Clearance of Bacteria Injected into the Hemolymph of the Penaeid Shrimp, *Sicyonia ingentis*

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Shrimp rapidly removed four strains of bacteria from their hemolymph. Total hemocyte counts (THC) dropped to 20% of preinjection levels during the 24 hr after injection and returned to preinjection levels by 72-96 hr. Shrimp with reduced THC were still able to clear bacteria from their hemolymph. Saline injections caused only a slight decrease in THC; endotoxin and laminarin injections mimicked the injection of bacteria. The mitotic index of hematopoietic stem cells was elevated during the recovery period. Use of radiolabeled bacteria showed that the gills are the main sites of clearance and that the digestive gland is not involved. Clearance mechanisms in the shrimp are compared to those of other crustaceans. © 1993 Academic Press, Inc.

**KEY WORDS:** Crustacea; shrimp; *Sicyonia ingentis*; bacteria; clearance; hemocytes.

**INTRODUCTION**

Decapod crustaceans are able to rapidly clear a variety of exogenous materials injected into their hemolymph including viruses (McCumber and Clem, 1977; Johnson, 1980; Johnson et al., 1981), bacteria (Corkin and Stewart, 1968; McKay and Jenkins, 1970; White and Ratcliffe, 1982), and dyes and latex particles (Merrill et al., 1979; Factor and Beekman, 1990). Mullainadh et al. (1984) showed that the clearance rates for dyes by the crab *Scylla* were the same after repeated injections and were not affected by charge or opsonization. Clearance rates increased with larger molecular weights and higher concentrations. The cells responsible for the removal of foreign material are thought to include the circulating hemocytes as well as the fixed phagocytes, primarily in the gills and digestive gland. The morphology and function of the fixed phagocytes have received considerable attention and it has been suggested that the two populations of cells are specialized to remove particles of different sizes (Strangways-Dixon and Smith, 1970; Foster and Howse, 1978; Doughtie and Rao, 1981; Johnson, 1987; Factor and Naar, 1990; Factor and Beekman, 1990; Sagrista and Durfort, 1990).

Circulating hemocytes have also been implicated in the clearance of foreign material for the following reasons: (1) they phagocyte foreign materials *in vivo* (Fontaine and Lightner, 1974; Smith and Ratcliffe, 1980) and *in vitro* (McKay and Jenkins, 1970; Paterson and Stewart, 1974; Tyson et al., 1974; Paterson et al., 1976; Smith and Ratcliffe, 1978; Goldenberg et al., 1984); (2) some types of hemocytes contain lysozyme enzymes (Hearing, 1969; Hose et al., 1987, 1990); (3) some hemocytes contain prophenoloxidase (Unestam and Söderhäll, 1977; Söderhäll, 1982; Smith and Söderhäll, 1989; Hose et al., 1990) which, when released, initiates the encapsulation and melanization of foreign particles too large to be phagocytosed by individual cells; and (4) hemocytes have been observed around foreign material in such sites as the gills (Smith and Ratcliffe, 1980; White and Ratcliffe, 1982). In addition, the circulating total hemocyte count (THC) drops following the injection of foreign materials (Paterson et al., 1976; Smith and Söderhäll, 1983; Smith et al., 1984). Corkin and Stewart (1968) and Tyson and Jenkins (1973), studying lobsters and crayfish, respectively, found decreased hemocyte counts 15 min following injection. The fate of these hemocytes is unclear. However, injections of bacteria or even endotoxin alone have been shown to initiate clot formation (Levin, 1967), which may account for the loss of some cells at the site of injection or by disseminated clot formation. Factor and Beekman (1990) injected latex beads into lobsters and suggested that some phagocytic hemocytes may leave circulation and enter the connective tissue. Several studies have described aggregations of hemocytes in the gills of animals that have been injected with foreign materials (Corkin and Stewart, 1968; Fontaine and Lightner, 1974; Johnson, 1976; Smith and Ratcliffe, 1980). Some of these hemocytes phagocyte the material while others seem to be involved in the encapsulation of the larger hemocyte–foreign material aggregations.

