The LILR family: Modulators of innate and adaptive immune pathways in health and disease

Article *in* Tissue Antigens · October 2004

Impact Factor: 2.14 · DOI: 10.1111/j.0001-2815.2004.00290.x · Source: PubMed

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Review Article

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The LILR family: modulators of innate and adaptive immune pathways in health and disease

Key words:
autoimmunity; ILT; LILR; MHC; tolerance

Acknowledgments:
We thank N. T. Young for his helpful discussions and advice compiling this review. Damien Brown is supported by the Leukaemia Research Fund, UK.

Abstract: Leukocyte immunoglobulin (Ig)-like receptors [LILRs, also known as Ig-like transcripts (ILTs)] are a family of inhibitory and stimulatory receptors encoded within the leukocyte receptor complex and are expressed by immune cell types of both myeloid and lymphoid lineage. Several members of the LILR family recognize major histocompatibility complex class I. The immunomodulatory role of LILR receptors indicates that they may exert an influence on signaling pathways of both innate and adaptive immune systems. LILR activity can also influence the antigen-presenting properties of macrophages and dendritic cells and may thus play a role in T-cell tolerance. The wide-ranging effects of LILR signaling on immune cell activity imply that these receptors are likely to play an important role in a range of clinical situations including pregnancy, transplantation, and arthritis (including the human leukocyte antigen B27-associated spondyloarthropathies). In this review, we summarize current knowledge on the nature and function of LILRs, focusing on their regulation of immune cell activity and their potential role in disease.

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When faced with infection, our immune system relies on a complex input of signals from its various receptors to direct an appropriate response. Members of the Immunoglobulin (Ig) superfamily play essential roles as antigen receptors, costimulatory proteins, adhesion molecules, and immunomodulatory receptors. One such group of Ig superfamily receptors, implicated in both innate and adaptive immunity, were named as Ig-like transcripts (ILTs) or leukocyte Ig-like receptors (LIR) (1, 2). Proteins of the ILT/LILR family are also known as myeloid inhibitory receptors, CD85, and, following a recent attempt to standardize the nomenclature, LILRs (Table 1) (http://www.gene.ucl.ac.uk/nomenclature/genefamily/lilr.html) (3, 4). For the purpose of this review, the new LILR nomenclature will be used as appropriate for receptors that have been assigned an LILR number.

LILRs are predominantly expressed on the surface of myelomonocytic cells such as macrophages and dendritic cells, where they are

Received and accepted for publication 19 May 2004

doi: 10.1111/j.1399-0039.2004.00290.x

Tissue Antigens 2004: **64:** 215–225

Not all of the leukocyte immunoglobulin (Ig)-like receptors (LILRs) have yet been assigned a LILR nomenclature. Official LILR designation can be found on the Human Genome Nomenclature Committees website http://www.gene.ucl.ac.uk/nomenclature/genefamily/lilr.html. The A or B suffix refers to either activating (A) or inhibitory (B) isoforms

Receptor	Alternative names
LILRA1	LILR6, CD85i
LILRA2	ILT1, LILR7, CD85h
LILRA3	ILT6, LILR4, CD85e
LILRB1	ILT2, LILR1, CD85, CD85j
LILRB2	ILT4, LILR2, CD85d, MIR10
LILRB3	ILT5, LILR3, CD85a
LILRB4	ILT3, LILR5, CD85k
LLIRB5	LILR8, CD85c
ILT7	CD85g
ILT8	CD85b
ILT9	CD85b
ILT10	CD85m
ILT11	LILR9, CD85f

ILT, Ig-like transcript; MIR, myeloid inhibitory receptor.

Table 1

thought to influence the signaling of other immune receptors. Activating or inhibitory receptor isoforms are defined by residues within their transmembrane and cytoplasmic domains, while extracellular Ig domains are responsible for ligand binding at the cell surface (Fig. 1). Protein sequence motifs known as immunoreceptor tyrosine-based activating motifs (ITAMs) and immunoreceptor tyrosine-based inhibitory motifs (ITIMs) are responsible for the activating or inhibitory signals transmitted by LILR. Similar signaling mechanisms are employed by the closely related killer cell inhibitory receptor (KIR). ITIMs recruit inhibitory phosphatases that dephosphorylate ITIM and ITAM domains in order to influence intracellular signaling cascades (Fig. 2) (1, 5–8). In contrast, activating LILRs, which lack any signaling domains of their own, rely on association with an adaptor protein such as FcεRIγ to transmit their signal through its intracellular ITAMs (7). Clustering of activating receptors at the cell surface triggers rapid phosphorylation of a tyrosine residue within the ITAM. Protein tyrosine kinases with Src homology 2 domains can then bind the phosphorylated tyrosine residue, leading to downstream signaling and gene modulation (Fig. 2) (9). LILR expressed on different cell types may recruit alternative protein tyrosine kinases, or phosphatases, and therefore modulate a range of intracellular signaling pathways.

