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Expression, purification, and refolding of the myeloid inhibitory receptor leukocyte immunoglobulin-like receptor-5 for structural and ligand identification studies

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Abstract

The leukocyte immunoglobulin-like receptors (LIRs, also known as ILTs, CD85, and LILRs) comprise a family of related immunoregulatory receptors encoded within the leukocyte receptor cluster (LRC) on human chromosome 19. LIRs are transmembrane proteins containing either two or four extracellular immunoglobulin domains, and most family members are expressed predominantly on myeloid cell lineages. Although the inhibitory receptors LIR-1 and LIR-2 are known to bind to a broad range of class I MHC molecules and are thought to play important roles in immune regulation, the majority of LIRs are currently of unknown structure and their ligands remain unidentified. In this study, we describe recombinant production and characterisation of the extracellular portion of LIR-5 (ILT3), a poorly understood inhibitory receptor that transduces tolerising signals to dendritic cells. The two extracellular immunoglobulin domains of LIR-5 were expressed in *Escherichia coli* to a high level and were found to accumulate in inclusion bodies. Inclusion bodies were purified, solubilised, and receptor then renatured by dilution refolding, with acceptable yields. Size exclusion chromatography and SDS–PAGE analyses confirmed the extracellular portion behaved as a monomer in solution, and purified protein was antibody-reactive. LIR-5 is representative of a subset of LIR receptors that on the basis of structural and sequence comparisons with LIR-1 seem unlikely to bind class I MHC molecules. Successful prokaryotic generation of correctly folded LIR-5 in high levels has implications for production of other LRC receptors and should greatly facilitate attempts to define the structure and ligands of this important regulator of dendritic cell function.

Keywords: LIR-5; Dendritic cells; Inhibitory; Refolding

Appropriate control of an immune response is essential to ensure that a balance is maintained allowing adequate pathogen-specific immunity whilst avoiding immunopathological damage to self tissues. Several classes of immune receptors have thus far been identified that exert inhibitory or activatory effects upon immune effector cells and as such are important elements of this control. The leukocyte receptor cluster (LRC)[1] on human chromosome 19q13.4 contains many such immunomodulatory genes, the majority of which encode receptor proteins that are members of the immunoglobulin superfamily [1]. These include the killer cell immunoglobulin receptors (KIRs), the leukocyte-associated immunoglobulin-like receptors (LAIRs), the IgA receptor FcαR, the natural killer cell activatory receptor NKp46, and the platelet collagen receptor GpVI [2]. Also encoded within the LRC is a family of related immunomodulatory receptors

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[1] *Abbreviations used:* LIR, leukocyte immunoglobulin-like receptor; KIRs, killer cell immunoglobulin receptors; hCMV, human cytomegalovirus; PBMC, peripheral blood mononuclear cell; DC, dendritic cell; Ig, immunoglobulin; ITIMs, immunoreceptor tyrosine-based inhibitory motifs; ITAMs, immunoreceptor tyrosine-based activatory motifs; PTKs, protein tyrosine kinases; TCA, trichloroacetic acid; LAIRs, leukocyte-associated immunoglobulin-like receptors; ILTs, immunoglobulin-like transcripts.

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known as the leukocyte immunoglobulin-like receptor (LIR) (or alternatively immunoglobulin-like transcripts (ILTs), CD85, or LILRs) [3,4].

The ILT/LIR receptor family was originally identified using two contrasting approaches. First, isolation of novel cDNAs on the basis of their homology to KIRs, FcαR, and murine gp49 led to the characterisation of two novel receptors designated ILT-1 and -2 [5]. A second group described a novel immunoglobulin (Ig) superfamily molecule that bound both cellular MHC class I and the class I homologue UL18 [6] and designated this prototypic receptor LIR-1 (also known as ILT2). The same two groups went on to clone and characterise several new members of the LIR/ILT family by probing peripheral blood mononuclear cell (PBMC) and dendritic cell (DC) cDNA libraries with cDNA probes.

