confirmed that the strongest were SNPs near the HLA-DRB1 locus. Notably, results of the study also revealed at least two regions of significant association in the HLA class I region, independent of the DRB1 genotype. The greatest non-DRB1 associations were found for SNP alleles in the conserved HLA A1-B8-DR3 haplotype, clustered around the HLA-C locus, with maximal signals found within the HLA-C locus itself. A subsequent study by Nordang et al included an additional study arm of $ACPA^-$ patients along with $ACPA^+$ patients and healthy controls (31). Eleven SNPs and one insertion/deletion were analyzed for association with ACPA. Subjects were all Norwegian (950 RA patients and 933 healthy controls) using Illumina's 550K Beadchip. The association of HLA-C with $ACPA^+$ RA showed statistical significance ($P = 1.10 \times 10^{-15}$) beyond the threshold used in GWAS studies. None of the SNPs analyzed were common to the two above studies.

## HLA-C AND NK CELL INTERACTIONS IN RHEUMATIC DISEASES

Interestingly, NK cells have been shown to play either pathogenic or protective roles in rheumatic diseases (32). They can contribute to pathogenesis by producing proinflammatory cytokines, releasing factors that induce osteoclast differentiation, contributing to B-cell activation and class switching, promoting dendritic cell maturation, co-stimulating T and B lymphocytes, activating monocytes, and stimulating proinflammatory cytokine production by synovial fibroblasts (32). However, NK cells can also regulate adaptive immune responses through cytotoxic deletion of activated T cells (33,34). Furthermore, NK cell production of interferon gamma appears to inhibit the differentiation of Th17 cells that contribute to rheumatic disease activity (35,36). NK cells can be distinguished into two groups: $CD56^{dim}CD16^+$ and $CD56^{bright}CD16^-$. Of these two types, only the $CD56^{dim}CD16^+$ cells express KIR (37), and thus would be subject to HLA-C activation or inhibition. NK cell activation is governed by the balance of activating and inhibitory receptors interacting with ligands expressed on a target cell (32). There is an apparent continuum of KIR and HLA genotypes that predispose an individual's NK cells toward inhibition or activation. An NK cell "activating" genotype is associated with better control of viral infections and tumorigenesis but also confers a greater risk of autoimmunity (38).

HLA-C and KIR interactions play a dominant role in controlling human NK cell activity, as shown through a number of evolutionary, genetic, and functional studies (39,40). The polygenic and polymorphic KIR are encoded on chromosome 19q13.4 (41). Because the inheritance of KIR is independent from that of HLA, this allows for inheritance of all possible acti-

vating/inhibitory receptor and ligand combinations in any given individual. Here we discuss the association between HLA-C and autoimmunity as well as response to therapies through its role as KIR ligand.

HLA-C molecules are divided into two mutually exclusive groups based on the presence of a lysine/asparagine residue at position 80 of the α-1 domain, which determines KIR receptor specificity. Group 1 HLA-C molecules with an asparagine at position 80 includes HLA-C alleles *01, *03, *07, *08, *12, *13, *14, and *16:01. Alternatively, group 2 HLA-C molecules with a lysine at position 80 includes HLA-C alleles *02, *04, *05, *06, *15, *16:02, *17, and *18 (42). Each individual inherits two HLA-C alleles that may belong to group C1, C2, or one to each. The important role of HLA-C is reinforced by the fact that KIRs recognize 100% of HLA-C types but only a minority subset of HLA-A and -B types (39). HLA-DRB1 are not ligands for NK receptors.

Yen et al conducted association studies to test the hypothesis that KIR receptors and their corresponding HLA ligands influence susceptibility to RA vasculitis (43). Genotypes of activating receptor genes KIR2DS1 and KIR2DS2 were evaluated in patients with uncomplicated RA, patients with rheumatoid vasculitis, and healthy controls. Although these activating KIR receptors were not significantly associated with overall risk of RA, KIR2DS2 was strongly associated with development of RA vasculitis. The investigators also compared HLA-C allele frequencies between RA vasculitis patients and healthy controls. Among RA patients with vasculitis, HLA-C*04 showed diminished frequency (odds ratio 0.21, $P = 0.033$), whereas HLA-C*05 alleles increased by four-fold (odds ratio 5.71, $P < 0.001$) and HLA-C*03 alleles almost doubled in frequency, with 60% of patients typing positive (odds ratio 3.74, $P = 0.003$).

