Phagocytosis of *Escherichia coli* by Insect Hemocytes Requires Both Activation of the Ras/Mitogen-activated Protein Kinase Signal Transduction Pathway for Attachment and β₃ Integrin for Internalization*

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Insect hemocytes in response to lipopolysaccharide (LPS) of Gram-negative bacteria facilitate binding and internalization of either cell-associated or cell-free LPS (Charalambidis, N. D., Foukas L. C., and Marmaras V. J. (1996) *Eur. J. Biochem.* 236, 200–206). An early event in LPS signaling in hemocytes involves protein tyrosine phosphorylation (Charalambidis N. D., Zervas C. G., Lambropoulou M., Katsoris P. G., and Marmaras V. J. (1995) *Eur. J. Cell Biol.* 67, 32–41). Here we report further data of LPS-mediated signal transduction responsible for *Escherichia coli* phagocytosis. We demonstrate that both adhesion of hemocytes to substrata and LPS stimulation can cause activation of p44MAPK in *Ceratitis capitata* hemocytes but with distinct kinetics indicating different functions. In addition, we showed that Drk, a homolog protein to the mammalian GRB2, is implicated in the transmission of LPS signaling, indicating that the Ras/mitogen-activated protein kinase pathway is involved. Either the cell-free or the cell-associated LPS appears to attach to the hemocyte surface by the same mechanism that is based on the cross-linking of LPS to membrane-associated p47 via the intermediary of tyrosine derivatives generated by the action of phenol oxidase. By contrast, the cell-free LPS internalization into the hemocytes differs from the cell-associated LPS internalization. For *E. coli* internalization integrin receptors as well as cytoskeletal rearrangements are required, as judged by inhibition of *E. coli* internalization in the presence of the RGD peptide, β₃-integrin antibodies, and cytochalasin D.

Phagocytosis is a process that involves binding and internalization of pathogens, and it is essential for host defense in higher eukaryotes. Phagocytosis in mammals is mainly achieved by neutrophils, monocytes, and macrophages, whereas in insects it is achieved by granular cells and plasmatocytes (1, 2). Even though phagocytosis is an event of major importance for insect immunity, the biochemical pathways involved in it are poorly understood.

Recently, substantial progress in understanding the mechanism of LPS binding and *Escherichia coli* entrapment as well as nodule formation has been achieved by *Ceratitis capitata* hemocytes. Upon LPS triggering, a protein, p47, is released from hemocytes, *in vitro*, which together with tyrosine and phenol oxidase cause aggregation of *E. coli* cells (3, 4). The aggregation is based on the cross-linking of LPS to p47 via the intermediacy of tyrosine derivatives generated by the action of phenol oxidase (5). This protein is also weakly associated with the hemocyte surface (4) and covalently binds to LPS with the same mechanism. The LPS:catechol:p47 complex is then internalized into hemocytes, as judged by proteinase K treatment (5). The key regulatory component for the complex formation is pro (phenol oxidase)-activators (serine proteases), which are released by hemocytes upon LPS triggering and activate the hemocyte surface phenol oxidase (6). The process of LPS-stimulated release of p47 and pro-activators (phenol oxidase) depends on protein tyrosine phosphorylation (4, 6).

Mammalian macrophages, on the other hand, utilize either the LPS-binding protein and membrane CD14 for LPS binding and internalization or the alternative complement C3 pathway. LPS forms a ternary complex, LPS binding protein: LPS membrane CD14 based on weak interactions. This complex is utilized for LPS internalization (7). LPS-stimulated macrophages cause the activation of MAP kinases and the release of active molecules, such as tumor necrosis factor-α and interleukin-1β (8, 9). However, it is not known whether the signal is transduced through G proteins or through the Ras protein (10). In insects, MAP kinases have only been detected in *Drosophila* and are implicated in the development of the compound eye (11). Recently, the *Drosophila* MAP kinase DJNK, which is homologous to the mammalian c-Jun amino-terminal kinase, has been reported to participate in multiple physiological processes. The DJNK signaling pathway mediates morphogenesis and initiates an insect immune response upon LPS triggering (12).

