Arthropod-transmitted Parasites: Mechanisms of Immune Interaction

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SYNOPSIS. Knowledge of arthropod immune mechanisms has been based to a large extent on studies of non-medically important arthropod–microbial interactions. Investigations involving arthropods and their immune responses against helminth and protozoan parasites they transmit to vertebrate hosts are relatively limited. Data available strongly suggest that effective responses against these parasites are hemocyte mediated reactions, with blood cells involved in parasite recognition and effector mechanisms. It is also apparent that parasites are very successful in evading immune destruction in compatible arthropod–parasite associations by avoiding immune detection and/or by actively inhibiting the immune processes. However, an understanding of immune evasion mechanisms operating on behalf of the parasite is dependent on a more thorough understanding of parasite recognition, signal transduction, and effector mechanisms involved in arthropod immunity. Limited data are available on hemocyte recognition events, and nothing is known about the transduction processes whereby cells convert extracellular signals to intracellular messages that activate effector mechanisms. Any significant progress in these important areas of research will be limited until successful in vitro hemocyte cultures are developed. Convincing data are available from a variety of arthropod-parasite systems that phenol oxidases play a major role in effector mechanisms of the immune response. However, critical biochemical studies are needed to clarify their substrate specificities and the uniqueness of these enzymes. It is essential that we identify specific substrates involved in effector mechanisms, and their potential storage forms, if we are to begin to understand immune processes in arthropods. The sensitivity of HPLC with electrochemical detection provides a valuable tool for these investigations. The next several years should prove exciting in regard to our understanding of molecular/biochemical processes of arthropod immunity.

INTRODUCTION

Arthropod-borne parasitic diseases pose a major medical and veterinary health problem throughout the world. As a result, a major research effort has been directed during the last century towards such diseases as malaria, filariasis, trypanosomiasis, leishmaniasis, babesiosis, anaplasmosis and other arthropod-transmitted parasites. The vast majority of the research on these heteroxenous parasites, however, has emphasized the vertebrate host–parasite association. Relatively little attention has been given to understanding parasite–host relationships within the arthropod vector/intermediate host. Considering the absolute requirement of the arthropod for the survival of these parasites, it seems apparent that understanding those factors that contribute to successful or unsuccessful arthropod–parasite associations would contribute significantly to our understanding of the epidemiology of arthropod-borne diseases.

It has become increasingly evident during the last 30 years that arthropods possess effective internal defense mechanisms designed to retain the integrity of self. This relatively new area of comparative immunology is providing information that demonstrates a complex, effective and surprisingly specific immune response against foreign material. The sophistication of these responses is only now being realized.
The recent increase in the number of significant articles and symposia reviewing our present knowledge of the immune capabilities of arthropods against foreign insults attests to the growing interest in this research area (Lackie, 1980, 1981a, 1986a; Bauchau, 1981; Rowley and Ratcliffe, 1981; Ratcliffe, 1982; Söderhäll, 1982; Hall, 1983; Ratner and Vinson, 1983; Götz and Boman, 1985; Brehélin, 1986; Dunn, 1986; Gupta, 1986; Boman and Hultmark, 1987; Nappi and Christensen, 1987). Little of this effort, however, has been directed toward those arthropods that function as vectors or intermediate hosts of parasites that infect vertebrates. The tremendous number and diversity of species within the phylum Arthropoda suggest that an equally diverse array of internal defense mechanisms likely exists within the group. Data presently available suggest that significant differences exist, not only between arthropods from different classes, orders, genera and developmental life stages, but also between different species within the same genus.

The immune response of arthropods against the parasites they transmit to vertebrate hosts primarily involves what has been termed an encapsulation/melanization reaction. As with other organisms, an effective immune response in arthropods is dependant on the ability of the host to recognize a parasite as foreign. These recognition signals must then undergo transduction in order to provide the appropriate effector (i.e., encapsulation/melanization) for parasite destruction.