A recent study of Crohn's disease in a Spanish population showed significantly higher frequency of KIR2DL2/2DL3 genotype in patients compared with healthy controls (44). Furthermore, the combination of KIR2DL3 and homozygosity of the corresponding ligand HLA C1 was significantly increased in the Crohn's disease patient cohort. In terms of inhibitory KIR genotypes, the KIR2DL3/HLA-C1 is a weaker inhibitory combination than KIR2DL1/HLA-C2 or KIR2DL2/HLA-C1 (45). The authors speculated that the weaker inhibition of NK cells combined with failure to clear the inciting organisms could result in chronic inflammation. Dysregulated responses to commensal enteric bacteria and defects in clearance of pathogenic enteric microbes have been implicated in the pathogenesis of Crohn's disease (46). Lowe et al showed that a particular HLA-C and KIR combination—absence of the inhibitory receptor KIR2DL2 combined with presence of the activating receptor KIR2DS2 and its specific HLA-C ligand—was a predisposing factor for primary Sjogren's syndrome (47).

NK cells, KIR receptors, and HLA-C ligands appear to play a role in response to pharmacologic therapies for rheumatic diseases. A 2015 study in RA patients demonstrated differences in the baseline NK cell subset proportions and subsequent changes during 3 months of anti-IL6 tocilizumab therapy (48). The investigators found that neither raw nor adjusted NK cell proportions differed between healthy controls and RA patients, implying that NK cell deficiency was not a risk factor for RA. However, among RA patients at baseline, the proportion of $CD56^{dim}CD16^{+}CD3^{-}$ NK cells correlated inversely with the 28-joint Disease Activity Score (DAS-28). The authors interpreted these findings to suggest that there may be deficient regulatory activity of the $CD56^{dim}CD16^{+}$ NK cells in the context of RA. Over three months of tocilizumab treatment, reduced DAS-28 correlated with a decrease in the proportion of $CD56^{bright}CD16^{-}$ NK cells, and a corresponding increase in perforin and granzyme content associated with $CD56^{dim}CD16^{+}$ NK cells (48). Thus, it appears that the $CD56^{dim}CD16^{+}$ subset of NK cells that express KIR and fall subject to HLA-C regulation may play an immunoregulatory role in RA and contribute to the efficacy of tocilizumab therapy. Additional studies have shown associations between KIR and HLA-C genotypes with response to methotrexate (49) and anti–tumor necrosis factor therapy (50) in RA patients.

## HLA-C ASSOCIATIONS WITH PsA

Approximately one-third of patients with psoriasis vulgaris (commonly referred to as psoriasis) develop the inflammatory condition PsA, which is more severe than the cutaneous subtype (51). HLA-C*06:02 has consistently shown the strongest association with overall risk for psoriasis (52–57). Notably, HLA-C has proven to be a useful pharmacogenetic marker in psoriasis response to treatment with ustekinumab, a fully humanized monoclonal antibody targeting the common p40 subunit of IL-12 and IL-23 cytokines. When treated with ustekinumab, patients carrying HLA-C*06 show earlier and more substantial reductions in disease activity scores than patients who are negative for HLA-C*06 (58). The finding is clinically relevant considering the high incidence of the HLA-C*06:02 allele in the psoriasis population, reaching 47% to 64% (59).

Although SNPs in the HLA-C region have demonstrated a statistically significant association with PsA when compared with healthy controls (Table 1), differential effects of risk alleles between PsA and psoriasis suggested heterogeneous contributions of HLA alleles on the disease subtypes (60–64). Using fine mapping of the MHC to study the association of causal variants with psoriasis and its clinical subtypes, Okada et al found that risk heterogeneity between PsA and cutaneous psoriasis might be driven by amino acid position 45 of the HLA-B (65). Initial analysis of the results confirmed previous associations of HLA-C*06:02 with increased risk of psoriasis and decreased risk of PsA (60,61). However, after adjusting for HLA-B amino acid position 45, the differential effect of HLA-C*06:02 lost significance. Bowes et al found HLA-C*06:02 to be less common in patients with PsA compared with psoriasis and that HLA-C*06:02 had significant association with younger age of psoriasis onset (66). However,