Internalization of a pathogen by a phagocyte requires multiple successive interactions between the phagocyte and the target (1). Internalization is largely achieved by members of the integrin family, a class of heterodimeric surface receptors expressed on almost all cells in higher eukaryotes. Integrins act not only as simple mediators of cell adhesion but can also transduce signals across the cell membranes (13). Integrin-mediated signals activate cellular proteins, including most prominently MAP and FAK kinases (14). In vertebrates, integrins are important for processes such as embryonic morphogenesis, leukocyte migration, platelet aggregation, and regulation-activated protein; ERK, extracellular signal-regulated protein kinase; Drk, downstream of receptor kinase; mAb, monoclonal antibody PAGE, polyacrylamide gel electrophoresis; Y, catechol; TBS, Tris-buffered saline; membrane CD14.

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The abbreviations used are: LPS, lipopolysaccharide; MAP, mito-
tion of cell proliferation and differentiation (1, 14). Concerning insects, integrins have so far only been characterized in Droso-
philal melanogaster and are known to be required for a host of morphogenetic units (2).

The above results prompted us to clarify further the signal transduction pathways leading to E. coli phagocytosis by he-
mocytes in response to LPS. In the present work we show that the Ras/MAP kinase signal transduction pathway plays a piv-
otal role in the immune responses of insect hemocytes upon LPS triggering. In addition, cell-free LPS is evidently internal-
ized into hemocytes by a receptor-mediated endocytosis, whereas E. coli is phagocytosed via a β3 integrin-dependent process.

**EXPERIMENTAL PROCEDURES**

**Materials**—LPS (from E. coli, serotype O111:B4) was purchased from Sigma. Anti-p47 and anti-pheno oxidase are rabbit polyclonal antisera specific for C. capitata p47 and phenol oxidase, respectively, raised at our laboratory, as described previously (5, 15). Rabbit polyclonal anti-
serum against the Drosophila Drk protein, was kindly provided by Dr. T. Pawson (University of Toronto). ERK1 (X-23), an affinity purified rabbit polyclonal antibody raised against a protein that corresponds to a range of amino acids 905–927 within the kinase domain of the rat ERK1 kinase, was purchased from Santa Cruz Biotechnology (Santa Cruz,
CA). The anti-phosphotyrosine monoclonal antibody PY20 and the anti-
β3, monoclonal antibody raised against a peptide corresponding to the amino acids 16–223 of the extracellular domain of the mouse β3 integrin subunit, as well as the A431 and human fibroblasts cell lines, were purchased from Transduction Laboratories (Lexington, KY). Anti-
human HLA DR monoclonal antibody was from Boehringer Mannheim (Mannheim, Germany). 96-Well cell culture plates were from Corning Glass Works (Corning, NY), and 8-well glass slides were from Nunc Inc. (Naperville, IL). Other materials were obtained as indicated and were of the highest grade available.

**Isolation, Culture, and Homogenization of C. capitata Larval Tis-
tures—**C. capitata were reared as described previously (16). Isolation and homogenization of larval hemocytes, fat bodies, and integuments were performed as described elsewhere (6). Protein was assayed by the method of Bradford (17).

Hemocyte monolayers were prepared by allowing 10^5 cells to adhere on cell culture wells for 15 min at 25 °C, followed by washing with 100 μl of insect Ringer’s solution (128 mM NaCl, 18 mM CaCl2, 1.3 mM KCl, 2.3 mM NaHCO3, pH 7.1, for 3 times to remove nonadherent hemocytes. Hemocyte monolayers were cultured in Grace’s insect medium (Sigma).

**Cell Attachment-mediated and LPS-induced Tyrosine Phosphoryla-
tion of Hemocyte Proteins—**Isolated hemocytes were suspended in Grace’s insect medium and then either incubated nonadherently in a rotator or plated onto cell culture wells (10^5 cells per well) at 25 °C for the indicated times. For LPS stimulation, hemocyte monolayers, which had been cultured for 2 h at 25 °C, were stimulated with 100 μg/ml LPS for 5 min at 25 °C. In both cases, after incubations, hemocytes were washed twice with cold Ringer’s solution, then lysed in boiling electrophoresis sample buffer (60 mM Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (v/v) SDS, 10 μM diithiothreitol), and processed for immunoblot analysis.

