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REVIEW

Various roles of β-glucan in invertebrates

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Abstract

Glucans have a long history as immunomodulators; their effects confirmed in every species tested-from bees to humans. In invertebrates, glucan binding receptors are involved mainly in starting of the prophenoloxidase system, representing one of the first defense mechanisms that evolved in phylogeny. Our review summarizes the current knowledge of the glucan and lipopolysaccharide-binding proteins in invertebrates and offers a new possibility of using these proteins in human medicine.

Key Words: invertebrates; glucan, receptors; GBP: lipopolysaccharide

Introduction

Various glucans have a long history as immunomodulators. However, for several decades, the focus of investigations was almost exclusively oriented towards vertebrates. Glucans, and most of all their β-1,3 configurations, are structurally complex homopolymers of glucose, isolated from yeast, grain, seaweed, and fungus.

More than 20,000 scientific papers describing the biological activities of glucans exist. Strong immunostimulating effects of β-glucans have been demonstrated in all tested animal species including earthworms (Beschin *et al.,* 1998), bees, (Mazzei *et al.,* 2016) shrimp (Duvic *et al.,* 1992), fish (Anderson 1992), mice, rats (Feletti *et al.,* 1992), guinea pigs (Ferencik *et al.,* 1986), sheep, pigs (Benkova *et al.,* 1992), cattle (Buddle *et al.,* 1988), and humans. Currently, there are at least 80 clinical trials underway in numerous countries. Clearly, glucan belongs the oldest molecules with significant immunomodulating processes, and its activity found throughout the evolutionary scale.

From an evolutionary point of view, three main principles (*i.e*., recognition, processing, and elimination) are common in all invertebrates, despite the fact that they might be based on varying molecular or biochemical backgrounds. Defense strategies of invertebrates lacking lymphocytes and antibodies are based entirely on innate immunity. The major defensive reactions involve phagocytosis, wound healing, graft rejection, production of various factors (e.g., agglutinins, precipitins, opsonins, clotting factors, lysozyme and many others), and humoral defense (Sima *et al.,* 1990). One of the major defensive mechanism of invertebrates is the use of distinctive molecular patterns, including β-glucans.

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Prophenoloxidase system

Invertebrates lack immunoglobulins and their immune system relies on the innate ability initiated by pattern recognition receptors and pattern recognition proteins. These receptors are involved in microbial recognition and initiate protein-ligand interaction. These proteins are able to bind to a variety of microbial cell wall components.

In crustaceans alone, at least 15 different types of pattern recognition receptors are known. In general, arthropods, molluscs, and deuterostomian tunicates recognize the microbial surface determinants that are conserved and ubiquitous among microorganisms but not present in the eukaryotic host. These structures-mainly lipopolysaccharide, peptidoglycan, mannan, β-1,3 glucan, Gram-negative-binding protein, and C-type lectin-are recognized by means of a group of germline encoded receptors, usually termed pattern recognition receptors. Lipopolysaccharide and glucan-binding protein (LGBP) genes involved in the activation of prophenoloxidase (proPO) system were identified in almost every invertebrate species studied. The expression of proPO and LGBP genes is different in individual cell hemocyte types (Yang *et al.,* 2015a). These pattern recognition proteins participate in both humoral and cellular aspects of defense reactions by facilitating pathogenic

recognition of pathogen-associated molecular patterns (PAMP) and by subsequent triggering of a cascade of reactions such as phagocytosis, production of antibacterial peptides, activation of clotting, and proPO cascade (Lai *et al.,* 2011).

