Tick lectins: structural and functional properties

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SUMMARY

Few papers have been published on tick lectins so far, and therefore more data are needed to complete the mosaic of knowledge of their structural and functional properties. Tissue-specific lectin/haemagglutinin activities of both soft and hard ticks have been investigated. Some tick lectins are proteins with binding affinity for sialic acid, various derivatives of hexosamines and different glycoconjugates. Most tick lectin/haemagglutinin activities are blood meal enhanced, and could serve as molecular factors of self/non-self recognition in defence reactions against bacteria or fungi, as well as in pathogen/parasite transmission. Dorin M, the plasma lectin of *Ornithodoros moubata*, is the first tick lectin purified so far from tick haemolymph, and the first that has been fully characterized. Partial characterization of other tick lectins/haemagglutinins has been performed mainly with respect to their carbohydrate binding specificities and immunochemical features.

Key words: Lectin, agglutinin, protein–carbohydrate interaction, tick, tick-borne pathogens.

INTRODUCTION

The main goal of this review is to provide an overview of our knowledge of tick lectins as tissue-specific carbohydrate-binding proteins of ticks with emphasis on their structural properties and functional role either in defence reactions or pathogen transmission. A few other lectin reviews have been published in the last decade including those dealing with tissue-specific lectins in blood-sucking arthropods (e.g. Ingram & Molyneux, 1991; Grubhoffer, Hypša & Volf, 1997; Grubhoffer & Jindrák, 1998). On the other hand, there are several publications in the last ten years which have drawn attention to plant and animal lectins as well as to lectins as tools in modern glycobiological research (e.g. Jacobson & Doyle, 1996; Rhodes & Milton, 1998). Research on lectins began near the end of 19th century in 1888, with the doctoral thesis of Herman Stilmark at the University of Dorpat, in Estonia on the agglutinins of the seeds of castor bean *Ricinus communis* (Sharon & Lis, 1988). Further investigations into proteins able to agglutinate red blood cells (RBCs) have been improved significantly by modern biochemical and molecular biological techniques during the past several decades (Sharon & Lis, 1989; Slifkin & Doyle, 1990; Doyle & Slifkin, 1994; Jacobson & Doyle, 1996).

The term lectin comes from latin verb '*legere*' and reflects an ability of particular proteins (haemagglutinins) to aggregate different kinds of RBCs (Boyd & Shapleigh, 1954). Several authors improved the original definition of lectins as haemagglutinins to make it more exact in terms of modern knowledge of the structural and functional properties of lectin molecules and their sugar binding sites (Goldstein *et al*. 1980; Kocourek & Hořejší, 1981; Barondes, 1988; Drickamer, 1988; Yoshizaki, 1990; Lee, 1992). According to Peumans & van Damme (1995), lectins are proteins or glycoproteins carrying at least one binding site, possessing a reversible specific interaction with a particular carbohydrate moiety. Lectins are ubiquitous proteins which are likely present in all eukaryotic and many bacterial species as well as in some viruses. This diverse group of proteins is of great importance for living organisms and some of them are often considered to be functional analogues of immunoglobulins in organisms that lack specific immunity (Vasta & Marchalonis, 1984). Lectins as proteins with binding affinity for carbohydrate molecules take part mainly in protein–saccharide interactions. These interactions participate in both effector and regulatory processes in organisms, including those of disease-transmitting blood-sucking arthropods.

The list of identified lectins has grown rapidly in the last two decades with more than ten thousand publications dealing with these molecules. Based on their overall structure, three major types of lectins may be distinguished: mero-, holo-, and chimerolectins (Peumans & van Damme, 1995). Merolectins are proteins that have only carbohydrate-binding domain; hololectins are those comprised of two or more either identical or highly homologous domains; and finally, chimerolectins are those that possess a sugar-binding domain associated with an unrelated

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one having different biological activity or another carbohydrate-binding specificity. Molecules with such combined functional activities have often been called lectinoids (Gilboa-Garber & Garber, 1989). Combining different binding activities in such molecules offers an effective way to function as regulatory tools for mechanisms of recognition and molecular identification by the receptor/co-receptor systems. On the other hand, proteins having an enzyme activity for the carbohydrate substrates, regulatory proteins (e.g. repressor in the lac operon), or antibodies with idiotypic specificity to carbohydrates/glycoconjugates are not considered as lectins (Kocourek & Hořejší, 1981).

Drickamer (1988) recognized lectins as both soluble and/or membrane-bound post-translationally modified proteins providing often multimolecular complexes built from a number of identical subunits. The author has distinguished two major types of lectins based on their structure: S-type (recently called galectins) with thiol group-dependent activity localized in both intra- and extracellular compartments, and C-type (some of them are called selectins) with Ca^{2+}-dependent activity in the extracellular space. The C-type lectins are more variable in structure and more specific to organs and tissues. However, their sequence data show a common and homologous region responsible for binding of carbohydrates, the carbohydrate region domain (CRD) (Lee, 1992). Collectins represent an important subgroup of C-lectins. They have a collagen domain, a neck region and a globular C-type lectin-binding domain (Epstein *et al*. 1996; Ezekowitz, Sastry & Reid, 1996).

