Multi-functional Lectin-Like Transcript-1: a new player in human immune regulation

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Keywords: Carbohydrate Recognition Domain, C-type lectin-like receptors, Lectin-Like Transcript 1, Natural Killer Gene Complex, Osteoclast inhibitory lectin

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Abbreviations

Carbohydrate Recognition Domain (CRD), C-type lectin-like receptors (Clec); C-Type Lectin-like Domain (CTLD); Killer cell Lectin-like Receptor (Klr), Lectin-Like Transcript 1 (LLT1), Natural Killer gene Complex (NKC), Osteoclast inhibitory lectin (OCIL).

Abstract

Lectins and C-type lectins are a heterogeneous group of proteins with a diverse range of functions. Two C-type lectins, found in the Natural Killer gene Complex (NKC), Lectin-like transcript 1 (LLT1) and CD161, have recently gained interest from a broad range of immunological and non-immunological fields. LLT1 in particular has a diverse functional repertoire, from NK cell and B cell regulation to bone metabolism. This review seeks to bring together nearly two decades of research on these receptors and describe their importance in the immune system.

Lectins and C-type lectins

Lectins are a heterogeneous non-enzymatic group of proteins present in a whole variety of organisms, from viruses, bacteria and yeast to plants and animals [1]. The origin of the word 'lectin' is found in the Latin *legere*, which means 'to select'. This phylogenetically ancient class of proteins has a wide variety of functions and possesses a distinctive structural motif. This singular structural signature known as the Carbohydrate Recognition Domain (CRD) is responsible for the shared feature amongst lectins, which is their ability to bind carbohydrates.

The archetypical CRD fold comprises a double loop structure, two antiparallel β-strands and two α-helices [2,3]. The presence of four conserved cysteine residues allows the formation of two disulphide-bonds that contributes to its overall stabilisation. The CRD structure of a subgroup of lectins comprises Ca^{2+} binding sites [3]. They are located in one of the most evolutionarily flexible

regions and some of them are essential for the binding of carbohydrates. This particular group of lectins are known as C-type (Calcium-dependent) lectins. They usually present a short cytoplasmic tail, followed by a transmembrane domain, a stalk region and the extracellular CRD (Figure 1A). Oligomerisation through the stalk region is common amongst C-type lectins, and they are often found as multimers *in vivo* [4].

The C-type lectin fold has been found in more than a 1000 proteins. However, despite their structural similarities, some of these proteins have lost their Ca^{2+} binding sites, thus losing their sugar-binding restriction. Consequently, C-Type Lectin-like Domains (CTLD) are able not only to recognise carbohydrates, but also proteins, lipids, nucleic acids, inorganic compounds and even ice [1].

Their ligand versatility results in a diverse range of functions, with several receptors being involved in cell adhesion and immune responses to pathogens [5,6]. Animal lectins are classified into at least 15 distinct structural families [7]. Amongst them, group V can be highlighted for its immunological importance and it comprises a wide variety of NK cell receptors. This group is evolutionarily recent, restricted to higher vertebrates, encoded in the Natural Killer gene Complex (NKC) and usually involved in protein recognition [2]. Although their expression is not limited to NK cells, they are mainly associated with the modulation of NK cell activity. Nonetheless, the precise role of some members of the family remains unknown.

Lectin-like transcript 1 (LLT1) is a C-type lectin-like protein that was first described by Boles *et al*. [8]. Its characterization as the ligand for the CD161

receptor [9,10] depicted another pair of lectins-proteins intended to play a prominent role in the immune system.

This article aims to review the published literature about LLT1, covering its structure, expression pattern, function, resemblance with its mouse homolog and relevance in immune responses.

The Natural Killer Gene Complex (NKC)

The NKC dates from more than 300 million years ago. It was first identified in mice, where the presence of several genes encoding structurally related type II (N-terminal facing the cytosol) C-type lectins was reported [11]. These C-type lectin-like receptors (CTLRs) are expressed in various cell types, but most commonly on NK cells.

There is an interesting paradox in the evolutionarily history of the NKC. Hao *et al*. studied the NKC complex of four major groups of mammals [12] and concluded that there is a high degree of heterogeneity in gene content, as well as abundant birth and death of such genes, mainly occurring through duplications and deletions. However, although there has been a remarkable process of expansion and contraction of rodent NKC genes, the genomic structure is extraordinarily conserved between mice, rats and humans [13,14].

The human NKC comprises around 30 genes encoding for C-type lectin-like proteins, as well as some poorly characterised orphan genes. A wide variety of immune-related cells, including NK cells, T cells and myeloid cells have been found to express CTLRs.

