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Review

Pattern recognition receptors in companion and farm animals – The key to unlocking the door to animal disease?

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Abstract

The innate immune system is essential for host defence and is responsible for early detection of potentially pathogenic microorganisms. Upon recognition of microbes by innate immune cells, such as macrophages and dendritic cells, diverse signalling pathways are activated that combine to define inflammatory responses that direct sterilisation of the threat and/or orchestrate development of the adaptive immune response. Innate immune signalling must be carefully controlled and regulation comes in part from interactions between activating and inhibiting signalling receptors. In recent years, an increasing number of pattern recognition receptors (PRRs), including C-type lectin receptors and Toll-like receptors (TLRs), has been described that participate in innate recognition of microbes, especially through the so called pathogen-associated molecular patterns (PAMPs). Recent studies demonstrate strong interactions between signalling through these receptors. Whereas useful models to study these receptors in great detail in the murine and human system are now emerging, relatively little is known regarding these receptors in companion and farm animals. In this review, current knowledge regarding these receptors in species of veterinary relevance is summarised.

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1. Introduction

The immune system has traditionally been divided into innate and adaptive components, each with a different function and role. The adaptive component is organised around two classes of specialised lymphocytes, T cells and B cells, each of which display a unique receptor with a specific antigen, leading to a large and diverse repertoire of antigen receptors within the T/B cell population. This diversity is necessary to ensure that each individual cell can encounter its specific antigen, thus inducing activation, maturation and proliferation of the cell. This process, called clonal expansion, forms the basis of the function of the adaptive immune system. Whereas clonal expansion is crucial in dealing with an invading pathogen, the process

itself is relatively slow, taking days before enough effector cells and their products can be produced. This delay provides the majority of invading pathogens with time to attach, invade and successfully replicate within the host.

In contrast, the effector mechanisms of innate immunity are activated immediately after infection, with the aim to control the replication of the infecting pathogen at the site of entrance. These mechanisms include direct bactericidal activities, such as phagocytosis and the production of reactive oxygen and nitrogen intermediates (ROI and RNI, respectively), secretion of antimicrobial peptides, activation of the alternative complement pathway and the production of pro-inflammatory immune modulators.

Thus, the main function of the innate immune system classically was thought to be to deal with any foreign material until the adaptive immune system is able and potent enough to take over. However, it has become increasingly clear that the innate immune system has a much more

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important and fundamental role in host defence. This article aims to outline the current knowledge about specific classes of receptors involved in stimulating the innate immune response in companion and farm animals and how stimulation of these receptors controls the adaptive immune responses.

2. Strategies of innate and adaptive immune recognition

The main distinction between the innate and adaptive immune systems is in the mechanisms and receptors used for immune recognition. In the adaptive immune system, T cell and B cell receptors are generated during their development by somatic recombination. This equips each cell with a structurally unique receptor, leading to an extremely diverse and randomly generated receptor repertoire. Cells expressing a fitting, antigen-specific receptor are subsequently multiplied by clonal expansion. The downside of this system, however, is that receptors have to be developed within an individual and, no matter how useful they are, they have to be developed over and over again in subsequent cell generations, as these receptors are not encoded in the germ line.

Since the binding sites of antigen receptors arise as a result of random genetic mechanisms, the receptor repertoire contains binding sites that can react not only with microorganisms but also with innocuous environmental antigens and self antigens. Activation of the adaptive immune response can be harmful to the host when the antigens are self or environmental antigens, since immune responses to such antigens can lead to autoimmune diseases and allergies. How does the immune system determine the origin of the antigen and subsequently decide whether to induce an immune response? Recent studies demonstrate that the innate immune system plays a major role in these decisions.

During evolution, the innate immune system appeared before the adaptive immune system and some form of innate immunity probably exists in all multi-cellular organisms. Innate immune recognition, as stated above, is mediated by germ line-encoded receptors, which means that the specificity of each receptor is genetically predetermined. One advantage of this is that innate immune receptors evolved by natural selection to have defined specificities for infectious microorganisms. The problem, however, is that every organism has a limit to the number of genes it can encode in its genome. The human genome, for example, contains only 75,000–100,000 genes, most of which have nothing to do with immune recognition. In comparison, there are approximately 10^{14} and 10^{18} different somatically generated immunoglobulin receptors and T cell receptors, respectively. The total number of receptors involved in innate immune recognition is thought to be in the hundreds. Moreover, microorganisms are extremely versatile and can mutate at a much higher rate than any of their hosts.

The strategy of the innate immune response may not be to recognise every possible antigen, but rather to focus on a

few, highly conserved structures present in large groups of microorganisms. These structures are referred to as pathogen-associated molecular patterns (PAMPs) and the receptors of the innate immune system that have evolved to recognise them are called pattern-recognition receptors (PRRs). The best-known examples of PAMPs are bacterial lipopolysaccharide (LPS), peptidoglycan (PGN), lipoteichoic acids (LTAs), mannans, bacterial DNA, double-stranded RNA and glucans. Although these structures are chemically quite distinct, all PAMPs have common features. PAMPs are produced only by microbial pathogens and not by their hosts. They are usually invariant structures shared by entire classes of pathogens. For example, all Gram-positive bacteria have LTA and, therefore, the LTA-PRRs of the host can detect the presence of virtually any Gram-positive bacterial infection. The structures recognised by the innate immune system are usually essential for the survival or pathogenicity of microorganisms.

3. Pattern-recognition receptors

The PRRs of the innate immune system differ from T and B cell receptors in many ways. PRRs are expressed on a variety of antigen presenting cells (APCs), particularly monocytes, macrophages, dendritic cells (DCs) and B cells, with recent data suggesting expression of PRRs on T cells and natural killer (NK) cells (Hornung et al., 2002). As the expression of PRRs is not clonal, all receptors displayed by a specific cell type recognise the same PAMP. Activation of PRRs by PAMP binding triggers the immediate response of the cell, in contrast to the necessary proliferation/maturation of cells of the adaptive immune response. This accounts for the rapid kinetics of innate immune responses.

