The Skeleton and Biomineralization Mechanism as Part of the Innate Immune System of Stony Corals

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Stony corals are among the most important calcifiers in the marine ecosystem as they form the coral reefs. Coral reefs have huge ecological importance as they constitute the most diverse marine ecosystem, providing a home to roughly a quarter of all marine species. In recent years, many studies have shed light on the mechanisms underlying the biomineralization processes in corals, as characterizing the calicoblast cell layer and genes involved in the formation of the calcium carbonate skeleton. In addition, considerable advancements have been made in the research field of coral immunity as characterizing genes involved in the immune response to pathogens and stressors, and the revealing of specialized immune cells, including their gene expression profile and phagocytosis capabilities. Yet, these two fields of corals research have never been integrated. Here, we discuss how the coral skeleton plays a role as the first line of defense. We integrate the knowledge from both fields and highlight genes and proteins that are related to biomineralization and might be involved in the innate immune response and help the coral deal with pathogens that penetrate its skeleton. In many organisms, the immune system has been tied to calcification. In humans, immune factors enhance ectopic calcification which causes severe diseases. Further investigation of coral immune genes which are involved in skeleton defense as well as in biomineralization might shed light on our understanding of the correlation and the interaction of both processes as well as reveal novel comprehension of how immune factors enhance calcification.

Keywords: stony corals, coral immune system, biomineralization, coral skeleton, immune genes, calicoblasts, calcification

INTRODUCTION

Stony corals are among the most important calcifiers in the marine ecosystem. They hold significant ecological importance as they are the main builders of one of the most diverse and productive ecosystems in the ocean, the coral reefs (1, 2). Corals belong to the eumetazoan ancestor phylum Cnidaria, which are among the earliest metazoans to have evolved (3). Hence, they are significant in understanding the evolutionary origin as the early evolution of innate immunity (4, 5). Even though cnidarians lack some of the components of the adaptive immune system that are found in vertebrates, the sequencing of the first cnidarian genomes revealed a surprising immune
complexity and a striking resemblance to bilaterian immune genes, with many ancestral immune components that have been lost in other invertebrates, such as C. elegans, and D. melanogaster (5, 6). Coral genome sequencing and comparative genomics have highlighted the immune gene repertoires of corals and underlined the evolution of specific immune genes in corals, such as an increased number of Toll-interleukin (TIR) proteins, and diversification of immune genes in different coral species, thus suggesting diverse adaptive roles for innate immune pathways in each species (7–9). Moreover, many studies showed up-regulation of immune genes following exposure to different stressors or pathogens (10–13), while others linked the immunity response to coral-algae symbiosis and showed the involvement of immune genes in the initiation of this symbiosis as well as in coral bleaching (14–17). Another rapidly evolving field is the coral microbiome which correlates coral health, resilience, and immune response to the holobiont and its microbiome (18–22). Even though the innate immune response of corals was extensively studied, the existence of immune cells in corals was an enigma. Although granular amoebocytes were observed in a few corals around wounds and lesions (16, 23), the genetic identification of specialized immune cells has only recently been described in the single-cell atlas of the coral Stylophora pistillata (24). The study revealed two distinct cell types with molecular signatures indicative of immune function. These cells express immune transcription factors such as NAFT and IRFs, and many genes involved in the innate immunity response such as the interleukin receptor, LSP binding proteins, Perforin, endonucleases, prosaposins, antimicrobial ApeC proteins, tyrosinase, and genes involved in the inflammatory response (24). Following these findings, Snyder et al. (25) identified and characterized phagocyte cells of the coral Pocillopora damicornis and the sea anemone Nematostella vectensis, and showed that the phagocytic cells engulf bacteria, fungal antigens, and beads. In addition to the immune cells, the innate immunity of corals involves other aspects of the defensive mechanism such as the cnidocytes (i.e., stinging cells), venom-producing gland cells (26–28), mucus secretion, and mucus-associated bacteria involved in the antimicrobial activity on the coral surface (21, 29–31). Another less studied aspect related to the stony coral’s immune system is their calcium carbonate exoskeleton that functions as an additional barrier to the external marine environment and hence might play a crucial role in the innate immune response. Stony coral polyps face the water column while the aboral epithelium, referred to as the calicoblastic layer, constantly produces the aragonite skeleton (Figure 1) (32). Corals grow continuously, by budding new polyps, and their aragonite skeleton expands accordingly. In addition, new layers of aragonite are continuously deposited and accumulate on top of the old layers. Although the exact mechanism of the biomineralization process remains elusive, our understanding of the molecular mechanism underlying this process has greatly advanced (33–37). Proteomic analysis of the skeletal organic matrix from three different coral species revealed an assemblage of adhesion and structural proteins, transmembrane proteins, proteins containing known extracellular matrix (ECM) domains, as well as highly acidic proteins that were suggested to play a role in calcium carbonate nucleation (38–41). Furthermore, the first stony coral single-cell atlas characterized the gene expression profile of the cells involved in the formation of the coral skeleton (calicoblasts) and revealed more than 700 genes that are specifically expressed in the calicoblasts of the juvenile primary polyp and the adult coral.