Nkrp1 and Clec families

CTLRs can be divided into two large groups based on their expression patterns. Killer cell Lectin-like Receptors (KLR and Klr in human and mouse, respectively) are mainly expressed on NK cells, whereas they ligands, C-type lectin-like receptors (CLEC and Clec in human and mouse, respectively), are usually expressed in other cell types as well as NK cells. The nomenclature used has been complex; however, it is now accepted that *KLRB1/Klrb1* genes encode NKRP1/Nkrp1 (NK cell receptor protein 1) proteins and *CLEC2/Clec2* genes encode Clr and human equivalent proteins; Clr proteins are specifically rodent, while their human equivalents, e.g. CD69 and LLT1, are encoded by Clec2 genes.

The Nkrp1 cluster is comprised within the NKC complex, which is located in chromosome 6 in mice, chromosome 4 in rats and chromosome 12 in humans [15–18]. Since its discovery, evidence suggests high conservation of the Nkrp1 family between rodents and humans [19–21]. It is a highly stable genomic region, comprising minimal diversity as well as limited and focused allelic polymorphism [22–24].

In contrast to the polymorphisms within the Nkrp1 family, their Clr cognate ligands, and human equivalents, are even more conserved, suggesting susceptibility to selection pressure on the former but not on the latter [25]. The need to keep self-integrity could explain the extreme stability of this family.

The *Clr* gene family was first identified by Plougastel and colleagues, who mapped this genetic region also within the NKC complex on mouse chromosome 6 [26]. The ability of Nkrp1 receptors to engage with Clr molecules

has been well described in detail elsewhere [27,28]. This grants the NKC complex a striking peculiarity, as the Nkrp1 genes are interspersed with the genes encoding their Clr ligands [29]. It has been suggested that they could all share a common ancestor that once engaged in homophilic interactions. Moreover, this tight genetic linkage could be a way of ensuring coinheritance, providing a mechanism for biological self-recognition.

Both the products of *Nkrp1* and *Clec2* genes are C-type lectin-like proteins. As mentioned above, C-type lectins have evolved to recognise carbohydrates. Iizuka and colleagues first showed the binding of the Nkrp1d receptors to the Clr-b proteins in 2003, which was also shown by Carlyle and colleagues in 2004. This suggests that Nkrp1 receptors are capable of protein recognition. However, there still remains the possibility that glycans present in Clec2 encoded proteins play a prominent role in receptor binding.

The NKC complex is an orthologous region in humans and mice. Although there is a large overlap in terms of genomic organisation and even function, some gene families have diverged. Despite abundant similarities between Nkrp1 and *Clec2* encoded receptors between man and mouse, there are no obvious orthologies, with very few exceptions (e.g. CD69).

A prototypic mouse Nkrp1 receptor (Nkrp1c or NK1.1) exists. In contrast, three members of the human Nkrp1 subfamily have been identified: CD161, NKp65 and NKp80. The human inhibitory NKR-P1A (KLRB1) and activating NKp80/65 (KLRF1/2) receptors present abundant similarities; they are not strictly classified as human NKR-P1, unlike the multi-gene rodent Nkrp1 (Klrb1) family, which has

both inhibitory and activating family members [30]. The corresponding ligands that belong to the Clec2 family are LLT1 for CD161, Keratinocyte-Associated C-type Lectin (KACL) for NKp65 and Activation-Induced C-type Lectin (AICL) for NKp80. These molecules have been reviewed in detail elsewhere [31–33]. This review specifically focuses on the CD161-LLT1 pair, which have the greatest homology between mouse and man [34].

CD161-LLT1 and the rodent homologs

The human (h) gene homologous to mouse (m) *Klrb1* encodes the NK receptor KLRB1/NKR-P1A/CD161 [19,35], sharing 46% homology at a protein level [31]. The product of the *Clec2d* gene (Ocil/Clr-b) was identified as the ligand of the Klrb1 receptor [27,28]. Comparing the mouse and systems, the mouse inhibitory Nkrp1b/d (Klrb1b/d) alleles, and the inhibitory Nkrp1g (Klrb1/Klrb1g) receptor, are most homologous to human NKR-P1A (KLRB1), as are their ligands (Clec2d or Clec2g/h/i vs CLEC2D2) [36].

It has been possible, therefore, to establish homologous C-type lectin-like pairs in mice, rats (r) and humans. In humans, CD161 receptor binds LLT1; in rats Nkrp1A receptor binds Clr11 and in mice, Nkrp1B/D engages with Clr-b. In the latter, *Nkrp1d* has been identified as an allele of the *Nkrp1b* gene [22]. All hLLT1, mClr-b and rClr11 have been shown to inhibit NK cell activity, as discussed below.

