

Chapter 5

Insect C-Type Lectins in Microbial Infections



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Abstract C-type lectins (CTLs) are a family of carbohydrate-recognition domain (CRD)-containing proteins that bind to ligands in a calcium-dependent manner. CTLs act as important components of insect innate immune responses, such as pattern recognition, agglutination, encapsulation, melanization, phagocytosis and phenoloxidase activation, as well as gut microbiome homeostasis maintenance, to defend against pathogens. Besides, some insect CTLs can facilitate pathogen infection and colonization. In this review, we describe the properties of insect CTLs and focus on explaining their role in viral, bacterial, parasitic and fungal infections.

Keywords C-type lectins · Infection · Insect

5.1 Introduction

Insects are the most diverse group of organisms on Earth, occupying an extremely important position in the ecosystem. Over long-term evolution in an adverse environment with various pathogens, insects have developed a unique innate immune system involving cellular immunity and humoral immunity (Sadd and Siva-Jothy 2006). Cellular immunity depends mainly on blood cells that engulf foreign antigens by phagocytosis. The humoral immune system is an open and complete defence system consisting of lectins, antimicrobial peptides (AMPs), antiviral factors, lysozyme and

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protease inhibitors, and multifunctional blood cells (Hillyer 2015). Because of their inability to synthesize specific antibodies, invertebrates rely solely on non-specific immune responses to defend against pathogens. Upon pathogen invasion, insect immunity utilizes pattern-recognition proteins (PRPs) to help recognize pathogen-associated molecular patterns (PAMPs). There are many types of PRPs in insects, including peptidoglycan-recognition proteins (PGRPs), beta-1,3-glucan recognition proteins (bGRPs), scavenger receptors (SCRs), and C-type lectins (CTLs). Within the PRPs, CTLs play one of the most important roles and form the most diverse family (Zhang et al. 2015).

CTLs are a superfamily of carbohydrate-binding proteins and have a characteristic carbohydrate-recognition domain (CRD) (Dodd and Drickamer 2001) that binds to carbohydrates in a calcium-dependent manner (Drickamer 1993; Weis et al. 1998; Drickamer 1999). Some CRD-containing proteins are referred to as C-type lectin-like domain (CTLD) proteins, and with the continuous growth of the family, a few have been found that do not depend on calcium for binding activity (Zelensky and Gready 2005; Pees et al. 2016). Insect C-type lectins have various functional domains, including single CRDs, double CRDs (the immulectin group) and other functional domains (the CTL-X group). In the CTL-X group, functional domains containing complement control protein (CCP), immunoglobulin modules (Ig), an extracellular domain (CUB), epidermal growth factor-like domain (EGFL) and chitin binding domain (CBM) have been identified (Rao et al., 2015a, b; Waterhouse et al. 2007; Xia et al. 2015). Most insect CTLs have signal peptides, indicating that they are secreted proteins, while some CTLs contain transmembrane domains.

Recently, many works have reported the important position of CTLs in insect innate immune defence against microorganisms. Some insect CTLs play important roles as pattern-recognition receptors by binding invading pathogens and mediating immune responses, such as agglutination, encapsulation, melanization, phagocytosis and prophenoloxidase activation, to clear the pathogens. Certain other CTLs, however, have the opposite function; they facilitate pathogen infection. Thus, we will mainly focus on explaining the role of insect C-type lectins in viral, bacterial, parasitic and fungal infections.

5.2 Insect C-Type Lectin and Viral Infections

Multiple C-type lectins have been identified as susceptibility factors for viral infection in mosquitoes. Arthropod-borne viruses (arboviruses) are named for their unique method of transmission, via the bite of an arthropod vector, among which dengue virus (DENV), Zika virus (ZIKV), yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV) and chikungunya virus (CHIKV) are transmitted by mosquito vectors and pose a major threat to human health (Zhu et al. 2017). These viruses enter the mosquito by an infectious blood meal and infect the vector in a sequential manner; first, the midgut epithelium is infected, and then the surrounding tissues become infected before the virus disseminates to the salivary gland (Guzman