From a functional point of view, it is important to emphasize that lectins are involved in the innate immunity of arthropods and vertebrates since they provide the primitive/ancient functions, such as aggregation of microorganisms and opsonizing them for phagocytosis (Ratcliffe & Rowley, 1987; Vasta, 1991; Vasta *et al*. 1994). During evolution, lectins gained more sophisticated functions in defence against pathogens, which is evident from their linkeage to the complement system (Matsushita, 1996; Vasta *et al*. 1999; Matsushita *et al*. 2001; Iwanaga, 2002). It has been reported that the lectin activation pathway of the complement system can be triggered not only by the mannose-binding lectin (MBL), a member of the collectins, but also by a group of other lectins called ficolins (Gadjeva, Thiel & Jesenius, 2001; Matsushita *et al*. 2001). In the lectin activation pathway, MBL with a collagen-like and carbohydrate recognition domains (CRD) (Taylor *et al*. 1990), activates the complement cascade in cooperation with a MBL-associated serine protease (Hoffmann *et al*. 1999). Ficolins occur in mammalian sera. Besides a collagen-like domain common in MBL, ficolins contain a fibrinogen-like domain (Lu & Le, 1998). Recent characterization of ficolins present in human, mouse and pig serum/plasma, as well as in the body fluids of ascidians, has revealed that they are lectins with common binding specificity for GlcNAc. Human serum ficolins have been shown to function as opsonins (Matsushita *et al*. 1996). Most likely, ficolins function as self/nonself recognition lectin molecules in haemolymph (Gokudan *et al*. 1999). Members of the fibrinogen-related protein family have been identified both from vertebrates and invertebrates (Xu & Doolittle, 1990; Adema *et al*. 1997; Kurachi *et al*. 1998; Dimopoulos *et al*. 2000; Kenjo *et al*. 2001; Leonard *et al*. 2001). To complete the list of known lectins, the I-type (belonging to the immunoglobulin superfamily), pentraxins (lectin-like molecules, such as the C-reactive protein) and heparin-binding proteins have to be mentioned (Vasta, Ahmed & Quesenberry, 1996). Lectins that recognize more complex structures at the cell surface, such as C-type lectins and galectins, are found in invertebrate organisms as well as in vertebrates, but the functions of these proteins have evolved separately in different animal lineages (Dodd & Drickamer, 2001).

Moreover, other proteins with carbohydrate-binding domains, which do not fall into the lectin family, can serve as scavenger receptors (Pearson, 1996). They occur also on the surface of haemocytes carrying a binding specificity to anionic polysaccharides (e.g. fucoidan, dextran sulfate, chondroitin sulfate). Scavenger proteins as well as proteins with binding affinity for oligo/polynucleotides seem to play a significant role in the receptor functions over the whole evolution of innate immunity. Together with both lipopolysaccharide (LPS) and β-1,3 glucan-binding proteins, scavenger proteins constitute an important group of molecules engage in non-self recognition in invertebrates.

ARTHROPOD LECTINS

Lectins of general arthropods

Lectins may interact either with 'self' glycoconjugates or with glycosylated components of viral, bacterial, protozoan and metazoan pathogens. Soluble or membrane-bound invertebrate lectins take part in the processes of cell adhesion, opsonization, phagocytosis and cytolysis (Vasta & Marchalonis, 1983). They are clearly important molecules involved in humoral and cellular reactions for recognition and defence by opsonization of non-self objects in the inner environment of arthropods. Lectins may also play a part in the transmission of pathogens by vectors, for instance as receptors, homing factors, differentiation factors, etc. A great number of lectins of various invertebrates have been described (Yeaton 1982*a*, *b*; Ratcliffe *et al*. 1985). From the viewpoint of the structure and function, the best known arthropod lectins are those from the horseshoe crabs,

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Limulus polyphemus and *Tachypleus tridentatus*, and from the scorpions *Centruroides sculpturatus* and *Parauroctonus* (Vasta & Marchalonis, 1984; see Olafsen, 1986, 1996 for reviews). Almost all plasma lectins isolated from the above mentioned group of chelicerates specifically bind N-acetyl neuraminic (sialic) acid and other N-acylamino-carbohydrates. Studies on insect immunity show many molecular aspects common to invertebrate and vertebrate immune responses. For instance, there is a great similarity in the clotting mechanisms of haemolymph and blood. Remarkably, one of the four characterized haemolymph clotting factors of *Limulus*, factor C (serine protease zymogen), resembles in structure and probable function the selectin family of cell-adhesion molecules. It belongs to C-type (Ca^{2+} ion dependent) chimerolectins (for review see Iwanaga, 1993; Hoffmann, 1995).

Recently, lectins purified from haemolymph of the Japanese horseshoe crab, *T. tridentatus*, and called tachylectins 5A/5B, have been characterized and cloned (Gokudan *et al*. 1999). Both tachylectins (Tl-5A/5B) are proteins of high structural and sequential similarity to mammalian ficolins. However, Tl-5A/5B have no effector collagen domains in their structure and no lectin-associated serine proteases, as found in mammalian ficolins. Tl-5s, together with other defence molecules released from haemocytes, is employed in effective innate immunity against invading pathogens (Kawabata & Tsuda, 2002).