Structurally, PRRs belong to two major groups; C-type lectin receptors (CTLRs) and Toll-like receptors (TLRs). Whereas the majority of CTLRs seem to be involved in antigen uptake, either by mediating the interaction of a PAMP with a specific receptor in a secreted or surface expressed form, TLRs play a major role in activating intracellular signalling cascades after PAMP engagement. However, similar to the murine and human systems, we may need to revise the above mentioned groups and include the intracellular expressed PRRs belonging to the family of NOD-like receptors and RIG-1-like receptors (Creagh and O'Neill, 2006). To date, NOD-like receptors have only been shown to detect bacteria, whereas RIG-1-like receptors only recognise viruses. Thus, in addition to the bacterial and viral ligands to surface PRRs, cells seem to have a second, redundant system, acting through similar signalling pathways, and probably acting co-ordinately in circumstances of TLR/CTLR activation.

3.1. C-type lectin receptors

A comprehensive review of the discovery and functional grouping of C-type lectins was recently published by Zelenky and Gready (2005). The superfamily of C-type lectin-

like domains (CTLDS) is a group of currently more than 1000 identified extracellular proteins. C-type lectins were among the first animal lectins discovered, with bovine conglutinin being known since 1906. The terms C-type lectin, carbohydrate recognition domain (CRD) and CTLD are often used interchangeably in the literature. The term C-type lectin was introduced to distinguish a group of Ca^{2+} -dependent (C-type) carbohydrate-binding proteins (lectins) from the Ca^{2+} -independent lectins (Drickamer, 1988). When the structure of C-type lectins was established and their functions defined, it was found that the carbohydrate-binding activity was mediated by a CRD which was present in all Ca^{2+} -dependent lectins but not in other types of animal lectins (Drickamer, 1988). Further information regarding C-type lectin groups in the human and murine system can be found at <http://www.imperial.ac.uk/animal-lectins/default.html> and <http://plab.ku.dk/tcbh/lectin-links.htm>.

The genes coding for C-type lectins that bear a single CTLD are localised in a single genomic region that shows conservation of synteny in rodents and humans (Kelley et al., 2005; Trowsdale et al., 2001). The NK gene complex (NKC) is located on human chromosome 12p13.2 and mouse chromosome 6. Nineteen C-type lectin and CTLR genes have been localised within the human NKC, including LY49L, the NKG2 family, CD94, CLEC-1, CLEC-2, OLR1, KLRF1, AICL, CD69, LLT1, NKR-P1A, MAFA-L, M6PR, DCIR, DLEC and DECTIN-1 (CLECSF12). The similarity of the genomic structure of these genes has led to the hypothesis that they are derived from a common ancestral gene. Some of these genes show a wide pattern of expression in the haematopoietic lineage (CD69, AICL), whereas others are restricted to NK cells and some T cell subpopulations (NKG2, CD94, NKR-P1A), DCs (CLEC-1, DLEC, DECTIN-1) or vascular endothelial cells (OLR1/OX-LDL, which codes for an oxidised low-density lipoprotein receptor) (Kelley et al., 2005; Trowsdale et al., 2001).

In recent years, a variety of surface-expressed CTLRs involved in pathogen detection have been identified (Cambi et al., 2005). Membrane-bound CTRLs involved in antigen uptake can be divided into type I and type II, depending on the orientation of their N-terminus (pointing outwards or inwards into the cytoplasm of the cell, respectively). Type II receptors have a single transmembrane domain with an extracellular carboxyl terminus containing the CRD and a cytoplasmic amino terminus. Typical examples of this group are the asialoglycoprotein receptor of hepatocytes, macrophage galactose/*N*-acetylgalactosamine-specific lectin and the NK cell low affinity IgE receptor (CD23). These receptors have a consensus amino acid motif in their cytoplasmic domains responsible for associating with clathrin-coated vesicles. Some type II receptors (e.g., asialoglycoprotein receptors) are involved in uptake of molecules into cells through the endocytic pathway. Others (e.g., NK receptor NKR-P1) are involved in signal transduction based on cell–cell recognition. Oligomerisation (into

Table 1

Summary of some lectins and C-type lectin receptors and their currently known ligands

Group	Members	Known ligands
MMR family	MMR	HIV, <i>Mycobacterium tuberculosis</i> , <i>Candida albicans</i>
	DEC205	Unknown
Collectins	MBL	HIV, <i>Staphylococcus aureus</i> , <i>Streptococcus pneumoniae</i> , <i>C. albicans</i> , <i>Aspergillus fumigatus</i>
	SP-A	RSV, HSV-1, <i>S. aureus</i> , <i>S. pneumoniae</i>
	SP-D	RSV, <i>M. tuberculosis</i> , <i>C. albicans</i> , <i>A. fumigatus</i> , <i>Pseudomonas aeruginosa</i>
C-type lectin R	DC-SIGN	HIV, HCV, Dengue, <i>M. tuberculosis</i> , <i>A. fumigatus</i> , <i>C. albicans</i> ,
	Dectin-1	<i>Pneumocystis</i> , <i>C. albicans</i>

dimers, trimers or hexamers, depending on the molecule) allows these receptors to bind to carbohydrate ligands with multivalent degrees of interaction.

Type I CTRLs have tandem extracellular CRDs and their extracellular amino terminus has a cysteine-rich domain, followed by a fibronectin type II domain, tandem CRDs, a transmembrane domain and a carboxyl terminal cytoplasmic domain. The most important type I CTRLs are the DC-specific ICAM-3-grabbing non-integrin 1 (CD209, DC-SIGN, C-type lectin family member 4L, CLEC4L), macrophage mannose receptor (macrophage-MR, CD206 antigen, CLEC13D), DEC205 (CD205, CLEC13B, gp200-MR6, GP200-MR6, lymphocyte antigen 75 precursor, Ly75), DC-associated lectin-1 (dectin-1, CLEC7A, BGR, C-type lectin superfamily member 12, CLECSF12) and dectin-2 (CLEC6A, CLECSF10).

