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Review

Insect immunorecognition

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Abstract

The mechanisms of the innate immunity in the insects have been reviewed. In particular, the cellular component (phagocytosis, encapsulation, melanization, nodule formation, wound healing, hemolymph clotting and transplantation) and the humoral component (lectins, cytokine-like molecules and anti-microbial peptides) of the hemolymph have been investigated.

Key Words: insects; immune and neuroendocrine responses

Introduction

The First line of defence in insects is the cuticle. This external barrier of carbohydrate-protein material protects the animal from both physical damage and pathogen attack. The second and more important line of defence is the internal defence system, with a well-developed capacity to discriminate between self and non-self. As other coelomatic animals, insects implement this recognition through the cellular and humoral components of the hemolymph.

Cellular component

With regard to the cellular component, there is a general problem in defining the number of immunocytes present in the invertebrate hemolymph and this is a subject of great debate. One reason for this situation is that in most cases there is no hemopoietic organ. When, however, the organ is present, as for example in insects, it supplies immunocytes during the animal's development, and these continue to differentiate in the circulation (Nappi and Carton, 1986). However, the immunocyte proliferation occurs mainly in circulating hemolymph, thus containing different stages of maturation of the same cell.

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To avoid describing these different stages of maturation as specific cell types, morphological studies should be performed in parallel with functional analyses. This problem is also seen in insect immunocytes. These derive from the mitotic division of circulating blood cells and hemopoietic organs (Jones, 1977). The hemopoietic organs have been described in different species and present peculiar locations and morphological characteristics. In particular, five pairs of lymph glands located along the anterior portion of the dorsal blood vessel are the sources for immunocytes in *Drosophila melanogaster* (Nappi and Carton, 1986). Hoffmann (1973) reported two possible organizations for hemopoietic organs. One is structured as dissociated accumulations of immunocytes that could be referred to as the "haemocyte reservoirs" described by Jones (1977), while the other is considered a truly differentiated hemopoietic organ. In their elegant treatise, Rowley and Ratcliffe (1981) reported the presence in the insect hemolymph of the following cell types: prohaemocytes, plasmatocytes, granular cells, cystocytes, spherule cells and oenocytoids. Subsequently Brehélin and Zachary (1986) proposed a new classification of blood cells, based mainly on the ultra-structural observations, and nine cell types are described: prohaemocytes, plasmatocytes, oenocytoids, spherule cells, thrombocytoids and four types of granular haemocytes. Among the different types of cells described from different species, some have an immune function, while in others either the function is unknown or it is not directly involved in defence. Prohaemocytes are present in all the species examined. They are small and rounded, with a large, round nucleus and seem to function as stem cells

(Brehélin and Zachary, 1986; Franchini *et al.*, 1996) (Fig. 1). Plasmatocytes share the typical functions of a macrophage, i.e. glass adhesion with the emission of pseudopodia allowing amoeboid movement, phagocytic capacity, encapsulation, nodule formation and wound repair (Rowley and Ratcliffe, 1981; Franchini *et al.*, 1996). Despite the morphology, granular cells also present the immune functions described in plasmatocytes, even if to a lesser extent. Spherule cells do not seem to be involved in immune functions, but the available data suggest a role in the synthesis of blood mucopolysaccharides (Gupta and Sutherland, 1967; Akai and Sato, 1973) and in silk production in *Bombix mori* (Nittono, 1960). The function of the oenocytoids is also as yet not established, even if the cytoplasmic presence of phenoloxidase (proPO) activity may support an involvement in melanization processes. The crystal cells containing a crystal of tyrosine belong to this cell type and are found in some Diptera (Rizki and Rizki, 1959; Drif and Brehélin, 1983).

Despite attempts to establish a correct, comparative classification of insect blood cell types, problems still remain. Apart from the prohaemocytes, probably only two immunocytes, plasmatocyes and granular cells, appear to play a role in immune functions. However, some exceptions exist. *D. melanogaster*, for instance, only shows the plasmatocyte together with the specialized crystal cells (Nappi and Corton, 1986). This result reflects findings in other invertebrates such as molluscs and annelids (Yoshino, 1976; Cheng and Guida, 1980; Ottaviani, 1992; Cooper *et al.*, 1995).