Lectins of disease vectors

Despite the great influence of vector-borne pathogens on humans and domestic animals, relatively little information is available on lectins of invertebrate vectors, with the exception of reduviid bugs, tsetse flies, mosquitoes and sand flies (Pereira, Andrade & Ribeiro, 1981; Wallbanks, Ingram & Molyneux, 1986; Maudlin & Welburn, 1988; Gomes, Furtado & Coelho, 1991; Volf, 1993; Hypša & Grubhoffer, 1995; Ratcliffe *et al*. 1996; Chen & Billingsley, 1999).

The best studied vector/parasite system are lectins of various tsetse flies (*Glossina* spp.) (Maudlin & Welburn, 1988; Welburn & Maudlin, 1990; Ingram & Molyneux, 1991). Lectins have been demonstrated in the gut and haemolymph of tsetse flies that can function as signalling factors of maturation for African trypanosome species or, on the contrary, lytic (killing) factors. In this case, an interaction between the gut lectin of the tsetse fly and symbiotic rickettsia-like organisms, or with products of their saccharide metabolism, is a major factor affecting the life cycle of trypanosomes in tsetse flies (Maudlin & Welburn, 1988). A similar interference between symbiotic rickettsia with protozoa was thought to be involved in ticks without considering lectin activities (Smith *et al*. 1976). Sand flies, the vectors of *Leishmania* spp., are another group of vector arthropods that have been well studied with regards to midgut lectins and their receptor function in the process of *Leishmania* transmission (Volf *et al*. 1994; Palánová & Volf, 1997; Volf, Škařupová & Man, 2002).

Lectin–carbohydrate interactions participate in the vector–pathogen/parasite relationship. Tissue-specific lectins of blood sucking arthropods interact with glycosylated molecules of receptors on the surfaces of pathogens or their cells within appropriate developmental stages and *vice versa*. For instance, bacteria or other intracellular parasites have often employed their surface lectins to enter into phagocytes by the mechanism of lectinophagocytosis (Ofek & Sharon, 1988).

Tick lectins in overview

The lectins of ticks have not received the same level of attention as those of insects. A role for tick haemolymph lectins in transmission of pathogens by ticks has been suggested (Munderloh & Kurtti, 1995). Lectin/haemagglutinin activites in the haemolymph of four tick species, *Ixodes ricinus*, *Ornithodoros tartakovskyi*, *O. tholozani* (*papillipes*) and *Argas polonicus* have been described (Vereš & Grubhoffer, 1990; Grubhoffer & Mařha, 1991; Grubhoffer, Vereš & Dusbábek, 1991). The partially characterized tick lectins have affinity for sialic acid and *N*-acetyl-D-glucosamine but differ in their binding specificity to other sugars. It is postulated that these lectins can interact with glycosylated structures of transmitted pathogens, but this has not yet been proved. *I. ricinus* haemolymph lectin was immunolocalized in tick tissues and its distribution supports the idea that the lectin is produced and/or stored in haemocytes and contributes to the immune system by recognition of foreign substances (Kuhn, Uhlíř & Grubhoffer, 1996). Like limulin, the sialic acid-specific lectin of the horseshoe crab, *Limulus polyphemus*, and other sialic acid-specific lectins of chelicerates, the haemolymph lectin of ixodid ticks might also recognize a wide range of Gram-negative bacteria due to its site specificities for *N*-acetyl-D-glucosamine, D-galactose and 2-keto-3-deoxy-octonate acid (Vasta & Marchalonis, 1983; Grubhoffer *et al*. 1991; Kuhn *et al*. 1996). The haemocytes of *I. ricinus* phagocytose spirochaetes of *Borrelia burgdorferi* by the coiling method, and coiling phagocytosis is thought to be a lectin-mediated process (Kuhn *et al*. 1994).

Kamwendo *et al*. (1993) reported haemagglutination activities in the haemolymph, gut homogenates and salivary glands in the tick *Rhipicephalus appendiculatus*, the vector of East Coast fever. This is a serious tick-borne disease of cattle caused by the protozoan parasite *Theileria parva* (see chapter by Bishop *et al*. in this Supplement). Bovine red blood cells were found as the most sensitive detection

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system for all tissue homogenates examined with no significant differences between those in extracts from unfed and fed ticks (Kamwendo *et al*. 1993). However, human erythrocytes of the ABO system and rabbit red blood cells showed significantly higher values of haemagglutination titres in tissue samples coming from fed than unfed ticks, with the highest increase recorded in the gut extract. Later, Kamwendo *et al*. (1995) focused particularly on a specific functional role of the tick salivary gland haemagglutinin in terms of the tick's capability to transmit *T. parva* parasites. *R. appendiculatus* gut lectin activity also influences transmission of the causative agent of theileriosis in Africa with agglutination of piroplasms (E. Sebitosi, personal communication). This has led to the conclusion that the protein–carbohydrate interactions of piroplasms in the inner environment of tick might be a target for the control of theileriosis. Many functional and morphological aspects of tick tissues are involved in the process of parasite transmission (Friedhoff, 1990). However, little is known about the molecular aspects of cell communication and tick interactions with the envelope components of transmitted pathogens. The specific part of the review which follows consists of two sections devoted to tissue specific lectins of the hard tick, *Ixodes ricinus*, and the soft tick, *Ornithodoros moubata*. Due to a lack of published data on tick lectins, most results presented are based on the research of the Tick Lectin Group in the Institute of Parasitology České Budějovice, Czech Republic (see Table 1).

