Immune response of *Drosophila melanogaster* to infection with the flagellate parasite *Crithidia* spp.

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Abstract

Insects are able to recognize invading microorganisms and to mount an immune response to bacterial and fungal infections. Recently, the fruitfly *Drosophila melanogaster* has emerged as a promising invertebrate model to investigate innate immunity because of its well-characterized genetics. Insects are also vectors of numerous parasites which can trigger an immune response. We have investigated the interaction of *Drosophila melanogaster* with the flagellate protozoan *Crithidia* spp. We show that a *per os* parasitic infection triggers the synthesis of several antimicrobial peptides. By reverse phase HPLC and mass spectrometry, peptides were shown to be present in the hemolymph and not in the gut tissue, suggesting the presence of immune messengers between the site of the infection, namely the gut, and the fat body, the main site of synthesis for antimicrobial peptides. Interestingly, we have identified one molecule which is specifically induced in the hemolymph after infection with *Crithidia*, but not with bacteria, suggesting that *Drosophila* can discriminate between pathogens. When flagellates were injected into the hemolymph, a low synthesis of antimicrobial peptides was observed together with phagocytosis of parasites by circulating hemocytes. The data presented here suggest that *Drosophila–Crithidia* spp. represents an interesting model to study host defense against protozoan parasites. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Insect immunity; *Drosophila melanogaster; Crithidia; Flagellate parasite; Antimicrobial peptides*

1. Introduction

Insects proliferate in diverse biotopes and are constantly exposed to various microorganisms. Consequently, during evolution they have developed efficient immune defenses to resist hostile environments. These defenses rely on different mechanisms: phagocytosis, activation of proteolytic cascades such as coagulation and melanization, and production of various antimicrobial peptides (for reviews, see Richman and Kafatos, 1995; Hoffmann and Reichhart, 1997; Boman, 1998). The *Drosophila* host defense has been particularly well investigated and has served to elucidate the phylogenetic basis of innate immunity (reviewed in Hoffmann et al., 1999). Indeed, in response to an experimental systemic infection with bacteria, *Drosophila* synthesizes in its fat body (a functional equivalent of the vertebrate liver) a large panel of antimicrobial peptides. Despite the many different structures, all the gene-encoded antimicrobial peptides studied to date are small cationic molecules. Their size is mostly below 10 kDa and interestingly, they possess a broad spectrum of potent antimicrobial activity. Drosomycin has exclusively antifungal properties, in contrast to drosocin, diptericin, cecropin and defensin which affect bacterial growth (Bulet, 1999). Several insect species are vectors of medically important parasitic diseases. Studies in mosquitoes have demonstrated that the presence of filarial worms or malaria parasites can induce an immune response in *Aedes* spp. (Beemtsen et al., 1994) and in *Anopheles* spp. (Dimopoulos et al., 1998), respectively. We were inter-
ested in developing a *Drosophila* model of parasitic infection to benefit from the genetic tools of this organism. In particular, we wanted to compare the effects of parasitic infection to the well-studied antibacterial and antifungal responses. *Drosophila* has been reported to harbor kinetoplastida flagellates which include several species of the genus *Crithidia* (Wallace, 1966; Ismaeel, 1994). *Crithidia* parasites exclusively infect invertebrates and predominantly insects (Wallace, 1979). In general, flagellates develop in the digestive tract and interact with the intestinal epithelium by their flagellum. This interaction induces the formation of hemidesmosomes (Molyneux and Killick-Kendrick, 1987) and frequently leads to damage of the intestinal cells (Schaub, 1994). *Crithidia* spp. parasites vary widely in their degree of host specificity (Wallace, 1966). As the species infecting *Drosophila* have not been identified, we have decided to use in this study *C. bombi* and *C. fasciculata*, isolated from bumblebee and mosquitoes, respectively. Here we report that *per os* infection of *Crithidia* triggers a defense reaction in *Drosophila*. In particular we have observed, by several biochemical techniques, the induction of several known antimicrobial peptides as well as the induction of new *Drosophila* Immune induced Molecules (DIMs — Bulet and Uttenweiler-Joseph, 1999) in the hemolymph of gut-infected *Drosophila* adults. As *Crithidia* parasites do not cross the gut barrier, our results suggest that this tissue can signal to the fat body, the principal site of antimicrobial peptide synthesis. In contrast to oral infection which did not affect fly survival, systemic infection had different effects on the flies depending on the *Crithidia* strain used: whereas *C. fasciculata* rapidly killed the flies, *C. bombi* was harmless. The role of phagocytosis by hemocytes and antimicrobial peptides in the hemolymph was investigated to try to explain this difference.

