Annals of Parasitology 2016, 62(3), 209–219
doi: 10.17420/ap6203.54

Original papers

Pre-exposure to faeces or saliva of *Triatoma dimidiata* decreases parasitemia in mice challenged with *Trypanosoma cruzi*: a description of the inflammatory reaction at the inoculation site

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ABSTRACT. Under natural conditions, *Trypanosoma cruzi* infection is transmitted to mammals when faeces contaminated with metacyclic trypomastigotes gain access through skin lesions, mucosa or bite wounds. Natural infection of bugs with *T. cruzi* can vary greatly from less than 1% up to 70%, depending on triatomine species: in the case of *Triatoma dimidiata,* the percentage of infection is around 30%. In this work uses biological fluids (saliva and faeces) from *Triatoma dimidiata* to inoculate experimental animals once or multiple times, before inoculation with faeces contaminated with metacyclic trypomastigotes discrete type unit Ia (TcI). The site of infection was analyzed for histological changes based on hematoxile-eosine technique and toluide blue stain for mast cells. Inoculation with saliva led to the recruitment of eosinophils and mononuclear cells at the inoculation site, whereas inoculation with faeces led to the recruitment of neutrophils. Mice inoculated multiple times exhibited a strong inflammatory reaction from the first hour. Mono- or multi-exposure to *T. dimidiata* fluids before inoculation with metacyclic trypomastigotes helped to control the level of parasitemia. Previous contact with saliva or faeces of *T. dimidiata* reduces parasitemia in *T. cruzi* I -infected mice.

Key words: *Trypanosoma cruzi*, saliva, faeces, *Triatoma dimidiata*, metacyclic trypomastigotes

Introduction

Triatomines are obligatory blood feeders, and are also vectors of the protozoan *Trypanosoma cruzi* parasite, the causative agent of Chagas disease. It is traditionally accepted that infection occurs via faeces contaminated with metacyclic trypomastigotes that are deposited on mucous membranes, through small wounds in the skin or even in the puncture made by the bite of the triatomine [1].

Cutaneous allergic reactions to triatomine bites are well known. In the first systematic study, 20% of test subjects developed immediate reactions to the bite of *Triatoma infestans* (localized urticarial reaction), and 90% experienced delayed reactions that were not ameliorated by treatment with corticosteroid or antihistamine creams [2]. Allergic reactions and occasionally fatal outcomes have been attributed to the bite of *Triatoma proctracta* [3], the major allergen of which is procalin, a 20 kDa

protein [4].

Triatomine saliva possesses a wide variety of bioactive molecules such as anticoagulants, vasodilators, an antihistamine immunosuppressor, an inhibitor of platelet aggregation induced by collagen, and apyrase [5]. Triatomine faeces include biotic and abiotic elements. It has been shown that the microbiota of triatomine guts is composed of a collection of bacterial species, i.e. *Serratia*, *Candidatus*, *Arsenophonus*, *Rhodnococcus*, *Gordonia* and *Wolbachia* [6]. The chemical composition of urine is based on sulphate, phosphate, potassium, sodium and chloride [7].

When *Trypanosoma cruzi* infection is transmitted by a triatomine, the host makes contact with faeces at the inoculation site, and the saliva could also be present. Our previous work based on the inoculation of mice with metacyclic vector-derived trypomastigotes found neutrophils to be the main infiltrating cells during the first three days at the inoculation site. [8,9]. Mesquita et al. [10] showed that vector saliva is a powerful inducer of cell chemotaxis.

Triatomines are usually numerous in regions that are endemic for Chagas disease, and inhabitants can be bitten several times before being infected. As a consequence, they are likely to have been inoculated with saliva and faeces more than once in their lifetime. *Triatoma dimidiata* includes three different haplogroups within the rubrofasciata subgroup. Its life cycle is longer than those of other species of triatomines: the develpment from egg to adult may take over a year, the adult lifespan is approximately 170 days and an adult can survive without a meal for up to two months. They can feed on very diverse range of mammal species; its natural rate of infection with *T. cruzi* in selvatic and peridomestic cycles is below 30%. In addition, their geographical distribution is wide, ranging from Mexico to Colombia and Ecuador, and this vector is very common in the Yucatan Peninsula, the region studied in the present paper [11]. The present work describes the inflammatory reaction to faeces or saliva of *Triatoma dimidiata* at the inoculation site in a murine model and the effect of these fluids on exposure to *T. cruzi*.

