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Rachel L. Allen[1*], Laura E. Hudson[1]

[1]Institute for Infection & Immunity, St George's, University of London, United Kingdom

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Leukocyte Ig-Like Receptors – a model for MHC class I disease associations

Laura Emily Hudson[1], Rachel Louise Allen[11*],

[1] Institute for Infection and Immunity, St George's, University of London, Cranmer Terrace, London. SW17 0RE.

*** Correspondence:**
Rachel Allen
rallen@sgul.ac.uk

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Abstract

MHC class I (MHC-I) polymorphisms are associated with the outcome of some viral infections and autoimmune diseases. MHC-I proteins present antigenic peptides and are recognised by receptors on Natural Killer cells and Cytotoxic T lymphocytes, thus enabling the immune system to detect self-antigens and eliminate targets lacking self or expressing foreign antigens. Recognition of MHC-I, however, extends beyond receptors on cytotoxic leukocytes. Members of the Leukocyte Ig-like receptor (LILR) family are expressed on monocytic cells and can recognise both classical and non-classical MHC-I alleles. Despite their relatively broad specificity when compared to the T Cell Receptor or Killer Ig-like Receptors, variations in the strength of LILR binding between different MHC-I alleles have recently been shown to correlate with control of HIV infection. We suggest that LILR recognition may mediate MHC-I disease association in a manner that does not depend on a binary discrimination of self/non-self by cytotoxic cells. Instead, the effects of LILR activity following engagement by MHC-I may represent a "degrees of self" model, whereby strength of binding to different alleles determines the degree of influence exerted by these receptors on immune cell functions. LILR are expressed by myelomonocytic cells and lymphocytes, extending their influence across antigen presenting cell subsets including dendritic cells, macrophages and B cells. They have been identified as important players in the response to infection, inflammatory diseases and cancer, with recent literature to indicate that MHC-I recognition by these receptors and consequent allelic effects could extend an influence beyond the immune system.

1 Introduction

MHC class I (MHC-I) proteins are characterised by a high level of polymorphism, with thousands of allelelic variants identified to date (1). Such extensive variation indicates powerful selection pressure to maintain a wide range of alleles. Disease associations for individual MHC-I alleles are well-documented. The most striking is that of HLA-B27, which is present in >90% of patients with ankylosing spondylitis (2). MHC-I polymorphisms have also been shown to be associated with the outcome of viral infections, including the control of HIV infection (3), clearance of HCV infection (4,5) and protection from dengue hemorrhagic fever following secondary infection with this virus (6).

Proposed mechanisms to explain classical MHC-I disease associations have focussed on the functional role(s) of these proteins. The best characterised of these roles is MHC presentation of short antigenic peptides for recognition by the T cell receptor (TCR) on cytotoxic T cells (CTL). Thus, many studies have examined the nature of the peptides presented by disease-associated alleles and of T cell responses restricted by these alleles (7, 8). For example, a number of studies have examined the peptide specificities of HLA-B27 subtypes (9). In the context of HIV infection, a dominant HLA-B27 restricted viral peptide is thought to play a key role in the association of this allele with control of infection. Immune escape from the response against the dominant peptide results in a decrease in HIV-1 replication (10).

In humans, classical MHC-I are also recognised by members of the Killer Ig-like Receptor (KIR) family, which are encoded in the Leukocyte receptor complex (LRC) on chromosome 19. KIR demonstrate allele (and in some cases peptide) specificity (11), albeit at a lower level of precision for individual peptide/MHC complexes than that shown by classical T cell receptors. KIR are expressed on natural killer (NK) cells and T cells where they inhibit the ability of these cytotoxic cells to lyse target cells that express self MHC-I alleles. As knowledge regarding their biology and MHC specificities has grown, KIR have been studied alongside MHC-I in conditions such as spondyloarthropathy, HIV and HCV infection (5,12,13). There is considerable variation in KIR haplotypes, such that any individual may not carry the relevant MHC ligand for every KIR receptor that they express and *vice versa*. A number of studies suggest that particular combinations of KIR and HLA alleles, believed to result in functional receptor/ligand interactions are associated with protection from progression to AIDS following HIV infection (14).

A lesser-studied family of proteins encoded within the LRC are also capable of recognising MHC class I. These Leukocyte Ig-like receptors (LILR) do not appear to be involved in the cytolytic removal of targets bearing non-self MHC-I protein complexes (15). Instead they are predominantly expressed on cells of the myelomonocytic lineage and some of them show a broad specificity encompassing both classical and non-classical MHC-I (16). The observation that LILR vary in the strength of their binding to individual MHC-I alleles, however, raised the possibility that these innate immune receptors may contribute in some manner towards MHC-I disease associations (17). In support of this theory, a recent study of a large cohort of HIV-1 infected patients demonstrated that the overall binding strength of LILRB2 for the MHC-I haplotypes expressed by these individuals was positively associated with the level of viraemia (18).

