FORM, FUNCTION AND PHYLOGENETIC RELATIONSHIPS OF MOSQUITO IMMUNE PEPTIDES

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INTRODUCTION

Insects represent one of the earth's most successful groups of organisms, colonizing essentially every niche possible, with the exception of the oceans. One factor contributing to this success in exploiting such diverse ecological niches is their ability to defend themselves against harmful pathogens and parasites (1,2). Insects can mount an extremely effective response against invasion by prokaryotic and eukaryotic pathogens involving both cellular and humoral factors (3,4). These responses may include phagocytosis of bacteria, formation of nodules containing large aggregates of bacteria, the melanotic encapsulation of metazoan parasites (5,6) or the use of potent antimicrobial peptides (7,8).

Phylogenetic Perspectives on the Vertebrate Immune System.
Edited by Beck *et al.*, Kluwer Academic/Plenum Publishers, 2001.

These inducible responses, including those that result in the production of immune peptides, have been incorporated into what is termed innate immunity in both animals and plants (9-11). Immune peptides are quickly expressed *de novo* and delivered to the appropriate site to defend the organism in a manner that is neither learned or acquired, unlike the hallmark characteristics of classical immunology (9). The innate immune system of insects is a complex interactive system used extensively by all genera to protect themselves from infection from bacteria, fungi, and parasites, and although the insects' innate system lacks the sophistication of the mammalian system, there are similarities between different taxa that would suggest common origins of some immune molecules.

The role and goal of the innate immune system is a rapid and effective elimination of invading pathogens. We can find examples of innate immunity from primitive and advanced organisms; genes encoding immune molecules have been isolated from plants, mammals, birds, amphibians, fish and many classes of invertebrates, and we would anticipate that such genes are present in every living organism as a mechanism of defense against pathogens. Because very similar molecules have been isolated from such diverse taxonomic groups, it has been proposed that the efficacy of these ancient molecules has perpetuated their presence through evolutionary diversification. An assessment of the innate immune response of invertebrates may lead to understanding of the origins and evolution of immune systems in higher organisms including the identification of precursor molecules and progenitor responses of vertebrates.

It is now becoming apparent, as more and more related immune peptides are being isolated from very diverse taxa, that antimicrobial peptides are key, and highly conserved elements of the innate immune system in both animals and plants (12-14). Since the first cecropin molecule was identified and characterized in the laboratory of Hans Boman (15), over 170 immune peptides have been isolated from insects alone. This potent arsenal of antimicrobial peptides results in hemolymph concentrations from 1-100 mM within 24h of pathogen stimulation (7). These peptides have been classified into specific families by their physical structure and several recent reviews have described in detail these families of compounds, their range of activity and the taxa from which they have been identified (7,8,10,16-20).

It is interesting to note that closely related insects often have the same peptides in their antimicrobial repertoire, but do not rely on the same peptides as their major response to microbial invasion. Although the origins and evolution of these peptides in invertebrates may have arisen from common ancestors before the divergence of the insects, specific peptides are preferentially expressed by different species.

This review will address the antimicrobial peptides used by insects in general, and specifically by mosquitoes, and will review the growing literature on the role of these immune peptides in determining the competence of mosquitoes to transmit parasites. Mosquitoes are unquestionably the most important arthropod vectors of diseases to humans and are responsible for the transmission of malaria (500 million cases, 3 million deaths, (21)), lymphatic filariasis (100 million cases, (21)), and numerous arboviruses including yellow fever and Dengue fever (21).

Over the last few years, several immune peptides have been isolated and characterized from mosquitoes. These include defensins (1,22-25), cecropins (26-28), and a specific peptide active solely against Gram negative bacteria (Lowenberger and Bulet

unpublished data). Other molecules found in mosquitoes that may have a role in antibacterial activity include insect transferrins (29). It generally is accepted that the site of production of antimicrobial peptides is the insect fat body, the functional equivalent of the mammalian liver. However, in some species immune peptides have been found in hemocytes (30,31) salivary glands (32), genital tracts, and the cuticle (33). As such many of the insect immune peptides may be produced as larger precursors that are transported to the hemolymph and subsequently to specific locations and activated by a cleavage of the precursor molecule.

INSECT DEFENSINS

Insect defensins were described first from the flesh fly *Sarcophaga peregrina* (19,20) and independently from *Phormia terranovae* (34). Since these initial reports, over 30 defensins have been reported from Diptera, Coleoptera, Hemiptera, Hymenoptera, Trichoptera, Odonata, and related molecules reported from scorpions and molluscs. Functional analogues also have been reported from mammals (35,36) and plants (14,37). Insect defensins contain 34-43 residues (with the exception of the defensins isolated from royal jelly (38) and the honey bee (39). A characteristic of the insect defensins is the presence of 6 cysteine residues that combine to form three intramolecular bridges to provide the characteristic 3-dimensional structure. The molecule consists of an N-terminal loop (residues 4-14, an α-helix structure, (residues 15-23), and a 2-stranded twisted antiparallel β-sheet (residues 27-31 and 35-39). The structure is stabilized by 2 of the disulphide bridges between the α-helix and the β-sheet (Cys2-Cys5, Cys3-Cys6) forming the "cysteine-stabilized α-β motif"described by Cornet et al. (40). This motif is present in other families of peptides, such as scorpion toxins (41) and plant g-thionins (40), and similarities have been described for the antifungal peptide, drosomycin (7). In insect defensins the disulphide bridges that maintain stability of the molecule are Cys1-Cys4, Cys2-Cys5, and Cys3-Cys6.

Defensins have been isolated from several orders of insects: from the higher insects such as the Diptera (*Drosophila* and mosquitoes), and Coleoptera, and also from ancient insects such as dragon flies (*Aeshna cyanea*), which evolved about 100 million years before the emergence of mosquitoes. Functional analogues also have been isolated from amoebae (42), nematodes (43), scorpions (44,45), molluscs (46,47) mammals (35,36) and plants (14) (Figures 1 and 2). The presence of defensin like molecules in such a diverse group of taxa suggests an ancient origin whose importance in protecting self against microbes has maintained the prominence of this peptide family through evolutionary divergence.

Insect defensins are active against many Gram positive, and a few Gram negative bacteria. Activity against Gram positive bacteria is carried out almost immediately with a lytic effect on the bacterial membranes. These membranes are permeabilized, resulting in a loss of cytoplasmic potassium, a depolarization of the inner membrane, reduced amounts of cytoplasmic ATP, and a reduction in respiration (48).