LIRs are type I transmembrane glycoproteins, expressed predominantly on monocytes, macrophages, and DCs, but with various other members expressed in B cells, natural killer cells, T cells, and granulocytes, suggesting they have evolved to regulate the function of a broad range of immune cells [3,4]. Their extracellular region is composed of either two or four Ig domains, the structures of which are stabilised by intrachain disulphide bonds between canonical cysteine residues [3,4,7,8]. A single hydrophobic region spans the lipid bilayer. Inhibitory members of the LIR family (LIR-1, LIR-2, LIR-3, LIR-5, and LIR-8) have a long cytoplasmic tail containing between two and four immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which upon phosphorylation recruit SH2-domain-containing phosphatases such as SHP-1 that mediate inhibitory signalling; activatory members of the family (LIR-6, LIR-7, LIR-11, and ILT7) have a short cytoplasmic tail but instead use a charged arginine residue within the transmembrane domain to associate with the common γ-chain of FcεRI [3,4,9]. This adaptor protein contains immunoreceptor tyrosine-based activatory motifs (ITAMs) within its cytoplasmic domain that upon phosphorylation recruit protein tyrosine kinases (PTKs) and thus transduce an activatory signal. A single receptor, LIR-4, lacks transmembrane and cytoplasmic domains and is thought to be expressed in a soluble, secreted form. In vitro studies on a range of LIR receptors, both inhibitory and activatory, have suggested that either ligand binding or receptor cross-linking can lead to significant alteration in effector cell function, consistent with broad and efficient regulation of immune responses [3,4].

Recently, production of recombinant forms of LIR-1 and LIR-2 has greatly facilitated studies addressing recognition of class I MHC molecules by these receptors. Ligand-binding fragments of LIR-1 and LIR-2 consisting of membrane-distal D1–D2 domains were used in surface plasmon resonance studies to show that LIR-1 and LIR-2 interact with a diverse range of classical and non-classical MHC class I molecules, with micromolar affinities [10]. Similar affinities were established by Shiroishi et al. [11]. In addition, use of recombinant proteins enabled a putative ligand-binding site on LIR-1 to be identified and helped establish that both LIR-1 and LIR-2 recognise class I MHC molecules in a novel peptide-independent manner distinct from other class I MHC receptors such as the KIRs [7]. Availability of recombinant *Escherichia coli*-derived receptor fragments have also enabled the first structural characterisations of the family, leading to crystal structures of LIR-1 in the absence [7] and presence [12] of a class I MHC ligand, and also LIR-2 [8]. These studies highlight the importance of obtaining high quality recombinant protein for both structural and binding studies of less well-characterised LIR receptors.

Here, we describe recombinant production of LIR-5 (also known as ILT3), an inhibitory receptor expressed on monocyte and myeloid lineages [13]. Recent studies on LIR-5 suggest it may be an important transducer of tolerising signals to DCs [14]. LIR-5 belongs to a subset of family members (including LIR-3, LIR-8, ILT10, and ILT11) that have low conservation with LIR-1 at amino acid positions comparable to those involved in contacting class I MHC [12]. On account of this low conservation, such “group 2” LIRs [12] are hypothesised to bind novel, non class I MHC ligands that do not associate with β2m. Our study documents successful recombinant production of a group 2 LIR receptor, detailing robust prokaryotic methods for the production of milligram quantities of the ectodomain of LIR-5 in soluble form, suitable for both crystallisation and ligand identification and binding studies. Availability of correctly folded soluble LIR-5 should greatly facilitate attempts to define the structure and ligands of this important regulator of DC function.

Materials and methods

Cloning of LIR-5 and LIR-5bt

DNA fragments encoding LIR-5 were amplified from plasmids kindly provided by David Cosman. PCR was carried out with *Pfu* polymerase, and utilised the 5′ primer BWBH22 (5′-GGAATTC CAT ATG GGT CCA CTT CCA AAA CCA ACT CTC TGG GCT GAG CCA GGC TC-3′), which introduced a unique *Nde*I site and non-coding alterations to destabilise RNA secondary structure and improve codon usage. For the non-tagged LIR-5 construct, BWBH22 was used in combination with the 3′ primer BWBH26 (5′-CCC AAGCTT CTA TGA GAC TAT GAG CTC CAG GGG GTC-3′), which introduced a stop codon and unique *Hin*dIII site. PCR products were digested with *Nde*I and *Hin*dIII, and ligated into pET23a vector digested similarly. For the biotinylation-tagged LIR-5 construct (LIR-5bt), BWBH22 was used in combination with BWBH117 (5′-CGC GGA TCC TGA GAC TAT GAG CTC CAG-3′, introducing an in-frame *Bam*HI site), PCR products digested with *Nde*I and *Bam*HI, and ligated into the plasmid JMB002 digested similarly, allowing incorporation of a biotinylation tag (GSGGGLNDIFEAQKIEWH) at the carboxy terminal of the LIR-5 chain. LIR-5 and