Although the clearance of foreign particles and the associated loss of circulating hemocytes have been de-
scribed in several crustaceans, the ensuing rise in the number of circulating hemocytes to preinjection levels has received less attention. The objectives of this study are to describe (1) the shrimp's ability to eliminate bacteria injected into the hemocoeal, (2) the effect of these injections on the circulating hemocyte count, (3) the ability of the shrimp to eliminate foreign material when the hemocytes are depleted by a prior injection, (4) the activity of the hematopoietic tissue during the depletion in the number of circulating hemocytes and their return to preinjection levels, and (5) the relative importance of various organs involved with clearance of bacteria. The clearance mechanisms of the shrimp are compared to those of other crustaceans.

MATERIALS AND METHODS

Collection and Maintenance of Animals

Ridgeback prawn (Stenopenaeus setiferus) were collected by trawl from depths of 100 m off the Palos Verdes Peninsula, California, and maintained in aquaria with flowthrough seawater at 33 ppt salinity and 17°C. Molt cycle staging was determined using the criteria of Anderson (1985) and only shrimp in molt stages C and D, were used in this study.

Preparation of Bacterial Solutions for Injection

The following bacteria were used: Bacillus cereus and Bacillus subtilis (Carolina 15-4870 and 15-4921) and Pseudomonas fluorescens (Carolina 15-5255) were cultured overnight in nutrient broth at 37°C, Vibrio alginolyticus (Occidental College isolate) grown on Marine broth and agar at 25°C overnight, and Aerococcus viridans (ATCC 10400) on S-110 agar at 37°C. The V. alginolyticus was originally isolated from a marine fish and was identified from biochemical characteristics listed in Bergey's Manual (Williams, 1989). To prepare the solution for injection, cultures were pelleted at 600g for 10 min, washed three times in filter (0.2 μm)-sterilized 3% saline, and resuspended in sterile saline to obtain a concentration of 5 × 10⁶. Bacterial counts were determined using a brightline hemocytometer at 1000×.

Tests for Bacterial Clearance

To determine how rapidly bacteria were cleared from the hemolymph, shrimp were injected with 0.1 ml bacterial solution (containing 10⁸ cells/ml) and small (0.050-ml) samples of hemolymph were removed from each animal after 5, 10, 30, and 60 min. The samples were immediately added to 2 ml 12.5% sodium citrate to prevent coagulation, mixed with 7 ml of melted agar, poured into petri dishes, and incubated at appropriate temperatures for 24 hr. Numbers of bacterial colonies per plate were counted and divided by the volume of hemolymph extracted to determine the number of colony-forming units per milliliter of hemolymph. The number of colony forming units per milliliter divided by the number of bacteria per milliliter injected times 100% equals the percentage of bacteria still in circulation. Ten to 15 shrimp were tested for their ability to clear each type of bacterium.

Two procedures were used to test for the presence of bacteriociains, chemicals in the hemolymph that might inhibit bacterial growth. In the first procedure, sterile filter discs (Difco, ¼ inch) were saturated with hemolymph and placed on culture dishes inoculated with each type of bacterium and incubated at 37°C. Hemolymph from preinjected shrimp as well as hemolymph from shrimp at various times after bacterial injection was used. Twenty-four hours later, each dish was examined for bacterial growth around the disc.

The second procedure was developed by White et al. (1985); 100 μl of a suspension of B. subtilis (1 × 10⁸/ml) was added to a tube containing 50 μl of plasma and 0.5 ml of sterile 3% saline. The tube was incubated for 1 hr at 15°C with intermittent shaking. Then 4 ml of marine broth was added to the tube and placed in a shaking water bath at 33°C for 8 hr. The turbidity was measured at 686 nm relative to a blank made of 4-ml broth and 0.65 ml of saline. For controls, 50 μl of sterile saline replaced the plasma. Five plasma and saline (control) samples were tested.

The Effect of Injections on the Total Hemocyte Count

To begin each experiment, approximately 0.050 ml of hemolymph was removed from the base of the second right pleopod and added to 0.500 g of fixative (2.5% glutaraldehyde containing 0.1 M sodium cacodylate, pH 7.4, and 24% glucose). Samples were weighed to the nearest 0.001 g; THC was then performed using a brightline hemocytometer. Immediately following the withdrawal of hemolymph, 0.1 ml of test solution was injected into the corresponding left pleopod and the animal returned to the aquarium. The few animals that showed signs of leakage were discarded. At 4, 24, 48, 72, and 96 hr shrimp were removed and small hemolymph samples were obtained for postinjection THCs. Individual shrimp were bled only two times; at the start and the end of an experiment in order to avoid the stress of multiple bleedings and postinjection counts were expressed as a percentage of the first THC.

