

# Evolution and Function of Innate Immune Receptors – Insights from Marine Invertebrates

Philip Rosenstiel<sup>a</sup> Eva E.R. Philipp<sup>a</sup> Stefan Schreiber<sup>a</sup> Thomas C.G. Bosch<sup>b</sup>

<sup>a</sup>Institute for Clinical Molecular Biology and <sup>b</sup>Zoological Institute, Christian-Albrechts University Kiel, Kiel, Germany

## Key Words

Innate immunity · Sea urchin · Cnidaria · Nucleotide-binding and oligomerization domain-like receptor · Toll-like receptor · Crohn disease · Asthma

## Abstract

Innate, nonadaptive immune receptors represent phylogenetically ancient first-line sensors of invariant non-self patterns and other cellular danger signals. From lower animal phyla to vertebrates, most pathogens are immediately detected by various recognition systems and are destroyed by induction of defense effectors like antimicrobial peptides. Toll-like receptors, nucleotide-binding and oligomerization domain-like receptors and scavenger receptor cysteine-rich proteins represent archetypes of the innate immune receptors, which mediate the complex interaction between the host and microbiota at the interface of epithelial barriers. In this review, we will use knowledge gained from marine invertebrates as a paradigm to describe how this constant molecular crosstalk within the holobiont, i.e. the animal with all its associated microorganisms, contributes to epithelial homeostasis, immunological integrity and maintenance of the resident microbial diversity. Copyright © 2009 S. Karger AG, Basel

## Introduction

Maintenance of the immunological integrity of organisms has been a driving force in evolution. Diversification of life forms has led to a vast number of heterogeneous non-self recognition strategies and defense effector mechanisms. However, selected principles of innate immunity seem to be molecularly conserved across animal phyla. These major functions of the innate immune system include a controlled host/microbial crosstalk at epithelial barriers, the recognition of danger signals, clearance of intracellular pathogens by autophagy, the recruitment of mesoderm-derived professional immune cells and the secretion of local or circulating effector molecules such as antimicrobial peptides and simple opsonic forms of complement.

In this review, we will discuss selected aspects of innate immune recognition strategies in marine invertebrates and demonstrate that this field is rapidly evolving by the advent of novel genomic techniques including ultrafast sequencing. Marine invertebrate species are especially suited to understand the evolutionary forces that shape genetic diversity of the innate immune armamentarium as they are constantly subjected to selective pressures from variable physical conditions of the aquatic habitat (e.g., light, salinity, dense microbial communities, trophic conditions). As some marine animals (e.g., the

bivalve *Arctica islandica*) are among the longest living animals on earth, we will debate the role of immunosenescence and longevity. We will use this knowledge to show that innate immune mechanisms play a crucial role in the broadening concept of the 'holobiont'. This term, which was first coined in corals to describe the functional entity of the animals together with their respective endosymbionts and associated microbiota [1], emphasizes the role of an intact host-microbial interaction for normal development and function of epithelial barrier organs conserved from marine invertebrates to humans. The general importance of these principles also for human health has become increasingly clear, as it was demonstrated that genetic variants in phylogenetically ancient innate immune genes are involved in the etiology of emerging chronic inflammatory diseases of epithelial barrier organs such as Crohn disease, atopic dermatitis and asthma [2].

### **Germline Genetic Diversity of Innate Immune Receptors: Toll-Like Receptors, Nucleotide-Binding and Oligomerization Domain-Like Receptors and Scavenger Receptor Cysteine-Rich Domain-Containing Proteins**

Conversely to the genetic plasticity of the adaptive immune system in vertebrates that allows maturation and selection of high-affinity responses towards a specific antigen, the innate immune system has its origin early in the evolution of metazoans and relies on a limited set of germline-encoded receptors for recognition of danger signals. These pattern recognition receptors (PRRs) sense invariant molecular signatures that are either present in potential pathogens (pathogen-associated molecular patterns, PAMPs, e.g., lipopolysaccharides or unmethylated CpG DNA) or that are derived from endogenous sources (e.g., extracellular heat shock proteins, oxidatively modified proteins) and attest profound cellular damage. Engagement of these receptors leads to a fast induction of protective programs, e.g., the induction of antimicrobial peptides or the elimination of the infected cell by means of apoptosis. Whereas the sets of innate immune receptors seem to be rather conserved, it must be emphasized that the molecular realization of protection downstream of receptor activation and their cognate pathways may vary considerably among phyla. Various families of conserved PRRs have been identified, which are either localized on the cellular surface or in intracellular compartments. In this review, we will focus on 3 major types of