LECTINS OF THE HARD TICK, *IXODES RICINUS*

Gut

The sheep tick, *Ixodes ricinus*, which transmits tick-borne encephalitis (TBE) virus and the Lyme disease-causing spirochaete *Borrelia burgdorferi* sensu lato is the most important tick vector in Europe. Gut haemagglutinating activity of *I. ricinus* has been found and partially characterized (Uhlíř, Grubhoffer & Volf, 1996). Native mouse erythrocytes were the most sensitive detection cell system in a wide pH range of 6·5–8·0. Binding specificity analysis showed that simple sugars *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, rhamnose, dulcit and glycoconjugates fetuin, hyaluronic acid, laminarin (β-1,3 glucan) and bacterial LPS strongly inhibit haemagglutination. Binding characteristics of the tick gut agglutinin are consistent with LPS-binding agglutinins in the gut tissue of other blood-sucking arthropods (Grubhoffer *et al*. 1997). It seems that the midgut agglutinin as a potential LPS-binding protein (LPS-BP) could (in co-operation with digestive enzymes) affect Gram-negative spirochaetes *B. burgorferi* passing through the gut epithelium. Midgut extracts from unfed ticks lack a haemagglutinating activity, which is consistent with observations of Kamwendo *et al*. (1993) who worked on *R. appendiculatus*. Mouse polyclonal antibodies raised against midgut haemagglutinating activity (Yeaton, 1982*a*) recognized, by western blotting, four proteins with molecular weights of 37, 60, 65, and 73 kDa as putative structural components of the lectin(s) (Uhlíř *et al*. 1996). Whereas the 37 and 60 kDa proteins are glycoproteins modified by both high mannose and complex *N*-glycans, the 70 kDa subunit is modified by complex type of glycans only (Uhlíř *et al*. 1994). Using specific mouse polyclonal antibodies, the midgut haemagglutinin/lectin complex was immunohistochemically localized solely in the midgut cells, and not in the gut content or other tick tissues including haemolymph (Uhlíř *et al*. 1996).

Two agglutinins/lectins of the gut haemagglutinating complex in *I. ricinus* were isolated from the gut homogenate after delipidation by acetone extraction (Durnová, 1998). Both agglutinins/lectins were purified by affinity chromatography either on immobilized bovine submaxillary mucine (BSM) or laminarin (β-1,3 glucan). Using SDS–PAGE, a 65 kDa protein was found as the main agglutinin with binding affinity to BSM, and a 37 kDa protein showed strong binding specificity for laminarin (β-1,3 glucan). Although crude gut homogenate had no binding affinity for free sialic acid in the haemagglutination inhibition assay, the delipidated proteinaceous 'acetone powder' prepared from the crude gut homogenate showed a strong ability to bind sialic acid. It seems to be likely that lipids, including glycolipids abundant in the gut tissue, hide the lectin binding site for sialic acid. The pure 65 kDa agglutinin/lectin also maintained the affinity for free sialic acid. Mouse antibodies raised against the entire haemagglutinating activity of the crude gut homogenate recognized both proteins. Because, the 37 kDa gut agglutinin/lectin functions as a β-1,3 glucan-binding protein, it might be part of the defence machinery protecting the tick against fungal infections.

Factors taking place in processes of pathogen/parasite transmission by ticks were reviewed by Friedhoff (1990). According to Friedhoff (1990), the midgut agglutinin/LPS-binding protein may belong to factors associated with the development of pathogens/parasites in the digestive tract of the tick. The process of blood digestion in ticks differs from that of haematophagous insects, where protein digestion proceeds rapidly and takes place in the lumen of the gut (Gooding, 1972). Instead, digestion in ticks is intracellular (Balashov, 1972). It proceeds slowly, and the pH optimum of proteases is about 3·0, while the pH of the gut content is 6·5 (Akov, Samish & Galun, 1976). In hard ticks, the blood meal is taken up by the digestive cells by means of fluid-phase endocytosis and via receptor-mediated endocytosis. Large particles such as whole cells are

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Table 1. Tick lectins/agglutinins and their basic characteristics

