

REVIEW

The immune responses of the coral**C Toledo-Hernández¹, CP Ruiz-Diaz^{1,2}**¹*Sociedad Ambiente Marino, PO Box 22158, San Juan Puerto Rico 00931*²*Department of Environmental Sciences, University of Puerto Rico, Río Piedras campus, PO Box 70377 Puerto Rico**Accepted November 3, 2014***Abstract**

Corals are among the most ancient extant animals on earth. Currently, coral viability is threatened, due in part to the increased number of diseases affecting them in recent decades. Understanding how the innate immune systems of corals function is important if we want to predict the fate of corals and their response to the environmental and biological changes they face. In this review we discuss the latest findings regarding the innate immune systems of corals. The review is organized following the chronology of steps taken by corals from the initial encounter with a potential pathogen and recognition of threats to the orchestration of a response. We begin with the literature describing the repertory of immune-related receptors involved in the recognition of threats and the subsequent pathways leading to an immune response. We then review the effector responses that eliminate the threats described for corals. Finally, we acknowledge the literature of coral microbiology to access the potential role of microbes as an essential constituent of the coral immune system.

Key Words: immune response; coral diseases; coral-associated microbes; afferent and effector arms

Introduction

Coral reefs are among the most ancient extant ecosystems. Evidence of their existence can be traced back to the Mesozoic era (Veron, 1995). They are also among the most persistent ecosystems as they have survived several mass extinction events over geological time, and continued to thrive. Ironically, during the last century reefs of the world have undergone unprecedented declines, primarily due to human derived stresses (McClanahan *et al.*, 2002). A combination of factors, which include water pollution (*i.e.*, nutrient and sediment influxes), overfishing, rising water temperatures, ocean acidification and disease are causing declines in coral growth and coral cover. Such degradation is readily observed in the Indo-Pacific region where reefs are disappearing at an average rate of 2 % per year (Bruno and Selig, 2007). In the Caribbean, reefs are being lost even faster, *i.e.*, at an average rate of 5.5 - 9.2 % per year (Buddemeier and Ware, 2003). Given increasing human impacts, these pressures are projected to exacerbate damages to coral reefs worldwide and

these ecosystems are likely to suffer even more extreme stress than those already observed in the recent past.

From the myriad of stressors threatening coral health, disease has perhaps, received the most attention by the scientific community during the last few decades. Reports of emerging coral diseases have increased since they were first described in the early 1970s (Antonius, 1973; Richardson, 2012). Currently, over 35 coral diseases have been reported (Kline and Vollmer, 2011), some of which have worldwide distribution, *i.e.*, Black Band Disease (BBD). Others have narrower distributions, such as Yellow Band Disease (YBD), which is reported exclusively in the Caribbean (Veron, 1995). Diseases have affected over 80 coral species and their impacts have been so dramatic as to alter the seascape and the community structures of reefs (Pandolfi and Jackson, 2006).

Studies addressing the causations of coral diseases, as well as their prevalence, incidence, and their impacts on vital life history traits such as growth and reproduction, have dominated the scientific literature. For instance, several long-term field studies have shown that diseased corals exhibit slower growth rates and a reduced fecundity when compared to healthy ones (Baird and Marshall, 2002; Petes *et al.*, 2003; Toledo-Hernández *et al.*, 2009; Weil *et al.*, 2009). Far less

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attention has been devoted to study the mechanisms by which corals recognize a threat and orchestrate an immune response to an insult. The fact that we do not fully understand how the immune system of corals works, undermines our capacity to comprehend the real causes and consequences of coral disease processes.

The innate immune system of corals, as in all invertebrates, is responsible for maintaining the integrity and stability of the internal environment (homeostasis). Adaptive immune-like systems have yet to be described in invertebrates. Corals are thought to pose a rudimentary and rather simplistic immune system (Alker *et al.*, 2004; Pollock *et al.*, 2011; Toledo-Hernández *et al.*, 2012). This notion has been supported in part by their primitive diploblastic body plan, typical of organisms from the basal Metazoa phylogeny, concomitant with their similar macroscopic immune responses toward different immune insults. However, new molecular findings have shown that coral immunity is surprisingly complex, with some immune components such as immune-related genes and proteins homologous to those from vertebrates (Miller *et al.*, 2007; Palmer and Traylor-Knowles, 2012). In this review we will present the latest findings regarding coral immune systems. For the purpose of this review, corals are identified as members of the Scleractinia and Gorgonacea orders. The review is organized following the chronology of steps taken by corals from the initial encounter with a potential pathogen and recognition of threats to the orchestration of a response. Accordingly, we begin by summarizing the current literature concerning the repertory of immune-related receptors involved in the recognition of threats and the subsequent pathways to mount a response. We then review the effector responses that eliminate or mitigate the threats. Finally, we acknowledge the literature of coral microbiology to access the potential role of microbes as integral constituents of the coral immune system.