receptors. Toll-like receptors (TLRs), which belong to the Toll/IL1R receptor family, are a prototype of the transmembrane PRR with an extracellular ligand-binding domain and an intracellular adaptor domain, where downstream signaling molecules are recruited upon activation [3]. The intracellular nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) family plays a pivotal role in the recognition of intracellular PAMPs [4] and is related to the apoptosis-inducing apoptosis-protease activating factor (APAF)-like molecules. The third group is the family of scavenger receptor cysteine-rich (SRCR) domain-containing proteins [5]. A complex set of transcripts featuring SRCR repeats can be found from sea urchins to humans, which may contribute to the diversity of innate immune responses.

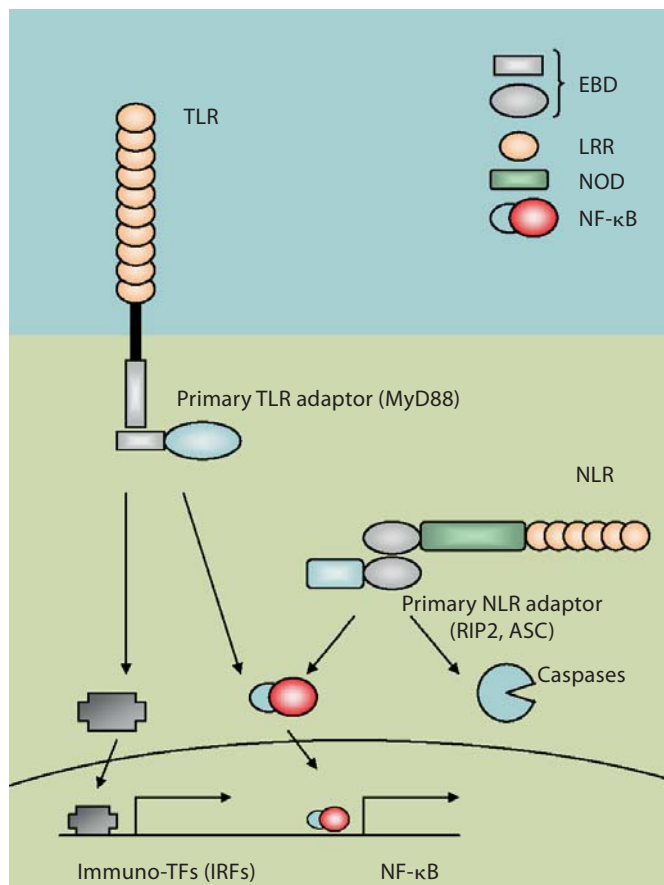
#### *Toll-Like Receptors*

The domain architecture of TLRs comprises extracellular 15–25 leucine-rich repeats (LRRs), a transmembrane domain and an intracellular Toll/IL-1 receptor signal-mediating domain [3]. It is thought that the extracellular domain senses the signal which it transmits to the nucleus via a complicated pathway of adaptor molecules leading to an activation of the transcription factors nuclear factor- $\kappa$ B (NF- $\kappa$ B) and different interferon-regulatory factors (fig. 1). This structure and cognate function is strikingly similar to functional homologs in plants [6] and has first been identified as an important receptor system in *Drosophila* dorsoventral patterning [7]. Since then, it has been recognized that besides this developmental role, which seems to be rather unique to insects, a major role of the ever-increasing family of TLRs is the recognition of immunological danger signals. Again, first clues were derived from the *Drosophila* model system [8] and were later extended to other phyla. The receptor system has been an archetype of our understanding of how invariant extracellular sensor structures may recognize threats induced by microbial assaults. Two main theories have been put forward. (1) The receptor directly binds to conserved minimal structures of pathogens. It was proposed by Janeway and Medzhitov [9] that the recognized PAMPs are so essential to survival and replication of the bacteria or other microbiota that they cannot be easily modified in order to escape immune recognition. This concept leaves us with the problem of how a pathogen can be distinguished from the abundance of commensals and symbionts, which are likely to harbor the same molecules (e.g., lipopolysaccharides, lipopeptides, certain nucleic acids) and, ultimately, with the unanswered question why a host that carries myriads of bacteria on its surfaces does

