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The immune response of cephalopods from head to foot

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A R T I C L E I N F O

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A B S T R A C T

Cephalopods are a diverse group of marine molluscs that have proven their worth in a vast array of ways, ranging from their importance within ecological settings and increasing commercial value, to their recent use as model organisms in biological research. However, despite their acknowledged importance, our understanding of basic cephalopod biology does not equate their ecological, societal, and scientific significance. Among these undeveloped research areas, cephalopod immunology stands out because it encompasses a wide variety of scientific fields including many within the biological and chemical sciences, and because of its potential biomedical and commercial relevance. This review aims to address the current knowledge on the topic of cephalopod immunity, focusing on components and functions already established as part of the animals' internal defense mechanisms, as well as identifying gaps that would benefit from future research. More specifically, the present review details both cellular and humoral defenses, and organizes them into sensor, signaling, and effector components. Molluscan, and particularly cephalopod immunology has lagged behind many other areas of study, but thanks to the efforts of many dedicated researchers and the assistance of modern technology, this gap is steadily decreasing. A better understanding of cephalopod immunity will have a positive impact on the health and survival of one of the most intriguing and unique animal groups on the planet, and will certainly influence many other areas of human interest such as ecology, evolution, physiology, symbiosis, and aquaculture.

1. Introduction

Cephalopods are marine invertebrates belonging to a class within the phylum Mollusca, the second largest phylum of animals after arthropods [1]. This metazoan group includes a variety of muscular, soft-bodied creatures broadly classified into octopi, cuttlefish, squid, and nautili. A generalized description of their anatomy divides their bodies into three regions: 1) the mantle, a bag-like muscular structure containing the majority of internal organs; 2) the head, which is dominated by two large eyes (one at each side) and enclosing the brain and mouth areas; and 3) the arms and tentacles, usually covered by suckers used for graving and attachment. Locomotion is provided by water propulsion through a funnel-like structure (the syphon) attached to the mantle, along with posterior fins in some species for swimming and directional movement. Inherent diversity within the distinct cephalopod groups allow them to occupy a variety of habitats worldwide; from the frigid waters of the poles, to the temperate and warmer temperatures found in the Mediterranean and tropics. Unlike other molluscs, which are mostly benthic, cephalopods can be found both in benthic and pelagic zones of seas and oceans, as well as close to the water surface, and as deep as 5000 m [2]. With the exception of the nautilus, they also differ from their molluscan relatives by lacking an external shell. They have highly developed visual and nervous systems, and an exquisite capacity to speedily adapt to their environment by means of camouflage, allowing them to be some of the most efficient marine predators [3].

Living members of the class *Cephalopoda* are classified into two subclasses: the *Nautiloidea* containing two genera, *Nautilus* and *Allonautilus* [4]; and the *Coleoidea* with four orders: Sepioidea, Teuthoidea, Octopoda, and Vampyromorpha [2]. Out of an estimated 1000 species of extant cephalopods, at present only 650–700 species have been described and named [5,6]. In the past, the classification of specimens was based on some of the most striking morphological differences among these animals, such as number of arms and tentacles, and the presence/absence of an external shell. Continuous study and recent technological advancements have incorporated new information such as data derived from fossil records, as well as developmental and genetic information that permit more accurate classification and identification [7]. Nonetheless, most people follow

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the original classification, grouping cephalopods into octopi (octopods, eight-armed), squids and cuttlefish (decapods, eight arms plus two tentacles), and the nautili (the only shelled cephalopods).

