Short communication

Microsporidia–insect host interactions: Teratoid sporogony at the sites of host tissue melanization

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Received 14 May 2006; accepted 16 August 2006
Available online 26 September 2006

Abstract

Microsporidia Paranosema locustae and Paranosema grylli infect fat bodies of orthopteran hosts Locusta migratoria and Gryllus bimaculatus, respectively, and cause formation of nodules consisting of deposits of melanin around heavily infected cells. Both species sporadically produce enlarged or malformed (teratoid) spores as a result of abnormal sporogony. Proportions of teratospores within melanized nodules were 6–10 times higher than in surrounding non-melanized tissues. The increased numbers of teratoid microsporidian spores within melanized regions may indicate the deteriorating effect of melanin metabolites on spore morphogenesis.

Keywords: Microsporidia; Melanization; Teratospores; Paranosema grylli; P. locustae; Gryllus bimaculatus; Locusta migratoria

1. Introduction

Sporadic production of giant or malformed spores is not uncommon among microsporidia. This phenomenon was observed in microsporidia infecting lepidopteran (Solter et al., 1997), hymenopteran (Knell et al., 1977) and orthopteran insects (Henry and Oma, 1981). Atypical spore formation also occurs in non-specific hosts (Solter et al., 1997). Incomplete divisions of Enterocytozoon bieneusi sporonts that lead to formation of teratoid spores, happened either spontaneously (Schwartz et al., 1998) or under the influence of antimicrosporidial drugs (Ditrich et al., 1994). Henry and Oma (1981) mentioned giant and triangular forms among Paranosema (Nosema) locustae spores in grasshoppers of the genus Melanoplus. Here we demonstrate that the number of atypical teratoid spores significantly increased in the melanized sites of the infected tissue, and presume, that teratoid sporogony is mediated by melanization.

2. Materials and methods

Gryllus bimaculatus Deg. and Locusta migratoria migratoria R.&F. were reared in the laboratory and were experimentally infected with Paranosema grylli and P. locustae, respectively, as described elsewhere (Dolgikh et al., 1997; Sokolova and Lange, 2002). The laboratory population of G. bimaculatus maintained in our lab originates from a mixed culture of crickets collected in southeastern Russia and Cyprus and maintained at the insectory of Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences (IEPB RAS), St. Petersburg, for more than 20 years. The laboratory culture of L. m. migratoria was presented by two isolates, referred to as a “laboratory” and a “wild” ones. The “laboratory” isolate is derived from the long-term laboratory culture maintained at the Moscow Zoo for more than 15 years. The “wild” isolate was the first generation of locusts hatched from eggs, collected in 2002 at the Astrakhan’ National Biosphere Reserve, Astrakhan’ Region, Russian Federation (southern European Russia). P. grylli is routinely cultivated in our lab colony of crickets for 10 years since it was first found in the cricket culture at

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IEPB RAS (Sokolova et al., 2003). P. locustae is maintained in our lab colony of locusts since 1997 when its spores were kindly provided by J.E. Henry and D.A. Streett from the United States Department of Agriculture-Agricultural Research Service, Rangeland Insect Laboratory, Bozeman, Montana (Sokolova and Lange, 2002).

Paranosema grylli and P. locustae infect fat bodies of their respective hosts and induce formation of “nodules”, the deposits of melanin around heavily infected cells (Tokarev and Sokolova, 2005), easily visible as brownish spots against the infected milky-white adipose tissue (Fig. 1a). Two hundred eight crickets and 100 locusts at the final (advanced) phase of the disease, and 100 uninfected insects of both species were dissected and examined for the presence of infection and the melanized nodules. Smears from nodules and non-melanized regions of infected tissues were observed under bright field and phase contrast optics of the microscope Axioscope-2 (Zeiss) with the attached digital camera. Length and width of spores were measured with the help of Carl Zeiss AxiosVision software version 3.1. For fluorescent microscopy, the smears were fixed with methanol and stained for 5 min either with Calcoflour White 2R (10 µM, in Tris-buffered saline, pH 7.0) or DAPI (10 µM in phosphate-buffered saline, pH 7.0) (both from Sigma, St. Louis, MO). The slides were examined with a Zeiss Axioscope equipped with epifluorescence and a digital camera.

