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Original papers

Gene expression to contaminated or not contaminated *Triatoma dimidiata* faeces with *Trypanosoma cruzi in vivo* and *in vitro* assay

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ABSTRACT. *Triatoma dimidiata* is considered one of the most important vectors in the spread of Chagas disease in Mesoamerica. Hematophagous insects may deposit faeces contaminated or not with *Trypanosoma cruzi* on the surface of the skin during the feeding process. In endemic areas humans can be in contact several times to this fluid before get infection. Due to limited work on the of immune response to insect's fluids in mammals. In this work, we explored the early response of chemokine and cytokines of skin tissue at the inoculation site against faeces from *T. dimidiata* contaminated or not with *T. cruzi* in murine model, also we explored the *in vitro* response of epitheloid cells line "HeLa cells" to this fluid. Mice were exposed intradermically to faeces free of metacyclic trypomastigotes, one or multiple times along the experiment before challenged with faeces contaminated with metacyclic trypomastigotes, mimicking natural conditions. In *in vitro* assay with HeLa cells only one exposure was carried out. It was observed that mice multi-exposed to faeces, induced the strong response of TNF-α and CXCL3 expression at the inoculation site; but faeces contaminated with metacyclic trypomastigotes lower the response of TNF-α, and CXCL3. In *in vitro* assay with HeLa cell line, the faeces induced a strong response of CXCL8 and IL-6, but the presence of faeces contaminated with metacyclic trypomastigotes lower the response of CXCL8. In contrast, to *in vivo* model null response of TNF α and IL-1-α was observed in HeLa cells. In conclusion, *Triatoma dimidiata* faeces induces at the inoculation site chemokines that mediate preferentially chemotaxis of neutrophils and macrophages but the presence of metacyclic trypomastigotes moderate their induction. Epithelial cells act as efficient sentinels producing chemokines that may recruit neutrophils and monocytes.

Keywords: *Triatoma dimidiata*, *Trypanosoma cruzi*, faeces, immune response, chemokines, inoculation site

Introduction

Triatoma dimidiata is known as one of the main vectors of *Trypanosoma cruzi* and it presents a wide distribution in countries of Central America and Mexico [1], occupying several domestic habitats, peri-domestic and wild in both rural areas and in peri-urban and urban dwellings of small towns and large cities [2]. The vector inoculates saliva, and during feeding may deposit feaces contaminated or not with metacyclic trypomastigote, the parasites are able to invade the mucous membranes or cells of the skin at the inoculation site. There are limited studies evaluating the effect of saliva or faeces on the immune system at the inoculation site. It has been recently published that the vector´s saliva is a powerful chemoattractant for monocytes and neutrophils at the site of inoculation [3,4]. Similar data have demonstrated that metacyclic vector-derived or faeces inoculated in animal model induces

an inflammatory reaction with the recruitment of neutrophils cells as soon as 1h post-inoculation, which turn on monocyte infiltration at 7 days later [5,6]. A recent paper published was stated that pre-exposure to faeces of *T. dimidiata* decrease parasitaemia in challenged mice inoculated with metacyclic trypomastigotes [7].

The action of the vector's saliva on the haemostatic system has been deeply studied but studies evaluating the effect of the saliva or faeces on cells of the immune system are still incipient. Recently, it was also published that dendritic cells population present in diverse tissues, including the skin, can be affected by saliva resulting in inhibition of the expression of class II molecules and the secretion of TNF-α, and IL-12 [8].

The skin, as the first barrier is composed of array of cells including keratinocytes, Langerhan cells, intraepithelial lymphocytes, fibroblasts, miofibroblasts, macrophages, adipocytes, dendritic cells, mast cells, endothelial cells, free nerve ending and mesenchymal stem cells. All of them are part of the network of the immune system, where epithelial cells are important sentinel cells in this system.

There are no studies describing the effect of faeces on skin cells at the inoculation site. The objective of this work was to analyse the expression of early response genes to exposure to faeces contaminated or not with metacyclic trypomastigotes in Balb/c mice and *in vitro* assay on epitheloid cell line " HeLa cells".

Materials and Methods

Five groups of animals were used in this experiment. Control mice group (five animals) was exposed exclusively to 5–10 µl of saline solution subcutaneous route. The mono-exposure group consisted of two set of animals (five animals/set); one set received 5–10 µl faeces inoculum free of metacyclic trypomastigotes, and the other set of animals received 5–10 µl faeces inoculum contaminated with metacyclic trypomastigotes both set of animals sacrificed at one hour and 24h later. The multi-exposure group consisted of two set of animals (five animals/set) one set received 5–10 µl faeces inoculum free of metacyclic trypomastigotes, and the other set of animals received 5–10 µl faeces inoculum contaminated with metacyclic trypomastigotes, weekly during the first month and monthly for the next three months. At the end of the respectively treatments, mice were sacrificed at one hour and 24h later.