Our intentions in this review article are (1) to briefly review the limited amount of data available on immune responses of arthropods against the parasites they transmit and on immune evasion tactics employed by parasites to avoid destruction, (2) to provide data emphasizing the necessity of evaluating individual species in regard to their immune capabilities and mechanisms against a particular parasite, and (3) to present our thoughts concerning those research areas that must be addressed in a more sophisticated and quantitative manner, if we are to begin to understand parasite recognition, signal transduction, and effector mechanisms involved in arthropod immunity. Our comments regarding this last area are open to debate and criticism. The ideas we express are based to a large degree on recent, and as yet unpublished, data generated in our laboratories. Our intent is to stimulate additional research activity and discussion in areas that we believe are vital for the elucidation of molecular and biochemical events involved in parasite destruction within arthropod hosts.

**DISCUSSION**

Arthropod immunity generally has been separated into cellular and cell-free or humoral immune responses. Humoral immune mechanisms are known to be directed against bacterial invaders and primarily involve antibacterial factors termed cecropins. This area of research has reached the highest level of sophistication in studies of arthropod immunity and is the subject of a thorough review by Boman and Hultmark (1987). Although cell-free immune responses also are likely involved in antiviral and antiprotozoan mechanisms of defense in arthropods, very little is known in these areas. Cellular responses involving the phagocytosis of bacteria, fungi and protozoans by hemocytes also constitute a major line of arthropod defense. Arthropod responses to metazoan parasites, however, usually are mediated by hemocytes in encapsulation reactions. Encapsulation of parasites is most often accompanied by the deposition of melanotic substances with the direct involvement of hemocytes. Humoral melanization of metazoan invaders likely requires at least an indirect involvement of hemocytes. Several valuable reviews that include aspects of cellular immunity in arthropods have recently been published (Götz and Boman, 1985; Götz, 1986a, b; Gupta, 1986; Nappi and Carton, 1986; Ratcliffe, 1986; Renwrantz, 1986; Smith and Söderhäll, 1986; Söderhäll and Smith, 1986; Nappi and Christensen, 1987).

Little of this available literature on arthropod immunity concerns medically important intermediate hosts and their defense responses against parasites. Adult mosquitoes have received the majority of
attention, especially regarding their immune capabilities against some of the filarial nematodes they transmit. Many of these studies on mosquito–filarial worm interactions have been highlighted in three recent reviews (Christensen, 1986; Stoffolano, 1986; Nappi and Christensen, 1987). Limited data also are available on mosquito–Plasmodium associations (Huff, 1927; Mayne, 1929; Weathersby, 1967; Collins et al., 1986). Because of this available literature and because of our own interest, much of the emphasis of this review will be on mosquito immune responses. Other literature available includes studies of insect–Hymenolepis systems (Ubelaker et al., 1970; Heyneman and Voge, 1971; Lackie, 1976, 1981a, b, c, 1986b), insects and the ancahocephalan, Moniliformis (Lackie and Lackie, 1979; Lackie, 1986b), Triatoma infestans–Trypanosoma cruzi relationships (Bitkowska et al., 1982), a Simulium–Onchocerca system (Ham, 1986), and insect–trypanosome models (Croft et al., 1982; East et al., 1983; Kaaya et al., 1985, 1986a, b, 1987; Molyneux et al., 1986), but essentially no published reports concern the response of non-insect arthropod intermediate hosts against parasites that infect vertebrates. Few of the above studies have been designed to assess the mechanistic basis of arthropod immune responses to parasites, but some of the recent work has provided clues regarding immune activation and effector processes in insects, as well as the ability of parasites to avoid and/or inhibit the immune response.

The vast majority of Trypanosomatidae develop within the intestinal tract of their arthropod vectors and therefore never come in contact with cellular or humoral components of the hemolymph. Although the gut serves as an effective barrier to most trypanosomes, a growing literature is developing regarding cellular and humoral components of Glossina and redivuid vectors of trypanosomes (see review by Molyneux et al., 1986 and articles by Kaaya et al., 1985, 1986a, b, 1987). The presence of a trypanosome inhibiting factor in cell-free hemolymph of G. morsitans morsitans that rapidly immobilized Trypanosoma brucei, T. congolense and T. vivax in vitro suggests that plasma factors might destroy these parasites if they would break the gut barrier (Croft et al., 1982; East et al., 1983). Although intrathoracic inoculation of G. morsitans morsitans with live Escherichia coli induced an increased production of two preexisting hemolymph proteins (17 kD and 70 kD), T. brucei brucei inoculations did not stimulate these proteins and usually resulted in the death of inoculated flies (Kaaya et al., 1986b). Subsequent studies have shown that E. coli can induce cecropin and attacin-like factors in G. morsitans morsitans hemolymph (Kaaya et al., 1987). The report by Bitkowska et al. (1982) provides evidence that infection of Triatoma infestans with Trypanosoma cruzi significantly inhibits the immune response, at least against xenograft tissue implants. These authors suggest that the production of exoantigens by the parasite is likely responsible for this immune inhibition in infected bugs. Presently it is difficult to determine how these hemolymph factors or influences might relate to vector-parasite associations when the parasite develops within the lumen of the gut; however, data reviewed by Molyneux et al. (1986) concerning the distribution of lectins within the gut of vectors as possible determinants of site selection of trypanosomatids deserves further attention.