**Osmotic Loading of Drk Antibodies and Evaluation of Its Effect in LPS-stimulated p47 Exocytosis—**Intracellular loading of Drk antibodies or control antibodies or bovine serum albumin was achieved by promoting uptake of extracellular proteins by incubation in a hypertonic me-
dium, followed by lysis of cytoplasmic pinosomes in a hypotonic solution by the method of Okada and Rechsteiner (18). Briefly, hemocyte mono-
layers, prepared as described above, were washed with Ringer’s solu-
tion and then incubated for 10 min in a hypertonic medium containing 0.5 M sucrose, 10% (v/v) polyethylene glycol 1,000, and Drk IgGs, DR antibody, or bovine serum albumin (30 μg/ml) in Grace’s insect medium. The monolayers were then rinsed with a hypotonic solution of diluted Grace’s medium/water (6:4) and incubated in the hypotonic medium for 2 min. After rinsing 3 times with normal Grace’s medium, the mono-
layers were allowed to recover for 2 h before further procedures were performed. The viability of the hemocytes was assessed by exclusion of trypan blue dye (Sigma).

After loading and cell recovery, hemocytes were stimulated with 100 μg/ml LPS for 5 min and then washed and allowed to secrete for 1 h. After that, culture media were collected, analyzed in SDS-PAGE, and immunoblotted against p47.

**RESULTS**

**Hemocytes Possess Putative Tyrosine-phosphorylated Proteins—**Insect hemocytes require protein tyrosine phosphoryla-
tion in order to attach and internalize cell-free or cell-associated LPS (3–5).

To elucidate the triggered LPS signaling pathway which leads to phagocytosis of bacteria, we identified the tyrosine-phosphorylated polypeptides present in hemocyte extracts. Immunoblot analysis using the monoclonal antibody PY20 and ECL as detection method showed a number of phosphorylated proteins (Fig. 1). Control experiments where antibodies were preincubated either with tyrosine phosphate or with a mixture of serine/threonine phosphates or with a mixture of serine/threonine phosphates demonstrated the specificity of tyrosine-phosphorylated proteins.

**Cell Adhesion and LPS-stimulated Activation of p44MAPK—**It is well known that hemocytes normally adhere to and spread upon foreign surfaces in protein-free medium (22). In our ex-
experiments, the adherence of hemocytes in the absence of any stimulus was relatively constant under the conditions of the assay, with an average of 70% in 15 min. Further, it is well known that cell adhesion affects the pattern of tyrosine-phosphorylated proteins in cells (23, 24). To study the putative pattern of tyrosine-phosphorylated proteins upon LPS triggering, three series of experiments were performed. In the first one series, hemocytes were kept in suspension (as control for plated cells); in the second series, hemocytes were plated in the absence of any stimulator (as control for LPS-triggered); and in the third series, monolayers were treated with LPS and incubated for the times indicated in the figure legends.

As shown in Fig. 2, only a few proteins were significantly phosphorylated on tyrosine, including a protein with molecular mass of 44 kDa (identified as MAP kinase, see below). Although some variation was observed between different experiments due to a rather low level of activation of hemocytes inherent in the preparation of isolated hemocytes, the most important difference, constant in all experiments, was that observed between hemocytes in suspension and attached cells. Cells in suspension showed a progressive dephosphorylation in relation to incubation time, presumably due to activation of hemocytes during the isolation procedure. By contrast, attached hemocytes showed a progressive increase in protein phosphorylation due to the adherence effect.

Focusing further on the 44-kDa protein, which shows a maximum of phosphorylation at 20 min, we succeeded in identifying a polypeptide homologue to ERK1 of mammals and ERK-A of D. melanogaster in hemocytes, fat body, and integument (epidermal cells plus cuticle) of C. capitata. The identification was based on immunoblot analysis using polyclonal antibodies against rat ERK1, which recognize both the 42- and 44-kDa forms of MAP kinases (Fig. 3, lane 1) and a MAP kinase of Drosophila (DmERK-A) (Fig. 3, lane 2). Indeed, anti-rat ERK1 antibodies recognize in hemocytes, fat body, and integumental extracts of C. capitata a 44-kDa polypeptide (Fig. 3, lanes 3–5, respectively) and in the integument a 42-kDa polypeptide as well.

