Crithidia deanei infection in normal and dexamethasone–immunosuppressed Balb/c mice

Dilvani Oliveira Santos1*, Saulo C. Bourguignon1, Helena Carla Castro1, Alice Miranda2,3, Rodrigo Tonioni Vieira1, Suzana Corte-Real4, Otilio Machado Pereira Bastos5

1Department of Cellular and Molecular Biology, Federal Fluminense University (UFF), Rio de Janeiro, Brazil; *Corresponding Author: santosdilvani@gmail.com; lacelauff@yahoo.com.br
2Department of Mycobacteriosis, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil
3Department of Pathology, FCM, University of State of Rio de Janeiro, Rio de Janeiro, Brazil
4Laboratory of Biology Structural, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil
5Department of Microbiology and Parasitology, Federal Fluminense University (UFF), Rio de Janeiro, Brazil

Received 18 October 2009; revised 21 December 2009; accepted 24 December 2009.

ABSTRACT

Monoxenous trypanosomatids protozoa are not believed to cause in vivo infection in vertebrate hosts throughout their life cycle. However, there are reports mentioning some cases of HIV-positive patients who have presented opportunistic infections caused by these protozoa. Recently, we have demonstrated the in vitro infection of mouse dermal fibroblasts by these protozoa. The aim of the present work is to investigate the possibility of Crithidia deanei, an endosymbiont-bearing monoxenous trypanosomatid, infect BALB/c mice under or not Dexamethasone treatment. To attend it, distinct groups of adult BALB/c mice were immunosuppressed with 50 mg/kg of Dexamethasone. This immunosuppressor was administered 24 hours before infection and daily, for 15 days after C. deanei inoculation. Control groups: C. deanei–inoculated animals but non-immunosuppressed and non-inoculated animals but immunosuppressed were also used. Light Microscopy analysis revealed an infection process characterized by the presence of the trypanosomatid inside dermal cells in the groups studied. The experimental inoculation resulted in a non-lethal infection characterized by the presence of the trypanosomatid inside dermal cells in the normal BALB/c mice, but notably, in the C. deanei–inoculated immunosuppressed group. These preliminary results lead to the following conclusions: 1) C. deanei is able to infect normal BALB/c mice; 2) the immunosuppressed mice seemed to be more susceptible to the C. deanei infection compared to the control group.

Besides C. deanei in dexamethasone-immunosuppressed mice provides a useful model for studies of monoxenous trypanosomatids ‘in vivo’ infection, resembling that one presumably occurring in immunodeficient individuals with AIDS.

Keywords: Monoxenous Trypanosomatid; ‘In Vivo’ Infection; Immunosuppression

1. INTRODUCTION

Trypanosomatids parasitize a diverse range of hosts including animals, plants and protists [1]. Some of them, such as Trypanosoma and Leishmania, are heteroxenous and are ethiological agents of serious diseases in humans and experimental animals. Others are monoxenous and are mostly found in insects [2]. Monoxenous trypanosomatids had never been confirmed as pathogenic in vertebrate host. However, there is one report of trypanosomatid, other than Trypanosoma and Leishmania, in some opportunistic cutaneous infections in immunocompromised individuals [3] or those without any previous history of immunodepression [4]. In addition, our group was pioneer in proving the infection of mouse dermal fibroblasts by two different monoxenous trypanosomatid species—Crithidia deanei and Herpetomonas roitmani [5]. Although some of these trypanosomatids were classified as a divergent member of the Leishmania genus [6], a visceral leishmaniasis—like infection was described in an HIV-positive patient as caused by Leptomonas pulexsimulantis, a monoxenous trypanosomatid found in dog’s flea [3], suggesting that monoxenous protozoa can be considered opportunistic agents in immunocompromised individuals. Therefore,
we investigated the ability of C. deanei to infect vertebrate host. For that purpose, we have used BALB/c mice under or not Dexamethasone treatment as an experimental model, based on a previous report of mouse dermal fibroblasts infection by C. deanei and H. roitmani [5].

