Chapter 7
The Immune System of Spiders

Lucia Kuhn-Nentwig and Wolfgang Nentwig

7.1 Introduction

Spiders, as all other arthropods, have an open circulatory system, and their body fluid, the hemolymph, freely moves between lymphatic vessels and the body cavities (see Wirkner and Huckstorf 2013). The hemolymph can be considered as a multifunctional organ, central for locomotion (Kropf 2013), respiration (Burmester 2013) and nutrition, and it amounts to approximately 20 % of a spider's body weight. Any injury includes not only immediate hemolymph loss but also pathogen attacks and subsequent infections. Therefore spiders have to react to injuries in a combined manner to stop fluid loss and to defend against microbial invaders. This is achieved by an innate immune system which involves several host defence systems such as hemolymph coagulation and the production of a variety of defensive substances (Fukuzawa et al. 2008).

In spiders, the immune system is localised in hemocytes which are derived from the myocardium cells of the heart wall where they are produced as prohemocytes and from where they are released as different cell types into the hemolymph (Seitz 1972). They contribute to the defence against pathogens by phagocytosis, nodulation and encapsulation of invaders. The humoral response includes mechanisms which induce melanin production to destroy pathogens, a clotting cascade to stop hemolymph loss and the constitutive production of several types of antimicrobial peptides, which are stored in hemocyte granules and released into the hemolymph (Fukuzawa et al. 2008) (Fig. 7.1).

The immune system of spiders is an innate immune system. It is hemolymph-based and characterised by a broad but not very particular specificity. Its advantage is a fast response within minutes to a few hours. This is in contrast to the adaptive immune system of vertebrates which can react to very specific pathogens, thus

L. Kuhn-Nentwig (✉) • W. Nentwig
Institute of Ecology and Evolution, University of Bern, Bern, Switzerland
e-mail: lucia.kuhn@iee.unibe.ch

W. Nentwig (ed.), *Spider Ecophysiology*,
DOI 10.1007/978-3-642-33989-9_7,

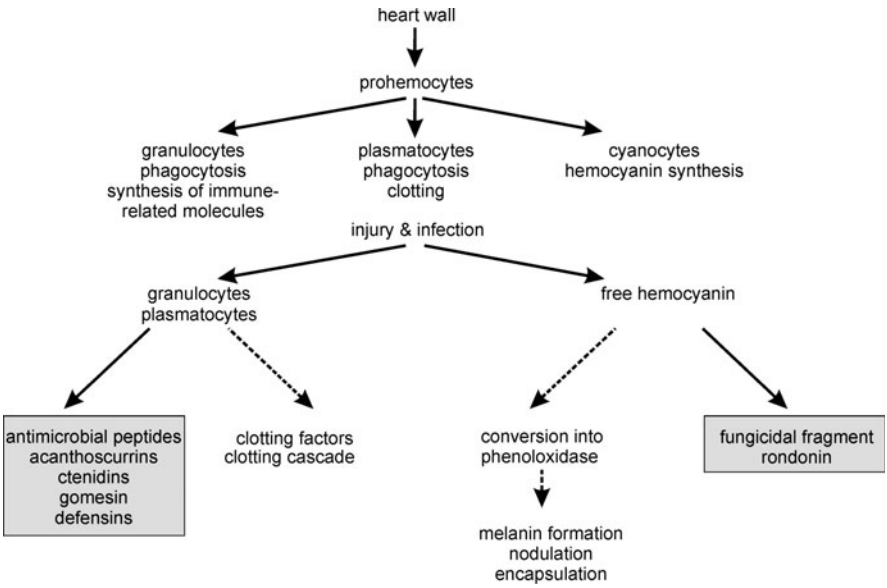

Fig. 7.1 Scheme of the immune system of spiders: origin and function of hemocytes (*upper part*) and reaction to injury and infection by microbial invaders (*lower part*). The reaction of spiders to injury and infection has not been well investigated and only the boxed components have been detected in spider hemocytes or hemolymph, whereas the clotting cascade and melanin formation pathway are adaptations from xiphosurans. For further details, see text

resulting in much more specific responses. Moreover, it creates an immunological memory during the lifetime of the species. The disadvantage is that it needs more time to react with antibody production, usually many hours to a few days, and needs to be built up during early ontogenesis.