Family	Species	Sequence
A		
Insect	*P. terranovae*	--ATCDLLS----GTGINHSACAAHCL-LRGNRGGYCNG--KGVCVC-RN
Insect	*Ae. aegypti* A	--ATCDLLS----GFGVGDSACAAHCI-ARGNRGGYCNS--KKVCVC-RN
Insect	*Ae. aegypti* B	--ATCDLLS----GFGVGDSACAAHCI-ARGNRGGYCNS--QKVCVC-RN
Insect	*Ae. aegypti* C	--ATCDLLS----GFGVGDSACAAHCI-ARRNRGGYCNA--KKVCVC-RN
Insect	*An. gambiae*	--ATCDLAS----GFGVGSSLCAAHCI-ARRYRGGYCNS--KAVCVC-RN
Insect	*A. mellifera*	--VTCDLLS---FKGQVNDSACAANCL-SLGKAGGHCE---KGVCIC-RKTSFKDLWDKRF
Insect	*T. molitor*	--VTCDILSVEAKGVKLNDAACAAHCL-FRGRSGGYCNG--KRVCVC-R
Insect	*P. apterus*	--ATCDILSFQSQWVTPNHAGCALHCV-IKGYKGGQCKI---TVCHC-RR
Insect	*A. cyanea*	-GFGCPLD--Q--------MQCHRHCQTITGRSGGYCSGPLKLTCTCYR
Scorpion	*L. quinquestriatus*	-GFGCPLN--Q--------GACHRHCRSIR-RRGGYCAGFFKQTCTCYRN
Scorpion	*A. australis*	-GFGCPFN--Q--------GACHRHCRSIR-RRGGYCAGLFKQTCTCYR
Mollusc	*M. edulis*	-GFGCPND----------YPCHRHCKSIPGRXGGYCGGRHRLRCTC
Mollusc	*M. galloprovinsialis*	-GFGCPNN----------YQCHRHCKSIPGRCGGYCGGXHRLRCTCYRC
B		
Amoeba	*E. histolytica*1	GEILCNLCTGLINTLENLLTTKGA-DKVKDYISSLCNKASGFIATLCTKVLDFGIDKLIQLIEDKVDANAICAKIHAC
Amoeba	*E. histolytica* 2	GAILCNLCKDTVKLVENLLTVDGA-QAVRQYIDNLCGKASGFLGTLCEKILSFGVDELVKLIENHVDPVVVCEKIHAC
Amoeba	*E. histolytica* 3	IPVLCPVCTSLVGKLIDLVLGGAV-DKVTDYLETLCAKADGLVETLCTKIVSYGIDKLIEKILEGGSAKLICGLIHAC
Nk-Lysin	*H. sapiens*	GYFCESCRKIIQKLEDMVGPQPNEDTVTQAASQVCDKL-KILRGLCKKIMRSFLRRISWDILTGKKPQAICVDIKICKE
Granulysin	*H. sapiens*	GRDYRTCLTIVQKLKKMVD-KPTQRSVSNAATRVCRTGRSRWRDVCRNFMRRYQSRVIQGLVAGETAQQICEDLRLCIPSTGP
C		
Plant	*A. hippocastanum*	LCNERPSQTWSGNCGNTAHCDKQCQDWEKASHGACHKRENHWKCFCYFNC
Plant	*C. ternatea*	NLC-ERASLTWTGNCGNTGHCDTQCRNWESAKHGACHKR-GNWKCFCYFNC
Plant	*D. merckii*	ELC-EKASKTWSGNCGNTGHCDNQCKSWEGAAHGACHVRNGKHMCFCYFNC

Figure 1. Comparison of the amino acid sequences of defensin like molecules from invertebrates (**A**), amoebae and mammals (**B**), and plants (**C**). Sequences were aligned for similarities, and dashes used to maintain optimal alignment.

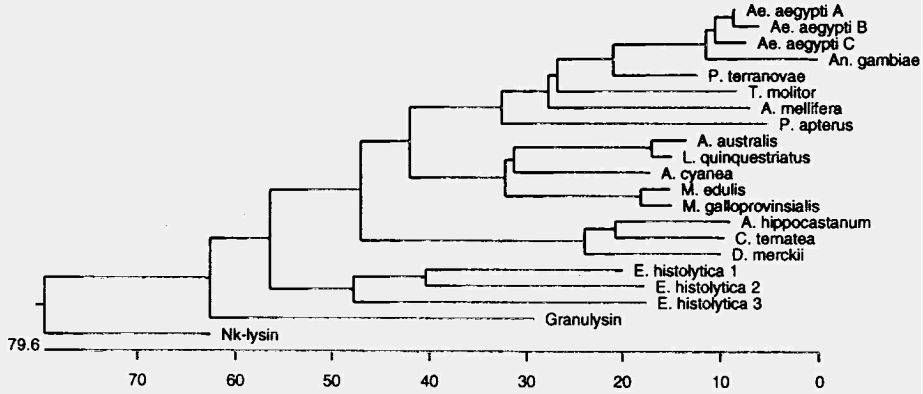

Figure 2. Phylogenetic analysis of the defensin amino sequences in Fig. 1. Constructions were done using the mature defensin sequences. The homology sequences were calculated using DNAStar software using the Jotun Hein method with PAM 250 residue table.

Three insect defensin isoforms have been isolated from *Ae. aegypti* (1,22,23), one from *An. gambiae* (25), and we have corresponding cDNAs from *Culex pipiens* (Lowenberger, Kamal, Solimen, Farid, and Christensen unpublished data). In *Ae. aegypti* the peptides were isolated from whole bodies of insects 24h after being inoculated with bacteria (23). Subsequent peptide analysis of the hemolymph of bacteria-inoculated mosquitoes confirmed that defensins are the predominant antibacterial peptide produced by this species.

Of the three isoforms of defensin isolated from *Ae. aegypti*, we believe that A and B are allelic variants of the same gene whereas isoform C likely is a separate gene. In the samples used in the initial study, the peptides corresponding to isoforms A and B were found in equal amounts in the insects whereas the peptide corresponding to isoform C was found at levels only 20% of A and B, and there are differences in the length and composition of the signal peptide region of the pre-pro defensins (1). Defensins are transcribed in the midguts of naïve mosquitoes, but only transcripts for isoform C were detected by RT-PCR, and these were up-regulated in the presence of a bloodmeal. The presence of lower amounts of isoform C in the mosquito, but its major presence in the midgut (1) and the much greater levels of isoforms A and B in the hemolymph (23) probably reflects tissue specific expression patterns. In immune activated mosquitoes, transcription for defensins continues for 21 days after inoculation with bacteria, and about 7-10 days after sterile injury (1).

If we consider the developmental stages of the mosquito, we only find transcription for defensin in the white or callow pupal stages, and no transcription in any of the larval stages (1). We thought initially that this may have been due to entry of bacteria through the soft white cuticle of the callow pupae during metamorphosis, or by a contamination of the hemocoel following the histolysis of the immature gut in the larva-pupa-adult transformation. However, because we do not see a similar transcriptional profile for cecropins (see below) in *Ae. aegypti*, which should occur if bacteria entered the hemocoel from the gut or through the cuticle, the transcription for defensin may be a developmentally regulated event independent of the presence of bacteria.

