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RESEARCH REPORT

The biological role of hemolymph lectins in *Episesarma tetragonum*

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Abstract

The hemolymph of the mangrove crab, *E. tetragonum* contains lectins specific for NeuGcα 2, 3 Gal β1-4 GluNAc β1 linkage and O-acetyl sialic acids. The role of sialic acid specific lectins on natural immunity of the crab is studied by using several kinds of mammalian erythrocytes as pathogen model. Injection of erythrocytes showing differential agglutinability with the lectins, induced augmentation of hemagglutinating activity suggesting an increase in the circulating lectins. A significant correlation was observed between *in vivo* clearances of exogenous erythrocytes with the extent of erythrocyte agglutination by the lectins. Another correlation was observed between the susceptibility of erythrocytes to lectin dependent hemocyte mediated hemolysis and the extent of lectin mediated erythrocyte agglutination. This study documents that opsonization of foreign pathogen by the native lectins is an important step in hemocyte recognition, hemolysis and clearance of the pathogen.

Key Words: innate immunity; opsonin; hemolysis; hemocytes; induction; clearance

Introduction

The great success of arthropods in diverse environments of earth must certainly rely on efficient immune defenses capable of protecting these animals against the microbial invasion. Lectins from the hemolymph of invertebrates have been regarded as potential molecules involved in immune recognition and phagocytosis of the microbes through opsonization (Marques and Barracco, 2000).

Lectins are proteins or glycoproteins usually without catalytic activity that have the ability to bind to specific carbohydrates expressed on different cell surfaces. Their specificity is always determined by the type of carbohydrate to which they bind.

Due to the fact that lectins have the ability to bind carbohydrate and promote the agglutination of different cells, such as bacteria and other invading pathogens, it is reasonable to assume that these molecules may be regarded as having a potential role in invertebrate nonself - recognition reactions. Many recent studies have emphasized the possible role of lectins as nonself - recognition molecules in invertebrate immunity (Zhu *et al.,* 2006; Vasta *et al.,* 2007; Yang *et al.,* 2011; Jin *et al.,* 2013). As with vertebrate immunoglobulins, lectins can agglutinate microorganisms and enhance their phagocytosis by acting as an opsonin (Arason, 1996; Gasparini *et al.,* 2008; Franchi *et al.,* 2011), and are apparently synthesized by invertebrate immune cells (hemocytes). However in contrast to immunoglobulins, the specificity of invertebrate lectins is restricted only to glycoprotein and sugar residues.

In spite of their apparent ubiquity, and the remarkable number of recent publications on their occurrence, structure, and specificity, the natural functions of lectins are still not fully understood. Interactions between lectins and carbohydrates have been shown to be involved in various activities. Therefore, different biological roles have been proposed for these molecules, including the cellular and tissue transport of carbohydrates, glycoproteins and calcium (Goldstein *et al*., 1980; Ravindranath and Coopper, 1984), correlation to insect development (Armstrong *et al*., 1996), cell adhesion, migration and apoptosis (Perillo *et al.,* 1995; Ni and Tizard, 1996).

Compared to other arthropodan groups, such as insects and horseshoe crabs, the current knowledge on lectin involvement in crustacean nonself - recognition is still much less well established. The hemolymph of the mud crab, *Scylla serrata* contains sialic acid specific lectin, which increased markedly on challenging with erythrocyte species that it agglutinated best (Mercy and Ravindranath, 1993). Similar augmentation of agglutinin activity after administration of erythrocytes was also observed in the freshwater crab *Paratelphusa jaquemontii* (Maghil, 2001), and

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anomuran crab *Emerita emeritus* (Jayasuriya, 2002). The enhancement of lectin activity in response to foreign cells suggests a specific lectin - foreign cell interaction.

Most crustacean lectins seem to share a common specificity to N-acetylated aminosugars, particularly sialic acids (Marques and Barracco, 2000). Sialic acids are a family of N- and O-acetyl or N-glycolyl derivatives of nine carbon monosaccharide neuraminic acid (Schauer, 1982), among which, N-acetyl neuraminic acid is the most common in nature. Sialic acid residues are commonly encountered in the cell membranes of almost all deuterostome animals and are virtually absent in protostomes including crustaceans (Schauer, 1982). Hence, the occurrence of sialic acid - lectins in the body fluids of crustaceans can indicate that these molecules could have a role in defense against exogenous sialylated pathogens (Cominetti *et al*., 2002). However, their precise role in the above function remains to be elucidated. Therefore, it was felt important to know the biological role of sialic acid binding lectins in the biology of crabs.

The lectins in the hemolymph of crabs are likely to form an innate immunological defense of organisms which can be verified by inducing immune response with model foreign antigens. To verify the above hypothesis, the mangrove crab *E. tetragonum*, was taken for the study and challenged with erythrocytes as an antigen model.

Materials and Methods

Sample collection

The mangrove crab, *Episesarma tetragonum* was collected for the study from the mangrove and fresh water regions of Manakudy, Kanyakumari district, Tamil Nadu. The crabs were maintained in plastic tubs with fresh water and mud. The water and mud were changed on alternate days and was fed with paddy grains.

Purification of lectin

As reported elsewhere (Devi *et al*., 2013), affinity purification of *Episesarma tetragonum* agglutinin-1 (EtA-1) was performed using cyanogen bromide activated sepharose 4B in an econo column (Bio-Rad) previously equilibrated with TBS at 4 °C. The elution of lectin (EtL-1) was done with elution buffer that contained 100 mM GluNAc and collected 1 ml fractions on ice in polypropylene tubes containing 10 µl of 100 mM calcium chloride at a rate of 0.3 ml/min. The fractions were vortexed immediately after collection and kept on ice. Fractions containing lectin were pooled on the same day and dialyzed against 1 mM $CaCl_2$, at 4 °C for 3 h and the dialysate was then aliquoted, lyophilized (speed-vac, Sawant), and stored at -20 °C.