et al. 2016). During this process, viruses are able to utilize mosquito C-type lectins to facilitate infection. A previous study identified an *Aedes aegypti* C-type lectin, *mosGCTL-1*, which facilitates WNV infection in vitro and in vivo. The expression of *mosGCTL-1* is upregulated by WNV, and it interacts with WNV in a calcium-dependent manner. *mosGCTL-1* also plays an important role in WNV infection by interacting with a mosquito homologue of human CD45 (*mosPTP-1*), which enables viral attachment to cells and facilitates viral entry (Cheng et al. 2010). In addition, Liu and colleagues first identified 9 *Aedes aegypti* *mosGCTL* genes as key susceptibility factors for DENV-2 infection, among which *mosGCTL-3* was found to most significantly enhance viral infectivity by interacting with the DENV-2 envelope protein in vitro and in vivo. In contrast, antisera against multiple lectins efficiently reduced DENV-2 infection during a mosquito blood meal (Liu et al. 2014). Other studies have also shown that *mosGCTL-7* can bind to the JEV envelope protein via an N-glycan at N154 in a calcium-dependent manner. After binding, cell surface *mosPTP-1* interacts with the *mosGCTL-7*-JEV complex and facilitates viral infection in vitro and in vivo (Liu et al. 2017).

In contrast to these viral infection facilitating roles in insects, C-type lectins can also induce protective responses during viral infections of insects. *Spodoptera exigua* C-type lectins (bacovirus-like lectins, Se-BLLs) are acquired by horizontal gene transfer and confer an adaptive advantage against baculovirus, a natural viral pathogen of *S. exigua*. Specifically, Se-BLL2 can reduce the pathogenicity of baculovirus, indicating that lectins can increase the resistance of *S. exigua* larvae to baculoviral infection (Gasmi et al. 2015). In addition, another lectin derived from *Spodoptera*, Se-BLL3, is able to reduce the mortality of *Spodoptera frugiperda* larvae during Junonia coenia densovirus (JcDV) infection, but no such effect is found in *S. exigua* larvae (Gasmi et al. 2018). Recently, two novel C-type lectins (MdCTLs) have been obtained from *Musca domestica* and show agglutination and antiviral properties in the laboratory. Interestingly, MdCTL-2 protein can inhibit the replication of influenza H1N1 virus, resembling the effect of ribavirin (Zhou et al. 2018).

Taken together, these studies show that insect C-type lectins play various roles in immune responses upon viral infection. Investigating the effect of C-type lectins during arbovirus infection may provide a better understanding of the replication of the virus in insects and shed light on new strategies for arbovirus transmission control. For instance, a transmission-blocking strategy may be feasible by blocking the C-type lectins that facilitate viral infection. Additionally, identifying novel insect C-type lectins may also lead to the discovery of new antiviral proteins.

5.3 Insect C-Type Lectins and Bacterial Infection

Insects utilize multiple immune systems to control bacterial overgrowth and maintain gut microbiome homeostasis. Specifically, C-type lectins are a large group of proteins in insects known for their ability to regulate bacterial survival and colonization. The role of insect C-type lectin in antibacterial immunity has been identified for *Manduca*