In *An. gambiae*, the major vector of malaria in sub-Saharan Africa, there is a similar pattern of transcription for defensins (25), but transcripts are short lived compared *to Ae. aegypti*, and there is not the similarly high level of defensin in the hemolymph of *An. gambiae* (1-5 μM) as is the case with *Ae. aegypti* (45 μM) (Table 1). Whether this reflects a more potent peptide in *An. gambiae* that is able to combat invading pathogens at a lower concentration, or the use of other, currently unknown, immune peptides is unknown. However, there are several differences in the transcription of insect defensins in these two mosquito species. In *Ae. aegypti* there is a low level of transcription for defensins in the midgut of naïve mosquitoes, that increases with the ingestion of a bloodmeal. In *An. gambiae*, an increase in transcription occurs when *Plasmodium* parasites are ingested in the bloodmeal, although not so with a non-parasite-infected bloodmeal (49). In addition, by RT-PCR, Dimopoulos et al. (32) demonstrated that defensin transcripts were found in the salivary glands 10 days after ingesting a *Plasmodium*-infected bloodmeal. In contrast, in *Ae. aegypti*, no transcripts were found by Northern analysis in whole body RNA extracts 10 days after ingesting a *P. gallinaceum*-infected bloodmeal. Whether these differences are due to different techniques used (northern analysis versus RT-PCR) or due to differences between mosquitoes of different genera remains to be elucidated.

CECROPINS

Cecropins were the first of the inducible antimicrobial peptides to be isolated and characterized (15). Approximately 20 cecropins have been reported from several Lepidoptera and Diptera, and from the intestine of pigs (50). These peptides are 31-39 residue proteins, devoid of cysteines, and generally amidated on the C-terminus. The helical structure of this molecule was described (51) as two helices joined by a Alanine-Glycine-Proline hinge. The N-terminal region forms an amphipathic α helix with equal hydrophobic and hydrophilic regions. The C-terminal region is more hydrophobic (8). Cecropins are active against Gr-negative and -positive bacteria: the mode of action seems to be through binding to the acidic components of the cell membrane, and inducing changes in cell permeability (52,53). Because of the amphipathic nature of the helices it has been proposed that the molecules bind to the cell membrane, inducing channel formation (54,55). In this "barrel-stave" mechanism trans-membrane amphiphilic α-helices form bundles that have outwardly facing hydrophobic surfaces that interact with membrane lipid regions, and inwardly facing hydrophilic regions that induce the pore (53,56-59).

Cecropins were originally isolated from Lepidoptera and, with the exception of cecropin D from *Bombyx mori* (60,61), all cecropins previously reported from insects have a tryptophan residue as the first or second amino acid in the mature peptide sequence. However, with the sequences we now have from mosquitoes (one obtained from immune-activated *Ae. aegypti* (26), one from a screen of a genomic library, and found expressed in immune activated mosquitoes (Lowenberger and Kamal unpublished data), 3 cDNA sequences found in bacteria exposed cells of an *Aedes albopictus* cell line (28,62), and one from *An. gambiae* (27)) no mosquito cDNA sequence has a tryptophan residue in this position. Another common feature of the cecropins is the blockage of the C-terminus by an amine group, a feature absent in the cecropins isolated from *Ae. aegypti,* but present in those isolated from *An. gambiae* (27) and *Ae. albopictus* (28).These features are considered significant because in *H. cecropia* the activity of a synthetic analogue deficient in the tryptophan residue was significantly reduced (63-65). Similarly in *S. peregrina*, an amidated cecropin molecule had a 4-fold greater antimicrobial activity than did the molecule with a free carboxylic group (66). Similar results implicating the importance of these two features have been reported by other authors (61,64,67,68). That the *Ae. aegypti* cecropin is not as active against bacteria as is the corresponding molecule from Drosophila (26), may reflect the lack of these two features in the mosquito cecropin.

From a phylogenetic perspective, cecropin like molecules are not limited to insects. Similar molecules have been isolated from tunicates, marine invertebrates belonging to the genus Chordata (69), from the intestine of a pig (50) and from bovine adrenal glands (70) once again exemplifying the diversity of taxa in which these immune peptides are found (Fig. 3). A comparison of the structure and phylogenetic relationships between cecropin like molecules identified from different organisms is presented in Figure 4.

TRANSFERRINS

Transferrins (TF) are molecules normally associated with transport of iron in vertebrates, and may play a role in limiting iron supplies to, and thus starving, parasites (71-74). Iron bound to lactoferrin found in mucosa and transferrin in blood serum and interstitial regions may lower the availability of free iron to levels below those needed by pathogens to survive. In insects transferrins have been isolated from *M. sexta* (75), *S. peregrina* (76), *D. melanogaster* (77) and the mosquito *Ae. aegypti* (29).The insect transferrins differ from those isolated from mammals in that the insects have the characteristic iron binding residues in the N-terminal region, but lack the iron binding domain in the C-terminal region (29). It has been speculated that this modification has arisen as a mechanism to reduce iron piracy by microorganisms (29). In *Ae. aegypti* there is an upregulation in TF transcripts after bacterial inoculation (29) or the injection of filarial worms (31). There is a similar increase in TF transcripts in *D. melanogaster* upon bacteria inoculation. When filarial worms were introduced into the hemocoel of *Ae. aegypti*, the transcripts were found in the hemocytes at the time when the mosquito was melanotically encapsulating the worms (31). However the process of injecting the worms also may have initiated the up regulation.

Family	Species	Sequence
Insect	*Ae. aegypti*	GGLKKLGKKLEGAGKRVFNAAEKALPVVA--G-AKALRK
Insect	*Ae. albopictus*	GGLKKLGKKLEGVGKRVFKASEKALPVAV--G-IKALG
Insect	*An. gambiae*	GRLKKLGKKIEGAGKRVFKAAEKALPVVA--G-VKAL
Insect	*D. melanogaster* A	GWLKKIGKKIERVGQHTRDATIQ-GLGIA--QQAANVAATAR
Insect	*D. melanogaster* B	GWLRKLGKKIERIGQHTRDASIQ-VLGIA--QQAANVAATAR
Insect	*D. melanogaster* C	GWLKKLGKRIERIGQHTRDATIQ-GLGIA--QQAANVAATAR
Insect	*S. peregrina* A	GWLKKIGKKIERVGQHTRDATIQ-GLGIA--QQAANVAATAR
Insect	*S. peregrina* B	GWLKKIGKKIERVGQHTRDATIQ-VIGVA--QQAANVAATAR
Insect	*C. capitata* A	GWLKKIGKKIERVGQHTRDATIQ-TIAVA--QQAANVAATARG
Insect	*H. cecropia* A	KW--KLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK
Insect	*H. cecropia* B	KW--KVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL
Insect	*H. cecropia* C	-W--NPFKELEKVGQRVRDAVISAGPAVATVAQATAL
Insect	*A. pernyi* B	KW--KIFKKIEKVGRNIRNGIIKAGPAVAVLGEAKAL
Insect	*M. sexta* B2	-W--NPFKELERAGQRVRDAVISAAPAVATVGQAAAIAR
Insect	*M. sexta* B4	-W--NPFKELERAGQRVRDAIISAAPAVATVGQAAAIAR
Insect	*B. mori* A	RW--KLFKKIEKVGRNVRDGLIKAGPAIAVIGQAKSL
Insect	*B. mori* B	RW--KIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAI
Insect	*B. mori* D	G---NFFKDLEKMGQRVRDAVISAAPAVDTLAKAKALGQG
Tunicate	*S. clava* E	GWLRKAAKSVGKFYYKHKYYIKAAWKIGRHALGDMTDEEFQDFMKEVEQAREEELQSRQ
Tunicate	*S. clava* D	GWLRKAAKSVGKFYYKHKYYIKAAWQIGKHALGDMTDEEFQDFMKEVEQAREEELQSRQ
Tunicate	*S. clava* C	GWFGKAFRSVSNFYKKHKTYIHAGLSAATLLGDMTDEEFQEFMQDIEQAREEELLSRQ
Pig		SWLSKTAKKLENSAKKRISEGIAIAIQGGPR
Bovine		AMDLELQKIAEKFSGTRG

Figure 3. Comparison of the amino acid sequences of cecropin molecules from insects, tunicates, and mammals. Sequences were aligned for similarities, and dashes used to maintain optimal alignment.