LILRs influence both innate and acquired immune systems. The best characterized receptors to date are the inhibitory receptors LILRB1 and LILRB2, which recognize major histocompatibility complex (MHC) class I. Studies are beginning to address the function of other inhibitory receptors such as LILRB4 and the activating receptors LILRA1 and LILRA2. Functions and ligands for the remainder of the LILR family have yet to be resolved, but some clues can be drawn from studies of the better-characterized receptors and from murine homologs. In this review, we address the relevance of LILRs to immune cell function. The immunomodulatory properties of LILR proteins suggest that they play a role in diseases ranging from viral infection to autoimmunity. We summarize clinical studies to date.

Genetics of the LILR Family

The leukocyte receptor complex

The leukocyte receptor complex (LRC) on human chromosome 19q13.4 encodes several families of closely related Ig superfamily proteins (Fig. 3) (10). KIRs show a high level of genetic variation. In addition to allelic diversity, the multiple KIR haplotypes vary in the number and nature of receptors that they encode. By contrast, analysis of the LILR gene cluster reveals two haplotypes, one containing 13 LILR loci, and the other only 12 complete loci, due to a 6.7 kb deletion encompassing part of the LILRA3 gene (10, 11). Members of

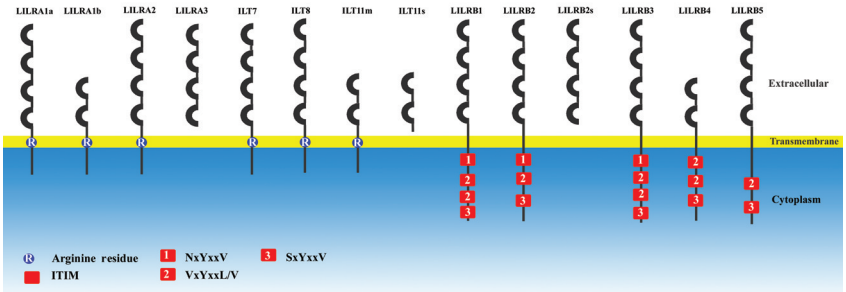

Fig. 1. **Schematic diagram of the leukocyte immunoglobulin (OryIg)-like receptor (LILR) family.** Activating LILRs have a short cytoplasmic tail and an arginine residue located within the transmembrane domain. Inhibiting LILRs do not express a charged arginine residue in the transmembrane region, instead they have a long cytoplasmic tail with two to four immunoreceptor tyrosine-based inhibitory motif (ITIM) domains and varying sequences as denoted in the figure. Ig-like transcript 9 (ILT9) and ILT10 are omitted from the figure as they are thought to be pseudogenes.

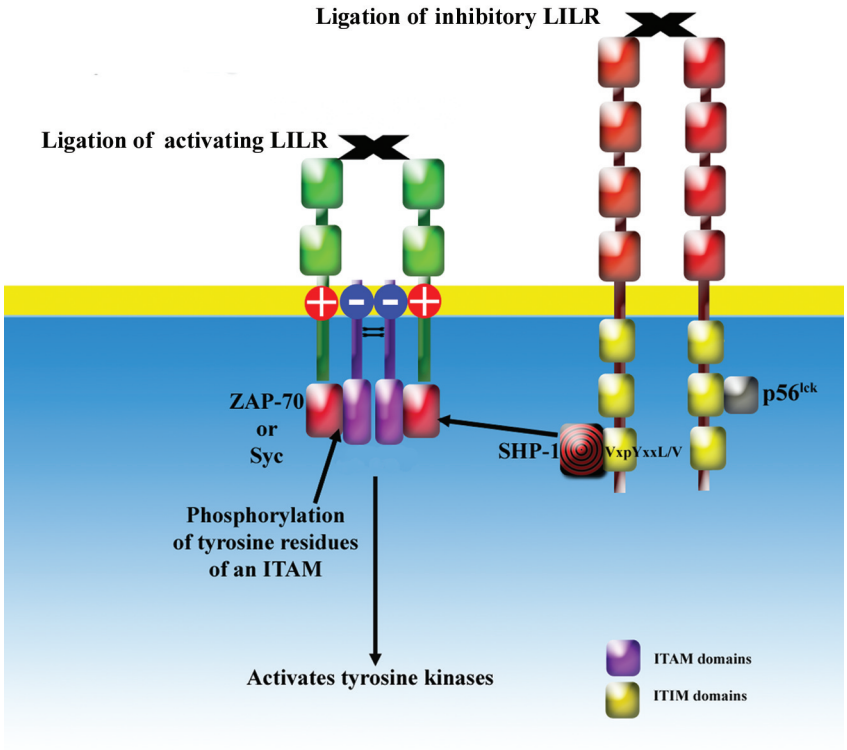

Fig. 2. **When activating leukocyte immunoglobulin-like receptors (LILRs) are stimulated, the coreceptor FcεRIγ (colored in purple) is recruited to the clustered receptors.** Immunoreceptor tyrosine-based inhibitory motif (ITIM) domains present on FcεRIγ are phosphorylated, allowing the recruitment of protein tyrosine kinases, leading to downstream events and eventually gene expression. When inhibitory LILRs are activated, their ITIM domains become phosphorylated and recruit $p56^{lck}$ and SH2-containing protein-tyrosine-phosphatase 1 SHP-1. This then results in the dephosphorylation of other ITIMs and immunoreceptor tyrosine-based activating motifs (ITAMs).

the LILR family are non-polymorphic when compared to the KIRs. LILRB1, LILRB2, LILRB4, LILRA3, and ILT8 are moderately polymorphic, and LILRB3 is more variable (10–12).

