Review

CellPress

Special issue on Avoidance, Resistance and Tolerance: Strategies in Host Defense

Rethinking the role of immunity: lessons from *Hydra*

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The ability of multicellular organisms to detect and respond to microorganisms is fundamental and has ancient evolutionary origins. In this review, I evaluate our current understanding of the evolution of epithelial-based innate immunity in *Hydra*, an apparently simple animal that shares deep evolutionary connections with all animals, including humans. I highlight growing evidence that the innate immune system with its host-specific antimicrobial peptides and rich repertoire of pattern recognition receptors has evolved in response to the need for controlling resident beneficial microbes rather than to defend against invasive pathogens. These findings provide new insight into how developmental pathways beyond those associated with the immune system, such as stem cell transcriptional programs, interact with environmental cues such as microbes.

Animal evolution is intimately linked to the presence of microbes

The interaction of bacteria with animal hosts is an interesting crossover topic of increasing importance [1,2]. Historically, bacteria are seen as pathogens. A significant leap forward in scientific understanding of the natural forces and risk factors affecting the patterns of illness and death came with Louis Pasteur's discovery of the link between germs and disease [3,4]. This led the way for Robert Koch to demonstrate in 1876 that a microorganism was the cause of an infectious disease. In the subsequent years, numerous microorganisms were identified as the causative agents of important human diseases; and bacteriologists, microbiologists, and immunologists have continued to focus on bacteria as pathogens for more than 100 years. This approach has led to enormous insights in the battle between the invading harmful microbes and the host, as well as enabled the development of efficient strategies to fight infections.

Bacteria have existed from very early in the history of life on Earth. They inhabit every environment on the planet. In fact, most bacteria are not harmful to plants or animals, and many of them are beneficial, playing a key ecological role. Bacteria fossils discovered in rocks date from at least the Devonian Period, and there are convincing arguments that bacteria have been present since early in the Precambrian Era, approximately 3.5 billion years ago [5]. Because animals diverged from their protistan ancestors some 3 billion years after bacterial life originated, and as much as 1 billion years after the first appearance of eukaryotic cells [6–8], relationships of animals with bacteria were likely to be already in existence when animals first appeared near the end of the Proterozoic Eon. Animal evolution, therefore, is intimately linked to the presence of microbes.

Eukaryotic cells *per se* appear to be the descendents of separate prokaryotic cells that joined together in an endosymbiotic event, with mitochondria being the direct descendents of a free-living bacterium that was engulfed by another cell [9,10]. Moreover, all animals, ranging from simple invertebrates to primates, are host to complex microbial communities [11–13] and, therefore, must be considered a meta-organism comprised of the macroscopic host and synergistic interdependence with bacteria, archaea, fungi, and numerous other microbial and eukaryotic species [14].

Living in the Ediacaran oceans, *Poriferal* (sponges) and *Cnidaria* (jellyfish, corals, and hydroids) evolved early during the phylogenesis of multicellular animals (Metazoa) [15–18] (Figure 1). Cnidarians not only are among the earliest known phyletic lineages to form natural symbiotic relationships with bacteria and eukaryotes [19–21] but also possess most of the gene families found in Bilaterians and have retained many genes that have been lost in *Drosophila* and *Caenorabditis elegans* [22–26]. For this reason, these 'basal metazoans' allow us to gain insights into the very early evolution of biological modules that may be involved in innate immune defenses.

In this review, I discuss the evolution and characteristics of the innate immune system in the early branching metazoan *Hydra*. I review recent findings that provide a new perspective on the relationship between bacteria and animal cells, and propose, in the context of the evolutionary time scale of bacterial and animal emergence, that innate immune system with its host-specific antimicrobial peptides and rich repertoire of pattern recognition receptors has evolved in response to the need for controlling resident beneficial microbes rather than to defend against invasive pathogens.

The innate immune system in *Hydra*

Hydra, like all other early emerging metazoans, has developed an effective innate immune system to detect and

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Keywords: Toll-like receptor (TLR) signaling; *Cnidaria*; innate immunity; antimicrobial peptides; orphan genes; commensal microbiota.

1471-4906/
 http://dx.doi.org/10.1016/j.it.2014.07.008

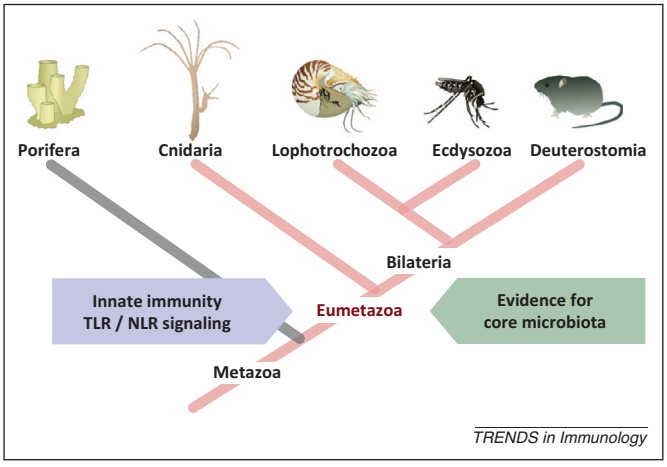

Figure 1. The early occurrence of *Cnidaria* on Earth. Cnidarians such as the freshwater polyp *Hydra* serve as models for studying the evolution of innate immunity and host–microbe interactions.

control microbial colonization [27,28]. *Hydra* uses two types of receptors and signaling pathways for microbial recognition – Toll-like receptors (TLRs) with MyD88 as a signal transducer, and nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs). Engagement of these receptors leads to the rapid induction of protective programs, for example, the induction of antimicrobial peptides (AMPs), or the elimination of the infected cell by means of apoptosis.