Innate immune system of corals

Physical barriers are the first line of defense of any organism. For the most part, physical barriers are anatomical structures that inhibit or prevent a threat from affecting or entering the internal environment of an organism. In corals, such barriers are the mucus and sclerite layers that primarily settle on top of and within the coral ectoderm. Coral mucus is a viscous fluid made of a complex mixture of compounds secreted by mucocytes. Sclerites, on the other hand, are carbon calcium carbonate crystals of variable shapes, sizes and secreted by sclerocytes from gorgonian corals. Coral mucus is involved in an array of functions ranging from feeding and sun protection to pathogen defense; the mucous layer hosts symbiotic microbes that prevent the settlement of noxious bacteria (Brown and Bythell, 2005). Sclerites may function as a shield that prevents or retards the recruitment of microbes. For instance, we have documented that a layer of sclerites from the sea fan *Gorgonia ventalina*, placed on top of culture media, delay the

colonization and further penetration into the culture media of fungi isolated from sea fans. However, once threats overwhelm these physical barriers, the immune system activates. Coral innate immune systems are no different from other invertebrates. It consists of two branches: 1) the afferent or sensing arm, and 2) the efferent or effector arm. The afferent arm is basically the component that recognizes the threat and activates an effector response, which will orchestrate a humoral and/or cellular response such as inflammatory and cytotoxic responses, plus phagocytosis, which will neutralize the threat.

Afferent component

Our knowledge regarding the recognition receptors of corals is very limited. Most of what we currently know is based on a few microarray and transcriptional studies from a few coral species, *i.e.*, *Acropora millepora* and *A. digitifera* (Miller *et al.*, 2007), or from extrapolation from closely related organisms such as *Hydra* or from more complex invertebrates such as the fruit fly, *Drosophila melanogaster*. Most of these studies report the presence of sequences that, due to their structural similarities with immune-related genes and proteins from other animals, are considered immune-related components of corals. Moreover, investigations working on recognition receptors found in other invertebrates suggest that numerous coral immune-related pathway components have yet to be identified.

Pattern recognition receptors (PRRs)

Like all animals, corals rely on their capacity to detect non-self entities by means of pattern recognition receptors (PRRs) to initiate an immune response. These receptors, which appear to be highly conserved across the animal kingdom, are complex proteins that bind to highly conserved cell wall structures from foreign entities collectively known as microbe-associated molecular patterns (MAMP), such as flagellin, lipopolysaccharide (LPS), lipoteichoic acid, and peptidoglycan. These are generally absent on hosts but are essential structural components in microbes (Murphy *et al.*, 2011). These bindings activate complex downstream signaling pathways that result in the expression, via transcription factors, of immune genes that initiate the proper effector responses, such as opsonization, phagocytosis, lysis, or the production of anti-microbial peptides. In corals, six distinct families of microbe detection receptors have been identified. Three of these receptors are trans-membrane sensors or receptors, *i.e.*, Toll-like receptors (TLRs), Lectins and Integrins. The remaining three belong to the families of intracellular receptors; the nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) and the complement-like system C3. However, it is important to highlight that, with few exceptions, the existing literature regarding PRRs is purely descriptive. Functional experiments are yet to be conducted, thus the roles of the majority of the PRRs as immune constituent are unknown.

Trans-membrane Receptors

Toll-Like Receptors (TLRs)

TLRs are perhaps the best-characterized of the PRRs and one of the principal innate immune components of invertebrates. TLRs consist of an outer leucine-rich repeat domain (LRR) responsible for pattern recognition, and a cytoplasmic component Toll/interleukin-1receptor (TIR), which mediates signal transmission. Complete TLRs (LRR and TIR) have been identified in *Acropora digitifera* (Miller *et al.*, 2007); while the cytoplasmic component TIRs have also been identified in *A. palmata*, *A. millepora* and *Montastraea faveolata* (Miller *et al.*, 2007; Shwarz *et al.*, 2008). Several signaling pathways associated with TLRs have also been identified in these corals. These include the homologues of the myeloid differentiation primary response adaptor protein (MyD88), which activates downstream signaling pathways, as well as homologues of the transcription factor NF- κ B, which lead to cell death. Mitogen-activated protein kinases (MAPKs), which are involved in the cellular responses to thermal stress among other roles, have been also identified in *A. digitifera* (Miller *et al.*, 2007; Shwarz *et al.*, 2008).