**Table 1.** Genetic associations of HLA-C with rheumatic diseases

| HLA-C SNP / Genotype | Allele | Odds Ratio | *P* Value | 95% CI | Notes | Ref |
|---|---|---|---|---|---|---|
| ACPA positivity in rheumatoid arthritis | | | | | | |
| HLA-C*03 | NP | 1.55 | $4.65 \times 10^{-7}$ | 1.31-1.84 | Controlled for HLA-DRB1 | (31) |
| HLA-C*12 | NP | 0.28 | $3.79 \times 10^{-8}$ | 0.18-1.46 | | (31) |
| rs3130531 | NP | 1.39 | 0.0025 | 1.13-1.72 | Odds ratios assume the risk allele is on the ancestral HLA A1-B8-DR3 haplotype | (21) |
| rs2844623 | NP | 1.56 | 0.00084 | 1.21-2 | | |
| rs2524077 | NP | 1.75 | $8.10 \times 10^{-5}$ | 1.33-2.29 | | |
| rs3130696 | NP | 1.43 | 0.007 | 1.11-1.84 | | |
| rs2524073 | NP | 1.75 | 0.00011 | 1.32-2.31 | | |
| rs2524044 | NP | 1.47 | 0.004 | 1.14-1.89 | | |
| rs3873385 | NP | 1.48 | 0.0034 | 1.15-1.92 | | |
| rs9264868 | NP | 1.79 | $5.00 \times 10^{-5}$ | 1.36-2.37 | | |
| rs9264869 | NP | 1.75 | 0.00011 | 1.32-2.31 | | |
| rs9264904 | NP | 1.51 | 0.00046 | 1.2-1.89 | | |
| rs1634747 | NP | 1.41 | 0.0015 | 1.14-1.73 | | |
| Vasculitis in rheumatoid arthritis | | | | | | |
| HLA-C*03 | NP | 4.15 | $P < 0.001$ | 2.14-8.08 | RA with vasculitis vs RA no hx extra-articular manifestations; controlled for HLA-DR shared epitope | (82) |
| Psoriatic arthritis | | | | | | |
| rs10484554 | T | 2.4 | $6.86 \times 10^{-11}$ | 1.8-3.1 | | (5) |
| rs13191343 | T | 2.48 | $2.63 \times 10^{-23}$ | 2.06-2.97 | | (57) |
| rs12191877 | T | 2.34 | $6 \times 10^{-62}$ | NP | | (52) |
| rs36058333 | –/C | 2.132 | $1.3 \times 10^{-29}$ | NP | | (83) |
| Ankylosing spondylitis | | | | | | |
| HLA-C*15:02 | NP | 6.58 | $1.52 \times 10^{-11}$ | 3.8-11.4 | Korean | (6) |
| Crohn's disease | | | | | | |
| rs114985235 | T/C | 2.25 | $8.71 \times 10^{-23}$ | 1.91-2.66 | Korean | (8) |
| Generalized osteoarthritis | | | | | | |
| HLA-Cw4 | NP | 3.04 | 0.0015 | 1.48-6.23 | Japanese | (84) |
| Systemic lupus erythematosus | | | | | | |
| HLA-C*17:01 | NA | 1.42 | $7.4 \times 10^{-6}$ | 1.21-1.65 | European and African American | (7) |
| HLA-C group 1 (C1) | NA | 6.47 | <0.0001 | 2.58-16.26 | Sicilian | (85) |

Abbreviation: ACPA, anticitrullinated peptide antibody; CI, confidence interval; HLA-C, human leukocyte antigen C; NA, not applicable; NP, not presented; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism.

once the data were controlled for the age of onset, there was no association of HLA-C*06:02 with PsA. Instead, the presence of an asparagine at amino acid position 97 of HLA-B demonstrated the strongest association with risk of PsA. Interestingly, an asparagine at position 97 of the HLA-B molecule is predominantly found on HLA-B27 alleles, and this same amino acid confers the greatest heritable risk for ankylosing spondylitis, suggesting significant genetic overlap between these spondyloarthropathies.

In a large case-control study, Chandran et al showed that genotypes positive for the activating KIR2DS2 in the absence of HLA-C 1 ligands for homologous inhibitory KIR2DL2/2DL3 conferred significantly higher risk of PsA (42). The study findings support a possible role for NK cells and innate immunity in the pathogenesis of PsA. Notably, these investigators found an increased risk for PsA with high-expression HLA-C alleles (HLA-C*02, *05, *06, *08, *12, *15, and *16). The association remained significant even after adjusting for HLA-C*06 in a logistic model. High-expression HLA-C alleles have been shown to confer enhanced control of HIV viremia (15,67), highlighting the delicate balance between innate immunity and autoimmunity.

In summary, there is substantial evidence linking HLA-C with PsA, although more work is needed to confirm and refine this association.