Most parasites transmitted by arthropods must penetrate the intestinal tract or cuticular covering of their intermediate host in order to reach their site of extrinsic development. These mechanical barriers are a means of defense for the arthropod and undoubtedly provide protection from infection by certain parasites. Orihel (1975) provided a review of the role the peritrophic membrane might play in limiting infection in arthropods that serve as biological vectors of parasites and concluded that it is not a barrier of any consequence. Physiological differences in the make-up of the midgut, however, do appear to limit parasite infections in certain species (Sutherlak et al., 1986). It also has been suggested that wound healing at the site of entry of a parasite into an arthropod might be the initiating event responsible for the
immune response seen against that parasite (Lackie, 1986c). But the specificity of the immune response seen in certain arthropods against parasites would argue against this hypothesis. Yamamoto’s laboratory has reported (Yamamoto et al., 1985), and we have subsequently verified in our laboratory (unpublished), that the mosquito, Armigeres subalbatus, encapsulates and destroys the majority of Brugia malayi microfilariae (mff) that penetrate the midgut and enter the hemocoel, but the biologically and morphologically similar Brugia pahangi mff, penetrating the midgut in essentially the same manner, successfully migrate to the thoracic musculature without eliciting a response, and there develop into infective-stage larvae. The major emphasis of research on arthropod immunity, however, has been directed towards an understanding of those humoral and cellular events that are initiated once a parasite penetrates into the body cavity and hemolymph environment of the host.

In the majority of insects, circulating hemocyte populations are quite large and these cells have been considered responsible for immune recognition of helminths and protozoans (Lackie, 1981b). However, humoral encapsulation of parasites with melanotic material, with no apparent involvement of hemocytes has been reported numerous times in those families of dipterans (especially Culicidae and Chironomidae) that harbor relatively few circulating hemocytes (reviewed by Götz, 1986b). Recent ultrastructural investigations of the encapsulation response of adult mosquitoes to filarioid nematodes provide convincing evidence that hemocytes are involved in the immune response (Chen and Laurence, 1985; Forton et al., 1985; Christensen and Forton, 1986). Data recently reported demonstrating surface changes on “immune activated” hemocytes from Aedes aegypti by Nappi and Christensen (1986) and on the requirement of intact hemocytes for in vitro melanization of mff by hemolymph of Anopheles quadrimaculatus by Chen and Laurence (1987) provide verification that hemocytes play a major role in recognition and effector mechanisms in the immune response of adult mosquitoes. The potential role of hemocyte surface membrane components (Yoshino, 1986), hemocyte-bound and nonmembrane-bound lectins (Renwrantz, 1986), surface charge and hydrophobic properties of hemocytes and parasites (Lackie, 1981b, 1983; Takle and Lackie, 1985), or other proposed immune recognition molecules (Smith and Söderhäll, 1983; Ratcliffe et al., 1984; Söderhäll and Smith, 1986) might play in parasite recognition by arthropod hemocytes is essentially unknown in medically important arthropods. Likewise, we have no direct evidence available that incriminates parasite excretory/secretory products as stimulators of arthropod immune responses, although observations of melanotic deposits in the area of the excretory pore of filarioid larvae within immune reactive mosquitoes suggest they may be important (Oothuman et al., 1974; Christensen, 1981).