2. Materials and methods

2.1. *Drosophila* strains

Oregon flies were maintained in the laboratory and used as a standard wild type strain of *Drosophila melanogaster*. The ILL 97 strain was collected from the field on fruit traps in the area of Strasbourg (France). The colony was amplified and maintained at 25°C on standard corn meal medium. ILL 97 flies were crossed with Oregon to demonstrate that they belong to the *melanogaster* species.

2.2. Parasite cultures

The two *Crithidia* species, *C. fasciculata* and *C. bombi* were received from the Swiss Tropical Institute (Basel, Switzerland). *C. bombi* was isolated by Dr Shykoff (Zoological Institute, Basel, Switzerland) from the rectal ampula of local bumblebees, *Bombus* spp. *C. fasciculata* was isolated from local mosquitoes, *Culex* spp. by Dr Stemmberger (Vienna, Austria). Parasites were maintained in Schneider’s medium supplemented with 10% Fetal Calf Serum (FCS) containing 60 mg/l penicillin and 100 mg/l streptomycin at 25°C. Dense parasite populations were subcultured twice weekly.

2.3. Parasite infections

For *per os* infection, small cotton balls were soaked with sterile parasite cultures. Adult flies from the two strains were fed on this medium for 24 h at room temperature. Control flies were maintained on sterile Schneider’s medium. Positive controls were provided by soaking a cotton ball with a mixture of bacteria, *Escherichia coli* (strain 1106) and *Micrococcus luteus* (strain A270). For systemic infection, parasites were washed with phosphate buffer saline (PBS) and pelleted by centrifugation at 3000 rpm for 10 min. Parasite suspensions (4.6 nl — around 5000 parasites) were injected into the thorax of adult flies using a nanoinjector (Nanoject, Drummond Scientific Co., USA). To quantify the physical effect of the injury, a PBS injection was performed under the same conditions as for control flies.

2.4. Northern blot analysis

Total RNAs from whole flies were extracted and Northern blot experiments performed according to the procedures of Lemaitre et al. (1996). The following cDNA probes were used: dipterericin, defensin, cercopin A1, drosomycin, drosocin and ribosomal protein 49 (rp49) as standard (Lemaitre et al., 1996). For each point, RNA was extracted from 20 flies. The intensity of the immune response was quantified by autoradiography for *per os* infection and with a Bio-Imaging Analyzer (BAS 2000, FUJIX, Japan) for parasitic injection.

2.5. Analysis of *Drosophila* hemolymph and extracts from digestive tract by RP-HPLC and MALDI-TOF MS

The procedure used was derived from Uttenweiler-Joseph et al. (1998). Briefly, hemolymph of 30 *Drosophila* adults infected *per os* with either (1) medium alone (negative control), (2) a bacteria mixture (*E. coli* and *M. luteus*) or (3) parasites, was collected with the help of a nanoinjector and directly transferred in 50 μl of acidified water (0.1% TFA) to reduce proteolysis. The sample was centrifuged for 10 min at 2000g to pellet hemocytes. Gut tissue of 30 *Drosophila* adults were dissected, washed in PBS and transferred to acidified water. To lyse cells, samples were ground, sonicated and centrifuged. The supernatants were subjected to RP-HPLC on an Aqua-
pore RP 300 C8 column (1×100 mm, Brownlee™). Hemolymph and gut extracts were separately analyzed by a linear gradient of 2–80% acetonitrile in 0.05% TFA over 80 min at a flow rate of 80 µl/min. The column effluent was monitored by absorbance at 214 nm and fractions were hand-collected. All HPLC purifications were performed with a Waters HPLC system equipped with a pump model 626, a controller model 600S and a detector model 486. For the subsequent analysis by MALDI-TOF-MS, fractions were selected according to the retention time of known antimicrobial peptides (Uttenweiler-Joseph et al., 1998) or according to major absorbance variations.