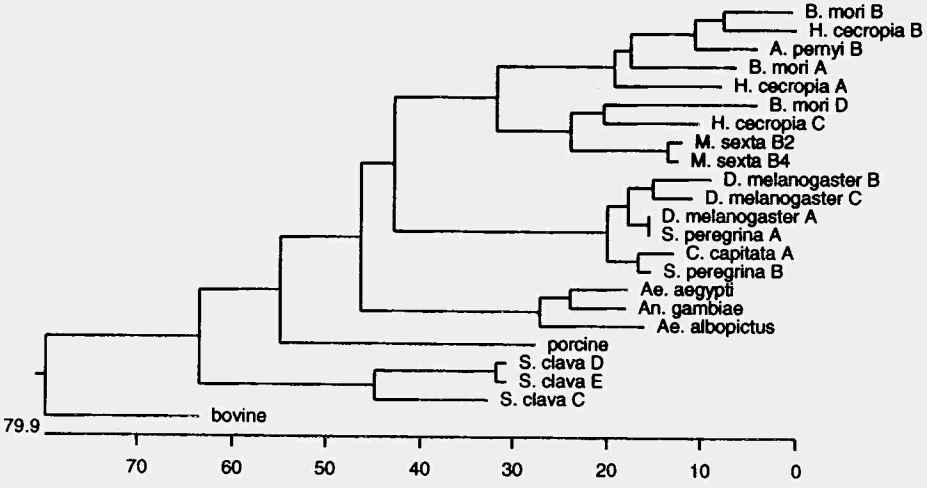

Figure 4. Phylogenetic analysis of the amino acid sequences of the mature cecropin sequences in Fig. 3. The homology sequences were calculated using DNAStar software using the Jotun Hein method with PAM 250 residue table.

We have been studying the immune response, including transferrin production, of another mosquito, *Armigeres subalbatus*, to infections with filarial worms. This is an ideal model to use because *Ar. Subalbatus* melanizes >95% of the microfilariae of *Brugia malayi* ingested in a blood meal. In this species the introduction of bacteria significantly increases transcription of TF, but TF transcripts also increase significantly during the melanotic encapsulation of *B. malayi* (Falk, Lowenberger and Christensen unpublished data). It is enticing to suggest that TF plays dual roles in the mosquito: that of iron transport in general, and as an immune response to pathogens or parasites to reduce their ability to establish and proliferate within the hemocoel during the period required for the production of other immune peptides.

One of the interesting aspects in looking at the different immune peptides in different insect species is the fact that even closely related Diptera do not produce the same molecules at the same concentration. Even making general references to the response of mosquitoes to a particular stimulus may be inappropriate because all species do not respond similarly. For instance, *Ae. aegypti* produces defensin in great amounts- about 45 μM in the hemolymph, and cecropin at about 1-5 μM, but *An. gambiae* produces defensin and cecropin at about 1mM, and *Drosophila* produces defensin at 1 μM and cecropin about 10 μM (Table 1). Whether these differences are related to the environments in which these different organisms live, and on the bacteria to which

they have been exposed through evolutionary time, remains to be examined. When we examine the phylogenetic relationships of the cecropins and defensins solely within the insects, it is common to make comparisons between taxonomic groups. However, environmental factors and specific niches inhabited by particular insects also may have played a role in the evolution and preponderance of use of specific peptides based upon the selective pressures to which these organisms have been exposed. In the analysis of cecropins, the terrestrial Diptera and Lepidoptera arise from one branch whereas the Diptera with aquatic immature stages, the mosquitoes, arise from a different branch (1). Similarly with the defensins, the terrestrial Diptera, as well as other terrestrial insect orders arise from a different branch than do the mosquitoes (1). To test the hypothesis of environmental effects on the preferential expression of particular immune peptides will require a more detailed analysis encompassing more species from different environments, and the microbial fauna in these diverse locations, than is currently feasible.

Table 1. Known concentrations of immune peptides in different Diptera[1]

	Aedes aegypti	*Anopheles gambiae*	*Drosophila melanogaster*
Defensin	45	1-5	1
Cecropin	1-5	1-5	20
Transferrin	1-5	*	?
Drosomycin	*	*	100
Drosocin	*	*	40
Attacin	*	*	?
Metchnikowin	*	*	10

[1]An asterisk indicates that the peptide has not been identified in the species and a question mark indicates no known concentration for the peptide.

PEPTIDE-PARASITE INTERACTIONS

Insect immune peptides generally have been considered ineffective against eukaryotic cells (16). However, there is a growing literature on the effects of these immune peptides on parasites normally transmitted by mosquitoes (Table 2). Gwadz et al. (78) injected cecropins and magainins into mosquitoes, and reported a significant reduction in development of *Plasmodium* sp. Since this first report, several laboratories have reported similar results. Jaynes et al (79) used a modified synthetic cecropin, Shiva, to reduce the development of *Plasmodium* sp. Further modifications of the cecropin molecule subsequently were used to prevent *Plasmodium* development (80-82). Chalk et al. (83) co-injected cecropins with the nematode *Brugia pahangi* into *Ae. aegypti*,

Table 2. Reports of the effect of immune peptides or immune activation on parasite development.