By whatever means initial recognition of a parasite is accomplished, it must involve some integral macromolecular component of the hemocyte surface membrane, i.e., a receptor. This extracellular signal must then be converted into an intracellular message that modifies cellular metabolism in such a way to activate effector mechanisms ultimately involved in encapsulating or otherwise destroying the parasite. The processes by which cells respond to extracellular signals is one of the most active areas of biochemical research. According to a widely accepted view, binding of an extracellular signal to the receptor activates an intracellular guanine nucleotide-binding protein, a G-protein, which transduces the signal into intracellular second messengers (Gilman, 1987). Candidate second messengers include 3',5'-cyclic AMP, inositol 1,4,5-triphosphate, and diacylglycerol (Berridge, 1987). These molecules in turn affect intracellular events such as mobilization of calcium and phosphorylation of proteins. There is convincing evidence that while differences exist between cell types, this basic format of signal transduction is ubiquitous among eucaryotic cells. Thus, participation of signal transduction mechanisms in the immune responses of arthropods can be predicted.
with some confidence, even though the exact components involved are presently unknown.

Probably the greatest hindrance to definitive studies capable of elucidating the molecular events involved in parasite–hemocyte interactions (i.e., parasite recognition and signal transduction) is our present inability to culture hemocytes in vitro or even to maintain them in culture for extended periods of time. The apparent recent success of maintaining and actually inducing proliferation of cockroach hemocytes in vitro (Howcroft and Karp, 1987; Dr. Richard D. Karp, personal communication) might well provide the clues necessary for culturing hemocytes of other insects.

Effector processes involved in parasite destruction within arthropods are most often associated with the production and deposition of melanotic substances that effectively sequester the parasite. The ultrastructural aspects of this process have been well described in adult mosquitoes reacting against filarioid larvae (Bradley and Nayar, 1986; Chen and Laurence, 1985; Forton et al., 1985; Christensen and Forton, 1986) and also for the response of a refractory strain of Anopheles gambiae against Plasmodium cynomolgi oocinetes (Paskewitz et al., 1988). The selection, by a short series of selective breedings, of a line of A. gambiae fully refractory to a number of species of Plasmodium suggested a relatively simple genetic control for refractoriness (Collins et al., 1986). The refractory mosquitoes encapsulate oocinetes in melanotic material after the parasites lodge between the basal membrane labyrinth and the basal lamina of the midgut. It is not known if this gene(s) for refractoriness controls the ability of A. gambiae to produce melanotic substances; but unpublished data (Dr. Susan M. Paskewitz, personal communication) show that uninfected midguts from refractory mosquitoes produce melanotic material upon incubation in dopa or dopamine, but midguts from the susceptible line of A. gambiae do not. Researchers at the NIH laboratories are actively pursuing molecular aspects of the genetic control of these mechanisms in this interesting model system (Dr. Robert Gwadz, personal communication). However, presently the biochemical mechanisms involved in the production of melanotic substances in medically important arthropods is essentially unknown.

The phenol oxidase-catalyzed conversion of o-diphenols (catechols), derived metabolically from the amino acid, tyrosine, to the corresponding o-quinones is generally accepted as an essential step in melanotic encapsulation. These chemically reactive quinones can both polymerize and bind covalently to nucleophilic protein residues to form colored protein–polyphenol complexes. Such pigmented material is commonly referred to as “melanin” or “sclerotin” (Nappi and Christensen, 1987), even though in no case has the molecular composition of melanotic capsules been determined. This model of the effector stage of arthropod immunity has been inferred largely from studies on cuticular sclerotization and responses of crustaceans (Smith and Söderhäll, 1986) and larval insects (Ashida et al., 1982) to bacteria and parasitoids. Little is known about the biochemistry of this process in adult vectors of medically important parasites. Ongoing studies in our laboratories on the responses of adult Aedes mosquitoes to filarial worms suggest that biochemical differences between closely related insect species probably determine species differences in the rate and intensity of the response. Thus, it is becoming apparent that a general description of “melanization” in arthropods is insufficient to explain why some insects are vectors while others are not. Answering this question will require identification of the specific substrates and enzymes involved in melanotic encapsulation and elucidation of the relevant regulatory mechanisms.