Although research of both biomineralization and immunity in corals have advanced considerably over recent years, as of yet, these two fields have not been integrated. Here, we underline the immunological basis of corals in a biomineralization context by reviewing and integrating the knowledge in both fields as well as highlighting immune genes expressed in the cells that form the coral skeleton or found in the skeleton itself.

THE CORAL SKELETON AS THE FIRST BARRIER AGAINST PATHOGENS

The coral life cycle involves a planktonic larva and a benthic adult. These two phases are separated by settlement and metamorphosis, two critical stages in coral development, during which some of the epidermal cells are transformed into calicoblast cells that immediately start with rapid skeleton deposition (32). This rapid process is important for coral adherence to the substrate, as well as in creating a protective environment, in the form of an aragonite skeleton, for the soft and vulnerable polyp (42). This process might be involved in the production and secretion of anti-microbial factors to clear the surface and prevent possible pathogenic infections. As in many other marine organisms, the exoskeleton is a physical barrier that protects the animal from the outside world and serves as the first line of defense. When coral polyps sense a physical threat (e.g., predators, strong currents, suspended sediment), they contract into their aragonite calyx in order to avoid the danger (43). In addition to physical protection, exoskeletons constitute biochemical protection, and in many organisms the exoskeleton is rich in antimicrobial molecules, enzymes, and toxins (44–47). Coral exoskeletons sustain diverse eukaryotic and prokaryotic microorganisms such as fungi, endolithic algae, viruses, and bacteria (Figure 1) (48–51). While these organisms are part of the holobiont and can produce metabolites and antimicrobial compounds that help the coral control its skeleton microbiota, others can be pathogenic and might use the skeleton to invade and penetrate the coral tissue (51, 52). Thus, the exoskeleton and the calicoblastic layer aligned to it (Figure 1B) might have an additional immune protection role against the invaders. One element of immune protection found in exoskeletons is melanin (53, 54). As a polymer, melanin can strengthen tissue and exoskeletons and improve their ability to act as physical barriers against the penetration of parasites (55). Furthermore, melanin can introduce potent antimicrobial activity by inhibiting lytic enzymes produced by microorganisms (56). In Arthropods, melanin deposits in the
Exocuticle play an important role in increasing the immune protection of the exoskeleton (53). In stony coral, melanin has been detected in granular cells and specifically in the tissue around wounds, during the healing of wounds in the coral *Porites cylindrica* (16). In the common sea fan *Gorgonia ventailina*, melanin has been observed around tissue lesions formed by the invasion of the pathogenic fungi *Aspergillus sydowii* (23). The fungal hyphae have been observed in the coral’s axial skeleton, with a thick melanin layer formed around the infection that serves as a barrier preventing the *A. sydowii* hyphae from contacting its tissue. In addition, an increase in pigmented calcium carbonate sclerites (the skeletal elements of soft corals) was observed, which gives the lesions their distinct, dark coloration (23). Proteins involved in melanin production such as tyrosinase have been detected in molluscan mantle transcriptomes and shells and were suggested to be involved in exoskeleton fabrication and hardening (57–59). In *S. pistillata*, tyrosinase genes were found to be expressed in the adult immune cells, while three different tyrosinase genes were expressed in the juvenile primary polyp calicoblastic cells (Figure 2B and Table S1) (24). One of these genes is a tyrosinase-like (XP_022797084.1) that possesses four ShK toxin domains, which are potassium channel blockers that were first isolated from the sea anemone *Stichodactyla helianthus* venom (60). While most genes with ShK domains showed high expression in *S. pistillata* cnidocytes (stinging cells) and gland cells, two extracellular genes containing ShK domains showed high specific expression in both adult and primary polyp calicoblasts (Figure 2) (24). One is a homolog of the protein meprin A (XP_022785469.1), a metallopeptidase with the ability to cleave various substrates, degrade ECM proteins, process proinflammatory cytokines, and promote leukocyte infiltration (61). Therefore, it might have similar functions in remodeling the corals’ skeletal organic matrix and initiating the inflammatory response once pathogens are detected. The second gene is a mucin-like protein with two Shk domains (XP_022806382.1), which could be secreted into the skeletal organic matrix and serve as a toxin. Another toxic candidate gene, highly expressed in the primary polyp calicoblasts, is Ntox44 (XP_022782305.1) (Figure 2B) (24). This gene is a homolog of a bacterial secreted RNase toxin with potential antimicrobial function. Hence, it might play a role in clearing the substrate while the larva metamorphoses into a primary polyp and starts calcifying its initial skeleton. Ramos-Silva et al. (38) reported on an additional toxin-like protein, (B7W114), found in the Acropora...
millepora skeletal proteome, which corresponds to a secreted protein with high similarity to the SE-cephalotoxin from the cephalopod Sepia esculenta.

