

REVIEW

Immunorecognition and immunoreceptors in the Cnidaria**SR Dunn***Center for Marine Studies, University of Queensland, Australia**Accepted January 29, 2009***Abstract**

Recent studies that are focused on cnidarians as model systems for cell biology are offering key insight into the complexities of higher metazoan biology. The innate immune system of these basal invertebrates is one of the cellular processes that until recently, was undescribed. The knowledge regarding both innate immunity and other cellular processes in cnidarians is far from complete. However, the evidence acquired so far, suggests highly conserved components of these cellular processes are more closely related to vertebrate homologues than more complex, but divergent invertebrate model systems. This review examines the immunorecognition and receptors that have been identified within the cnidarians so far. The complement of receptors and pathways already identified indicates that these basal invertebrates are far from "simple" in the array of methods they possess for dealing with potential invading microbes and pathogens.

Key Words: Cnidaria; immunity; symbiosis; pathogen; *Symbiodinium*; PAMP; PRR

Introduction

Gene function and cellular pathways in higher vertebrates, including humans, have increasingly shown to be highly conserved through metazoan evolution from the discovery of homologues in basal metazoans, such as sponges and cnidarians (Kortschak *et al.*, 2003; Kuo *et al.*, 2004; Kusserow *et al.*, 2005; Dunn *et al.*, 2006; Hemmerich *et al.*, 2007). The recent use of cnidarians as a metazoan model system (for review see Weis *et al.*, 2008), has brought about a wealth of new information that is unravelling the cellular processes involved in symbiosis, immunity regulation, cell death and organism longevity. There are, of course, considerable differences between the lower and higher metazoans: Cnidarians lack a vertebrate-like adaptive immune system in so far as immunological memory is absent. Furthermore, whilst amoebocytes have been shown to play a role in cnidarian cell defenses (Olano and Bigger 2000; Mullen *et al.*, 2004; Mydlarz *et al.*, 2008), specialization of cnidarian cells into components of an adaptive immune system is not evident.

The ability of cnidarians to distinguish between self and non-self has previously been shown to occur in anthozoans and hydrozoans (Frank *et al.*,

2001; Rinkevich 2004; Bosch, 2008). The capacity of allorecognition may vary across and between classes, colonies, such as *Anthopleura* sp. (class: Anthozoa) (Lubbock, 1980) and solitary individuals, such as *Hydra* sp. (class: Hydrozoa) (Kuznetsov and Bosch, 2003). The molecular triggers that lead to allorecognition in cnidarians may utilize the innate immune response, however at present, the cellular mechanisms remain unknown and beyond the scope of this review. This review focuses upon the complexity of cnidarian immunorecognition. A cellular process that was once thought of as 'basic' in these simple metazoans is in fact complemented by an array highly conserved defenses, which offer key insight into the foundation of the higher metazoan innate immunity.

One of the first lines of defense of the invertebrate immune system against potential invasive microbes are the pattern recognition receptors (PRR's). The host PRR's detect and bind to highly conserved components of microbe cell walls, such as proteins, lipids, carbohydrates and lipoteichoic acids (Gram-positive bacteria) or lipopolysaccharides (LPS; Gram-negative bacteria), which form a recognisable matrix or pattern, known as pathogen-associated molecular patterns (PAMP) or microbe-associated molecular patterns (MAMPS) (Murphy *et al.*, 2008). The activation of signal responses following the binding of host PRR's to PAMP's is rapid and can operate in three ways: Firstly, to stimulate microbe ingestion through phagocytosis and enzymatic degradation. Secondly,

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through chemotactic directives for molecules to move to sites of infection, and thirdly, the induction of effector molecules that leads to a cell signal cascade and ultimately an immune response (Murphy *et al.*, 2008). At present, the complement of cnidarian PRR's appears to be diverse across classes, although the limited number of descriptive immunoreceptor studies still hinders a full evaluation and requires much more in-depth research.