Phagocytosis

This phenomenon is of pivotal importance for nutrition and defence, and it is present throughout the animal kingdom. As mentioned before, both plasmatocytes (Fig. 2) and granular cells are involved in phagocytosis. The process is characterized by various steps: recognition (chemotaxis and attachment), ingestion and killing. Chemotaxis, i.e. non-random locomotion, allows cells to move towards a chemoattractant which has been recognized. The data on insect chemotaxis are controversial: some authors have demonstrated a chemotactic action of *Aspergillus flavus* conidia towards immunocytes from *Galleria mellonella* (Vey et al., 1968; Vey, 1969), while others failed to see such an action (Salt, 1970; Jones, 1956). Furthermore, plasmatocyte chemotactic activity has been seen to be involved in encapsulation, nodule formation and wound repair (Nappi, 1973; Ratcliffe and Gagen, 1977; Rowley and Ratcliffe, 1978).

The study of the mechanism by which invertebrate immunocytes recognize non-self material is also still in its infancy. It should be remembered that the immune system involves two types of response: innate (or natural) and adaptive (or acquired). Innate immunity is conserved from invertebrates to vertebrates, while adaptive immunity, based on antigen-specific T and B cells, only appears in vertebrates. In the latter, the old, innate immune system is able to discriminate between self and non-self by means of pattern-recognition receptors (PRRs), able to recognize conserved pathogen-associated molecular patterns (PAMPs) produced by microorganisms (Fearon, 1997; Medzhitov and Janeway, 2000). A similar scenario could also be present in invertebrates. Mammalian immune cells express several Toll-like receptors that are considered PRRs (Akira, 2001). In *Drosophila*, the innate immune recognition of micro-organisms is mediated by signalling through Toll receptors (Hoffmann *et al.*, 1999; Takeda and Akira, 2001). Peptidoglycan recognition proteins (PGRFs) are able to recognize bacteria and their cell wall component, the peptidoglycan, and are well conserved from insects to mammals (Dziarski, 2004). The mosquito *Anopheles gambiae* secretes a thioester-containing protein (TEP), αTEP1, which is related to vertebrate complement factors and α_2-macroglobulins (Levashina *et al.*, 2001). The reported studies are some examples of the extensive literature seeking to complete the mosaic of mechanisms involved in invertebrate innate immunity. Immunocytes also recognize abiotic material suggesting that these cells exert their recognition not only on micro-organism PAMPs, but it is unknown how this happens. Lavine and Strand (2002) surmise the presence in invertebrate immunocytes of receptors with promiscuous capacities that can interact with a wide range of molecules normally not encountered in the hemocoel.

In summary, as suggested by Medzhitov and Janeway (1997), innate immunity is based on the recognition of invariant modules in different bacterial and viral species. Consequently, the limited number of recognition systems is mirrored by a limited number of molecular structures, which are recognized. Paradoxically, from an invertebrate point of view, few are the microorganisms making the small number of recognition units (an extremely restricted repertoire) perfectly adequate for the small number of foreign modules to be recognized. The small number of recognition units induces a complex and efficient reaction by the ancestral defence based on the immune-neuroendocrine effector system present in invertebrates.

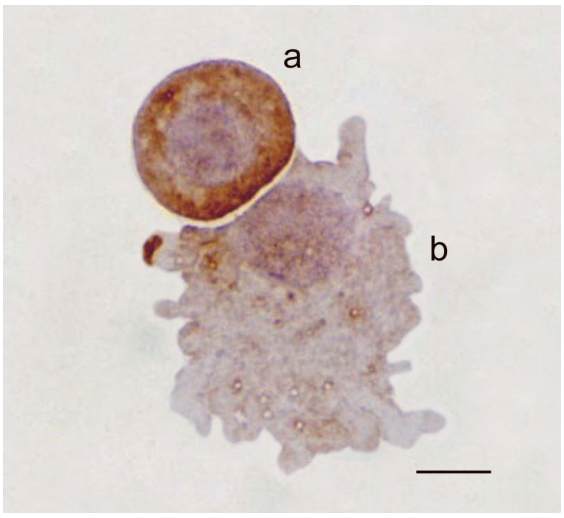

Fig. 1 Cytospin preparation of immunocytes from *Calliphora vomitoria*: a) plasmatocyte, b) granular cells (Bar = 10 μm).