One of the following solutions was injected (n = number of shrimp tested at each time point): (1) live bacteria (10⁸/ml; n = 10–15), (2) filter-sterilized 0.85% saline (n = 20–40), (3) filter-sterilized saline containing endotoxin (Sigma, 10 ng/ml; n = 10), and (4) filter-sterilized saline containing laminarin (Sigma, 10 μg/ml; n = 10). A sham injection was also performed where nothing was injected into 10 shrimp but the shrimp were handled and bled as before.
Bacterial Clearance in Hemocyte-Depleted Shrimp

Bacterial injections caused a severe depletion in circulating hemocytes with the lowest THCs at 24 hr after the injection. To determine if bacteria could still be cleared from the hemolymph of shrimp with very low levels of circulating hemocytes, a second set of injections was made 24 hr after the initial injection and bacterial clearance was determined as described above. Seventeen shrimp were injected and then challenged with B. cereus, 11 with Pseudomonas, 9 with Aerococcus, and 6 with Vibrio. Five shrimp were injected with Vibrio and then challenged with Aerococcus.

Activity of the Hematopoietic Tissue

At the end of the B. cereus and saline-control experiments, the epigastric hematopoietic tissue was removed and fixed (glutaraldehyde solution described above). After 3 hr fixation, the tissue was rinsed in 0.1 M sodium cacodylate containing 24% sucrose and post-fixed in 1% osmium tetroxide in cacodylate buffer for 1 hr. Tissue was then stained en bloc in 3% uranyl acetate in sodium acetate buffer for 1 hr, dehydrated through a graded series of ethanol, infiltrated, and embedded in Spurr’s (1969) low-viscosity plastic. Thick (0.5 μm) sections were stained with methylene blue. The number of dividing hematopoietic cells per high-power field (1000×) was determined using an Olympus BH-2 light microscope. The resulting mitotic index was an average from 10 counts per shrimp. At each time point, five bacteria-injected and three saline-control shrimp were evaluated.

Organs Involved with Initial Clearance of Bacteria

Radiolabeled bacteria were injected into shrimp. At specific times several organs were removed, digested, and the level of radioactivity was determined using a scintillation counter. Specifically, B. subtilis were cultured overnight at 37°C in nutrient broth containing 6 μCi/ml 14C-labeled algal hydrolysate (Amersham). The bacteria were washed three times and resuspended in sterile 3% NaCl solution. Using a 26-g needle, 0.1 ml of this solution containing approximately 1 × 10^6 bacteria and 12,800 counts per minute (CPM) was injected into the hemolymph by threading the needle through the second pleopod. This site was selected because it avoided direct injection into the thoracic organs and it eliminated leakage from the body. The animals were placed in individual plastic bags containing 1 liter of seawater and after 15 min, 1 hr, 6 hr, and 24 hr the following samples were collected: seawater, hemolymph (collected from the heart), heart, epigastric hematopoietic (lymphoid) organs, digestive gland, abdominal musculature, and gills. Five shrimp were used for each time. The weight of each sample was determined and then placed in NCS tissue solubilizer (Amersham) overnight at 40°C. The larger organs had to be divided into smaller pieces to facilitate solubilization. Samples were added to Scintiverse scintillation fluid (Fisher) and the level of radioactivity was expressed as CPM. Five shrimp were used as controls. These animals received an injection on nonradiolabeled bacteria and their tissues were processed as described above. Background levels of radioactivity in each organ of these animals were subtracted from counts on the experimental shrimp.

Are Fixed Phagocytes Present in the Digestive Gland?

In some crustaceans fixed phagocytes are attached to blood vessels in the digestive gland and are involved in clearance of foreign material. To determine if such cells are present in shrimp, the digestive gland was fixed in Davidson’s solution, dehydrated in ethanol, cleared in Hemo-D (Fisher), and infiltrated with paraffin. Serial sections (8 μm) were stained with hematoxylin and eosin and examined using light microscopy.

