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Crayfish immunity – Recent findings

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ABSTRACT

Freshwater crayfish is an important commodity as well as a successful model for studies on crustacean immunity. Due to the ease with which they are kept and the available methods for hemocyte separation and culture they have proven to be very useful. Here, recent progress regarding pattern recognition, immune effector production and antiviral mechanisms are discussed. Several cases of functional resemblance between vertebrate complement and the crayfish immune reactions are highlighted.

1. Introduction

Freshwater crayfish is an important commodity as well as a key component of many freshwater ecosystems. They are relatively easy to keep and breed in captivity and found very useful for research in various fields such as immunology and neurobiology. Here some recent findings aimed at unravelling the immune mechanisms of these important crustaceans are discussed. Three species namely *Pacifastacus leniusculus*, *Procambarus clarkii* and *Cherax quadricarinatus* have been widely used in aquaculture and research and, therefore, research done on these three have provided most of the data discussed here. For earlier research in this area see e.g. Cerenius et al. (2010), Jiravanichpaisal et al. (2006). Additionally, Söderhäll (2016) deals with crayfish – and general crustacean-hematopoiesis in greater detail than the present paper. Although such a division admittedly, is somewhat arbitrary this review was split into three parts: first recognition, second signalling and propagation of an immune response and third immune effectors including some antiviral reactions. As will be evident, several of these crayfish immune factors bear in several cases a striking functional resemblance with the vertebrate complement system in spite of not being structurally related to the analogous vertebrate complement factors.

2. Recognition and the initialising of the immune response

Some of the early classical work in this field precisely defined the structural requirements for the recognition of β-1,3-glucans and thus how important pathogens such as fungi and oomycetes are initiating the host defence (Unestam and Söderhäll, 1977; Söderhäll and Unestam, 1979). The molecular mechanisms for detecting bacteria and other disease causing organisms have proved to be harder to elucidate in crustaceans. Peptidoglycans (PGNs), ubiquitously present in almost all bacterial cell walls, do not seem to be recognised by PGRPs in crayfish and many other crustaceans. In *P. leniusculus*, with the use of a variety of methods including recombinant techniques, phenoloxidase (PO) activation by Lys-type PGN was demonstrated by Liu et al. (2011) to be mediated by a complex of two serine protease homologues (SPHs) and the lipopolysaccharide- and β-1,3-glucan-binding protein (LGBP). Thus, the latter protein has somewhat more diverse binding activities than its name implies. The formation of a molecular complex between LGBP and SPH1 plus SPH2 for the initiation of proPO activation is noteworthy given the prominent role of SPHs in proPO-activation and hemolymph clotting in other arthropods. The obvious absence of *PGRP* genes in most crustaceans is supported by whole genome sequencing of the amphiopod *Parhyale hawaiiensis* (Kao et al., 2016) and the cladoceran *Daphnia pulex* (McTaggart et al., 2009). It seems that PGRPs have been replaced by an

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extended family of LGBPs in the crustaceans with the exception of the remipedes (Kao et al., 2016). An LGBP with capacity to bind to bacterial cell walls was recently cloned from *Cherax quadricarinatus* (Hou et al., 2015) although it is not known whether immune system activation took place in this case.

In *P. leniusculus* two ficolin-like proteins (FLPs) act as pattern recognition factors as shown by numerous in vitro and in vivo assays (Wu et al., 2011). Strikingly, the crayfish FLPs have structural similarities with some of the lectins mediating complement activation in vertebrates such as human L-ficolin. C3-like proteins were not detected but instead the FLPs are strong candidates for mediating complement-like activities in crayfish (Wu et al., 2011). Recently, from another crayfish species, *Procambarus clarkii*, an FLP that in contrast to the *P. leniusculus* FLPs is lacking a collagenous region was isolated and found to bind and agglutinate bacteria (Chen et al., 2016a). Other candidates for bacterial detection and immune system activation are a series of C-type lectins (PcLec1-5) found in *P. clarkii* (Zhang et al., 2013 and references therein). Among these is PcLec3 a 252 amino acid protein with, in tandem, an immunoglobulin-like domain and a C-type lectin (Zhang et al., 2013). Studies with recombinant PcLec3 domains revealed that the Ig-like domain mediated hemocyte binding whereas the full protein promoted phagocytosis of bacteria (Zhang et al., 2016). A somewhat similar role in, i.e. bacterial binding and bacterial growth inhibition in this case in the crayfish *C. quadricarinatus* has been suggested for the hypervariable molecule Dscam (Li et al., 2015). Further detailed data confirming this possible host-pathogen interaction is eagerly waited. One recombinant *P. clarkii* C-type lectin, PcLT, has been shown to enhance phagocytosis of *Vibrio alginolyticus* in vitro and clear this bacterium from the hemolymph in vivo (Chen et al., 2013). A 290-kDa lectin obviously structurally unrelated to the above-mentioned lectin was reported to activate a hemocyte oxidative burst response (Sánchez-Salgado et al., 2014). No mechanisms for this possible lectin-mediated activation were presented though. Lectin-carbohydrate interactions may sometimes serve the opposite purpose, i.e. they instead prevent excessive immune system activation as demonstrated by *P. leniusculus* mannose-binding lectin (Pl-MBL), a protein capable to prevent lipopolysaccharide-induced proPO-system activation and to function as a scavenger for LPS to regulate proPO-system activation (Wu et al., 2013). In Table 1 data for some well-characterised crayfish pattern regognition proteins are displayed.