## PUTATIVE ROLES FOR ALTERNATE HLA-C CONFORMATIONS

HLA-C molecules are predominantly found anchored in the cell membrane in a trimeric structure consisting of the HLA-C heavy chain, bound peptide, and β2m. However, the heavy chain can dissociate from β2m and peptide to assume an open conformation (68). As free heavy chains on the cell membrane, HLA class I molecules have been shown to form homodimers

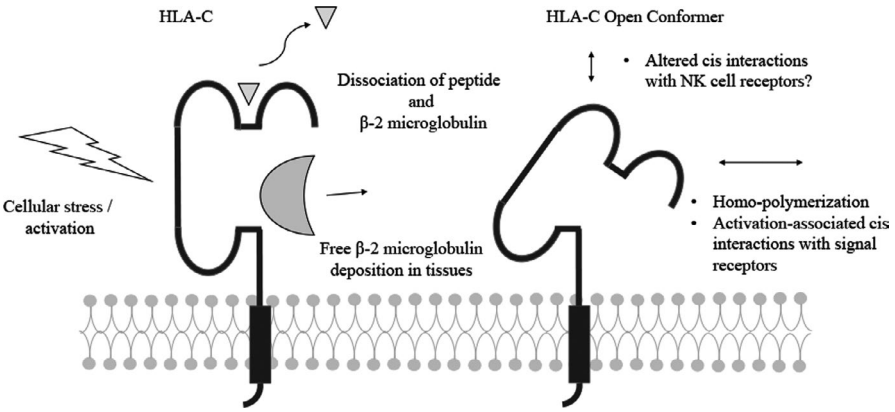


**Figure 1.** HLA-C open conformers may be associated with autoimmunity.

and homotrimers and take on new functions, interacting in *cis* with themselves and other surface receptors such as epidermal growth factor receptor and insulin receptor and in *trans* with NK cell receptors (16). Activating KIR including KIR2DS4 have been observed to specifically recognize the nonclassical class I molecule HLA-F in an open conformation (69). HLA class I open conformers are observed more frequently on metabolically active and rapidly dividing cells, including activated T cells (70).

In recent years, increased attention has been focused on HLA-C open conformers because of an observed potential influence on HIV infectivity (71). The monoclonal antibody L31 has been used to detect HLA-C open conformers. Parolini et al demonstrated that HLA-C alleles vary in stability of binding with β2m and expression levels as free heavy chains (72). HLA-C has a weaker association with β2m than HLA-A or -B (73), making dissociation from β2m and peptide to assume an open conformation more energetically favorable [**Figure 1**]. The increased tendency of HLA-C to dissociate from β2m is similar to that of HLA-B27, a molecule that is strongly associated with spondylarthritides (74). Raine et al showed that MHC HLA class I free heavy chains are present at higher levels on the peripheral blood leukocytes of reactive arthritis patients compared with healthy controls (75). Rysnik et al detected elevated levels of nonconventional forms of HLA-B27 (including free heavy chains and homodimers) in the joint and gut tissue of patients with spondyloarthritis (76). HLA-B27 homodimers have demonstrated altered binding to NK cell receptors including KIR-3DL1, KIR-3DL2, and leukocyte immunoglobulin-like receptors (LIRs) LIR-B2 (74,77). Payeli et al generated a phage display antibody HD6 with specificity for HLA-B27 homodimers (78). It was demonstrated that HD6 abrogated the binding of HLA-B27 dimers to KIR-3DL2 and inhibited the survival and proliferation of KIR-3DL2–positive NK cells (78). Furthermore, HD6 inhibited production of the proinflammatory cytokine IL-17 by peripheral blood leukocytes from patients with ankylosing spondylitis.

In addition, several studies have shown that free β2m, as would be released by HLA-C assuming an open conformation, can trigger synoviocytes to release mediators of inflammation and tissue destruction (79–81).

HLA-C is an attractive candidate risk locus for autoimmune rheumatic diseases given its pivotal role in both innate and adaptive immunity and the proposed infectious etiology of rheumatic diseases. Genetic studies have begun to reveal an independent association of HLA-C with rheumatic diseases and phenotypes. In some diseases, the association of HLA-C is quite clear, whereas others will require more sophisticated analytical approaches to validate genes and related pathways. In its role as a ligand for NK cells, HLA-C is associated with rheumatic and related diseases, including PsA, RA, ankylosing spondylitis, Sjogren's syndrome, and Crohn's disease. The intriguing potential role of β2m-free HLA-C open conformers in autoimmunity remains to be investigated. Overall, the association of HLA-C with rheumatic diseases merits further study considering its potential clinical application in precision medicine, such as predicting response to therapy.

## AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication.

**Study conception and design.** Siegel, Ahmed.
**Acquisition of data.** Siegel.
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