Materials and Methods

Triatomas. *Triatoma dimidiata* insects were captured from rural dwellings in the State of Campeche or the suburbs of Campeche City, Mexico. Before being used as a source of saliva or faeces, they were checked for the presence of *T. cruzi* by direct observation and PCR three different times over a period of two months before use; only *T. dimidiata* free of *T. cruzi* infection were used. The insects were kept at 28ºC in plastic bottles and fed on healthy mice every two weeks.

Mice. Seven experimental groups of Balb/c mice were formed (five mice/group). The first and second group of mice received saliva inoculum, one of them only once (mono-exposure) and the other seven times: weekly during the first month and monthly for the next three months (multi-exposure). At the end of the inoculation scheme, the mice were euthanized after one hour, four hours, and 24 hours and processed for hematoxylin-eosine staining and toluidine blue for mast cells. The third and fourth groups of mice were inoculated with faeces following the same scheme of inoculation. The fifth, sixth and seventh groups consisted of animals pre-exposed to saliva or faeces (mono or multi-exposure) and then challenged with 20, 000 metacyclic trypomastigotes TcI obtained directly from infected triatomines; they were sacrificed either one hour, 24 hours, 48 hours or 50 days later. The *T. cruzi* strain used in this study was Camp-9, isolated from *T. dimidiata* in Campeche State, Mexico. This strain belongs to the Tc I group [12]. All animals received the inoculum at the same site. The protocol was performed according to the "Comite de Seguridad e Higiene Universidad Autonoma de Campeche" guidelines of animal care.

Inoculation. Saliva inoculation was achieved via natural means. Briefly, non-infected *T. dimidata* were allowed to feed on the footpad of immobilized mice for five minutes. Faeces from non-infected triatomine were obtained after a bloodmeal, and the sample was collected in sterile microtubes and used to inoculate experimental mice (2–5 µL/mouse) intradermically into the footpad.

The control group only received faeces contaminated with metacyclic trypomastigotes of *T. cruzi* inoculum without pre-exposure to triatomine fluids. Another control group only received saline solution.

Parasitemia was monitored in the experimental and controls, and in those pre-exposed to triatomine fluids and challenged with *T. cruzi* parasites. In brief, parasitemia was followed from the 10^{th} day post infection (PI) through to the 50^{th} day PI. Five cubic millimeters of fresh blood sample taken from mouse's tail were compressed between a slide and a

Table 1. Histological description at the inoculation site to *Triatoma dimidiata* saliva

Times to exposition/ time of analysis post exposition to saliva	Grade of inflammation and mean findings	Cellular composition at the inflammatory foci	Collagen integrity and Fibroblast state	Mast cell degranulation (%) and size of largest foci at 400× (cell/field)
Monoexposed/1h	Light 1+ inflammation exclusively in the dermis with haemorrhage	Eosinophils 50% Neutrophils 50%	Light fragmentation (1+) at the site of inflammation light activated fibroblast(1+)	Mast cell degranulation (60%) (16 cells/field)
Monoexposed/4h	Moderate 2+ inflammation exclusively in the dermis with haemorrhage	Eosinophils (60%) Neutrophils (20%) Macrophages (20%)	Light fragmentation (1+) and light activated fibroblast (1+)	Mast cell degranulation (70%) (36 cells/field)
Monoexposed/24h	Intense 3+ inflammation exclusively in the dermis	Eosinophils (60%) Macrophages (25%) Neutrophils (15%)	Separation of fibers and light activated fibroblast (1+)	Mast cell degranulation (10%) (19 cells/field)
Multiexposed 8 times/1h	Intense 4+ inflammation in dermis and muscle tissue with haemorrhage	Neutrophils (60%) Eosinophils (30%) Macrophage (5%) Lymphocyte (5%)	Intense fragmentation (4+) Intense activated fibroblast (3+)	Mast cell degranulation (60%) (45 cells/field)
Multiexposed 8 times/24h	Intense 4+ inflammation in dermis and muscle tissue with haemorrhage	Eosinophils (60+) Neutrophils (30%) Lymphocyte (5%) Macrophage (5%)	Intense fragmentation (4+) with separation of fibers and intense activated fibroblast (3+)	Mast cell degranulation (70%) (18 cells/field)
Multiexposed 8 times/48h	Intense 3+ inflammation in dermis and muscle tissue	Lymphocyte (50%) Neutrophils (25%) Eosinophils (25%)	Separation of fibers and activated fibroblast (3+)	Light mast cell degranulation (10%) (20 cells/field)

Mice were exposed to *Triatoma dimidiata* biting for 5 minutes and the site of inoculation was analysed at different times after last stimulus. The grade of inflammation was graded as follow: **1+** (0-10 cells/field 1000×); **2+** (11-30 cells/field 1000×); **3+** (31-50 cells/field 1000×); **4+** (>51 cells/field). Collagen integrity: 1+ light fragmentation and 4+ intense fragmentation. Fibroblast with rounded shape was considered activated. Mast cell degranulation stained with toluidine blue.