Recognition of PAMP provides an essential step for the activation of the proPO cascade (Amparyup *et al.,* 2008). Using a highly selective recognition process, signaling cascades are activated. These cascades regulate production of defense substances, agglutinins, opsonins, nonagglutinin factors, inducible or constitutive antibiotic peptides, and the components of the proPO complex in the host (Ratcliffe *et al.,* 1979). Specific nonself-recognition mechanisms of the proPO system, as a basic part of immune defense of invertebrates, are involved during a row of hierarchized processes like cell cooperation and communication in the course of phagocytosis, nodule and capsule formation, melanin and sclerotization, hemocyte locomotion, and coagulation of blood (Vetvicka *et al.,* 2004). Although the mechanism of the proPO system has been determined, the precise contribution of β-glucan-binding protein (GBP) integration with glucan for the activation remains to be fully elucidated. Some studies show that the GBP can be specifically degraded following the activation of proPO with glucan, suggesting that the variations in the GBP levels after specific challenge are an important regulation mechanisms to immune response (Zhang *et al.,* 2016).

Glucan-binding protein

Invertebrates are using innate immune mechanisms which are well conserved throughout the evolution of immunity. A heterologous group of hemolymph proteins serves as a surveillance mechanism by binding to the surface of invading microbes. One member of this group is GBP, which plays a critical role in triggering the innate immune response by detecting glucan found on the surface of microbes. GBP comprises of proteins that share sequence homology to β-glucanase of bacteria (Juncosa *et al.,* 1994) and of sea urchins (Bachman *et al.,* 1996). The first family of GBP was discovered in the hemolymph of *Bombyx mori* (Ochiai *et al.,* 1988). Subsequently, proteins binding to the β-glucan have been identified in numerous invertebrate species; their activity is usually stimulating the proPO activation cascade. Subsequent purification and identification revealed similar properties-proteins containing carboxyl-terminal glucanase-like domain without any enzymatic activity. Glucan is bound via less conserved amino-terminal domain. Older review studies of the GBP-protein binding are available (Vetvicka and Sima, 2004). Interestingly, in earthworms, the coelomic cytolytic factor shares strong homology with GBP isolated from numerous invertebrates (Bilej *et al.,* 2000).

Glucan-binding protein isolated from mangrove crab *Episesarma tetragonum* and its interaction with pathogens was evaluated. Molecular recognition showed the specific binding affinity towards β-glucan molecule. This bindings triggers the innate immunity inside the host (Sivakamavalli *et al.,* 2014). A molecular cloning of the LGBP isolated from Chinese mitten crab *Eriocheir sinensis* showed significant homology with the same protein in shrimp. Additional experiments showed that the highest level of expression is in hemocytes, but the protein is also expressed in hepatopancreas, muscles, gills, stomach, and intestines. The expression is upregulated after bacterial infection (Zhao *et al.,* 2009). Subsequent study showed that the recombinant LGBP triggers the whole hemolymph-dependent melanization and stimulates the proPO cascade (Zhang *et al.,* 2016). Additional study of GBP isolated from river crab *Paratelhupsa hydrodromus* confirmed anti-inflammatory, antioxidant, and antibiofim properties (Iswarya *et al.,* 2017a).

In shrimp, GBP plays the vital role in the recognition mechanisms against PAMP found in the membrane of fungi. Recognition of PAMP leads to the widespread innate immune activation including cellular and humoral components of defense (Sritunyalucksana *et al.,* 2000). Formation of GBP-glucan complex induces degranulation of hemocytes and activation of the proPO system. As the gene encoding GBP is abundant in white spot syndrome virus (WSSV)-resistant shrimp, it can be hypothesized that GBP is involved in antiviral response. Purification, characterization, and functional analysis of GBP from green tiger shrimp *Penaeus semisulcatus* showed it has a bifunctional role in proPO-enhancing activity and agglutinating activity. This GBP not only recognizes PAMP, but also induces intracellular signaling (Sivakamavalli *et al.,* 2013).