LILR homologues and orthologues

The paired inhibitory receptors (PIRs) are probably LILR orthologues. PIRs correspond to LILRs in terms of their sequence, expression pattern, ligands, signaling, and function. Chimpanzee LILR sequences correspond closely to those of humans, and orthologues of individual receptors can be found. Analysis of human and chimpanzee sequences indicates that there are two distinct groups of LILRs. The first group (LILRA2, LILRB4, ILT7, and LILB5) is represented by orthologues in chimpanzee, while the remaining LILRs are thought to have evolved more recently through intergenic recombination and gene duplication (13).

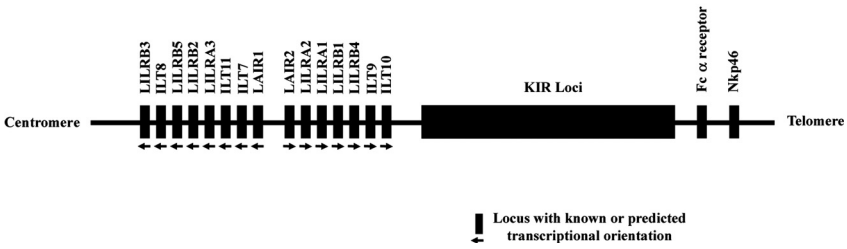

Fig. 3. **The leukocyte receptor complex (LRC) is located on human chromosome 19q13.4.** Within the LRC are a large number of immunoglobulin (Ig)-like receptors including the leukocyte Ig-like receptor (LILR), killer cell inhibitory receptor (KIR), and Leukocyte associated Ig-like receptor (LAIR) families. The LILR family is separated into two clusters by the LAIR molecules, with transcription of each of these LILR clusters being in a head to tail fashion.

LILR ligands

A viral ligand for LILRB1

Intracellular pathogens such as human cytomegalovirus (HCMV) render their host cell susceptible to lysis by cytotoxic T cells (CTLs) and have evolved mechanisms to subvert the MHC class I antigen presentation. However, a complete loss of MHC class I at the surface will increase susceptibility to natural killer (NK) cell-mediated attack (14). UL18, an HCMV protein that resembles an MHC-I protein was originally thought to act as a decoy molecule to prevent NK recognition (15). However, recombinant UL18 was found to bind various B-cell and monocytic lines, and subsequent immunoprecipitations identified LILRB1 as its ligand (1). The interaction between UL18 and LILRB1 has since been studied in detail using cellular and molecular techniques (1, 16, 17). Although the extracellular domain of LILRB2 shares 82% identity with LILRB1, it binds UL18 with 1500-fold lower affinity ($K_D \sim 14\,\mu M$) (18).

Classial and non-classical MHC class I ligands of LILR

Experiments using transfectants and recombinant protein showed that the products of a broad range of MHC class I genes and their alleles interact with LILRB1 and LILRB2 (Table 2). Independent studies using different systems revealed discrepancies for the interaction ($K_D \leq 10^{-5}$) of some alleles with LILRB1 and LILRB2 (Table 2) (2, 17, 19–21). Using the sensitive surface plasmon resonance (SPR) technique, Chapman et al. (17) demonstrated weak interactions for certain receptor/ligand pairs. Such weak binding could explain previous inconsistencies for some alleles of HLA-A, HLA-C, and HLA-E (Table 2). LILRA1 binds HLA-B27 in both classical and β_2-microglobulin (β_2m) independent forms (22). Although HLA-A2 and HLA-F do not interact with LILRA1 (Allen, unpublished), it may yet be found to bind other MHC molecules. Similarly, binding studies for other LILR have yet to detect any significant interactions with MHC class I, suggesting that either these LILRs do not bind to the alleles tested or that their affinities are too low to be detected by the method used (8, 20, 23).

Known major histocompatibility complex (MHC) class I interactions with leukocyte immunoglobulin-like receptor B1 (LILRB1), LILRB2, and LILRA1

MHC class I transfectants	LILRB1 binding	LILRB2 binding	LILRA1 binding	Reference
HLA-A0101	+	+	N/A	[19]
HLA-A0201	–	+/–	N/A	[19]
HLA-A0301	+	+	N/A	[2, 19, 20]
HLA-A2	N/A	N/A	–	Allen, unpublished
HLA-B0702	+	+	N/A	[17, 19]
HLA-B0801	+	+	N/A	[19, 20]
HLA-B0802	–	–	N/A	[19]
HLA-B1501	+	+	N/A	[19]
HLA-B2702	+	+	N/A	[2, 17, 19]
HLA-B2705	+	+	+	[2, 20, 22]
HC-B27 (Heavy chain form of B27)	–	+	+	[22]
HLA-Cw0301	+/–	+/–	N/A	[2, 17, 20]
HLA-Cw0304	+	+	N/A	[19]
HLA-Cw0401	–	–	N/A	[19]
HLA-Cw602	+	–	N/A	[17]
HLA-Cw0702	+/–	–	N/A	[17, 19]
HLA-E	+/–	N/A	N/A	[17, 21]
HLA-F	+	+	–	[25]
HLA-G1	+	+	N/A	[2, 17, 20, 21, 23]
UL18 viral homolog	+	+/–	N/A	[1, 16–18, 72, 73]

N/A indicates that there is no data available for this allele. HLA, human leukocyte antigen.