Author	Peptide	Parasite
Gwadz *et al.*, 1989	cecropin / magainin	*Plasmodium*
Jaynes *et al.*, 1988	cecropin	*Plasmodium*
Jaynes *et al.*, 1988	cecropin	*Trypanosoma cruzi*
Shahabuddin *et al.*, 1998	defensin	*Plasmodium*
Lowenberger *et al.*, 1996	immune activation	*Brugia malayi*
Lowenberger *et al.*, 1999a	immune activation	*Plasmodium*
Chalk *et al.*, 1995	cecropin	*Brugia pahangi*
Albuquerque *et al.*, 1996	defensin	*Brugia pahangi*
Rodriguez *et al.*, 1995	cecropin / Shiva	*Plasmodium*
Possani *et al.*, 1998	cecropin / Shiva	*Plasmodium*
Boisbouvier *et al.*, 1998	cecropin / Shiva	*Plasmodium*

and Albuquerque et al. (84) co-injected mosquito defensins and *B. pahangi* into *Ae. aegypti*. Both studies reported a reduction in parasite development. Lowenberger et al. (85) reported that the inoculation of bacteria into the hemocoel (a process normally done to activate the immune system of mosquitoes) and then subsequent feeding of these mosquitoes on a gerbil infected with *Brugia malayi*, resulted in significant reductions in both parasite prevalence (percent of mosquitoes that became infected) and mean intensity of infection (mean number of parasites/infected mosquito). Depending on the parasite burden to which the mosquitoes were exposed, prevalence was reduced to 50-57% in immune activated mosquitoes while non-inoculated controls had a prevalence of 92-97%. Similarly the mean intensity of infection was reduced from 8-16 worms / mosquito in the controls to 2.3-2.5 worms/immune activated mosquito. When the mosquitoes were exposed to extremely high numbers of parasites the hosts apparently were overwhelmed in their efforts to eliminate the parasites, and numbers were no different from naïve controls. Interestingly, in this study the microfilariae that were ingested by the mosquito, and which subsequently penetrated the midgut and entered the hemocoel, were found dead in the immune-activated mosquitoes. This killing apparently occurred after entering the hemolymph, and before the nematodes could reach the thoracic musculature where they normally develop. However, there was no evidence of melanotic materials being deposited on the worms, thus killing them, as is in the case of some mosquito-nematode relationships.

In a subsequent study (2) we studied the effect of immune activation of *Ae. aegypti* on the development of *Plasmodium gallinaceum*. As was the case with *B. malayi*, the immune activation process had a significant effect on the number of parasites that developed within the mosquito. However, the timing of events was critical: Only those mosquitoes immune activated before, or immediately after bloodfeeding demonstrated this reduction in parasite development. Mosquitoes immune activated 1-5 days after bloodfeeding demonstrated no reduction in oocysts on the midgut as compared to naïve controls. These results implicated the ookinete as the susceptible parasite stage

that was being killed. Approximately 24h after bloodfeeding, the ookinetes leave the blood bolus, migrate through the midgut wall, and establish on the hemolymph side of the midgut, beneath the basal lamina, where they form oocysts. As this study found very few oocysts, the authors assumed that the parasite killing factors had acted on the ookinetes. These data appear to conflict with those of Shahabuddin et al. (86) who injected purified defensins isolated from *P. terranovae* and *A. cyanea* into *Ae. aegypti*. In this study there was no effect of defensins *in vitro* or *in vivo* on the ookinetes or early oocysts, whereas later oocysts and sporozoites were killed by the defensins. However, whereas bacteria inoculation produced a significant reduction in prevalence, as well as in mean intensity of infection in one study (2) injection of purified defensins did not reduce the prevalence of infection in another study (86)The results of these two studies may differ because of the methodologies used: we (2) injected bacteria into the hemocoel, a process that turns on the expression of many immune peptides, some of which, alone or acting in concert, may have had an effect on the developing ookinetes, whereas in the study by Shahabuddin et al.(86) one individual peptide, defensin, was evaluated.

In order to determine what specific peptides are involved in the parasite killing effects reported, we need a mechanism by which we can express a particular peptide within the mosquito host, without expressing the complete arsenal of immune factors. The development of the Sindbis virus as a transient transducing vector for the expression of specific peptides has been developed for use by the AIDL at Colorado State University (87-89). Although the original viral construct had to be injected into the mosquitoes, the development of a double subgenomic construct that is orally infectious to *Ae. aegypti* (90) will prove invaluable in assessing the effects *in vivo* of particular peptides on developing parasites within mosquitoes.

There is no doubt that there are many more immune peptide molecules produced by mosquitoes and other insects, and the search continues to isolate, identify, and characterize them. With the recent announcement to sequence the entire genomes of *Ae. aegypti* and *An. gambiae* (91,92) future discoveries of genes coding for immune peptides will be able to probe these microchip libraries to find large flanking regions of these genes and such advances will no doubt increase the speed with which we can define the regulatory elements and promotor regions of specific genes. Similarly, with the ever expanding databases of sequences from diverse organisms, database searches will rapidly be able to discern the same genes within related species, members of the same gene family in diverse taxa, or even propose ancient precursor or ancestral genes that have evolved differently through different taxa.

However, gene sequence data alone will not allow us to determine what biological factors within mosquitoes act alone or in concert to induce or enhance the expression of particular peptides encoded by these genes or what signalling molecules are used for chemical communication between cells and tissues. The regulation of several immune peptides through Toll/ IMD pathways has been described in Drosophila (17,93,94), and these are, in all likelihood, similar in mosquitoes. However, the mechanisms, molecules, and means by which mosquitoes in particular, and insects in general, recognize non-self and then initiate the chain of events that results in the high concentrations of immune factors in the hemolymph is the holy grail of insect immunity today.

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REFERENCES

1. Lowenberger, C. A., Smartt, C. T., Bulet, P., Ferdig, M. T., Severson, D. W., Hoffmann, J. A., and Christensen, B. M. (1999) Insect immunity: molecular cloning, expression, and characterization of cDNAs and genomic DNA encoding three isoforms of insect defensin in *Aedes aegypti*. *Insect. Mol. Biol.* 8, 107-118
2. Lowenberger, C. A., Kamal, S., Chiles, J., Paskewitz, S., Bulet, P., Hoffmann, J. A., and Christensen, B. M. (1999) Mosquito-*Plasmodium* interactions in response to immune activation of the vector. *Exp. Parasitol.* 91, 59-69
3. Dunn, P. E. (1986) Biochemical aspects of insect immunology. *Annu. Rev. Entomol.* 31, 321-329
4. Lackie, A. M., and Vasta, G. R. (1988) The role of galactosyl-binding lectin in the cellular immune response of the cockroach *Periplaneta americana* (Dictyoptera). *Immunology* 64, 353-357
5. Christensen, B. M., and Forton, K. F. (1986) Hemocyte-mediated melanization of microfilariae in *Aedes aegypti*. *J. Parasitol.* 72, 220-225
6. Li, J. Y., and Christensen, B. M. (1990) Immune competence of *Aedes trivittatus* hemocytes as assessed by lectin binding. *J. Parasitol.* 76, 276-278
7. Bulet, P., Hetru, C., Dimarcq, J. L., and Hoffmann, D. (1999) Antimicrobial peptides in insects; structure and function. *Dev. Comp. Immunol.* 23, 329-344
8. Hetru C., H., D., and Bulet, P. (1998) Antimicrobial Peptides from Insects in *Molecular Mechanisms of Immune Responses in Insects* (Brey, P. T. and Hultmark, D. eds), pp. 40-66, Chapman and Hall, London
9. Boman, H. G. (1998) Gene-encoded peptide antibiotics and the concept of innate immunity: an update review. *Scand. J. Immunol.* 48, 15-25
10. Boman, H. G. (1991) Antibacterial peptides: key components needed in immunity. *Cell* 65, 205-207
11. Zasloff, M. (1992) Antibiotic peptides as mediators of innate immunity. *Curr. Opin. Immunol.* 4, 3-7
12. Boman, H. G. (1995) Peptide antibiotics and their role in innate immunity. *Annu. Rev. Immunul.* 13, 61-62
13. Ganz, T., and Weiss, J. (1997) Antimicrobial peptides of phagocytes and epithelia. *Semin. Hematol.* 34, 343-354
14. Broekaert, W. F., Terras, F. R., Cammue, B. P., and Osborn, R. W. (1995) Plant defensins: novel antimicrobial peptides as components of the host defense system. *Plant Physiol.* 108, 1353-1358
15. Steiner, H., Hultmark, D., Engstrom, A., Bennich, H., and Boman, H. G. (1981) Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature* 292, 246-248
16. Cociancich, S., Bulet, P., Hetru, C., and Hoffmann, J. A. (1994) The inducible antibacterial peptides of insects. *Parasitology Today* 10, 132-139
17. Hoffmann, J. A., Reachhart, J. M., and Hetru, C. (1996) Innate immunity in higher insects. *Curr. Opin. Immunol.* 8, 8-13
18. Hetru, C., Bulet, P., Cociancich, S., Dimarcq, J. L., Hoffmann, D., and Hoffmann, J. A. (1994) Antibacterial Peptides/Polypeptides in the insect host defense: a comparison with vertebrate antibacterial peptides/polypeptides in *Phylogenetic perspectives in immunity: the insect host defense* (Hofmann, J. A., Janeway, C. A., and Natori, S., eds), pp. 43-66, R.G. Landes, Austin
19. Matsuyama, K., and Natori, S. (1988) Molecular cloning of cDNA for sapecin and unique expression of the sapecin gene during the development of *Sarcophaga peregrina*. *J. Biol. Chem.* 263, 17117-17121
20. Matsuyama, K., and Natori, S. (1988) Purification of three antibacterial proteins from the culture medium of NIH-Sape-4, an embryonic cell line of *Sarcophaga peregrina*. *J. Biol. Chem.* 263, 17112-17116