not succumb to constantly high levels of inflammation. (2) The pathogen leads to an alteration or unmasking of hidden 'self' structures by cellular damage. Normally, these molecules, e.g., extracellular heat shock proteins released by lysed cells, are not seen by the TLR system. A theoretical advantage of this recognition strategy is that simple mutations in microbial genomes, which do not interfere with pathogenic virulence, may not easily lead to a direct evasion of recognition of cellular danger in itself.

Which traces of TLR pathways can be found in genomes of aquatic invertebrates and what can we learn about the early roots of TLRs in host defense?

Until a few years ago, the diversity of *TLR* genes throughout animal phyla seemed rather limited and TLRs were hypothesized to play a major role in mesodermal cells, i.e. fat body cells or circulating professional immune cells. Both views were undermined by genomic data from marine invertebrates. In the genome of the purple sea urchin, *Strongylocentrotus purpuratus*, more than 200 *TLR* gene models have been identified which can be classified into a number of distinct subgroups [10, 11]. This strikingly high diversity was in contrast to the narrow repertoire of the classical invertebrate model organisms *Caenorhabditis elegans* and *Drosophila melanogaster* and other marine invertebrates, e.g., the bivalve *Chlamys farreri* [12]. Work by Rast and colleagues has pointed out that TLRs in the sea urchin display characteristic features of a rapidly evolving gene family with more than 30% pseudogenes, tandem gene structures and a high rate of nonsynonymous polymorphisms especially in the LRR-containing ectodomain [10, 13, 14]. Interestingly, the majority of TLRs in *S. purpuratus* have a high homology to vertebrate-like TLRs, and only few genes display sequence similarity to protostome TLRs (e.g., Toll from *D. melanogaster*) pointing to an early bilaterian divergence of TLR subgroups [14]. Why has this expansion of TLR genes occurred in echinoderms? It is of course tempting to speculate that the genetic repertoire of receptors is reflecting the diversity of ligands, and thus threats, which can be recognized as danger signals. This could be interpreted as an evolutionary response to generate diversity and specificity of antigen sensing under the selection pressure by vast amounts of benthic microbes. However, this positive selection clearly is hypothetical and remains to be rigorously proven, e.g., by molecular identification of ligands activating specific TLRs and detailed phylogenetic analysis including population analysis of different sea urchin species and other echinoderms from different habitats.



**Fig. 1.** Conserved principles of innate immune recognition: signaling pathways of TLRs and NLRs. TLR proteins are integral membrane proteins with an ectodomain comprised of LRRs and an intracellular Toll/IL-1 receptor signal-mediating domain as effector-binding domain (EBD). NLR proteins typically comprise 3 domains with a C-terminal ligand recognition domain consisting of LRRs, a central NOD and the N-terminal effector-binding domain, which can be realized as a DEATH, CARD (caspase recruitment) or PYD. Upon activation, the receptors recruit primary adaptor molecules to engage downstream signaling pathways including NF- $\kappa$ B, interferon-regulatory factors (IRFs) and caspases. RIP2 = Receptor interacting protein 2; ASC = apoptosis-associated speck-like protein containing a CARD; MyD88 = myeloid differentiation factor 88; TFs = transcription factors.