Phenol oxidases have been the most intensively investigated component of the arthropod immune effector mechanism; but there is still much to be learned about these enzymes and the regulation of their catalytic activity. Phenol oxidases have been detected in both hemocytes and hemolymph plasma as catalytically inactive proenzymes (Ashida, 1971; Iwama and
Ashida, 1986). These can be activated by a variety of agents including proteases (Dohke, 1973a, b; Ashida and Dohke, 1980; Söderhäll, 1982; Yoshida and Ashida, 1986a), organic solvents (Rizki et al., 1985) and microbial products such as β-1,3-glucans (Söderhäll, 1982; Ashida et al., 1983), although the latter may act on protease zymogens to activate them rather than acting directly on prophenol oxidase (Söderhäll, 1982). Activation correlates with the immune response and it has been suggested by Söderhäll (1982) that the activation process itself may be the basis of non-self recognition. This may be true for bacteria and protozoa that can be phagocytosed, but for metazoan parasites, it seems more likely that prophenol oxidase activation is a consequence of the release of proteases from hemocytes resulting from transduction of parasite recognition by hemocyte surface receptors. (There may be differences in the exact pathway of prophenol oxidase activation depending on whether the organism is "phagocytosable.")

It is not known how many distinct phenol oxidases participate in the formation of melanotic substances. These enzymes are operationally defined in terms of the substrate used to detect activity. Monophenol oxidase or tyrosinase activity is assayed by measuring oxidation of tyrosine to dopa (Chen and Cavin, 1965; Pentz et al., 1986; Nappi et al., 1987), while diphenol oxidase activity is usually measured with dopa or dopamine, following appearance of chromophore at 475 nm (Pentz et al., 1986), or by darkening of a band in a polyacrylamide gel exposed to dopa (Rizki et al., 1985). Yet, what evidence is there that these are distinct proteins? A careful examination of the literature reveals that in no case have two distinct enzymes been shown to participate in the immune response. Determination of enzyme activity with two substrates using crude insect preparations is not proof of the existence of multiple phenol oxidases. On the contrary, a single enzyme form is probably sufficient. Mammalian phenol oxidase (tyrosinase) clearly can recognize both tyrosine and o-diphenols as substrates and can catalyze all three enzymatic reactions involved in the synthesis of melanin from tyrosine (Korner and Pawalek, 1982). Mushroom phenol oxidase (tyrosinase) also will catalyze these reactions (unpublished observations).

Lepidopterans such as Bombyx mori seem to have a single phenol oxidase (Ashida, 1971). There is some evidence from genetic studies on Drosophila melanogaster larvae that these dipterans do contain multiple forms of prophenol oxidase (Ashida et al., 1983). These forms were detected in polyacrylamide gels of whole larval homogenates after treatment of the gel with either a natural activator from the larvae (presumably a protease) or 50% 2-propanol (Rizki et al., 1985). Three separate phenol oxidases were detected using dopa as the substrate. When activity was checked with tyrosine (darkening of the gel due to melanin formation), one band was found after activation with the larval activator, but no activity was found if the gel was treated with 2-propanol (Rizki et al., 1985). These data suggest that some forms of Drosophila phenol oxidase can in fact recognize both tyrosine and o-diphenols. However, these experiments were done using whole larval extracts. There is no evidence that indicates which of these isoenzyme actually participates in an immune response. It seems conceivable that one form of the enzyme functions in cuticular sclerotization, while another is involved in the immune response.

The most important implication of this work to the field of arthropod immunity is that these results suggest that the phenol oxidase activity one detects (i.e., its substrate specificity) depends on the mechanism by which proenzyme is activated. It would seem that solvent-induced conformational changes in prophenol oxidase allows the Drosophila enzyme to recognize o-diphenols but not tyrosine. To recognize tyrosine, proteolytic cleavage appears necessary. If progress is to be made toward understanding the number and types of phenol oxidases involved in melanotic substance formation, it will be important to determine the biologically relevant mechanism of proenzyme activation that results during responses to par-
asites. This suggests that one should be cautious in interpreting the biological relevance of studies on prophenol oxidase activation using solvents and protein denaturants.

Another study on *Drosophila* indicates that mutations in the *Dox-A2* locus reduce diphenol oxidase activity without affecting monophenol oxidase activity (Pentz et al., 1986). This again supports the existence of multiple phenol oxidases in this dipteran, but does not provide any evidence for an role of multiple phenol oxidases in the immune response. In the end the burden of proof for participation of multiple distinct forms of phenol oxidase in the immune response lies with the investigators proposing such involvement. Such proof would require physical separation of distinct isoenzymes by biochemical means or appropriate genetic experiments demonstrating that alteration in the immune response correlates with changes in enzyme activity due to mutation. In the absence of such proof there is no compelling reason to postulate a role for more than one phenol oxidase.