Table 2

Non-classical MHC class I molecules such as HLA-E, HLA-F, and HLA-G associate with β_2m but perform alternative functional roles and show limited polymorphism compared to their classical counterparts (24). LILRB1 and LILRB2 have been shown to engage HLA-F and HLA-G (2, 23, 25, 26). There are conflicting results for the affinity of LILRB1 and LILRB2 for HLA-G (17, 26). The potential role of HLA-G in the regulation of immune cells during pregnancy will be discussed in more detail below. Recombinant HLA-F stains cell types that are known to express LILRB1 and LILRB2, which remain the only known receptors for this protein, and whose natural function is unsure (25).

Molecular interaction between LILR and their ligands

T-cell receptors (TCRs) and KIRs recognize subsets of MHC class I via their α1 and α2 domains. The broad recognition patterns described for LILRB1 and LILRB2, however, indicate a very different mode of binding. A combination of molecular studies have been used to investigate fine details of the interaction between LILRB1 and its ligands (17, 27). Four separate Ig domains designated D1–D4 make up the extracellular portion of LILRB1. SPR analyses comparing domain groupings demonstrated that the primary ligand binding sites are contained within the membrane-distal D1 and D2 domains (17, 27). A recent cocrystal structure for the D1–D2 region of LILRB1 complexed with HLA-A2 (27) revealed two major contact surfaces; D1 engages the α3 region of the MHC, while the D2 domain of LILRB1 interacts with β_2m (Fig. 4). In order to allow these interactions, a conformational change takes place in the hinge region between D1 and D2 when ligand binding occurs (27). While no major binding sites have been identified in the membrane-distal D3 and D4 domains, it remains possible that they form minor contacts with the upper regions of the LILRB1 ligand.

The broad specificity of LILRB1 may be explained by the conserved nature of its MHC class I contact site. While the polymorphic α1 and α2 domains of the class I heavy chain form a complex for antigen-specific TCR or allele-specific KIR, α3 and β_2m together form a structural scaffold where receptors such as CD8 or LILRB1 bind. Within the α3 region, residues 193–200 and 248 of the class I heavy chain are highly conserved between the classical and non-classical MHC class I and form a direct contact with six residues present on domain 1 of LILRB1 (27). Recognition of β_2m (a subunit shared by all human MHC class I and the viral UL18 homolog) by 14 residues present in domain 1 and domain 2 further contributes to the broad specificity of binding (27). β_2m plays a major role in the interaction between LILRB1 and its ligands; in the crystal complex 70% of the buried surface of the receptor is in contact with β_2m (27). One feature that may prove relevant to future studies of LILRB1 and related receptors is the importance of binding affinity for their various ligands. SPR identifies a hierarchy of interactions for LILRB1, the strongest being that seen for the viral homolog UL18 (17).

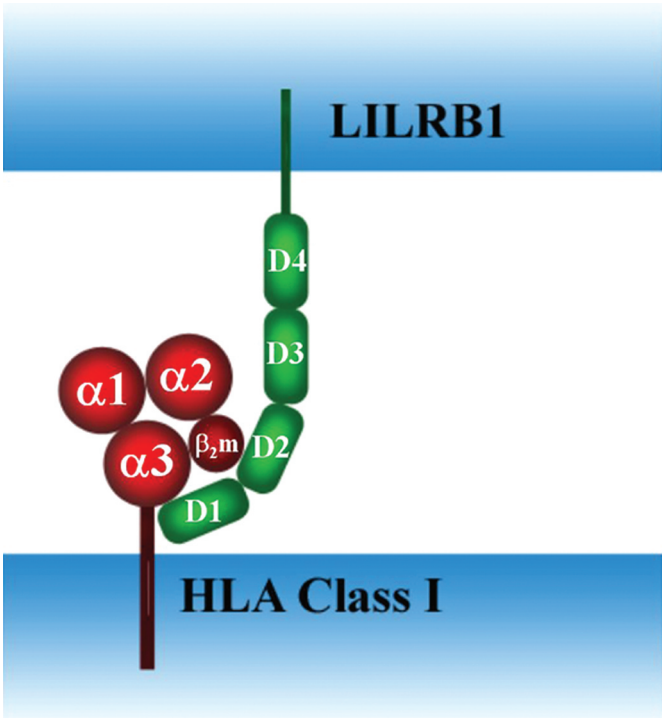

Fig. 4. **Leukocyte immunoglobulin-like receptor B1 (LILRB1) interacts with human leukocyte antigen (HLA) class I molecules at two interfaces.** The first interaction is between domain 1 (D1) of the LILRB1 molecule and the non-polymorphic α3 domain of HLA class I molecules. The second interaction is between D2 of LILRB1 and β_2-microglobulin (β_2m) complexed with HLA class I molecules.