HA assays to determine physicochemical parameters

The physicochemical properties of the purified lectin was determined by hemagglutination assays with serum samples under conditions of varying pH, temperature, bivalent cation of diverse concentration, EDTA and some chemical agents.

Table 1 Differences in the time taken for clearance of erythrocytes from circulation before and after coating the erythrocytes with sub agglutinating concentrations of the hemolymph lectins of *E. tetragonum*

Erythrocytes	HA titer (N = 9)	Time taken for clearance (min)	
		Before	After
Dog	64	45 ± 4.98	20 ± 2.29
Horse	32	60 ± 3.31	30 ± 3.05
Human O	4	240 ± 4.52	180 ± 2.81

N = number of crabs studied

HA assays were performed as described by Devi *et al*., 2013.

Hemagglutination inhibition (HAI) assay

Hemagglutination inhibition assay was performed to test the ability of various glycoproteins (fetuin, transferrin, porcine thyroglobulin, alpha acid glycoprotein, porcine and bovine submaxillary mucin, apotransferrin, bovine thyroglobulin) and sugars (GluNAc, galactose, NeuGc, maltose, mannose, lactose, GalNAc, NeuAc) to inhibit agglutination. Hemagglutination inhibition titer was reported as the reciprocal of the lowest dilution of inhibitors giving complete inhibition of agglutination after 1 hour.

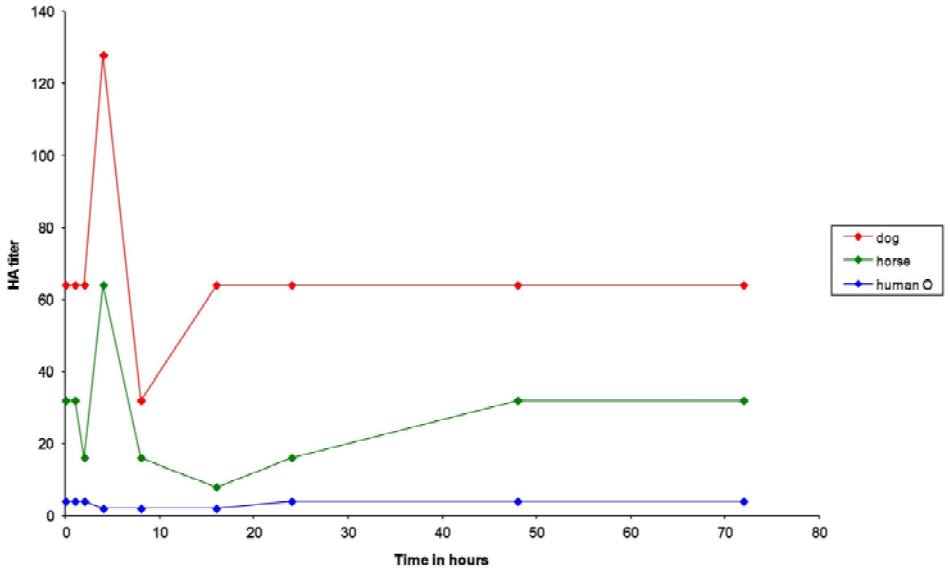
Sialidase treatment of sialoglycoprotein

Asialo fetuin was prepared by incubating 2 mg of glycoprotein (fetuin) with 0.1 unit of *Clostridium perfringens* sialidase (Sigma type X) in 400 µl of 5 mM acetate buffer, pH 5.5 for 2 h at 37 °C. As a control, fetuin was treated similarly without sialidase. HAI assay was performed with purified lectin for sialidase treated and untreated fetuin against 1.5 % dog erythrocyte suspension.

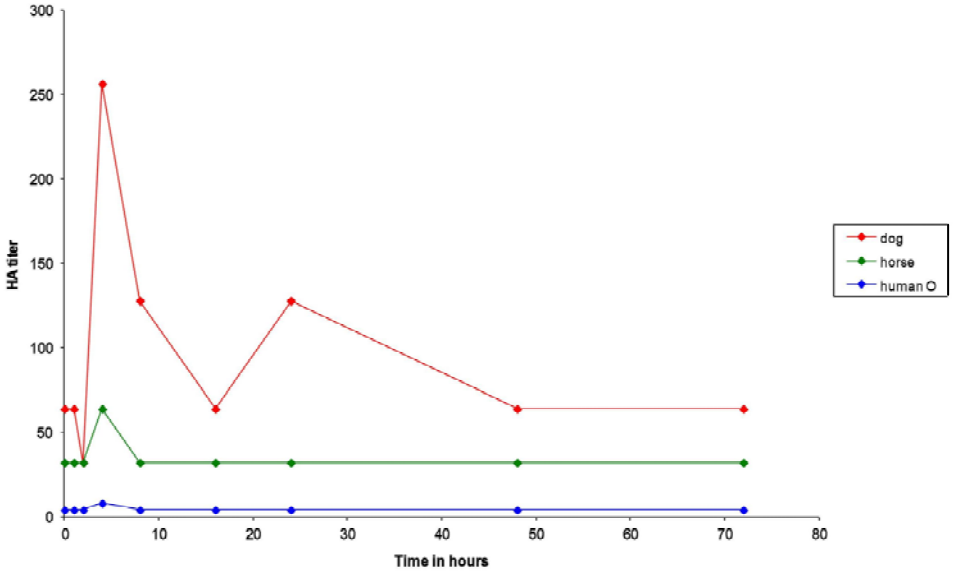
Erythrocyte collection

The erythrocytes selected for the experiments included dog, horse and human O. Human O erythrocytes were obtained with thanks from Kanya blood bank (Nagercoil). Equine blood was collected from the jugular vein and canine blood from cephalic vein. The blood samples were directly collected in sterile modified Alsevier's medium (30 mM sodium citrate, 77 mM sodium chloride, 114 mM glucose, 100 mg neomycin and 330 mg chloramphenicol). Before use, the erythrocyte types were suspended and washed thrice in Tris Buffered Saline (TBS: 50 mM Tris-HCl, pH 7.5, 100 mM sodium chloride, 10 mM calcium chloride) and resuspended in the same buffer to get required cell suspension.