Data from our laboratory also point out the importance of assessing biochemical mechanisms within specific vector-parasite systems. Although we have already demonstrated that mff can increase monophenol oxidase activity in cell-free hemolymph from *A. aegypti in vivo* (Nappi et al., 1987), we have not been able to demonstrate a similar phenomenon in *A. trivittatus* exposed to the same parasite (Li et al., 1989). We also have data (Li et al., 1989) that illustrate hemocyte monophenol oxidase activity in both *A. aegypti* and *A. trivittatus* inoculated with *D. immitis* mff. Enzyme activity, however, is at least four-fold higher in *A. trivittatus* as compared to *A. aegypti*. These distinct biochemical differences between two species within the same genus are of special interest because of the significantly greater immunocompetence of *A. trivittatus* noted earlier by our laboratory (Christensen et al., 1984).

A final area of interest with respect to the role of phenol oxidase in melanotic encapsulation is: what keeps the reaction localized to the surface of the parasite? Phenol oxidases tend to aggregate and have been described as "sticky" proteins (Söderhäll, 1982). Such behavior would be expected to contribute to the restriction of the reaction to parasite surfaces and could also explain the build up of layers or aggregates of pigmented material. What prevents the activation of prophenol oxidase from causing generalized melanization in bulk phase hemolymph? Obviously the tendency of phenol oxidase to aggregate would help. In addition, Sugumaran's group has reported the purification of a protease inhibitor from hemolymph of *Manduca sexta* by affinity chromatography on a trypsin-agarose column (Saul and Sugumaran, 1986). This inhibitor prevents activation of prophenol oxidase by a cuticular activator from the same species. Such a mechanism makes more sense if the protease (activator) is released from hemocytes during the response. No studies have been published regarding the existence of such protease inhibitors in the hemolymph of medically important insects, although their existence can be reasonably predicted.

Although a key role for phenol oxidase in the immune effector mechanisms seems apparent, the specific substrates involved and the biochemical pathways by which they are converted to melanotic substances have not been characterized. Tyrosine, dopa, dopamine, and N-acetyldopamine have all been implicated as substrates for cuticular melanization and sclerotization (Brunet, 1980; Hopkins et al., 1984) and by extrapolation in the immune response (Nappi and Christensen, 1987). However, direct experimental evidence is lacking. These are not the only candidate substrates. For example, N-β-alanyl derivatives of dopamine and norepinephrine (Morgan et al., 1987) also have been suggested as intermediates in cuticular sclerotization. During experiments to look for changes in catechol levels in hemolymph of *A. aegypti*, we found that cell-free hemolymph contains a novel catecholamine-like molecule in high concentrations. This compound, designated Peak I, was detected...
by HPLC after treatment of hemolymph with a mild alkaline buffer. It has been tentatively identified as a catecholamine based on its pH-dependent chromatographic behavior and its electrochemical reactivity. Peak I does not co-chromatograph with any known insect catechol or with a number of other standards. Efforts are now being made to isolate sufficient amounts of Peak I for determination of its chemical structure. The biological significance of Peak I is suggested by the fact that it was not detected in hemolymph of saline-inoculated *A. aegypti*, but was present in substantial amounts in mosquitoes inoculated with mff. Although Peak I is present in alkaline buffer treated hemolymph samples from *A. gambiae* and *A. subalbatus* as well as from two strains of *A. aegypti*, we can find no evidence for the existence of this catechol in *A. trivittatus*. This further emphasizes the potential for distinct biochemical mechanisms operating in the production of melanotic substances in *A. trivittatus* as compared to *A. aegypti*.