22x22 mm cover-slip. The number of parasites was determined by scoring 100 microscope fields and then multiplied by a factor of 35 [13].

Statistics. ANOVA was used to analyse parasitemia data. Descriptive statistics such as rates and percentage were used for the histological description. Calculations were performed using GraphPad Prism 5 TD software.

Results

Description of inflammatory reaction to saliva at the inoculation site

Our results indicated a light to moderate inflammatory reaction at the site of inoculation with saliva in the mono-exposed group, with a mixture of eosinophils, neutrophils and macrophages constituting the inflammatory cells. Eosinophils and mast cell degranulation reached a peak four hours after inoculation. However, in the multi-exposure group, an intense inflammatory reaction had already taken place one hour after exposure; the main infiltrating cells were eosinophils and neutrophils, accompanied by intense mast cell degranulation and collagen fragmentation. The type of infiltrating cells changed to eosinophils at 24 hours post-inoculation, whereas lymphocytes peaked at 48 hours, suggesting the presence of a delayed hyper -

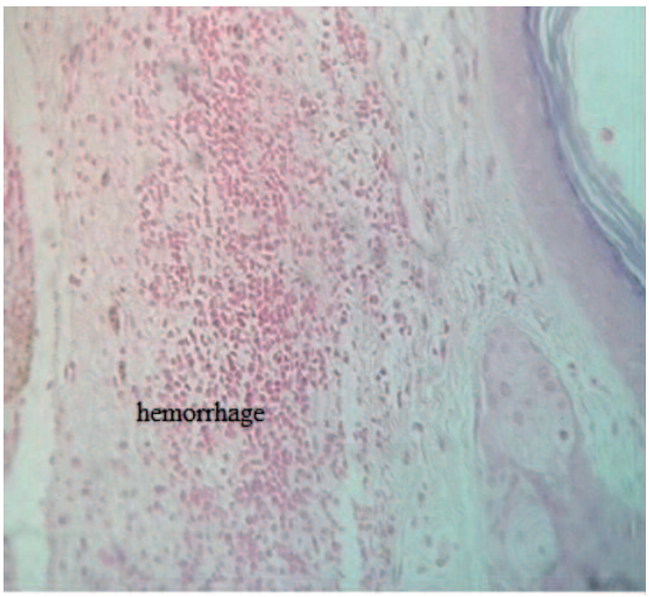

Fig. 1. Inflammatory reaction and hemorrhage at the site of inoculation with saliva in the mono-exposed group 1 h after inoculum. Hematoxilin-eosin stain.

sensitivity reaction (Table 1). An interesting finding was the presence of haemorrhage in this group of animals when exposed to biting (Fig. 1).

Inflammatory reaction to faeces at the inoculation site

The inflammatory reaction to faeces in mono-exposed mice was marked by the presence of neutrophil cells that persisted from one hour to 50 days after inoculation (Fig. 2). Low levels of inflammation were seen at one hour PI, but became more intense after 48 hours post-inoculation. This was accompanied by fragmentation of collagen fibres and mast cell degranulation. However, in the multi-exposed mice, high levels of inflammation appeared within the first hour after inoculation. Although neutrophil cells were predominant at this stage, lymphocyte cells were dominant after 48 hours, suggesting the presence of a delayed hypersensitivity reaction. Intense collagen fibre fragmentation and mast cell degranulation was observed from the first hour after inoculation, and collagen fibre fragmentation persisted for up to 48 hours (Table 2).

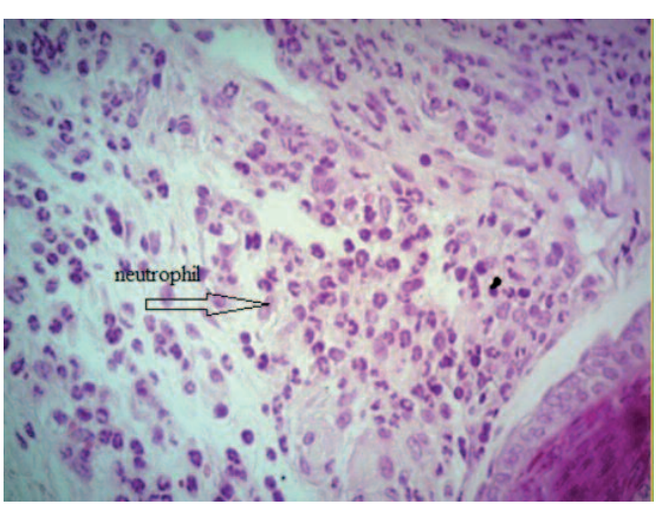

Fig. 2. Inflammatory reactions to feces in mono-exposed mice 48 h post-inoculation. Feces from non-infected triatomine was inoculated intradermically into the footpad (2–5 µL/mouse). Hematoxilin-eosin stain.