A)


HA titer
Time in hours
dog
horse
human O

B)


HA titer
Time in hours
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horse
human O

C)

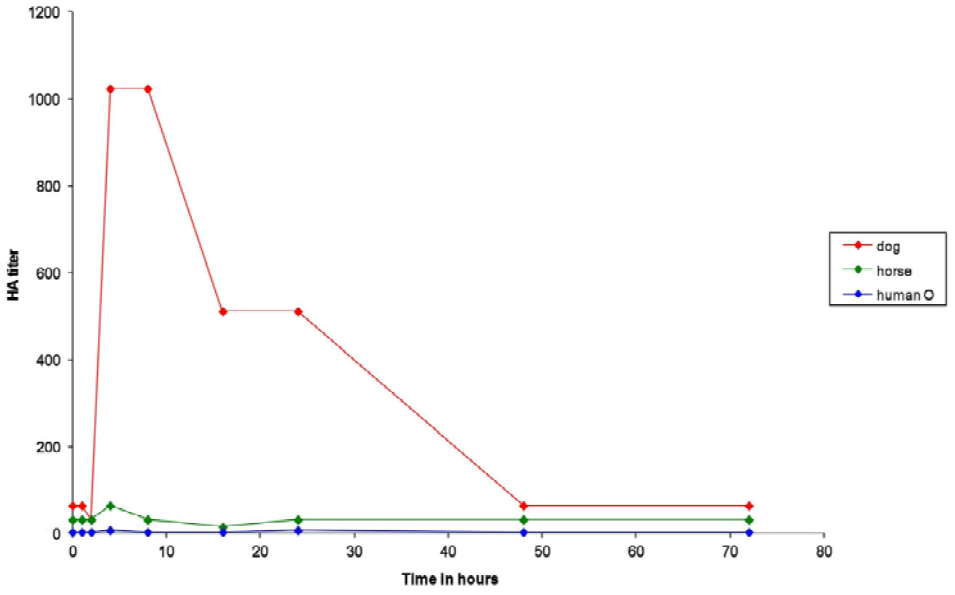

HA titer
Time in hours
dog
horse
human O

Fig. 1 Effect of injection of 1.5 % (**A**); 2.5 % (**B**); 5 % (**C**) dog erytrocytes on the HA titer of the hemolymph agglutinins of *E. tetragonum*.

Induction Experiments

a) Injection of erythrocytes

In each experiment 0.1 ml of 1.5 %, 2.5 % and 5 % of dog, horse and human O erythrocyte suspension in 0.9 % sterilized saline, was injected into the crabs separately. All injections were made slowly into the soft arthrodial membrane between the coxa of the fourth preopod and the dorsal surface of the carapace. The injection site was blotted with cotton both before and after the injection of the erythrocytes. Those crabs that bled at the injection site, which occurred if they moved vigorously during injection, were discarded. Care was taken to ensure complete injection of the erythrocytes.

b) Collection of hemolymph samples

To study the effect of erythrocytes injected into the hemocoel of crabs, on humoral agglutinin activity, hemolymph was collected at regular intervals (1, 2, 4, 8, 16, 24, 48 and 72 h) after the injection and HA assay was done with dog, horse and human O erythrocytes.

Clearance Experiments

a) Injection of erythrocytes

The erythrocytes selected for the study on clearance also included dog, horse and human O erythrocytes. In each experiment 100 µl of 1.5 % erythrocyte suspension in saline was injected into the hemocoel of crabs. The method of injection was same as stated for the experiment on induction.

b) Pretreatment of erythrocytes with lectins

To find out whether the clearance of erythrocytes was enhanced consequent to lectin binding, erythrocytes were coated with purified lectin diluted to sub agglutination concentration. The sub agglutination concentration differed for different erythrocytes, as shown in table 1. Resuspended 200 µl of washed and packed erythrocytes in 20 volumes of lectin (diluted to sub agglutinating concentration) and incubated the mixture for 1 h at 30 °C. The lectin coated erythrocytes were washed and resuspended in sterilized saline and were injected as stated earlier. The erythrocyte suspensions were examined under the microscope to ensure the presence or absence of clumps of erythrocytes. Clumps were disrupted by gentle vortexing. The rate of clearance before and after lectin coating was compared.

c) Collection of hemolymph samples

To study the clearance of injected erythrocytes from the circulation, hemolymph (100 µl) was collected with a micropipette at regular time intervals (5 minutes) until the injected erythrocytes completely disappeared from the circulation. Hemolymph was added to 700 µl of double distilled water, and after mixing, the total volume was adjusted to 1 ml with double distilled water.

Estimation of hemoglobin

The amount of hemoglobin was estimated using the cyanmethhemoglobin method, a hemoglobin kit manufactured by Sigma Diagnostics (India) Pvt. Ltd. Baroda. Product No. 72431 of Qualigens diagnostics designed for in vitro estimation of hemoglobin. The cyanmethhemoglobin technique is the method of choice selected by the International Commission for Standardization in Hematology (ICSH). The method measures all hemoglobin derivatives except sulfhemoglobin.

Hemolysis

To study the interaction of crab hemocytes on lectin - treated erythrocytes, the hemocytes were separated using the method of Soderhall and Smith (1983). The hemocytes were washed twice to remove the contaminating proteins and used for hemolysis experiments. The hemocyte suspension was added to the lectin - coated or uncoated erythrocytes and incubated for 1 h at 30 °C, and then the erythrocyte - hemocyte mixture was centrifuged at 200 x g for 5 min. The supernatant was collected for estimation of hemoglobin content, which was measured following the cyanmethhemoglobin method. The control included lectin coated or uncoated erythrocytes without hemocytes.