The fact that Peak I cannot be detected in hemolymph from naive mosquitoes without mild alkaline treatment suggests that normally it is present as an inert storage form. Storage forms of tyrosine and catecholamines have been found in numerous insect species (tyrosine-O-phosphate in *D. melanogaster* [Mitchell and Lunan, 1964], tyrosine-O-glucoside in *Manduca sexta* [Ahmed *et al.*, 1983a]) and *D. busckii* [Chen *et al.*, 1978], dopamine-O-phosphate in *Periplaneta* [Sloley and Downer, 1987], and O-glucoside derivatives of N-acetyldopamine in *Tenodera* [Kawasaki and Yago, 1983]), although no role in the immune response has been shown. These storage forms are not recognized as substrates for phenol oxidase and this is presumably a mechanism for regulating enzyme activity in a melanization reaction. In the praying mantid, release of N-acetyldopamine glucosides from the left colleterial gland is accompanied by release of β-glucosidase from the right gland (Kawasaki and Yago, 1983). The presence of tyrosine-O-phosphate in *D. melanogaster*, but tyrosine-O-glucoside in the closely related species, *D. busckii*, again emphasizes the need to determine molecules participating in immune responses in specific insect vectors.

Assuming that storage forms of phenol oxidase substrates exist in mosquitoes, then there must necessarily be one or more enzymes that catalyze the hydrolysis of the storage form to generate free substrate for melanization reactions. These enzymes would be sulfatases, phosphatases or glucosidases and would provide still another point of regulation of the immune response. One would expect that activation of phenol oxidase and hydrolysis of substrate storage forms will be shown to be coordinately regulated. Because of the requirement for hemocyte involvement in encapsulation reactions, these putative hydrodases could likely be lysosomal and thereby released upon hemocyte activation. Although there is no information about the participation of such hydrodases in arthropod immune responses, it has been shown that β-glucosidase, that cleaves tyrosine-O-glucoside, seems to be under endocrine control in *Manduca*, and its activity correlates with ecdysis (Ahmed *et al.*, 1983b).

Another area of the biochemistry of arthropod immunity that has received no attention is the involvement of enzymes other than phenol oxidase in effector mechanisms. For example, if enzymatic decarboxylation of dopa to dopamine is an obligatory step in melanotic substance formation, regulation of that L-aromatic amino acid decarboxylase may be crucial for the response. In fact, there may not be a single pathway for melanotic substance formation even within a single insect species. If there are multiple metabolic pathways leading from a single initial substrate (tyrosine) to melanotic substances, then each branch point enzyme becomes a possible target of metabolic regulation. Thus, it is clearly important to delineate substrates and biochemical pathways involved in immune effector mechanisms.

It has been well established that even an insect that is highly susceptible to parasite infection often possesses an inherent ability to encapsulate and destroy that parasite. This has been demonstrated in our laboratory using *A. aegypti* Liverpool strain mosquitoes and filarioid nematodes (Chris-
tensen et al., 1984; Sutherland et al., 1984). Although we originally believed that exposure to the mosquito midgut provided Brugia mff with protection from immune destruction (LaFond et al., 1985; Christensen et al., 1987), data now available (unpublished) suggest that these parasites possess an inherent ability to avoid immune recognition. It still seems apparent, however, that once B. pahangi begin developing within the thoracic musculature, they suppress the immune capabilities of the vector (Christensen and LaFond, 1986). The opposite situation may exist in blackflies infected with Onchocerca lienalis. In this model system, data suggest a parasite-induced or derived factor confers protection from subsequent infection in Simulium spp. (Ham, 1986). A similar situation seems to exist in the amphipod, Gammarus pseudolimnaeus, infected with the acanthocephalan, Pomphorhynchus bulbocoi. Infected amphipods elicit a much stronger encapsulation against a challenge infection than do uninfected controls (Dr. Larry Gleason, personal communication). The response of parasites to their arthropod intermediate hosts, either through immune evasion strategies or by stimulation of the immune response, is a very important component of vector-parasite associations and has been highlighted in several recent reviews (Lackie, 1986; Bauchau, 1981; Bozicevic and N. A. Ratcliffe, 1987). However, it is apparent from the data presented herein, and from other recent reviews, that we have an insufficient understanding of immune processes in arthropods to be able to determine mechanisms whereby parasites circumvent the immune response to their advantage.

ACKNOWLEDGMENTS

This work was supported in part by the National Institutes of Health, NIAID, through Grant No. AI19769.

REFERENCES


Christensen, B. M. 1986. Immune mechanisms and


Arthropod Immunity to Parasites


Sleeloy, B. D. and R. G. H. Downer. 1987. Dopamine,