Inflammatory reaction in pre-exposed animals with saliva or faeces and challenged with *Trypanosoma cruzi*

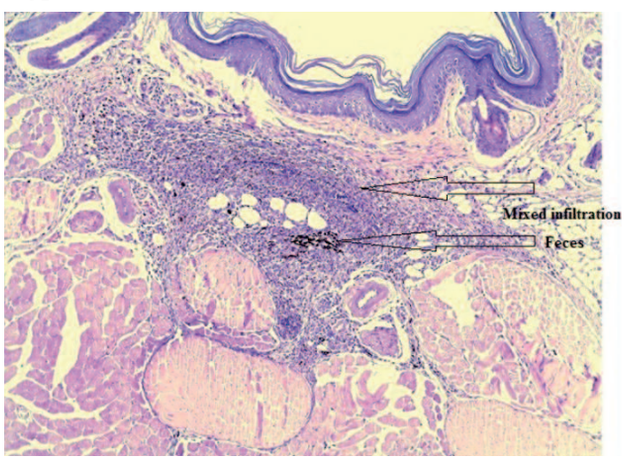

Fig. 3. Inflammatory reaction observed 1 h after challenge in the group of mice pre-exposed once to feces and challenged with metacyclic trypomastigotes. Hematoxilin-eosin stain. Toluidine blue stain.

A moderate inflammatory reaction was observed one hour after challenge in the group of mice pre-exposed once to faeces and challenged with metacyclic trypomastigotes (Fig. 3). Neutrophil cells were dominant in the infiltrate even 48 hours later. Intense collagen degradation was observed but there was no evidence of mast cell degranulation. Intense inflammation was also observed in the multi-exposed group, with macrophage and neutrophil cells predominating during the first 48 hours (Table 3). Strong inflammation was also observed one hour after inoculation in the group of mice multi-exposed to saliva and challenged with metacyclic trypomastigotes. Eosinophil and neutrophil cells dominated the infiltrate in parallel with active collagen degradation and intense mast cell degranulation, but inflammation became less intense at 48 hours, with the infiltrate being dominated by lymphocyte and eosinophil cells,

Table 2. Histological description at the inoculation site to *Triatoma dimidiata* faeces

Times to exposition/ time of analysis post exposition to faeces	Grade of inflammation and mean findings	Cellular composition of inflammation (%)	Collagen integrity and Fibroblast state	Mast cell degranulation and size of largest foci 400× (cell/field)
Monoexposed/1h	Light 1+ inflammation and exclusively in the dermis with haemorrhage	Neutrophils (90%) Macrophages (6%) Eosinophils (4%)	Fragmentation (1+) at the zone of inflammation and activated fibroblast(1+)	Mast cell degranulation (5%) (15 cells/field)
Monoexposed/48h	Intense diffuse inflammation 4+ in dermis and muscle	Neutrophils (80%) Macrophages (20%)	Extended fragmentation (3+) and light activation of fibroblast(2+)	Mast cell degranulation (50%) and (10 cells/field)
Monoexposed /50 days	Light and diffuse (<10 cells/field)	Macrophages (90%) Lymphocyte (10%)	conserved	Mast cell degranulation (17%) (39 cells/field)
Multiexposed 8 times/1h	Intense diffuse 4+ inflammation and exclusively in the dermis with haemorrhage	Neutrophils (50%) Macrophages (50%)	Fragmentation at the zone of inflammation (3+) Light activation of fibroblast (1+)	Mast cell degranulation (10%) (28 cells/field)
Multiexposed 8 times/48h	Intense 4+ inflammation in dermis and muscle tissue with haemorrhage	Lymphocyte (70%) Macrophage (20%) Neutrophils (10%)	Intense fragmentation (4+) with separation of fibers and intense activated fibroblast (3+)	Mast cell degranulation (40%) (18 cells/field)
Multiexposed 8 times and 50 days analysed	Intense (3+) inflammation in dermis and muscle tissue	Macrophage (80%) Lymphocyte (20%)	Separation of fibers and activated fibroblast (3+)	Mast cell degranulation (5%) (10 cells/field)

Mice were inoculated with 5-10 µL of *Triatoma dimidiata* faeces intradermically and the site of inoculation was analyzed at different times after last stimulus. The grade of inflammation was graded as follow: **1+** (0-10 cells/field 1000×); **2+** (11-30 cells/field 1000×); **3+** (31-50 cells/field 1000×); **4+** (>51 cells/field). Collagen integrity: 1+ light fragmentation and 4+ intense fragmentation. Fibroblast with rounded shape was considered activated. Mast cell degranulation stained with toluidine blue.