21. WHO. (1999) *Disease Statistics. The World Health Report*, WHO, Geneva
22. Chalk, R., Albuquerque, C. M., Ham, P. J., and Townson, H. (1995) Full sequence and characterization of two insect defensins: immune peptides from the mosquito *Aedes aegypti*. *Proc. R. Soc. Lond. B* 261, 217-221
23. Lowenberger, C., Bulet, P., Charlet, M., Hetru, C., Hodgeman, B., Christensen, B. M., and Hoffmann, J. A. (1995) Insect immunity: isolation of three novel inducible antibacterial defensins from the vector mosquito, *Aedes aegypti*. *Insect. Biochem. Mol. Biol.* 25, 867-873
24. Cho, W. L., Fu, T. F., Chiou, J. Y., and Chen, C. C. (1997) Molecular characterization of a defensin gene from the mosquito, *Aedes aegypti*. *Insect. Biochem. Mol. Biol.* 27, 351-358
25. Richman, A. M., Bulet, P., Hetru, C., Barillas-Mury, C., Hoffmann, J. A., and Kafalos, F. C. (1996) Inducible immune factors of the vector mosquito *Anopheles gambiae*: biochemical purification of a defensin antibacterial peptide and molecular cloning of preprodefensin cDNA. *Insect. Mol. Biol.* 5, 203-210
26. Lowenberger, C., Charlet, M., Vizioli, J., Kamal, S., Richman, A., Christensen, B. M., and Bulet, P. (1999) Antimicrobial activity spectrum, cDNA cloning, and mRNA expression of a newly isolated member of the cecropin family from the mosquito vector *Aedes aegypti*. *J. Biol. Chem.* 274, 20092-20097
27. Vizioli, J., Bulet, P., Charlet, M., Lowenberger, C., Blass, C., Muller, H.-M., Dimopoulos, G., Hoffmann, J. A., Kafatos, F. C., and Richman, A. (2000) Cloning and analysis of a cecropin gene from the malaria vector mosquito, *Anopheles gambiae*. *Insect Mol. Biol.* In Press
28. Sun, D., Eccleston, E. D., and Fallon, A. M. (1998) Peptide sequence of an antibiotic cecropin from the vector mosquito, *Aedes albopictus*. *Biochem. Biophys. Res. Commun.* 249, 410-415
29. Yoshiga, T., Hernandez, V. P., Fallon, A. M., and Law, J. H. (1997) Mosquito transferrin, an acute-phase protein that is up-regulated upon infection. *Proc. Natl. Acad. Sci. U S A* 94, 12337-12342
30. Dimarcq, J. L., Zachary, D., Hoffmann, J. A., Hoffmann, D., and Reichhart, J. M. (1990) Insect immunity: expression of the two major inducible antibacterial peptides, defensin and diptericin, in *Phormia terranovae*. *Embo J.* 9, 2507-2515
31. Beerntsen, B. T., Severson, D. W., and Christensen, B. M. (1994) *Aedes aegypti*: characterization of a hemolymph polypeptide expressed during melanotic encapsulation of filarial worms. *Exp. Parasitol.* 79, 312-321
32. Dimopoulos, G., Seeley, D., Wolf, A., and Kafatos, F. C. (1998) Malaria infection of the mosquito *Anopheles gambiae* activates immune- responsive genes during critical transition stages of the parasite life cycle. *Embo J.* 17, 6115-6123
33. Brey, P. T., Lee, W. J., Yamakawa, M., Koizumi, Y., Perrot, S., Francois, M., and Ashida, M. (1993) Role of the integument in insect immunity: epicuticular abrasion and induction of cecropin synthesis in cuticular epithelial cells. *Proc. Natl. Acad. Sci. U S A* 90, 6275-6279
34. Lambert, J., Keppi, E., Dimarcq, J. L., Wicker, C., Reichhart, J. M., Dunbar, B., Lepage, P., Van Dorsselaer, A., Hoffmann, J., Fothergill, J., and et al. (1989) Insect immunity: isolation from immune blood of the dipteran *Phormia terranovae* of two insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. *Proc. Natl. Acad. Sci. U S A* 86, 262-266
35. Ganz, T., and Lehrer, R. I. (1995) Defensins. *Pharmacol. Ther.* 66, 191-205
36. Evans, E. W., Beach, F. G., Moore, K. M., Jackwood, M. W., Glisson, J. R., and Harmon, B. G. (1995) Antimicrobial activity of chicken and turkey heterophil peptides CHP1, CHP2, THP1, and THP3. *Vet. Microbiol.* 47, 295-303
37. Penninckx, I. A., Eggermont, K., Terras, F. R., Thomma, B. P., De Samblanx, G. W., Buchala, A., Metraux, J. P., Manners, J. M., and Broekaert, W. F. (1996) Pathogen-induced systemic activation of a plant defensin gene in Arabidopsis follows a salicylic acid-independent pathway. *Plant Cell* 8, 2309-2323
38. Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T., and Kobayashi, K. (1990) A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *J. Biol. Chem.* 265, 11333-11337
39. Casteels, P., Ampe, C., Jacobs, F., and Tempst, P. (1993) Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (*Apis mellifera*). *J. Biol. Chem.* 268, 7044-7054
40. Cornet, B., Bonmatin, J. M., Hetru, C., Hoffmann, J. A., Ptak, M., and Vovelle, F. (1995) Refined three-dimensional solution structure of insect defensin A. *Structure* 3, 435-448