7.2 Hemocytes

Seitz (1972) described the development of hemocytes in the heart wall of *Cupiennius salei* (Ctenidae), but did not recognise their immunological function. He rather thought that they were mainly storage cells, involved in moulting and yolk production. Sherman (1973) analysed the hemocytes of the theraphosid *Aphonopelma marxi* and realised the context to immune defence and clotting. He also reviewed the confusing nomenclature of blood cells (Sherman 1981) and distinguished three mature hemocyte types and a precursor cell type.

- Granulocytes are the most important hemocyte cell type. They are filled with granules and the most prominent feature of this hemocyte type, which contain several antimicrobial peptides to be released into the hemolymph (degranulation), is to kill pathogens. The nucleus is smaller than in plasmatocytes and they

also eliminate invaders via phagocytosis (Fukuzawa et al. 2008). Granulocytes vary in shape, including irregular, trapezoidal, triangular, oval and spindle forms, and their size is about 10 × 25 μm.

- Plasmatocytes are the most common hemocyte type and they have no granular inclusions, i.e. their cytoplasm is generally agranular or finely granular. They are polymorphic and show spindle, oval to ellipsoidal size, some are triangular or irregular. Plasmatocytes have approximately the same size as granulocytes (5–10 × 20–30 μm) and they are involved in phagocytosis of pathogens and in clotting (Sherman 1981).
- Cyanocytes represent the least common hemocyte type in the hemolymph and correspond to oenocytes (Sherman 1981). They are rare in the hemolymph (<5 % according to Millot 1949) but are frequently found in the heart (Sherman 1973). Their cytoplasma is dense and contains many ribosomes and protein crystals which were identified as hemocyanin in the case of *Aphonopelma marxi*. Cyanocytes are usually rounded or elongated, and they are the largest hemocytes with 20 × 50 μm (Sherman 1981). Cyanocytes synthesise hemocyanin which then is released into the hemolymph (Kemper 1983).
- Prohemocytes are small, mostly round cells with a large, compact nucleus and thin homogenous cytoplasm. As precursor cells of the other hemocytes, they are stem cells (Sherman 1981) and are mainly found in the heart wall (Fukuzawa et al. 2008; Seitz 1972).

Leberidocytes have also frequently been mentioned among blood cells. They are obviously only involved in the moulting process (Sherman 1981) and correspond to the moulting hemocytes described by Seitz (1976).

After an injury occurs, hemocytes migrate to the infection site. They directly fight against pathogens by phagocytosis, the formation of nodules to entrap bacteria (nodulation), and by the formation of capsules to entrap larger objects (encapsulation). Most importantly, hemocytes release components of the coagulation cascade to induce clotting and antimicrobial peptides to kill the invading microorganisms. The direct importance of phagocytosis has probably been overestimated but presumably it is important for the removal of cellular debris and for remodelling damaged tissues (Fukuzawa et al. 2008). While the overall functions of hemocytes in spiders are understood, it is unclear which hemocyte type is responsible for which part and up to which degree they are specialised for specific tasks.

7.3 The Humoral Response

The humoral response starts with the recognition of invading microorganisms, triggered by compounds from the bacterial surface, and leads to the initialisation of complex processes. They include (1) the start of cascades to induce clotting, (2) the start of cascades to induce melanisation and (3) the release of several antimicrobial peptides. Clotting is an important step to reduce further hemolymph

loss and to enable subsequent wound healing. Melanin production is an unspecific but, nevertheless, very efficient system to destroy bacterial invaders. Antimicrobial peptides, also described as killing factors, are effective against a wide spectrum of pathogens. They are known from most major taxonomic groups of organisms and they differ considerably in structure and specificity. In spiders, so far four groups of antimicrobial peptides have been identified: defensins, glycine-rich peptides, a small open-ended cyclic peptide, and a hemocyanin fragment.

7.3.1 The Clotting and Agglutination System

Hemocytes circulating in the hemolymph react very sensitively to contact with pathogens and release the content of their granules into the hemolymph immediately via rapid exocytosis. This process has not as yet been investigated in spiders but well investigated in the horseshoe crab *Tachypleus tridentatus* (Xiphosura) (Kawabata 2010), which represents the most ancestral extant chelicerate group, and thus might be a model which could at least be partially valid for spiders.

