Developmental and Comparative Immunology 46 (2014) 480–488

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

TIR-domain-containing protein repertoire of nine anthozoan species reveals coral–specific expansions and uncharacterized proteins

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ARTICLE INFO

Article history:
Received 21 March 2014
Revised 4 June 2014
Accepted 5 June 2014
Available online 13 June 2014

Keywords:
TIR domain
TLR
IL-1R
Anthozoa

ABSTRACT

The intracellular toll/interleukin-1 receptor (TIR) domain plays an important role in vertebrate immunity, but the evolution and function of invertebrate TIR-domain-containing proteins is not fully understood. This study characterized and compared the TIR-domain-containing protein repertoire of nine cnidarians in class Anthozoa. A diverse set of proteins, including MyD88 (myeloid differentiation primary response protein 88), toll-like receptor (TLR)-like, interleukin-1 receptor (IL-1R)-like, and TIR-only proteins are present in the species surveyed. Increased numbers of TIR-only proteins were observed in corals compared to anemones, especially in the Acroporid and Pocilloporid coral families. This expansion could be linked to diversity of the microbial community on or in hosts and managing both positive and negative associations. Phylogenetic analysis indicates there are two groups of proteins with IL-1R-like domain architecture in anthozoans that potentially evolved independently of the vertebrate family. Bacterial-like TIR_2 domain proteins are also present, including one sequence with novel domain architecture. Overall this work promotes a better understanding of the anthozoan immune repertoire, which is important in the context learning about ancestral immune pathways and host–microbe interactions.

1. Introduction

The intracellular TIR domain is found in many proteins that participate in innate immune pathways, including TLRs, the IL-1R family, and the TIR adaptor proteins (Bowie and O'Neill, 2000; Leulier and Lemaitre, 2008). These immune signaling pathways are generally initiated by cytokines or microbial surface molecules binding to the extracellular regions of either TLRs or IL-1R family members. Ligand binding results in conformational changes that allow the TIR domain of the receptor to interact with the TIR domain of adaptor proteins through heterotypic protein–protein interactions (Dunne et al., 2003; O'Neill and Bowie, 2007). The TIR adaptor proteins then recruit downstream signaling molecules to ultimately activate transcription factors (O'Neill and Bowie, 2007; Sims and Smith, 2010).

In vertebrates, there are two major families of TIR-domain-containing receptors, the TLRs and IL-1R (Fig. 1). TLRs are pattern recognition receptors that detect a variety of microbial and viral signatures, such as lipopolysaccharide, dsRNA, flagellin, and non-CpG DNA (O'Neill and Bowie, 2007; Uematsu and Akira, 2008). Most TLRs localize to the plasma membrane, while others are present in the membranes of endosomes (Trinchieri and Sher, 2007). They are characterized by extracellular LRRs, which dictate ligand specificity, while the intracellular TIR domain initiates downstream signaling events through interactions with TIR adaptor proteins (Fig. 1) (Coscia et al., 2011; Takeda et al., 2003).

In vertebrates, signaling downstream of ligand binding and TLR activation operates through two distinct mechanisms: MyD88-dependent and MyD88-independent pathways (Kawai et al., 2001; O'Neill, 2003). MyD88 is a TIR adaptor protein and contains a death domain that recruits downstream signaling molecules that lead to activation of NF-κB and AP-1, resulting in expression of pro-inflammatory genes (Akira, 2006; Kenny and O'Neill, 2008; Trinchieri and Sher, 2007). In contrast, MyD88-independent

Abbreviations: TIR, toll/interleukin-1 receptor; MyD88, myeloid differentiation primary response protein 88; TLR, toll-like receptor; IL-1R, interleukin-1 receptor; Ig, immunoglobin; LRR, leucine rich repeat; NF-κB, nuclear factor kappa light chain enhancer of activated B-cells; AP-1, activator protein 1; TRIF, TIR-domain-containing adaptor-inducing interferon-β; IL-18R, interleukin-18 receptor; IL-1RAP, interleukin-1 receptor accessory protein; IL-18RAP, interleukin-18 receptor accessory protein; TIRAP, TIR-domain-containing adaptor protein; TRAM, TRIF-related adaptor molecule; SARM, sterile-α and heat armadillo motif containing protein; SAM, sterile-α motif; ARM, armadillo/beta catenin-like repeat; DD, death domain; PTK, protein tyrosine kinase; NLR, NOD-like receptor; TNF, tumor necrosis factor.

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http://dx.doi.org/10.1016/j.dci.2014.06.002
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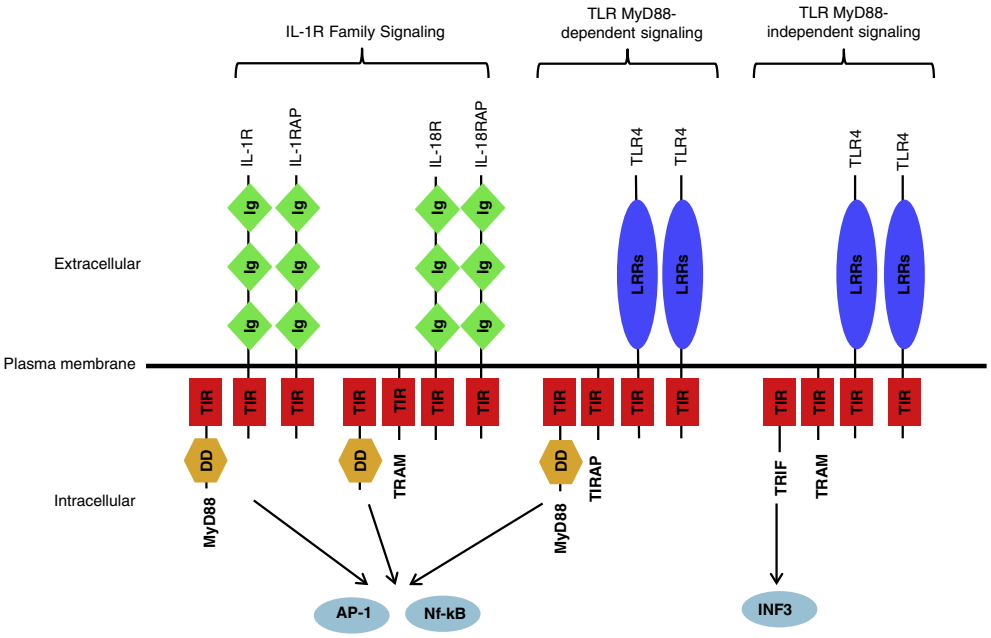