Recent extensive cnidarian Expressed Sequence Tag (EST) and genome sequencing projects have highlighted the broad range of highly conserved biological processes within all metazoans that are also found in cnidarians, including the innate immune system (Miller *et al.*, 2007). Across animal evolution, there has also been significant gene loss, and cnidarians are no exception. Gene loss and duplication has occurred across all of the cnidarian classes, and suggests that the Hydrozoa are divergent from basal Anthozoa, and the Scyphozoa from Hydrozoa (Miller *et al.*, 2007; Bosch, 2008). Several key domains and conserved components of pathways associated with innate immune receptors that have already been identified are: 1) Toll-like receptors (TLR's) containing Leucine rich repeats (LRR's), which recognise PAMPs, 2) components similar to that of the complement cascade, and 3) lectins (for review see Hemmerich *et al.*, 2007; Miller *et al.*, 2007; Kvennefors *et al.*, 2008).

TOLL-like and LRR receptors

Toll and Toll-like Receptors (TLR) are part of a metazoan receptor superfamily that all share a Toll interleukin-1 receptor domain (TIR). In the cnidarians, TLR-domain conserved proteins vary in number and structure. In the hydrozoan, *Hydra magnipapillata*, there are four TLR-domain proteins described so far. Two of which, HmMyD88-1 and HyMyD88-2 are related to the downstream MyD88, each displaying an additional characteristic death domain. The two remaining *Hydra* TIR-domain proteins, HyTRR-1 and HyTRR2 have a typical transmembrane and short extracellular scaffold, and are likely to be pathway initiator receptors. Yet unusually, they lack the typical LRR-domains, and may be devoid of canonical structure (Miller *et al.*, 2007). However, this structural variation may not be uncommon or necessary for the same function (Sun Jin and Lee, 2008). Recent work by Bosch *et al.* (in press) has revealed additional TLR-related LRR domain proteins in *Hydra* may function synergistically with HyTRR-1 and HyTRR-2 in PAMP recognition, indicating multiple receptor responses to an immune challenge operate in the cnidarians.

The Anthozoa, in comparison to hydrozoans, have a broader selection of TLR-domain proteins reflecting their basal phylogeny in the group. The estuarine sea anemone, *Nematostella vectensis* have at least 5 TLR-domain proteins from the predicted structures. One of the TLR-domain proteins, NvMyD88 has a similar structure to the HmMyD88-1 and 2 and is thought to function in the same manner to induce expression of immune response genes through activation of the

transcription factor, NFkB. A second TLR-domain protein, NvTLR-1, has multiple LRR domains and both carboxy and amino-terminal flanking cysteine rich motifs, which is characteristic of an ancestral domain structure (Miller *et al.*, 2007). The remaining identified predicted TLR-domain protein structures all contain immunoglobulin (Ig) domains. The Ig domains, which may function in cell-cell recognition, form a distinct clade away from the higher vertebrate structures. In comparison to other anthozoan sequences so far screened, *N. vectensis* has the highest complement of TLRs. At present, only one TLR-domain protein has been identified in the corals, *Acropora palmata* and *Acropora millepora*. Both of the coral TLR-domains were similar to the *N. vectensis* Ig-domain TLR, NvL-1R1. However, no extracellular domains were detected in the coral receptors (Miller *et al.*, 2007). In addition to the TLR-domain structures described by (Miller *et al.*, 2007), downstream components of the Toll/TLR pathways were also described from the EST/ genome analysis, such as those associated with the c-Jun N-terminal kinases (JNK)/Mitogen-activated protein kinase (MAPK) pathway and NFkB transcription that can lead to cell death. The depth of the known pathway homology indicates that it is not just Toll/TIR receptors that are highly conserved, but a complete representative PRR immune response pathway.

Antimicrobial peptides and metabolites

An important contribution to the cnidarian immune response is the array of anti-microbial peptides. Peptides are small signalling molecules that control a variety of processes, such as development, muscle contraction and the control of target gene expression (for review see Bosch and Fujisawa, 2001). Antimicrobial peptides secreted by microbes within the mucus of corals are known to be a potent inter-specific microbial regulator of the epiphytic coral microbial community (Ritchie, 2006). The composition of the cnidarian microbial community structure is important to host health (Ritchie 2006; Fraune and Bosch, 2007), yet the roles of host anti-microbial peptides in host immunity until recently were unknown. Host peptides have a stable structure that allows translocation to different areas around the cnidarian diploblastic body structure via the interepithelial space or mesoglea (Fraune and Bosch, 2007). The antimicrobial properties of peptides such as, aurelin, from the scyphozoan, *Aurelia aurita* may focus activity on removing invasive Gram-positive or Gram-negative bacteria (Ovchinnikova *et al.*, 2006). Host peptides may play a role in regulating associated microbial community populations to benefit the host, such as observed in the hydrozoan, *Hydra oligactis* (Fraune and Bosch, 2007). In *Hydra*, the induction of antimicrobial peptides is mediated by the interaction of a LRR domain protein with a TIR-domain protein lacking LRR's. The anti-microbial peptides from *Hydra*, such as Hydramacin-1 (Jung *et al.*, 2009), which is upregulated in the presence of LPS and the peptide, Perculin-1, upregulated in the presence of LPS and flagellin, are important components of the