2.6. Electron microscopy

A parasite suspension of C. bombi or C. fasciculata (4.6 nl corresponding to approximately 5000 parasites) was injected into Drosophila larvae of stage 3. At different time points following the injection (1 and 3 h), larvae were pricked to collect hemolymph and the hemolymph was centrifuged to pellet hemocytes and parasites. The pellet was fixed in a 4% solution of glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Preparations were post-fixed in 1% osmic acid for 1 h, then dehydrated and embedded in epoxy resin. Ultrathin sections were contrasted with uranyl acetate–lead citrate and processed for transmission electron microscopy.

3. Results

3.1. Per os infection with flagellates induces expression of antimicrobial peptide genes in a field-strain of Drosophila but not in Oregon flies

Adult Oregon flies were orally infected with bacteria and parasites. Twenty four hours later, infection was confirmed by the presence of parasites, first detected in the diverticulum, then in the gut (data not shown). Infection persisted for approximately 4 days after which the parasites were eliminated with the feces. To determine whether a per os infection induced an immune response, we extracted RNA from whole flies 24 h post infection and probed Northern blots with cDNAs coding for Drosophila antimicrobial peptides. As shown in Fig. 1A, the bacteria which had been orally administered induced a strong expression of drosocin in flies, whereas the two Crithidia species did not induce a detectable expression of drosocin messenger RNA in the Oregon strain. Similar results were obtained with cecropin, diptericin, defensin and drosomycin probes (data not shown). As the Oregon flies had been maintained in laboratory culture conditions for several years, we suspected that they might have lost the capacity to react to the presence of parasites in the digestive tract. We therefore collected flies in the field, and established a new line, ILL 97, that we exposed to per os parasite infection. As shown in Fig. 1B, the per os administration of bacteria induced a strong expression of the genes encoding cecropin, drosocin, diptericin and drosomycin and to a lesser extent defensin. Significantly, these peptides were also induced by oral administration of Crithidia parasites in this field strain, although the level of induction was lower than with bacteria. Induction by parasite infection was strongest for drosocin and diptericin, whereas defensin was only marginally induced. We have subsequently used the ILL 97 flies to study the response of Drosophila to Crithidia spp. infection.

Fig. 1. Antimicrobial peptide gene expression in flies following experimental per os infection. Flies from the Oregon (A) or ILL 97 (B) strains were orally infected with a mixture of Gram-positive and Gram-negative bacteria (bact.) or with two Crithidia species, C. bombi (C.b.) or C. fasciculata (C.f.). Total RNA was prepared 24 h post-infection from control (c) or challenged adult flies, and analyzed by Northern blot for the induction of defensin (Def), cecropin A1 (CecA), diptericin (Dipt), drosocin (Drc) and drosomycin (Drom). The ribosomal protein 49 (rp49) was used as quantitative control. A representative experiment is shown.
3.2. Per os infection with flagellates triggers a systemic immune response

Surface epithelia have been shown to synthesize antimicrobial peptides both in mammals (reviewed in Ganz and Lehrer, 1998) and in various insect species, namely *Bombyx mori* (Brey et al., 1993), *Manduca sexta* (Russell and Dunn, 1996), *Stomoxys calcitrans* (Lehane et al., 1997), *Anopheles gambiae* (Dimopoulos et al., 1998) and *D. melanogaster* (Ferrandon et al., 1998; Ohresser and Imler, unpublished data). To analyze if *Crithidia* induces antimicrobial peptide synthesis in the digestive tract (local response) or in the fat body (systemic response), we performed a separate analysis by reverse phase HPLC (RP-HPLC) and mass spectrometry of acidic extracts of gut tissue and hemolymph from individual flies after per os infection with *Crithidia* spp. or with bacteria. As shown in Fig. 2, infection by both *C. fasciculata* and bacteria induced a systemic immune response illustrated by the appearance of drosocin and drosomycin (peaks 1 and 2, respectively) in the hemolymph of infected flies, 24 h after infection. The absence of defensin and cecropin in Fig. 2 may be explained by their early synthesis which reaches a maximum 7 h after infection (Bulet, unpublished data). We have made a similar analysis with the Oregon flies: drosomycin and drosocin were detected at considerably lower levels which confirmed the poor detection obtained for the Northern blot experiment (see above, Fig. 1A).