The term ‘LLT1’ will only be used in this work to refer to the human CLEC2D, and the term ‘OCIL’ will be used in the context of bone.

The number of genes encoding the Nkrp1 and Clec2-family proteins is different between mouse, rat and man (Figure 1B). The alternative names for LLT1 in humans as well as its rodent homologs are specified in Figure 1C. Within the human NKC complex, CD161 and its binding partner LLT1 are only separated by a 100 kilobases. Thus, the human receptor-ligand pair CD161-LLT1 and their closest homolog in mouse (Nkrp1B/D-Clr-b) share the genetic linkage that allows coinheritance as well as their gene organisation, orientation and copy number. Evidence is less clear when it comes to their expression pattern, particularly regarding hLLT1 and mClr-b (see *Expression pattern: mClr-b vs hLLT1*). The members of the Nkrp1 and both Clr and human equivalent families, as well as their binding partners and signalling motifs are detailed in Figure 1D. To date, there are still a few orphan receptors such as Nkrp1A and Nkrp1C and some promiscuous ligands (e.g. both Clr-d/g bind to Nkrp1f/g). While true orthology is not clear and there are expression differences in the ligands, the rodent Nkrp1b/d/g receptors most likely do represent homologs of the human NKR-P1A receptor. Therefore, it is difficult to establish a simplified model for the study of human CD161-LLT1 interactions.

Human LLT1

LLT1 was first identified as a member of the C-type lectin-like protein family in 1999 [8]. Alternative names are CLEC2D (C-type lectin domain family 2 member D), OCIL (Osteoclast inhibitory lectin) and CLAX (Lectin-like NK cell receptor). Its sequence and domains are specified in Figure 2.

Using an expressed sequence tag database, together with human and mouse consensus sequences of C-type lectin receptors, Boles *et al*. described the

molecular characterisation and the chromosomal mapping of human LLT1. AICL and CD69, both also encoded in the NKC, were identified as the structurally closest receptors, sharing 59% and 56% of sequence similarity, respectively. Furthermore, AICL (CLEC2B), CD69 (CLEC2C), and KACL (CLEC2A) are all structurally similar to LLT1 (CLEC2D2).

At a genomic level, southern blot analyses suggested a single gene for LLT1, or a small number of genes [8,37]. A traditional or inverted TATA promoter sequence is missing in the LLT1 gene; however, a GAATCA sequence can be found upstream of the transcription start site [37]. Expression of the rat OCIL gene is regulated by Sp1 transcription-factor binding sites [38]. At the expression level, northern blot analysis has described the existence of several splice variants [8]. This was later confirmed and described in detail by Germain and colleagues [39]. *LLT1* comprises five exons and four introns, with five alternatively spliced variants as the result of exon skipping, a part from a nonsense-mediated RNA decay product (isoform 3). Variant 1, or LLT1, was identified as the only surface protein; while, variants 2 and 4 were retained in the endoplasmatic reticulum (ER), forming homodimers or heterodimers with LLT1. Finally, isoforms 5 and 6 were identified as the soluble versions of LLT1 and variant 4 respectively, lacking the transmembrane domain encoded in exon 2.

The crystal structure of LLT1 was recently solved [36,40]. One monomeric and one hexameric structure, as well as two dimeric structures with different glycosylation patterns were characterised. For the first time, the two α-helices, the two antiparallel β-sheets and the disulphide bonds that help to stabilise the

structure could be experimentally observed. Furthermore, a detailed analysis of the dimeric interface suggested the existence of a hydrophobic core responsible for leading the interaction. The study concluded a key role for glycosylation in both structure and flexibility in the interface. The LLT1 extracelullar domain, responsible for receptor recognition, was also crystalised by Kita and colleagues, who cast further light on the binding surface and acknowledging the conservation within CTLRs at the structural, binding and signalling levels [36].

The homodimeric nature of LLT1 as well as its large polysaccharide content have been described [39,41]. It is now accepted that the most common presentation of LLT1 is as a highly glycosylated homodimer located on the cell surface.

The C-type lectin-like protein family still comprises abundant orphan receptors. LLT1 left this category in 2005, when two simultaneous studies characterised it as the ligand for the CD161 receptor [9,10]. As previously mentioned, genetic linkage between receptor-ligand pairs of C-type lectin-like proteins is not uncommon. Amongst all the previously described isoforms, LLT1 is the only one able to interact with CD161 [39]. The molecular basis of CD161 and LLT1 interaction is characterised by low affinity (K_d = 48 μM) and fast kinetics, which is very common within cell-cell recognition receptors [42]. This implies that only small conformational changes may be necessary to allow binding.