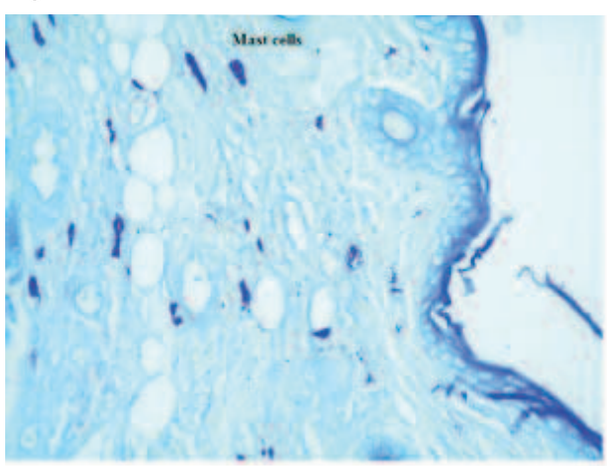

Fig. 4. Mast cells in the dermis of multiexposed mice to *Triatoma dimidiata* feces and challenged with metacyclic trypomastigotes 1 h post inoculum. Toluidine blue stain.

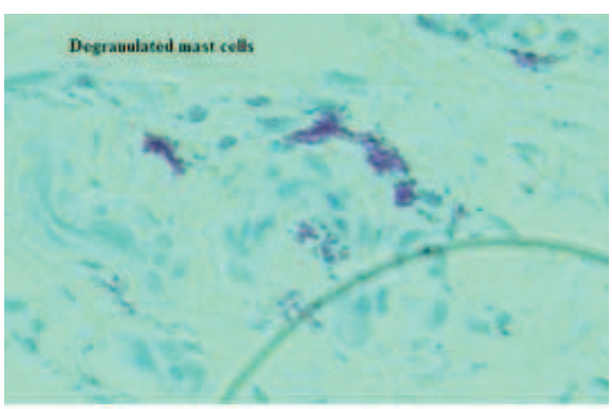

Fig. 5. Mast cells in the dermis of multiexposed mice to *Triatoma dimidiata* saliva and challenged with metacyclic trypomastigotes 1 h post inoculum.

Table 3. Histological description at the inoculation site in monoexposed or multiexposed mice to *Triatoma dimidiata* faeces and challenged with metacyclic trypomastigotes

Times to exposition/time of analysis post exposition to faeces	Grade of inflammation and mean findings	Cellular composition at the inflammatory foci	Collagen integrity and Fibroblast state	Mast cell degranulation and size of largest foci 400× (cell/field)
Monexposed/1h	Moderate focal inflammation (3+) at site of inoculation with haemorrhage	Neutrophils (94%) Macrophages (6%)	Moderate fragmentation (2+) at the zone of inflammation Light activated fibroblast (1+)	Mast cell degranulation (10%) (10 cells/field)
Monexposed/48h	Intense focal inflammation (4+)	Macrophages (50%) Neutrophils (40%) Lymphocyte (5%) Eosinophils (5%)	Moderate fragmentation (3+) Light activated fibroblast (2+)	Mast cell degranulation (10%) (40 cells/field)
Monoexposed/50 days	Light focal inflammation (1+)	Macrophages (80%) Lymphocytes (20%)	Light fragmentation (1+) Light activated fibroblast (1+)	Mast cell degranulation (50%) (34 cells/field)
Multiexposed 8 times/1h	Intense diffuse inflammation (4+)	Neutrophils (50%) Lymphocytes (30%) Macrophages (20%)	Moderate fragmentation (2+) Moderate activated fibroblast (2+)	Mast cell degranulation (20%) (19 cells/field)
Multiexposed 8 times/48h	Intense diffuse inflammation (4+)	Neutrophils (40%) Macrophages (40%) Lymphocytes (17%) Eosinophils (3%)	Intense fragmentation (4+) Moderate activated fibroblast (2+)	Mast cell degranulation (80%) (38 cells/field)
Multiexposed/50 days	Moderate focal inflammation (2+)	Macrophages (70%) Eosinophils (25%) Lymphocytes (5%)	Light fragmentation (1+) Light activated fibroblast (1+)	Mast cell degranulation (70%) (33 cells/field)