The clotting process is triggered by pathogen-associated molecular patterns, resulting in a proteolytic cascade and hemolymph coagulation (Iwanaga and Lee 2005). Compounds from the bacterial surface, such as lipopolysaccharides (major components of the cell wall of Gram-negative bacteria) and β-1,3 glucans (typical for fungi), initiate the start of a coagulation cascade in horseshoe crabs and probably also in spiders. In the presence of lipopolysaccharides, all the components of the cascade are released from hemocytes by degranulation. After binding of lipopolysaccharides to factor C, the serine protease zymogen is autocatalytically activated to factor C′, which itself activates factor B to B′. Activation of the proclotting enzyme occurs through factor B′ and results in the conversion of coagulogen into noncovalent coagulin homopolymers (Osaki and Kawabata 2004).

In a cDNA library of hemocytes from the theraphosid *Acanthoscurria gomesiana*, immune related transcripts have been identified as factor C precursor, coagulation factor B precursor and proclotting enzyme precursor-like components (Lorenzini et al. 2006). For *Cupiennius salei*, these three components of the clotting system have also been identified in a hemocyte cDNA library (Kuhn-Nentwig, unpublished). Transcripts concerning coagulogen precursor or stablin and proxin precursors involved in crosslinking of the coagulin homopolymers via hemocyte-derived transglutaminase, as verified for horseshoe crabs, have not been detected so far in spiders (Kawabata 2010; Kawabata et al. 2002; Matsuda et al. 2007). However, ESTs concerning a protein-glutamine gamma-glutamyltransferase (EC2.3.2.13) are known from *A. gomesiana* and *C. salei* (Lorenzini et al. 2006; Kuhn-Nentwig, unpublished results). No information is available concerning the β-1,3 glucan initiated clotting cascade via activation of factor G, as reported for horseshoe crabs (Kawabata 2010).

In horseshoe crabs, pathogens can also be recognised and agglutinated by a series of compounds present in the hemolymph and circulating hemocytes. Besides

several agglutinins, five types of lectins and three types of C-reactive proteins have been described (Kawabata 2010). Lorenzini and co-workers identified for *A. gomesiana* a transcript encoding tachylectin 5a and 5b-like components, hinting to similarities in these systems (Iwanaga and Lee 2005; Lorenzini et al. 2006).

7.3.2 The Phenoloxidase System and Melanin

During the early evolution of arthropods, hemocyanins evolved from a phenoloxidase-like enzyme. Hemocyanin, one of the major components of spider hemolymph, is usually known as an oxygen carrier (see Burmester 2013), while phenoloxidases are key enzymes in the melanin-producing pathway of arthropods that plays an important role in the sclerotisation of the cuticle. Since melanin is also toxic to microorganisms, additional functions in wound healing and in the humoral immune system evolved at the same time, and it has been argued that the simultaneous appearance of the arthropod exoskeleton and of an effective immune system obviously fostered the evolution of arthropods remarkably (Burmester 2002).

In contrast to other arthropods, chelicerates do not have a true phenoloxidase and hemocyanin itself acts also as a phenoloxidase after limited proteolysis with trypsin or chymotrypsin, as shown for the theraphosid *Aphonopelma hentzi* (sub *Eurypelma californicum*, see Nentwig 2012) (Decker and Rimke 1998). During the evolution of spider hemocyanin, two hemocyanin subunits not only conserved the original properties of phenoloxidases but also gained the property of the other subunits to bind oxygen reversibly (Decker and Tuczek 2000; Jaenicke and Decker 2004). For horseshoe crabs, the above described coagulation cascade is linked to prophenoloxidase activation in which the clotting enzyme transforms hemocyanin to phenoloxidase without proteolytic cleavage (Nagai and Kawabata 2000). Furthermore, the functional conversion of hemocyanin to phenoloxidase is also mediated by the binding of the antimicrobial peptides tachyplesin and tachystatins to the hemocyanin α-subunit (Nagai et al. 2001).

In arthropods in general, the activity of phenoloxidase follows a similar pattern as in the above described clotting process with recognition of compounds from bacterial membranes by recognition molecules. They react on serine proteases which activate a prophenoloxidase to a phenoloxidase (Söderhäll and Cerenius 1998). This is performed by hemocyanin and it leads to the production of melanin, a polymer of dihydroxyindole carboxylic acids, the monomers of which are derived from the amino acid tyrosine. The intermediary compounds of this process and melanin itself are toxic to microorganisms. Within minutes after infection, melanin is deposited either onto the surface of the microorganisms or onto a capsule of microorganisms and this process is called melanisation. Microorganisms are engulfed in a hardened gel and this leads finally to their destruction (Söderhäll and Cerenius 1998; Iwanaga and Lee 2005).