Results

Physicochemical properties of purified lectin (EtL-1) specific to dog erythrocytes

The HA activity of the purified lectin was sensitive to pH and temperature. The HA was stable between pH 6.5 - 9.5 and at temperature ranging from 10 - 40 °C. Among the cations tested, divalent calcium ions did not have any effect on HA titer, however magnesium at 10 - 0.1 mM concentration reduced the HA titer to 32 while at 100 mM concentration gave the normal HA titer. The metal ion chelator - EDTA at very low concentrations (1 - 0.01 mM) decreased the HA activity (HA = 32). At concentrations from 1 - 5 mM, the HA activity increased one fold from the normal HA (128) and at concentration from 10 - 20 mM there was sudden decrease in HA activity, after which the HA activity was completely lost. The agglutinating activity was completely inhibited by chloroform while the activity was greatly inhibited by incubation with denaturing agents such as HCl and NaOH.

Hemagglutination inhibition (HAI) assay

The inhibitory potency of assayed compounds on HA by EtL-1 was as follows: Fetuin > porcine thyroglobulin = transferrin > α-acid glycoprotein > PSM > apotransferrin = bovine thyroglobulin. The purified EtL-1 showed a remarkable inhibitory potency with fetuin containing sialic acids with α, 2-3 linkages. On the other hand, BSM and lactoferrin failed to inhibit the HA activity of EtL-1. To further define the possible role of sialic acids as potent inhibitor of lectin, the sialoglycoprotein, fetuin was enzymatically modified and its derivative was examined for HAI. Sialidase treatment of fetuin reduced its inhibitory properties at 20 h. In order to find out if EtL-1 was NeuAc or NeuGc specific, free sialic acids NeuAc and NeuGc were tested as

inhibitors of hemagglutination. NeuAc did not inhibit hemagglutination whereas NeuGc inhibited. However NeuGc linked glycoproteins were more inhibitory than free NeuGc. Galactose that showed

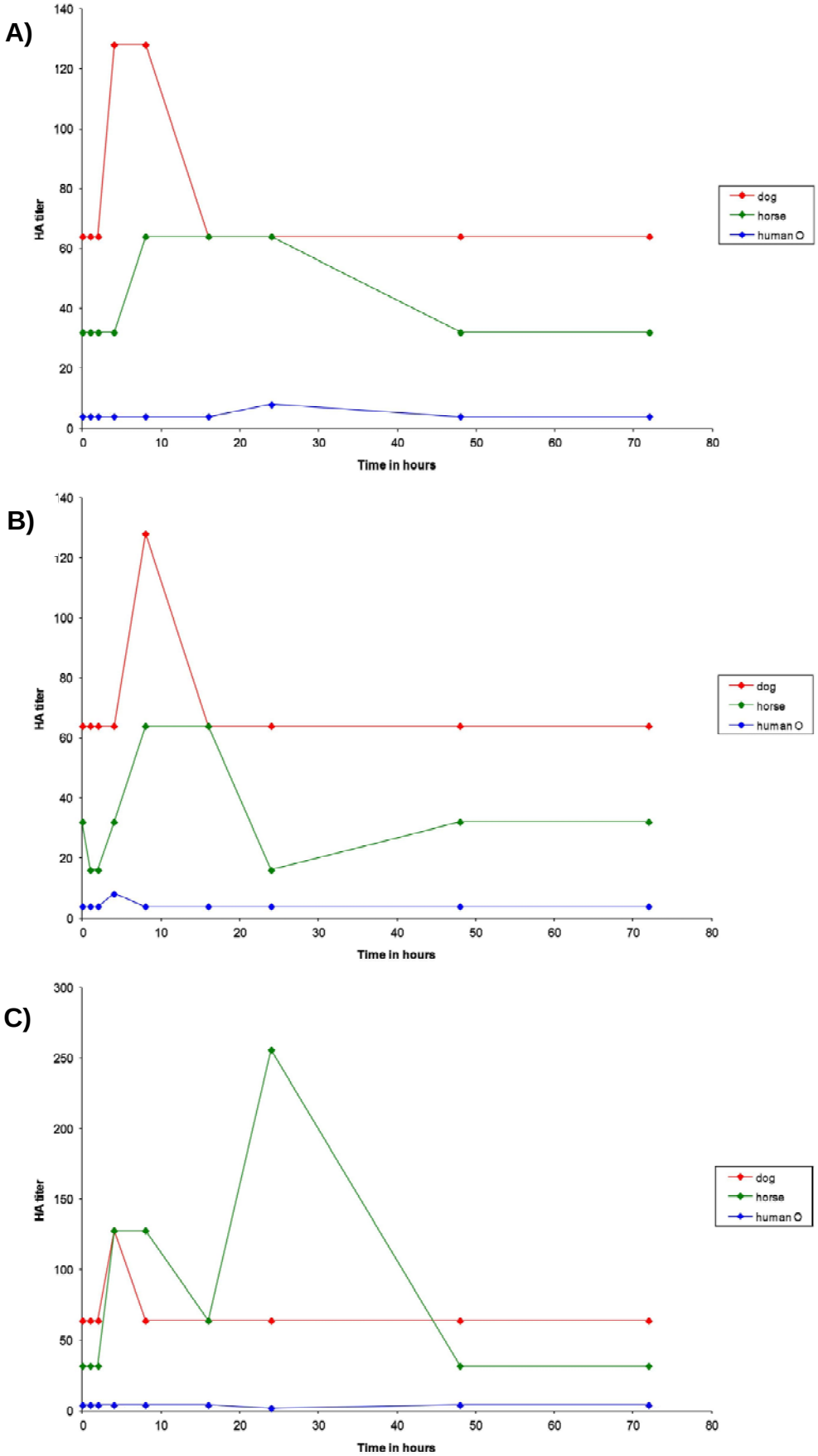


Fig. 2 Effect of injection of 1.5 % (**A**); 2.5 % (**B**); 5 % (**C**) horse erytrocytes on the HA titer of the hemolymph agglutinins of *E. tetragonum.*

very low inhibitory potency with crude agglutinin was a potent inhibitor of purified EtL-1.