3. Signalling and the propagation of the immune response

Pattern recognition will, among other things, lead to the initiation of proteolytic cascades in the hemolymph and to the activation of receptors on immune-active cells such as hemocytes. The results will be both short term, such as release by exocytosis and other mechanisms of immune effectors from the hemocytes and long-term effects on hematopoiesis and differentiation (Söderhäll, 2016). One important immediate response is the activation of the proPO-system resulting in melanisation, parasite enclosure, production of cytotoxic and lytic activities etc. Immune active hemocytes exhibit activities very similar to inflammasome activation such as the production of caspase-1 like activities (Jearaphunt et al., 2014). In crayfish caspase-1 catalysed limited proteolysis will accompany the leaderless release of proPO into the extracellular milieu demonstrating a connection between inflammation and melanisation (see also below for immune effector activities). As indicated above, an infection has significant effect on hemocyte numbers and replenishment. Hematopoietic cells do not produce proPO in contrast to mature circulating hemocytes (Fig. 1). It is evident that one of the last import changes to accompany hemocyte release and maturation from the hematopoietic tissue is the onset of proPO expression. It has been established in *P. leniusculus* that reactive oxygen species (such compounds may be produced by several different immune reactions) can serve as a signal to activate renewal of the hemocyte population by inducing increased hematopoiesis (Junkulo et al., 2016). This effect on hematopoiesis is via regulation of extracellular transglutaminase, an enzyme activity that keeps immature hemocyte precursors inside the hematopoietic tissue (Lin et al., 2008). Long-term effects of immune signalling on hemocyte differentiation may even ramify further as recent

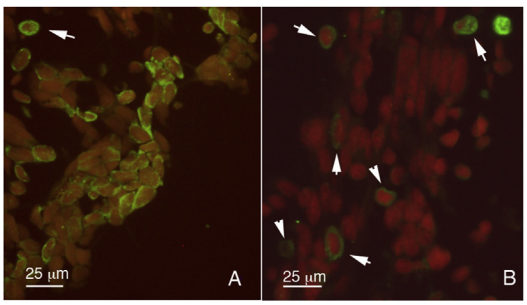

Fig. 1. Section from the posterior part of the hematopoietic issue in *Pacifastacus leniusculus* stained with (A) astakine 1 antibodies (white arrows) and (B) with proPO antibodies (also here white arrows). Only free circulating hemocytes are staining for proPO whereas both hematopoietic cells and free hemocytes express astakine 1. Nuclei are stained with propidium red (red). Figures courtesy of Irene Söderhäll, Uppsala University. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Some pattern recognition proteins in freshwater crayfish.

Protein	Molecular size (kDa)	Ligands/binding activities	Physiological function
Lipopolysaccharide and glucan-binding protein (LGBP)[a]	39	LPS, β −1,3 glucans	proPO-system activation
Masquerade-like protein (mas-like)[b,c]	134 + 129	LPS, β −1,3 glucans hemocyte membranes	Opsonin
Serine proteinase homologue 2 (SPH2)[d]	30	Lys-type PGN in complex with LGBP, SPH1	proPO-system activation
PcLectin 1 (PcLec1)[e]	16.6	LPS, PGN, LTA	Clearance of bacteria
PcLectin 2 (PcLec2)[f]	16.4	Gram negative bacteria	proPO-system activation
PcLectin 3 (PcLec3)[g]	27.8	LPS, PGN, LTA Hemocyte membranes	Opsonin
PcLectin 4 (PcLec4)[h]	18.6	LPS, PGN, LTA	Clearance of bacteria
C-type lectin PcLT[i]	16.8	Gram-positive and-negative bacteria, mannose	Clearance of bacteria
Mannose-binding Lectin (Pl-MBL)[j]	28.3	LPS	proPO-system regulation
Ficolin-like protein 1 (FLP1)[k]	58	Gram-positive and -negative bacteria	Clearance of bacteria
Ficolin-like protein 2 (FLP2)[k]	56	Gram-positive and-negative bacteria	Clearance of bacteria
Fibrinogen-related protein (PcFBN1)[l]	50.6	Gram-positive and -negative bacteria	Clearance of bacteria

References: [a]Lee et al. (2000), [b]Lee and Söderhäll (2001), [c]Huang et al. (2000), [d]Liu et al. (2011), [e]Zhang et al. (2011), [f]Wang et al. (2011), [g]Zhang et al. (2016), [h]Zhang et al. (2013), [i]Chen et al. (2013), [j]Wu et al. (2012), [k]Wu et al. (2011), [l]Chen et al. (2016a).

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