sexta (Yu et al. 1999a, b). The *M. sexta* genome encodes 34 C-type lectins, among which is *M. sexta* immuelectin-1 (*MsIML-1*), which was first cloned from the larval fat body. *MsIML-1* expression is induced by the injection of inactivated gram-positive and gram-negative bacteria or yeast, and it is highly expressed in insect immune tissues, such as fat body and haemolymph, after bacterial infection. Recombinant *MsIML-1* protein impairs bacterial growth by agglutinating bacteria and stimulating the activation of prophenoloxidase (PPO) (Yu et al. 1999a). Immuelectin-2 from *M. sexta* (*MsIML-2*) was also isolated from the larval fat body and is induced by *Escherichia coli* and *Saccharomyces cerevisiae*. However, *MsIML-2* has not been measured in haemocytes after bacterial stimulation. *MsIML-2* can agglutinate *E. coli* in a calcium-dependent manner but has no effect on the agglutination of *S. cerevisiae* (Yu and Kanost 2000). *M. sexta* immuelectin-3 (*MsIML-3*) and immuelectin-4 (*MsIML-4*) cDNA has also been cloned from the larval fat body. By coating the proteins onto agarose beads, it has been shown that *MsIML-4* can facilitate encapsulation and melanization by haemocytes in vitro, while *MsIML-3* only enhances encapsulation in vitro (Yu et al. 2005, 2006). The silkworm *Bombyx mori* genome encodes 23 CTL genes (Rao et al. 2015b). A recent study revealed that C-type lectin-S3 from silkworm *B. mori* (*BmCTL-S3*) is expressed in various tissues, including the fat body, haemocytes, midgut, Malpighian tubule, ovary and embryo. The *BmCTL-S3* protein facilitates the rapid clearance of *E. coli*, *S. aureus*, *Serratia marcescens* and *Bacillus subtilis* by binding to bacterial cell wall components (Zhan et al. 2016). Shahzad and colleagues also cloned a C-type lectin-S2 (*BmCTL-S2*) from the silkworm *B. mori* and performed functional analyses, which suggested that *BmCTL-S2* is a pattern-recognition receptor with antibacterial activity (Shahzad et al. 2017). In the mosquito field, Schnitger and colleagues identified two CTLs from *Anopheles gambiae*, which are required for the clearance of *E. coli* but not *S. aureus* (Schnitger et al. 2009). These results reveal that multifunctional CTLs in insects can promote the interaction between host and bacteria. A better understanding of the molecular mechanism of the antibacterial activity of CTLs may provide novel strategies for bacterial defence.

In addition, CTLs function as pattern-recognition receptors and exhibit antimicrobial activity in invertebrates other than insects. A recent study identified a novel soluble and bacteria-inducible PRR, Leulectin, containing a CTLD from the hepatopancreas of kuruma shrimp *Marsupenaeus japonicus*. This CTLD can agglutinate bacteria and promote haemocytic phagocytosis by recognizing LPS (Wang et al. 2017a, b). Another CTL, PmCLec, from the black tiger shrimp *Penaeus monodon*, was characterized. Purified recombinant PmCLec protein binds and agglutinates gram-positive bacteria, *Staphylococcus aureus* and *S. haemolyticus*, in the presence of calcium (Wongpanya et al. 2017). A mannose-binding CTL called FmLC3 was isolated from *Fenneropenaeus merguensis* and exhibits agglutination activity against various microbial strains in a calcium-dependent manner (Runsang et al. 2017). Moreover, a CTL named *MjCC-CL* in the kuruma shrimp (*Marsupenaeus japonicus*) contains both a CTLD and a coiled-coil domain (CCD). In an antibacterial response, the CTLD recognizes bacterial glycans leading to the interaction of the CCD with

the surface receptor Domeless and subsequent activation of the JAK/STAT pathway and upregulation of AMP expression (Sun et al. 2017).

Most CTLs serve as immune factors suppressing bacterial growth in insects. However, some mosquito C-type lectins function as immune factors facilitating bacterial growth. Pang and colleagues revealed that blockade of mosGCTLs suppresses the growth of gut bacteria in *Aedes aegypti* and *Culex pipiens pallens*. It has been demonstrated that mosGCTLs function by directly coating bacterial cells and neutralizing antimicrobial peptide (AMP)-mediated elimination (Pang et al. 2016). In summary, insect C-type lectins mediate the regulation of insect microbiome homeostasis via various mechanisms and show pleiotropic effects in insect–microbial interactions.

5.4 Insect C-Type Lectins and Parasite Infections

Malaria parasites must go through a complex life cycle in an *Anopheles* mosquito to establish an infection before being transmitted to a new host. Osta and colleagues conducted a large-scale functional screen of mosquito genes and identified two C-type lectins (CTL4 and CTLMA2), which act as protective agonists for the development of *Plasmodium* ookinetes to oocysts and facilitate parasite susceptibility by inhibiting parasite melanization (Osta et al. 2004). Recently, Simoes and colleagues found that protection against parasite melanization by CTL4 and CTLMA2 in *A. gambiae* mosquitoes is dependent on infection intensity. Interestingly, CTL4 and CTLMA2 exert agonistic and antagonistic parasite regulation effects in different *Anopheles* species. (Simoes et al. 2017). However, silencing of CTL4 and CTLMA2 has no effect on *Plasmodium falciparum* development in humans (Cohuet et al. 2006). Additionally, Shin and colleagues identified a novel gene, *CLSP2*, encoding C-type lectin domains. Knockdown of *CLSP2* activates prophenoloxidase and thus inhibits parasite growth, suggesting that *CLSP2* is an antagonist of *Plasmodium* parasite infection in mosquitoes (Shin et al. 2011).