Profile of HA after injection of dog erythrocytes

Injection of dog erythrocytes enhanced the agglutinin activity of the hemolymph. The HA titer of induced agglutinins varied with time and concentration of dog erythrocytes administered. Injection of 1.5 % dog erythrocytes augmented the HA activity to two fold with dog and horse erythrocytes at 4 hour post injection. Human O erythrocytes showed insignificant variation in HA titer (Fig.1A). After injection of 100 µl of 2.5 % dog erythrocytes the HA activity increased four fold with dog erythrocytes at 4 h, which showed gradual decrease and then heightened activity at 24 hours post injection (Fig.1B). Injection of 5 % dog erythrocytes showed six fold increase in HA activity with dog erythrocytes within 4 - 8 h of post injection (Fig.1C).

Profile of HA titer after injection of horse erythrocytes

Injection of 1.5 % horse erythrocytes showed an enhancement of agglutinin activity between 8 - 24 hours with horse erythrocytes and between 4 - 8 hours with dog erythrocytes (Fig. 2A). Injection of 2.5 % erythrocytes, slightly depressed the HA activity with horse erythrocytes at 2 hours and then it showed a consistent increase and the peak of activity was observed between 8 - 16 h post injection. However with dog erythrocytes HA activity showed an augmentation only at 8 hours post injection (Fig. 2B). Injection of 5 % horse erythrocytes enhanced the HA activity with two peaks, one at 4 - 8 h and another at 24 h post injection with horse erythrocytes. But only a single peak of HA activity occurred with dog erythrocytes at 4 h post injection. Human O showed no augmentation in HA activity (Fig. 2C).

Profile of HA titer after injection of Human O erythrocytes

Injection of 1.5 % human O resulted in four fold and two fold increase in agglutinin activity with dog and horse erythrocytes respectively at 4 h of post injection. However HA activity of dog erythrocytes was depressed at 8 - 16 h post injection, and regained its preinjection level within two days (Fig. 3A). Injection of 2.5 % human O erythrocytes induced and augmented agglutinin activity as evidenced in four fold increase in HA titer with dog erythrocytes and two fold increase with horse erythrocytes. Human O showed very low HA titer (Fig. 3B). Similarly on injection of 5 % human O erythrocytes, six fold increase in HA activity was observed with dog erythrocytes after 4 h of post injection. However the animals were unable to counter the challenge and crumbled after 48 h of post injection (Fig. 3C).

Clearance of erythrocytes

With a view to elucidate the biological role of lectins in the defense strategy of the crab, *E. tetragonum*, an attempt was made to find out whether there is any correlation between erythrocyte clearance and their respective HA titers. A positive correlation was observed between lectin agglutinability of different erythrocytes and the time taken for complete clearance of the respective erythrocytes. The hemoglobin level in the hemolymph indirectly measures clearance of erythrocytes at different time intervals after injection of different erythrocytes. Dog and horse erythrocytes that were strongly agglutinated by the agglutinins were cleared faster than the human O erythrocytes (Table 1). Faster clearance of lectin coated erythrocytes was also observed. The lectin coated dog erythrocytes were cleared within 20 min whereas uncoated dog erythrocytes were cleared only by 45 min from the hemolymph. Similarly, the lectin coated horse erythrocytes were cleared in less than 30 min whereas uncoated erythrocytes cleared only by 60. On the contrary, the crabs required 240 min to clear the low agglutinating human O erythrocytes, 180 min to clear the lectin coated human O erythrocytes (Table 1).