In the sea urchin, expression of TLRs seems to be restricted to coelomocytes, i.e. to simple forms of professional immunocytes derived from the mesenchyme [10, 14]. Is the primordial migratory immune cell the origin of TLR pathways as a defense tool in animals? This perspective has been challenged by studies in early branching metazoans that lack the mesodermal layer (e.g., sponges, corals, sea anemones and polyps). Recent data point to a pivotal role for TLRs in epithelial immune de-

fense in these animals. In the demosponge *Suberites domuncula*, a receptor with high homology to bilaterian TLRs and a cognate adaptor kinase similar to IL-1 receptor-associated kinase have been cloned [15] and demonstrate that the phylogenetically oldest extant metazoan phylum already comprises this important signaling pathway of the antimicrobial host defense system in the absence of a mesodermal layer. Unequivocal evidence for a functional TLR system in early eumetazoans has been gathered in Cnidaria. Data from expressed sequence tag archives have clearly shown the presence of several orthologs of TLR genes in the classes of Anthozoa and Hydrozoa [16, 17]. Although in the genome of *Hydra magnipapillata* conventional TLRs are absent, TLR function is realized by the interaction of an LRR domain containing protein with a Toll/IL-1 receptor signal-mediating domain containing protein lacking LRRs. Coexpression of both membrane proteins is linked to antimicrobial peptide induction in vivo, and heterologous overexpression of the 2 *Hydra* proteins in mammalian cell lines leads to a sensitization to the PAMP flagellin supporting the hypothesis that the epithelium represents the ancient system of host defense [18].

#### *NOD-Like Receptors*

*NLR* genes encode for cytosolic proteins that comprise a trimodular domain structure, characterized by a central NOD, and an N-terminal DEATH-fold-like effector-binding domain, e.g., a Pyrin domain (PYD), DEATH or caspase recruitment domain (CARD) [4]. The NOD, also termed 'NACHT domain' (a domain present in neuronal apoptosis inhibitor protein, the major histocompatibility complex transactivator (CIITA), HET-E and TP1) [19], is a member of the recently defined superfamily of P-loop NTPases. Sequence homology exists with the nucleotide-binding core of APAF-1, which is responsible for the dATP/ATP-dependent oligomerization of APAF-1 upon cytochrome c recognition, which activates the initiator caspase-9. Upon ligand sensing via the C-terminal LRRs, which can be regarded as the intracellular counterpart of the ectodomain of the TLRs, the molecules have propensity to form self-oligomers, thereby recruiting proximity-activated binding partners (fig. 1). This leads to either activation of proinflammatory signaling pathways (e.g., NF- $\kappa$ B transcription factor via canonical RIP2/IKK signals) [20, 21] or direct activation of proinflammatory caspases via formation of a large protein complex, which in turn enables the processing of IL-1 $\beta$ -like substrates [19]. Here, the same question has been posed as with the TLRs: do NLR-type LRRs directly bind to their cognate PAMPs

or are they indirect sensors of cellular danger? Whereas in TLRs there is evidence for the realization of both principles [22–26], in NLRs, the situation is less clear. Minimal structures from bacterial peptidoglycan have been shown to activate human NOD1- and NOD2-dependent signaling. Human NOD1 sensitizes cells to a unique diaminopimelate-containing N-acetylglucosamine-N-acetylmuramic acid (GlcNAc-MurNAc) tripeptide motif present in the peptidoglycan of Gram-negative bacilli [27, 28] and, particularly in Gram-positive bacteria, NOD2 detects the more generalized motif muramyl dipeptide (MurNAc-L-Ala-D-isoGln, MDP) [29, 30]; however, no convincing direct binding assays have so far been provided. For the inflammasome-forming NALP3, a variety of exogenous (e.g., asbestos, viral DNA and MDP) and endogenous danger signals (e.g., monosodium urate, ATP) have been identified as elicitor molecules [31–34]. Unifying principles how all these different compounds are integrated into a specific NALP3-inflammasome signal may involve the activation of potassium ion fluxes through P2X7-gated ion channels [33, 35] or sensing of reactive oxygen species [31] which are induced downstream of the aforementioned elicitors.

It must be emphasized that all of this knowledge has been gained from studies of vertebrate NLRs. Until a few years ago, *NLR* genes were thought to have evolved in teleost fish probably by means of domain shuffling [36, 37], as only single domains of *NLR* genes with low homology had been identified in the genomes of the classical invertebrate model organisms *D. melanogaster*, *Ciona intestinalis* and *C. elegans*.

