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

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ABSTRACT

Monoxenous trypanossomatids 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*, a endosymbiont-bearing monoxenous trypanossomatid, infect BALB/c mice under or not Dexamethasone treatment. To attend it, distinct gro- ups 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 trypanossomatid inside dermal cells in the groups studied. The experimental inoculation resulted in a non-lethal infection characterized by the presence of the trypanossomatid 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 immunosupressed 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 imunodeficient individuals with AIDS.

Keywords: Monoxenous Trypanossomatid; 'In Vivo' Infection; Immunosuppression

1. INTRODUCTION

Trypanossomatids 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 a 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,

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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 experimenttal 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. Auxiliadora de Souza (Trypanosomatids Collletion 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 (Azium®) [8] 24 hours before infection with *C. deanei*. After infection with 10^7 2-day-old promastigotes *C. deanei* by subcutaneous route (hind foot pad) —day 0, dexameth-asone 50 mg/kg was administered daily, for 15 days. Four BALB/c mice group were used: control without dexamethasone; control with dexamethasone; *C. deanei*–inoculated 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 microtome. 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 heaptomegaly.

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 reations of the foot pad were observed until d15 (**Figure 2(e1)**).

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 immunosupressed 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*–infection might be through sincicious 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 mice (**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 histologycal and inflammatory reactions of the foot pat were observed until day 15 (**Figure 2(e1)**).

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*–infection 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

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Table 1. Distribution of the experimental groups according to the animals number e the respective data of necropsy.

Groups	Animals number / time of necropsy					
	6 h	24 h	48 h	72 h	day 7	day 15
I (*C. deanei*–inoculated DMT treated mice)	2	2	2	2	2	2
II (*C. deanei*–inoculated mice)	2	2	2	2	2	2
III (DMT treated mice)	1	1	1	1	1	1
IV (not DMT treated and not *C. deanei* inoculated mice)–Control Groups	1	1	1	1	1	1
Total	6	6	6	6	6	6