2. MATERIALS AND METHODS

Parasite culture. Crithidia deanei was kindly provided by Dr. M. Auxiliarida de Souza (Trypanosomatids Collection of the Oswaldo Cruz Institute, Rio de Janeiro, Brasil). The monoxenous were kept at 28°C with serial passages at 48 h intervals in Warrens’ medium [7] containing 10% fetal calf serum.

Experimental animal infection. Female 8-week old BALB/c mice (Nau, Instituto de Biologia /UFF) were used. Animals housed in standard conditions were treated with Dexamethasone (Aziume) [8] 24 hours before infection with C. deanei. After infection with 10^5 2-day-old promastigotes C. deanei by subcutaneous route (hind foot pad) —day 0, dexamethasone 50 mg/kg was administered daily, for 15 days. Four BALB/c mice group were used: control without dexamethasone; control with dexamethasone; C. deanei—incubated with dexamethasone and C. deanei—inoculated without dexamethasone (Table 1). A determined number of mice from each group were euthanasiated at 6 h, 1 d, 2 d, 3 d, d 7 and d 15 after C. deanei inoculation. At each control point, mice were weighted and parasite burdens were determined in foot pad by histological analysis.

Histological analysis. Specimens of foot pad were fixed in 10% buffered formalin. After dehydration in graded ethanol, the tissues were embedded in paraffin and, then, processed routinely as previously reported [9]. 5 µm thick sections were obtained with a Leica microscope. After that, they were collected on glass slides for Hematoxilin-Eosin (HE) staining. The tissues samples infected or not were observed at least 400 randomly selected cells at 1000 × magnification, using a Zeiss photomicroscope.

3. RESULTS

Clinical finding’s. No mortality, weight loss or clinical signs were observed in mice infected with either dexamethasone or not.

Macroscopy findings. Both groups C.deanei—inoculated immunosuppressed mice and not inoculated immunosuppressed mice displayed splenomegaly and hepatomegaly.

Histological analysis. Through light microscopy the morphological analysis just of the foot pad was done. At necropsy, parasites were found in the foot pad from the mice inoculated with C. deanei, regardless immunosuppressed or not. In the Dexamethasone treated-controls groups (in the absence of C. deanei inoculation), no histological and inflammatory reactions of the foot pad were observed until d15 (Figure 2(c1)).

Surprisingly, in both experimental design-in the presence or not of dexamethasone, C. deanei was infective to BALB/c mice (Figure 1 and 2), but, notably, in the immunosuppressed BALB/c mice (Figure 2).

Using light microscopy, it observed C. deanei—infected mouse dermal cells after 24 h infection (Figures 1(a1) and (a2)). On the 2nd post infection day, C. deanei was also observed within mice dermal cells (Figures 1(b1) and (b2)). and, between whiles, extracellular parasites were seen (Figure 1(b1)). A large numbers of parasites were clearly present in the dermal cells after the third post-infection day (Figures 1(c1) and (c2)). Although it was possible to observe C. deanei within the dermal cells, their mechanism of entrance is still not clear as it can involve phagocytosis, penetration in the cell or inducing membrane invagination. Anyway, one mechanism of the C. deanei—infestation might be through saccipodic formation from the host cells as the image of the Figures 1(c1) and (c2) suggest. After 7 days of infection it still can observe parasites present in dermal cells C. deanei—infected (Figures 1(d1) and (d2)). At this time, some extracellular parasites were still seen (Figures 1(d1) and (d2)).

After 15 days of infection, the light microscopy still revealed intracellular forms of C. deanei as well as some extracellular forms of this parasite attached to the dermal cells surface (Figures 1(e1) and (e2)).

In the controls groups (in the absence of C. deanei inoculation and presence of dexamethasone) no histological and inflammatory reactions of the foot pad were observed until day 15 (Figure 2(c1)).