LGBP from shrimp *Fenneropenaeus chinensis* was cloned. Sequence analysis and comparison revealed a high identity of 94 %, 90 %, 87 %, and 72 % with *Paneaus monodon* β-1,3-glucan binding proteins, *Litopenaeus stylirostris* LGBP, *Marsupenaeu japonicus* β-1,3-glucan binding proteins, and *Homarus gammarus* β-1,3-glucan binding proteins, respectively (Liu *et al.,* 2009). In all cases, the transcription of LGBP increased at 6hrs postinfection, suggesting that this gene is not only a constitutive expression gene, but and an inducible acute-phase expression gene, the product of which is necessary to amplify the activity of proPO. Subsequent study named this peptide FcβGBP-HDL and showed that the full-length cDNA of 6713 bp has an open reading frame with two glucanase-like motifs and one arginylglycylaspartic acid motif. The expression was upregulated upon infection, but the level of changes was different in each tissue, suggesting that this protein performs its role differently in different tissues (Lai *et al.,* 2011). Similar homology between individual prawn species was found between LGBP genes from *Macrobranchium nipponense* and *M. rosenbergii* (89 % identity), *M. japonicus* (76 %), and *Fenneropaneus chinensis* (74 %). Again, expression of LGBP mRNA was elevated in the hepatopancreas (Xiu *et al.,* 2014). Similar data were found for LGBP gene from Indian white shrimp *F. inducus* (Valli *et al.,* 2012) and giant freshwater prawn *M. rosenbergii* (Yeh *et al.,* 2009). A recent study not ony cloned and characterized LGBP from *Fenneropeaeus merguiensis*, but showed its wide

specificity towards both Gram-positive and Gram-negative bacteria and yeast (Chaosomboon *et al.,* 2017). Similar studies performed in *F. chinensis,* however, showed binding activity towards Gram-negative bacteria only and expression of LGBP mRNA in hemocytes only (Du *et al.,* 2007).

In crayfish *Astacus leptodactylus*, there exists three apolipoproteins, all translated as a large precursor. Cleavage at the furin-type side results in high density LGBP (Stieb *et al.,* 2014), which is directly involved in innate immunity of Crustaceans (Schmidt *et al.,* 2010).

A GBP isolated from hemocytes of blue swimmer crab *Portunus pelagicus* was shown to have a multifunctional role in defence reactions including agglutination, proPO-enhancing activity, phagocytosis, and encapsulation. In addition, GBP reaction product exhibited antibacterial and antibiofilm activity against both Gram-positive and Gram-negative bacteria (Anjugam *et al.,* 2016).

Molecular cloning and characterization of GBP from *Plutella xylostella* showed significant similarities with β-glucan recognition proteins of other insects. The transcription levels were found upregulated by microbial challenges in all life stages; tissue distribution was mainly expressed in fat body (Huang *et al.,* 2015).

Rather different effects of GBP were found in experiments using ZnO nanoparticles coated by the crustacean GBP. These particles had significant antibacterial activities and showed cytotoxicity against HepG2 cancer cells (Iswarya *et al.,* 2017b), but the mechanisms remain unclear. Rather similar and more detailed data were found using peptides derived from GBP isolated from Pacific abalone *Haliotis discus hannai* (Nam *et al.,* 2016).

Lectins and opsonins were routinely found in molluscs, but only limited information about GBP is available. The first study found and isolated a GBP from the plasma of marine mussel *Perna viridis*. Further characterization showed a 510 kDa protein with ability to activate the proPO cascade via inherent serine protease activity (Jayaraj *et al.,* 2008).

In scallop *Chlamys farreri*, the LGBP has significant polymorphism, enhancing the binding activity of lipopolysaccharide and glucan, This protein has a direct association with disease resistance (Siva *et al.,* 2012). In Zhikong scallop *Chlamys farreri,* mRNA expression of LGPB in hemocytes was strongly upregulated by stimulation of lipopolysaccharide and β-glucan and moderately stimulated by peptidoglycan. A recombinant LGBP showed strong agglutination activity towards *Escherichia coli, Bacillus subtilis,* and *Pochia pastoris* (Yang *et al.,* 2010). These results suggest that LGBP plays not only a significant part in the response against Gram-negative bacteria as in other invertebrates (Lee *et al.,* 1996), but also against infection with Gram-positive bacteria. LGBP with vastly diverse specificities might function as a nonclonal effector for animal immune system. An excellent review of LGBP molecules in bivalves was published in 2015 (Allam *et al.,* 2015). One group managed chromosomal localization and molecular marker development of the LGBP gene in the Zhikong scallop *Chlamys farreri* (Huan *et al.,* 2010).