Recent structural studies of LILRB1 allow comparisons to be made regarding ligands and binding sites for other LILR. On the basis of residues in the ligand binding domain, members of the LILR family can be split into two subgroups (Table 3) (27). Group one LILRs are predicted to recognize MHC class I, an interaction that has already been confirmed for three of the five receptors that fall into this category. LILRB1, LILRB2, and LILRA1 exhibit overlapping but distinct repertoires of MHC class I ligands. There are likely to be subtle differences in the way that these receptors engage MHC class I. Like LILRB1, LILRB2 displays a broad specificity for class I proteins and might therefore be predicted to bind class I at similar conserved sites. Comparison of the crystal structure for the D1–D2 domains of LILRB2 and LILRB1/HLA-A2 structure indicates that some predicted contact residues would be rotated away from the class I ligand (18).

Studies using free heavy chains (FHCs) of MHC class I also indicate differences in major contact sites for LILRB2 and LILRA1 compared to LILRB1. FHCs of HLA-B27 form *in vivo* and act as a ligand for various KIR and LILR (28). As described above, β_2m forms a major contact site between LILRB1 and its ligands, so it is not

Amino acid residues of leukocyte immunoglobulin (Ig)-like receptor B1 (LILRB1) that with interact major histocompatibility complex (MHC) class I were aligned with the other members of the LILR family. This enabled the LILRs to be divided into two distinct groups; those that share conserved residues with LILRB1 and those LILRs that did not. Based upon this, it has been speculated that group 1 LILRs will interact with MHC class I molecules. Three LILR members of group 1 have known interactions with MHC class I, while no members of group 2 have been shown to interact with MHC class I. This may indicate that the other members of group 1 with unknown ligands may recognize MHC class I (27).

Group 1	Group 2
LILRA1	LILRB3
LILRA2	LILRB4
LILRA3	LILRB5
LILRB1	ILT7
LILRB2	ILT8
	ILT11

ILT, Ig-like transcript.

Table 3

surprising that LILRB1 does not recognize these FHCs of HLA-B27 (22). Because LILRB2 recognizes both normal and FHCs of HLA-B27, β_2m may not form a major contact site for this receptor (22). The broad specificity of LILRB2 may result from interaction with conserved residues in the α3 region of the class I heavy chain. A possible contact site in the α3 domain is suggested by the observation that like LILRB1, LILRB2 competes with CD8 for MHC class I binding (26). Residue 184 of LILRB1 is one of the main contact residues for β_2m. This amino acid is non-conservatively substituted for valine in all other group 1 and group 2 receptors, which may explain the lesser importance of β_2m in their binding interactions (27). HLA-B27 is the only ligand known to engage LILRA1 (22). Recognition of both normal and FHC structures again implies that β_2m does not form a major contact site. However, it is not yet possible to determine whether the main contact sites lie in the α3 region of the HLA-B27 heavy chain or within the less-conserved peptide binding domain or whether other domains in the LILRA1 receptor exert a greater influence on binding.

The nature of the predicted contact residues for group two LILRs is sufficiently different to indicate that these LILRs may not recognize MHC class I (27). To date, experiments have failed to show such recognition (8, 20, 23). Likely candidates for group two LILRs include MHC class II proteins or MHC class I homologs, and we should therefore consider the possibility that these receptors may recognize alternative ligands.

Expression and function of LILR

All LILRs are expressed on myelomonocytic cells, and some on cells of the lymphoid lineage. Within the myelomonocytic lineage, different cell types express different repertoires of LILR. LILRB1 is expressed on B cells, NK cells, myelomonocytic cells and is the only member of the LILR family to be expressed on T cells (1, 2, 4, 29). Cell-surface expression of LILRs can be regulated during cellular differentiation, as illustrated by LILRB3, which becomes up-regulated on $CD14^+CD33^+$ monocytes and $CD14^-CD16^+$ granulocytes as the cells mature (30). Similarly, the examination of bone marrow samples reveals low-level LILRB1 expression on pre-B I cells ($CD34^+CD19^+$), increasing as they differentiate through pre-B II ($CD19^+CD10^+$) and immature B-cell stages, with highest expression on mature B cells ($CD10^-CD19^+$) (30).

Most cell types of both the innate and acquired immune systems express at least one member of the LILR family. Generation of specific monoclonal antibodies has enabled the expression of some to be studied at the protein level, and cross-linking experiments to be performed as a means to study the effects of receptor signaling on immune cell activity. Further insights can be gained from studies of PIRs, the murine LILR orthologues. The effects of LILR and PIR activity on various cell types are discussed in more detail below.