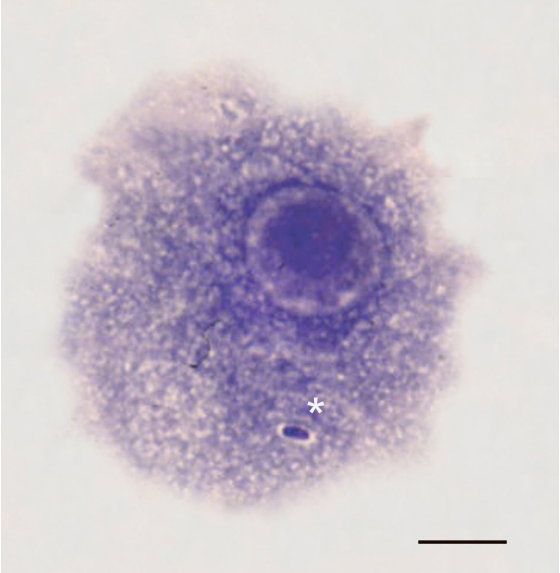

Fig. 2 *In vitro* bacterial phagocytosis from *Calliphora vomitoria* plasmatocyte (asterisk) (Bar = 10 μm) (Modified from Franchini *et al.*, 1996).

Innate immunity, stress and inflammation are interconnected in this system that has been conserved throughout evolution (Ottaviani and Franceschi, 1997; Ottaviani *et al.*, 1998). The last steps in phagocytosis are ingestion and killing. As in vertebrates, the ingested phase shows the formation of phagosomes and subsequent lysosomal fusion, as reported in the plasmatocytes of *G. mellonella* (Rowley and Ratcliffe, 1979). Studies in the fruit fly *Ceratitis capitata* suggest that signal transduction pathways that modulate phagocytosis are conserved in insects and mammals (Foukas *et al.*, 1998; Metheniti *et al.*, 2001). Furthermore, TEPs seem to have an opsonic effect on the *Drosophila* phagocytosis in a similar manner to mammalian complement factor C3 (Lagueux *et al.*, 2000). Similar to mammals, the ingested phase needs energy that derives from the glycolitic pathway, as demonstrated by Anderson *et al.* (1973) in the *Blaberus craniifer* immunocytes. The authors also report an antimicrobial system. Indeed, reactive oxygen intermediates (ROI), reactive nitrogen intermediates (RNI) and nitric oxide (NO) are found in immunocytes of insects (Whitten and Ratcliffe, 1999; Luckhart *et al.*, 1998; Nappi *et al.*, 2000). In *D. melanogaster* and *D. teissieri*, NO activates the gene encoding the anti-microbial peptide Diptericin (Nappi *et al.*, 2000).

Encapsulation

Cellular encapsulation is a mechanism present in all the insect groups examined (Rowley and Ratcliffe, 1981). This means of defence involves immobilizing insect parasites, fungi and large protozoans that escape the phagocytic activity of the single immunocyte. The reaction is strong against living organisms and is followed by melanization, while it is weak against abiotic material and not always followed by melanization. The encapsulated material is surrounded by multi-cellular sheaths, hence the term capsule. Götz (1986) defines ten steps in this cellular defence, with granular cells and plasmatocytes as the main actors. Briefly, the first event in the encapsulation process is the contact of the immunocytes with foreign material and the degranulation of the granular cells. The material released from granules is sticky and adheres to foreign surfaces. The degranulation and disintegration of granular cells activate the plasmatocytes that participate in the formation of the capsule. Subsequently, the process of melanization, originated in the granular cells, takes place. The approximate time of the encapsulation is about 1-3 days (Fig. 3).