In addition to the secretion of toxins and antimicrobial compounds into the skeleton, the biomineralization process can act as a direct immune defense mechanism. The most familiar example is the pearl formation in mollusks, in which the animal uses its calcification ability against irritant foreign bodies, parasites, or other pathogens by creating a calcium carbonate structure (62). As for corals, there is only one document (as per our knowledge) of a calcification defense mechanism shown in response to a fungal invasion (63). Le Campion et al. demonstrated that the stony coral Porites lobata responds to fungi penetrating its skeleton by the deposition of calcium carbonate, to form skeleton thickness that will prevent the fungi from reaching the polyp tissue (63). Further investigation of this interesting phenomenon in corals might reveal the correlation between the self and non-self-recognition, the innate immune response, and the calcification processes. Additionally, further molecular investigation of this phenomenon could shed light on genes which are involved in both processes, the innate immune response and calcification.

**GENES WITH POTENTIAL DUAL FUNCTION IN THE CORAL’S INNATE IMMUNE RESPONSE AND IN BIOMINERALIZATION**

In recent years, increasing evidence regarding the integration of biomineralization and immunity has come to light, including
proteins with dual function in both (64–66). In humans and other mammals, immunity and calcification have been tightly connected, as many studies have shown that immune cells are closely associated with ectopic calcification as in the development of atherosclerosis, vascular calcification, chronic kidney disease, breast cancer, etc (67–69). It has been demonstrated that vascular calcification is part of the immune response and involves many factors and genes of the innate and adaptive immune system (69–71). The lack of effective therapy for ectopic calcification is an indicator of the complexity of its mechanism as well as the significance of understanding the interaction between the immune response and calcification (69, 72). Uncovering the individual contribution of immune genes to enhanced calcification, would improve our understanding of the inflammation dependent mechanisms of ectopic calcification, and could offer new diagnosis tools as well as therapeutic treatments for the involved diseases.