Polymorphonuclear leukocytes

Neutrophils, basophils, and eosinophils (together known as granulocytes or polymorphonuclear leukocytes) exert their major functions through the release of secretory granules to provide a rapid response in the early stages of immune challenge. Eosinophils express LILRB1, LILRB2, LILRB3, and LILRA2 (31). Cross-linking of LILRA2, an activating receptor triggers eosinophil degranulation accompanied by the secretion of interleukin-12 (IL-12) (31). Neutrophils express high levels of the ILT11/LIR9 transcript (32). Expression patterns of the other LILRs have not yet been examined on other polymorphonuclear leukocyte subsets. Studies of the homologous mouse PIRs provide us with an idea of the influence that LILR might exert on granulocyte functions. Both activating and inhibitory isoforms of PIR are expressed on the surface of murine mast cells (33). Unless the cell is activated, the inhibitory PIR-B isoform predominates and is constitutively phosphorylated. Colligation of this inhibitory receptor with Fcε receptor inhibits mast cell activation and serotonin release (33). It is therefore likely that inhibitory LILR plays a similar role to control the activity of human mast cells.

NK cells

NK cells lyse targets that are deficient for surface MHC class I expression (14). LILRB1 has been shown to inhibit killing by NK cells, as recognition of MHC class I on the target cell was shown to inhibit CD16-dependent lysis (21). This may complement and enhance the inhibitory effects of KIR on NK cell activity. In addition

to its influence on cytotoxicity, LILRB1 appears to inhibit the adhesion of NK cells to their targets in the initial recognition process (34).

Mononuclear phagocytes

Macrophages and monocytes function in pathogen clearance and as professional antigen-presenting cells (APCs) in adaptive immune responses. Monocytes undergo differentiation to become macrophages upon entering the periphery. The differentiation process may be accompanied by an altered LILR expression pattern. We have found that U937 cells up-regulate cell-surface LILRB4 in response to maturation stimuli (Brown, unpublished). Activation of monocytes (by cross-linking HLA-DR, FcγRI, and FcεRI) can be inhibited through colligation of LILRB1, LILRB2, and LILRB4 (2, 8, 20).

Macrophages and dendritic cells recognize microbial structures using pattern recognition receptors such as the toll-like receptors (TLR) on their surfaces. The recent demonstration that monocytes down-regulate LILRA2, LILRA4, LILRB1, and LILRB4, following TLR activation indicated that LILR might exert some influence upon innate response pathways (35, 36). LILRA2 activation reduces TLR-mediated antimicrobial activity (35). LILR signaling on monocytes can direct the nature of a downstream adaptive immune response by skewing the cytokine secretion profile of peripheral blood monocytic cells (35) or modulating the expression of costimulatory molecules on the APC surface (37).

Dendritic cells

Circulating dendritic cells (DCs) ($CD14^-$ $HLA\text{-}DR^{Bright}$) can be divided into two distinct populations ($LILRB4^+/LILRA2^+$ or $LILRB4^+/LILRA2^-$) on the basis of their LILR expression pattern (38). The $LILRB4^+/LILRA2^+$ subset express CD33, CD13, and CD11c and represent the classical myeloid population (30, 38). The second subset ($LILRB4^+/LILRA2^-/CD11c^-$) are devoid of myeloid lineage markers and display cellular characteristics similar to plasmacytoid monocytes (30, 38). In contrast to myeloid DCs (mDCs), which enter lymph nodes via the afferent lymphatics, plasmacytoid DCs (pDCs) access secondary lymphoid organs from the blood stream via high endothelial venules (30). Within a few hours of appropriate stimulation, pDCs secrete type I interferons, whereas mDCs produce homeostatic chemokines (39). Different DC subsets can thus direct the development of different types of T-cell response. Mice deficient for PIR-B showed impaired maturation of DCs (40).

Receptors in the LILR family can also influence the antigen-presenting functions of DCs. Effective T-cell proliferation and differentiation requires a costimulatory signal delivered via B7 molecules on the surface of a professional APC (41). TCR signaling in the absence of costimulation results in T-cell anergy (42). Inhibitory LILR may be involved in T-cell anergy. High-level expression of LILRB2 or LILRB4 inhibited the expression of the costimulatory proteins B7-1 (CD80) and B7-2 (CD86) with a consequent effect on $CD4^+$ T-cell proliferation (37). IL-10 has also been implicated in the anergy of T cells and induces up-regulation of LILRB2 on monocytes and lipopolysaccharide matured DCs (43, 44). However, soluble LILRB2 is present in the supernatant of DCs and can down-regulate IL-10, while enhancing T-cell proliferation (43). Taken together, these findings indicate that IL-10 in association with soluble and surface forms of LILRB2 act to modulate costimulator expression on APCs and thus control T-cell proliferation.