Role of melanization

Melanization is involved not only in encapsulation, but also in cuticular defenses, e.g. against bacteria, wounding and sclerotization, as well as in other defense responses such as the killing of bacteria or parasites and cytotoxicity (Söderhräll and Smith, 1986; Ashida and Brey, 1995; Nappi *et al.*, 1992; Nayar *et al.*, 1992; Nappi and Ottaviani, 2000). The melanin is synthesized by the activation of the proPO-activating system (proPO System) (Söderhräll, 1982) comprising a complex cascade of serine proteases that allows the conversion of proPO to phenoloxide (PO), which, in turn, acts on substrates such as tyrosine and its derivatives (DOPA and dopamine) to form melanin. This process was extensively studied for the first time in crustacean *Astacus astacus* (Söderhräll, 1982) and in the insect *Bombyx mori* (Ashida, 1971, Ashida and Ohnishi, 1967, Ashida *et al.*, 1983). The activation of proPO has now been described in several invertebrate taxa (Arizza *et al.*, 1995; Beschin *et al.*, 1998; Frizzo *et al.*, 1999; Tujula *et al.*, 2001). The proPO System has also been seen as a recognition system activated by different foreign materials, such as β-1,3-glucans from fungal and algal cell walls, and lipopolysaccharides and peptidoglycans from microbial cell walls (Söderhräll and Cerenius, 1998). The cDNA encoding PGRPs has been cloned from the silkworm *B. mori* fat body cDNA library (Ochiai and Ashida, 1999). The specific binding of PGRP to the peptidoglycan induces the proPO cascade. Furthermore, these studies show that the means of extracellular recognition of microorganisms is conserved from insects to mammals.

With regard the role of the proPO system in the cuticular site, it has been demonstrated that proPO originates from the circulating immunocytes in the silkworm *B. mori* and from immunocytes and epidermal cells in the caterpillar *Calpodes ethlius*. Subsequently, it is actively transported across the epidermis to the cuticular matrix (Ashida and Brey, 1995; Sass *et al.*, 1994). The proPO cuticular silkworm is activated through a limited proteolysis by the serine proteinase, termed proPO activating enzyme, which itself exists as a zimogen (Ashida and Brey, 1995). Cuticular PO is normally considered to be injury PO, however, other two types of PO are present in the cuticle of insects: granular PO involved in the body colour and laccase-type PO involved in sclerotization of a newly ecdysed cuticle (Barrett, 1991). Recently,

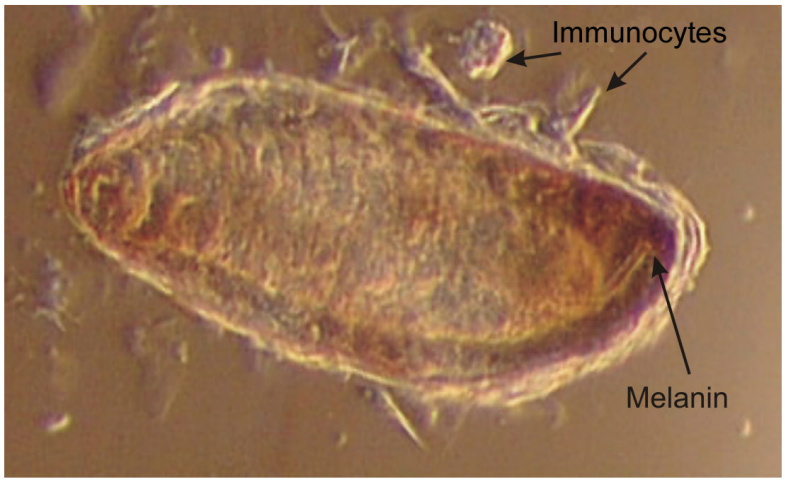

Fig. 3 Encapsulation of the egg of the wasp parasitoid *Leptopilina boulardi* in *Drosophila melanogaster* host (Courtesy Prof. Nappi AJ).

