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ARTÍCULO DE REVISIÓN

MECHANISMS OF IMMUNE RESPONSES IN CNIDARIANS

Mecanismos de respuesta inmune en cnidarios

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ABSTRACT

The immune system maintains the integrity of the organisms through a complex network of molecules, cells, and tissues that recognize internal or external antigenic substances to neutralized and eliminate them. The mechanisms of immune response have evolved in a modular fashion, where members of a given module interact strongly among them, but weakly with members of other modules, providing robustness and evolvability to the immune system. Ancestral modules are the raw material for the generation of new modules through evolution. Thus, the study of immune systems in basal metazoans such as cnidarians seeks to determine the basic tool kit from which the metazoans started to construct their immune systems. In addition, understanding the immune mechanisms in cnidarians contributes to decipher the etiopathology of coral diseases of infectious nature that are affecting coral reefs worldwide.
Keywords: coral diseases, cnidarian immunity, evolutionary immunology.

RESUMEN

El sistema inmune mantiene la integridad de los organismos vivos por medio de una red compleja de moléculas, células y tejidos que reconocen sustancias antigénicas internas o externas para neutralizarlas y eliminarlas. Los mecanismos de respuesta inmune han evolucionado de una manera modular, en donde miembros de un módulo dado interactúan fuertemente entre sí, pero débilmente con componentes de otros módulos, otorgando así robustez y potencial evolutivo al sistema inmune. Módulos ancestrales representan el material básico para la generación de nuevos módulos durante el proceso evolutivo. Así, el estudio de sistemas inmunes en metazoarios basales como los cnidarios busca determinar cuales son los módulos ancestrales a partir de los cuales se constituyen los sistemas inmunes de animales derivados. Adicionalmente, el entendimiento de los mecanismos de respuesta inmune en cnidarios eventualmente contribuirá a descifrar la etiopatología de las enfermedades de corales de carácter infeccioso que está afectando los corales en el mundo.
Palabras clave: enfermedades de corales, inmunidad en cnidarios, inmunología evolutiva.

INTRODUCTION

The cnidarians are a basal metazoan group, sister of all bilaterian animals. In this group are included corals, anemones, and hydras, and they are the structural and functional basis of coral reef ecosystems, one of the most diverse of the world (Sheppard *et al.*, 2009). Indeed, coral reefs have an immense biological diversity only comparable to that of the tropical rain forest (Jackson, 2008). Yet, about one third of reef-building corals worldwide are facing extinction (Carpenter *et al.*, 2008) due in large part to an increased incidence of coral diseases of infectious nature (Harvell *et al.*, 2007). Current efforts have been primarily focused to determine the contribution of local and global environmental factors (Sokolow, 2009) as well as to identify the etiological agents of coral diseases (Rosenberg *et al.*, 2007). However, the mechanisms of immune response in cnidarians are just beginning to be studied systematically (Palmer *et al.*, 2012a). Genomic and transcriptomic studies in the Hydrozoans *Hydra magnipapillata* (Chapman *et al.*, 2010; Wenger *et al.*, 2013), and *Hydractinia echinata* (Soza-Ried *et al.*, 2010), the sea anemone *Nematostella vectensis* (Miller *et al.*, 2007; Putnam *et al.*, 2007) and a few corals (Miller *et al.*, 2007; Schwarz *et al.*, 2008; Shinzato *et al.*, 2011; Vidal-Dupiol *et al.*, 2011), have revealed several immune response genes conserved from cnidarians to vertebrates. This review presents a description of immune response mechanisms described in cnidarians.