Lectins

Lectins are a family of trans-membrane receptors ubiquitous throughout the animal kingdom. Lectins play an important role in cell recognition, including recognition of bacteria and viruses. These receptors contain one or more carbohydrate recognition domains, which allow binding to carbohydrate components from foreign entities in a calcium-dependent manner. Lectin-like genes have been identified in several scleractinian corals. *Millectin* for instance, has been identified and described in the scleractinian coral *A. millepora* (Kvennefors *et al.*, 2008). *Millectin* contains a peptide recognition site and a carbohydrate-binding site, which under Ca^{2+} dependent conditions has an affinity toward N-acetylglucosamine and other simple sugars such as mannose, glucose, and fructose. Several studies have confirmed the role of *Millectin* in recognizing non-self structures. For instance, functional experiments have revealed *Millectin* binding to and agglutinating *Vibrio harveyi* and *V. corallilyticus* as well as other potential pathogens (Kvennefors *et al.*, 2008). *Millectin* has also been detected bound to zooxanthellae, further emphasizing its function in the recognition of non-self entities (Kvennefors *et al.*, 2008). Immunohistochemistry studies have detected *Millectin* encapsulated within nematocysts in the epithelial tissue lining the gastrodermis of the oral and aboral regions of *A. millepora* (Kvennefors *et al.*, 2010). As these regions are constantly in contact with potential pathogens, the authors hypothesized that *Millectin* would be secreted upon pathogen recognition, preventing further penetration of the pathogen into the internal environment of the host. At the gene expression level, up-regulation of *Millectin* has been detected after *A. millepora* fragments were exposed to bacterial LPS (Kvennefors *et al.*, 2008). In this study, up-

regulation of *Millectin* peaked 45 min. after LPS exposure and gradually decreased within the next twelve hours, suggesting that overexpression of *Millectin* and perhaps other C-type lectin genes primarily occurred during the first few minutes after pathogen recognition (Kvennefors *et al.*, 2010; Brown *et al.*, 2013).

An additional lectin-like gene, namely *Techylectin* has been described from the scleractinian coral *Oculina* sp. (Hayes *et al.*, 2010). *Oculina* tachylectin-2 is remarkably similar to the Tachylectin-2 originally isolated from the Japanese horseshoe crab *Tachypleus tridentatus*, which has been shown to have broad-spectrum anti-microbial activity as well as a role in no-self recognition (Kawabata and Iwanaga, 1999). Due to these similarities, *Oculina* tachylectin-2 has been suggested to play a role in the coral immune system; however, functional experiments are yet to be conducted to confirm this argument.

Integrins

Integrins are surface receptors highly conserved across the animal kingdom, and employed in multiple functions, such as development and growth, wound repair and immune response. Functional integrins always consist of at least two glycoprotein subunits, the α and β chains. These α β heterodimers are involved in cell-to-cell intra or extra cellular binding. In corals, specifically in *A. millepora*, several integrins have been identified (Brower *et al.*, 1997; Knack *et al.*, 2008). However, these have been associated to developmental roles, i.e. coral gastrulation (Knack *et al.*, 2008). The role of integrins as constituents of coral immunity has yet to be demonstrated.

Intracellular receptors

NLRs

Microbes eluding trans-membrane surveillance encounter a second line of sensors with the capacity to recognize MAMPs at the intracellular level. These receptors are collectively known as nucleotide oligomerization domain (NOD)-like receptors (NLRs). Similar to extracellular receptors, NLR activation triggers multiple proinflammatory signaling pathways, which result in the eradication or inhibition of the invaders. The architecture of NLRs essentially consists of a C terminal LRR domain for microbe pattern recognition, an intermediary NOD domain for nucleotide binding and modulation of NLR activity, and a N-terminal effector domain (Kanneganti *et al.*, 2007). Genomic analyses have revealed that NLRs of *A. digitifera* are surprisingly diverse within the central domain containing a death-effector domain (DED), death domain (DD) and Caspase recruitment domain (CARD), all of which play pivotal roles in apoptosis (Hamada *et al.*, 2012).