microbe and stress host response. These particular peptides have been shown to have important therapeutic qualities as potent antibiotics against drug resistant human pathogens (Bosch *et al.*, in press). The initial descriptions of the antimicrobial peptide structures indicate that they are unique, but also, as is the case with aurelin, have similarity to defensins and K⁺-channel blocking toxins (Ovchinnikova *et al.*, 2006).

A current rapidly expanding area of research is that of biodiscovery or bioprospecting. One target of this research are peptides, the other are secondary metabolites and their important roles in cellular homeostasis and defense against predation, parasites and disease (Newman and Hill, 2006; Dunlap *et al.*, 2007). The roles of many peptides and secondary metabolites as part of the cnidarian innate immune system is now being explored and have been shown to function as antioxidants and as antimicrobial compounds (Mydlarz and Jacobs 2004, Shapo *et al.*, 2007). However, the pathways associated with the synthesis and receptor mediation of these metabolites remain unknown.

Complement and lectins

The Complement signalling cascade is a major part of the vertebrate innate immune system, whereby microbes and foreign cells that are detected undergo opsonisation, phagocytosis and lysis. Complement is composed of four pathways. Three of the pathways are involved in activation and contain a thiolester C3 component, which leads to the fourth membrane attack complex (MAC) lytic pathway (Murphy *et al.*, 2008). In vertebrates the primary function of C3, C4 and other members of the alpha-2-macroglobulin (A2M) paralogous gene family is opsonisation of microbes or immune complexes (Armstrong and Quigley, 1999).

There are complement or precursors of the complement pathways, in the form of C3-like thiolester-containing proteins (TEP) that predate the protostome-deuterostome split identified in cnidarians (Dishaw *et al.*, 2005; Miller *et al.*, 2007). The first C3-like, TEP cDNA to be identified from the gorgonian coral, *Swiftia exserta*, had high conservation to vertebrate C3. There was an overall similarity with mammalian C3, C4 and C5 sequences, with a characteristic anaphylatoxin region which is absent in other A2M proteins, and cleavage sites of vertebrate C4 (Dishaw *et al.*, 2005). This basal, multiple 'attribute' domain protein, which may predate a later divergence into a larger protein family with individual domains and specific function, has previously been suggested to be a feature of cnidarians, such as caspases and Bcl-2 family members of the apoptotic pathway (Dunn *et al.*, 2006).

A similar C3-like protein has also identified in the anthozoans, *Acropora* and *Nematostella*, and although the counterpart in *Hydra* was absent, *Hydra* was shown to contain an A2M-like protein. It is interesting to note that all forms were restricted to the endoderm/gastrodermal tissues respectively (Miller *et al.*, 2007). Although they have different structures, all forms may play a role in opsonisation

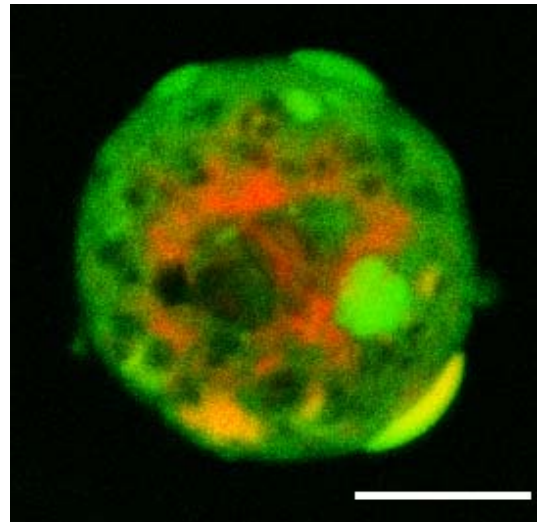


Fig. 1 A laser confocal microscopy image of the surface of a cultured *Symbiodinium* sp. labelled with FITC-labelled concanavalin-A lectin (green), which binds specifically to glycans, including α -mannose. (FC: 100 μ g/ml). Sample prepared by D Logan and S Davy, University of Wellington, New Zealand in accordance with Wood-Charlson *et al.* (2006). Image taken by SRD. Key: Red = Chlorophyll autofluorescence. Bar = 5 μ m.