Tick species	Tissue	Binding specificity[a]	Agglutination of RBC	Structural subunit[b] (molecular size, kDa)	Reference
Ixodes ricinus	Haemolymph (plasma, haemocytes)	Sialic acid *N*-acetyl-D-glucosamine Fetuin; Asialofetuin; BSM	Mouse (pronase treated)	85	Kuhn, Uhlíř & Grubhoffer (1996)
	Gut	*N*-acetyl-galactosamine *N*-acetyl-glucosamine Rhamnose; Dulcit; Fetuin; Hyaluronic acid; Laminarin; LPS	Mouse	37; 60; 65; 73 (hemagglutinating complex)	Uhlíř, Grubhoffer & Volf (1996)
	Gut (after acetone extraction of lipids)	Sialic acid BSM; Laminarin	Mouse	37 (Laminarin[c]) 65 (BSM[c])	Durnová (1998)
	Salivary glands	Sialic acid; Fructose; Rhamnose; Trehalose Fetuin; Asialofetuin; BSM; Laminarin; Heparin	Mouse	70	Unpublished observations
Rhipicephalus appendiculatus	Haemolymph Gut	n.d.	Bovine Human Rabbit	n.d.	Kamwendo *et al*. (1993)
	Salivary glands	Mannose[d]; Turanose[d]	Bovine Human Rabbit	n.d.	Kamwendo *et al*. (1995)
Ornithodoros tartakovskyi	Haemolymph/plasma	Sialic acid *N*-acetyl-D-glucosamine *N*-acetyl-D-galactosamine Fetuin; Asialofetuin; BSM	Mouse (pronase treated)	30; 35	Grubhoffer, Vereš & Dusbábek (1991)
	Haemocytes			35	
Ornithodoros tholozani (*papillipes*)	Haemolymph/plasma	Sialic acid *N*-acetyl-D-glucosamine D-galactose Fetuin; BSM	Mouse (pronase treated)	37; 40	Grubhoffer, Vereš & Dusbábek (1991)
Ornithodoros moubata	Haemolymph/plasma	*N*-acetylneuraminyl-lactose Sialic acid *N*-acetyl-D-galactosamine *N*-acetyl-D-glucosamine *N*-acetyl-D-mannosamine Fetuin; Asialofetuin; BSM; PSM-I	Mouse (pronase treated)	~37 Dorin M GenBank: AY 333989	Grubhoffer & Kovář (1998) Kovář, Kopáček & Grubhoffer (2000) Unpublished observations
Argas polonicus	Haemolymph/plasma	*N*-acetyl-D-glucosamine D-galactose Fetuin; BSM	Mouse	n.d.	Grubhoffer, Vereš & Dusbábek (1991)

Abbreviations: [a] – determined by the haemagglutination inhibition assay; [b] – determined by SDS–PAGE and Western blotting; [c] – ligands in affinity chromatography; [d] – inhibition of *T. parva* transmission *in vivo*; BSM – bovine submaxillary mucine; PSM-I – porcine submaxillary mucine; n.d. – not determined.

phagocytized. The binding activity of the midgut agglutinin/LPS-BP is pH dependent. At pH 3·0, which is an optimum value for the gut proteases, the gut agglutinin does not work at all whereas at pH 6·5, which corresponds to the gut content, a haemagglutinating activity of this binding protein reaches its optimum (Uhlíř *et al*. 1996). Therefore, the midgut agglutinin/LPS-BP could be involved in the preparatory phase of blood meal digestion, in addition to its role in defence.

Haemolymph (*plasma and haemocytes*)

Most lectins isolated from arthropods are constituents of the haemolymph, the extracellular circulating fluid that fills the body cavity or haemocoel. The haemolymph, especially haemocytes and large protein complexes, is physically isolated from direct contact with body tissues by basal laminae. However, haemocytes, which are cells within the haemolymph, are in direct contact with this fluid (Mullins, 1985). Haemocytes are immunocytes of arthropods (Gupta, 1991); however, they contribute to nutritional functions, too (Gupta, 1985). Haemolymph lectins and other humoral immune factors in the haemolymph are produced and secreted into the haemolymph mainly by haemocytes and fat body cells. Recently, Gudderra *et al*. (2002) have published a comprehensive review on haemolymph proteins in ticks emphasizing the structural and functional features of vitellogenin and other tick proteins such as macroglobulins, heme-lipoproteins, antimicrobial proteins and lectins.

Haemagglutinating activity from the haemolymph of *I. ricinus* was characterized as Ca^{2+}-dependent binding activity of the lectin with a subunit molecular size of 85 kDa by non-reducing SDS–PAGE, and binding affinity to sialic acid as well as *N*-acetyl-D-glucosamine and D-galactose (Grubhoffer *et al*. 1991, and unpublished observations). Sialic acid-binding lectins are common in chelicerates (Mandal & Mandal, 1990; Wagner, 1990; Zeng & Gabius, 1992), and recognize Gram-negative bacteria. Mouse polyclonal and monoclonal antibodies prepared toward a lectin haemagglutinating activity (Yeaton, 1982*a*) of the tick haemolymph identified a protein of 85 kDa as a structural lectin subunit. A semipurified plasma fraction highly enriched with a haemagglutinating activity was obtained by the gel filtration FPLC on Superose 12 (unpublished observations). The lectin was localized to the haemocytes in *I. ricinus* (Kuhn *et al*. 1996). Immunoreactivity with poly- and monoclonal antibodies was detected in the granules of both types of granular haemocytes, on the plasma membrane of haemocytes, and on the basal laminae surrounding the haemocoel (Figs. 1, 2). Furthermore, cells attached to the midgut invaginations of Géné's organ and granular inclusions of nephrocytes were labeled. The haemolymph- and