Mice were inoculated with 5-10 µL of *Triatoma dimidiate*`s faeces intradermically and the site of inoculation was analysed at different times after last stimulus. The grade of inflammation was graded as follow: **1+** (0-10 cells/field 1000×); **2+** (11-30 cells/field 1000×); **3+** (31-50 cells/field 1000×); **4+** (>51 cells/field). Collagen integrity: 1+ light fragmentation and 4+ intense fragmentation. Fibroblast with rounded shape was considered activated. Mast cell degranulation stained with toluidine blue.

indicating delayed hypersensitivity but with the presence of an allergic reaction (Table 4). In addition, a different grade of mast cell degranulation was observed in conjunction with fibroblast activation; this reaction was more evident 48 hours after the stimulus (Figs. 4 and 5).

Inflammatory reaction in animals inoculated with *Trypanosoma cruzi* without pre-exposure to triatomine fluids

The inflammatory reaction ranged from light to moderate (Fig. 5). Neutrophil cells were dominant in the infiltrate. Collagen degradation and fibroblast activation was light with poor mast cell degranulation. In the case of the control group that received only saline solution, a very light inflammation could be observed at 24 hours after inoculum, which practically disappeared 48 hours later (data not shown).

Parasitemia in mice pre-exposed to triatoma´s fluids and challenged with *Trypanosoma cruzi*

A very interesting finding was observed in mice pre-exposed to faeces or saliva and challenged with *T. cruzi* parasites; the parasitemia was lower and statisctially different in this group of mice compared with the control group that only received metacyclic trypomastigotes. There were no differeces among

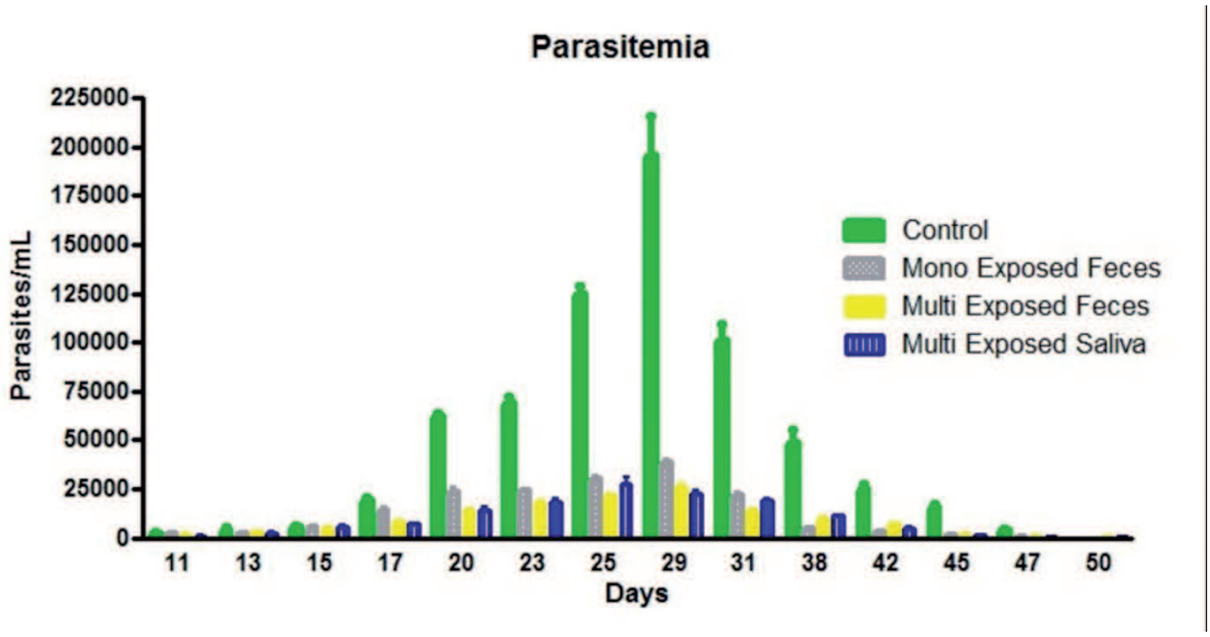

Fig. 6. Parasitemia in mice pre-exposed to saliva o feces before challenged with 20,000 metacyclic trypomastigotes. Control mice received only metacyclic trypomastigotes.

pre-exposed mice to triatoma´s fluids and challenged with metacyclic trypomastigotes (Fig. 6).