41. Bontems, F., Roumestand, C., Gilquin, B., Menez, A., and Toma, F. (1991) Refined structure of charybdotoxin: common motifs in scorpion toxins and insect defensins. *Science* 254, 1521-1523
42. Leippe, M. (1999) Antimicrobial and cytolytic polypeptides of amoeboid protozoa—effector molecules of primitive phagocytes. *Dev. Comp. Immunol.* 23, 267-279
43. Kato, Y., and Komatsu, S. (1996) ASABF, a novel cysteine-rich antibacterial peptide isolated from the nematode *Ascaris suum*. Purification, primary structure, and molecular cloning of cDNA. *J. Biol. Chem.* 271, 30493-30498
44. Cociancich, S., Goyffon, M., Bontems, F., Bulet, P., Bouet, F., Menez, A., and Hoffmann, J. (1993) Purification and characterization of a scorpion defensin, a 4kDa antibacterial peptide presenting structural similarities with insect defensins and scorpion toxins. *Biochem. Biophys. Res. Commun.* 194, 17-22
45. Ehret-Sabatier, L., Loew, D., Goyffon, M., Fehlbaum, P., Hoffmann, J. A., van Dorsselaer, A., and Bulet, P. (1996) Characterization of novel cysteine-rich antimicrobial peptides from scorpion blood. *J. Biol. Chem.* 271, 29537-29544
46. Charlet, M., Chernysh, S., Philippe, H., Hetru, C., Hoffmann, J. A., and Bulet, P. (1996) Innate immunity. Isolation of several cysteine-rich antimicrobial peptides from the blood of a mollusc, *Mytilus edulis*. *J. Biol. Chem.* 271, 21808-21813
47. Hubert, F., Noel, T., and Roch, P. (1996) A member of the arthropod defensin family from edible Mediterranean mussels (*Mytilus galloprovincialis*). *Eur. J. Biochem.* 240, 302-306
48. Cociancich, S., Ghazi, A., Hetru, C., Hoffmann, J. A., and Letellier, L. (1993) Insect defensin, an inducible antibacterial peptide, forms voltage- dependent channels in *Micrococcus luteus*. *J. Biol. Chem.* 268, 19239-19245
49. Richman, A. M., Dimopoulos, G., Seeley, D., and Kafatos, F. C. (1997) *Plasmodium* activates the innate immune response of *Anopheles gambiae* mosquitoes. *Embo J.* 16, 6114-6119
50. Lee, J. Y., Boman, A., Sun, C. X., Andersson, M., Jornvall, H., Mutt, V., and Boman, H. G. (1989) Antibacterial peptides from pig intestine: isolation of a mammalian cecropin. *Proc. Natl. Acad. Sci. U S A* 86, 9159-9162
51. Holak, T. A., Engstrom, A., Kraulis, P. J., Lindeberg, G., Bennich, H., Jones, T. A., Gronenborn, A. M., and Clore, G. M. (1988) The solution conformation of the antibacterial peptide cecropin A: a nuclear magnetic resonance and dynamical simulated annealing study. *Biochemistry* 27, 7620-7629
52. Shai, Y. (1995) Molecular recognition between membrane-spanning helices. *TIBS* 20, 460-464
53. Shai, Y. (1998) Mode of Action of Antibacterial Peptides in *Mode of action of antibacterial peptides in Molecular mechanisms of immune responses in insects* (Brey, P. T., and Hultmark, D., eds), pp. 111-134, Chapman and Hall, London
54. Christensen, B., Fink, J., Merrifield, R. B., and Mauzerall, D. (1988) Channel-forming properties of cecropins and related model compounds incorporated into planar lipid membranes. *Proc. Natl. Acad. Sci. U S A* 85, 5072-5076
55. Cruciani, R. A., Barker, J. L., Durell, S. R., Raghunathan, G., Guy, H. R., Zasloff, M., and Stanley, E. F. (1992) Magainin 2, a natural antibiotic from frog skin, forms ion channels in lipid bilayer membranes. *Eur. J. Pharmacol.* 226, 287-296
56. Ehrenstein, G., and Lecar, H. (1977) Electrically gated ionic channels in lipid bilayers. *Q. Rev. Biophys.* 10, 1-34
57. Inouye, M. (1974) A three-dimensional molecular assembly model of a lipoprotein from the *Escherichia coli* outer membrane. *Proc. Natl. Acad. Sci. U S A* 71, 2396-2400
58. Oiki, S., Danho, W., Madison, V., and Montal, M. (1988) M2 delta, a candidate for the structure lining the ionic channel of the nicotinic cholinergic receptor. *Proc. Natl. Acad. Sci. U S A* 85, 8703-8707
59. Lear, J. D., Wasserman, Z. R., and DeGrado, W. F. (1988) Synthetic amphiphilic peptide models for protein ion channels. *Science* 240, 1177-1181
60. Hara, S., and Yamakawa, M. (1995) Moricin, a novel type of antibacterial peptide isolated from the silkworm, *Bombyx mori*. *J. Biol. Chem.* 270, 29923-29927
61. Hara, S., Taniai, K., Kato, Y., and Yamakama, M. (1994) Isolation and alpha-amidation of the non-amidated form of cecropin-D from larvae of *Bombyx mori*. *Comp. Biochem. Physiol. B* 108, 303-308
62. Sun, D., Eccleston, E. D., and Fallon, A. M. (1999) Cloning and expression of three cecropin cDNAs from a mosquito cell line. *FEBS Lett* 454, 147-151