The per os infection with *Crithidia* spp. or bacteria also induced molecules distinct from the known antimicrobial peptides (Fig. 2A). Mass spectrometry analysis of peak B yielded several masses ranging from approximately 3 kDa to 7 kDa, one of which (mol. wt 3175 Da) was specific for *Crithidia* infection (Table 1). The corresponding molecules are still under investigation. Interestingly, peak B was observed only in per os infected flies, and was not detected in flies injected with bacteria. Peaks A and C of Fig. 2A were only induced by bacteria, both after per os administration and injection. These data demonstrate that not only the nature of infecting microorganisms but also the route of infection induce different molecules in the hemolymph.

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**Fig. 2.** Differential study of *Drosophila* (ILL 97 strain) hemolymph after systemic or per os infection. (A) Hemolymph from ILL 97 flies was collected 24 h post infection and analyzed by RP-HPLC. Flies were either injected with a mixture of Gram-positive and Gram-negative bacteria (inj. bact.), or infected per os with Schneider medium (control), a mixture of Gram-positive and Gram-negative bacteria (bact.) or *C. fasciculata* (C.f.). The numbers (1,2) indicate peaks containing induced antimicrobial peptides, and the letters (A,B,C) indicate peaks containing induced molecules. (B) The fractions corresponding to peaks 1 and 2 were subjected to MALDI-TOF mass spectrometry to demonstrate that they contain drosocin and drosomycin, respectively. The results of the MALDI-TOF mass spectrometry analysis of peaks A, B and C are presented in Table 1.
Table 1
Specificity of the immune response in *Drosophila* injected into the thorax with bacteria or infected *per os* with different pathogens (bacteria and *Crithidia* spp.).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Bacteria injection</th>
<th><em>Per os</em> bacteria infection</th>
<th><em>Per os</em> C. <em>bombi</em> infection</th>
<th><em>Per os</em> C. <em>fasciculata</em> infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DIM 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DIM 1</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>DIM 4</td>
<td>DIM 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>–</td>
<td>2899.0</td>
<td>2899.4</td>
<td>2899.0</td>
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<tr>
<td></td>
<td></td>
<td>3176.6</td>
<td>3175.2</td>
<td></td>
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<td></td>
<td></td>
<td>4347.3</td>
<td>4347.8</td>
<td>4347.3</td>
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<td></td>
<td></td>
<td>7193.0</td>
<td>7193.5</td>
<td>7193.0</td>
</tr>
<tr>
<td>C</td>
<td>DIM 13</td>
<td>DIM 13</td>
<td>–</td>
<td>–</td>
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</table>

<sup>a</sup> The RP-HPLC fractions corresponding to induced peaks A, B and C were analyzed by MALDI-TOF-MS. DIMs 1 (1666 Da), 4 (1722 Da), 13 (2651 Da) were previously described (Uttenweiler-Joseph et al., 1998). Measured molecular masses (MH<sup>+</sup>) are indicated for unknown induced molecules.

<sup>b</sup> DIM — *Drosophila* Immune-induced Molecules.

...of *Drosophila*. Using a similar methodology, we have analyzed gut tissue from bacteria- and *Crithidia*-infected field strain *Drosophila*. We were unable to detect the presence of antimicrobial peptides in this type of extract 24 h after a *per os* infection. These data were confirmed by reverse transcription-PCR (RT-PCR) analysis which showed expression of drosocin and diptericin in dissected fat bodies from *per os* infected flies, but not in dissected guts (data not shown).

### 3.3. Injection of C. *fasciculata* kills flies

Some flagellate parasites such as certain *Trypanosoma* species naturally undergo part of their lifecycle in the insect hemolymph (Kaaya et al., 1986; Molyneux and Killick-Kendrick, 1987). Others invade accidentally hemolymph like *Herpetomonas* spp. or *Blastocrithidia* spp. (Schaub, 1994). This has prompted us to analyze the response of flies to injection of parasites. First, we recorded the survival of field strain flies to intrathoracic injection of *C. fasciculata* and *C. bombi* over 6 days (Fig. 3). In contrast to *per os* administration, which did not induce any lethality, injection of *C. fasciculata* killed all flies within 4 days post-injection. Injection of *C. bombi* was not harmful and up to 80% of injected flies had survived 6 days after injection of the parasite or PBS. Interestingly, dissection of *C. bombi*-infected flies showed few viable (e.g. motile) parasites 4–6 days after injection while *C. fasciculata*-infected flies contained large numbers of highly motile parasites.