Test to Determine Phagocytic Activity of Hemocytes in Suspension

Hemolymph (5 ml pooled from 10 shrimp) was added to 5 ml of Sieyonia culture medium (Brodie and Chang, 1989) in a polyethylene culture tube. A 0.1-ml aliquot of A. viridans or B. cereus (5 × 10^9 bacteria/ml) was added and gently rotated at 25 rpm for 20 min. Samples of the solution were then pipetted onto glass slides and examined with phase optics. The percentage of hemocytes that had phagocytized bacteria was determined.

Test to Determine if Hemocytes Return to Circulation Laden with Bacteria

A. viridans (5 × 10^9/ml) was injected into the hemocoel of six shrimp. After 48 hr, 0.1–0.2 ml of hemolymph was removed and mixed with an equal amount of fixative. Samples were examined by light microscopy and the percentage of hemocytes containing bacteria was determined.

RESULTS

1. Tests for Bacterial Clearance

All four strains of bacteria are rapidly eliminated from shrimp hemolymph (Figs. 1–4). B. cereus and A. subtilis were completely removed from circulation within 10 min. Vibrio and Pseudomonas were also rapidly removed from circulation but not eliminated; 23 and 7.8%, respectively, of the bacterial inoculum was still free in the hemolymph 1 hr later.
Tests for bacteriocidins in cell-free hemolymph were negative. Hemolymph-saturated discs did not inhibit bacterial growth; in fact, growth occasionally appeared heavier around and over the discs. Results from the turbidometric method also showed that there was no reduction in bacterial growth following exposure to plasma. The initial concentration of bacteria in nutrient broth was $1 \times 10^6$ ml. After 8 hr, the final concentration of bacteria following plasma or saline (control) treatment was $2.8 \times 10^6$ ml and $2.02 \times 10^6$ ml, respectively.

2. Effect of Injections on the Total Hemocyte Count

Preinjection THCs were similar between saline-injected ($27 \pm 2 \times 10^6$, $X \pm$ SEM, $n = 34$) and bacteria-injected ($23 \pm 2 \times 10^6$, $n = 34$) shrimp. Figure 5 shows that THCs rapidly declined following injections of *Bacillus* and reached a minimum 24 hr after injection. At this time, THCs averaged only 23% of their initial levels with some individuals as low as 1%. After 24 hr, THCs began to rise and most shrimp regained preinjection THCs by 72 or 96 hr after injection.

A slower and less severe decline in the THC followed the injection of filter-sterilized saline; THCs returned to normal levels by 96 hr (Fig. 5). Shrimp receiving sham injections, that is, when blood was removed for THC but nothing was injected back into their hemolymph, showed very slight declines in their THC and returns to preinjection levels by 24 hr after bleeding (Fig. 5).

Injections of solutions containing endotoxin and laminarin caused a decline in THCs similar to injections of intact bacteria and more rapid and severe than that caused by saline alone (Fig. 6). These experiments examined only the early effects of these chemicals on THC and were terminated after 48 hr. However, all
five shrimp injected with laminarin and not bled were dead at 48 hr.

3. Bacterial Clearance by Hemocyte-Depleted Shrimp

Circulating hemocytes were at their lowest level 24 hr after bacterial injection. When these shrimp were given a second challenge, it was cleared as rapidly as the first injection (Figs. 1–4; in Figs. 1 and 2 only one line is seen on each graph because the initial and secondary clearance data were exactly the same). The extent of bacterial clearance at 1 hr was similar for both injections.

When shrimp were injected with Aerococcus 24 hr after initially receiving Vibrio, the Aerococcus was cleared and completely eliminated from circulation within 10 min (Fig. 7).

4. Mitotic Activity in the Hematopoietic Nodules

Figure 8 shows the mitotic index in the epigastric hematopoietic tissue following injection of bacteria (B. cereus) or sterile saline. In the saline control group, the mitotic index was similar throughout the 96-hr period, with means ranging between 2.3 and 4.0 mitoses/high-power field. In comparison, the bacteria-injected shrimp exhibited a significantly elevated mitotic index at 6 hr of 8.4 mitoses/field. At 24 hr, the mitotic index decreased to 3.7 mitosis/field. Thereafter the mitotic index remained stable around 5% mitoses/field, a significant increase over control rates by 20–50%.