Complement system

The complement system is a major effector mechanism of the innate immune system. Its function varies from opsonizing pathogens for

further phagocytosis to lysis, tissue regeneration, or the clearance of immune complexes and apoptotic cells (Trouw and Daha, 2011). In higher vertebrates, complement activation proceeds via: 1) antibody-antigen binding; 2) mannose-binding lectin (MBL) and 3) spontaneous binding of a complement component to the pathogen surface (Murphy *et al.*, 2011). These pathways lead to the activation of complement factor C3, the main component of this system. As corals lack adaptive immunity, complement activation hypothetically should proceed either via MBL or spontaneously (Cerenius *et al.*, 2010). Thus far, complement-like genes have been identified in three coral species, including the gorgonian corals *Swiftia exserta* (Dishaw *et al.*, 2005) and *Gorgonia ventalina* (Burge *et al.*, 2013), and the scleractinian coral *A. millepora* (Miller *et al.*, 2007). Given the over-expression of complement-like genes from *A. millepora* and *G. ventalina* in response to challenge with bacterial pathogens and its localization in the epithelium, as in the case of C3 from *A. millepora*, these genes have been linked to the immune systems of corals (Kvennefors *et al.*, 2010; Brown *et al.*, 2013; Burge *et al.*, 2013).

Effector components

Humoral response

Humoral response in corals consists of the synthesis and release, upon activation, of a variety of chemical compounds, such as melanin, reactive oxygen species (ROS), antimicrobial peptides (AMP), and secondary metabolites whose main function is to kill microbes by: 1) opsonizing and agglutinating invaders, 2) permeabilizing the invader's cell membrane, causing lysis, or 3) disrupting of their metabolism (Ellis *et al.*, 2011). These compounds may be constitutively present and stored in granules within epithelial cells or contained in free-living cells, in which case they can be recruited to the afflicted areas (Palmer *et al.*, 2008). Alternatively, these compounds can be synthesized and released *in situ* upon local induction by mechanical stress, injuries or pathogen detection (Destoumieux *et al.*, 2000; Ganz, 2003; Geffen *et al.*, 2009). Antimicrobial agents have no functional specificity, meaning that these agents have a broad spectrum of activity against Gram-positive and Gram-negative bacteria, fungi, viruses and protists (Otero-González *et al.*, 2010; Ellis *et al.*, 2011). That is not to say, however, that all antibacterial agents are equally effective against microbes (Gochfeld and Aeby, 2008). The size of the antimicrobial agent may impact structural components of the microbes differently. For instance, small peptides (< 23 amino acids long) mainly disrupt the integrity of the cell membrane of the invading entities, while larger peptides pose lytic properties, or may be proteins with specific domains that sequester essential nutrients from microbes (Ganz, 2003).

Melanization

Melanin is notorious among invertebrates, including corals. Melanization, the synthesis and deposition of melanin around foreign entities or wounded areas, is a relatively well-understood process in invertebrates, particularly in arthropods

and crustacea (Cerenius and Soderhall, 2004). The enzyme phenoloxidase (PO) is essential to the synthesis of melanin. PO and its inactive form prophenoloxidase (ProPO), belong to the tyrosinase group of oxidases whose main role is to oxidize phenols (Gonzalez-Santoyo *et al.*, 2012). Melanin is derived from phenylalanine, which is hydroxylated to form tyrosine. Upon activation, the proteolysis of proPO activates PO. Once activated, PO catalyzes the oxidation of mono-phenol (tyrosine) and di-phenols (DOPA) to form quinones. Ultimately, non-enzymatic polymerization of quinones forms melanin (Palmer *et al.*, 2011). Synthesis of melanin also leads to the formation of proteases, cytotoxic quinones and reactive oxygen and nitrogen intermediates that not only kill pathogens but also inflict self-damage. In summary, regulatory mechanisms of PO such as the production of phenoloxidase inhibitor (POI), serine protease inhibitor, radical scavengers and anti-oxidant enzymes are produced to control the synthesis of melanin (Cerenius and Soderhall, 2004; Cerenius *et al.*, 2010).

Activation of PO in response to DOPA has been demonstrated in *A. millepora* and *Porites* sp. (Palmer *et al.*, 2008). Multiple melanin-synthesis pathways (tyrosinase-type and laccase-type pathways), and multiple POs (*i.e.*, catecholase, cresolase and laccase), have also been identified in corals from several families, (*i.e.*, *Pocilloporidae*, *Acroporidae*, *Merulinidae*, *Mussidae*, *Fungidae*, *Faviidae* and *Poriferidae*) (Mydlarz and Palmer, 2011; Palmer *et al.*, 2012). Melanin synthesis in corals has also been shown during wound healing, pathogenic interactions and sustained elevated water temperatures (Petes *et al.*, 2003; Mydlarz *et al.*, 2008; Palmer *et al.*, 2008). Furthermore, base-line PO activity and the size and abundance of melanin-containing granular amoebocytes (the cells where melanin synthesis takes place), have been compared among several Indo-Pacific and Caribbean corals (Palmer *et al.*, 2010). In fact, base-line levels of these immune constituents have been found to be good predictors of disease and bleaching susceptibility and thus have been related to coral immunocompetence. In other words, corals exhibiting lower base-line concentrations of the aforementioned melanization constituents are more susceptible to disease and bleaching than those with higher levels (Palmer *et al.*, 2011).