The total number of spores, and the number of giant/malformed spores were counted on smears prepared from three sources: nodules (Var. 1), non-melanized sites of infection from the same insects (Var. 2), or from infected insects that did not exhibit signs of melanization (Var. 3). Average percentage of atypical spores was calculated in 10–20 view fields of the microscope at 400× per one infected insect considered as one replicate. Eight to 10 crickets, and 2–6 locusts, were examined for each variant. The obtained numbers were compared by t-test for independent variables (Statistica 7.0, StatSoft Inc.).

3. Results and discussion

Crickets infected with 10⁶ spores at 3rd instar reached the advanced phase of microsporidiosis in 60–90 days post infection (dpi), approximately 20–50 days after their last ecdysis. This phase of pathogenesis was characterized by intensive spore formation and the appearance of melanized nodules in fat bodies of 87 ± 2.33% of the examined crickets (N = 208) (Fig. 1b). The earliest appearance of granules of melanin was always associated with the masses of microsporidian spores, while the tissues invaded with prespore stages remained unmelanized. Uninfected insects never displayed signs of melanization.

Locusts infected with the 10⁶ P. locustae spores at 3rd instar reached the advanced phase of pathogenesis in 14–20 dpi. Interestingly, the rates of fat body melanization due to microsporidiosis in locusts depended on the source of the locust isolate: 30.0 ± 10.25% (N = 20) of the infected “wild” locusts contained numerous nodules associated with the sites of spore formation, whereas only 2.5 ± 1.75% (N = 80) of “laboratory” locusts contained sporadic melanin granules.

Normal P. grylli spores measured 4.66 ± 0.063 ± 2.69 ± 0.029 (μm ± SE, n = 50), P. locustae spores – 5.12 ± 0.049 µm × 3.02 ± 0.040 μm (n = 50). Spore suspensions of both microsporidia prepared from infected tissues contained also a small proportion of teratoid spores, which occurred to be either enlarged, trianguate, irregularly curved, or extremely

Fig. 2. Teratospores of Paranosema grylli and P. locustae, stained with fluorochromes. (a) DAPI-stained spores of P. grylli. Small arrow indicate unstained layer of endospore. (b) DAPI-stained spores of P. locustae. (c) Calcoflour-stained spores of P. grylli. (d) Calcoflour-stained spores of P. locustae. N, normal spores. Arrow indicates teratospores. Scale bar: 5 μm.

Fig. 1. Melanization in insects as a response to microsporidian spore formation. (a) Dissected adult female of a cricket infected with Paranosema grylli. FB, fat body; OV, ovaries; arrows indicate melanin granules. (b) Melanization rates (% ± m% of insects with melanin granules at the advanced phase of disease) in crickets (N = 208) and locusts (N = 80) from laboratory colonies and locusts from a natural population (N = 20). Different letters indicate values different significantly at p < 0.01.
broad (Fig. 2). The most typical teratoid spores were just much bigger than the normal and measured 7.91 ± 0.226μm × 3.50 ± 0.189μm (n = 16) and 8.61 ± 0.217μm × 3.39 ± 0.083μm (n = 20) in P. grylli and P. locustae, respectively. DAPI staining demonstrated 3–5 nuclei or 2 diplokarya in each spore (Fig. 2a and b). DAPI extensively stained nuclei and faintly stained spore cytoplasm and exospore. In some cases, this differential staining revealed an unstained endospore layer between the stained cytoplasm and exospore (Fig. 1a). Calcofluor staining suggested the presence of chitin in the spore walls (Fig. 2c and d). Taken together, these observations indicate that the atypical spores have resulted from abnormal mitosis or cytokinesis, and that the spore wall had matured before the sporoblast divided.

The average percentage of atypical spores on smears from the tested P. grylli-infected crickets in Var. 1 (smears from nodules) reached 4.1% ± 0.29% (N, number of insects = 10, n, number of spores = 4724); in Var. 2 (non-melanized tissues)—0.9% ± 0.21% (N = 10; n = 2290); in Var. 3 (melanin-free insects)—0.74% ± 0.18% (N = 8, n = 1633) (Table 1).