T cells

Only one member of the LILR receptor family, LILRB1, is expressed on T lymphocytes (1, 2, 4). Within the T-cell population, cytotoxic T-cell clones and $CD4^+$ T lymphocytes show variable cell-surface expression, as not all T cells examined expressed cell-surface LILRB1 (4, 29). Irrespective of surface expression, all $CD4^+$ and $CD8^+$ clones may be positive for intracellular LILRB1 (4). LILRB1 inhibits signaling through the TCR, through dephosphorylation of the TCRξ ITAM domains, thus reducing the activity of ZAP70 and the linker for activation of T cells (6). Cross-linking of either CTLA4 or LILRB1 on the T-cell surface causes a decrease in T-cell proliferation, accompanied by a decrease in IL-2 and interferon-γ production, but an increase in IL-10 and transforming growth factor-β (TGF-β) production. Proliferation could be restored by the addition of exogenous IL-2 which blocks IL-10 and TGF-β production (45).

In addition to intrinsic signaling pathways, LILRs can exert an effect on T cells by influencing the behavior of other cell types. These effects can be mediated through cell-surface proteins (costimulatory proteins), soluble factors (cytokines), and a failure to control APC differentiation/proliferation. For example, triggering of LILRA2 on monocytes alters their cytokine expression profile by pushing the IL-10/IL-12 ratio in favor of IL-10, thus favoring a Th2 humoral type response (35). Skewing towards the Th2 phenotype is also seen in PIR-B deficient mice as a result of impaired DC maturation (40).

B cells

Inhibitory LILR expressed on B cells may control cellular proliferation and signaling through the B-cell receptor. Ligation of PIR-B maintains a negative regulatory effect on murine B cells (46). In the absence of inhibitory receptor control, PIR-B knockout mice show constitutive B-cell activation (40). The negative influence of PIR-B on B-cell activity can be overcome by addition of IL-4, which

down-regulates the inhibitory isoform, while up-regulating activating PIR-A receptor. IL-4 can therefore free B cells from PIR-B inhibitory receptor-mediated suppression to enhance B-cell and Th2 responses (47).

Activating LILR

Most studies of LILR function have focused on control mechanisms exerted by inhibitory receptors. Less is known about the functional role of activating LILRs compared to the inhibitory receptors. Activating LILRs studied in detail to date are LILRA2, which is implicated in the activation of eosinophils, and ILT11/LIR9 (membrane bound form), which is expressed exclusively on myelomonocytic cells (31, 32). Receptor cross-linking results in the secretion of cytokines [IL-1β and tumor necrosis factor-α (TNF-α)], and other factors associated with the early stages of an inflammatory response, suggest that activating LILR may play a role in the modulation of inflammatory responses (31, 32).

The clinical relevance of LILR

Given the role played by LILR in the modulation of immune cell activity, they would be predicted to show some association with immune-related pathology. Functional studies demonstrate that inhibitory LILRs can exert control over T-cell responses, as modulation of these receptors can potentially trigger a state of anergy or uncontrolled activation. A similar range of effects could occur for myelomonocytic cells where LILRs are thought to play an important role in regulating cellular responses to a range of immunological stimuli.

Viral infection

The identification of LILRB1 as receptor for a viral MHC class I homolog points to a role for LILRs in viral infection. A recent study has shown that when patients are immunosuppressed to prevent transplant rejection, cytomegalovirus can become reactivated, accompanied by an increase in LILRB1+ cells (48). It is not yet clear whether this phenomenon is a symptom or a cause of pneumonitis. It may result from ineffective cytotoxic responses or an exploitation of the interaction between LILRB1 and UL18 (49).

HIV^+ subjects show increased LILRB2 expression on $CD14^+$ monocytes and elevated levels of IL-10 in their serum compared to HIV^- subjects (44). Blocking with anti-IL-10 caused a decrease in LILRB2 expression, indicating that IL-10 was at least partly responsible for receptor up-regulation (44). Proliferation of $CD4^+$ T cells was also inhibited when cocultured with monocytes and HIV^+ (i.e., elevated IL-10) sera compared to normal sera. These results imply that increased levels of IL-10 in the sera of HIV^+ subjects impair the antigen presentation function of monocytes via up-regulation of LILRB2 (44).

Bacterial infection

LILR signaling on macrophages can trigger cytokine secretion to skew subsequent adaptive responses in favor of either Th1 or Th2 profiles. During infection with *Mycobacterium leprae*, a dominant Th1 response results in the tuberculoid form of disease, with few bacteria and a strong cell-mediated immune response (35). In contrast, during lepromatous leprosy, high bacterial loads and disseminated skin lesions are accompanied by the expression of Th2 cytokines and the dominance of humoral immunity (35). Analysis of skin samples from lepromatous leprosy patients confirmed the expression of the type 2 cytokines TGF-α, IL-4, IL-5, and IL-10, with elevated expression of LILRB5, LILRB3, the activating receptor LILRA2, and the soluble receptor LILRA3. The greatest differential expression between lepromatous and tuberculoid lesions was seen for the activating receptor LILRA2 (35).