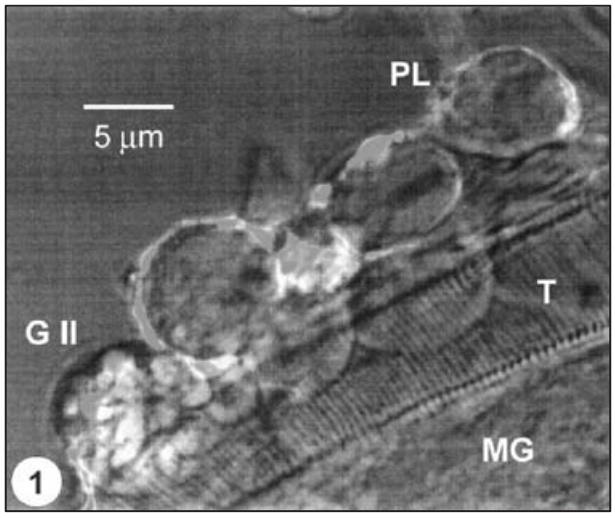


Fig. 1. Indirect immunofluorescence detection of the plasma lectin in the haemocytes *Ixodes ricinus* tick using mouse monoclonal antibody 3/D3/C5 to the plasma lectin activity of *I. ricinus* (diluted 1 : 100). GII – type II of granular haemocytes; PL – plasmatocyte; T – trachea; M – midgut. Laser confocal microscopy (courtesy Dr K.-H. Kuhn).

haemocyte-associated immunoreactivity supports the idea that this haemolymph lectin may function as a recognition molecule in the immune system of *I. ricinus*. Therefore, the haemolymph lectin could be involved in transmission of *Borrelia burgdorferi* as a defence molecule in tick haemolymph/haemocytes. Johns *et al*. (2001*a*, *b*), studying differences in vector capability of various tick species for *Borrelia* transmission, have suggested a potential role of immune molecules. Several studies have shown that *B. burgdorferi* migrate from the midgut across the haemocoel to the salivary glands during tick feeding (Ribeiro *et al*. 1987; Zung *et al*. 1989). However, less than 5% of midgut population of spirochaetes survived to reach salivary glands (Coleman *et al*. 1997). Another function could be in the protection of eggs that are coated with secretions by Géné's organ (Kuhn *et al*. 1996).

The distribution of lectin immunoreactivity, especially the labeling of membranes and granular inclusions of haemocytes and of the basal laminae surrounding the haemocoel, strengthens the assumption that the haemolymph lectin is produced or stored in haemocytes and contributes to the immune system of *I. ricinus*. Like limulin, the sialic acid-specific lectin of *Limulus polyphemus*, and other sialic acid-specific lectins of chelicerates, the haemolymph lectin of *I. ricinus* might also recognize a wide range of Gram-negative bacteria due to its additional specificities for *N*-acetyl-D-glucosamine, D-galactose, and unrelated molecules such as 2-keto-3-deoxyoctonate (Vasta & Marchalonis, 1983; Grubhoffer *et al*. 1991). Moreover, it was shown in *in vitro* assays that *B. burgdorferi* is a target for the immune system of

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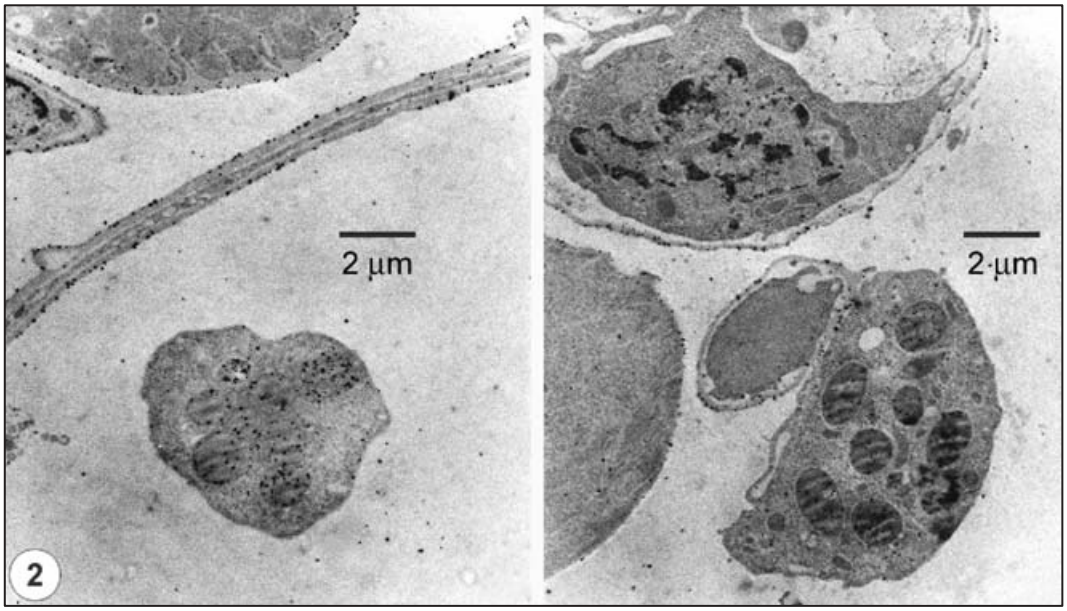


Fig. 2. Electron micrographs of immunogold-silver labeling of haemocytes with mouse polyclonal antibodies to the plasma lectin activity of *Ixodes ricinus* tick. Granular inclusions of type-II granular haemocytes (courtesy Dr K.-H. Kuhn).