Epithelia as immune barriers

Cnidarians are essentially epithelial organisms. They are constituted by two epithelial layers, the ectoderm (epidermis) and the endoderm (gastrodermis), separated by an acellular layer known as the mesoglea (Kozloff, 1990). The epithelial cells play a fundamental role in immunity as they display phagocytic activities and secrete mucus, which acts as a physicochemical barrier preventing or slowing down the proliferation of potential pathogens (Augustin *et al.*, 2011). The mucus contains several protector factors, including serine protease inhibitors with bactericidal activity and antimicrobial peptides (AMPs) (Augustin *et al.*, 2009). Some cnidarian species, like the octocoral *Gorgonia ventalina*, have granular amebocytes specialized in phagocytosis, constituting a primary line of defense against the fungus *Aspergillus sydowii*, a common pathogen in this species (Mydlarz *et al.*, 2008). Additionally, these amebocytes activate the prophenoloxidase enzymatic pathway that promotes the deposition of melanin in the affected zone, forming a barrier against the dispersion of pathogens (Mydlarz *et al.*, 2006). Scleractinian or stony corals also have granular amebocytes that activate the melanization processes in response to thermal stress (Palmer *et al.*, 2011) and near tissues with skeletal anomalies (Domart-Coulon *et al.*, 2006). Cnidarians have an immense capacity to regenerate their tissues as a consequence of the continuous proliferation of stem cells (Fautin, 2002); this could be considered as an additional arm of immune defense in these organisms since the cells infected intracellular parasites are quickly removed in a programmed way (apoptotic processes) and they are immediately replaced by non infected cells (Augustin *et al.*, 2011). Cnidarians posses a complex set of symbiotic bacteria inhabiting the epithelial surfaces that compete with potential pathogens to colonize the tissues (Bosch, 2013). Alterations in the structure of the symbiotic bacterial communities due to environmental changes, might promote the proliferation of opportunistic microorganisms that can cause disease (Cárdenas *et al.*, 2012). Hence, bacterial communities associated to the epithelia can also be considered part of an efficient immune barrier in cnidarians.

Deconstruction of immune response in cnidarians: recognition, signaling and effector modules

The defense against potential pathogens is one of the most important factors for the organism survival and the immune systems have evolved to maintain the integrity of the tissues against these challenges. There are several molecular mechanisms that mediate the recognition of potentially dangerous agents and the response to neutralize and eliminated them. These molecular mechanisms can be grouped into three modules, the recognition, the intracellular signaling and the effector modules. The recognition module is perhaps the most dynamic evolutionarily, where antigen receptors diversify rapidly to keep pace with the highly diverse microorganisms, while the signaling and effector modules are much more conserved. In the following sections we present some components of these three modules characterized in cnidaria.

The Immune recognition module of cnidarians

The recognition module of the immune response is perhaps the most dynamic, due to the high diversification of receptors for antigen binding. In this module are grouped the pattern recognition receptors (PRRs), which recognize molecules unique for a given microorganism but are absent in the host (pathogen-associated molecular patterns–PAMPs). PAMPs include components of microbial cell walls like zymosan, lipoteichoic acid (in Gram-positive bacteria) and lipopolysaccharides (LPS) (in Gram-negative bacteria), or different classes of bacterial proteins, like flagellin (Dunn, 2009). PRRs can recognize also damage-associated molecular patterns (DAMPs), which are self molecules or debris from altered cells, to initiate their removal (Takeuchi *et al.*, 2010). The interaction between PRRs and PAMPs (or DAMPs) induce a quick response acting at three different levels (Dunn, 2009): a) stimulating the microbial ingest through phagocytosis and enzymatic degradation, b) stimulating the mobilization of molecules to places where the infection is produced, and c) activating effector molecules through intracellular signaling transduction cascades. According to their location, at least three classes of predicted PRRs have been identified in cnidarians: membrane (mPRRs), soluble (sPRRs) and cytploasmic (cPRRs). The mPRRs and sPRRs recognize non-self (bacteria, viruses and fungi) and altered-self molecular patterns either immobilized on cell surfaces or soluble in extracellular space, while the cPRRs play an important role recognizing viruses and intra-cellular bacteria (Takeuchi *et al.*, 2010). A summary of the cnidarian PRRs can be seen in Figure 1.