and therefore, their presence in cells associated with food particle /microbe selection and uptake is not surprising. In addition, Miller *et al.* (2007) described a suite of predicted proteins with a membrane attack complex (MAC) and perforin domains associated with the final phase of the complement cascade, indicating that multiple components from different stages of the complement cascade pathways exist in the cnidarians.

Activation of complement-like pathways and opsonization through the formation of a lectin-binding complex has been indicated in cnidarians during the onset of the complement-like cascade. The lectin-binding complex may also be of particular relevance in the onset and specificity of the prolific symbiosis with the dinoflagellate, *Symbiodinium* sp., found in many cnidarians. The role of lectins in cell surface recognition of potential pathogens or symbionts is the important first step in cnidarian cell surface recognition. In previous studies using glycosides and concanavalin-A lectin to bind and mask the surface sugars of *Symbiodinium* sp. (Fig. 1) prior to infection of aposymbiotic hosts, have shown differential uptake and onset of symbioses (Jimbo *et al.*, 2000; Lin *et al.*, 2000; Koike *et al.*, 2004; Wood-Charlson *et al.*, 2006).

At present, only one cnidarian lectin has been characterised, *millectin* from the scleractinian anthozoan, *A. millepora* (Kvennefors *et al.*, 2008). *Millectin* has sequence homology to the lectin domain of a range of c-type lectins and is a relative of both the collectins, mannose binding lectin (MBL) and the surfactant, Sp-A. In addition, millectin has the unusual characteristic of having extensive variability in the substrate binding region, indicating

a potential broad range of PAMP's that may be recognized and bound by *millectin/s*, and in part, may be due to a dual role in pathogen/potential symbiont recognition of this receptor (Kvennefors *et al.*, 2008).

Intracellular Immune receptors

So far, this review has covered receptors associated with intercellular-mediated innate immune recognition, which act as a first line of defense and gateway to phagocytosis and entry into the host. Cnidarian innate immunorecognition is not limited to the cell surface or just the 'gate' into the cell. The cnidarian cell is well equipped with intracellular receptors and pathways that act as a second line of defense to recognise and remove the "Trojan horse" that has managed to slip through the first line of defense and is now inside the walls. This multilevel approach to microbe recognition for removal of the microbe/pathogen and/or retention of the symbiotic 'rent payer' is key to the onset and specificity of symbiosis known as the 'winnowing process' that was first described in the squid, *Euprymna scolopes* and *Vibrio fischeri* bacterium, symbiosis (Nyholm and McFall-Ngai, 2004).

One of the intracellular recognition immune response found in cnidarians is the increased enzyme driven production of melanin (Petes *et al.*, 2003; Mydlarz *et al.*, 2008; Palmer *et al.*, 2008). The phenoloxidase (PO) cascade that leads to melanin production, is known to play an active associated role with phagocytic aggregation and microbe/pathogen removal in many other invertebrates (Johansson and Söderhäll, 1996). Initiation of the PO cascade through Pro-form cleavage in other invertebrate systems may vary according to substrate, suggesting a functioning role in recognition. In Arthropods, activation occurs through contact with specific polysaccharides such as β -1,3-glucan (β 13g), LPS and peptidoglycan, in urochordates the inducers are LPS and β 13g, and in echinoderms only β 13g has been shown to activate the cascade (Johansson and Söderhäll, 1996). In cnidarians, differential PO activity was shown by experimental substrate addition to samples of both a healthy and compromised branching acroporid coral species, and to a lesser degree in the massive *Porites* spp. (Palmer *et al.*, 2008). However, although previous studies have detected increased melanin within coral tissues (Petes *et al.*, 2003; Mydlarz *et al.*, 2008; Palmer *et al.*, 2008), it is important to note that similar host pigmentation occurs in response to a multitude of different stimuli (Roff *et al.*, 2008), and quite normally is often more visible in areas of healthy new growth or in areas of varying symbiotic dinoflagellate density. Therefore, defining and attributing a cause to the different areas of host pigmentation is important to resolving the extent to which the PO-melanin pathway is activated and associated with inflammation and immunity across a broad spectrum of cnidarian hosts.