Recently, another CTL was found in the cotton bollworm *Helicoverpa armigera* (*HaCTL3*), which is involved in defence against parasites. *HaCTL3* enhances haemocyte encapsulation and melanization by interacting with β -integrin. The expression of *HaCTL3* is induced by 20-hydroxyecdysone (20E) treatment while silencing of the 20E receptor can abolish this response (Wang et al. 2017a, b).

Indeed, C-type lectins play various roles in host–parasite interactions. Investigating and understanding these dramatically different CTL mechanisms during the parasite defence process may lead to novel strategies to inhibit parasite transmission. Notably, Yoshida and colleagues generated transgenic mosquitoes expressing the C-type lectin from sea cucumber *Cucumaria echinata*, which impairs *Plasmodium berghei* oocyst formation. It is the first established *Anopheles* mosquito engineered to block parasite transmission (Yoshida et al. 2007).

5.5 Insect C-Type Lectins and Fungal Infection

Upon fungal infection, the innate immune system of insects must perform all defence functions because of the lack of adaptive immunity. Therefore, C-type lectins act as an important component in the defence against fungal infections in insects. Tian and colleagues identified a C-type lectin from *Helicoverpa armigera* (Halectin), which promotes haemocyte phagocytosis of pathogens and protects the insect from fungal infection (Tian et al. 2009). In addition, C-type lectin 14 (*CTL14*) from *H. armigera* is induced in the fifth larval stage by entomopathogen *Beauveria bassiana* infection specifically. Recombinant CTL14 protein can aggregate *B. bassiana* in vitro while silencing of CTL14 decreases the resistance to fungal infection in *H. armigera* (Cheng et al. 2018). Recently, to investigate the role of the JAK/STAT signalling pathway in the antifungal immune response of silkworm *Bombyx mori*, Geng and colleagues revealed that *B. mori* C-type lectin 5 (*BmCTL5*) is induced by *Beauveria bassiana* infection and showed that BmCTL5 might be a pattern-recognition receptor for the JAK/STAT signalling pathway in silkworms (Geng et al. 2016).

5.6 Conclusion

Insects utilize lectins to recognize pathogens, gain access to invaders through in vivo interactions, and eliminate exogenous invaders. Understanding the role of insect lectins in the insect immune system may provide insight into the interactions between pathogens and their hosts. Insect CTL genes regulate various innate immune responses, including pattern recognition, agglutination, encapsulation, melanization, phagocytosis and prophenoloxidase activation, as well as the maintenance of gut microbiome homeostasis. Some CTLs have specialized functions, such as stimulating cell proliferation (Table 5.1).

Mosquitoes are one of the major insect vectors of several human diseases in the world. The mosquito immune response regulates pathogen infection and transmission via multiple pathways. Specifically, different C-type lectins from *A. aegypti* have been found to facilitate the infection of mosquitoes by various arboviruses, including WNV, DENV and JEV (Cheng et al. 2010; Liu et al. 2014, 2017). Furthermore, other CTLs that regulate flavivirus infection in mosquitoes may be identified by using bioinformatics methods to analyse homologous functional domains of the CLTs in mosquitoes. The development of strategies to inhibit certain C-type lectins in the flavivirus life cycle may be a promising strategy for flavivirus transmission control. A better understanding of the role of C-type lectins in insect gut ecology, maintaining gut microbiome homeostasis or dysregulating the gut microbiota, may offer a novel target for vector-borne disease control in nature (Pang et al. 2016).