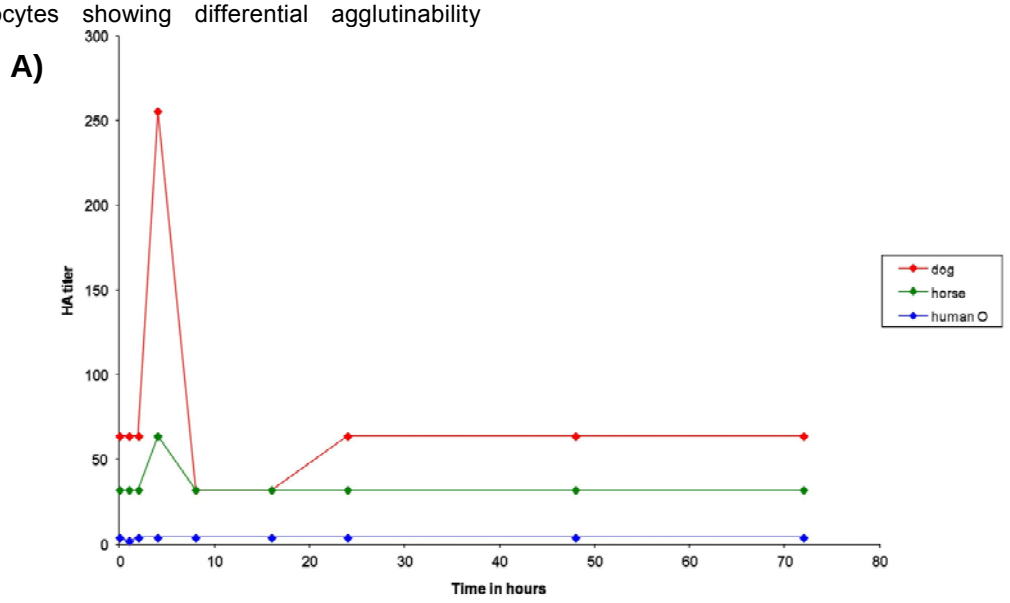
Hemocyte mediated hemolysis

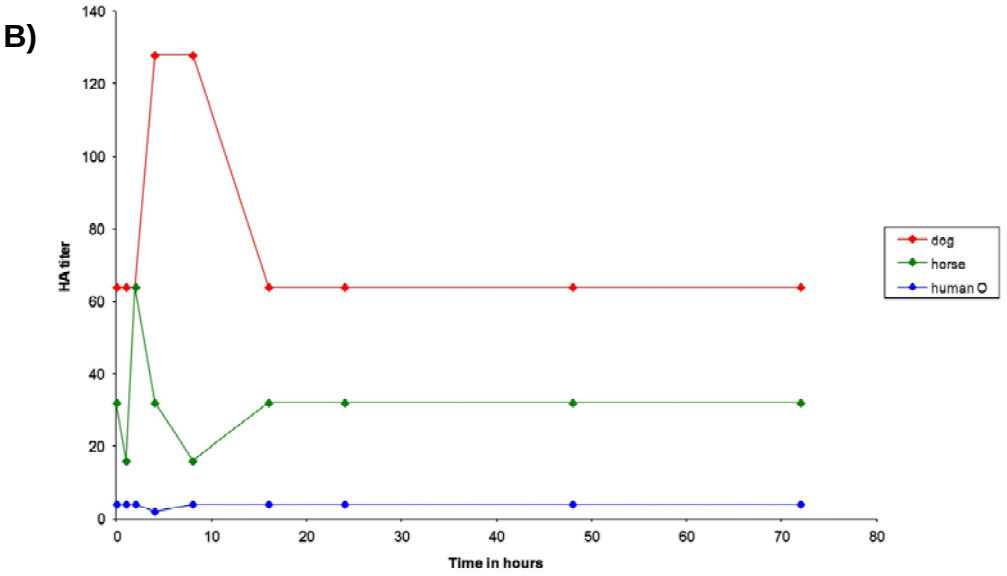
Hemolysis was measured after mixing hemocytes with lectin coated or uncoated erythrocytes. Lectins or hemocytes alone had very weak effect to bring about hemolysis of any of erythrocytes, whereas the hemocytes with lectin markedly facilitated hemolysis of erythrocytes (Table 2). The results clearly established a positive correlation between hemocyte mediated hemolysis of erythrocytes and their respective HA titers. Lectin coated dog and horse erythrocytes showed marked lysis when compared to lectin coated human O erythrocytes.

Discussion

The ability to distinguish self from nonself is the fundamental aspect of immunity. Lectin - carbohydrate recognition represents a ligand - receptor interaction that is universal in organisms (Cambi and Figdor, 2003). Arthropods display both cellular (hemocyte - mediated) and non - cellular (humoral) responses against a wide variety of antigens and recognize self from non - self molecules (Gallo *et al.*, 2011). Lectins, non - enzymic proteins that bind mono - and oligosaccharides reversibly and with high specificity, occur widely in nature (Sharon and Lis, 1995). Lectins are receptor specific proteins (Muramoto *et al*., 1995) whose biological role is meagerly understood. Considering the report that crustaceans, like other protostomians, are not capable of synthesizing sialic acids (Segler *et al.,* 1978), any physiological role for the lectin should be implied with caution. In the absence of any interaction of the lectin with crab tissue components due to paucity of sialic acids in the tissues, the lectin may function as a defensive tool, protecting the host by binding with pathogens such as bacteria and virus that may contain sialoglycoproteins or sialopolysaccharides.

In this study, the biological role of hemolymph lectin of the crab *E. tetragonum* is elucidated. Erythrocytes showing differential agglutinability with the lectin are used as pathogen model. The lectin demonstrates specificity for the erythrocytes of





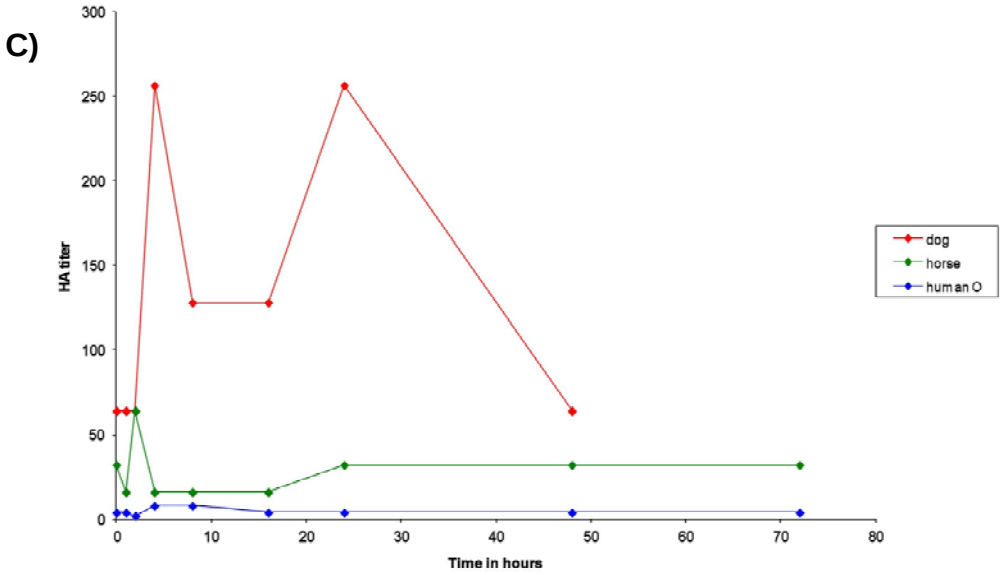


Fig. 3 Effect of injection of 1.5 % (**A**); 2.5 % (**B**); 5 % (**C**) human O erytrocytes on the HA titer of the hemolymph agglutinins of *E. tetragonum.*