Cephalopods have always captured human interest and imagination, as evidenced by their depictions in ancient art and the many stories and myths about marine giants and monsters found in the writings of classical authors such as Jules Verne and Victor Hugo [8]. Nowadays, cephalopods maintain their popularity as fantastic creatures, but additionally, they have gained a modern interest for their potential commercial value as food sources [2,3,6], and for their scientific importance as research organisms [e.g. [9–17]. These contemporary interests and their demands have made evident the fact that we know very little about basic cephalopod biology, and that knowledge in this field is lagging behind in comparison to other commercially important species in aquaculture such as bivalves, shrimp, and fish. In recent years, with the aid of modern scientific and technological advances and the efforts from scientists around the world, this gap in knowledge is being bridged, and although a lot more work remains to be done, we are slowly beginning to understand these remarkable animals. A topic of growing interest and significant importance is the basis and workings of the cephalopod immune system. Driving this interest are diverse underlying reasons, which include basic scientific inquiry to improve our understanding of cephalopod biology, phylogeny, and interspecific relationships, as well as other complex and anthropocentric pursuits. Because cephalopods are both predators and prey, changes in their health and population dynamics may have consequences and unknown repercussions on other animal populations, including those that serve as major sources for human consumption. For example, certain species of octopus and squid are important sources of nourishment and income in many countries of Southeast Asia, Europe, and South America [18–24]. Additionally, the popularity and demand of these animals have been increasing steadily worldwide in recent years [25–29]. The Fisheries and Aquaculture Department (FAO) reported an increase of cephalopod catches from approximately one million metric tonnes in 1970 to about 3.6 million metric tonnes in 2010 [30]. This upsurge is, in part, a consequence of changes in capture methods in the fishing industry and efforts trying to overcome the reduced productivity resulting from the overharvesting of popular fish species. As a consequence of these changes, several cephalopod species are being seriously considered as excellent candidates for aquaculture enterprises due to their rapid growth and high protein content [2,31], and for this purpose, new cultivation methods are currently being developed, which aim at improving the health and long-term maintenance of cephalopods in enclosed environments [26,27,32–34]. As researchers study the quality of octopus and squid for human consumption, several species of bacteria, viruses, and parasites have been found to infect cephalopods, some of which are known or have the potential to be transmitted to humans and cause disease [reviewed in [35–38]. To this effect, in order to ensure a responsible utilization of cephalopods as a sustainable human commodity, while at the same time learn from their unique biology, it is imperative to study more about the composition and functioning of their immune system. In the following paragraphs, we present a review containing the current knowledge in the field of immune defenses in cephalopods, focusing on the internal immune components of these animals and their associated functions when this information is available. A summary of this information can be found Table 1 and Figs. 1 and 2 that accompany this review.

2. Invertebrate immunity

Cephalopods, along with all other members of the phylum Mollusca, are invertebrates and thus, lack an adaptive immune system [39,40]. By this we mean that the invertebrate equivalents for B- and T-lymphocytes, as well as their highly diversified antigen-receptors (immunoglobulins and T-cell receptors), have not been found in these organisms [41]. Regardless, invertebrates have an efficient immune system comprised of cellular and humoral components that allow them to interact with microorganisms, discern and remove pathogens, and repair wound and tissue damage [42]. The invertebrate immune system is composed of innate immune cells and molecules which have homologs present in all major metazoan groups. These ancient immune players have regained the appeal and importance they once had when Metchnikoff discovered phagocytosis in starfish and the concept of non-self recognition was conceived [43]. Indeed, the extent and influence the innate immune system has in the development and the efficiency of adaptive responses is now fully recognized [44,45]. In addition, the study of immunity in invertebrate models also experienced a renewed interest when immune-related molecules discovered in these animals, such as the toll receptor in *Drosophila*, were found to have homologs in vertebrates [46]. As a consequence, the study of immunity in lower taxa has been intensified and expanded in the last few decades, not only because of the implications these findings may have in the understanding of the evolution of immune mechanisms, but also because of the potential applications that these comparative studies can have on vertebrate research.

Innate immune functions are phylogenetically ancient and based on the recognition of non-self, missing-self, and the presence of danger signals [47,48]. This recognition is carried out by a variety of receptors that immediately induce the activation of defense effectors, which in turn will kill, remove, or neutralize the foreign invader or offensive material. These immune sensors are collectively called pattern recognition receptors (PRRs) and pattern recognition molecules (PRMs), and include proteins like the toll and toll-like receptors, nucleotide-binding and oligomerization domain-like receptors, scavenger receptors, and lectins. These sensor molecules interact with pathogen/microbial associated molecular patterns (PAMPs/MAMPs) [49,50], which are in their majority invariant microbial surface components such as lipopolysaccharide (LPS), peptidoglycan (PGN), flagellin, and molecules containing conserved glycan residues. Upon recognition and binding of PRRs with their appropriate PAMPs/MAMPs ligands, there will often be changes in the receptors' structural conformation that will allow them to interact with other host proteins involved in signal transduction, gene expression, or activation of effector molecules. Immune effectors are the host's factors performing the killing, neutralization, or removal activities that will protect, prevent infection, or repair damage to the host. These effectors are diverse and include molecules involved in phagocytosis and lysis, secretion of reactive oxygen and nitrogen species (ROS, RNS), antimicrobial peptides, proteinase inhibitors. Similarly, as with adaptive immune responses, the components of innate immunity can be classified as cellular or humoral. In most cases, the cells involved in immune reactions are those with higher probabilities to interact with pathogens, including epithelial cells that cover the host's surfaces exposed to the environment, and those found in the circulatory system and therefore able to infiltrate tissues. The latter are motile, actively circulating cells which, in invertebrate animals are commonly called hemocytes, immunocytes, coelomocytes, or phagocytes. Since circulating cells play pivotal roles in immune surveillance as well as in effector functions, they are of utmost importance; this is especially true if they also contain the sensors, the signaling components, and the capacity to express effector molecules.

Immune reactions have been studied in a handful of cephalopods, mainly in commercially-relevant octopus and squid, making it difficult to make generalizations across species. The information from those studies is presented in the following paragraphs and has