7.3.3 Antimicrobial Peptides

The contact of hemocytes with bacterial membrane compounds leads to an immediate release of a variety of antimicrobial peptides. The main location of these peptides seems to be the granulocytes but they contain different granule types which, in turn, may each contain different sets of antimicrobial peptides (Fukuzawa et al. 2008). While several compounds with antimicrobial activity were found for the horseshoe crab *Tachypleus tridentatus* (Iwanaga and Lee 2005), only a few substances have been described so far for spiders (Silva et al. 2000; Lorenzini et al. 2003; Baumann et al. 2010a, b).

7.3.3.1 Defensins

Spider defensins are small, amphipathic cysteine-rich peptides and consist of 37 amino acid residues. The peptides exhibit six conserved cysteines and follow most likely the cysteine bonding pattern of arthropod defensins (C1–C4, C2–C5, C3–C6), which characterises them as members of the "ancestral group" of invertebrate defensins (Froy and Gurewitz 2003). Their precursors reveal a putative signal peptide of 23–24 amino acid residues, followed by the peptide precursor of 37 amino acid residues and the stop signal. Defensins are primarily active against Gram-positive bacteria.

Defensins are known from several groups of araneomorph spiders such as Araneidae (*Argiope* sp.), Tetragnathidae (*Meta menardi*), Agelenidae (*Tegenaria atrica*), Sparassidae (*Polybetes pythagoricus*), and Ctenidae (*Cupiennius salei* and *Phoneutria reydyi*). Within spiders, defensins are rather similar with 75–100 % similarity at amino acid level. The overall similarity with defensins from ticks and scorpions is in the range of up to 70 %, while the similarity with insects is much lower (Baumann et al. 2010a).

In *C. salei*, it could be shown that defensins are constitutively expressed not only in hemocytes but also in other organs such as ovaries, subesophageal nerve mass, hepatopancreas and muscles of uninfected spiders. This underlines the important role defensins play, not only for defence in the hemolymph, but also in various tissues. Despite analysis of a hemocyte cDNA library of the theraphosid *Acanthoscurria gomesiana* (Lorenzini et al. 2006), up to now no defensin could be identified on the mRNA or peptide level for mygalomorph spiders.

7.3.3.2 Glycine-Rich Peptides

These antimicrobially acting peptides are cationic and characterised by an unusually high glycine content of 71–73 % and only 6–8 further amino acids. This results in repeats of 3–6 glycines, mostly interrupted by a different amino acid. Two very

similar peptide families are known so far, acanthoscurrins from mygalomorph and ctenidins from araneomorph spiders.

Two acanthoscurrin isoforms have been isolated from the hemocytes of *Acanthoscurria gomesiana*. They are 130 and 132 amino acids long, with molecular masses of 10.1 and10.2 kDa. Acanthoscurrins have a positive net charge of +8 at physiological pH and are characterised by a three-fold repeat of 26 amino acids: GGGLGGGGLGGGGLGGGKGLGGGGLG. These peptides are active against the Gram-negative bacterium *Escherichia coli* SBS363 (Minimal inhibitory concentration MIC = 2.3–5.6 μM) and the yeast *Candida albicans* (MIC = 1.15–2.3 μM), but however no activity could be measured against the Gram-positive *Micrococcus luteus* up to a concentration of 5.6 μM. Acanthoscurrins are constitutively expressed in hemocytes, stored in their granules and released into the hemolymph upon infection (Fukuzawa et al. 2008; Lorenzini et al. 2003).

In *Cupiennius salei* three ctenidin isoforms have been isolated, which act against Gram-negative *E. coli* (MIC = 2.5–5 μM) but not against the Gram-positive bacteria *Staphylococcus aureus*. Up to a tested concentration of 5 μM, no activity against *C. albicans* was detectable. Growth patterns of bacteria suggest that ctenidins act bacteriostatically rather than bactericidally. The three mature peptides are 109, 119 and 120 amino acid residues long, with molecular masses between 8.8 and 9.6 kDa. Ctenidins are distinguished from acanthoscurrins by a central sequence of 10 amino acids which interrupts the glycine repeat: VIDGKDDVGL. The function of this sequence is still unknown. Ctenidins are constitutively expressed mainly in hemocytes, to a small amount also in the subesophageal nerve mass, but not in six other tissues so far investigated (Baumann et al. 2010b).