Common to all these receptors is that they act as co-receptors in the uptake of important human pathogens, such as human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis*. No data are currently available for CTRLs of companion animals, while some have been cloned in farm-animals. Table 1 summarises the main C-type lectin receptors and their ligands.

3.1.1. DEC205

Although the exact function of DEC205 is not known, it is thought to play a role in the capture, phagocytosis and subsequent processing and presenting of antigens (Jiang et al., 1995). Previous studies have identified a 210 kDa molecular weight molecule expressed at a high level on the surface of DCs in bovine afferent lymph and evident on cells with the morphology of DCs in lymphoid tissues (Howard et al., 1996). Expression in other immune cells is either absent or present at a lower level. The cDNA sequence of the bovine orthologue, originally termed WC6, shares 86% and 78% nucleic acid identity with human and mouse DEC205, respectively. COS-7 cells transfected with a plasmid containing the bovine DEC205 coding region expressed a molecule that stained with a WC6-specific monoclonal antibody (Gliddon et al., 2004). Two-colour flow cytometry of bovine

mononuclear cells from afferent lymph draining skin and from blood, confirmed the high level of expression of DEC205 (WC6) on large cells in lymph that were uniformly positive for DC-lysosome-associated membrane glycoprotein (DC-LAMP) and major histocompatibility complex class II (MHC II). Within this DEC205⁺ DC-LAMP⁺ population were subpopulations of cells that expressed the macrophage-MR or CD172a, implying that DCs in afferent lymph all express this CTLR but, within this population, different subsets with potentially different functions may be present (Gliddon et al., 2004).

3.1.2. Dectin-1 and dectin-2

Dectin receptors have specificity for β -glucans predominantly found in fungal cell walls. Dectin-1 has a non-classical immunoreceptor tyrosine-based activation motif (ITAM) on its cytoplasmic tail, clearly indicating that signalling through this molecule leads to cellular activation, resulting in phagocytosis and production of tumour necrosis factor (TNF) α . Hence, unlike many of the lectins and scavenger receptors involved in pattern recognition, dectin-1 has a clear role in both the recognition and subsequent induction of a pro-inflammatory response to fungi. Recently, two dectin receptors were identified in cattle (Bonkobara et al., 2006; Willcocks et al., 2006) and partial sequences for ovine dectin-1 and -2 have been determined (GenBank AM167930 and AM167931, respectively).

Dectin-1 and -2 seem to be present in different isoforms (see Fig. 1). Transcripts for bovine dectin-1 were consistently detected in monocytes, macrophages and DCs, whereas both T cells and B cells were negative for bovine

dectin-1 (Willcocks et al., 2006), contrasting with results in humans (Taylor et al., 2002; Willment et al., 2003). Furthermore, low bovine dectin-1 expression was consistently observed in NK cells. Interestingly, in all samples analysed, independent regulation of both dectin-1 isoforms was never detected. In contrast with murine dectin-2 mRNA, the recently identified bovine dectin-2 is abundantly expressed by Langerhans' cells compared to macrophages (Bonkobara et al., 2006). The highest expression of dectin-2 mRNA was detected in lymph node samples, while the spleen and lung had the highest levels of expression of mouse and human dectin-2 (Bonkobara et al., 2006; Willcocks et al., 2006). In cattle, dectin-2 expressed by DCs may be clinically involved in the recognition of invading antigens in lymph nodes.

3.1.3. Macrophage mannose receptor

The macrophage-MR is a type I membrane protein predominantly expressed by macrophages and non-vascular endothelial cells. It is a constantly recycling endocytic receptor, containing eight CRDs which mediate recognition of terminal mannose/fucose-linked *N*-acetyl glucosamine and sulphated-*N*-acetylgalactosamine via their cysteine-rich domains. Macrophage-MR has been implicated in the recognition of numerous microbial pathogens, including *Candida albicans*, *Pneumocystis carinii*, *Leishmania* spp. and HIV (Gordon, 1998; Linehan et al., 2000). Functional characterisation of the putative bovine macrophage-MR (GenBank NM_175779) showed that this receptor is involved in mannosyl-rich glycoprotein-induced mitogenesis in bovine airway smooth muscle cells and the

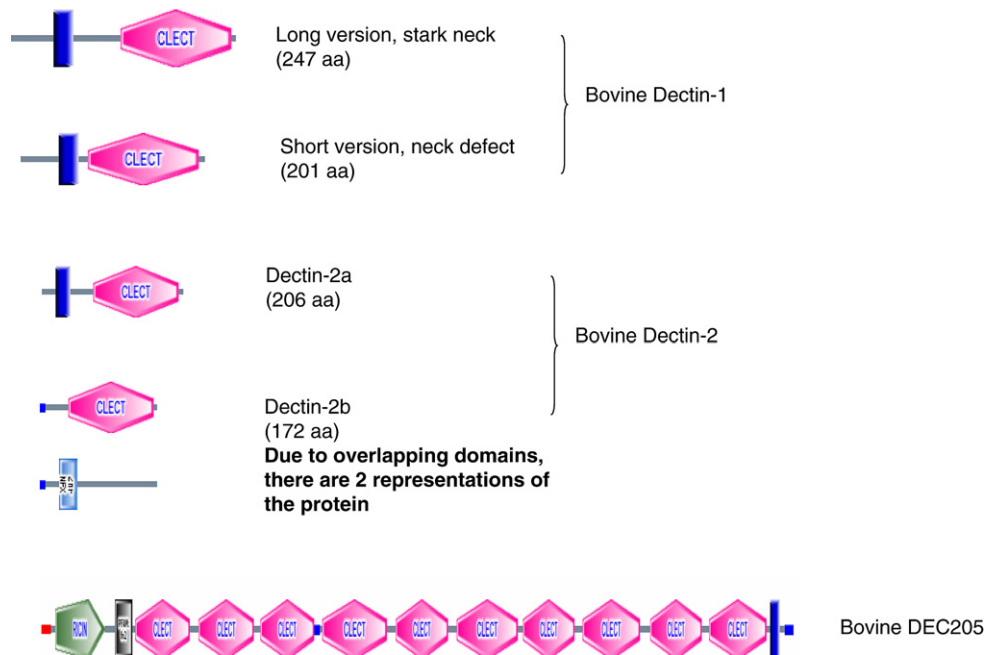


Fig. 1. SMART representation of bovine C-type lectin receptors. Each receptor has several extracellular C-type lectin binding domains, followed by a neck region and the transmembrane region. The function of the neck region is not yet fully understood.