The intracellular immune receptors by which

cnidarian cells detect previously 'undetectable' or persistent invading microbes are only now being identified and may also operate to remove dysfunctional symbionts under stress (for review see Weis, 2008). Two key components of this intracellular innate immune repertoire can lead to cell death activation: Firstly, the upregulation of nitric oxide (NO) in the anthozoan, *Aiptasia pallida* in response to LPS, and hyperthermic stress (Perez and Weis, 2006). Although inducible nitric oxide synthase is still yet to be identified in cnidarians, nitric oxide up-regulation has been shown to play an active role in the removal of symbiont *Symbiodinium* sp. under symbiosis stress through cell death pathway activation (Trapido-Rosenthal *et al.*, 2001; Perez and Weis, 2006).

Secondly, expression of a member of the CD36 family, the scavenger receptor SR-B1, is upregulated in the symbiotic anthozoan, *Anthopleura elegantissima* compared to aposymbiotic individuals, (Rodriguez-Lanetty *et al.*, 2006). CD36 family members including SRB-1, act in host defense through the lipid metabolism. Members of the CD36 family are known to be manipulated by invasive pathogens to gain entry into the host cell, (Stafford *et al.*, 2002) and may be controlled through bridging molecules, such as thrombospondin-1, C1q collectins and β 2 glycoproteins (for review see Májai, 2006). Although EST's to homologs of a number of bridging molecules have been identified in *A.millepora* (Meyer *et al.*, 2007), a direct link between either lipids or bridging molecules and CD36 in host defense is yet to be shown in cnidarians.

In all metazoans the destruction or removal of microbes/pathogens through host cell apoptosis, autophagy or induced microbe programmed cell death (PCD) can occur at initial contact stage to prevent infection, or at an intracellular level to mitigate damage (James and Green, 2004). The initiation of cnidarian host apoptosis, autophagy and / or *in situ* symbiont PCD /necrosis, is known to occur during development (Cikala *et al.*, 1999), allrecognition (Seipp *et al.*, 2001; Kuznetsov and Bosch, 2003), in response to disease (Ainsworth *et al.*, 2007), in response to hyperthermic oxidative stress (Dunn *et al.*, 2002, 2004, 2007; Franklin *et al.*, 2004; Richier *et al.*, 2006), and as a post-phagocytic removal mechanism of *Symbiodinium* sp. during the onset of symbiosis (Dunn and Weis, 2009). The success of symbiosis in cnidarians appears to stem from the ability to restrain or prevent autophagy/apoptotic cell death pathways (Rodriguez-Lanetty *et al.*, 2006; Dunn *et al.*, 2007), and corresponds with similar cell death inhibition in other invertebrate symbioses (Pannebakker *et al.*, 2007). Intracellular parasites (the "Trojan horse") of vertebrates host cells, such as *Leishmania donovani* and *Mycobacterium tuberculosis*, also avoid immuno-detection by retarding apoptotic and autophagic pathways (for reviews see Dermine *et al.*, 2000; Koul, 2004; Gutierrez *et al.*, 2004). The pathogenic control over these pathway occurs by manipulating Ca^{2+} signalling, phosphoinositide metabolism, phosphatidylinositol 3-kinase signalling