Table 2 Effect of hemocytes on hemolysis (measured as equivalent of µg of hemoglobin released) from erythrocytes coated with or without lectins purified from *E. tetragonum*

Treatment (N = 12)	Hemoglobin content in erythrocytes (µg)		
	Dog	Horse	Human O
Control	28	32	30
Uncoated erythrocytes + hemocyte suspension	1± 0.24	0.86± 0.036	0.92 ± 0.05
Lectin coated erythrocytes + hemocyte suspension	20.5±2.31	18±1.34	4.5±4.2
Lectin coated erythrocytes without hemocyte suspension	0.5 ± 0.04	0.5 ± 0.07	0

different species. The high HA titer with dog and horse cells indicates the preponderance of the lectin specific sugar moieties on dog and horse erythrocytes. The low HA titer for human O indicates that the sugar residues on these erythrocytes may not have the proper conformation to be recognized by the hemolymph lectin. Injection of dog erythrocytes preferentially augmented the HA activity with dog erythrocyte and, of horse erythrocyte augmented the HA activity with horse erythrocyte. This clearly revealed the induction of two different lectins (as reported in Devi *et al*., 2013) in the hemolymph of crab, *E. tetragonum*. Following injection of 1.5 %, 2.5 % and 5 % dog erythrocytes, the hemolymph lectin exhibited a slight decrease in the HA from the initial titer of 64 with dog erythrocytes to 32 between 1 - 2 h and an increase to 2, 4 and 16 fold respectively between 4 - 24 h. The normal titer value was resumed by 48 - 72 h. Similarly injection of horse erythrocytes resulted in normal and low HA followed by an enhanced hemagglutinin activity, but at 16 h of post injection the hemagglutinin activity declined and then enhanced to attain the second peak, suggesting that the adaptive survival of the crabs depended mainly on the inductive property of the lectin. Subsequent increase in HA activity and the peak activity at 4 - 8 h suggest that some other tissue may be secreting the lectin when the hemolymph lectins are used up for elimination of pathogen or injected erythrocytes. Similar patterns of augmentation in lectin activity after administration of erythrocytes is also reported in earthworms (Stein *et al.,* 1987), insects (Jayalakshmi, 2005), oyster, *Crassostrea gigas* (Hardy *et al.,* 1977) fresh water snail, *Planorbarius corneus* (Ottoviani *et al.,* 1986), millipedes (Basil Rose, 1999) and crabs (Mercy and Ravindranath, 1994; Jayasuriya, 2000; Maghil, 2001).

In crabs an enhancement in agglutinin activity occurred at a short duration after the injection of erythrocytes. This suggests the possibility of release of the lectin from other tissues. Presence of hemagglutinating activity in the hepatopancreas of the sand crab *Emerita emeritus* (Jayasuriya, 2000), fresh water crab, *P. jacquemontii* (Maghil, 2001) and the mud crab, *S. serrata* (Mullainadhan, 1984) support such a possibility.

The important finding that emerges from this study is that, there is a distinct positive correlation between clearance time and HA titers with different erythrocytes. This correlation suggests that the efficiency of clearance of injected erythrocytes is reflected in the HA titer against different erythrocytes. The rate and time taken for clearance of erythrocytes coated with lectin revealed that the clearance is faster with both high agglutinating dog and horse erythrocytes. Hence lectins coated on the erythrocytes made them to be easily recognized by the hemocytes and, clear them by hemolysis. Thus lectins have been known as playing a central role in nonself - recognition and clearance of invaders in invertebrate immunity. Faster clearance and more effective hemolysis of lectin-coated erythrocytes clearly indicate that the hemolymph lectins may function as a recognition mediator between the foreign cells

Conclusions

From these observations it can be summarized that the crab, *E. tetragonum* is physiologically adapted to challenge foreign cells by enhancing the production of lectins in the hemolymph. These lectins may now act as "opsonins" by coating the foreign cells and makes them to be easily recognized by hemocytes, followed by hemolysis and rapid clearance from the animal. Our investigations on induction, clearance and hemolysis, strongly support the role of lectins as recognition molecule in the defense strategy of the crab *E. tetragonum*.

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References

Arason GJ. Lectins as defense molecules in vertebrates and invertebrates. Fish Shellfish Immunol. 6: 277-289,1996.

Armstrong PB, Swarnakar S, Srimal S, Misquith S, Hahn EA, Aimes RT *et al*. A cytolytic function for a sialic acid binding lectin that is a member of the pentraxin family of proteins. J. Biol. Chem. 271: 14717-14721, 1996.

Basil Rose MR. Millipede hemolymph lectin: Nature, source and possible function. Ph.D Thesis, Manonmaniam Sundaranar University, Thirunelveli, India, 1999

Cambi A, Figdor CG. Dual function of C-type lectin-like receptors in the immune system. Curr. Opin. Cell. Biol. 15: 539-546, 2003.

Cominetti MR, Marques MR, Lorenzini DM, Löfgren SE, Daffre S, Barracco MA. Characterization and partial purification of a lectin from the hemolymph of the white shrimp *Litopenaeus schmitti.* Dev. Comp. Immunol. 26: 715-721, 2002.

Devi RV, Basil Rose M, Mercy PD. Sialic acid specific lectins from *Episesarma tetragonum* (Decapoda, Grapsidae): isolation, purification and characterization. Int. J. Aqu. Biol. 1: 150-157, 2013.

Franchi N, Schiavon F, Carletto M, Gasparini F, Bertoloni G, Tosatto SC, *et al.* Immune roles of a rhamnose-binding lectin in the colonial ascidian *Botryllus schlosseri*. Immunobiology 216: 725-36, 2011.

Gallo C, Schiavon F, Ballarin L. Insight on cellular and humoral components of innate immunity in *Squilla mantis* (Crustacea, Stomatopoda). Fish Shellfish Immunol. 31: 423-431, 2011.