I. ricinus and is readily ingested by phagocytic haemocytes (Kuhn *et al*. 1994). Additionally, Kurtti *et al*. (1988) have reported that *B. burgdorferi* spirochaetes readily adhere to tick cells in tissue culture. Therefore, the lectin might mediate the interaction of pathogens like *B. burgdorferi* or tick-borne encephalitis (TBE) virus with the tissues of the vector. The main binding specificity of the haemolymph lectin is for sialic acid (Grubhoffer *et al*. 1991), which is present in the wall of *B. burgdorferi* (Hulínská, Volf & Grubhoffer, 1992). In addition, the haemolymph lectin of the tick can bind complex oligosaccharides lacking sialic acid. Therefore, the protein gpE of TBE virus, the only structural glycoprotein that exhibits biantennary complex oligosaccharides (Grubhoffer *et al*. 1990) could interact with the haemolymph lectin. The localization of lectin immunoreactivity in diverse tissues and cells of the tick does not imply that all labeled proteins are associated with the same function. Probably, polyclonal antibodies recognize common epitopes in functionally diverse proteins with multiple domains. Calcium-dependent globular lectin domains (C-type lectins) (Drickamer, 1993; Drickamer & Taylor, 1993) that are components of vertebrate haemostasis and complement systems (Thiel & Reid, 1989; Sastry & Ezekowitz, 1993; McEver, 1994) and a related protein, factor C (Muta *et al*. 1991), and an immune protein of the complement–coagulation system of *L. polyphemus* (Iwanaga, 1993), are examples of this phenomenon.

A cDNA library was constructed from unfed *I. ricinus* females and screened using a probe obtained from the lectin gene of *B. burgdorferi* B31 (unpublished observations). Clones showing the strongest hybridization signals were selected and sequenced. The amino acid sequence of a putative carbohydrate recognition domain of the recombinant clone from an *I. ricinus* cDNA library was compared with those of other lectins (Fig. 3). The predicted amino acid sequence of one clone, termed r58, revealed strong homology (81%) with four lectins from *Periplaneta americana* cockroach haemolymph (Kawasaki, Kubo & Natori, 1996).

Salivary glands

Kamwendo *et al*. (1995) were the first to study the role of the tick salivary gland haemagglutinin of the tick *R. appendiculatus* in relation to the mechanism of transmission of the protozoan parasite *Theileria parva*. A significant increase was observed in *T. parva* acinar infection rates in the salivary glands of *R. appendicalatus* fed on ears of rabbits infused with melibiose and raffinose (haemagglutination inhibiting sugars). In contrast, mannose and turanose (non-inhibitory sugars) did not affect of *T. parva* acinar infection rates. Haemagglutinating activity in *I. ricinus* salivary gland (SG) extracts was highest against mouse red blood cells, and was inhibited by sialic acid, D-fructose, L-rhamnose, D-trehalose and several glycoconjugates (unpublished observations). Mouse polyclonal antibodies raised against the haemagglutinating activity were used in western blotting to identify a 70 kDa protein responsible for SG haemagglutinating activity. It is likely that the lectins have a significant role in the entry of pathogens into the salivary gland and, hence, the salivary gland lectin in *I. ricinus* will be important in the transmission of TBE and *B. burgdorferi*.

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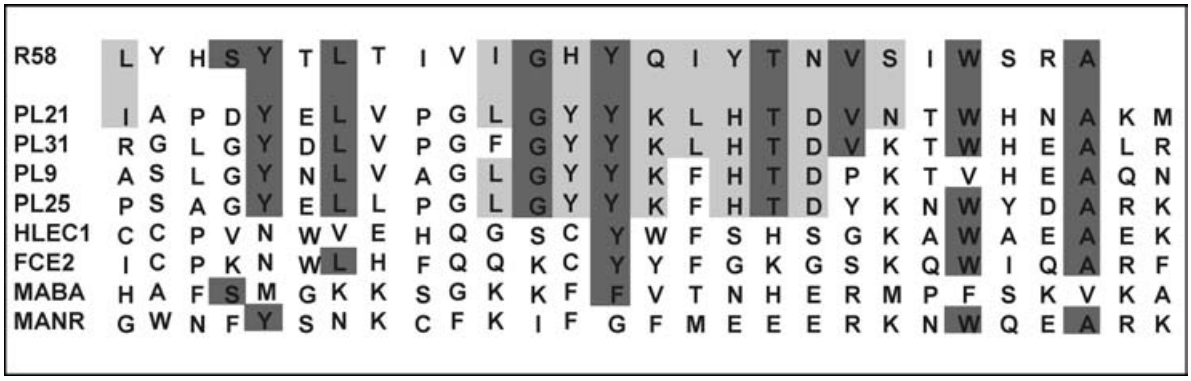


Fig. 3. Comparison of carbohydrate recognition domain of *I. ricinus* lectin (r58) with those of different lectins. The lectins compared are: r58, clone from *I. ricinus* cDNA library (unpublished observations); PL21, 31, 9 & 25, four lectins from *Periplaneta americana* cockroach hemolymph (Kawasaki *et al*. 1996); HLEC, human hepatic lectin (Halberg *et al*. 1987); FCE2, mucose immunoglobulin Fc receptor (Bettler *et al*. 1989); MABC, rat mannose-binding protein A (Drickamer, Dordal & Reynolds, 1986); and MANR, human macrophage mannose receptor (Taylor *et al*. 1990). Dark grey represents identitical amino acid residues and light grey represents similar residues.