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

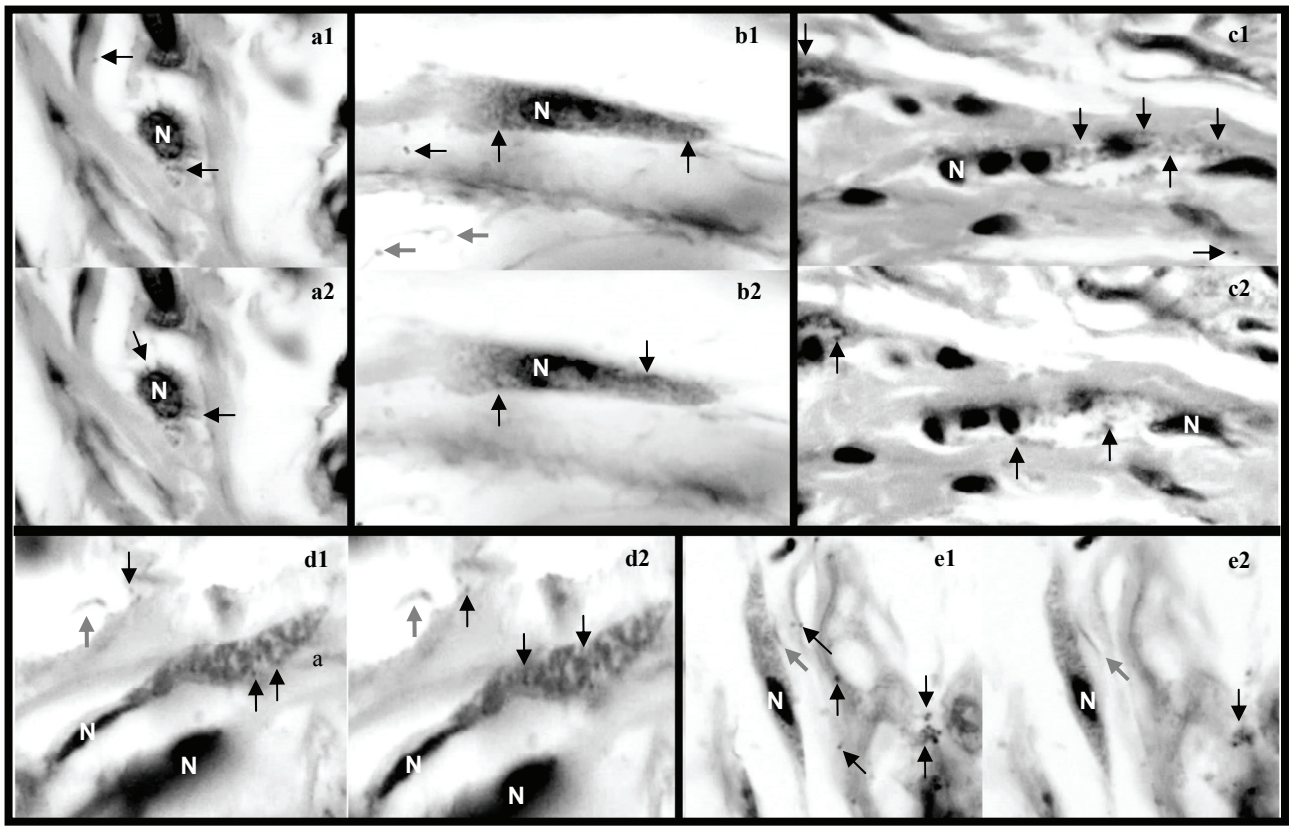

Figure 1. Analysis by light microscopy showing *C. deanei* interaction with Balb/c mouse. Pictures were taken in a two different plans from the same field (e.g. a1 and a2, etc…) in order to show whole extension of cell infection. Representative sections from skin samples of 24h (a1 and a2); 48h (b1 and 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 sincicious formation in c1 and c2. N = Dermal cells nucleous.