The genome sequence of the purple sea urchin again revealed surprising evidence that *NLR*-like genes were already present in echinoderms. A multigene family of more than 200 *NLR*-like genes has been identified clustering into different subfamilies [10, 14]. While in many of the gene models the exact structure of the C-terminal sensor region has not yet been convincingly elucidated, the N-terminal effector-binding domain is realized as a DEATH domain, rather than as a CARD or PYD motif. It is hypothesized that all of these domains have evolved from a common DEATH-fold-like ancestor [38]. As the cognate adaptors in *S. purpuratus* (RIP2-like kinase and orthologs of proinflammatory caspases) also carry DEATH domains as homotypic interaction motifs, it will be interesting to reveal at which point of evolution the whole system has switched to a higher complexity of CARDS and PYDs as homotypic interactors.

In vertebrates, 2 major themes of NLR-mediated immunity have been proposed. (1) NLRs are involved in

shaping immune responses induced by mesodermal professional immunocytes (e.g., dendritic cells and monocytes) [39–41]. It has been shown that NOD2 triggers a potent antigen-specific immune response with a Th2-type polarization profile, demonstrated by the induction of IL-4 and IL-5 by T cells and IgG1 antibody responses. These findings together with other data pointing to a role of NLRs as negative regulators for Th1-type immune responses show that NLRs are involved as microbial-induced polarization principles and provide a link between the innate and the adaptive immune system. (2) The NLRs NOD1 and NOD2 have been shown to play a pivotal role in intestinal epithelial cells as a first line of immune defense [42–47]. Dysfunction of NOD2 has been associated with an impaired expression of antimicrobial peptides at the intestinal barrier [48–50]. In the sea urchin, the gut seems to be a major site of NLR expression [13], which may point to a primordial function of NLRs in protective epithelial responses and shaping of associated microbial communities. Thus, functional insights from lower invertebrates are urgently needed to understand the evolutionary origins of NLRs as epithelial sensors involved in the maintenance of commensal diversity and defense against pathogens.

#### *SRCR Domain-Containing Proteins*

SRCR-containing proteins constitute a group of conserved receptors playing a role in innate immunity and development. The SRCR domain is phylogenetically ancient and is defined through a cysteine-rich protein module of approximately 100–110 amino acids, which was first described as a scavenger receptor in macrophages [51]. SRCR proteins can be found throughout all animal phyla from poriferans to chordates. The SRCR motif in scavenger receptors expressed in mammalian macrophages provides direct binding to microbial motifs [52] and aberrant self such as altered lipid structures [5]. The role of other SRCR motif-bearing proteins is heterogeneous: while some genes encode classical type 1 membrane proteins, others code for secretory proteins that may be involved in immune exclusion [52, 53]. Multi-domain SRCR proteins were shown to be an immensely large family in sea urchin coelomocytes, where they were shown to be regulated upon immune challenge [11, 14, 54–56]. In the marine sponge *Petrosia ficiformis*, the SRCR gene *PfSym2* is differentially expressed in relation to its symbiotic state [57]. DMBT1, a protein structurally similar to the sea urchin multidomain SRCR factors, is linked with NLR and TLR signaling in vertebrates [53]. Deletion variants in the gene have been associated with

epithelial cancers and inflammatory diseases, pointing to a crucial role in both epithelial defense mechanisms and proliferative homeostasis. Given the highly dynamic and variable expression patterns of multidomain SRCR transcripts in cell populations of the sea urchin, the family of proteins may serve as an interesting starting point to functionally dissect the evolution of the direct interaction between host and microbiota.