In P. locustae-infected locusts we observed the following proportions of teratoid spores: in Var. 1 it was 5.89% ± 0.737% (N = 2, n = 1019) in “laboratory” locusts and 6.70% ± 0.629% (N = 4, n = 1581) in “wild” locusts; in Var. 2—0.60% ± 0.27% (N = 2, n = 827) and 1.05% ± 0.524% (N = 4, n = 380); and in Var. 3—0.47% ± 0.164% (N = 5, n = 1719) and 0.32% ± 0.225% (N = 6, n = 727). In all three cases the percentage of teratoid spores produced inside melanized nodules was significantly higher (Table 1).

It is well known that melanin synthesis pathway comprises a major part of invertebrate innate immunity. It is mediated mainly by cellular and plasma components of the haemolymph and is directed to the elimination of material recognized as non-self (Marmaras et al., 1996; Lavine and Strand, 2002; Kanost et al., 2004; Christensen et al., 2005). Free radicals, quinones and other intermediates and derivatives of melanin biosynthetic pathway produce toxic effects on various insect pathogens—viruses, bacteria, fungi, protists and helminthes, and thus improve defensive function of phagocytosis and encapsulation reactions which trigger prophenoloxidase activation upon non-self recognition process (Götz and Boman, 1985; Gillespie et al., 1997). The role and consistency of melanization in pathogenesis of microsporidian infection is unclear. Previously it has been shown that microsporidia-infected insects exhibited decreased levels of melanization level and phenoloxidase activity of haemolymph (Sokolova et al., 2000; Vorontsova et al., 2004; Tokarev and Sokolova, 2005). This study shows that nodule formation regularly takes place at the final phases of microsporidiosis in crickets and occasionally in locusts. Elevated rates of melanization detected in “wild” locusts may reflect their upraised immune status contrasting to poorer immunity of laboratory insect cultures, which in fact are easily susceptible to various infections (Vavra and Maddox, 1976). The increased numbers of teratoid microsporian spores in nodules may indicate the deteriorating effect of melanin metabolites on spore morphogenesis, that can be regarded as a defensive mechanism against microsporidian infection (though there are no data proving efficacy of this defense reaction). If so, the ability of some microsporidia to suppress phenoloxidase activity may be considered as an evolutionary adaptation of microsporia to parasitism in insects. Testing insect tyrosine metabolites for antimicrosporidal activity might be promising from the point of potential expanding the spectrum of antimicrosporidial drugs.

Acknowledgments

We are thankful to Leelen Solter (Illinois Natural History Survey, USDA) for advice, to Michael Berezin (Moscow Zoo) for his gift of locust eggs, to Georgii Lednev and Maxim Levchenko (All-Russian Institute for Plant Protection, St. Petersburg) for their help in collecting locusts in nature and to Margaret C. Henk for checking English.

The research was supported by a personal DAAD grant to Y. T. (Ref 325 PKZ A/03/01385) and by Russian Foundation for Basic Research (Grants #03-04-49629, #04-04-49314, and #06-04-90814).

References


Table 1

Percentage of teratospores among spore samples of Paranosema sp. from melanized and non-melanized tissues

<table>
<thead>
<tr>
<th>Microsporidia and its host species</th>
<th>% of atypical spores among microsporidian spores, %± SE%</th>
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<tr>
<td></td>
<td>Melanized tissues Var. 1</td>
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<tr>
<td>Paranosema grylli–Gryllus bimaculatus (lab colony)</td>
<td>4.13 ± 0.290 (a)^a</td>
</tr>
<tr>
<td>P. locustae–Locusta migratoria (lab colony)</td>
<td>5.89 ± 0.737 (a)^b</td>
</tr>
<tr>
<td>P. locustae–Locusta migratoria (wild population)</td>
<td>6.70 ± 0.629 (a)^b</td>
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Same letters in one row indicate values not different significantly.

^a Var. 1 significantly differs from Var. 2 at p < 0.05 and from Var. 3 at p < 0.01.

^b Var. 1 significantly differs from Var. 2 and Var. 3 at p < 0.01.