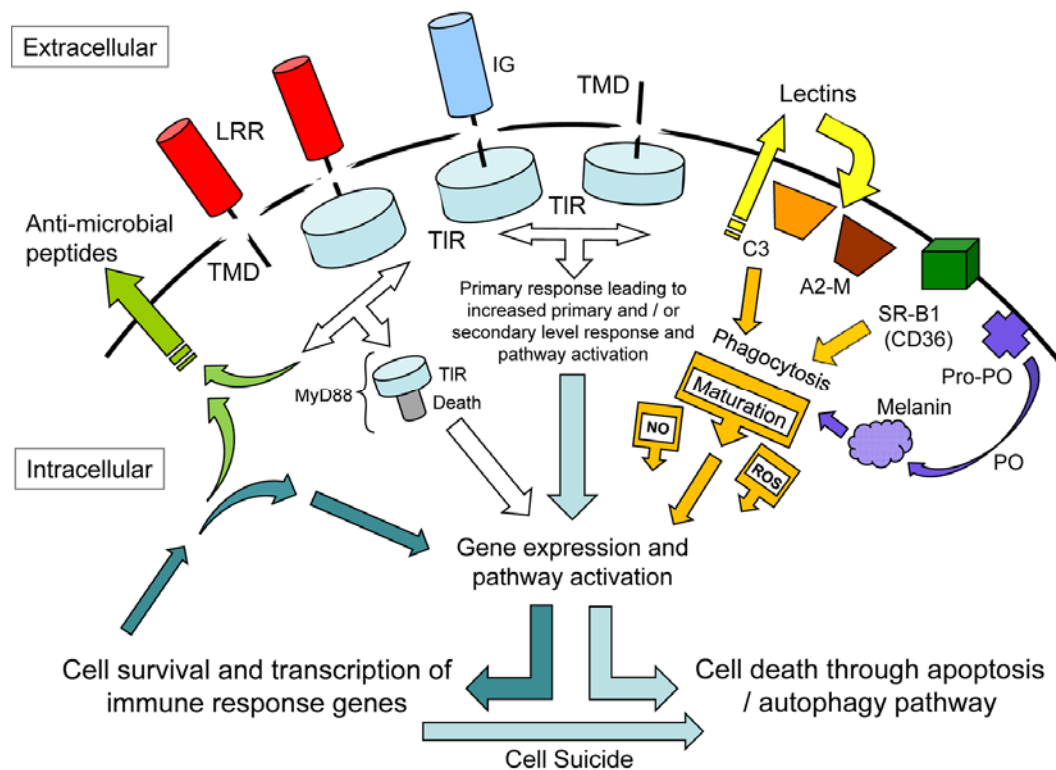


Fig. 2 A schematic representation of the innate immunoreceptors and associated protein domains, and peptides found in cnidarians that are prosed to contribute to the innate immune response. Some receptors are part of the primary response at the cell surface leading to increased secretion of peptides and lectins or to a secondary intracellular response, gene expression and pathway activation. Key to abbreviations are reported in Table 1.

cascade and inhibiting pro-cell death molecular triggers (Fratti *et al.*, 2001; Chua *et al.*, 2004; Koul *et al.*, 2004; Hilbi 2006). Whether the cnidarian-dinoflagellate symbiosis is controlled by the host, or if there is a symbiont directed manipulation of host immunity and associated cell-death pathways at present remains unresolved.

In conclusion, there is now strong evidence that cnidarian innate immunorecognition is far from simple and members of all classes display a diverse armory of innate immune receptors that operate at both an inter-cellular and intra-cellular level. The interaction between TIR-domain and LRR domain proteins and the production of peptides (possibly

with the addition of secondary metabolites) is complemented with lectin secretion that may interact with a C3-like complement phagocytic signalling cascade (Fig. 2). This multiple attribute defense system leading to an immune response, offers a key insight into the basal function of more complex innate immune pathways found in vertebrates. There is no doubt that the discovery of homologous, highly conserved cell pathways, and their functioning molecular components within cnidarians, will continue to promote members of the phylum as an ideal model system for the study of not only innate immunity, but much broader areas of higher metazoan cell biology.

Table 1 Different classes of receptors retrieved in cnidarians and their conserved domains

	Abbreviation	Domain Name
Cell Surface Receptor Signalling	LRR	Leucine Rich Repeat
	TMD	Trans Membrane Domain
	TIR	(Toll-Like) Toll interleukin-1 Receptor
	IG	Immunoglobulin
	A2M	Alpha-2-macroglobulin
	SR-B1	Scavenger Receptor B1
	Pro-PO	Prophenoloxidase
Intracellular Signalling	TIR/ Death	TIR (as above) /Death Domain
	PO	Phenoloxidase
	NO	Nitric oxide
	ROS	Reactive Oxygen Species

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