#### **Stable Host-Microbiota Interactions – Towards an Understanding of the Evolution of the Holobiont**

We are beginning to understand that the complex interaction between commensal communities of microbiota residing on the surfaces of epithelial barriers and their respective host is required for normal development, epithelial regeneration and maintenance of immunological integrity in general [58–60]. The microbial flora attached to epithelial interfaces plays an important role in nutrition physiology, and disturbed diversity of microbiota is associated with a wide range of pathologies from obesity to atherosclerosis to emerging chronic inflammatory diseases [61]. Body surfaces are colonized by microorganisms from their first days of life. This principle is true for all animal life forms, not only for mammals, where the maternal flora serves as the predominant source of initial colonization [62]. Most epithelial surfaces are covered by physical barrier substances, e.g., mucus or chitin. Character and composition of these layers are pivotal for the spatiotemporally controlled microbial colonization. It has been shown in a variety of species that despite a high degree of individual variation in microbiota composition over time, the core microbial communities remain highly stable and species specific. This argues for a strong host genetic control and coevolution of the host and its associated microflora [58, 62, 63]. The longitudinal variance of microbial diversity on interface organs during the entire lifespan and the passive modification of the flora by environmental influences, including nutrition and hygiene, are unclear [64]. Only few studies have focused on the ‘resilience’ phenomenon, i.e. the capacity of the microflora to regain homeostasis of diversity after environmental challenges such as infections. This phenomenon involves the process of ‘quorum sensing’, i.e. the communication between microorganisms by specific ligands such as homoserine lactones, but also the creation of ecological niches beneficial for the epithelial barrier and immune system of the host [65, 66]. Vice versa, a requirement of tonic recognition of bacterial components

through TLRs and NLRs seems to exist in order to create a normal regenerative capacity of the epithelium and to drive a normal development of underlying mesodermal immune cells. On the bacteria side, the Mazmanian group was first to reveal the identity of a molecular entity, polysaccharide A, involved in this process in mice [67, 68]. Interestingly, the symbiotic relationship between epithelial cells and microbes has been emphasized by several studies in marine invertebrates, where the recognition of tracheal cytotoxin from *Vibrio fischeri* is required for epithelial light organ formation in squids [69–71]. Although the receptors involved in the recognition of *V. fischeri* have not been identified yet, it must be noted that tracheal cytotoxin represents the specific elicitor for the murine NLR NOD1 [72], making it tempting to speculate that innate immune signaling is involved. The role for epithelial homeostasis and proliferation is also supported by data from the basal eumetazoan *Hydra viridis* demonstrating that bacteria-derived factors are needed for proper budding. Aposymbiotic animals that do not reproduce asexually under sterile conditions regain their budding ability after addition of nonsterile *Artemia* larvae or bacteria per se [73].

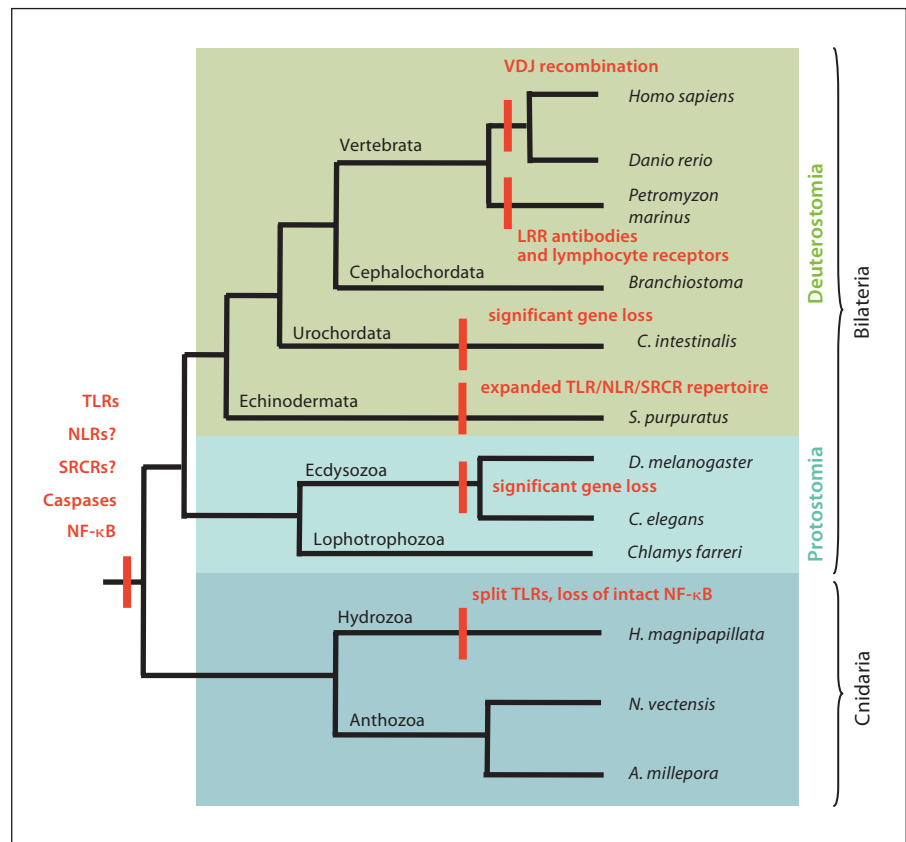
From all the cumulated data on host-microbial interactions, a mutual dependency between host and associated microbiota can be hypothesized, in which the metabolic activities of the bacterial flora are responsible for gene regulation necessary for homeostasis of the host barriers [58]. This homeostasis and the gene expression programs specifically induced via innate immune receptors in turn stabilize the diversity of the associated microbiota. This principle of a ‘holobiont’ is conserved from humans to early branching eumetazoans [1]. Although the term was first used in corals to describe the complete animal with its endosymbionts and associated microbiota, we propose to broaden the holobiont concept, as the dynamic communities of microbiota on body surfaces must be understood as an integral part of the functionality of the respective organism itself, regardless of the animal phylum.