### 3.4. Injection of *Crithidia* parasites induces a weak systemic antimicrobial response

We next examined whether the antimicrobial peptides identified in the *Drosophila* host defense against bacteria or fungi could participate in the humoral immune response after flagellate injection. Indeed, several authors in different parasite–insect systems have reported that antimicrobial peptides, when induced by a septic injury (Lowenberger et al., 1996) or when injected into the thorax (Shahabuddin et al., 1998), can control the establishment of parasite infection present in the hemolymph. It might be speculated that the difference in susceptibility of *Drosophila* to infection with either *C. fasciculata* or *C. bombi* results from a difference in the level of induction of antimicrobial peptides. To investigate this, we analyzed the expression of the corresponding genes in flies 1 and 2 days after injection of both parasites, and compared this response with that...
induced by injection with bacteria (Lemaitre et al., 1996). As shown in Fig. 4, both parasites induced a similar low level of expression of genes encoding diptericin, drosomycin and drosocin genes 24 h post injection. The levels were markedly lower than those induced by injections of bacteria, and had returned to basal level or lower 48 h post infection.

3.5. Phagocytosis of Crithidia parasites by hemocytes

In addition to antimicrobial peptides, circulating and sessile hemocytes contribute to the encapsulation of microorganisms during insect immune response (reviewed in Lackie, 1988). We therefore investigated whether C. bombi parasites, which disappear rapidly after injection into the thorax, were better internalized by phagocytic cells than C. fasciculata parasites. Larvae were used in this experiment since the hemocyte number is significantly higher in larvae than in adults. As shown in Fig. 5A, 1 h after injection, several C. bombi parasites were internalized by the blood cells, unambiguously identified by the presence of the mitochondrial DNA or kinetoplast, a structure characteristic of this group of flagellate parasites, and by the presence of the flagellum.

The efficient recognition and phagocytosis of C. bombi suggested that it might account for the resistance of Drosophila to this strain of parasite. We performed a similar experiment with the lethal C. fasciculata species. As shown in Fig. 5B, phagocytosis of C. fasciculata by blood cells was also observed in infected larvae. Therefore, the difference in pathogenicity between C. fasciculata and C. bombi cannot be explained by differential recognition and phagocytosis by blood cells.

4. Discussion

In this paper, we describe for the first time a model based on the flagellate protozoan Crithidia to study host defense against parasite infection in D. melanogaster. Using antimicrobial peptides as markers to monitor immune response, we present evidence that Crithidia is recognized by Drosophila upon either systemic infection or local digestive tract infection. Induction is stronger for drosocin (at least ten fold) and it will be interesting to test if this antimicrobial peptide also has antiparasite
activity, as has been reported for other insect antimicrobial peptides (Lowenberger et al., 1996; Shahabuddin et al., 1998). In addition, we have shown that depending on the route of infection (e.g., intrathoracic injection vs per os) and the nature of the infecting microorganism (e.g., bacteria or *Crithidia*), different molecules were induced in the hemolymph of infected flies. The differential induction of these peptides by bacterial or *Crithidia* infection strongly argues that these molecules are involved in insect immunity and are not simply stress-related factors.