Discussion

Our results indicate that the composition of inflammatory responses in naïve animals depends on the type of fluid inoculated. Saliva induces a light infiltration composed of eosinophil and neutrophil cells whereas faeces preferentially

Table 4. Histological description at the inoculation site in multiexposed mice to *Triatoma dimidiata* saliva and challenged with metacyclic trypomastigotes

Times to exposition/ time of analysis post exposition to saliva	Grade of inflammation and mean findings	Cellular composition at the inflammatory foci	Collagen integrity and Fibroblast state	Mast cell degranulation and size of largest foci 400× (cell/field)
Multiexposed 8 times/1h	Moderate diffuse inflammation (3+)	Eosinophils (40%) Neutrophils (35%) Lymphocytes (20%) Macrophages (5%)	Moderate fragmentation (2+) Moderate activated fibroblast (2+)	Mast cell degranulation (60%) (43 cells/field)
Multiexposed 8 times/48h	Light focal inflammation (2+)	Eosinophils (37%) Lymphocytes (30%) Neutrophils (20%) Macrophages (13%)	Moderate fragmentation (3+) Moderate activated fibroblast (2+)	Mast cell degranulation (70%) (26 cells/field)
Multiexposed/50 days	Light focal inflammation (1+)	Neutrophils (66%) Eosinophils (30%) Lymphocytes (4%)	Light fragmentation 1(+) Light activated fibroblast (1+)	Mast cell degranulation (71%) (40 cells/field)

Mice were exposed to *Triatoma dimidiata* biting for 5 minutes and the site of inoculation was analysed at different times after last stimulus. The grade of inflammation was graded as follow: **1+** (0-10 cells/field 1000×); **2+** (11-30 cells/field 1000×); **3+** (31-50 cells/field 1000×); **4+** (>51 cells/field). Collagen integrity: 1+ light fragmentation and 4+ intense fragmentation. Fibroblast with rounded shape was considered activated. Mast cell degranulation stained with toluidine blue.

Table 5. Histological description at the inoculation site in mice exposed to *Triatoma dimidiata* feces with metacyclic trypomastigotes without pre-exposure to triatomine fluids

Time of analysis post exposition	Grade of inflammation and mean findings	Cellular composition at the inflammatory foci	Collagen integrity and Fibroblast state	Mast cell degranulation and size of largest foci 400× (cell/field)
1 h	Moderate 2+ inflammation and exclusively in the dermis	Neutrophils (95%) Macrophages (5%)	Fragmentation (1+) at the zone of inflammation and activated fibroblast(1+)	Mast cell degranulation (5%) (8 cells/field)
48 h	Light 1+ local inflammation in dermis	Neutrophils (70%) Macrophages (30%)	Moderate fragmentation (2+) and light activation of fibroblast (2+)	Mast cell degranulation (20%) and (10 cells/field)
50 days	Light and diffuse (<10 cells/field)	Macrophages (80%) Lymphocyte (20%)	conserved	Mast cell degranulation (10%) (20 cells/field)

Mice were inoculated intradermically with 10 µL of *Triatoma dimidiata* feces contained 20,000 metacyclic trypomastigotes. The site of inoculation was analyzed at different times after inocolum The grade of inflammation was graded as follow: **1+** (0-10 cells/field 1000×); **2+** (11-30 cells/field 1000×); **3+** (31-50 cells/field 1000×); **4+** (>51 cells/field). Collagen integrity: 1+ light fragmentation and 4+ intense fragmentation. Fibroblast with rounded shape was considered activated. Mast cell degranulation stained with toluidine blue.

induce neutrophil cells. Multi-exposed animals experience a very strong inflammatory reaction that takes place immediately after inoculation.

The inflammatory cell infiltrate arising following inoculation with saliva has previously been described in other genera of hematophagous insects such as *Lutzomya longipalpis* in a canine model [13]. The study found minimal inflammation in naïve animals, but a dramatic increase in inflammation composed of macrophages, lymphocytes and eosinophils in multi-exposed animals. Similar results were observed in pre-exposed mice to *L. longipalpis* bites, in which neutrophils, eosinophils, and macrophages dominated the infiltrate, with the modest presence of mast cells [14]. However in humans from a Leishmaniasis-endemic area, the inflammatory reaction at 48 hours post-challenge was found to be composed mainly of T lymphocytes and to a lesser extent, macrophages: neutrophils and eosinophils are almost completely absent [15].