2 Leukocyte Ig-Like Receptors (LILR):

The various members of the LILR family are broadly categorised as inhibitory (LILRB) or activating (LILRA), according to the presence or absence of tyrosine-based signalling motifs in their cytoplasmic tail. In some cases, putative activating receptors have been shown to elicit inhibitory effects and *vice versa* for inhibitory receptors (19). Receptor engagement results in intracellular phosphorylation of the tyrosine-based motifs within the receptors themselves (LILRB), or on associated adaptor molecules (LILRA) (19). Downstream signaling events can be mediated by phosphatases such as SHP-1, SHP-2 and SHIP (20, 21) and vary according to the receptor and/or cellular context. For example, SHP-2 may mediate production of IL-6 via the NF-kB pathway following LILRB2 engagement on dendritic cells (22) or inhibition of the mTOR pathway following LILRB1 engagement on T lymphocytes (23).

There are multiple similarities between KIR and LILR in terms of Ig-domain based structure, gene location within the leukocyte receptor complex and ability to recognise MHC-I (15). Unlike their NK receptor counterparts, however, LILR orthologues (known as PIR) are found in rodents, where they demonstrate similar ligand binding, expression and functional profiles (24,25). This may indicate a higher degree of evolutionary conservation for LILR than for KIR, with bovine orthologues also identified (26) and similar proteins documented in chickens and fish (27,28). Within the murine system there is a single inhibitory receptor, PIR-B and multiple activating receptors (PIR-A). PIR are involved in the regulation of lymphocyte, antigen presenting cell and granulocyte functions (29) and their study has enabled the identification of functions for both these receptors and their human counterparts such as the regulation of synaptic plasticity (30) and platelet activation by PIR-B and LILRB2 (31).

Figure 1 shows the known expression profiles of LILR on leukocyte subsets according to current literature. The known expression profiles for LILR are not exhaustive; expression of individual members of the family has been documented for macrophages, B-cells, NK cells and other non-immune cells (32-40). These receptors are therefore likely to have far-reaching effects on a range of immunological functions. Immune cells which have yet to be characterised in full for LILR expression include Invariant Natural Killer (iNKT), Gamma-Delta ($\gamma\delta$), Regulatory (T_{reg}) and T helper 17 (T_h17) T-cells, B-cell subsets, as well as the various APC subsets and granulocytes.

LILR activity can result in the upregulation or downregulation of both innate and adaptive functions with a range of effects on different cell types. For example, LILR and PIR have been shown to inhibit TLR-mediated functions of antigen presenting cells such as inflammatory cytokine secretion (38, 41-43). Inhibitory LILR have been shown to inhibit the upregulation of co-stimulatory proteins on antigen presenting cells (36, 44-46), thus favouring regulatory T cell responses (47-50). On lymphocytes, inhibitory LILR have been shown to inhibit T and B cell receptor signaling and downregulate antibody and cytokine production (51-53). Activating LILR have been shown to mediate monocyte activation and secretion of inflammatory cytokines (54) and on basophils to trigger release of histamine (55).

3 MHC recognition by LILR:

Following the initial identification of LILRB1 as a receptor for self and viral MHC-I (56), structural studies predicted that several other members of the family would also recognise MHC-I (57). Members of the family were allocated into two groups on this basis, with Group 1 containing

receptors predicted to bind MHC-I and Group 2 containing receptors that were not predicted to bind MHC-I (57). It was confirmed subsequently that the Group 1 members LILRA1, LILRA2, LILRA3, LILRB1 and LILRB2 can engage MHC-I (17, 58). Members of the LILR family vary in their MHC-I binding preferences. LILRB2 demonstrates the broadest specificity, with the ability to recognise all classical and non-classical self MHC-I alleles and forms tested to date. Although LILRB2 binds to both the α3 & β2m regions of the MHC-I antigen presenting structure, the major portion of its binding site lies within the highly conserved α3 domain (59). The degree of interaction between this receptor and the α3 domain is sufficient to allowing LILRB2 to bind open conformers of MHC-I, which lack β2m. In contrast, the major LILRB1 binding site lies within β2m, thus this receptor can only associate with β2m-associated MHC-I. Recognition of open MHC-I conformers has also been observed for LILRA1 and LILRA3, which were shown in one study to have stronger binding to open confomers than to β2m-associated MHC-I (17). These findings indicate that alternatively folded forms of MHC-I may play a functional role in the immune response. It is also important to note that members of the LILR family may interact *in cis* with MHC-I on the cell surface, as has been demonstrated for PIR-B and LILRB1 (60, 61).