Unfortunately, research on insect lectins is still extremely limited. While a few studies have focused on certain lectins from a specific species of insect, the molecular mechanisms underlying the interactions between pathogens and most lectins are

Table 5.1 Function of C-type lectin in microbial infection in insect

Microbial	Species	Name	Functions	Calcium-dependent	References
Virus	WNV	mosGCTL-1	Facilitates WNV infection by interacting with mosPTP-1 to enhance viral entry	+	Cheng et al. (2010)
	DENV	mosGCTL-3	Interacts with DENV-2 envelope protein to facilitate virus infection	+	Liu et al. (2014)
	JEV	mosGCTL-7	Binds to JEV envelope protein by a N-glycan at N154, promotes JEV infection	+	Liu et al. (2017)
	JcDv	Se-BLL2	Increases the resistance of <i>S. exigua</i> larvae to baculovirus infection	Unknown	Gasmi et al. (2015)
	JcDV	Se-BLL3	Reduces the mortality of <i>S. frugiperda</i> larvae	Unknown	Gasmi et al. (2018)
	H1N1 virus	MdCTL-2	Inhibition of H1N1 virus replication	Unknown	Jing et al. (2018)
Bacterial	Gram-negative bacteria	MsIML-1	Impaired bacteria growth by agglutinating bacteria and stimulating the activation of prophenolox	+	Yu et al. (1999a)
	<i>E. coli</i>	MsIML-2	Agglutinates <i>E. coli</i> in a calcium-dependent manner	+	Yu and Kanost (2000)

(continued)

Table 5.1 (continued)

Microbial	Species	Name	Functions	Calcium-dependent	References
<i>E. coli</i>	<i>M. sexta</i>	MsIML-3	Agglutinates <i>E. coli</i> via a calcium-dependent manner and does not facilitate melanization	+	Yu et al. (2005)
	<i>M. sexta</i>	MsIML-4	Binds to LPS in a calcium-dependent manner and enhances encapsulation and melanization	+	Yu et al. (2006)
	<i>E. coli</i> , <i>S. aureus</i> , <i>S. marcescens</i> , <i>B. subtilis</i>	BmCTL-S3	Binds to bacterial cell wall to facilitate rapid clearance of bacteria	Unknown	Zhan et al. (2016)
	<i>S. aureus</i> , <i>B. subtilis</i>	BmCTL-S2	Inhibits the growth of <i>B. subtilis</i> and <i>S. aureus</i>	+	Shahzad et al. (2017)
	<i>E. coli</i>	mosGCTLs	Required for the clearance of <i>E. coli</i>	+	Schnitger et al. (2009)
Symbiotic microbiome	<i>A. aegypti</i>	mosGCTLs	GCTLs directly coat the bacterial cells and inhibit antibacterial function of AMPs	+	Pang et al. (2016)
Parasite	<i>P. berghei</i>	CTL4 and CTLMA2	Inhibit melanization to enhance parasite infection	+	Osta et al. (2004)
	<i>P. gallinaceum</i>	CLSP2	Enhances parasite infection by impairing prophenoloxidase	+	Shin et al. (2011)

(continued)

Table 5.1 (continued)

Microbial	Species	Name	Functions	Calcium-dependent	References
	<i>Parasite</i>				
	<i>H. armigera</i>	HaCTL3	Regulated by the steroid hormone ecdysone to protect against parasites	Unknown	Wang et al. (2017a, b)
Fungi	<i>B. bassiana</i>	HaCTL14	Aggregates with <i>B. bassiana</i> to impair fungal infection	Unknown	Cheng et al. (2018)
	<i>B. bassiana</i>	BmCTL	A pattern recognition receptor for JAK/STAT pathway in fungal infection	Unknown	Geng et al. (2016)

poorly understood. Current knowledge of CTLs suggests that, in addition to mediating innate immune responses, some insect CTLs have unique functions, such as antimicrobial activity and stimulating cell proliferation, while other CTLs have multiple functions. Current research on CTLs mainly focuses on the functional analysis of individual CTLs, while a general and extensive CTL functional investigation in insects should be considered. A better understanding of insect CTLs may promote the development of novel antibacterial or viral transmission-blocking strategies. Further investigation into the functions and mechanisms of insect CTLs in innate immunity is expected because it may contribute to the protection of beneficial insects as well as the biological control of pests and disease vectors.

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Competing Interests The authors declare that they have no competing financial interests.

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