To correlate the 44-kDa tyrosine-phosphorylated polypeptide of Fig. 2 to p44MAPK, the polyvinylidene difluoride membrane of Fig. 2 was stripped from the various antibodies after the ECL treatment and was incubated with anti-rat ERK1. As shown in Fig. 2B the band of 44 kDa is aligned perfectly with that recognized by rat ERK1 antibodies. Furthermore, by comparing A and B of Fig. 2, in regard to p44MAPK, it can be concluded that although the quantity of p44MAPK remained rather unchanged (Fig. 2B), the adhesion-induced MAP kinase activation was increased up to 20 min and then declined progressively (Fig. 2A). Thus, cell adhesion can promptly and effectively activate MAP kinase, a situation already well known in mammalian systems (25, 26).

LPS-triggered hemocytes in suspension culture also caused a strong activation of p44MAPK (Fig. 4A), whereas the quantity of ERK1 remained rather unchanged (Fig. 4B). However, the kinetics of adhesion-mediated activation and LPS activation are distinct. The adhesion-mediated activation of p44MAPK reached maximum at 20 min (Fig. 2), whereas the activation by LPS reached maximum at 5 min (Fig. 4). Similar results have already been obtained with integrin and growth factors (26). Therefore, it appears that even though both LPS and cell adhesion stimulate MAP kinase activities they seem to demonstrate important regulatory differences. Furthermore, we sought to determine whether factors affecting cytoskeleton rearrangements, e.g. cytochalasin D, might influence the activation of MAP kinase by LPS. Treatment of hemocytes with...
cytochalasin D, an inhibitor of actin filament formation, had no effect on LPS-induced MAP kinase activation (Fig. 4), suggesting that these kinases are not associated with cell shape changes including cell spreading and phagocytosis.

Drk Plays an Essential Role in Immune Responses—As MAP kinases are considered to be key molecules for the convergence of extracellular signals, we sought to elucidate an upstream component in the pathway leading to the MAP kinase activation. A key component that functions downstream of the tyrosine kinases and upstream of the MAP kinase pathway is Drk, which is homologous to the mammalian GRB2 (27).

We initially tried to identify the presence of the Drk protein in C. capitata larval tissues. Immunoblot analysis by using polyclonal anti-Drk antibodies (Fig. 5) clearly showed a 24-kDa polypeptide recognized by anti-Drk in the tissues tested. Furthermore, to determine whether this SH2-SH3 adaptor protein is implicated in the transmission of LPS signaling, we used osmotic lysis of pinocytic vesicles to load hemocytes with anti-Drk. Fig. 6A showed the successful uptake of anti-Drk IgGs by the hemocytes. Furthermore, to determine whether the LPS signaling is directly affected by Drk, we examined whether the p47 immune protein was released or not after LPS stimulation of the antibody-loaded hemocytes. Fig. 6B showed that in hemocytes loaded with anti-Drk, the LPS-induced release of the immune protein p47 was largely inhibited (Fig. 6, lanes 5 and 10) compared with the unloaded hemocytes (Fig. 6, lanes 3 and 4), which reached the level of basal secretion (Fig. 6, lanes 1 and 2). The specificity of the anti-Drk effect was checked by loading the hemocytes with bovine serum albumin (Fig. 6, lanes 7 and 8) or with the monoclonal anti-DR, an irrelevant antibody (Fig. 6, lanes 5 and 6). Consequently, in hemocytes the Ras/MAP kinase pathway is activated in response to endotoxic LPS.

Phagocytosis and β Integrin—Phagocytosis is a multistep process including, among others, attachment and internalization of invaders. In the following experiments, an attempt was undertaken to record some of the requirements needed for the process of E. coli phagocytosis by hemocytes and to compare it with the known requirements for cell-free LPS internalization into hemocytes (4–6). The phagocytic activity of the hemocytes against both latex beads and heat-fixed E. coli cells was evaluated by a fluorescence quenching assay with trypan blue (21). In both cases about 13% of the cells kept at 31 °C were phagocytically active.