Membrane pattern recognition receptors (mPRRs)

Toll-like receptors (TLRs) are among the most conserved mPRRs (Augustin *et al.*, 2010), and perhaps the best studied in invertebrates (Franzenburg *et al.*, 2012). The TLRs are transmembrane proteins composed by an extracellular N-terminal domain having leucine rich repeats (LRRs), which is responsible for the recognition process, a flanking cysteine-rich domain, a transmembrane domain, and an intracellular Toll/Interleukine-1 receptor (TIR) domain that initiates the transmission of intracellular signals leading to the translocation of transcription factors from NF-kB family (Franzenburg *et al.*, 2012). In *Drosophila,* these transcription factors activate genes coding for antimicrobial peptides, while in mammals they induces the expression of pro-inflammatory cytokines. Nine *Toll* genes have been identified in the *D. melanogaster* genome, 10 TLRs in *Anopheles gambiae*, one in horseshoe crab *Tachypleus tridentatusza*, 214 in the sea

urchin *Strongylocentrotus purpuratus* and 222 in amphioxus *Branchiostoma floridae* (Huang *et al.*, 2008). The number and structure of TLRs vary in cnidarians (Dunn, 2009). In *Hydra,* two transmembrane proteins have been characterized having extracellular LRRs similar to those present in vertebrate TLRs (HyLRR-1 and HyLRR-2). However, these two proteins do not possess the intracellular TIR domain typical of vertebrate TLRs (Fig. 1). In addition, *Hydra* has two other transmembrane proteins, HyTRR-1 y HyTRR-2, having intracellular a TIR domain with no recognizable extracellular domains. Immune-challenge assays suggest that HyLRR-2 and Hy-TRR-1 are functionally linked to recognize molecular patterns and to transduce the signal from the cell membrane to the nucleus (Augustin *et al.*, 2011). The recognition of bacteria by TLRs is not only an immune process but it also contributes to recolonization of commensal bacteria (Franzenburg *et al.*, 2012). In addition, silencing the *HyTRR-1* and *HyLRR-2* genes leads to a drastic reduction in the synthesis of antimicrobial peptides such as Hydramacin-1, Arminin-1a, and Periculin-1, indicating that the TLR pathway in this hydrozoan activates an antimicrobial state (Augustin *et al.*, 2010). A dual TLR structure similar to that of *Hydra* might also be present in other cnidarians such the corals *Montastraea cavernosa*, *Pocillopora damicornis,* and *Seriatopora hystrix,* as they do not posses canonical TLRs (Poole *et al.*, 2014). Yet, canonical TLRs have been identified in other Anthozoans, like the anemone *Nematostella vectensis* and the staghorn coral *Acropora millepora* (Miller *et al.*, 2007). In *N. vectensis* one TLR has been identified, which is structurally similar to the vertebrate TLRs having an extracellular LRRs domain and an intracellular TIR (Miller *et al.*, 2007). Furthermore, in this anemone three other transmembrane TIR-containing proteins have been identified, having a variable number of extracellular immunoglobulin (Ig) domains, similar in architecture to mammalian interleukin receptors (ILR) (Fig. 1). In *A. digitifera*, four TLRs and 19 ILRs have been identified, suggesting the immune recognition repertoire of this coral is more complex than that of *Nematostella* (Shinzato *et al.*, 2011). Other important group of mPRRs is the scavenger receptors (SR), which recognize a wide variety of molecular patterns, and in vertebrates, have been classified into 8 types, A-H (Plüddemann *et al.*, 2007). EST analysis have reveled the presence of transcripts encoding SR in the corals *M. faveolata* and *A. palmata,* including various from the B family that are characterized by the presence of scavenger receptor cystein rich (SRCR) domains (Schwarz *et al.*, 2008). The mechanisms of recognition and the signal transduction pathways that these receptors activate are yet to be explored in cnidarians.