uptake of dextran into monocyte-derived DC (Lew et al., 1994; Werling et al., 1999). More recently, both cytopathic and non-cytopathic types of bovine viral diarrhoea virus (BVDV) were shown to affect the mechanisms of antigen uptake by monocytes. Endocytosis in BVDV-infected monocytes, unlike uninfected cells, was MR-independent and mediated by fluid phase uptake after 1 h of infection, but became MR- and fluid phase uptake-dependent after 24 h of infection, independent of the BVDV biotypes (Boyd et al., 2004). Thus, by influencing the early antigen uptake function of APCs, BVDV might disrupt the function of monocytes as professional APCs and contribute to specific immunotolerance to BVDV (Boyd et al., 2004). Interestingly, a variety of recent publications indicate that CTLRs collaborate with TLRs in the recognition and uptake of pathogens, as well as the induction of the innate immune response (Underhill, 2003).

Fig. 1 shows the domain architecture of the currently sequenced bovine CTLRs, clearly indicating the similarity of the molecules. Fig. 2 shows the evolutionary relationships of the three known bovine molecules with their human and murine orthologues, based on currently available GenBank accession numbers of full length molecules (Table 2). Amino acid sequence alignments were generated with CLUSTALW. Molecular distances and the tree were

computed using the Phylodraw programme. This figure also shows the relationships between the different dectin isoforms in the bovine, murine and human systems, indicating that, in most cases, the bovine molecules seem to be more closely related to the human than to the murine counterparts.

3.2. Toll-like receptors

TLRs are type I transmembrane proteins that serve as a key part of the innate immune system. These proteins have been found in animals as well as plants and are thus believed to have an ancient evolutionary origin, representing the oldest components of the immune system (Beutler and Rehli, 2002). Their name derives from sequence homology to the *Drosophila melanogaster* gene Toll; “Toll” is German for “amazing” or “mad”.

In flies, Toll was first identified as a gene important in embryogenesis, involved in establishing the dorsal-ventral axis (Anderson et al., 1985a,b). Subsequently, Toll was shown to have a role in immunity to fungal infections (Lemaitre et al., 1996). This discovery was soon followed by the first description of a TLR in humans, which, together with the CD14 molecule, forms the LPS receptor complex (Medzhitov et al., 1997).

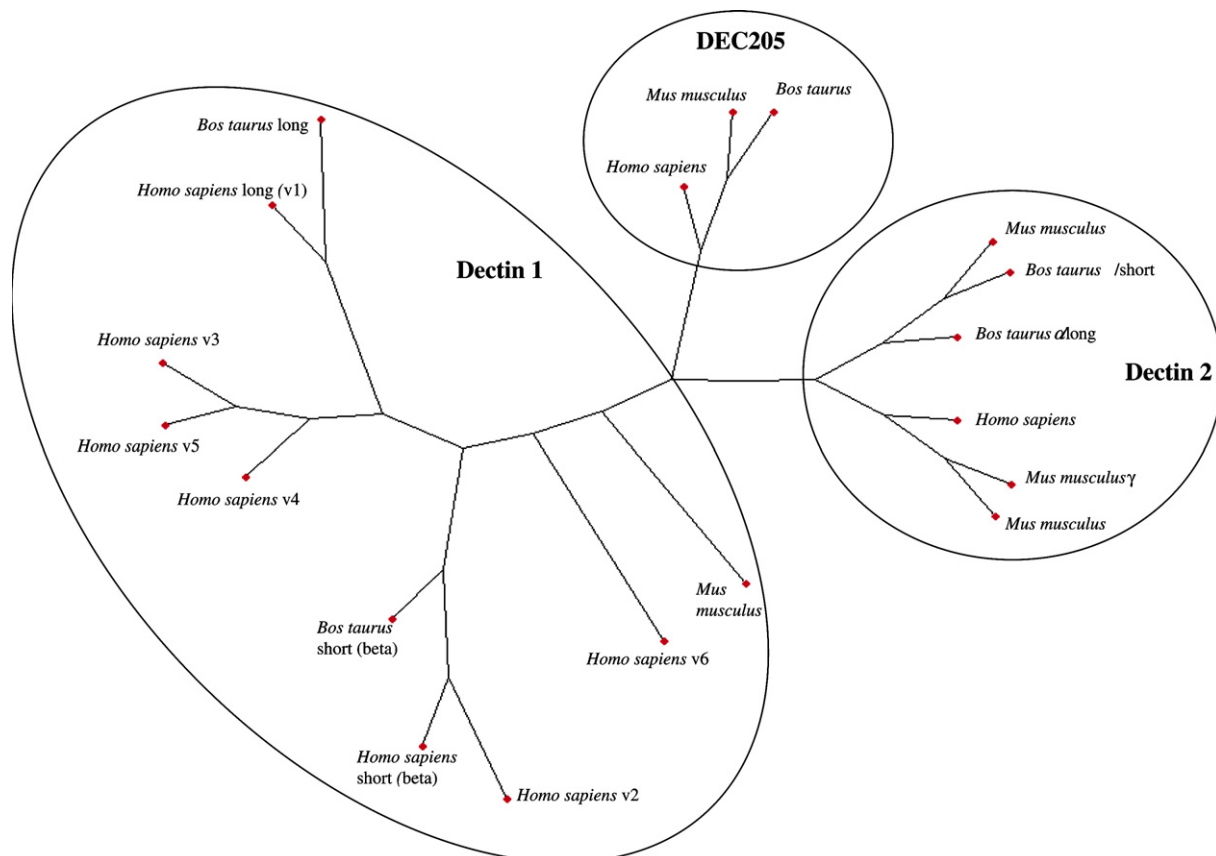


Fig. 2. Radial tree of published bovine C-type lectin receptor sequences with their human and murine counterparts. Each major family is shown in a circle. Note that for DEC205 and dectin-2, the bovine molecule seems to be more closely related to the murine than the human counterpart.