Expression pattern: mClr-b vs hLLT1

Despite the existence of CD161-LLT1 homologs in rodents, large differences in expression pattern have been acknowledged between mice and humans,

particularly on the Clr-b side. Such singular expression could translate into distinct functionality of these C-type lectins, depending on the host.

Expression of different Clr genes has been shown to be highly variable as well as regulated. Zhang and colleagues studied the expression of different members of the NKrp1 and Clr families in a wide range of mouse tissues, reaching the conclusion that the majority of Clr genes are specifically expressed in certain tissue; for examples Clr-d, the expression of which is restricted to the eye, or Clr-f, found in kidney, liver and intestine (particularly, in differentiated intestinal epithelial cells), which may imply tissue-specific functions [34,43,44]. However, expression of certain Clr, such as Clr-b, did not follow the rule.

Clr-b expression was found in nearly all haematopoietic cells, although absent on erythrocytes, and in a wide range of mouse tissues [34]. This broad expression pattern resembles that of MHC class I molecules, although Clr-b expression is β2-microglobulin independent. Thus, the Nkrp1b:Clr-b axis broadens the missing-self hypothesis beyond self-MHC class I molecules (see below) [27,45,46].

The expression pattern of Clr-b still presents some controversies, however. Whereas its broad expression has been reported several times in the literature, some studies suggest a more restricted expression pattern, predominantly on DCs and macrophages [28] .

The literature about LLT1 is limited, partially due to a lack of extensive and validated tools for its study and characterisation (ie. antibodies). This issue has

been previously addressed by several groups, which have generated their own anti-LLT1 antibodies [39,47,48].

However, there are still many inconsistencies in the published data regarding the distribution of LLT1 in human tissues and cell types (Table 1). Such discrepancies might be due to differences in techniques used (e.g. measuring LLT1 mRNA through Northern blot/RT-PCR versus LLT1 protein using flow cytometry/Western blot). LLT1 was first characterised by Northern blot and was found predominantly expressed on NK cells but also on T and B cells, as well as in thymus, lymph node and spleen [8]. Some future studies questioned and expanded these results, bringing new insights into the expression pattern of LLT1. Mathew *et al*. reported LLT1 expression in all monocytes, most B cells and a few T and NK cells, as well as in Phorbol 12-Myristate 13-Acetate (PMA) and Interleukin (IL)-2-activated Peripheral Blood Mononuclear Cells (PBMCs) [47]. Other groups could not detect LLT1 in resting PBMCs but only after pathogen, Interferon-γ (IFN-γ) [49] or PMA stimulation [50], as well as on activated B cells and DCs [10]. Enhanced levels of LLT1 could be detected on B cells following TLR9, BCR and CD40 stimulation [41,48]. Several studies have shown changes in LLT1/Clr-b expression levels due to viral infection, malignant transformation or cellular stress (see bleow). Also, LLT1 has been detected in the inflammatory setting. Synovial fluid monocytes from Rheumatoid Arthritis (RA) patients, as well as in tissue resident macrophages from RA joints, have been described as LLT1+ [51]. This study was also the first to confirm soluble LLT1 (sLLT1) in serum and to describe its increased abundance in this autoinflammatory condition.

One plausible explanation for all the aforementioned discrepancies regarding the pattern of expression of LLT1 is the variety of anti-LLT1 antibodies used in different studies, as well as the potential distinct states of cell activation amongst the samples tested. Further research on LLT1 expression is needed in order to fully understand its distribution [48].

OCIL in the context of bone

Bone homeostasis involves the constant removal of mature bone tissue (bone resorption) as well as the continuous formation of new bone tissue. Osteoclasts, from the Greek *osteo* (bone) and *clasts* (break) are responsible for the former, whereas osteoblasts, *blast* meaning to germinate, lead the formation of new bone cells.

Osteoclasts are derived from the monocyte-macrophage lineage and they are large, multinucleate cells. On the contrary, osteoblasts are terminally differentiated cells derived from mesenchymal stem cells and present a single nucleus.

In parallel to the mapping of the Clr region by Plougastel *et al.*, Zhou and colleagues cloned and expressed the murine OCIL [26,52]. Its expression was described in osteoblasts and osteoblast-derived cell lines in mice, presenting a very similar expression pattern to that of the Receptor activator of NF-κB ligand (RANKL) [52], which, in contrast to OCIL, promotes the differentiation and formation of osteoclasts. OCIL expression levels are susceptible to hormone treatment, as they are increased by parathyroid hormone, calcitriol, IL-1α, IL-11

and retinoic acid [52]. IL-4 does not seem to play a role in the regulation of OCIL [53], nor mechanical stimuli [54].