Interestingly, the kinetics of infection in foot pad from C. deanei—inoculated Dexamethasone immunosuppressed mice showed parasites as early as 6h in the subcutaneous tissues (Figures 2(a1) and (a2)). Notably, the most exuberant C. deanei—infestation was observed in the presence of Dexamethasone on the first day of infection (Figures 2(b1) and (b2)). In the meanwhile, it can clearly observe a C. deanei within a vacuole (Figure 2(b2)). On the following day, it can still observe a large numbers of C. deanei inside the cells (Figures 2(c1) and (c2)). In this time of infection, similar to the findings on the previous day, it can see that each parasite occupies its own vacuole (Figures 2(c1) and (c2)). The image of C. deanei inside
### Table 1. Distribution of the experimental groups according to the animals number e the respective data of necropsy.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Animals number / time of necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
</tr>
<tr>
<td>I (C. deanei–inoculated DMT treated mice)</td>
<td>2</td>
</tr>
<tr>
<td>II (C. deanei–inoculated mice)</td>
<td>2</td>
</tr>
<tr>
<td>III (DMT treated mice)</td>
<td>1</td>
</tr>
<tr>
<td>IV (not DMT treated and not C. deanei inoculated mice–Control Groups)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
</tr>
</tbody>
</table>

- DMT-Dexamethasone 50 mg/kg.
- In a total 36 animals were used.
- The animals were euthanazed according to the rules of ethical comitê (Comissã o de ética no uso de animais (CEUA- FIOCRUZ) P8317 ação: 1201 no. P024705).

**Figure 1.** Analysis by light microscopy showing *C. deanei* interaction with Balb/c mouse. Pictures were taken in a two different plans from the same field (e.g. a1 and a2, etc…) in order to show whole extension of cell infection. Representative sections from skin samples of 24h (a1 and a2); 48h (b1 and b2); 72h (c1 and c2); 7 days (d1 and d2) and 15 days (e1 and e2) *C. deanei* post-infection (original magnification x 100). Grey Arrow shows some free *C. deanei* extracellular forms (b1; d1 and d2) as well as *C. deanei* extracellular forms attached to the dermal cells surface (e1 and e2). Dark arrow show multiple *C. deanei*-infected dermal cells. Note the presence of sincicicious formation in c1 and c2. N = Dermal cells nucleous.
vacuoles continues to be seen in the third day of infection (Figures 2(d1) and (d2)). Here some tissue degradation was also observed (Figures 2(d1) and (d2)). Interestingly, at the last days of infection time (7 and 15 days) in immunosuppressed BALB/c mice, no parasites were found contrasting to some tissue alterations which were observed (data not shown).

4. DISCUSSION

Several clinical cases suggesting that monoxenous trypanosomatids could be implicated in human infections have been described in the last years. They have been emerging as possible opportunistic pathogens in immunocompromised individuals. An unusual Leishmania-like parasite was found in a HIV-positive patient with symptoms of Leishmania infection [10]. Despite the previously-mentioned data, genotypic and phenotypic characterization showed that a flagellate parasite, found in the bone marrow of a Brazilian HIV-positive patient presenting a visceral leishmaniasis-like reaction, was indeed a monoxenous trypanosomatid, although no tissue invasion could be detected [3]. Surprisingly, a new case of cutaneous infection by a presumed monoxenous trypanosomatid was reported in the island of Martinique; however, the individual had no history of immunosuppression, particularly HIV infection [4].