### **A Role for the Innate Immune System as a Determinant of Longevity?**

For a long time the concept was iterated that evolution of the adaptive immune system as a more effective immune defense was needed to meet the requirements of the increasingly complex bauplan and a long life [9]. This would imply that animals with only the innate immune

system at hand are simple and short lived. However, many marine invertebrates are extremely complex (e.g., the giant squid) and are record holders of longevity. Species of sea urchins and crustaceans show life spans of over 100 years (AnAge database) and, among bivalves, 2 species have been found that represent 2 of the longest lived animals on earth, the freshwater pearl shell *Margaritifera margaritifera* (maximum life span potential 190 years) [74] and the marine mud clam *A. islandica* with a lifespan over 375 years [75, 76]. Furthermore, sponges and the freshwater polyp *Hydra* are assumed to be nearly immortal. This demonstrates that also with an innate immune system, extrinsic mortality due to infections or cancer development are prevented and longevity can be achieved. Studies directly linking immune responses and longevity in such long-lived invertebrates are still scarce and completely absent on a molecular basis. Whereas the genome sequence of the purple sea urchin may be used to extrapolate the diversity of the immune gene repertoire in longer-lived echinoderms, genomic data for bivalves are very limited. Interestingly, a high genetic variability has been shown in oysters [77] (approximately 1 single nucleotide polymorphism per 40 bp), which may contribute to plasticity of immune response in populations spread across different habitats with distinct selective pressures. One first line of evidence of how an effective program for tissue repair and defense against infection is associated with longevity in marine invertebrates has been demonstrated in the long-lived bivalve *M. margaritifera*. Ziuganov et al. [74] tested the regenerative capacity after experimental injury in populations displaying a different maximum lifespan. Individuals from a southern Spanish population reach 30–40 years, whereas specimens from the Arctic can live up to 200 years. Upon mutilation, it was found that the longer-lived arctic population showed higher shell and wound healing as well as survival rate compared with the shorter-lived population from Spain. On a population level, no diseases, parasites or tumors were observed, even in old individuals. Furthermore, the biological principle seems to be transmissible, as infestation of the Atlantic salmon *Salmo salar* with parasitic *M. margaritifera* larvae, of the arctic population, which develop inside the fish gills, resulted in higher resistance of the fish to burns and wounds, prevention of cancer development and an increase in lifespan beyond the first spawning event after which the fish normally dies [74]. Although it must be pointed out that this observation is purely coincidental and may not be directly linked to the longevity principle of the bivalve in itself, the larvae can somehow ‘switch off’ the death program of the fish and enhance its stress resistance.

**Fig. 2.** Scheme of important immune-related events during animal evolution. The phylogenetic tree represents model organisms where genomic and/or transcriptomic data are available. Note that the immune-related changes in genome architecture in the diagram represent the current knowledge. The advent of ultra-fast sequencing and other genomic tools allows for a fast and systematic exploration of additional model organisms which may result in a comprehensive view on the origins and distribution of the immune gene repertoire in the animal kingdom. VDJ = Variable (V), diversity (D) and joining (J) gene rearrangement for antigen receptor diversification.