By employing the above assay, it was possible to demonstrate that the phagocytic activity was largely inhibited when hemocytes were first incubated with antibodies against phenol oxidase or an inhibitor of phenol oxidase activity (phenylthiourea) (Table I). These results strongly support the hypothesis that, in vivo, the cross-linking of LPS (E. coli) to the hemocyte surface p47 via the intermediacy of tyrosine derivatives generated by the action of phenol oxidase (5) is a prerequisite for the phagocytosis of E. coli cells by hemocytes. In addition, genistein, as well as disodium 4-acetamido-4'-isothiocyanato-2,2'-disulfonate, an inhibitor of cell secretion, also blocked E. coli phagocytosis (Table I), further supporting the above hypothesis. Both inhibitors for different reasons blocked the LPS-dependent release of immune proteins p47 and pro-activators (phenol oxidase) (4, 6) responsible for the activation of the system and the attachment of E. coli at the hemocyte surface (6). As a result, the attachment of either E. coli or cell-free LPS at the hemocyte surface depends on the same...
Insect Hemocytes Phagocytosis and Signal Transduction

Hemocyte monolayers were incubated in 100 μl of Grace’s insect medium containing the indicated dilutions of anti-β3 mAb, cytochalasin D, RGD, or RGE peptides for 30 min at 25 °C. After the completion of the incubation the cells were washed and further incubated with labeled LPS-Y (20,000 cpm/well) for 60 min at 25 °C or were incubated with E. coli cells for 60 min at 31 °C. Finally, the cells were washed and treated with lysis buffer, and the radioactive incorporation was measured. To determine protease-resistant LPS attachment, the cells after the LPS-Y attachment were treated with proteinase K. The phagocytic activity was determined by a fluorescence quenching assay with trypan blue, as described under “Experimental Procedures.” Anti-PO indicates anti-phenol oxidase. Values are means of three different experiments (S.D.).

Table I

<table>
<thead>
<tr>
<th>Effector molecule</th>
<th>Attachment</th>
<th>Internalization</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPS-Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Anti-p47 (1/100)</td>
<td>37±2</td>
<td>30±5</td>
<td>20°</td>
</tr>
<tr>
<td>Anti-PO (1/100)</td>
<td>33±4</td>
<td>30±5</td>
<td>35±5</td>
</tr>
<tr>
<td>Phenylthiourea (1 mM)</td>
<td>32±6</td>
<td>23±5</td>
<td>17±5</td>
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<tr>
<td>Genistein (4 mM)</td>
<td>27±5</td>
<td>25±2</td>
<td>30±3</td>
</tr>
<tr>
<td>SITS (10 μl)</td>
<td>27±5</td>
<td>23±3</td>
<td>25±3</td>
</tr>
<tr>
<td>Anti-β3</td>
<td>93±5</td>
<td>95±5</td>
<td>ND</td>
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<tr>
<td>RGD (2 mM)</td>
<td>97±5</td>
<td>ND</td>
<td>18±2</td>
</tr>
<tr>
<td>RGES (2 mM)</td>
<td>ND</td>
<td>ND</td>
<td>70±5</td>
</tr>
<tr>
<td>Cytochalasin D (10 μM)</td>
<td>95±3</td>
<td>92±2</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Data from Ref. 5.
† Data from Ref. 6.
- SITS, disodium 4-acetamido-4’-isothiocyanostiboline-2,2’-disulfonate.
* ND, not determined.

To clarify further whether both forms of LPS share the same requirements for their internalization into hemocytes, we studied the effect of the anti-β3 integrin as well as that of the RGD and RGE peptides on internalization of both forms of LPS. Treatment of hemocyte monolayers with antibodies against β3 integrin and RGD peptides resulted in blockage of E. coli phagocytosis indicating the participation of integrins and possibly of other molecules containing RGD recognition sequence in this process. By contrast, this treatment does not affect the attachment and internalization of cell-free LPS. The presence of β3 integrin on the surface of hemocytes was further supported by an immunoblot analysis. Fig. 7 demonstrated that a monoclonal antibody against the β3 integrin subunit recognizes a band at 90 kDa, characteristic of β3 integrin, on membranes of hemocytes and fat body. By contrast, integumental proteins are not recognized by the same monoclonal antibody. To our knowledge, this is the first report concerning the expression of β3 integrin on the hemocyte surface of insects and its involvement in the process of insect hemocyte phagocytosis. Internalization of cell-free LPS also differs from cell-associated LPS internalization. Cytochalasin D completely inhibited any phagocytic activity but not LPS-cell-free internalization (Table I), indicating once again the association of cytoskeleton rearrangement with phagocytosis.