Fig. 1. Vertebrate TIR-domain-containing protein signaling pathways. IL-1R family signaling and MyD88-dependent TLR signaling both lead to activation of transcription factors AP-1 and NF-κB, while MyD88-independent TLR signaling leads to activation of interferon regulatory factors (IRF). LRR, leucine rich repeat; TIR, TLR/IL-1R domain; Ig, immunoglobulin domain; DD, death domain.

signaling is unique to TLR3 and TLR4 and utilizes another TIR adaptor protein, TRIF, to activate downstream interferon production (Oshiumi et al., 2003a; Yamamoto et al., 2003). Although they use different adaptor proteins, the end result from either TLR signaling mechanism is the expression of pro-inflammatory cytokines.

The second group of TIR-domain-containing receptors is the IL-1R family. In humans, this consists of nine proteins that are composed of extracellular Ig domains and an intracellular TIR domain (O'Neill, 2008; Subramaniam et al., 2004). Most members of this family have three extracellular Ig domains including IL-1R, IL-18R and their receptor accessory proteins (IL-RAPs) (Fig. 1) (Subramaniam et al., 2004). In order to be activated, IL-1R and IL-18R need to bind not only their respective cytokines (IL-1 and IL-18), but also receptor accessory proteins (IL-1RAP and IL-18RAP) (Subramaniam et al., 2004). Once these complexes have formed, downstream signaling similar to that of TLRs is initiated, resulting in production of pro-inflammatory cytokines that influence T-cell development and functioning (Akira, 2000; Sims and Smith, 2010).

Although TLR and IL-1R signaling is best understood in vertebrate systems, these pathways are also of interest in invertebrates. TLR-like sequences are present in most invertebrate groups, and functional studies have revealed roles for these proteins in both immunity and development (Coscia et al., 2011; Halfon et al., 1995; Rosetto et al., 1995; Tenor and Aballay, 2007; Wang et al., 2011; Yuan et al., 2009). Unlike TLRs, there is little evidence for the existence of true homologs to IL-1R family members in invertebrates. There have been descriptions of IL-1R-like molecules in several phyla, but the evidence is either indirect, or based only on protein domain structure (Beck et al., 2000; Huang et al., 2008; Shinzato et al., 2011). Therefore, the evolutionary history of the Ig-TIR domain combination has not been fully resolved and requires further investigation.

In addition to the TIR-domain-containing receptors, there are several intracellular TIR adaptor proteins that act downstream of TLR or IL-1R family activation. In mammals, there are five TIR-domain adaptor proteins: MyD88 and TRIF (both described above), TIRAP, TRAM, and SARM (O'Neill, 2003). Each of these adaptor proteins plays a specific role in intracellular signaling cascades. MyD88, as previously discussed, is recruited to activated TLRs, IL-1R, and IL-18R at the plasma membrane to propagate downstream signaling cascades (Adachi et al., 1998; Medzhitov et al., 1998; Muzio et al., 1997). In some cases, a second adaptor protein, TIRAP, acts as a bridge between TLRs and MyD88 (Horng et al., 2001; Sheedy and O'Neill, 2007; Yamamoto et al., 2002). In a similar manner to TIRAP, TRAM acts as a bridging protein for TRIF in the TLR MyD88-independent pathway, (Oshiumi et al., 2003b) and MyD88 in IL-18R signaling (Ohnishi et al., 2012). Finally, the only adaptor that negatively regulates TLRs is SARM, which specifically blocks TRIF-dependent signaling (Carty et al., 2006).

Several of the TIR adaptor proteins are present in invertebrates, while others likely evolved after or at the emergence of chordates. MyD88 homologs have been identified in basal metazoans such as sponges and cnidarians and are also present in all invertebrate groups investigated, with the exception of *Caenorhabditis elegans* (reviewed in Coscia et al., 2011). SARM has homologs in many invertebrate groups, but the function is not entirely conserved. For example, in cephalochordates and horseshoe crab, SARM homologs negatively regulate TLR signaling, while in *C. elegans* they promote antimicrobial peptide production, and therefore have the opposite function of their vertebrate counterpart (Belinda et al., 2008; Couillault et al., 2004; Yuan et al., 2010). At present, the role of the ancestral SARM protein remains unclear, and it may have been co-opted for a variety of functions throughout evolution. The remaining TIR adaptors, TRIF, TRAM, and TIRAP have been found only in chordates (Wu et al., 2011b).

Research on TIR-domain-containing proteins in cnidarians is of interest in the context of learning about the function of ancestral immune pathways and the role of immunity in cnidarian–dinoflagellate symbiosis. Cnidarians serve as hosts to a diversity of microbes including symbiotic dinoflagellates, bacteria, viruses, and apicomplexans (Kirk et al., 2013; Reshef et al., 2006; Thurber and Correa, 2011). It is unknown how cnidarians manage this balance of positive and negative associations, and characterization of the cnidarian immune repertoire will allow for the generation of