7.3.3.3 Small Open-End Cyclic Peptide

Gomesin is a small, open-ended cyclic peptide consisting of 18 amino acids (2.3 kDa) isolated from hemocytes of *Acanthoscurria gomesiana* (Silva et al. 2000). This peptide forms two internal disulphide bridges and adopts a β-hairpin like fold, thus strongly resembling antimicrobial peptides known from other arachnids (tachyplesin and polyphemusin from horseshoe crabs, androctin from scorpions). Gomesin is constitutively expressed in hemocytes, it is stored in the granules and released into the hemolymph after lipopolysaccharide stimulation in a concentration-dependent manner (Fukuzawa et al. 2008). Gomesin has been tested against a variety of microorganisms and it strongly affects the growth of 24 out of 27 tested strains of Gram-positive and Gram-negative bacteria, as well as the development of nine filamentous fungi species and five yeast species. Also eukaryotic cells, such as *Leishmania amazonensis* cells or human blood cells, are affected (Silva et al. 2000). Although gomesin is so far only known from one theraphosid spider, it can be assumed that comparable peptides are more widely spread among spiders, especially since related substances are found in other arachnid groups. Gomesin also exhibits in vitro and in vivo cytotoxicity on tumour cells (Rodrigues et al. 2008), and it is supposed that the cytotoxic effect of this peptide involves L-type calcium channel ion influx, intracellular signalling as well as the generation of reactive oxygen species (Soletti et al. 2010).

7.3.3.4 Hemocyanin Fragments

Very recently, the isolation of rondonin, an antifungal peptide from the hemolymph of the theraphosid *Acanthoscurria natalensis* (sub *A. rondoniae*) has been reported. The amino acid sequence of this small peptide (IIIQYEGHKH) corresponds to a C-terminal fragment of the 'd' subunit of hemocyanin identified from *Acanthoscurria gomesiana* and *Aphonopelma hentzi*. This protein fragment is not active against several Gram-negative and Gram-positive bacteria at a tested concentration up to 67 μM, but exerts a fungicidal activity against fungi and yeasts in micromolar concentrations (fungi: MIC = 1.1–2.1 μM; yeasts: MIC = 8.37–33.5 μM) (Riciluca et al. 2012).

7.4 Hemocytes Derived Compounds Involved in the Immune Response

Mygalin is an acylpolyamine isolated from the hemocytes of mygalomorph and araneomorph spiders (Baumann 2009; Pereira et al. 2007). The compound was identified as bis-acylpolyamine *N*1,*N*8-bis(2,5-dihydroxybenzoyl) spermidine with a molecular mass of 417 Da. Mygalin is only active against *E. coli* in a concentration of 85 μM, but not active against the Gram-positive bacteria *M. luteus* and the yeast *C. albicans*. Pereira et al. supposed an antibacterial activity mediated through the production of hydrogen peroxide. Contrary to this, mygalin isolated from the hemocytes of *Cupiennius salei* exhibits only weak activity in the millimolar range against *E. coli* and *S. aureus* (MIC = 0.5–1 mM). This discrepancy between the results from both publications is not yet understood and needs further investigations (Baumann 2009). Recently, mygalin was also identified as a potent modulator of vertebrate innate immune responses (Mafra et al. 2012).

A single insulin-like growth factor binding domain protein (SIBD-1) was isolated from the hemocytes of *C. salei*. SIBD-1 (8.7 kDa) is characterised by six disulfide bridges and carries a sugar moiety at Thr 2 (Fuc-GlcA-GalNAc-Thr). The peptide is mainly expressed in hemocytes. After infection, the peptide content in the hemocytes decreases and also the temporal SIBD-1 expression seems to be downregulated. While SIBD-1 itself does not affect pathogens, these results indicate that it may be involved in regulatory processes of the immune system (Kuhn-Nentwig et al. 2011; Trachsel et al. 2012).

From the hemocytes of the theraphosid *Lasiodora* sp. the first elastase inhibitor showing antibacterial activity against *Enterococcus faecalis* has been described (Soares et al. 2011).