Table 2
GenBank accession numbers of companion and farm animal C-type lectin receptors and TLRs

	TLR1	TLR2	TLR3	TLR4	TLR5	TLR6	TLR7	TLR8	TLR9	TLR10
Cat	n/a	n/a	n/a	BAB43947	n/a	n/a	n/a	n/a	NP_001009285	n/a
Dog	n/a	BAD42423	XP_540020	NP_001002950	XP_545722	n/a	BAE79271	n/a	NP_001002998	n/a
Swine	BAD91798	BAD91799	n/a	CAF31361	BAD91800	NP_998925	n/a	BAC66635	AAW50956	NP_001025705
Horse	n/a	AAR08196	n/a	AAF91076	n/a	n/a	n/a	n/a	n/a	n/a
Cow	AAT48486	AAT48487	NP_001008664	AAT48488	AAT48489	NP_001001159	NP_001028933	ABA39705	NP_898904	AAT48490
Chicken	NP_001007489	BAB16843 (type 1) BAB16842 (type 2)	NM_001011691	NP_001025864	NP_001019757	n/i	NP_001011688	n/i	n/a	n/a
			Dectin-1			Dectin-2				DEC205
Cow			AAY22121 long AAY221120 short			ABA08411 α /long ABA08412 β /short				AAP93901

This table shows the accession numbers for the companion and farm animal protein sequences used to create the phylogenetic trees shown in Figs. 2 and 3. It contains only those molecules for which a full length coding sequence has been identified. n/a – Not available, n/i – not included.

TLRs are expressed by many cell types, such as monocytes, macrophages, DCs, vascular endothelial cells and epithelial cells. They are often, but not exclusively, found on the cell surface.

In addition to their function as single PRRs, some TLRs form dimers. Although most TLRs appear to function as homodimers, TLR2 forms heterodimers with TLR1 or TLR6, each dimer having different ligand specificity, thus increasing its binding repertoire. TLRs may also depend on other co-receptors for full ligand sensitivity, such as recognition of LPS by TLR4, which requires MD2, CD14 and LPS-binding protein (LBP) (Underhill, 2003; Werling and Jungi, 2003). Since the specificity of TLRs (and other innate immune receptors) cannot be changed, these receptors must recognise patterns that are constantly present on infectious non-self microorganisms, not subject to mutation and highly specific (e.g., not normally found in the host where the TLR is expressed). These PAMPs are usually evolutionarily conserved, critical to a pathogen's function and cannot be eliminated or changed through mutation.

Activation of TLRs can stimulate two different signalling pathways via the cytoplasmic Toll/interleukin-1 receptor (TIR) domain, a myeloid differentiation factor (MyD88)-dependent-interferon regulatory factor (IRF-3)-independent pathway and a MyD88-independent-IRF3-dependent pathway, both resulting in activation of nuclear factor (NF) κ B. In both cases, signalling leads to the production of cytokines and modulation of surface co-stimulatory molecules, forming a bridge between innate recognition and the acquired immune system (O'Neill, 2006). Up to 14 TLRs have been discovered in different species, either enabling the host to recognise bacterial components (TLRs 1, 2, 4, 5, 6 and 11), RNA/DNA components (TLRs 3, 7, 8 and 9) or with no known function (TLRs 10, 12, 13 and 14). Numerous reviews have been published within the last years, covering all aspects of TLR-ligand interaction and several websites offer comprehensive information (e.g., <http://www-personal.umich.edu/~ino/List/TOLLRE.htm>).

Fig. 3 summarises our current knowledge about TLRs and their ligands, including the main signalling pathways,

and provides information about the cellular localisation of these receptors. However, it has to be stated that the localisation of these receptors is not fixed, but rather dynamic, and may change with cellular activation.

Fig. 4 shows the evolutionary grouping of all confirmed sequences of farm and companion animal TLRs compared to the corresponding human orthologues. The figure illustrates the relationships between receptors from different species, highlighting the potential for differences in pathogen recognition. It also indicates that TLRs of farm animals may have developed from a common ancestor, which is different to the corresponding human TLR. GenBank accession numbers for full length molecules used in this analysis are shown in Table 2. Amino acid sequence alignments were generated with MUSCLE. Molecular distances and the tree were computed using the PhyloDraw programme.

3.2.1. Chicken TLRs

In the last two years, research on the chicken innate immune response has generated a wealth of information regarding TLRs. At present, full length coding sequences or genes for chicken TLRs 1, 3, 4, 5 and 7 have been identified. In addition, chickens possess two types of TLR2 (types 1 and 2), which appear to be generated by gene-duplication and may represent homologues to human TLRs 2 and 4, whereas the TLR8 gene is disrupted. TLR15 (GenBank DQ267901) has no known orthologue in other species and is thought to be an avian-specific TLR (Higgs et al., 2006). A putative chicken TLR6 (GenBank XM428184) has been predicted from the genome sequence. However, sequence analysis suggests that this is not accurate and may be a misassembled part of the genome.