Gasparini F, Franchi N, Spolaore B, Ballarin L. Novel rhamnose-binding lectins from the colonial ascidian *Botryllus schlosseri*. Dev.Comp. Immunol. 32: 1177-1191, 2008.

Goldstein IJ, Hughes RC, Monsigny M, Osawa T, Sharon N. What should be called a lectin? Nature 285: 66, 1980.

Hardy SW, Grant PT, Fletcher TC. A haemagglutinin in the tissue fluid of the Pacific oyster, *Crassostrea gigas*, with specificity for sialic acid residues in glycoproteins. Experientia 33: 767-769, 1997.

Jayalakshmi M. Coconut pest *Oryctes rhinoceros* lectin: Natural purification and ecophysiological significance. PhD thesis, Manonmaniam Sundaranar University, Tirunelveli, India, 2005.

Jayasuryia S. Identification, Purification, Characterization and biological role of a sialic acid specific lectin from the hemolymph of the anomuran crab *Emerita emeritus* (Linnaeus). PhD Thesis, Manonmaniam Sundaranar University, Thirunelveli, 2002.

Jin KX, Li S, Guo XN, Cheng L, Wu MH, Tan SJ, *et al.* Two antibacterial C-type lectins from crustacean, *Eriocheir sinensis*, stimulated cellular encapsulation *in vitro.* Dev. Comp. Immunol. 41: 544-552, 2013.

Maghil D. Purification, Characterization and biological role of a sialic acid specific lectin from the hemolymph of the fresh water crab *Paratelphusa jaquemontii* (Rathbun). PhD Thesis, Manonmaniam Sundaranar University, Tirunelveli, 2001.

Marques MRF, Barracco MA. Lectins as non-self recognition factors, in crustaceans. Aquaculture 191: 23-44, 2000.

Matsushita M. The lectin pathway of the complement system. Microbiol. Immunol. 40: 887-893, 1996.

Mercy PD, Ravindranath MH. Purification and characterization of N-glycolyneuraminic-acid-specific lectin from *Scylla serrata*. Eur. J. Biochem. 215: 697-704, 1993.

Mullainadhan P, Ravindranath MH, Wright RK, Cooper EL. Crustacean defense strategies. I. Molecular weight dependent clearance of dyes in the mudcrab *Scylla serrata* (Forskal) (Portunidae: Brachyura). Dev. Comp. Immunol. 8: 41-50, 1984.

Muramoto K, Matsuda T, Nakada K, Kamiya H. Occurrence of multiple lectins in the hemolymph of kuruma prawn, *Penaeus japonicus*. Fish. Sci. 61: 131-135, 1995.

Ni Y, Tizard I. Lectin-carbohydrate interaction in the immune system. Vet. Immunol. Pathol. 55: 205-223, 1996.

Ottaviani E, Aggazzotti G, Tricoli S. Kinetics of bacterial clearance and selected enzyme activities in serum and haemocytes of the freshwater snail *Planorbarius corneus* (L.) (Gastropoda, Pulmonata) during the primary and secondary response to *Staphylococcus aureus*. Comp. Biochem. Physiol. 85A: 91-95, 1986.

Perillo NL, Pace KE, Seilhamer JJ, Baum LG. Apoptosis of T cells mediated by galectin-I. Nature 378: 736-739,1995.

Ravindranath MH, Cooper EL. Crab lectins: receptor specificity and biomedical applications. Prog. Clin. Biol. Res. 157: 83-96, 1984.

Renwrantz L. Lectins in mollusks and arthropods. Their occurrence origin and roles in immunity. Symp. Zool. Soc. Lond. 56: 81-93, 1986.

Schauer R. Chemistry, metabolism and biological functions of sialic acids. Adv. Carbohydr. Chem. Biochem. 40: 131-234, 1982.

Segler BA, Wang JL, Edelman GM.. Isolation of lectins of different specificities on a single affinity absorbent. J. Biol. Chem. 250: 7535-7538, 1978.

Sharon N, Lis H. Lectins as cell recognition molecules. Science 246: 227-234, 1989.

Sharon N, Lis H. Lectins--proteins with a sweet tooth: functions in cell recognition.

Soderhall K, Smith VJ. Induction of degranulation and lysis of haemocytes in the freshwater cray fish. *Astacus astacus* by components of the prophenoloxidase activating system in *vitro.* J. Biol. Chem. 235: 2860-2869. 1983.

Stein EA, Younai S, Cooper EL. Hemagglutinins and bacterial agglutinins of_earthworms. Prog. Clin. Biol. Res. 233:79-89, 1987.

Vasta GR, Quesenberry M, Ahmed H, O'Leary N. C - type lectins and galectins mediate innate and adaptive immune functions: their roles in the complement activation pathway. Dev. Comp. Immunol. 23: 401-420, 1999.

Vasta GR, Ahmed H, Tasumi S, Odom EW, Saito K. Biological Roles of Lectins in Innate Immunity: Molecular and Structural Basis for Diversity in Self/Non-Self Recognition. Adv. Exp. Med. Biol. 598: 389-406, 2007.

Wilson R, Chen C, Ratcliffe NA. Innate immunity in insects: The role of multiple, endogenous serum lectin in the recognition of foreign invaders in the cockroach, *Blaberus discoidalis*. J. Immunol. 162: 1590-1596, 1999.

Yang J, Wang L, Zhang H, Qiu L, Wang H, Song L. C-Type Lectin in Chlamys farreri (CfLec-1) Mediating Immune Recognition and Opsonization. PLoS ONE 6(2): e17089, 2011.

Zhu Y, Ng PML, Wang L, Ho B, Ding JL. Diversity in lectins enables immune recognition and differentiation of wide spectrum of pathogens. Int. Immunol. 18:1671-1680, 2006.