Asano and Ashida (2001) studying cuticular PO and its zymogen (proPO) in *B. mori* purified two proPO isoforms. In the same animal, the authors also found two proPO isoforms in the hemolymph. These latter isoforms differ from the cuticular isoforms for the presence of five aminoacid residues. All the isoforms are activated by specific enzymes, and the hemolymph isofoms are transported to the cuticle.

Nodule formation

This type of cellular defence takes place when phagocytosis is inadequate in the face of a large number of small non-self particles such as bacteria. Rowley and Ratcliffe (1981) divide nodule formation into two phases. In the first, the bacteria are entrapped in the material released by exocytosis from the granular cells and melanization then occurs externally. In the second, plasmotocytes, adhere and flatten the necrotic core forming the typical multicellular sheath.

Wound healing, hemolymph clotting and transplantation

Invertebrates respond to an external injury in order to avoid the loss of biological liquids. Different mechanisms, such as fat or intestine extrusion, muscular contraction, hemolymph clot formation, cellular aggregation and melanin deposition, have been described (Theopold *et al.*, 2004, 2002; Bidla *et al.*, 2005). The hemolymph clot is well-known in the American cockroach, *Leucophaea maderae* (Bohn and Barwig, 1984). The process involves clotting proteins present in the hemolymph plasma (plasma coagulogen) that are released from immunocytes (hemocyte coagulogen). The immunocytes migrate and aggregate around the altered region, and after their rupture the clotting starts. The process involves an enzymatic cascade which is activated by Ca^{2+}. The clotting reaction is very fast and in less than 3 minutes the clot is completely insoluble. A different clotting mechanism is described for *Locusta migratoria* (Brehélin, 1979). In this case, coagulation involves only the plasma (by means of the coagulogen), and this seems to be a functional equivalent in clotting of mammalian fibrinogen.

Lackie (1986) raised the question of how the damaged self is recognized, but following a study of the possible mechanisms involved, i.e. a non-specific response to the altered physiochemical surface proprieties of the connective tissue layer, a specific response via receptor-ligand interactions and the release of wounding factors by immunocytes, found no conclusive answer.

Tissue graft transplantation is a technique to test immunological specificity and memory in insects and, in general, in invertebrates, even if the mechanisms by which the host recognizes self and non-self implanted material have not yet been clarified. Auto- and allo-grafts of integument in the insects *B. craniifer and Extatosoma tiaratum* were accepted, while xenografts were rejected after melanization. All these phenomena showed an accumulation of immunocytes around the grafts which was more marked in the xenografts (Thomas and Ratcliffe, 1982). A different result was obtained with allogeneic cuticle and xenogeneic cuticle from *Blatta orientalis*. Both grafts were recognized as foreign by *Periplaneta americana*, while implanted xenogeneic tissue from *B. craniifer* was not rejected by *P. americana* (Lackie, 1983). The transplantation experiments by Karp and Meade (1993) using the same species, i.e. *P. americana* and *B. orientalis*, found that *P. americana* recognizes *B. orientalis* as foreign and mounts a rejection response against integumentary grafts.

Humoral component

Although lacking immunoglobulins, insects, as all other invertebrates, possess a variety of factors of varying degrees of specificity, including lectins, cytokine-like molecules and anti-microbial peptides.