Role of LILRs in tumors

HLA-G is predominantly expressed by trophoblast, although ectopic expression is observed in certain situations such as IL-10-secreting tumor cells (50, 51). Such expression has been proposed as a potential immune evasion mechanism and has been observed for melanoma, lung, renal, and breast carcinomas (52–58). HLA-G is expressed on some but not all myelomonocytic and T cells that infiltrate lung and breast carcinomas (53, 55). HLA-G is not expressed in epithelial tissue in lung carcinomas, but is expressed in epithelial tissue in breast carcinomas, and in the nodular area of primary melanoma and lymph node metastases (52, 53, 55). A study of breast carcinoma found an apparent correlation between increased HLA-G expression and the inflammatory grade of the lesions (53). Breast carcinomas, $CD8^+$ and $CD56^+$ lymphomas have also been examined for the expression of LILRB1, a known receptor for HLA-G (53, 59). Immunohistochemistry revealed the expression of LILRB1 on all $CD8^+$ and $CD56^+$ lymphomas and breast carcinomas examined, with expression levels proportional to the inflammatory grade of the lesions (53, 59). However, LILRB1 is normally expressed on subsets of $CD8^+$ CTLs and $CD56^+$ NK cells, so it is unclear whether expression contributes to tumorigenesis or evasion from cytolysis. Interaction between HLA-G and LILRB1 on lymphocytes and NK cells is likely to result in reduced cytolytic activity. Similarly, the recognition of HLA-G by LILRB2 on infiltrating macrophages or LILRB1 on NK cells could skew cytokine profiles to influence subsequent immune activity.

Arthritis

Rheumatoid arthritis (RA) involves synovial inflammation and destruction of the articular surfaces. Pathology is characterized by an increase in neutrophils and macrophages in synovial fluid, with macrophages the predominant source of proinflammatory cytokines IL-1β and TNF-α (60). Activation of ILT11/LIR9 on monocytes results in calcium flux and secretion of IL-1β and TNF-α (32). This cytokine secretion profile could indicate a link between ILT11/LIR9 signaling and the pathology of RA. Other activating and inhibitory LILRs have been examined in relation to the pathobiology of RA; extensive expression of LILRA2 and LILRB2 on neutrophils and macrophages observed in this disease is attributed to the increased presence on these cell types during early to intermediate stages of RA (61). This is in contrast to extremely limited expression of these LILRs seen for subjects with chronic RA, negligible in subjects with osteoarthritis (OA), and no expression in control tissue (61). LILRB3 was also examined in subjects with RA and OA. However, the expression was variable and limited, with LILRB3 exclusively expressed by macrophages in subjects with RA (61). Future studies should reveal whether there is an up-regulation of LILRA2 and LILRB2 expression at the cell surface of the neutrophils and macrophages, or if this increase is due simply to the numbers of lymphocytes infiltrating the synovium.

A closely related group of arthritic diseases known as spondyloarthopathies have long been known to be associated with the HLA-B27 allele (62). HLA-B27 is recognized by LILRB1, and in both normal and FHC forms acts as a ligand for LILRB2 and LILRA4 (22). A growing interest in FHC biology and recognition of HLA-B27 by alternative immune receptors has led to the suggestion that receptors such as KIR and LILR may be involved in disease pathogenesis (63).

Coeliac disease

Coeliac disease is a chronic heritable disorder, characterized by atrophy of villi within the small bowel mucosa, with malabsorption caused by abnormal reaction to ingestion of prolamins. Irish and UK studies have linked the disorder to chromosomal region 19q13.4, the location of the LRC. Moodie et al. (64) examined LILRA3 in coeliac disease, with the rationale that LILRA3 is the only gene within the LRC to exhibit a presence or absence variability. No association was found between coeliac disease and the deletion of LILRA3 (or through the single-nucleotide polymorphism in exon 3). LILRA3 is a putative soluble protein, but there is no evidence that it is translated or secreted. Further studies are need to be performed to determine whether LILRA3 is secreted before genetic screening for the presence/absence of LILRA3 in disease can be effectively examined.

Transplantation

Triggering of mouse inhibitory receptor PIR-B resulted in prolonged allogeneic graft survival (65). In an *ex vivo* model of xenoreactivity, porcine endothelial cells were transfected with HLA-G. Interaction between HLA-G and LILRB1 reduced rolling adhesion of human NK cells (34). A partial inhibition of NK cell lytic activity was also observed, although the percentage inhibition did not correlate with the LILRB1 expression levels indicating that another receptor might confer a separate protective function (34, 66).

HLA-G and pregnancy

Expression of classical fetal MHC class I is limited during pregnancy, presumably to evade an allo-specific maternal response. Thus, to avoid the problems of a 'missing self' response associated with a lack of MHC class I expression, non-classical alleles HLA-C, HLA-E, and HLA-G are expressed by trophoblast during pregnancy (63, 67, 68). Unlike HLA-E, HLA-G expression is restricted to trophoblast, where its expression may be enhanced through the action of IL-10 (50). Four immunomodulatory receptors – LILRB1, LILRB2, KIR2DL4 and p49 recognize HLA-G (1, 16, 18, 69). Of these, LILRB1 is expressed at high levels on NK cells isolated from maternal decidua during pregnancy, and both LILRB1 and LILRB2 on maternal decidual macrophages (70, 71). Recognition of HLA-G by LILRB1 causes the partial inhibition of NK directed cell lysis (34, 71).

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