5. Organs Involved with Initial Clearance of Bacteria

Carbon 14-labeled B. subtilis were injected into shrimp and the radioactivity in each organ was measured between 15 min and 24 hr later. Figure 9 shows the amount of recovered radioactivity as a percentage of the total injected. Fifteen minutes after the bacterial injections, 77% of the injected radioactivity could be accounted for in the six organs studied. Most of the radioactivity was located in the gill (75%), 17% in the hemolymph, 6% in the heart, and <3% in each of the remaining organs. The percentage of injected radioactivity decreased to about 53% by 1 hr and dropped to 27% at 24 hr. The relative percentage of 14C in the gills was similar throughout this period but percentages in the hemolymph were somewhat lower. By 24 hr, the relative percentage of 14C in the heart decreased from 6–8% to 1% and values for the remaining organs were 6% for hematopoietic tissue, 2% for the digestive gland, and 10% for the abdominal musculature. Leakage of radioactivity from the shrimp into the seawater did not occur to any detectable level. Although the digestive gland is the largest organ examined, it accounts for the smallest amount of radiolabeled bacteria.

6. Test to Determine Phagocytic Activity of Hemocytes in Suspension

When hemocytes were kept in suspension in culture medium containing either A. viridans or B. cereus only 21 of 1063 hemocytes (1.98%) had phagocytized bacteria.

7. Test to Determine if Hemocytes Return to Circulation Laden with Bacteria

When shrimp were injected with A. viridans and their hemocytes examined 48 hr later, only 6 of 2595 hemocytes (1.4%) contained bacteria.
DISCUSSION

Our results demonstrate that while the shrimp, *Stenopus hispidus*, possesses many of the bacterial clearance mechanisms common to the crustaceans, there are several important differences. Like other decapods, shrimp rapidly remove large numbers of injected bacteria from their hemolymph (Cuénot, 1905; Read, 1968; McKay and Jenkins, 1970; Smith and Ratcliffe, 1980; McCumber and Clem, 1983; Factor and Beekman, 1990). Shrimp eliminated all three gram-positive bacteria (*B. cereus*, *B. subtilis*, and *A. viridans* var. *homari*) from circulation. Concentrations of both gram-negative bacteria (*P. fluorescens* and *V. alginolyticus*) were rapidly reduced, but not eliminated. Mechanisms underlying differential clearance of gram-positive and -negative bacteria have not been elucidated but probably reflect different sensitivities to various types of infection. For example, *A. viridans* is completely eliminated from the circulation of the shrimp and is not pathogenic (Lightner, 1983). However, it freely circulated throughout the hemolymph in the lobster (*Homarus americanus*) where it causes the fatal disease gaftkemia (Cornick and Stewart, 1968; Johnson et al., 1981).

Bacteria appear to be physically removed from shrimp circulation rather than through bactericidal activity within the hemolymph. These results agree with the reported absence of effective bactericidins in the shore crab (Smith and Ratcliffe, 1978) and in *Aerococcus*-infected lobster (Paterson et al., 1976). However, the latter investigators did find a bactericidin to *Pseudomonas perolens* in the shore crab and its activity was enhanced following challenge with *Aerococcus* or *P. perolens* endotoxin.

Bacterial clearance in shrimp, as in all decapods studied, is associated with a rapid and profound decline in the number of circulating hemocytes (Cornick and Stewart, 1968; Tyson and Jenkins, 1973; Paterson et al., 1976; Smith and Söderhäll, 1983; Smith et al., 1984). Injections of endotoxin or laminarin alone also cause similar declines in the abundance of circulating hemocytes (Paterson et al., 1976; Smith and Söderhäll, 1983; Smith et al., 1984). The fate of these hemocytes is not clear. Factor and Beekman (1990) theorize that some may migrate into connective tissue, others (Fontaine and Lightner, 1974; Smith et al., 1984) found hemocyte clumps in blood sinuses of several organs and especially in the gills, and Johnson (1987) suggested that circulating hemocytes may give rise to the fixed phagocytes. Regardless of their fate, when a second bacterial challenge is given to a hemocyte-depleted shrimp, the bacteria were cleared as effectively as before. Clearance rates remained similar for each bacterium tested, regardless of the type of bacteria originally injected. How can foreign material be removed in crustaceans with very low levels of circulating hemocytes? Perhaps the removal of xenogenic particles such as bacteria occurs in steps with the first step independent of circulating hemocyte action and accomplished instead by physiochemical adherence to the lining of the hemocoel (Ratner and Vinson, 1983). Circulating hemocytes, primarily small-granule granulocytes, would then attach at sites of bacterial adherence and phagocyte the foreign material. Indeed, sequential histological sections of shrimp show bacteria attached to the hemocoel surface with small-granule hemocytes surrounding the bacteria shortly afterwards (unpublished).
The experiments described here support the requirement for attachment of hemocytes to a surface prior to phagocytosis. Less than 2% of shrimp hemocytes were able to bind bacteria if constantly agitated. Previous studies of hemocytic phagocytosis dealt with attached hemocytes in vitro (Paterson and Stewart, 1974; Tyson et al., 1974; Schapiro et al., 1977; Smith and Ratcliffe, 1978; Söderhäll et al., 1986; Hose and Martin, 1989) and studies of hemocyte aggregations in narrow vessels within the gills (Cornick and Stewart, 1968; Smith and Ratcliffe, 1980). It may be that hemocytes are only capable of phagocytosis after adherence to a surface similar to the required activation of macrophages in vertebrates (see Goldenberg et al., 1984). If hemocytes settle out of circulation onto sites of bacterial attachment on the hemocoeal lining they would continue to be available for elimination of the secondary bacterial challenge.