To the best of our knowledge, there are no studies that link between the immune response and calcification in corals. To create a database of genes with potential immune and biomineralization functions, we explored the genes that are expressed in the calicoblastic cells, (24), the proteins found in the skeletal organic matrix proteomes of several corals (38, 40, 73) and searched the available literature for known functions of their homologs in other organisms. This data can serve as a database for further investigations of the molecular mechanisms that underlie the response of corals to pathogens that penetrate their skeleton. The whole gene list is available in Table S1.

We found that genes that are known to be involved in vascular calcification, such as CD36, DOCK1, DSPP, and Perforin (74–76), are expressed in the coral immune cells but not in the calicoblasts. This might imply that these genes do not play a role in coral calcification. However, immune cells that express these genes might enhance calcification in corals in a similar manner to the enhancement of vascular calcification by macrophages (69, 71). It will be interesting to investigate this issue in corals, for example, during the wound healing processes, in which immune cells might migrate toward the wound (16), help repair the tissue and protect it from pathogens and additionally enhance skeleton precipitation to repair the damaged skeleton.

In addition, we found that many cathepsin genes are expressed in both calicoblasts and in the immune Cells (Figure 2 and Table S1). Cathepsins are multifunctional enzymes involved in many biological processes such as lysosomal protein recycling, digestion, wound healing, bone remodeling, reproduction, and innate immune response (77). Cathepsin L, which is expressed in both immune and calicoblast cells, is known to be a multifunctional protein involved in the immune response of fish and mollusks (78–80), in biomineralization (81), and in bone and cartilage resorption in humans (82); Cathepsin D, which is expressed only in the immune cells of S. pistillata, is a membrane-associated acidic protease, familiar with macrophage endosomes (83), also involved in cardiovascular calcification (84); and Cathepsin V, which is expressed only in the calicoblasts, is known to promote vascular calcification in humans (85).

Another interesting protein is the ovotransferrin that was found to have a dual role in avian eggshell formation (86). It was first identified as an antibacterial and antifungal protein (87, 88) and later was found to have a role in the biomineralization processes as it was expressed in the initial stage of shell biomineralization and was localized to the sites of calcite nucleation (44). Furthermore, the addition of the purified protein in-vitro results in a large modification of the calcium carbonate crystals morphology. In corals, we found a homolog ovotransferrin gene (XP_022780954.1) with high expression in both adult and primary polyp calicoblasts (Figure 2 and Table S1) (24). Therefore, we suggest that this gene potentially can be involved in coral biomineralization and serves as a bacteriostatic filter.

Another protein that might have a dual function is Peroxidasin (XP_022794431.1). In the human myofibroblasts, peroxidase is secreted into the extracellular space where it becomes organized into a fibril-like network and colocalizes with fibronectin to form the ECM (89). It catalyzes sulphylamine bond formation in collagen IV and catalyzes hydrogen peroxide (H₂O₂) into hypochlorous acid (HOCl). An excessive peroxidase activity, allows free oxidizing hypohalous acid to accumulate and produce intended or unintended toxicity (90). The high reactivity of the hypochlorous acid toward a variety of biological molecules, cause oxidative damage to pathogens proteins and contribute to the killing of pathogens as was demonstrated in neutrophils (91). Since in S. pistillata peroxidase is specifically expressed in the adult and primary polyp calicoblasts (Figure 2) (24), we suggest that it is secreted into the skeletal organic matrix where it might generate fibril-like network and in addition, produce hypohalous acids with toxic activity.