Because several parasites, including flagellates, carry out part of their development in the insect hemolymph, we have analyzed the immune response of *Drosophila* after a systemic infection with *Crithidia*. Surprisingly, one of the *Crithidia* species assayed, *C. fasciculata*, was found to kill *Drosophila* flies within 5 days after infection in the hemolymph. Similarly, *C. fasciculata* was lethal when injected into the hemolymph of *Glossina* spp., the vector of trypanosomiasis (Ibrahim and Molyneux, 1987). By contrast, *C. bombi* did not seem to affect *Drosophila*, thus suggesting a differential recognition of the two parasite species. The reason for this difference is still not clear at present. The fact that we could not observe any significant differences between *C. fasciculata* and *C. bombi* for the induction of antimicrobial peptides strongly argues that these molecules are not involved in the control of *Crithidia* infection in the hemolymph. Humoral immunity also relies on proteolytic cascades leading to melanization (Hoffmann and Reichhart, 1997). In this study, melanization of injected parasites was not observed. Lectins are also involved in insect immunity and they have been shown to play a major role against flagellates (Pereira et al., 1981; Welnburn et al., 1994; Pimenta et al., 1992; Mello et al., 1999). As with Leishmania and Trypanosoma parasites, the membrane of *Crithidia* spp. is covered uniformly with carbohydrates (Schneider et al., 1996). Hemolymph lectins could interact with parasite carbohydrates and play a role as opsonins to facilitate phagocytosis. Phagocytosis was observed in our experiments with both *Crithidia* species, confirming previous results obtained upon injection of *C. fasciculata* into *Drosophila virilis* (Schmittner and McGhee, 1970). However, we did not observe significant differences in the phagocytosis of *C. fasciculata* and *C. bombi* which could explain the difference in the outcome of systemic infection by the two species. One possibility is that hemolymph lectins may interact differently with the two parasites due to surface carbohydrate variability. Indeed, lectins can also act as cytotoxic agents as shown in *Rhodnius prolitus* infected with *Trypanosoma cruzi* (Mello et al., 1996).

Using RP-HPLC, we have shown that upon per os infection antimicrobial peptides were induced in the hemolymph and not locally in the digestive tract. This is somehow surprising since it is well known that in vertebrates, epithelia participate in the host defense by secreting locally active antimicrobial peptides (Ganz and Lehrer, 1998). Similarly, in invertebrates, a local constitutive expression of defensin has been demonstrated in the digestive tract of *Stomoxys* spp., and expression of this antibacterial peptide increased following a lipopolysaccharide-containing blood meal (Lehane et al., 1997).

In *Drosophila*, the study of transgenic flies expressing the Green Fluorescent Protein under the control of several antimicrobial peptide promoters revealed local immune responses in several surface epithelia including the digestive tract (Ferrandon et al., 1998; Ohresser and Imler, unpublished data). Similarly, defensin expression is locally induced in the anterior midgut of *Anopheles gambiae* after infection with *Plasmodium berghei* (Richman et al., 1997) although *Plasmodium gallinaceum* infection in *Aedes aegypti* did not induce defensin in the digestive tract of the mosquito (Lowenberger et al., 1999). The fact that we did not observe induction of known antimicrobial peptides in the digestive tract in our RP-HPLC and RT-PCR experiments does not exclude that other, yet-to-be-discovered molecules are induced in the gut following *Crithidia* infection. This raises the question of the mechanism by which digestive tract infection triggers antimicrobial peptide expression by the fat body. One possibility is that heavy infection occasionally results in tissue damage or migration of parasites into the hemolymph (Schaub, 1994). This hypothesis was investigated by collecting hemolymph from flies infected per os with *Crithidia* spp. After microscopic observation, no *Crithidia* spp. was detected in the hemolymph 24 h after injection (data not shown). Another possible mechanism could involve cytokine-like molecules. In this model, interaction of flagellate parasites with the digestive tract epithelium, possibly through the formation of hemidesmosomes (Ismaeel, 1994), would induce the synthesis of such cytokine-like molecules, that would then activate cell surface receptors on fat body cells and induce antimicrobial peptide synthesis. Interestingly, we report here the identification of three molecules of molecular weight 2898 Da, 4346 Da and 7192 Da which are induced in the hemolymph after per os infection with bacteria or *Crithidia*, but not after injection of bacteria. These molecules represent good candidates for such signaling messengers between the gut and the fat body and their biochemical characterization is under progress.

In conclusion, this paper describes the immune response of *Drosophila* to *Crithidia* infection and provides a new model to better understand the molecular basis of host defense against flagellate parasites in insects using the powerful genetics of *Drosophila*. Our results support previous work showing that insects can discriminate between pathogens during an infection (Lemaître et al., 1997). We are in parallel extending this study by working with two other flagellates, *Trypano-
soma spp. and Leishmania spp., which are transmitted to humans and animals by Glossina spp. and Phlebotomus spp., respectively. Altogether, these experimental models should allow us to identify and characterize novel molecules which may be involved in the control of parasitic infections in Diptera. In this regard, the identification of a molecule of 3175 Da which is exclusively induced in the hemolymph of Drosophila after a per os infection with Crithidia is particularly encouraging.

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References