In contrast to other species, functional characterisation of chicken TLRs is relatively advanced. At the cellular level, activation of chicken TLRs expressed on heterophils and monocytes/macrophages activated NF κ B, p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase 1/2 (ERK 1/2) and c-Jun NH2-terminal kinase (JNK), resulting in the production of nitric oxide (NO), pro-inflammatory cytokines and chemokines

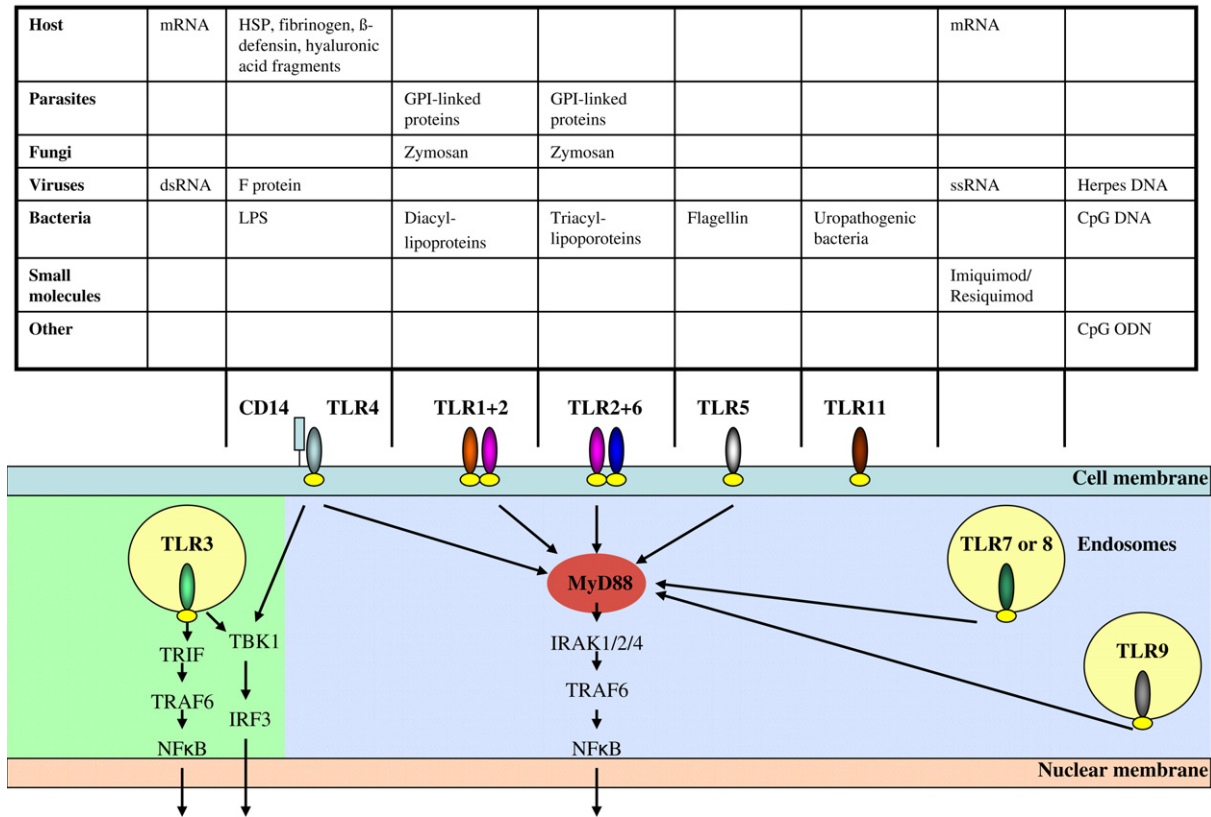


Fig. 3. Diagram illustrating the current knowledge regarding TLRs, their known agonists and the signalling pathways activated by the interaction of the receptor with an agonist.

(Farnell et al., 2003a,b,c; He et al., 2006; Kogut et al., 2005a,b). In addition, allelic variations in TLR4 (Leveque et al., 2003) and single-nucleotide polymorphisms (SNPs) in the MD2 gene are linked to susceptibility to *Salmonella enterica* serovar Typhimurium infection in chicken and bacterial load in the spleen, respectively (Leveque et al., 2003; Malek et al., 2004). In addition to these allelic variations, different chicken strains seem to be able to mount a stronger or weaker TLR-dependent immune response to bacterial LPS (Dil and Qureshi, 2002a,b).

3.2.2. Ruminant TLRs

In the ruminant system, TLRs 1–10 have recently been mapped to chromosomes in the bovine genome (McGuire et al., 2006). Overall, bovine TLRs seems to show a greater sequence homology to their humans orthologues than those found in mice (Griebel et al., 2005; Werling et al., 2006). Expression of these TLRs has been monitored in selected bovine tissues (Menzies and Ingham, 2006), as well as in subsets of bovine APCs (Werling et al., 2006). Based on these data, it is evident that the majority of tissues/cells analysed so far do express TLRs. However, the expression pattern varies between tissues, between cell subsets within cattle and the TLR repertoire varies in different tissues/cells compared to those analysed from other species, such as mice and humans. Several SNPs have already been identified in bovine TLRs between different breeds (White et al.,

2003a,b). Whether these SNPs are linked to functional differences remains to be clarified.

Despite lacking bovine-specific or convincingly cross-reacting reagents, several functional studies have been conducted over the last few years. Expression of TLRs 2 and 4, but not TLR9, was demonstrated in an experimental mastitis model in vivo (Goldammer et al., 2004), whereas TLR expression seemed to be largely unaffected when analysing primary mammary gland cells exposed in vitro to LPS and LTA (Strandberg et al., 2005). Similarly, TLR4 expressed by either airway or endometrial epithelial cells was shown to be involved in a bacterial response by these cells, despite the fact that they do not express CD14 (Herath et al., 2006; Legarda et al., 2005).