LECTINS OF THE SOFT TICK, *ORNITHODOROS MOUBATA*

Haemolymph (*plasma and haemocytes*)

The soft tick *Ornithodoros moubata* is a vector of African swine fever virus and *Borrelia duttoni*. Laboratory colonies of this species are valuable experimental models that have been employed to answer questions about the tick physiology and morphogenesis, such as vitellogenin synthesis (Chinzei, Chino & Takahashi, 1983; Chinzei & Yano, 1985; Chinzei, 1988; Gudderra *et al*. 2002, for review), defensins as immunopeptides (Nakajima *et al*. 2001), and peritrophic matrix synthesis (Grandjean, 1984). Recently, the immune response of the argasid tick *O. moubata* induced by infection with the filarial worm *Acanthocheilonema viteae* has been studied by Hutton, Reid & Towson (2000). A plasma/haemolymph lectin of *O. moubata* has been thoroughly studied (Grubhoffer & Kovář, 1998; Kovář, Kopáček & Grubhoffer, 2000), though we also have evidence of some lectin activity in the salivary glands and gut of *O. moubata* (unpublished observations).

A lectin with high haemagglutinating activity, called Dorin M, was identified in the plasma of *O. moubata* (Grubhoffer & Kovář, 1998; Kovář *et al*. 2000). The activity of the plasma lectin could be efficiently inhibited by sialic acid, *N*-acetyl-D-hexosamines and acid (sialo)glycoproteins. Dorin M was purified to homogeneity using two different isolation systems: affinity chromatography on a column of bovine submaxillary mucin conjugated to Sepharose 4B elution with *N*-acetyl-D-glucosamine (Grubhoffer & Kovář, 1998), or chromatography on Blue-Sepharose followed by anion exchange FPLC on a MonoQ column (Kovář *et al*. 2000). Dorin M is a glycoprotein with *N*-type of glycosylation that, in the native state, forms aggregates with molecular mass of about 640 kDa. Non-reducing SDS–PAGE revealed that the lectin consists of two non-covalently bound subunits migrating closely around 37 kDa. After chemical deglycosylation, only one band of about 32 kDa, without haemagglutinating activity, was detected. A tryptic cleavage of purified Dorin M was performed and the amino acid sequence of three resulting internal peptides fragments were determined by Edman degradation. Using degenerate primers and a haemocyte cDNA template, a 290 bp PCR product was cloned and sequenced. The haemocyte origin of the cDNA template demonstrated that haemocytes are a site of Dorin M synthesis (unpublished observations). The sequence of the complete Dorin M cDNA was completed by the method of RACE. The derived amino acid sequence clearly showed that the Dorin M has a fibrinogen-related domain and exhibited the highest significant similarity with tachylectins 5A (38%) and 5B (32%) from the horseshoe crab. Since tachylectins function as non-self recognizing molecules, we believe that Dorin M may play a similar role in innate immunity of the tick and possibly also in pathogen transmission (unpublisahed observations).

More than ten years ago the research on tick haemolymph lectins was initiated with the study of *O. tartakovskyi* and *O. tholozani* (*papillipes*), species closely realated to *O. moubata* (Vereš & Grubhoffer, 1990; Grubhoffer *et al*. 1991). Sialic acid-specific lectins are present in *Ornithodoros* tick plasma/haemolymph with minor differences in binding affinity to other free carbohydrates, such as *N*-acetyl-D-glucosmine, D-glucosamine and D-galactosamine. Two bands of 30 and 35 kDa in tick haemolymph and a single component of 35 kDa in the haemocyte lysate were distinguished by immunoblotting with polyclonal antibodies. In whole body homogenates, in addition to the 30 kDa protein, a 150 kDa band was identified. Both structural units of haemolymph lectin activity were found to be glycoproteins in

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the lectin affinoblotting, The 30 kDa protein was modified by the complex type of glycans with terminal D-galactose, whereas the 35 kDa protein was complexing with high mannose type of glycans (Grubhoffer *et al*. 1991).

FUTURE DIRECTIONS

Protein–carbohydrate interactions are known to participate in vector–pathogen/parasite relationships. One of the most important issues implicating lectins in immune responses to pathogens is the inducibility/upregulation of their binding (haemagglutinating) activities after blood feeding or pathogen invasion. To examine this question, subtractive cDNA libraries of *I. ricinus* tick were analysed for the occurrence of upregulated clones and a gene with high homology with Jacalin, a plant lectin, was found (unpublished observations). Another important issue of pathogen/parasite transmission by ticks is the reciprocity of lectin–carbohydrate interactions. Here the lectins of transmitted pathogens/parasites (e.g. tick-borne viruses, borrelia spirochaetes, piroplasma, etc.) may recognize carbohydrate moieties of receptors on tick cells. This may facilitate binding of the pathogen to the tissues where they can evade antimicrobial attacks by the host. A lectin/haemagglutinin from the relapsing fever spirochete, *Borrelia recurrentis*, has been described as well as two different lectins/haemagglutinins from *B. burgdorferi* spirochaetes (Grubhoffer, Uhlíř & Volf, 1993; Leong *et al*. 1995; Parveen & Leong, 2000; Rudenko, Golovchenko & Grubhoffer, 2000). With the increasing evidence of the existence of protein–carbohydrate interactions between tick tissues and pathogens, as discussed in this review, the next goal is to elucidate their biological roles and significance in tick–pathogen relationships and, ultimately, to design potential disease transmission-blocking strategies based on our understanding of the tick or tick-borne pathogen lectins.

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