Next, we explored all proteins found in scleractinian skeleton proteomes (38, 40, 73) and looked for proteins with a possible immune function. One such protein is the sacsin protein (40), that acts as a regulator of the Hsp70 chaperone machinery (92, 93). While the mammalian sacsin was studied in association with a neural disorder (92), the sacsin homolog in fish was reported to be involved in the innate antiviral immune response in several fish species (94–96).

A second protein is thioredoxin reductase 1, cytoplasmic (XP_022804785.1), its human homolog mediates cell death induced by a combination of interferon-beta and retinoic acid (97). It also induces actin and tubulin polymerization, leading to the formation of cell membrane protrusions (98). Cell membrane protrusions were observed in the coral calicoblastic cell layer and are thought to be essential structures for coral skeleton formation (99, 100). In addition, this protein might be involved in the induction of calicoblasts apoptosis, in case of infection.

Furthermore, a few proteases were found as well (38, 40, 73). These proteases are thought to be involved in digestion and modeling the skeletal organic matrix as a scaffold for the calcium carbonate skeleton and in processing and activating other bioactive molecules and proteins involved in the biomineralization process (101, 102). Proteases and specifically serine proteases are also known to be key mediators of the innate immune response as they act as processing enzymes of pro-inflammatory cytokines and other enzymes related to the
inflammatory response (103–105). Additionally, many proteases, including matrix metalloproteinases and protease inhibitors are expressed in *S. pistillata* calicoblasts as well (Figure 2 and Table S1) (24). Metalloproteases, such as MMP-25, regulate the innate immune response through the NF-kB signaling in mice (106). A homolog of MMP-25 is highly expressed in the adult calicoblasts of *S. pistillata* (Figure 2A) (24). Since metalloproteinases hydrolyze and process a large number of substrates, they might be involved in remodeling the skeletal organic matrix scaffold of the coral skeleton or in the interaction of the calicoblastic cell layer with the skeleton ECM. In addition, some of the metalloproteases might be involved in processing and activating factors involved in biomineralization such as the acidic proteins involved in nucleation or factors involved in the innate immune response such as pro-inflammatory cytokines and chemokines, growth factors and other receptors’ ligands.

DISCUSSION

The coral skeleton serves as a firm structure for animal protection, and as the first line of defense against invaders and pathogens. In order to protect the animal from these parasites and pathogens, the exoskeleton must include antimicrobial molecules and toxins. Some are produced by the symbionts inside the skeleton and help the coral control its skeleton biota. Others, most likely, are extracted by the coral itself, through the tissue that forms the skeleton, the calicoblastic layer. In this review, we highlighted genes and proteins that might serve as toxins or bacteriostatic molecules as well as genes and proteins that are known to play a role in the immune response and are found either in the calicoblastic cells or in the skeleton itself (Figure 2 and Table S1). Further exploration of the role of these genes along the process of biomineralization can illuminate how corals deal with pathogens that penetrate their skeleton as well as reveal immune genes that might be involved in the biomineralization process or enhance calcification.

Stony corals belong to the Anthozoa class in the Cnidaria phylum, a sister group of Bilateria. As stony corals are the only cnidarians that build an exoskeleton, they hold an interesting and important key position in our understanding of the evolution of the immune system and its involvement in calcification. Understanding the mechanisms that correlate immunity and calcification, and revealing the role of genes shared by both, is a valid point that may help shed light on these complex mechanisms. It can reveal novel etiologies of ectopic calcification involved in severe diseases and chronic disorders such as vascular calcification, atherosclerosis, osteoarthritids, kidney stones and several cancers. Thus, it can provide new tools for diagnosis and treatments for these common pathologies.

As a whole, we tried to review and integrate the data obtained in two important and enhanced fields in coral research and create a valuable database for further research to better understand how biomineralization and the innate immune system are involved, and which factors are shared by both.

AUTHOR CONTRIBUTIONS

SL and TM contributed to the conception and design of the article and interpreting the relevant literature. SL designed the figures and wrote the first draft, with inputs from TM. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.850338/full#supplementary-material

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