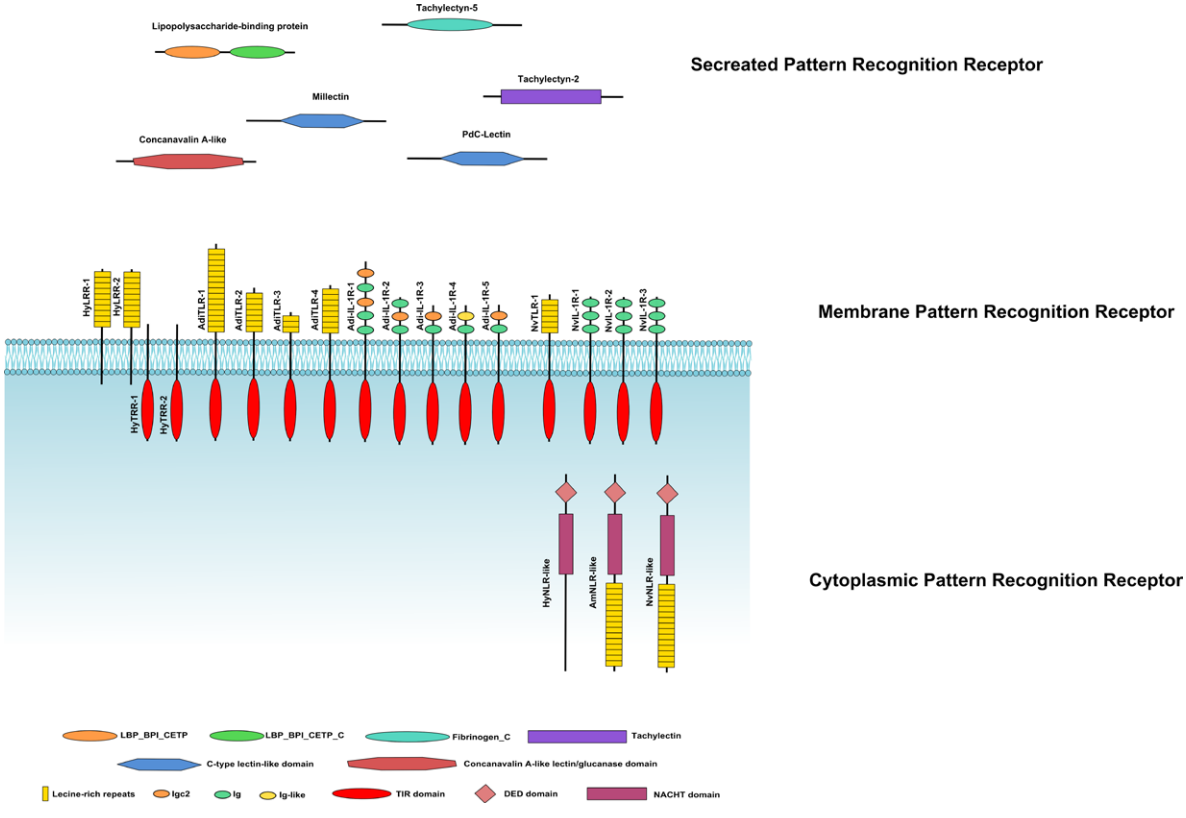

Figure 1. Predicted proteins from the immune recognition module identified in cnidarians.

Cytosolic pattern recognition receptors (cPRRs)

One of the most prominent families of cPRRs found in cnidarians is the NOD-like receptors (NLRs). They are cytosolic receptors that form a signaling scaffold to activate cytokines or inflammatory caspases, and are composed of a central nucleotide-binding domain NACHT, a C-terminal domain with several LRRs, and an N-terminal effector domain such as Pyrin, CARD, DED or BIR (Proell *et al.*, 2008). Complex repertoires of NACHT-containing proteins have been identified in *H. magnipapillata, N. vectensis,* and *A. millepora* (Lange *et al.*, 2011). Classical NLR with tripartite domain structure (DED/NATCH/LRRs) are present in both *N. vectensis,* and *A. millepora*, but not in *Hydra*, indicating that this hydroid has modified secondarily its NLR receptors gene set (Lange *et al.*, 2011).