Functional studies have shown that murine and human OCIL inhibit osteoclast formation and function, therefore diminishing bone resorption [37,52]. In order to analyse the physiological function of OCIL, *ocil*$^{-/-}$ mice were generated [55]. They were healthy and fertile and the abnormalities presented were restricted to the bone environment. They presented reduced bone formation and increased osteoclast formation compared to their wild-type counterparts, which resulted in the animals suffering from osteopenia. Therefore, this study confirmed OCIL as a negative physiological regulator of bone metabolism. There were no obvious immunological defects, although immunological function of these animals was not specifically assessed. In the human *LLT1*, a naturally occurring Single-Nucleotide Polymorphism (SNP) that results in a non-synonymous mutation (Asn19Lys) has been associated with a decrease of bone mineral density in post-menopausal women [56] (Figure 2).

Interestingly, the role of OCIL inhibiting osteoblast differentiation has also been reported [57]. In this study, Nakamura *et al*. described not only the reduction in osteoblastic function but also the blocking of adipocytic differentiation. This is a remarkable fact, as it provides the first evidence of the involvement of NK cell receptors in the regulation of mesenchymal cell differentiation and function. Up until that point, none of the aforementioned studies had addressed the question of OCIL ligand in the context of bone metabolism. Nakamura and colleagues were the first ones to approach it, and they found that OCIL bound to high

molecular weight sulfated glycosaminoglycans (GAG), as is typical of the C-type lectin family. However, the binding was not Ca^{2+}-dependent.

Neither Nkrp1D nor Nkrp1A were detected in osteoblasts and KUSA O cells, suggesting the sulfated GAG may be the primary ligand for OCIL within the bone marrow, or another yet unidentified receptor.

LLT1 signaling and function

C-type lectin-like proteins can bind to a wide variety of ligands. This results in an equally versatile response, including different processes such as endocytosis, pro-inflammatory and anti-inflammatory responses. Such variety of outcomes is a consequence of the multiple signalling pathways that can be involved in CLR signalling, which has been reviewed elsewhere [58]. Nonetheless, all these pathways share a prominent role for tyrosine-based motifs.

Up until now, no consensus signalling motifs or charged transmembrane residues have been described in Clr and human equivalent proteins. The published literature regarding LLT1 signalling is very limited. The lack of intracellular Immunoreceptor Tyrosine-based Inhibition Motifs (ITIM) has been described [8], which suggests that LLT1 may trigger activation signals. Currently, there is only one study that has addressed the full signalling pathway triggered by LLT1 [59]. In this work, LLT1 signalling was investigated on NK92 cells (which naturally express LLT1 but not CD161) stimulated with a transfectant K562-CD161 cell line. Pharmacological inhibitors were employed to treat NK92 cells and elucidate the LLT1 signalling mechanism; Bambard *et al*.

concluded that this particular C-type lectin uses Src-PTK, p38 and ERK signalling pathways, but not PKC, PI3K or calcineurin.

Data assessing the consequences of LLT1-signaling is mostly limited to NK cells. Resting and IL-2 activated NK cells increased their IFN-γ production upon LLT1 engagement, but their cytotoxic potential remained unchanged [47]. The same was true for the NK cell line YT. Ligation of LLT1 in another NK cell line, NK92, also resulted in a higher release of IFN-γ, but it did not activate their cytolytic function [59]. The presence of the blocking anti-CD161 antibody DX12 had no impact on cytotoxicty [59]. Further investigation is required to elucidate the precise consequences of LLT1-CD161 interaction on both sides. Beyond NK cells, we have recently shown that LLT1 cross-linking acts in a co-stimulatory manner on B cells stimulated through their BCR or CD40, promoting activation and the expression of CD83; while, signalling through LLT1 only on germinal centre B cells led to a down-regulation of CXCR4 [48].

LLT1 cross-linking CD161

Unlike LLT1 expression, CD161 expression appears to be relatively restricted to T lymphocytes and NK cells [19,60,61]. There is conflicting evidence regarding the consequences of LLT1 binding to CD161 expressing T cells. Several studies have suggested a costimulatory role for CD161, as it increases cytokine production upon TCR engagement [10,61,62]. However, cross-linking CD161 was shown to inhibit Tumor Necrosis Factor α (TNF-α) production in TCR-activated CD8+ T cells [41].