Reactive oxygen species (ROS)

ROS are a group of highly reactive, relatively short-lived compounds *i.e.*, $^1\text{O}_2$, O_2 , H_2O_2 , $\text{HO}\cdot$, $\text{NO}\cdot$, which are by products of oxidation-reduction (REDOX) reactions of living organisms. In biological systems, ROS have a diverse and dose-dependent impact. For instance, at relatively low doses, ROS can function as signaling molecules, such as in immune defense and apoptosis (Nappi and Ottaviani, 2000). Yet, at higher doses and since ROS are not target-specific compounds, they produce oxidative stress (if the accumulation of ROS exceeds the threshold of organisms to suppress them), which can inflict damage to important cell components of both host and invader, such as DNA, RNA, proteins and lipids (Lesser, 2006).

ROS synthesis has been studied in corals as a way to measure environmental stress. For instance, Mydlarz and Jacobs (2006) studied the production of ROS in the gorgonian corals *Pseudopterogorgia elisabethae*, *P. americana*, *Eunicea fusca* spp., and *Lophogorgia chilensis* after subjecting them to physical injury and thermal stress. They found that these stressors induced an oxidative stress that varied among species. In another study, Saragosti *et al.* (2010) measured the production of ROS in non-bleached and bleached *Stylophora* sp and found that both produced a comparable ROS concentration when maintained in the dark. However, under light regimes non-bleached corals increased ROS production by two-fold when compared with bleached fragments, demonstrating that light increments induced the production of ROS. On the other hand, Tchervno *et al.* (2011) experimentally established that ROS produced by zooxanthellae from the scleractinian corals *Seriatopora hystrix* and *Stylophora pistillata* and from the corals themselves maintained under thermal stress conditions, activated the caspase cascade, which promoted bleaching and apoptosis. They also demonstrated experimentally that exogenous ROS activates the caspase pathway and apoptosis of *Montipora capitata* fragments under conditions of low light and temperature, further showing the role of ROS as a signaling pathway.

Antimicrobial peptides (AMPs)

According to Ganz (2003) AMPs are peptides with less than 100 amino acid residues, encoded by genes and synthesized by ribosomes from animal hosts. Thus, this definition excludes AMPs synthesized by potential symbionts such as bacteria, fungi or protists. In addition to their antimicrobial properties, AMPs show immunomodulatory activities such as apoptosis and regulation of gene transcriptions (Otero-González *et al.*, 2010). However, in general, little is known regarding AMPs of corals. In fact, Damicornin from *Pocillopora damicornis* is the only AMP characterized for any coral thus far (Vidal-Dupoil *et al.*, 2011). Damicornin is a cationic, 40 amino acid AMP, which inhibits Gram-positive bacteria and fungi *in vitro* experiments. Moreover, it is constitutively transcribed and stored in granular cells from the epithelium where it is released upon non-pathogenic immune challenge.

Transcript analyses have also revealed the presence of genes encoding potential anti-microbial peptides in corals. For instance, Burge *et al.* (2013) working with the sea fan coral *G. ventalina*, identified gene homologues to the *arenicin-2* gene from a polychaete worms and the *royalisin* gene from honeybee. Furthermore, Burge and co-workers have also observed an over expression of *areicin-2* in response to the parasite *Aplanochytrium* sp. parasite suggesting a role in host/pathogen interactions. The *royalisin*-homolog, on the other hand, was suggested to be active against gram-positive bacteria and fungi.

Peroxidase enzymes

The super family of peroxidase enzymes is another well-known enzyme family with multiple

roles in the immune systems of organisms. For instance, peroxidase enzymes have been linked to the formation of ROS (Mydlarz and Harvell, 2007), detoxification of active forms of oxygen (Lesser, 2006), phagocytosis (Rodríguez *et al.*, 2003) and synthesis of melanin (Mydlarz and Harvell, 2007). Peroxidases activity has also been linked to stress responses and has been identified as an immune constituent in corals. For instance, Mydlarz and Harvell (2007) were able to induce the production of Peroxidases in *G. ventalina* colonies in response to fungal pathogen exposure. They also were able to show experimentally that commercial and sea fan extracted peroxidases can inhibit *A. sydowii* growth. However, they were unable to describe the mechanisms by which peroxidases acts against the fungal pathogen.