Soluble or secreted pattern recognition receptors (sPRRs)

C-type lectins (CTLs) participate in important immune functions across the animal kingdom, including opsonization (Takeuchi *et al.*, 2010) and activation of the complement system (Fujita *et al.*, 2004). In the coral *Pocillopora damicornis* two CTLs have been characterized, the mannose binding lectin PdC-Lectin and concanavalin (Vidal-Dupiol *et al.*, 2011) (Fig. 1). These two molecules increase their expression after a challenge with a virulent strain of *Vibrio coralliilyticus* and also are involved in the molecular interactions between the coral and the algal symbionts during thermal stress events (Vidal-Dupiol *et al.*, 2009). Another immune-type lectin family found in several cnidarian species is the Tachylectins (TLs). These lectins were originally isolated from horseshoe crab *Tachypleus tridentatus* and were shown to induce an anti-microbial activity through the recognition of PAMPs, such as LPS and peptidoglycans (Beisel *et al.*, 1999). TL-2 proteins have been characterized in some corals species, including those from genera *Acropora, Montastraea* and *Oculina* (Fig. 1). In addition, a TL-like molecule was characterized in the hydrozoan *Hydractinia echinata*, but despite to its similarity to TL, it has no immune function (Mali *et al.*, 2006). A different type of lectin, a mannose-binding lectin (MBL) called Millectin, has been isolated from the coral *A. millepora*. Millectin binds bacteria and the algal symbiont *Symbiodinium,* and is involved in the process of immune response and symbiont acquisition (Kvennefors *et al.*, 2008). Finally, the Lipopolysaccharide (LPS)-binding proteins (LBPs) is a family of sPRRs that recognize LPS from Gram-negative bacteria, leading to the activation of NF-kB pathway in both vertebrates and invertebrates (Fraser *et al.*, 2008). Homologues of LBPs have been identified in the genomes of *H. magnipapillata* and *N. vectensis* (Miller *et al.*, 2007).

The immune signaling module of cnidarians

This module includes components of signaling pathways involved in the activation of immune effector molecules. Several signaling genes homologous to those of vertebrates have been identified in cnidarians; yet, there is very little functional information that can confirm their actual role in those processes. The TLR signaling pathway is well conserved in metazoans, and in the cnidarians *H. magnipapillata, N. vectensis* and *A. millepora,* genes encoding the universal adaptor protein MyD88, and kinases that participate in the signal delivery, such as IRAK, TRAF and TAK, have also been identified (Palmer *et al.*, 2012b). Indeed, MyD88-knockdown of a *Hydra vulgaris* line was generated to demonstrate that TLR recognize bacteria to subsequently induce the synthesis of AMPs (Franzenburg *et al.*, 2012). Furthermore, the gene coding for the transcription factor NF-kB, which activates the expression of various genes involved in a wide range of immune processes, has also been characterized in wild populations of *N. vectensis* and *A. millepora* (Palmer *et al.*, 2012b). In *Hydra*, it is known that the activation of NF-kB leads to the expression of AMPs (Augustin *et al.*, 2012). Finally, components of other signaling transduction pathways triggered by PRRs appear to be conserved in corals and other cnidarians, for example, those involved in the Interferon and ECSIT signaling pathways (Miller *et al.*, 2007).

The immune effector module of cnidarians

Research on immune effector mechanisms in cnidarians is in its beginnings, and it has been suggested that the main effector molecules are proteases, serine protease inhibitors, antimicrobial proteins, and the Complement system (Dunn, 2009). Among proteases, the lysosomic cathepsins are playing an important role in phagocytosis, and have been identified in the genomes of *Hydra* (Chapman *et al.*, 2010) and *N. vectensis* (Putnam *et al.*, 2007). Protease inhibitors with putative immune activity have also been characterized in some cnidarians. For example, the kazal-type protease inhibitor isolated in *Hydra* shows strong *in vitro* bactericidal activity against *Staphylococcus aureus* (Augustin *et al.*, 2009). Other protease inhibitors, the kunitz-type protease inhibitor and the alpha-2-macroglobulin, have been identified in anemones and are thought to play a role in immunity by inactivating virulence factors from bacteria (Fujito *et al.*, 2010; Kimura *et al.*, 2009; Peigneur *et al.*, 2011). AMPs have been identified in *Hydra* (Bosch, 2013) and in the coral *P. damicornis* (Vidal-Dupiol *et al.*, 2011). In the latter, the AMP is called damicornin, and it is expressed in the ectodermal granular cells and displays antimicrobial activity *in vitro* against Gram-positive bacteria and fungi (Vidal-Dupiol *et al.*, 2011). In the former, three AMPs have been characterized, Hydramacin-1, Arminin-1a and Periculin-1 (Augustin *et al.*, 2010; Jung *et al.*, 2009). The synthesis of AMPs in *Hydra* is triggered by the interaction between TLR-like molecules and a ligand, and they show some degree of specificity to different PAMPs. For example, Hydramacin-1 increases in presence of LPS (Augustin *et al.*, 2010), while the expression of Periculin-1 increase in presence of both LPS and flagellin