In contrast, the data for LLT1 cross-linking CD161 receptor is much clearer. Cross-linking of CD161 on NK cells results in inhibition of cytotoxicty [9,10,63] and cytokine production [9,41]. Inhibition of NK cells through Clr-b/LLT1 has also been described by tumours, both in mice [27] and humans [47,64]; however, one study postulates an activating role for rClr11 [65]. Nonetheless, these experiments were based on studying NK:DC interactions, which are hampered in the rat system, as Clr11 binds both Nkrp1b (inhibitory) and Nkrp1a (activating). Also, strain specificity in the rodent systems is polymorphic and complex.

A detailed characterisation of the antibodies used when studying LLT1/CD161 interactions and their consequences is lacking (ie. agonistic effects, antagonistic effects, redirected activation through antibody-dependent cell-mediated cytotoxicty, etc.). This could explain the underlying mechanisms for the varied effects observed when employing them in different settings.

Overall, it is the induction of this inhibitory signal that has led to the suggestion that LLT1 acts like MHC Class I forming part of the missing-self hypothesis [27,45,46,66]. Thus, understanding the expression and function of both LLT1 and CD161 is important in both infectious disease and cancer settings.

LLT1 expression during infection

Epstein-Barr virus (EBV), Human immunodeficiency virus (HIV), influenza, Human herpesvirus (HSV)) has also been identified as a cause for LLT1 upregulation [41,49]. Recently, increased expression of LLT1 was described in the lung following Respiratory Syncytial Virus (RSV) infection on primary human

bronchial epithelial cells after stimulation by TLR3 and TLR2/6 ligands led to a rapid increase in LLT1 mRNA in respiratory epithelial cells, as did the proinflammatory cytokines type I interferons, interleukin 1β (IL-1β), and tumour necrosis factor alpha (TNF-α) [67] In a study published by Fine *et al*. [68], genotoxic stress caused rapid downregulation of Clr-b, enhancing the killing capabilities of Nkrp1B-expressing NK cells. Clr-b surface expression was lost in murine cells upon poxvirus infection resulting in increased susceptibility to NK cell lysis [69]. In rats, down-regulation of Clec2d11 (Clr11), as a consequence of Rat Cytomegalovirus (RCMV) infection, has also been described [23]. Potentially, lower Clec2d11 (speculated to be an ortholog of Clr-b in mouse) surface levels could make viral-infected cells a better target for NK killing. Interestingly, RCMV encodes a rat C-type lectin-like protein (RCTL) homologous to Clr ligands that confers protection to infected cells. RCTL interacts with NK cells through Nkrp1B receptor, thus preventing killing. These three examples suggest a role for Clr-b in the negative regulation of NK cells, consistent with the missing-self hypothesis. This has been further addressed and confirmed by others.

LLT1 in tumours

Several studies have reported the expression of LLT1 on B cell and monocyte-derived tumour cell lines protecting them from NK cell activity [8,27,39,41,49]. Indeed, Tran *et al*. [70] used a lentiviral transduction system to overexpress Nkrp1B on primary mouse NK cells. This resulted in increased sensitivity to inhibitory signals delivered by Clr-b-expressing target cells.

In vivo, several studies have reported the expression of LLT1 on B cell lymphomas, in particular those derived from germinal centre cells where LLT1 is already highly expressed [48,71], as well as gliomas [64]. Furthermore, a recent study by Rahim and colleagues supported the idea that the Nkrp1B:Clr-b system plays a role in NK tolerance, tumour surveillance and immune escape in an MHC-I-independent manner [45]. Moreover, in a metadata study of ~18,000 human tumours across 39 malignancies, Gentles et al recently described KLR1B (CD161) expression as the most favourably prognostic marker [72]. Further implicating this receptor pair's role in tumour control.

Conclusions

There has been an increasing interest in LLT1, as it can be seen by numerous recent publications. The uniqueness of the CD161-LLT1 pair and the abundant unanswered questions regarding its functionality has made its study attractive within the immunological field. The genomic stability of the NKC complex as a whole, as well as the tight genetic linkage between receptors and their ligands suggests an essential role for these C-type lectins in immune cell interactions and functions. Indeed, LLT1 appears to be a multi-functional receptor, involved in a broad range of biological activities, from bone formation to the germinal centre reaction (Table 2).

Further research will help clarify these established roles and potential reveal new ones. Murine models will bring useful insight into the LLT1 functionality; however, caution will be needed when extrapolating this data to humans due to the differences describe in this review.

However, the role of LLT1 and its the genetically-linked receptor, CD161, in regulating NK cells and B cells make these receptors attractive candidates as therapeutic targets in malignant transformation, as well as autoimmunity and self-tolerance.

ACKNOWLEDGEMENTS

This work was supported by grants from the NIHR Biomedical Research Centre, Oxford, and NIHNIAID U19 Bio-defense Programme (NIH NIAID 1U19AI082630-01) (CBW); Oxford Martin School, Wellcome Trust Senior Fellowship WT091663MA, NIHR Senior Investigator award (PK); and Obra Social La Caixa (AL).