As stated earlier, Santos et al. (2004) first reported that endosymbiont-bearing trypanosomatid C. deanei and Herpetomonas roitmani are able to infect mouse dermal-derived fibroblasts while Crithidia fasciculate and Herpetomonas samuelpessoai (trypanosomatid...
endosymbiont free) did not infect. It is also of interest to observe that both C. deanei and H. roitmani can be resistant to lysis mediated by the complement system. In contrast, H. samuellpessoa and C. fasciculata displayed 100% of lysis after incubation with the complement system [5]. The symbionts of C. deanei can influence the phagocytosis of these parasites by macrophages as have been presented by [11]. And, most recently, [12], reported the infection of HIV-1-infected primary human macrophages by Blastocritidia culicis (another endosymbiont-bearing monoxenous trypanosomatid). Our present data further emphasize the large capacity of C. deanei to infect vertebrate host and reinforce the idea that monoxenous trypanosomatids present low host specificity [2,13,14]. As demonstrated by our work, C. deanei can readily infect normal BALB/c mice by subcutaneous route and infection persist in the dermal cells for 15 days. These are very interestingly results, since we have previously reported the “in vitro” C. deanei–infection of dermal cells obtained from a different species of mouse-the Swiss mouse [5]. Besides, as observed in our present work, extracellular forms of C. deanei are displayed in dermal tissue of the BALB/c mice (Figures 1(b1), (d1) and (d2)). This fact is interesting to be mentioned since it might suggest that, after intracellular C. deanei cycle, these parasites leave the host cell and, after that, appear in the extracellular medium (in a flagellate form) to re-infect others dermal cells. Taken together, these evidences reinforce the idea that monoxenous trypanosomatids are able to infect and to survive once reaching the vertebrate host. Over and again, we demonstrated the infection of BALB/c mice, but, a much more pronounced C. deanei–infection in a different experimental design: in Dexamethasone-immunodepressed mice (Figure 2). Through its lipophenic activity, specially about T cell production [15], the dexamethasone can reduce the mechanisms of anti-parasite effect of immune system and it might explain the increase of susceptibility to C. deanei infection observed in all immunosuppressed animals. The important survival of the parasite in the murine experimental host contrast strikingly with the weak clinical-pathological effects observed with absence of lymphocytic infiltrates in parasitized footpad. This can be paralleled to that observed during human visceral leishmaniasis where patent infections with parasite dissemination are frequently associated with T cell unresponsiveness to Leishmania antigen [16], while cure is accompanied with restoration of the cellular response [17,18]. Although monoxenous trypanosomatids in humans are more correlated to opportunist parasites, our work is pioneer in demonstrating that C. deanei is able to infect normal mice (withouth dexamethasone treatment). Our findings corroborate to the reports of [4], who also found monoxenous tripanosomatids in a non-immunocompromised individual though in a localized skin lesion. Besides, our previous report demonstrated the monoxenous trypanosomatid infection by dermal cells isolated from skin of normal Swiss mice [5]. Nevertheless, our data shows that the infection of C. deanei by dexamethasone-treated mice, although earlier prominent at the beginning of the time of infection (Figures 2(a),(b)), could not be followed longer, since the dermal cells seemed to be degenerated (data not shown). These results suggest that C. deanei might induce dermal cells degeneration. Most recently, [19] reported that C. deanei was able to induce fibroblasts lysis.

Besides the interaction of monoxenous trypanosomatids with vertebrate cells, the literature have also mentioned some results obtained from the interaction of these trypanosomatids with invertebrate cells. Then, [20,21], reported the colonization of Aedes aegypti midgut by the endosymbiont-bearing trypanosomatid Blastocritidia culicis and C. deanei respectively.

Considering the colonization of hematophagous insects by monoxenous trypanosomatids and their low host specificity, human cases of infection with lower trypanosomatids could have been largely underestimated until now due to their morphological similarity with Leishmania species. This emphasizes the relevance of enzymatic characterization, whenever possible, of all Leishmania-like parasites isolated from skin or visceral lesions of patients with or not immunosuppression history. Taken together, these reports reinforce the idea of the urgent need of elucidating the epidemiology of these lower trypanosomatids that so far remains poorly known.

5. ACKNOWLEDGEMENTS

We thank FAPERJ, CNPq, UFF and FIOCRUZ for the financial support.

REFERENCES


tinique (French WEst Indies). Transactions of the Royal Society of Tropical Medicine and Hygiene, 94(1), 51-52.


Noyes, P., Pratlong, F. and Chance, M. (2002) A previously unclassified trypanosomatid responsible for human cutaneous lesions in Martinique (French West Indies) is the most divergent member of the genus Leishmania ss. Parasitology, 124(Pt 1), 17-24.