(Augustin *et al.*, 2011). Finally, the Complement system is an important effector mechanism in animals functioning in opsonization, regulation of inflammatory responses, and bacterial lysis. The Complement system is activated by three parallel proteolytic cascades, known as the classical, alternative, and lectin pathways, which converge in the cleavage of the Complement component 3 (C3) to generate inflammatory factors and bacterial lysis (Sarma *et al.*, 2011). The various components of the Complement system are grouped into five families (Nonaka *et al.*, 2006): the C3 family (C3, C4, and C5), the factor B (Bf) family (Bf and C2), the mannan-binding lectin-associated serine protease (MASP) family (MASP-1, -2, -3, C1r, and C1s), the C6 family (C6, C7, C8A, C8B, and C9), and the factor I (If) family. The C3 family is part of the thioester bond-containing protein (TEP) superfamily, which also includes the serine protease inhibitor alpha-2 macroglobulin (A2M) and GPI-linked glycoprotein CD109. Complement-encoding genes identified in cnidarians include those for C3, factor B (Bf), and Mannose-binding lectin-associated serine proteases (MASP) (Dishaw *et al.*, 2005; Kimura *et al.*, 2009; Miller *et al.*, 2007; Fujito *et al.*, 2010; Kenkel *et al.*, 2011). Functional studies on these complement molecules are needed to understand their actual role in cnidarian immunity.

CONCLUDING REMARKS

Cnidarians are the structural and functional basis of coral reefs, one of the most biodiverse ecosystems in the planet, and are also responsible in large extent for maintaining the physicochemical properties of the ocean in the tropics. In the last few decades, global and local environmental changes have generated an unprecedented crisis in the health of coral reefs characterized by frequent bleaching episodes and high incidence of coral diseases (Weil *et al.*, 2006). This latter phenomenon has attracted the attention of the scientific community and important contributions have been generated directed to identify possible etiological agents involved in the pathology of such diseases. Yet, a detailed understanding of the phenomenon, and, perhaps more importantly, the generation of preventive and corrective measures, requires a more integrative approach involving, among other things, knowing how the immune system of corals, and of cnidarians in general work. Thanks to the advantages that provide the current methods of genome and transcriptome sequencing, it is now possible to identify cnidarian genes homologous to those of model organisms for which functional studies have been performed (Dheilly *et al.*, 2014). Most cnidarian immunity studies have focused on Hydrozoa and Anthozoa taxa, yet, to have a better understanding of the origin and evolution of the immune mechanisms in cnidaria, it is necessary to analyze basal groups, e.g. Ceriantharia and Cubozoa, that due to their large phylogenetic distances (Miller *et al*., 2007; Stampar *et al*., 2014), will prove to be highly informative. Beyond comparative analyses of conserved immune-type molecules, the study of cnidarian immunity needs to be focused on functional studies of the genes identified by sequencing. However, a general panorama can be seen from the available data revealing a vast diversity of immune recognition molecules, associated with some signaling pathways and effector mechanisms conserved throughout metazoans. Much more of such molecules are yet to be discovered, and, together with functional analyses, will provide the opportunity to generate a general description of the immune system of cnidarians.

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