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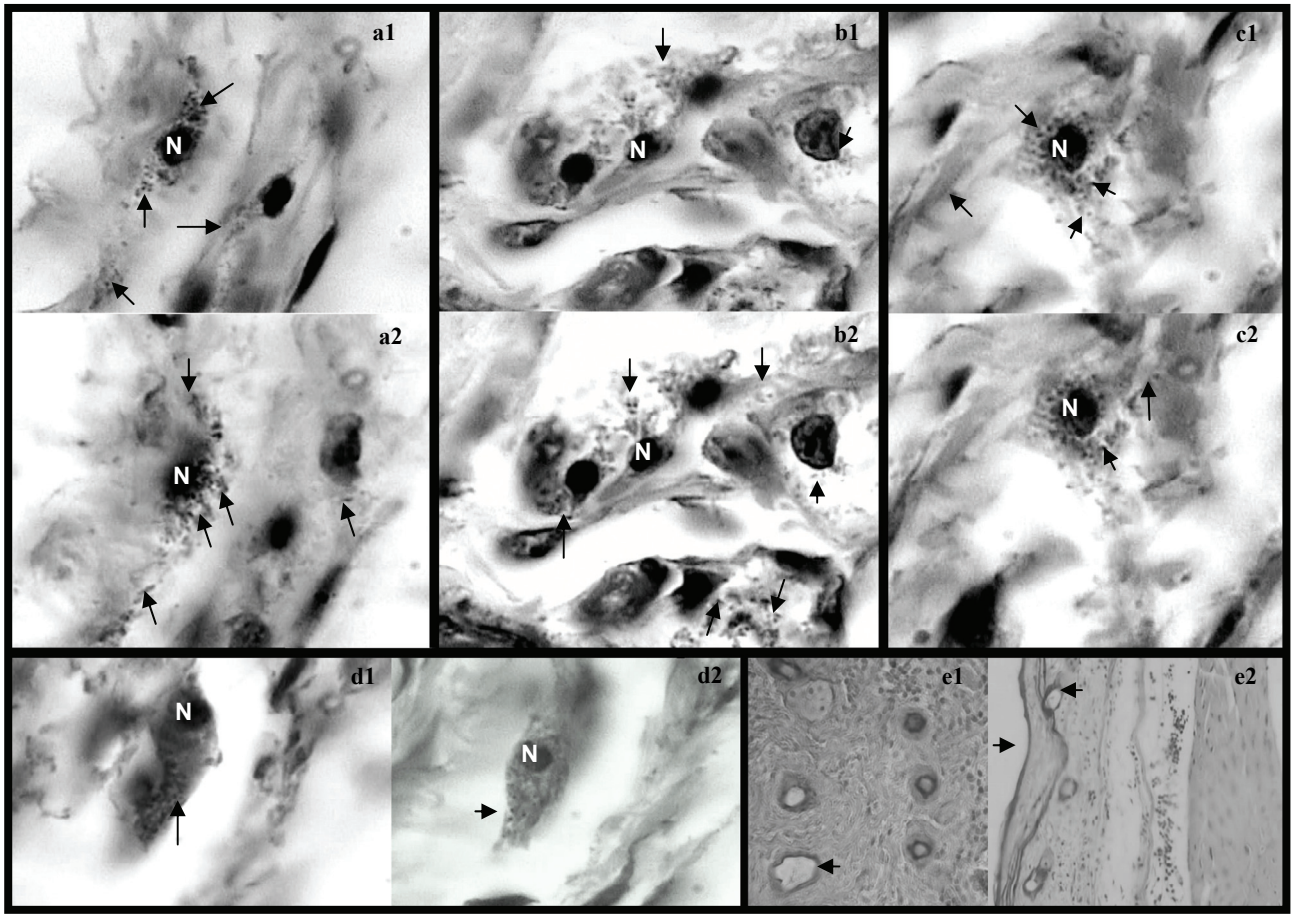

Figure 2. Analysis by light microscopy showing *C. deanei* interaction with dexamethasone immunosuppressed Balb/c mouse. Pictures were taken in a two different plans from the same field (e.g. a1 and a2, etc…) in order to show whole extension of cell infection. Representative sections from skin samples of 6h (a1 and a2); 24h (b1 and b2); 48h (c1 and c2); 72 h(d1 and d2) *C. deanei* post-infection (original magnification x 100). Dark arrow show multiple strongly *C. deanei*–infected dermal cells. Occasionaly, some parasites seemed to be clearly envolved by a vacuole (a2, b2, c1, c2 and d2). Controls groups 15 days after dexamethasone treatment or not (e1: dexamethasone-immunosuppressed Balb/c mouse; and e2: normal Balb/c mouse; original magnification (e1) × 50 and (e2) × 10). N = Dermal cell 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 immunosupressed 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 imunocompromised individuals. An unusual Leishmania-like parasite was found in a HIV-positive patient with symptoms of Leishmania infection [10]. Despite the previous-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 leishamniasis-like reaction, was indeed a monoxenous trypanosomatid, although no tissue invasion could be detected [3]. Surprinsingly, 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* (trypanossomatid

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endosimbiont 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. samuelpessoai* and *C. fasciculate* 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 prim- ary human macrophages by *Blastocrithidia 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 specifity [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 specie 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 limphopenic 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 susceptibily 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 foot pad. 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 opportunistic parasites, our work is pioneer in demonstrating that *C. deanei* is able to infect normal mice (whithout 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]. Neverthless, our data shows that the infection of *C. deanei* by dexamethasone-treated mice, although earlier proeminent 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 trypanossomatids with vertebrate cells, the literature have also mentioned some results obtained from the interaction of these trypanossomatids with invertebrate cells. Then, [20,21], reported the colonization of *Aedes aegypti* midgut by the endosymbiont-bearing trypanosomatid *Blastocrithidia 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.

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