Molecular characterization and gene expression analysis of LGBP was also successfully achieved in the hard clam, *Meretrix meretrix*. The expression was observed in six different tissues, the highest levels in the gill and digestive gland tissue (Liu *et al.,* 2014).

Direct effects of glucan in invertebrates

In earthworms injected with glucan, an increase in celomic cytolytic factor and lysozyme-like activity was reported. Is seems that this action is caused by direct binding of glucan to hemocytes (Kohlerova *et al.,* 2004; Vetvicka and Sima 2004).

Diet supplementation of sea cucumber *Apostichopus japonicus* with glucan resulted in strong enrichment of intestinal-dominant classes and in increased proliferation of the *Rhodobacteraceae* and *Verrucomicrobiaceae* families. In addition, glucan addition had significant impact on immune response of the intestine via NF-κB signaling pathway (Yang *et al.,* 2015b). In scallops, glucan treatment increased the expression of cf/TEP, leading to higher survival of those infected by *Vibrio* (Xue *et al.,* 2017).

One of the most common targets of glucan are shrimp, as the negative impact of WSSV can be commercially significant. A detailed study revealed how the pattern recognition protein binds to glucan and subsequently activates the proPO system (Amparyup *et al.,* 2012). Besides general immunostimulating activity, various glucans were found to offer protection in *Penaeus monodon* post larvae against WSSV infection by changing the immune gene expression (Wilson *et al.,* 2015). A study (Bae *et al.,* 2012) of flesh shrimp *F. chinensis* showed that glucan supplementation in absence of pathogen challenge increased total hemocyte counts. Single administration of glucan prior to the WSSV challenge resulted in strong activation of the proPO cascade and reduced shrimp mortality up to 50 % (Thitamadee *et al.,* 2014). However, the second application of glucan led to a significant increase in mortality, most probably through the combination of WSSV infection and overproduction of reactive oxygen species. These data suggest that with prolonged application of glucan in shrimp aquaculture, caution should be prudent as more is not always better (Wang *et al.,* 2013).

Conclusions

Many of the genes for GBP and LGBP are highly expressed in the digestive glands, particularly in bivalves, suggesting that the filter-feeding life might show biased pattern recognition towards digestive system as a first line of defense (Allam and Raftos, 2015). Clearly, the importance of these binding molecules in invertebrates is extremely high, with some authors even suggesting that the functional diversity of these molecules is comparable to antibodies in vertebrates (Fisher *et al.,* 1991).

However, the current rush to clone and characterize the LGBP in various invertebrates is reminiscent of the golden age of competitive immunology, when the scientists happily evaluated phagocytosis or other immune attributes in one

species after another. A plethora of reports on LGBP in invertebrates concludes that this protein plays an important role against infection in crustaceans. Although the detailed characterization of this protein in individual species adds to the mosaic of our knowledge of the immune reaction in invertebrates, the rush to be first to describe it in an additional species is counterproductive to the real needs in this field.

Anticancer activities of either GBP or GBP-derived peptides offer a new window for GBP research. Particularly important may be the peptides, as they can be produced in a cost-effective manner. If more data confirm these results, it might potentially offer a new way how to obtain anticancer drugs.

In addition to the use of GBP, externally added glucan was also found to significantly improve immune reactions of invertebrates. However, due to the limited available data, our knowledge of possible mechanisms is still lacking.

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