7.5 Conclusions

The immune system of spiders is localised in their hemocytes and reacts very fast to an invasion by pathogens and to injuries by releasing several compounds into the hemolymph. The immune response comprises (1) phagocytosis, nodulation and encapsulation of invaders, (2) the regulation of hemocyanin/phenoloxidase to produce melanin which destroys pathogens, (3) a clotting cascade to stop hemolymph loss and immobilise invaders and (4) the constitutive production of antimicrobial peptides. So far four different antimicrobial peptide groups have been identified in spiders. Firstly, six similar defensins have been detected in different spider groups. A second group of antimicrobial peptides comprises several glycine-rich peptides (acanthoscurrins and ctenidins), a third group of small cysteine-rich peptides contains gomesin, an 18 amino acid residue peptide, and as a fourth group, a hemocyanin fragment has been identified.

References

Baumann T (2009) Immune defence of *Cupiennius salei*. PhD thesis, University of Bern

Baumann T, Kämpfer U, Schürch S, Schaller J, Largiadèr C, Nentwig W, Kuhn-Nentwig L (2010a) Ctenidins: antimicrobial glycine-rich peptides from the hemocytes of the spider *Cupiennius salei*. Cell Mol Life Sci 67:2787–2798

Baumann T, Kuhn-Nentwig L, Largiadèr C, Nentwig W (2010b) Expression of defensins in non-infected araneomorph spiders. Cell Mol Life Sci 67:2643–2651

Burmester T (2002) Origin and evolution of arthropod hemocyanins and related proteins. J Comp Physiol B 172:95–107. doi:10.1007/s00360-001-0247-7

Burmester T (2013) Evolution and adaptation of hemocyanin within spiders. In: Nentwig W (ed) Spider ecophysiology. Springer, Heidelberg (this volume)

Decker H, Rimke T (1998) Tarantula hemocyanin shows phenoloxidase activity. J Biol Chem 273:25889–25892

Decker H, Tuczek F (2000) Tyrosinase/catecholoxidase activity of hemocyanins: structural basis and molecular mechanism. Trends Biochem Sci 25:392–397

Froy O, Gurewitz M (2003) Arthropod and mollusk defensins-evolution by exon-shuffling. Trends Genet 19:684–687

Fukuzawa AH, Vellutini BC, Lorenzini DM, Silva PI Jr, Mortara RA, da Silva JMC, Daffre S (2008) The role of hemocytes in the immunity of the spider *Acanthoscurria gomesiana*. Dev Comp Immunol 32:716–725

Iwanaga S, Lee BL (2005) Recent advances in the innate immunity of invertebrate animals. J Biochem Mol Biol 38:128–50

Jaenicke E, Decker H (2004) Functional changes in the family of type 3 copper proteins during evolution. Chembiochem 5:163–169. doi:10.1002/cbic.200300714

Kawabata S (2010) Immunocompetent molecules and their response network in horseshoe crabs. Adv Exp Med Biol 708:122–136

Kawabata S, Osaki T, Iwanaga S (2002) Innate immunity in the horseshoe crab. In: Ezekowitz RAB, Hoffmann JA (eds) Infectious disease: innate immunity. Humana, New York

Kemper B (1983) Site of hemocyanin biosynthesis in the tarantula *Euryplema californicum*. Naturwissenschaften 70:255–256

Kropf C (2013) Hydraulic system of locomotion. In: Nentwig W (ed) Spider ecophysiology. Springer, Heidelberg (this volume)

Kuhn-Nentwig L, Largiadèr CR, Streitberger K, Chandru S, Baumann T, Kämpfer U, Schaller J, Schürch S, Nentwig W (2011) Purification, cDNA structure and biological significance of a single insulin-like growth factor-binding domain protein (SIBD-1) identified in the hemocytes of the spider *Cupiennius salei*. Insect Biochem Mol Biol 41:891–901

Lorenzini DM, da Silva PI, Fogaça AC Jr, Bulet P, Daffre S (2003) Acanthoscurrin: a novel glycine-rich antimicrobial peptide constitutively expressed in the hemocytes of the spider *Acanthoscurria gomesiana*. Dev Comp Immunol 27:781–791

Lorenzini DM, Da Silva PI Jr, Soares MB, Arruda P, Setubal J, Daffre S (2006) Discovery of immune-related genes expressed in hemocytes of the tarantula spider *Acanthoscurria gomesiana*. Dev Comp Immunol 30:545–556

Mafra DG, Da Silva PI Jr, Galhardo CS, Nassar R, Daffre S, Sato MN, Borges MM (2012) The spider acylpolyamine mygalin is a potent modulator of innate immune responses. Cell Immunol 275:5–11