Despite their broad specificity, LILRB1 and LILRB2 both show variation in their strength of binding to different MHC-I alleles (17). Binding occurs predominantly through the D1-D2 domains of the receptor (57), but it has been suggested that secondary binding sites in the D3 and D4 domains may contribute to allelic variations in the strength of LILR binding (62). The potential importance of such variations was first highlighted by the observation that MHC-I complexes differing by only one amino acid in the bound peptide showed different affinities for LILRB2, which corresponded with the extent of LILRB2-mediated modulation of antigen presenting cell phenotype (63). A subsequent comparison of binding strength for different MHC-I alleles to LILRB1 and LILRB2 identified distinct preferences (17). LILRB1 has a lower affinity for some HLA-A alleles; those with Ala^{193} and Val^{194} have shown lower binding ability. Ser^{207} and Gln^{253} alleles also show weaker binding to LILRB1, and are in linkage disequilibrium with Ala^{193} and $Val^{194.}$ LILRB2 has been shown to bind most strongly to HLA-A, and weakest to HLA-B alleles, but with greater variability for these alleles than LILRB1. Its binding is weakest to a subset of alleles including HLA-B27 and HLA-B*5701. Some of these outliers were MHC-I alleles with known disease associations, leading to the suggestion that LILR recognition of MHC-I might influence susceptibility to, and outcome of, some viral infections or autoimmune diseases.

4 LILR, MHC and infection

Viral infection may be regarded as the primary pathology in which MHC-I recognition is essential to achieve a successful immune response. MHC-I proteins present fragments of intracellular proteins to T cells in order to enable the lysis of infected cells, and the peptide binding specificity of particular MHC-I alleles may thus influence the course of disease. There is evidence to suggest that LILR expression is induced in response to infection (64) and can be regarded as an indicator of an effective adaptive immune response (65). Studies are now beginning to highlight the relevance of LILR in particular infections and the influence of MHC-I recognition in the process.

Distinct LILR expression profiles were found to be associated with dendritic cell dysfunction during acute HIV-1 infection (66) and with ‘elite’ control of infection (39). As there are well-characterised associations for different MHC-I alleles with either HIV viral control or progression to AIDS (67) and given that LILR have been implicated in its disease pathology, this viral infection represented a suitable model for testing the hypothesis that LILR may mediate MHC-I disease associations. Support for this theory was provided by studies which demonstrated that MHC-I alleles and

complexes associated with disease progression were preferential ligands for the inhibitory receptor LILRB2 whereas those associated with delayed onset of AIDS showed weaker binding to the receptor (17, 63, 68, 69). It could therefore be hypothesised that weaker affinity for LILRB2 would result in a lack of inhibition of dendritic cell functions, resulting in a more effective anti-HIV immune response. One study sought to examine the MHC-I haplotype of HIV-1 patient cohorts in combination with the strength of their LILR binding in order to assess whether LILR recognition might influence the course of disease. An association with LILRB2 but not LILRB1 binding strength was observed, indicating that the strength of MHC-I recognition correlates with control of viral load (18). This study provided the first strong evidence that despite the broad specificity of LILR, the strength of their binding preference for different MHC-I alleles could represent a novel mechanism for an MHC-I association during infection.

Binding of MHC-I by 'Activating' members of the LILR family may also be relevant in HIV-1 infection. LILRA1 and LILRA3 preferentially bind HLA-C open confomers (17) and HLA-C variants have been associated with different outcomes of HIV infection. One particular polymorphism, *-35C/T*, lies 35kb upstream of the HLA-C locus. The -35C allele corresponds with increased HLA-C expression, which in turn is associated with delayed onset of AIDS (70). HLA-C proteins are more stable in open conformer form than their HLA-A, and –B counterparts and are upregulated following immune cell activation. It is therefore possible that LILRA1 or LILRA3 recognition of HLA-C might provide a further mechanism for MHC-I disease associations during HIV infection.