However, the inflammatory reaction to *T. cruzi* at the inoculation site has been poorly studied. In previous data obtained from a murine model, it was found that neutrophil cells predominated for the first week, which change to MN cells in the second week with persistence of DNA parasites [8,9]. A similar type of infiltration was observed in a Rhesus monkey model [16]. In pioneer studies by Romaña in 1943 described the histology of different mouse groups exposed to culture metacyclic parasites or IMT contaminating faeces inoculated on intact skin, scraped skin, oral mucosa, olfactory mucosal membrane or administered percutaneously. In all these experiments, the concentration of parasites was not controlled, and the inoculum size was usually excessively large, because they wanted demonstrate the most probable and efficient route of contamination [17].

To the best of our knowledge, this is the first study to examine immune responses when the host has experienced contact with faeces or saliva prior to infection with metacyclic trypomastigotes. Interestingly, a reduced level of parasitemia was observed in naïve animals pre-exposed to *T. dimidiata* fluids and challenged with *T. cruzi.* This finding can be interpreted as a consequence of the action of the innate immune response, such as infiltration of neutrophils and eosinophils and a complement system which can control the size of the *T. cruzi* inoculum. In natural conditions in endemic areas, individuals can be multi-exposed to faeces before being infected, and as a result can develop a memory response to immunogens

contained in the composition of the faeces, thus allowing the inflammatory reaction to become faster and more intense in the event of a secondary stimulus compared to naïve animals, probably resulting in better control of the *T. cruzi* inoculum. Our data shows that neutrophils are the predominant lineage of phagocytes recruited at the inoculation site in mono or multiexposed animals, but infiltration is more intense in multiexposed animals. A very recently published paper demonstrated that neutrophils play an important role in inducing the production of leukotriene B_4 and TNF by macrophages, leading to enhanced killer capability of Leishmania-infected macrophages [18].

Our findings differ from those reported by Mesquita et al. [10] who used *R. prolixus* saliva and purified lysophospholipidylcholine (LPC) in a murine model. They found higher levels of parasitemia in mice that had received saliva or LPC prior to *T. cruzi* infection. The discrepancies observed can be explained by different factors. Firstly, a different saliva vector was used, as the present study uses *T. dimidiata*: other studies have demonstrated that sialome of triatomines differ among species [19–22]. Secondly, our model more closely resembles mimicked natural conditions in terms of the parasite phase and saliva inoculation. Thirdly, the *T. cruzi* strain used in our model is a Mexican strain that belongs to the TcI group, this factor could influence the outcome of infection; there is enough published data that could support this point. It was recently published that TcI and TcII strains have a distinct impact on human cells during early infection [23]. Despite this factor, it is clear that saliva and/or its components have a direct impact on immune responses, likely depending on their concentration.

Finally, another interesting finding was the intense collagen degradation observed as early as one hour post-infection in the group of animals pre-exposed several times to triatoma fluids. Our observation is clearly at the descriptive and histological levels. Further studies on mechanism are necessary. However, we can speculate that this histological alteration may promote parasite escape from an aggressive inoculation site that is rich in nonspecific and specific immune mediators. It is known that the parasite can navigate through the basal lamina thanks to several *T. cruzi* surface proteins, such as Gp83, Tc45, TcCRT, Tcgp83 and Tc85 [24]. These conditions may favour the escape of some parasites from the inoculation site. In a pioneering work, it was demonstrated that the parasite can be disseminated as soon as five minutes after initial contact [25].

Taking this information together, we can hypothesize that metacyclic trypomastigotes are vulnerable to innate inflammatory mediators at the site of inoculation, resulting in the reduction of inoculum size. However, the susceptibility of *T. cruzi* parasites to innate immune responses depends on the *T. cruzi* strains used. The insect-transmitted metacyclic trypomastigotes appear resistant to the complement system and destruction by phagocytosis, but this is not a strict characteristic of the metacyclic trypomastioges; rather there are strains which are sensitive to the complement system and others are resistant, thus not all metacyclic trypomastigotes succeed in infecting the host [26]. We have found a variable degree of susceptibility to complement system *in vitro* with Mexican strains [27].

Conclusions

The inflammatory reaction depends on the type of biological fluids of *T. dimidiata*. Faeces induce strong neutrophil and mononuclear cell infiltration, whereas saliva recruits these cells and also eosinophils in multi-exposed animals. This inflammatory reaction may help to control the size of the metacyclic trypomastiogte inoculum at the site of inoculation, leading to a reduction in parasitemia when mice are infected with TcI.

Acknowledgements

Financial Support by Consejo Nacional de Ciencia y Tecnologia (CONACYT) Project CB-2010-01 153764.

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Received 28 March 2016
Accepted 15 June 2016