Matsuda Y, Osaki T, Hashii T, Koshiba T, Kawabata S (2007) A cysteine-rich protein from an arthropod stabilizes clotting mesh and immobilizes bacteria at injury sites. J Biol Chem 282: 33545–33552

Millot J (1949) Appareil circulatoire. In: Grasse P (ed) Traité de Zoologie, vol VI. Masson, Paris, pp 639–646

Nagai T, Kawabata S (2000) A link between blood coagulation and prophenol oxidase activation in arthropod host defense. J Biol Chem 275:29264–29267

Nagai T, Osaki T, Kawabata S (2001) Functional conversion of hemocyanin to phenoloxidase by horseshoe crab antimicrobial peptides. J Biol Chem 276:27166–27170

Nentwig W (2012) The species referred to as *Eurypelma californicum* (Theraphosidae) in more than 100 publications is likely to be *Aphonopelma hentzi*. J Arachnol 40:128–130

Osaki T, Kawabata S (2004) Structure and function of coagulogen, a clottable protein in horseshoe crabs. Cell Mol Life Sci 61:1257–1265

Pereira LS, Silva PI Jr, Miranda MT, Almeida IC, Naoki H, Konno K, Daffre S (2007) Structural and biological characterization of one antibacterial acylpolyamine isolated from the hemocytes of the spider *Acanthocurria gomesiana*. Biochem Biophys Res Commun 352:953–959

Riciluca KCT, Sayegh RSR, Melo RL, Silva PI Jr (2012) Rondonin an antifungal peptide from spider (*Acanthoscurria rondoniae*) haemolymph. Res Immunol 2:66–71

Rodrigues EG, Dobroff AS, Cavarsan CF, Paschoalin T, Nimrichter L, Mortara RA, Santos EL, Fazio MA, Miranda A, Daffre S, Travassos LR (2008) Effective topical treatment of subcutaneous murine B16F10-Nex2 melanoma by the antimicrobial peptide gomesin. Neoplasia 10: 61–68

Seitz KA (1972) Zur Histologie und Feinstruktur des Herzens und der Hämocyten von *Cupiennius salei* KEYS. (Araneae, Ctenidae). Zool Jb Anat 89:351–384

Seitz KA (1976) Zur Feinstruktur der Häutungshämocyten von *Cupiennius salei* KEYS (Araneae, Ctenidae). Zool Jb Anat 96:280–292

Sherman RG (1973) Ultrastructurally different hemocytes in a spider. Can J Zool 51:1155–1159

Sherman RG (1981) Chelicerates. In: Ratcliffe NA, Rowley AF (eds) Invertebrate blood cells, vol 2. Academic, London

Silva PI Jr, Daffre S, Bulet P (2000) Isolation and characterization of gomesin, an 18-residue cysteine-rich defense peptide from the spider *Acanthoscurria gomesiana* hemocytes with sequence similarities to horseshoe crab antimicrobial peptides of the tachyplesin family. J Biol Chem 275:33464–33470

Soares T, Ferreira FRB, Gomes FS, Coelho LCBB, Torquato RJS, Napoleao TH, Cavalcanti MSM, Tanaka AS, Paiva PMG (2011) The first serine protease inhibitor from *Lasiodora* sp. (Araneae: Theraphosidae) hemocytes. Prog Biochem 46:2317–2321

Söderhäll K, Cerenius L (1998) Role of the prophenoloxidase-activating system in invertebrate immunity. Curr Opin Immunol 10:23–28

Soletti RC, del Barrio L, Daffre S, Miranda A, Borges HL, Moura-Neto V, Lopez MG, Gabilan NH (2010) Peptide gomesin triggers cell death through L-type channel calcium influx, MAPK/ERK, PKC and PI3K signaling and generation of reactive oxygen species. Chem Biol Interact 186:135–143

Trachsel C, Widmer C, Kämpfer U, Buhr C, Baumann T, Kuhn-Nentwig L, Schürch S, Schaller J, Baumann U (2012) Structural and biochemical characterization of native and recombinant single insulin-like growth factor-binding domain protein (SIBD-1) from the Central American hunting spider *Cupiennius salei* (Ctenidae). Proteins 80:2323–2329

Wirkner C, Huckstorf K (2013) The circulatory system of spiders. In: Nentwig W (ed) Spider ecophysiology. Springer, Heidelberg (this volume)