Secondary metabolites

Natural chemical compounds with antimicrobial properties, other than melanin, AMP and peroxidase enzymes, have also been extensively documented in gorgonian and scleractinian corals. In fact, gorgonians are rich in secondary metabolites with anti-microbial properties. For instance, Ospina and Rodríguez (2006) isolated 9 polycyclized diterpenes from the gorgonian coral *Briareum polyanthes*, all of which showed antimicrobial activities against human pathogens such as *Plasmodium falciparum* and *Mycobacterium tuberculosis*. Similarly, Shapo *et al.* (2007) reported that crude extracts from the gorgonian coral *Leptogorgia virgulata*, likely containing homarine, showed inhibitory activity against *Echerichia coli* and *Vibrio harveyi* as well as other bacteria. Uncharacterized antimicrobial agents have been also documented in over a dozen members of the *Plexauridae*, *Gorgonidae* and *Ellisellidae* families (Kim, 1994; Kim *et al.*, 2000a). Some of these extracts, such as lipid metabolites extracted from *G. ventalina* colonies, have been shown to be active against *A. sydowii* (Kim *et al.*, 2000b; Alker *et al.*, 2001; Dube *et al.*, 2002). Furthermore, the effectiveness of these extracts against pathogens appears to be dependent on the temperature and the location within the colony. For instance, crude extracts from the edges of *G. ventalina* colonies have shown higher inhibitory activity against *A. sydowii* than extracts from the center (Kim *et al.*, 2000b). On the other hand, crude extracts taken from colonies at temperatures over 30 °C inhibit the growth of *A. sydowii* less effectively than extracts at lower temperature colonies (Alker *et al.*, 2001). The effectiveness of extracts has also been linked to ontogenic factors. That is, extracts from sea fan recruits show higher activity against *A. sydowii* than extracts from mature sea fan colonies (Dube *et al.*, 2002). However, other studies contradict this argument (for detail see Couch *et al.*, 2013).

Scleractinian corals also possess secondary compounds with antimicrobial properties, though they have been less well studied than those derived from the gorgonians (Gochfeld and Aeby, 2008). Gochfeld and Aeby (2008) were able to show that crude extracts from three Hawaiian corals *i.e.*, *Montipora capitata*, *Porites lobata* and *Pocillopora meandrina*, had some degree of antibacterial activity

against coral pathogens such as *Serratia marcescens*, *Vibrio coralliityticus* and *V. shilo*. However, the antibacterial activity of extracts varied among species and as a function of the state of health of the host. In another study, the scleractinian corals *Pocillopora damicornis* and *Stylophora pistillata* were shown to continuously release chemical compounds that inhibit the growth of *Vibrio coralliityticus* upon sequential stress inductions (Geffen and Rosenberg, 2005; Geffen *et al.*, 2009).

Cellular response

Phagocytosis is a fundamental cellular response in innate immunity. Phagocytosis is the process by which cells, some of which contain phagosomes, engulf foreign entities. The process is initiated upon recognition via actin-mediated mechanisms such as opsonization or in another instance, by cell-cell recognition (Stuart and Ezekowitz, 2008). Phagosomes are organelles composed of over 140 proteins, including hydrolytic enzymes that neutralize the engulfed entity (Garin *et al.*, 2001). Phagocytic cells target two main entities: pathogens and self material such as dead cells targeted for recycling. In addition, phagocytes also produce and release cytotoxic compounds such as ROS, cytokines and melanin upon activation (Rabinovitch, 1995). Thus, phagocytosis is not only a cornerstone of host defense but also essential to tissue remodeling.