Lectins

Lectins are sugar-binding proteins or glycoproteins that agglutinate cells and/or precipitate glycoconjugates (Goldstein *et al.*, 1980). In insects and other invertebrates, the lectins are also called agglutinins and hemagglutinins. Many insect species contain natural agglutinins (Yeaton, 1981; Ratcliffe and Rowley, 1983; Chen *et al.*, 1993) that can be induced by antigenic stimulation (Komano et al., 1980; Ratcliffe and Rowley, 1983). These are mainly produced by immunocytes (Whitcomb *et al.*, 1974) and fat body (Komano *et al.*, 1983). Biomolecular studies have allowed the cDNA from lectins of *Arachis hypogaea* (Shanker and Das, 2001) and from *Pinellia ternata* (Yao *et al.*, 2003) to be cloned. Even if conflicting data have been reported, these substances appear able to agglutinate foreign material (Komano *et al.*, 1980; Ratcliffe and Rowley, 1983), they present opsonic properties (Pendland *et al.*, 1988; Kawasaki *et al.*, 1993), they increase phagocytic activity (Wilson *et al.*, 1999) and they have a toxic effect (Powell *et al.*, 1998). With regards this latter effect, *Galanthus nivalis* agglutinin (GNA) added to the diet of the insect pest, *Nilaparvata lugens*, has been seen to cross the mid-gut epithelial barrier and pass into the circulatory system of the insect, so exerting its toxic effect. Lectins from *P. ternata* and *Pinellia pedatisecta* present insecticidal activity towards cotton aphids (*Aphis gossypii*) and peach potato aphids (*Myzus persicae*) when incorporated into artificial diets (Huang *et al.*, 1997; Pan *et al.*, 1998).

Cytokine-like molecules

Cytokines belong to the broad family of soluble factors that by communicating among different cell types induce the complex interactions that allow immune responses. These molecules have been called by different names according to their origin or function. Nowadays, the general term cytokines is used for these signal molecules. Interleukins (IL), tumour necrosis factor (TNF), interferons (IFN), transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), etc. are the most common examples in mammals. Cytokine-like molecules have been reported in various tissues of a number of invertebrates, including insects (Table 1). The first findings on cytokine-like molecules in invertebrates reported a so-called "growth promoting substance" in insects and, in particular, in *B. mori* (Aizawa and Sato, 1963; Vaugghn and Louloudes, 1978) and *Samia cynthia* (Williams and Kambysellis, 1969). Cherbas (1973) indicated haemokinin as a fraction partially purified from both plasma and epidermal tissue of saturniid pupae and able to stimulate immunocyte activation. Subsequently, the presence of epidermal growth factor (EGF), fibroblast growth factor (FGF) and others has been demonstrated in different cells and tissues (Table 1). Particular attention has been paid to insulin-like growth factors, which have been described in different taxa, including insects (Joosse *et al.*, 1983; Ebberink *et al.*, 1989). Moreover, genomic DNA clones encoding brain secretory peptides that have been isolated from insects share substantial homologies with vertebrate preproinsulins. (Adachi *et al.*, 1989; Iwami *et al.*, 1989; Lagueux *et al.*, 1990). IL-1α-, TNF-α-, PDGF-AB- and TGF-β1-like molecules have been detected in the plasmatocytes and granular cells of *Calliphora vomitoria* and *G. mellonella* (Franchini *et al.*, 1996; Wittwer *et al.*, 1998). Similar results have also been reported in the cell lines derived from the hemolymph of *Estigmene acraea* and from the fat body cell of *Lymatria dispar* (Wittwer *et al.*, 1998; Ottaviani *et al.*, 2000).

The presence in insects of pro-inflammatory cytokines, i.e. IL-1 and TNF-α, suggest an important role of these molecules in animal host defence responses. Even if few functional data are present in the literature regarding insects, studies from our laboratory dealing with cytokines in invertebrates (mainly molluscs) have demonstrated that mammalian cytokines affect immune and neuroendocrine functions, wound repair and programmed cell death, while, at the same time, presenting the pleiotropicity, functional redundancy and receptor promiscuity seen in vertebrates (Ottaviani *et al.*, 2004). These findings suggest that the same frame may also exist in insects. Furthermore, the insect cytoplasmic domain of the Toll family proteins is homologous with the cytoplasmatic domain of the IL-1 receptor family (Kopp and Medzhitov, 1999).

Given the lack of molecular biology data, it has been hypothesized in the literature that a correlation between invertebrate and vertebrate cytokine genes does not exist (Beschin *et al.*, 2001). However, to claim that mammalian cytokines intervene in the main invertebrate functions merely as a result of functional convergence does seem reductive. Different research groups have found that the main molecules involved in the basic and fundamental functions of cell survival, for example adenocorticotropic hormone (ACTH), corticotrophin-releasing hormone (CRH) and nitric oxide synthase (NOS), show a gene homology with their vertebrate counterparts (Duvaux-Miret and Capron, 1991; Yuda *et al.*, 1996; Salzet *et al.*, 1997).