LILR binding preferences for MHC-I alleles may influence the outcome of other viral infections. Expression of HLA B27 is associated with spontaneous clearance of Hepatitis C virus infection (71), and by analogy with HIV-1 it could be hypothesised that the low binding preference of LILRB2 for this allele might influence disease outcome. Another viral infection where LILR may be responsible for MHC-I associated protective effects is Dengue. Large case-control studies have identified MHC-I alleles with protective effects in Dengue infection (72). Antibody opsonised Dengue has recently been shown to co-ligate the inhibitory receptor LILRB1 when engaged by FcγR, leading to inhibition of FcγR signaling (73) and indicating that LILRB1 may play a role in antibody dependent Dengue. Infection with DENV is highly inflammatory and results in a large influx of activated B-cells.

5 Autoimmunity

Individual LILR have been implicated in autoimmunity and their preferences for MHC-I alleles may be relevant in these conditions. Of the receptors known to recognise MHC-I, LILRA3 has been found to be associated with a number of inflammatory conditions. Expressed only in a soluble form, LILRA3 possesses no known signalling capacity of its own, but can bind ligands of cell-associated LILR. Some individuals do not express LILRA3 due to a large 6.7kbp sequence deletion. The prevalence of this deletion polymorphism is population-dependent and ranges from 6-84% (74, 75), with a particularly high relevance in the Japanese population, where a number of non-functional spliced isoforms have also been identified (76). The deletion has been associated with increased susceptibility and early onset of Multiple Sclerosis (MS) symptoms in a number of studies (77, 78), although conflicting data have been observed in other populations (74).

LILRA3 deficiency may also be a risk factor for Sjögrens syndrome (SS), with increased prevalence of null allele homozygous individuals (79) in certain populations, whilst the functional allele is a suggested risk factor in others (75). More recent studies have linked LILRA3 to Rheumatoid Arthritis (RA). In contrast to MS, increased serum levels of functional LILRA3 is a proposed genetic risk

factor for RA, with serum levels correlating directly with disease severity (80). Of further note is the prominent expression of LILRA2, A5, B2 and B3 in synovial tissues of RA patients (81) and the reduction of LILRA2, LILRB2 and LILRB3 in patients responsive to disease-modifying anti-rheumatic drugs (DMARDs) (82). Functional LILRA3 has also been suggested as a risk factor for Systemic lupus erythematosus (SLE) following a genotyping study in Han Chinese populations, which also found higher levels of LILRA3 mRNA in SLE patients (75).

6 Other ligands and functions of LILR

Direct recognition of Dengue virus by LILRB1 highlights the relevance of future studies to characterise the full range of ligands for these receptors and compare their relative binding strengths. As described above, LILRB2 is known to be the most promiscuous receptor in the family in terms of its broad specificity for classical and non-classical MHC-I in folded and unfolded forms. LILRB2 has also been shown to bind a range of non-MHC ligands including Angiopoietin-like proteins (32) and NOGO, a myelin component (30). More recently, LILRA3 has also been shown to bind NOGO (83). These findings extend the relevance of LILR beyond immune responses to situations such as neurodegeneration, neural plasticity, angiogenesis and other as yet unidentified scenarios where MHC-I may compete with other ligands for receptor binding (84). In the future, comparative binding assays may indicate how MHC-I allelic preferences might influence the ability of LILR to bind alternative ligands. Such investigations could cast light on previous observations regarding the relevance of MHC-I in neural plasticity and regeneration (85, 86) and associations with non-immune conditions such as Alzheimer's disease.

7 Future Directions

Studies on HIV-1 have provided proof of concept that LILR binding preferences for MHC-I alleles could represent a novel mechanism to explain some of the associations of MHC-I alleles with autoimmune diseases and the outcome of certain viral infections. According to this model, the influence of LILR can vary according to the strength of their binding to MHC-I alleles, representing a "degrees of self" model. MHC polymorphisms could, therefore, determine the degree of LILR signaling and consequent regulation of functions for a range of immune cell subsets as indicated in Figure 2. However, identification of the underlying mechanisms through which LILR might alter disease outcomes will require an enhanced understanding of LILR biology. It will be necessary to obtain a full characterisation of the LILR expression repertoire on immune cell subsets, and identify the functional effects of LILR on each cell type. For example, in the context of dengue infection, LILR expression on B cell subsets may also be relevant in viral uptake and/or generation of non-neutralising antibodies. It will also be necessary to characterise LILR expression and function on non-immune cells. Comparative binding assays between MHC-I alleles and alternative ligands should then help explain the wide-ranging influence of these proteins.