In corals, cells capable of phagocytosis are collectively known as amoebocytes. These cells are the putative immune cells of cnidarians (Mullen *et al.*, 2004; Ruiz-Diaz *et al.*, 2013). Amoebocytes are motile cells, primordially found spread across the mesoglea (Mullen *et al.*, 2004). A distinctive feature of amoebocytes is the presence or absence of granules. Granules are amoebocyte organelles that synthesize and store PO and ROS (Olano and Bigger, 2000; Petes *et al.*, 2003). The roles and tasks of amoebocytes are variable and not yet fully understood, although they have been linked to wound healing and protection from infection, as shown in studies conducted mostly in gorgonian corals. For instance, during the wound healing process in the gorgonian coral *Swiftia exserta*, amoebocytes gathered as in an inflammatory response in the wounded area to clean up the cellular debris (Olano and Bigger, 2000). Meszaros and Bigger (1999) show that, amoebocytes from the gorgonian *Plexaurella fusifera* streamed and lined-up at the edge of a wounded areas fusing and subsequently differentiating or dedifferentiating to form new epithelial cells. In the sea fan *G. ventalina*, a higher abundance of granular amoebocytes has been observed in tissue areas infected by *A. sydowii* as compared to healthy tissues (Petes *et al.*, 2003; Midlarz *et al.*, 2008). Similarly, diseased tissue fragments of *G. ventalina* placed on healthy corals induced an increase in the area occupied by amoebocytes when compared to healthy grafts (Couch *et al.*, 2013). Direct evidence of the phagocytic capacities of amoebocytes has been tested in functional experiments. For instance, Olano and Bigger (2000) documented phagocytosis in disassociated cells from *S. exserta* after being

challenged with India ink and carmine. In this study, phagocytosis was also observed occurring in granular amoebocytes and to a lesser extent in other cells such as scleroblasts, epithelial cells and gastrodermal cells, after India ink and carmine were introduced to *S. exserta* via tissue trauma. Likewise, phagocytosis has also been observed in disassociated cells from diseased and healthy tissue of diseased, as well as in healthy colonies *G. ventalina* (Ruiz-Diaz *et al.*, 2013). In this study, disassociated living cells were incubated for several hours with fluorescent beads and the number of cells containing fluorescent beads was quantified. Interestingly, the phagocytic index (defined as the number of cells containing fluorescent beads with respect to the total number of cells within 1 cm² of sea fan tissue) was higher in diseased tissue when compared to healthy tissue from diseased colonies and from healthy colonies. These results suggested that diseased tissue was immunologically active previous to the experimental challenge.

The ecological function of amoebocytes in scleractinian corals has been also studied. However, as scleractinian mesoglea is thinner than in gorgonians and amoebocytes are smaller and fewer, the identification of amoebocytes and their roles has been challenging (Mullen *et al.*, 2004; Work and Aeby, 2010). Nonetheless, as in gorgonians, increased abundance of amoebocytes in some scleractinians has been observed in immunologically challenged individuals. For instance, infiltrated granular amoebocytes have been observed in pigmented tissues from *Porites* species. In fact, the mean number of granular amoebocytes per 100 μm² of epidermal layer from pigmented *Porites* was fourfold greater than from the epidermal layer of non-pigmented tissue, suggesting a role as an immune constituent (Palmer *et al.*, 2008). Moreover, amoebocyte infiltration, plug formation due to the releasing of melanin from agranular amoebocytes, and amoebocyte differentiation to form epithelial tissue has been observed during the wound healing process in *Porites cylindrica* (Palmer *et al.*, 2011). However, in other scleractinian corals the role of amoebocytes as an immune constituent seems to be less prominent than in *Porites*. For instance, infiltration of amoebocytes has seldom been observed or not observed at all in tissues from *Monastrea carvervosa* under sedimentary-stress conditions (Vargas-Angel *et al.*, 2007), in pigmented tissues from *Acropora millepora* (Palmer *et al.*, 2008), or during wound repair in *Montipora capitata* (Work and Aeby, 2010).

Role of coral-associated microbial community

Numerous studies have revealed that corals harbor a rich and diverse microbial assemblage that includes viruses, bacteria, archaea and several microbial eukaryotes. This microbial assemblage, referred to as a coral holobiont, varies widely as a function of coral species and the health conditions of an individual coral organism. Furthermore, the microbial assemblage may vary within different parts of a single coral colony *i.e.*, the microbial assemblage of the coral mucus layer is different from that of the internal coral tissue and the

skeleton (Bourne and Munn 2005; Koren and Rosenberg 2006; Rosenberg *et al.*, 2007; Shnit-Orland and Kushmaro, 2009). Interestingly, microbe assemblages seem to be species-specific, suggesting that a beneficial relationship exists between corals and their holobiont. Yet, in most cases, the roles of coral-associated microbes are unknown, due in part, to the difficulties in performing functional studies using culturing techniques. Nonetheless, experimental evidence, much of which comes from cultured bacteria associated with coral mucus, suggests that certain microbes play a role as coral immune constituents. In this regard, several bacteria-associated mechanisms that play important roles in the protection of corals have been described, two of which we discussed below 1) compounds with anti-microbial properties, and 2) quorum sensing.