Our results and those using other decapods (Smith and Söderhäll, 1983) found that original THCs were restored by 72 or 96 hr after challenge. Recovery of the THC could be due to either (1) settled hemocytes returning to circulation or (2) the production and/or release of hemocytes from hemopoietic tissue. In shrimp, only 1.4% of the hemocytes in circulation contained bacteria 24 hr after infection. This suggests that very few hemocytes that drop out of circulation to phagocytize bacteria are able to return. In addition, we have shown that new hemocytes are produced in shrimp during the recovery period. Mitotic indices of the hemopoietic tissue are elevated soon after the injection of foreign particles. Although the level of mitotic activity appears low, the 4-hr value of 8.4 mitoses/field actually represents a two- to threefold increase over values for saline-injected controls and un.injected shrimp at comparable molt stages. Of interest is the observation that hemopoiesis was not enhanced in saline-injected controls, despite the fact that their THCs were depressed slightly between 1 and 72 hr. A second unexplored possibility is a large release of hemocytes including immature forms from the hemopoietic tissue such as that observed around molting (Hose et al., 1992).

Although hemocytes are involved in bacterial clearance, radiotracer studies reveal that the gills and the digestive gland are the organs responsible for recognition and removal of foreign materials in most crustaceans that have been studied. The relative importance of each organ seems to vary with regard to the size of the injected particles (Fontaine and Lightner, 1974; Smith and Ratcliffe, 1980; White and Ratcliffe, 1982; Mullainadhan et al., 1984). In this penaeid shrimp, we were unable to histologically demonstrate fixed phagocytes bound to the outer wall of vessels in the digestive gland (unpublished observations), like those previously described in the lobster (Factor and Naar, 1990; Factor and Beekman, 1990). This may explain the low clearance rate of bacteria by the digestive gland in the shrimp. Fixed phagocytes called branchial podocytes do occur within the gills of decapods (see Johnson, 1987), including this shrimp. Although these cells actively accumulate small materials such as xenogenic proteins, bacteriophages, and dye molecules by pinocytosis, they do not phagocytize bacteria.

A final mechanism involved in clearance of injected bacteria is the formation of aggregations of hemocytes and bacteria. These aggregations have been observed in the gill vasculature of shrimp although the large, organized, melanized nodules typical of many decapods have not been observed. In shrimp, these aggregations could result from physical factors such as decreased hemolymph pressure or reduction in vessel size. The rapid (>50% in 24 hr) reduction of radioactivity in the gills of challenged shrimp suggests that bacteria are broken down and ultimately externalized but the underlying mechanisms are unknown. The lack of significant accumulations of radiolabeled bacteria in large, well-vascularized organs such as the abdominal musculature and digestive gland suggests that dispersed nodule formation is not a major means of fighting bacterial infection in shrimp. However, in other crustaceans, hemocyte aggregations (nodules) have been reported dispersed throughout the body as well as in the antennal gland, the heart, and the gills (Bauchau, 1981; Johnson et al., 1981; Ratner and Vinson, 1983; White et al., 1985). The results presented here underscore both the physiologic and anatomical diversity of the decapod immune response and suggest several avenues for further research on clearance of foreign materials.

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