Disclosure of Conflicts of Interest

The authors declare no financial or commercial conflict of interest.

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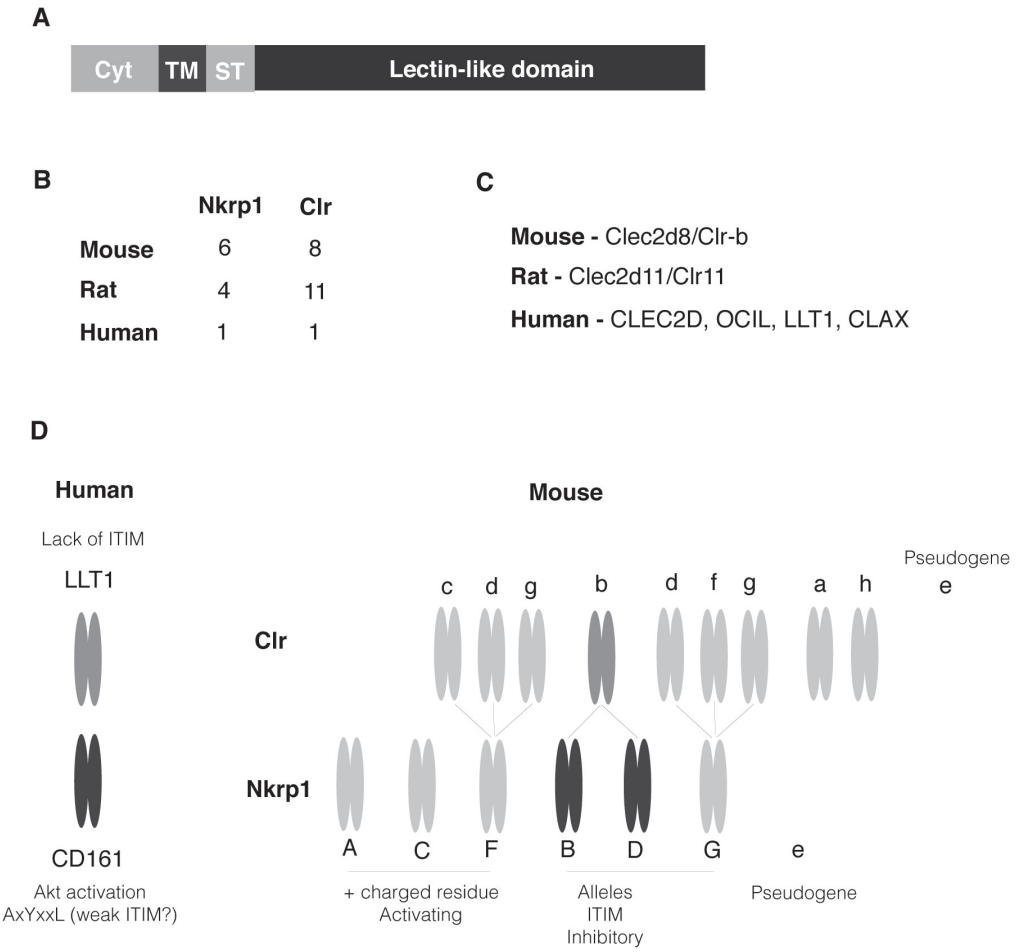

Figure 1. LLT1 and its homologs. (A) shows the general structure of a C-type lectin-like protein, with a short cytoplasmic domain (Cyt), a transmembrane region (TM) a stalk region (ST) and the lectin-like shaped domain. (B) shows the numbers of Nkrp1 and Clr genes in mice, rats and their equivalents in humans. (C) shows the closest homolog to human LLT1 in both mice and rats, as well as its alternative names. Although CLEC2D and LLT1 were used indistinctively for a long time, LLT1 is equivalent to CLEC2D2, as defined by Hao et al. [12], as CLEC2D1 is a pseudogene. (D) shows the characteristics of LLT1-CD161 interaction and their mouse homologs, as well as their described signalling motifs, orphan receptors and pseudogenes.