63. Andreu, D., Merrifield, R. B., Steiner, H., and Boman, H. G. (1983) Solid-phase synthesis of cecropin A and related peptides. *Proc. Natl. Acad. Sci. U S A* 80, 6475-6479
64. Andreu, D., Ubach, J., Boman, A., Wahlin, B., Wade, D., Merrifield, R. B., and Boman, H. G. (1992) Shortened cecropin A-melittin hybrids. Significant size reduction retains potent antibiotic activity. *FEBS Lett* 296, 190-194
65. Andreu, D., Merrifield, R. B., Steiner, H., and Boman, H. G. (1985) N-terminal analogues of cecropin A: synthesis, antibacterial activity, and conformational properties. *Biochemistry* 24, 1683-1688
66. Li, Z. Q., Merrifield, R. B., Boman, I. A., and Boman, H. G. (1988) Effects on electrophoretic mobility and antibacterial spectrum of removal of two residues from synthetic sarcotoxin IA and addition of the same residues to cecropin B. *FEBS Lett* 231, 299-302
67. Merrifield, R. B., Vizioli, L. D., and Boman, H. G. (1982) Synthesis of the antibacterial peptide cecropin A (1-33). *Biochemistry* 21, 5020-5031
68. Callaway, J. E., Lai, J., Haselbeck, B., Baltaian, M., Bonnesen, S. P., Weickmann, J., Wilcox, G., and Lei, S. P. (1993) Modification of the C terminus of cecropin is essential for broad- spectrum antimicrobial activity. *Antimicrob. Agents Chemother.* 37, 1614-1619
69. Zhao, C., Liaw, L., Lee, I. H., and Lehrer, R. I. (1997) cDNA cloning of three cecropin-like antimicrobial peptides (Styelins) from the tunicate, *Styela clava*. *FEBS Lett* 412, 144-148
70. Strub, J. M., Garcia-Sablone, P., Lonning, K., Taupenot, L., Hubert, P., Van Dorsselaer, A., Aunis, D., and Metz-Boutigue, M. H. (1995) Processing of chromogranin B in bovine adrenal medulla. Identification of secretolytin, the endogenous C-terminal fragment of residues 614-626 with antibacterial activity. *Eur. J. Biochem.* 229, 356-368
71. Britigan, B. E., Lewis, T. S., McCormick, M. L., and Wilson, M. E. (1998) Evidence for the existence of a surface receptor for ferriclactoferrin and ferrictransferrin associated with the plasma membrane of the protozoan parasite *Leishmania donovani*. *Adv. Exp. Med. Biol.* 443, 135-140
72. Svistunenko, D. A., Patel, R. P., Voloshchenko, S. V., and Wilson, M. T. (1997) The globin-based free radical of ferryl hemoglobin is detected in normal human blood. *J. Biol. Chem.* 272, 7114-7121
73. Wilson, M. E., Vorhies, R. W., Andersen, K. A., and Britigan, B. E. (1994) Acquisition of iron from transferrin and lactoferrin by the protozoan *Leishmania chagasi*. *Infect. Immun.* 62, 3262-3269
74. Wilson, M. E., and Britigan, B. E. (1998) Iron acquisition by parasitic protozoa. *Parasitology Today* 14, 348-353
75. Bartfeld, N. S., and Law, J. H. (1990) Isolation and molecular cloning of transferrin from the tobacco hornworm, *Manduca sexta*. Sequence similarity to the vertebrate transferrins. *J. Biol. Chem.* 265, 21684-21691
76. Kurama, T., Kurata, S., and Natori, S. (1995) Molecular characterization of an insect transferrin and its selective incorporation into eggs during oogenesis. *Eur. J. Biochem.* 228, 229-235
77. Yoshiga, T., Georgieva, T., Dunkov, B. C., Harizanova, N., Ralchev, K., and Law, J. H. (1999) *Drosophila melanogaster* transferrin. Cloning, deduced protein sequence, expression during the life cycle, gene localization and up-regulation on bacterial infection. *Eur. J. Biochem.* 260, 414-420
78. Gwadz, R. W., Kaslow, D., Lee, J. Y., Maloy, W. L., Zasloff, M., and Miller, L. H. (1989) Effects of magainins and cecropins on the sporogonic development of malaria parasites in mosquitoes. *Infect. Immunol.* 57, 2628-2633
79. Jaynes, J. M., Julian, G. R., Jeffers, G. W., White, K. L., and Enright, F. M. (1989) In vitro cytocidal effect of lytic peptides on several transformed mammalian cell lines. *Pept. Res.* 2, 157-160
80. Rodriguez, M. C., Zamudio, F., Torres, J. A., Gonzalez-Ceron, L., Possani, L. D., and Rodriguez, M. H. (1995) Effect of a cecropin-like synthetic peptide (Shiva-3) on the sporogonic development of *Plasmodium berghei*. *Exp. Parasitol.* 80, 596-604
81. Possani, L. D., Zurita, M., Delepierre, M., Hernandez, F. H., and Rodriguez, M. H. (1998) From noxiustoxin to Shiva-3, a peptide toxic to the sporogonic development of *Plasmodium berghei*. *Toxicon* 36, 1683-1692
82. Boisbouvier, J., Prochnicka-Chalufour, A., Nieto, A. R., Torres, J. A., Nanard, N., Rodriguez, M. H., Possani, L. D., and Delepierre, M. (1998) Structural information on a cecropin-like synthetic peptide, Shiva-3 toxic to the sporogonic development of *Plasmodium berghei*. *Eur. J. Biochem.* 257, 263-273
83. Chalk, R., Townson, H., and Ham, P. J. (1995) *Brugia pahangi*: the effects of cecropins on microfilariae in vitro and in *Aedes aegypti*. *Exp. Parasitol.* 80, 401-406
84. Albuquerque, C. M., and Ham, P. J. (1996) In vivo effect of a natural *Aedes aegypti* defensin on *Brugia pahangi* development. *Med. Vet. Entomol.* 10, 397-399

85. Lowenberger, C. A., Ferdig, M. T., Bulet, P., Khalili, S., Hoffmann, J. A., and Christensen, B. M. (1996) *Aedes aegypti*: induced antibacterial proteins reduce the establishment and development of *Brugia malayi*. *Exp. Parasitol.* 83, 191-201
86. Shahabuddin, M., Fields, I., Bulet, P., Hoffmann, J. A., and Miller, L. H. (1998) *Plasmodium gallinaceum*: differential killing of some mosquito stages of the parasite by insect defensin. *Exp. Parasitol.* 89, 103-112
87. Beaty, B. J., and Carlson, J. O. (1997) Molecular manipulation of mosquitoes. *Curr. Opin. Infect. Dis.* 10, 372-376
88. Rayms-Keller, A., Powers, A. M., Higgs, S., Olson, K. E., Kamrud, K. I., Carlson, J. O., and Beaty, B. J. (1995) Replication and expression of a recombinant Sindbis virus in mosquitoes. *Insect. Mol. Biol.* 4, 245-251
89. Olson, K. E., Higgs, S., Gaines, P. J., Powers, A. M., Davis, B. S., Kamrud, K. I., Carlson, J. O., Blair, C. D., and Beaty, B. J. (1996) Genetically engineered resistance to dengue-2 virus transmission in mosquitoes. *Science* 272, 884-886
90. Seabaugh, R. C., Olson, K. E., Higgs, S., Carlson, J. O., and Beaty, B. J. (1998) Development of a chimeric sindbis virus with enhanced per Os infection of *Aedes aegypti*. *Virology* 243, 99-112
91. NIAID. (1999) http://www.niaid.nih.gov/ncn/microbes.htm.
92. NIAID. (1999) http://www.niaid.nih.gov/facts/minutes/0599min1.htm.
93. Hoffmann, J. A., and Reichhart, J. M. (1997) *Drosophila* Immunity. *Trends Cell Biol.* 7, 309-316
94. Engstrom, Y. (1998) Insect Immune Gene Regulation in *Molecular mechanisms of immune responses in insects.* (Brey, P. T., and Hultmark, D., eds), pp. 211-244, Chapman and Hall, London