In addition to these studies involving bacterial antigens, two recent publications reported the involvement of TLRs in the immune response to viral infection (Franchini et al., 2006; Zhang et al., 2006). Surprisingly, BVDV-infection of macrophages resulted in a modulated NO response when cells were stimulated with TLR2 or TLR4 ligands, suggesting that viral infection may modulate TLR-function without inducing differences in expression of mRNA transcripts (Franchini et al., 2006). This observation may become even more important when looking at different APC subsets (Werling et al., 2004) or in cells isolated from animals exposed to stress or growth hormones (Eicher et al., 2004). However, whether differential expression of

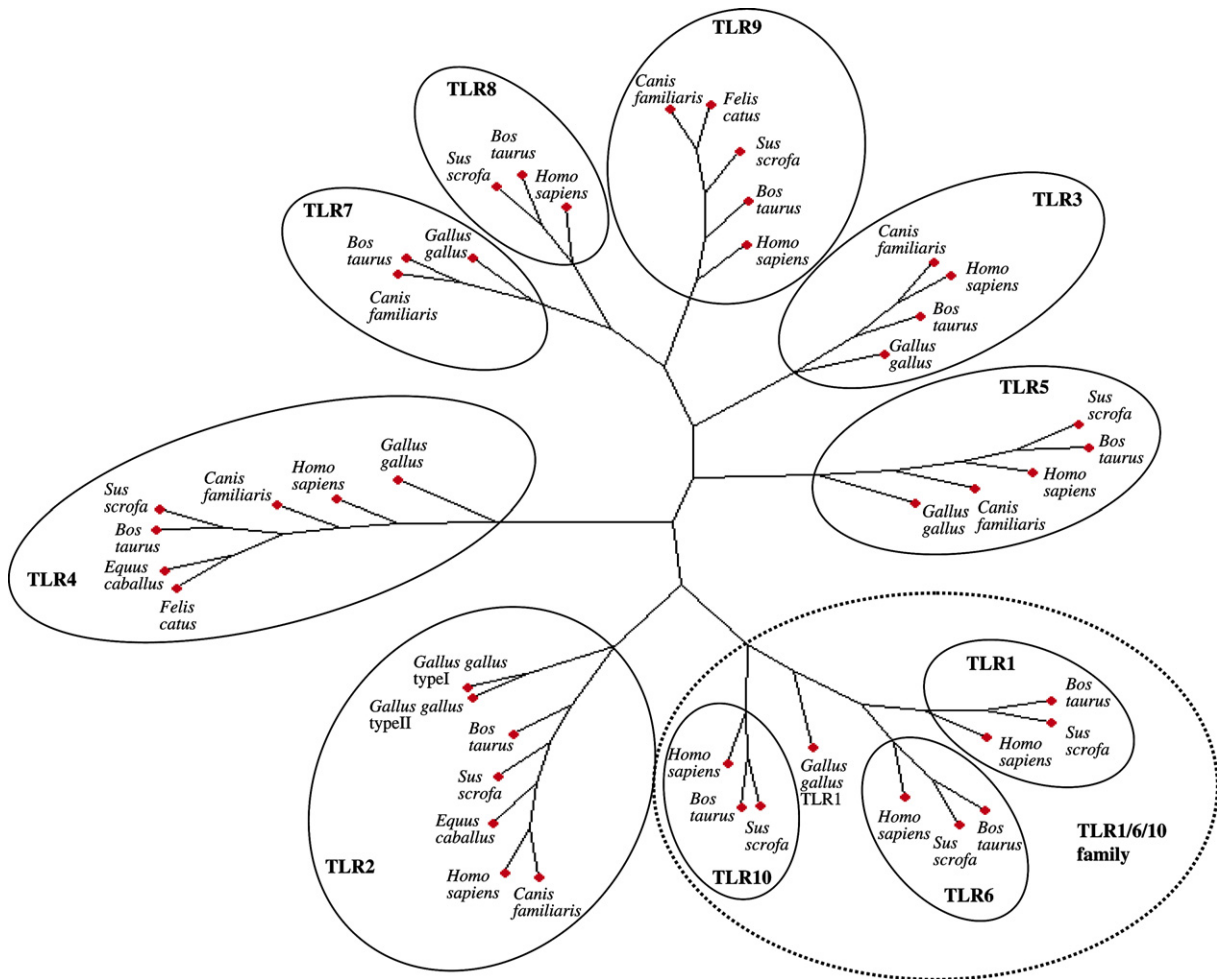


Fig. 4. Radial tree of companion and farm animal TLRs, compared to their human orthologues. Each TLR group is shown in a circle, with the TLR 1/6/10 family highlighted. Note the branch structure within a TLR group can be variable (e.g., TLR9), which may have implications for differences in pathogen recognition between TLRs from different species. Some canine TLR sequences were omitted from the tree due to concerns over the validity of the sequences (see text for further explanation).

TLR mRNA is based on the interaction of a ligand with the TLR, or whether these effects are just a “side-effect” based on cellular stress in general, requires further study. One approach is to analyse the expression of bovine TLRs in human embryonic kidney (HEK) cells, a cell line devoid of some TLRs, allowing direct analysis of TLR-ligand interaction by gene reporter assays or chemokine production. Recently, this has been used successfully to study activation of NF κ B by bovine TLRs (Yang et al., 2006).

3.2.3. Equine TLRs

Currently, full length sequences are only available publicly for equine TLR2 (GenBank AY429602) and TLR4 (Vychodilova-Krenkova et al., 2005). In addition, a partial sequence for TLR9 was recently deposited in GenBank (DQ157779). At the gene-level, four SNPs with unknown biological relevance were identified within the equine TLR4 (Vychodilova-Krenkova et al., 2005). Recently, Werners et al. (2006) investigated the inter-individual variation within equine TLR4 in the response to

LPS. Analysis of the cDNA sequence encoding members of the LPS-receptor complex (TLR4, MD2 and CD14) revealed sequence mutations in TLR4, but these could not be related to an altered response to LPS. In addition, no alterations were found in either the MD2 or CD14 gene.

3.2.4. Porcine TLRs

The genes for TLRs 1, 2, 4, 6 and 10 have been identified in pigs (Shinkai et al., 2006; Thomas et al., 2006) and full length coding sequences are also available for porcine TLRs 5 (GenBank AB208697), 8 (AB092975) and 9 (Raymond and Wilkie, 2005). The mRNA expression of these TLRs in different tissues/APC subsets has been investigated by reverse transcriptase polymerase chain reaction (RT-PCR) (Alvarez et al., 2006; Raymond and Wilkie, 2005). In addition, TLR2 and TLR6 have been shown to be involved in *Mycoplasma hyopneumoniae*-induced production of TNF α by porcine alveolar macrophages (Muneta et al., 2003).