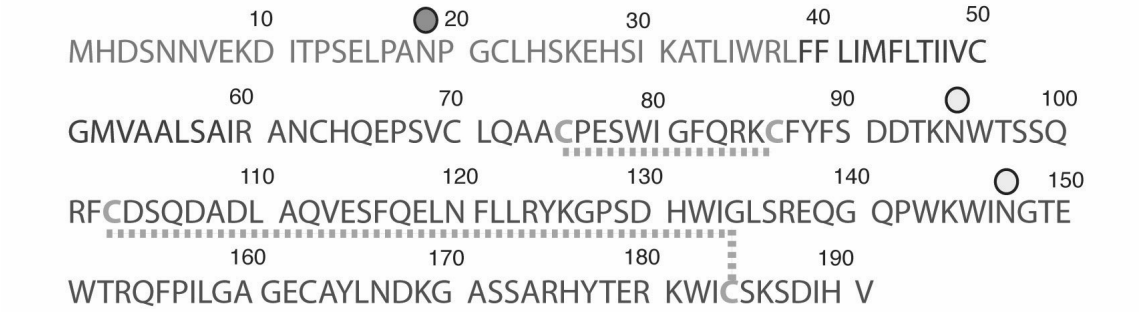

Figure 2. LLT1 aminoacid sequence. LLT1 aminoacid sequence with its cytoplasmic (blue), transmembrane (red) and extracellular (purple) domains specified. The disulphide bridges are depicted by orange dashed lines, the N-glycosylation sites with yellow dots and the described Single Nucleotide Polimorphism (SNP) with a green dot.

Reference	Method	Antibodies	Cell type / Tissue	Function
Boles 1999	Northern Blot	-	T, B and NK cells; Lymph node, thymus, spleen, LAK	-
Eichler 2001	RT-PCR	-	PMA-stimulated PBMCs	-
Zhou 2001	Northern blot, IHC, *In situ* hybridization	-	Osteoblasts; Liver, kidney, gut, heart, skeletal muscle	-
Iizuka 2003	RT-PCR	-	DCs, Macrophages (from bone marrow)	-
Carlyle 2004	FACS	mAb 4A6 (rat IgMκ)	Hematopoietic cells (no erythrocytes); Down-regulation in tumour cell lines	Inhibition of NK cytotoxicity
Mathew 2004	FACS	mAb L9.7	All monocytes, most B cells, few T and NK cells; PMA and IL-2 activated PBMCs	Induction of IFN-γ in NK (in LLT1 expressing cells)
Hu 2004	RT-PCR	-	Osteoblasts, Osteoblast cell line (MG63); Upregulated with IL-1α and PGE_2	-
Aldemir 2005	LLT1 Multimer	-	-	Inhibition of NK cytotoxicity via CD161
Rosen 2005	LLT1 containing liposomes	-	-	Inhibition of NK cytotoxicity and IFN-γ; enhancement of CD3-triggered IFN-γ in T cells
Roth 2007	RT-PCR, FACS	mAB 4C7 (Abnova)	Glioma cells (Upregulated with TGF-β)	Immunosupressive
Rosen 2008	FACS, WB, RT-PCR	2F1, 4F68; mAb 402624 and 402659 (R&D); Rabbit anti-LLT1 antisera	Activated B cells and DCs; B cell-derived cell lines(*)	Inhibition of NK cytotoxicity and cytokine production
Bambard 2010	FACS, RT-PCR	402659 (R&D) and 4C7 (Abnova)	NK92 cells	IFN-γ production (in LLT1 expressing cells)
Germain 2010	FACS, RT-PCR, Real Time RT-PCR	2F1, 4F68; mAb 3480 (R&D); 4C7 (Abnova)	T and B cells, NK cells (low); B cell-derived cell lines(**), THP-1 (low)	-
Germain 2011	FACS, WB, Real time RT-PCR	2F1, 4F68; 3480 (R&D), 4C7 (Abnova)	No in resting PBMCs – upregulated with pathogen/IFN-γ stim; Raji	Inhibition of NK cell function; Costimulation of T cells
Satkunanathan 2014	RT-PCR, IHC, Live cell confocal imaging	4C7 (Novus Biologicals)	RSV-infected/TLR and cytokine stimulated respiratory epithelial cells	-
Chalan 2015	FACS, IHC	FACS: 402659 (R&D) IHC: 4C7 (Abnova)	Monocytes and macrophaes from RA joints	-

Table 1. LLT1 expression and function in the published literature.

	Cell type	Potential sites for interaction	Potential function
CD161 expression	T cells NK cells FDCs cells	Bone Lymph nodes Inflamed tissues Germinal centres	NK cell inhibition T cell inhibition/ activation
LLT1 expression	Osteoblast PBMCs GC B cells Early Plasmablasts B cell lymphomas RSV-infected lung epithelia RA joints		NK IFN-γ production Osteoclast inhibition B cell activation DZ to LZ transition

Table 2. CD161 and LLT1 expression and function. Cell types expressing the C-type lectins CD161 and LLT1 are specified, as well as potential sites for their interaction and functions.