DISCUSSION

Phagocytosis of bacteria usually elicits a more complex and thus different response than LPS alone. Therefore, it was interesting to examine whether the biochemistry of cell-free and cell-associated LPS attachment and internalization differ.

Our current findings demonstrated that initially, both cell-free LPS and E. coli cells recognize p47 on the hemocyte surface (5). This process is associated with phosphorylation of intracellular proteins (4, 5), representing a potential signal recognition pathway leading to the release of intracellular p47 and pro-activators (phenol oxidase) from hemocytes (4, 6), and presumably also the release of certain effector molecules responsible for both cell adhesion and cell spreading. The release upon LPS triggering p47 and pro-activators (phenol oxidase) is responsible for the association of both cell-free and cell-associated LPS (E. coli) with hemocyte surface (4–6). The process of attachment, therefore, involves a signal transduction pathway (4, 5). In mammals, at present, there is no evidence that signaling is required for LPS attachment and/or internalization nor there is evidence that it is not dependent upon some sort of signal to the cell (8, 9).

The fact that intracellular signaling pathways are required for LPS attachment to the hemocyte surface prompted us to investigate further, in order to clarify the intracellular LPS-induced signal transduction pathways. We have demonstrated that LPS can cause a strong and prompt activation of p44MAPK in hemocytes (Fig. 4). This activation of p44MAPK differs from that caused by adhesion of hemocytes to substrata (Fig. 2), as the kinetics of adhesion-mediated activation of p44MAPK and LPS-mediated activation are distinct indicating different functions. Therefore, it appears that LPS-mediated activation are distinct indicating different functionalities. The notion that the Ras/MAP kinases pathway is considered to be a key pathway for the convergence of extracellular signals, and their transmission throughout the cell, encouraged us to search for an upstream component in the pathway leading to MAP kinase activation, in order to establish the hypothesis that this pathway is involved in the outside-in signaling by LPS. Indeed, we succeeded in demonstrating that Drk, a downstream receptor kinases molecule, is present in C. capitata hemocytes (Fig. 3). In addition, hemocytes loaded with anti-Drk inhibited the release of immune proteins (3, 5), suggesting the involvement of Drk in LPS signaling. This molecule has so far only been characterized in Drosophila and is homologous to...
GRB2 of mammalian cells (27).

These results strongly support that p44MAPK and the effector molecule Drk are evidently involved in insect hemocyte immune responses. Therefore, it can be concluded that LPS signaling for the release of immune proteins is transmitted via the Ras/MAP kinases pathway. The Ras/MAP kinases pathway has not been implicated in any aspect of the invertebrate immunity nor in macrophage immune responses (2). Evidently, much more work is needed to elucidate in detail the signaling pathways leading to the release of immune proteins. Furthermore, it would be essential to see whether Ras/MAP kinases pathway is normally involved in the hemocyte immune responses challenged by other elicitors or other unrelated microbes.

The LPS signaling for the release of immune proteins transmitted via the Ras/MAP pathway is essential for the attachment of both cell-free or cell-associated LPS at the hemocyte surface. The mechanism of attachment for both LPS forms, as can be evaluated from the results of Table I, is the same. That is, both LPS forms cross-link to hemocyte surface p47 via the intermediacy of tyrosine derivatives generated by the action of phenol oxidase, as is the case for cuticular protein-chitin cross-links during sclerotization (28). This attachment is a prerequisite for both LPS forms internalization into hemocytes (5).

The sum of our data (Refs. 4–6 and the present results), however, strongly supports that cell-free LPS internalization into hemocytes differs from cell-associated LPS internalization. As criteria for the process of internalization were the resistance of cell-free LPS binding to dissociation by proteinase K and the phagocytic activity of the hemocytes against E. coli cells, as evaluated by an established fluorescence-quenching assay.

The fact that the phagocytic activity was largely inhibited, when hemocytes were preincubated with RGD peptides or an integrin 3 integrin might be responsible for E. coli internalization without affecting attachment. Similar results have already been obtained in other systems. Integrins in insects have so far been implicated in insect immunity. Integrins in insects so far have not been implicated in any aspect of the invertebrate immunity. Integrins in insects so far have not been implicated in any aspect of the invertebrate immunity. Integrins in insects so far have not been implicated in any aspect of the invertebrate immunity. Integrins in insects so far have not been implicated in any aspect of the invertebrate immunity.

REFERENCES
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