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9 Figure Legends

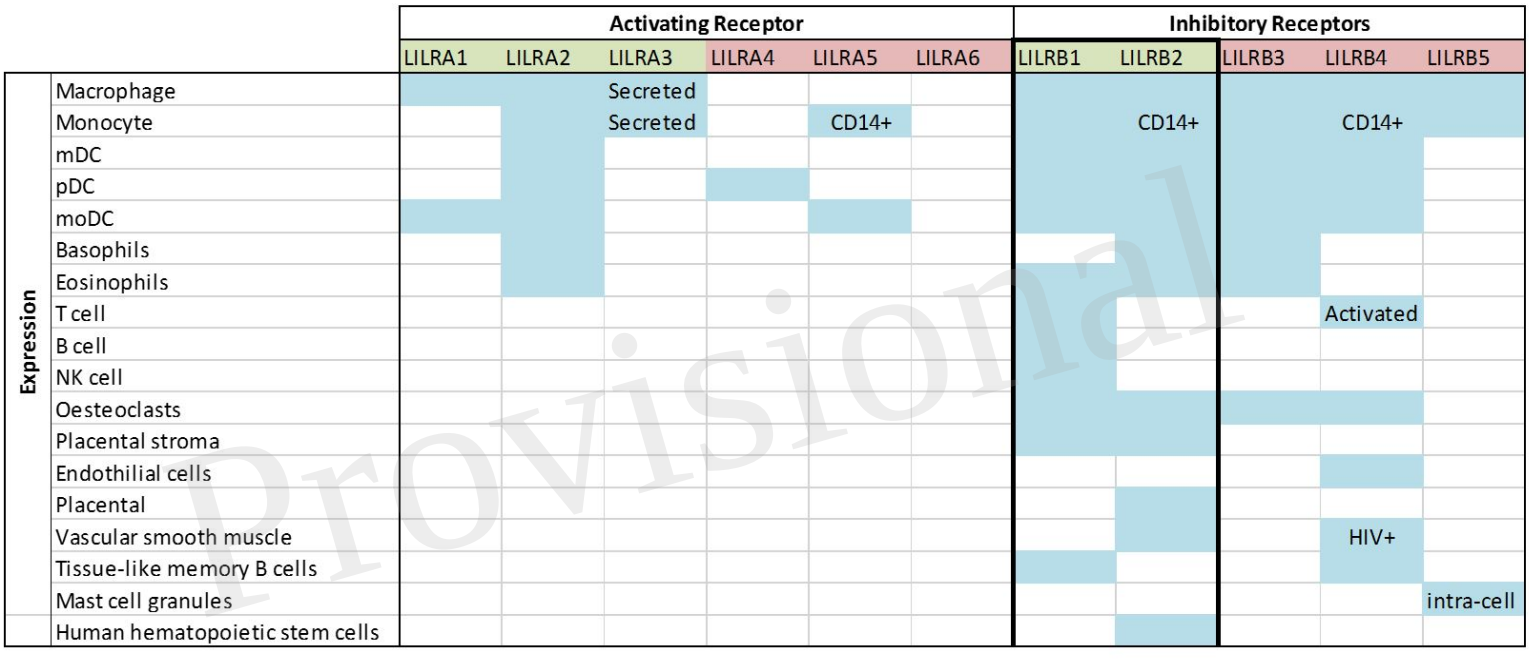
Figure 1: LILR expression profile, according to literature.

Blue shaded squares indicate expression according to the literature (32-40); annotation within boxes indicates expression specifics (for example, observed during in HIV Infection or for a particular cell phenotype). Green denotes Group 1 LILR, and Red, Group 2 LILR.

Figure 2*:* Immunoregulatory Receptor Mechanisms & Functions

A) T-cell mediated non-self killing through non-self MHC-I peptide presentation.
B) NK mediated non-self killing through Missing-self, Non-self and stress/damage-induced lysis
C) LILR mediated regulation of immune cells. LILR may regulate cell phenotype and functions, in a variety of ways, which have yet to be determined in full.

Figure 01.JPEG



		Activating Receptor						Inhibitory Receptors				
		LILRA1	LILRA2	LILRA3	LILRA4	LILRA5	LILRA6	LILRB1	LILRB2	LILRB3	LILRB4	LILRB5
Expression	Macrophage			Secreted								
	Monocyte			Secreted		CD14+			CD14+		CD14+	
	mDC											
	pDC											
	moDC											
	Basophils											
	Eosinophils											
	T cell										Activated	
	B cell											
	NK cell											
	Oesteoclasts											
	Placental stroma											
	Endothilial cells											
	Placental											
	Vascular smooth muscle										HIV+	
	Tissue-like memory B cells											
	Mast cell granules											intra-cell
	Human hematopoietic stem cells											

Figure 02.JPEG