Anti-microbial peptides

Anti-microbial peptides that are expressed constitutively or are readily inducible have been extensively studied in insects (Boman, 1995; Bulet *et al.*, 1999). The main production site is the fat body, a functional equivalent of the mammalian liver (Fehlbaum *et al.*, 1994), but immunocytes (Boman, 1991), the cuticular epithelial cells (Brey *et al.*, 1993) and the reproductive tract (Rosetto *et al.*, 1996) are also involved. Bulet *et al.* (1999) classified the anti-microbial peptides into three classes on the basis of the sequence and structural characteristics:

1. linear peptides forming α-helices and devoid of cysteine residues;
2. cyclic peptides containing cysteine residues;
3. peptides with an over-representation in proline and/or glycine residues.

The different anti-microbial peptides are named attacins, cecropins, defensins, drosomicins, etc. According to the Bulet *et al.* (1999) classification, cecropins belong to the first class of anti-microbial

Table 1

Insecta	Cytokine-like molecules	References
Bombyx mori	growth promoting factor	Aizawa and Sato (1963); Vaughn and Louloudes(1978)
Samia cynthia	growth promoting factor hemokinin	Williams and Kambysellis (1969) Cherbas (1973)
Antheraea polyphemus	hemokinin	Cherbas (1973)
Hyalophora cecropea	hemokinin	Cherbas (1973)
Drosophila melanogaster	EGF TGF-α, β neurotrophic factor imaginal disc GFs	Wharton *et al.* (1985); Kelley *et al.* (1987); Padgett et al. (1987); Bryant (1988); Kopczynski et al. (1988); Hayashi et al. (1992); Neuman-Silberberg and Schupback (1993); Kutty *et al.* (1998); Kawamura *et al.* (1999)
Manduca sexta	hemolymph trophic factor	Wielgus *et al.* (1990)
Calliphora vomitoria	TNF-α, PDGF-AB, TGF-β1	Franchini *et al.* (1996)
Galleria mellonella	IL-1α, TNF-α	Wittwer *et al.* (1999)
Estigmene acraea	IL-1α, TNF-α	Wittwer *et al.* (1999)
Lymantria dispar	PDGF-AB, TGF-β1	Ottaviani *et al.* (2000)

peptides, while defensins are ascribable to the second class.

Cecropins were the first anti-microbial peptides to be isolated and characterized (Hultmark *et al.*, 1980). They are small 4 kDa peptides found in Diptera and Lepidotera and present an anti-bacterial activity against both Gram positive and Gram negative bacteria. Cecropins share common features, i.e. molecular size (31-39 aminoacids), a strong basicity in the N-terminal region and a hydrophobic portion in C-terminal part. They are induced by bacterial infection and are synthesized as preproproteins of 62-64 residues (Boman, 1991). The precursors are first cleaved by a signal peptidase, and the pro-portion is then removed by a dipeptidyl aminopeptidase (Boman *et al.*, 1989).

In contrast to cecropins, defensins mainly attack Gram positive bacteria (Boman, 1991). They are 4 kDa cationic peptides with a characteristic six cysteine/three disulfide bridge pattern and three domains, a flexible amino-terminal loop, a central α-helix and a carboxy-terminal anti-parallel β-sheet (Hanzawa *et al.*, 1990; Bonmatin *et al.*, 1992; Bulet *et al.*, 1999). Several reports have described the presence of defensins in different insect species (Bulet *et al.*, 1999), but not in Lepidoptera. In our laboratory we recently identified for the first time the presence of a defensin active against Gram positive bacteria in Lepidoptera (IZD-MB-0503 cell line derived from immunocytes of *Mamestra brassicae*) (Mandrioli *et al.*, 2003). The biomolecular study revealed the presence of the defensin gene of 294 pb (Fig. 4).