3.2.5. Carnivore TLRs

Of the canine and feline TLRs, TLRs 2–5, 7 and 9 have been cloned from dogs (Asahina et al., 2003; Hashimoto et al., 2005; Ishii et al., 2006), while only TLRs 4 and 9 have been identified so far in cats (Ignacio et al., 2005), with some TLRs only being partially cloned. Analysis of canine TLR cDNA in a variety of tissues by RT-PCR revealed differences in the pattern of expression. Furthermore, the response of canine peripheral blood mononuclear cells to LTA, a TLR2-ligand, induced CXCL-8 secretion by these cells (Bazzocchi et al., 2005). However, further analysis of the published canine TLR 2 and 4 sequences has suggested that they are not full length as claimed (Catchpole and House, personal communication). Expression of feline TLRs 1–9, as analysed by quantitative PCR, found expression of these TLRs in all cell types analysed (Ignacio et al., 2005). The level of expression of each TLR varied between tissues and expression was further affected by infection with feline immunodeficiency virus (Ignacio et al., 2005). Similarly, the U3-long terminal repeat of feline leukaemia virus was recently shown to activate NF κ B via a TLR3-dependent mechanism (Abujamra et al., 2006).

4. Pathogen recognition by PRRs: protection or evasion?

There is increasing evidence to suggest that pathogens use several strategies to evade host immune surveillance (Zelensky and Gready, 2005). There are several examples of non-metazoan C-type lectins, found in parasitic bacteria and viruses. They are thought to be involved in interactions with the host by either hijacking host proteins, such as CTLRs, or by imitating them. This group contains members such as pertussis toxin (Stein et al., 1994), *Escherichia coli* intimin (Luo et al., 2000) and *Yersinia pseudotuberculosis* invasin (Hamburger et al., 1999). Several viral C-type lectins have been identified, including in African swine fever virus (Galindo et al., 2000) and cowpox virus (Shchelkunov et al., 1998).

DC-SIGN enhances the infection of T cells with HIV by helping to establish the formation of an infectious synapse between HIV-1-bearing DCs and resting CD4⁺ T cells (Arrighi et al., 2004). It is becoming clear that other viruses besides HIV-1, such as hepatitis C virus (Wang et al., 2004) and severe acute respiratory syndrome coronavirus (Yang et al., 2004) target DC-SIGN to avoid lysosomal degradation. This appears to promote viral dissemination in the body and modulate DC function to enable establishment of chronic infections. *Helicobacter pylori* has also been shown to target DC-SIGN, blocking a polarised T helper type 1 (Th1) response (Bergman et al., 2004).

Yersinia enterocolitica, vaccinia virus and *C. albicans* have recently been shown to enhance immune evasion by either directly blocking TLRs or by interfering with the intracellular TLR-signalling cascade (Haga and Bowie, 2005; Heesemann et al., 2006; Murciano et al., 2006). Despite these examples of immune evasion by pathogens through CLRs, these receptors have a fundamental role

in limiting the early proliferation of infectious microorganisms. The discovery of bacterial and viral C-type lectins and increased recognition of the use of CTLRs by pathogens to avoid immune recognition may lead to new routes of either vaccine application or new vaccine formulations.

5. Conclusion

In the recognition of pathogens by the host, several levels of complexity can be distinguished that might determine the destiny of the pathogen and the outcome of an immune response. Firstly, a specific PAMP, or a combination of different PAMPs, are expressed on the surface of a pathogen, dictating recognition by specific host receptors. Secondly, the set of PRRs expressed varies among different cells, resulting in a PRR repertoire that is different for each cell type of the immune system. Thirdly, changes in PRR cell-surface distribution adds yet another level of complexity, modulating binding of PAMPs to certain carbohydrate moieties and thus regulating the affinity for different ligands. Finally, these receptors have been shown to interact with each other, thereby giving rise to a network of multimolecular complexes that add an extra level of complexity to pathogen recognition.

Unravelling the precise mechanisms regulating the formation and exact function of these receptor networks is the current challenge. This challenge will become even bigger with the discovery of intracellular PRRs, the NOD-like and RIG-1-like receptors, for which no homologue has been described as yet in companion and farm animals.

Will knowledge about these PRRs help us unlock the key to animal diseases? Clearly, knowledge about these PRRs will aid in the understanding of host–pathogen interactions and may lead to the revision of current concepts of pathogenesis. Even more importantly, knowledge about the functions of these receptors, their ligands, and subsequent gene-activation after receptor–ligand interaction may lead to the development of new therapeutic approaches by interfering at three points in the PRR activation cascade. Ligands can be used as adjuvants, providing the first point to regulate PRR activation. Secondly, modulation of PRR activity can be achieved by the addition of soluble forms of PRRs or PRR-blocking molecules. Finally, PRR signalling can be positively regulated by the adapters or negatively regulated by their inhibitors. Recent findings suggest that there are small synthetic molecules that block TLR–adapter interaction (Ii et al., 2006). More information about TLR agonists in association with adjuvants has recently been published (Del Vecchio et al., 2005; Mbow and Sarisky, 2005).

However, it is also important to mention that in nearly all articles published so far on TLR expression in farm and companion animals, expression has only been analysed by RT-PCR or quantitative PCR. Two groups have analysed expression of TLR2 and TLR4 in dogs by immunohisto-

chemistry or flow cytometry (Bazzocchi et al., 2005; Wassef et al., 2004), but further evidence is required that the antibodies used are genuinely cross-reacting (e.g., western blotting, multi-colour flow cytometry or HEK cells expressing full length clones). Thus, as for all other species, the generation of TLR-specific reagents in non-human, non-rodent animal models remains a primary target. Two major initiatives (Immunological Toolbox, funded by the BBSRC in the UK and a similar initiative funded by the USDA in the USA) aim to address these issues and provide the necessary tools.

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