A deficient response to infections has been described as a hallmark of human aging and is among the major mortality factors in elderly people. For a long time this was mainly attributed to a deregulation of the adaptive immune system resulting in reduced immunoglobulin secretion, a decrease in B cell numbers and a diminished apoptotic clearance rate of peripheral blood lymphocytes [78, 79]. The process has been called ‘immunosenescence’, and the dysfunction of the adaptive immune system was thought to result from the general decline in repair and proliferation capacity of the high-aged organism [80]. We could recently demonstrate that human aging is also accompanied by complex changes of innate immune mechanisms at epithelial barriers [81]. This included not only a diminished expression of recognition molecules (both TLRs and NLRs), but also a downregulation of molecules involved in negative feedback loops (e.g., SARM1) and an augmentation of inflammation processes in the elderly. It may be hypothesized that the innate immune system has evolved to quickly control pathogens, and therefore, efficient induction of proinflammatory pathways via innate immune receptors is a necessary

and important component of a host’s defense mechanism early in life and in adulthood. However, it may become detrimental later in life as the overproduction of inflammatory molecules together with the decline in pathogen recognition contributes as ‘inflammaging’ to age-related diseases. Investigating the innate immune system and its change with age in long-lived marine invertebrates like the ocean quahog *A. islandica* or potentially immortal cnidarians may help to gain insight into mechanisms responsible for sustained and effective immune responses despite high age.

### Future Prospect or What Can We Learn from Invertebrate Immunity for Emerging Human Chronic Inflammatory Diseases?

The pivotal role of NLRs, TLRs and SRCRs for the physiological immune response is paralleled by a remarkable series of association findings of polymorphisms in the cognate genes with human chronic inflammatory diseases of epithelial barrier organs. Polygenic diseases

associated with loss-of-function variations in *NLRs*, *TLRs* and *SRCRs* include Crohn disease (NLR, TLR and SRCR), atopic disease and asthma (NLR and TLR), which are characterized by chronic relapsing-remitting inflammation of barrier organs (intestine, lung, skin). A suggestive association of epithelial cancer entities (breast and colonic carcinoma) with germline mutations in *NOD2* and *NOD1* supports the hypothesis that chronic inflammation induced by epithelial barrier dysfunction significantly contributes to the etiology of malignant diseases.

Most chronic inflammatory diseases have seen a steep rise in incidence over the last 100 years; e.g., Crohn disease was essentially unknown before the 1930s and it was thus hypothesized that environmental factors and changes in lifestyle conditions (e.g., nutrition or hygiene) in modern industrial societies are key pathophysiological triggers. Evidence has been obtained that the inappropriate immune responses observed in inflammatory barrier disorders are driven by the resident microbiota and their metabolic structure [82, 83]. In contrast to the short history of this class of diseases, most of the genetic profiles conferring risk have arisen some 40,000 years ago [84]. It is unlikely that this genetic variability would have been conserved over such a long period of time without any evolutionary pressure favoring it. It is thus tempting to speculate that the specific sequence variability may have had protective function in the past and only recently has become a risk factor under today's living conditions [2].

The present insights into the evolutionary origins of the innate immune system in aquatic invertebrates (fig. 2) may prove to be especially useful to understand how selective pressures form genetic diversity and sequence variability to cope with different immune challenges. The advent of novel ultra-fast sequencing technologies will allow building comparative single nucleotide polymorphism maps of innate immune receptors together with an inventory of the associated microbiota in different populations of cosmopolitan invertebrate species to describe the interaction between barrier and environment. Dissecting the primordial cellular programs of innate immunity, the 'Ur-defensome', in simple eumetazoans is alleviated through transgenic technologies allowing for a stable genetic manipulation of these animals under laboratory conditions. Early branching metazoans as novel animal models may contribute to the emerging concept of evolutionary medicine in order to identify novel targets for therapeutic augmentation of barrier function and epithelial drug delivery.

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