Alignment of *M. brassicae* defensin with homologous sequences from the insect defensin A genes revealed identity ranging from 43 % to 59 % (Fig. 5). The analysis of the putative protein indicated the presence of 98 aminoacids, including 8 cysteine residues. In particular, cysteine residues 3-8 were highly conserved, suggesting their involvement in the formation of three disulfide bridges. Northern blotting experiments showed a constitutive expression of the defensin gene in *M. brassicae* cells. Its expression was increased by Gram positive, but not by Gram negative bacteria (Fig. 6).

Fig. 4 Complete sequence of *M. brassicae* defensin gene. Upper lines show the nucleotide sequence, while the putative amino acidic sequence is indicated in the lower lines (Modified from Mandrioli *et al.*, 2003).

Fig. 5 Defensin peptide alignment of *Mamestra brassicae* (AF465486) (**A**) with *Aedes aegypti* (AF156093) (**B**), *Apis mellifera* (D17670) (**C**), *Phormia terranovae* (AF182164) (**D**), *Drosophila melanogaster* (AC007414) (**E**) and *Anopheles gambiae* (AF063402) (**F**). Highly conserved aminoacids are boxed (Modified from Mandrioli *et al.*, 2003).

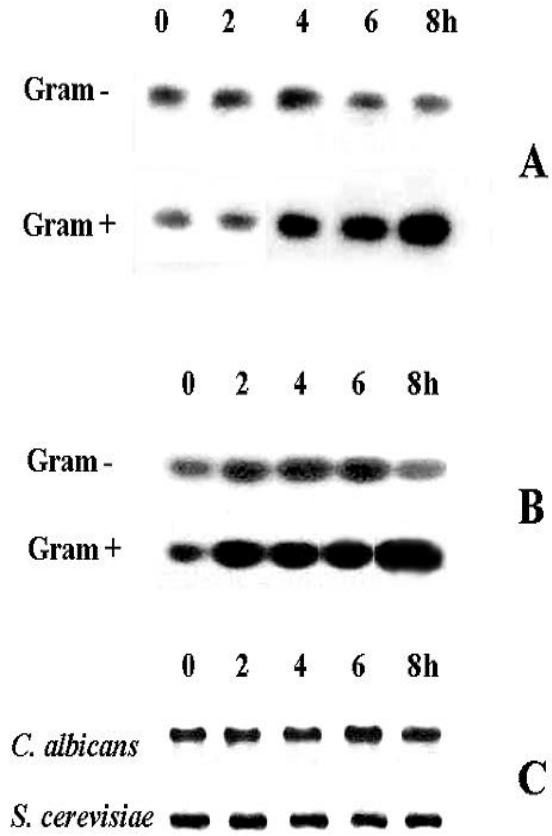

Fig. 6 Northern blotting with the defensin probe on RNA samples extracted at different times from *M. brassicae* cells after induction with heat-killed (**A**) and live bacteria (**B**). Northern blotting indicates that defensin expression is increased by Gram positive bacteria, in particular by live specimens, but not by *Candida albicans* and *Saccharomyces cerevisiae* (**C**) (Modified from Mandrioli *et al.*, 2003).

Defensin induction was greater when using live bacteria rather than heat-killed specimens (Fig. 6). No defensin gene induction was observed in the presence of *Candida albicans* and *Saccharomyces cerevisiae* (Fig. 6). Microbial killing by antimicrobial peptides involves both the charge and the structure of the latter. In cecropins and defensins, the mechanism is thought to relate to the peptides' ability to assemble in the target membrane and form a pore (Boman, 1991). The means by which cecropins associate with the plasma membrane is called "carpet-like" and involves: i) peptide arrangement parallel to membrane surface by binding to the phospholipid head groups; ii) rotation of peptides in order to re-orientate their hydrophobic residues toward the membrane hydrophobic core; iii) disruption of the membrane bilayer (Shai, 1999).

Concluding remarks

From an evolutionary point of view, insects, together with molluscs and annelids, are characterised by a body cavity, the coeloma, in which immunocytes appear with morpho-functional properties similar to mammalian blood cells. The new structure allows the immune system to develop more defined strategies to discriminate between self and non-self and to build up complex types of immune responses.

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