Antimicrobial peptides as host-derived regulators of the microbiota

AMPs are known as prominent effector molecules of the innate immune system in vertebrates and invertebrates, where they act by disrupting the structure or function of the microbial cell membranes [29]. To date, three families of potent AMPs have been identified in *Hydra*: the hydramacin, periculin, and arminin families of peptides [27,30]. Species-specific variability and constitutive high-level expression have made the arminin peptide family, in particular, an excellent candidate for investigating the role of AMPs in shaping the host-specific microbiota of *Hydra*.

Bacteria in *Hydra* are specific for any given species [11]. Closely related *Hydra* species, such as *Hydra vulgaris* and *Hydra magnipapillata*, are associated with a similar microbial community. In line with this, comparing the phylogenetic tree of the *Hydra* species with the corresponding cluster tree of associated bacterial communities reveals a high degree of congruency [31].

To examine the impact of arminin function on the resident microbial population in *Hydra*, and to broadly interfere with the host's arminin expression, we generated transgenic *Hydra vulgaris (AEP)* polyps that expressed a hairpin cassette containing *arminin* antisense and sense sequences fused to a reporter gene [31]. The resulting double-stranded RNA (dsRNA) triggered the RNA interference (RNAi) machinery, which led to a 97% decrease in the endogenous *arminin* transcript. A tissue extract of arminin knockdown polyps showed a 50% decrease in bactericidal activity. For functional analysis, germfree control and *arminin*-knockdown polyps were generated and subsequently recolonized by foreign bacterial consortia provided by co-cultivation with other *Hydra* species, such as *Hydra oligactis* or *Hydra viridissima*. Four hundred and fifty-four pyrosequencing trials revealed [31] that *arminin*-deficient *Hydra vulgaris (AEP)* polyps have a decreased ability to select suitable bacterial partners from a pool of foreign potential colonizers, because they are colonized differently than control polyps, which select for bacterial types partially resembling their native microbiota. These findings suggest that AMPs shape the stable associated microbiota, acting as host-derived regulators of microbial diversity, rather than unspecific bactericides.

The ancestral function of TLR-signaling

TLRs are conserved throughout animal evolution, but appear to serve different functions in different model organisms [32,33]. The Toll pathway was initially identified to be essential in early embryonic development in *Drosophila* [34]. In addition to its essential crucial role in the establishment of the dorsal–ventral axis, *Drosophila* Toll-1 was shown to be involved in muscle development [35] and heart formation [36]. Later, it was discovered that Toll-1-signaling in *Drosophila* also contributes to defense reactions against bacteria as well as to antifungal defense by regulating, among others, the expression of the antifungal peptide drosomycin [37,38]. In addition, Toll-8 was recently shown to be involved in the control of the immune response in respiratory epithelia in *Drosophila* [39]. All other *Drosophila* Toll family members (Toll-2–9) act in embryonic and larval development and seem to have no clear *in vivo* function in immunity [40]. Other invertebrates such as the nematode *Caenorhabditis elegans* lack central proteins of the canonical TLR-signaling cascade [41]). Only one Toll-homologue termed TOL-1 was identified in *C. elegans* [42]. The fact that TOL-1 mutants show strong developmental defects, despite the fact that mutants for the putative signaling cascade do not show any developmental abnormalities, led to the belief that TOL-1 in *C. elegans* might function as a cell–cell adhesion protein in neurons [41]). By contrast, the vertebrate homologues of Toll, the TLRs, are predominantly pattern recognition receptors (PRRs) of the innate immune system and are involved in the elimination of pathogens and controlling commensal colonization [43–45].

The wide range of known functions of TLR-signaling, from development in insects and cell adhesion in nematodes to immunity in vertebrates, prompted us to search for the putative ancestral function of TLR-signaling. Using the basal metazoan *Hydra* we simply asked: which function – regulation of embryonic development or immune defense – was first in evolution? To address this question, we performed a MyD88 loss-of-function study in *Hydra vulgaris* (AEP). A stable transgenic *Hydra* line with reduced expression level of the universal adapter protein MyD88 was generated by RNAi-induced knockdown, and microarray analyses were performed to identify effector genes downstream of the TLR-signaling cascade [46]. In parallel, we analyzed the gene expression profile of germfree animals to directly investigate the connection between TLR-signaling and bacterial recognition. We found that more than 75% of the MyD88-responsive transcripts appeared to be also