Microbes as producer of anti-microbial compounds

Coral mucus not only harbors a rich and diverse micro-flora assemblage, but also contains a diverse array of chemical compounds, some of which are produced by the coral itself, while others are produced by the microbes inhabiting the mucus. It has been rather difficult to track the source of bioactive compounds, *i.e.*, host corals vs. symbionts, as most of the antimicrobial activity assays have used whole-colony extracts including other living organisms associated with corals. These data are essential to complement data information about the chemical structures of the antimicrobial compounds, their stereochemistry and estimates of their effective concentrations in the wild. These knowledge gaps, and the fact that the majority of the microbes associated with coral can not be cultured, precluded our ability to determine the roles of microbes as a constituent of the coral immune system. Nonetheless, when researchers have been able to culture these microbes, a wealth of information has been obtained. For instance, in a functional experiment using cultured organisms, nearly 8 % of native bacteria from *A. palmata* mucus interfered with the ability of *S. marcescens*, the causal agent of white pox disease, to swarm and form biofilms in coral mucus thereby preventing the disease. The mechanism was reported to be inhibition of the enzyme activity involved in the breakdown of nutrients (Krediet *et al.*, 2013). However, further research must be conducted to elucidate the molecules involved in this inhibition. On the other hand, numerous studies have shown that bacteria associated with coral mucus produce anti-microbial compounds. For instance, Ritchie (2006) found that 20 % of cultured bacteria isolated from the mucus of healthy *A. palmata* exhibited anti-microbial activity against Gram-positive and Gram-negative bacteria, including *S. marcescens*. Snit-Orland and Kushmaro (2009) and Kvennefors *et al.* (2011) also showed, using agar-plating experiments, that *Vibrios* and *Pseudoalteromonas* bacteria isolated from several scleractinian and gorgonian corals, produced anti-bacterial agents against Gram-positive and Gram-negative bacteria.

Quorum-sensing (QS)

QS is a density dependent communication mechanism that coordinates the collective behavior of bacteria (Fuqua and Greenberg, 2002; Chen *et al.*, 2011; Golbert *et al.*, 2013). The QS communication mechanism in bacteria relies on hormone-like compounds that control the expression of genes involved with certain processes such as virulence, swarming, biofilm formation and others (Skindersoe *et al.*, 2008; Tait *et al.*, 2010; Golberg *et al.*, 2011, 2013; Hunt *et al.*, 2012; Krediet *et al.*, 2013). QS can be detrimental to hosts, if the behavior promoted by QS signaling activates the expression of virulence factors. However, the QS system may be beneficial to the host when the QS signals promote the production of compounds that regulate the growth of other noxious microbes. QS signals from bacteria associated with coral mucus have been demonstrated in numerous studies. For instance, Golberg *et al.* (2011) reported that nearly 30 % of bacteria associated with coral mucus from several scleractinian and gorgonian corals, exhibited the production of QS signals. Tait *et al.* (2010) also reported QS signaling in *Vibrios* associated with diseased and healthy scleractinian corals. However, only one study has documented the ability of coral associated bacteria to inhibit quorum-sensing systems and thereby the progression of *S. marcescens* in sea anemone *Aiptasia pallida* as a result of QS inhibition (Alegely *et al.*, 2011).

The roles of other microbes found associated with corals such as viruses, Archaeans, protozoans and fungi are unknown or undescribed, other than saying that members of some of these groups are known coral pathogens (Cróquer *et al.*, 2006; Toledo-Hernandez *et al.*, 2012). Fortunately, as culturing and molecular techniques continue to improve, so does our knowledge regarding microbes and their relevance to coral immune system function.

Conclusions

Corals are by no means simple creatures in terms of immune systems functionality. On the contrary, they possess a rather complex immune system that integrates a diverse array of recognition receptors and genes whose expression mediates the synthesis of countless cytotoxic compounds and cell responses that ultimately protect the coral from threats. It is also true that we are still ignorant of much of the detail of the coral's immune system, although many advances have been made thanks to modern molecular techniques. Clearly, future studies need to address, how climate changes will influence the coral immune system and its holobiont. In this regard, studies focusing on how environmental stressors such as elevated water temperature, and ultra violet radiation among others affect: 1) the capacity of corals to recognize threats, 2) the expression of immune-related genes and thus the synthesis of chemical compounds with microbial activities; 3) the base-line levels of immune constituents and 4) the microbial community associated. Answering these questions will allow us to better understand the fate of corals under the current global changes scenario.

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