Animals were maintained accordance with the Guide for the Care and Use of Laboratory Animals and the Ethical Principles for Animal Experimentation established by the "NORMA Oficial Mexicana NOM-062-ZOO-1999".

Cell culture. HeLa human cell line (ATCC® CCL-2™) was grown in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco) supplemented with 10% heat-inactivated foetal bovine serum (FBS) (Gibco) at 37°C in a 5% CO_2 atmosphere. We include HeLa cells in the experiment in order to know the possible reaction of epithelial cells to faeces contaminated or not with metacyclic trypomastigotes.

Obtaining triatomes. *T. dimidiata* insects were caught in rural areas of the state of Campeche, Mexico, in the communities of Xcalot Akal, Bolonchén de Rejón, Tenabo and Campeche, during the period of March–July 2012, 2015 and 2016 in each respective year. The majority of the insects were captured in the peridomicile and were analysed for the presence of *T. cruzi* by direct observation and PCR three different times over a period of two months before use to ensure bugs are infected or not. After two months of observation, the bugs were divided in two group, infected and not infected with *T. cruzi* and used for the collection of faeces. The insects were kept at 28°C in plastic bottles, and they fed on healthy mice every two weeks.

Inoculation. Faeces from non-infected and infected triatomine were obtained after a blood meal, and the sample was collected in sterile microtubes and used to inoculate five experimental mice (5–10 µl/mouse) into the footpad subcutaneous route. In the case of faeces contaminated with *T. cruzi* the inoculum was adjusted to 1000 metacyclic trypomastigotes. The control group only received a saline solution.

Cell treatment. 1×10^6/ml HeLa monolayers were seeded in 96 multiwell plates (200 ml final volume). After 24h, monolayers were treated with 2 ml of faeces/urine with or without parasites (500 parasites/well) and incubated at 37°C, in humid conditions, and 5% CO_2. After 3, 6, 18, and 24 hours, supernatants of the treated cells were harvested and stored at –80°C and the cells were recovered in Trizol and stored at –80°C until use.

Determination of cytokine and chemokine pattern by ELISA. Interleukin 1-beta (IL-1β), IL-6, tumour necrosis factor-alpha (TNF-α); and the

chemokines CCL5, and CXCL8 were quantified by ELISA in culture supernatants of HeLa cells under different conditions of stimulation, according to the manufacturer's protocol. Briefly, 96-well flat-bottom plates were coated overnight with a capture antibody at a final concentration of 2 μg/ml, and then plates were blocked with 10% PBS-FCS, washed three times, and incubated with the cell culture supernatant samples or control antigens overnight at 4°C. After washing, plates were incubated with the respective biotinylated anti-cytokine antibodies (R&D System) at 1 μg/ml for 1 h in the dark. Plates were washed and streptavidin-Alkaline Phosphatase at 1:2000 was added for 30 min. in the dark, then washed, and 100 μl of ABTS (2,2,-azino-bis(3-ethylbenzthiazoline)-6-sulphonic acid) (Zymed) was added as substrates and the reaction was allowed to proceed for 20 min. at room temperature (RT); the reaction was stopped with 2% sulphuric acid, and absorbance was read at 415 nm by an ELISA reader (Multiscan MS, Labsystem).

RNA extraction, cDNA synthesis and RT-PCR.The RNA of the hind foot pad at the inoculation site of five experimental animals or from culture cells was isolated using the TRIzol system (Invitrogene, Life Technologies). One microgram of RNA was reverse transcribed to cDNA with an oligonucleotide (poly(dT)16) using the SuperScript II reverse transcriptase (Invitrogene, Life Technologies) and the cDNA was used as a template for PCR.

a) PCR for animal tissues. From the cDNA of tissue inoculated with dejections, we performed gene amplification for chemokines and cytokines. The set of primers used for IL-1β, TNF-α, gamma-Interferon (IFN-γ), CXCL2, CXCL3, and CCL5 were as follow: IL-1β, (5'-ATG GCA GAA GTA CCT AAG CTC GC-3' and 5'-CAC AAA TTG CAT GGT GAA GTC AGT T-3'), TNF-α (5'-TTC TGT CTA CTG AAC TTC GGG GT-3' and 5'-GTA TGA GAT AGC AAA TCG GCT GAC GG-3'), IFN-γ (5'-GAC CAG AGC ATC CAA AAG A-3' and 5'-CCT TTT TCG CTT CCC TGT TTT A-3'), CXCL2 (5'-TGG GTG GGA TGT AGC TAG TTC C-3' and 5'-AGT TTG CCT TGA CCC TGA AGC C-3'), CXCL3 (5'-TGC CCC TTC CTC AGT CAT

Table 1. Oligonucleotides used for gene amplification in HeLa cells

Gene	Primer (5´- 3´)	Size [pb]	Reference
IL-1β	Forward - ATGGCAGAAGTACCTAAGCTCGC Reverse - ACACAAATTGCATGGTGAAGTCAGTT	802	[21]
TNF-α	Forward -TTCTGTCTACTGAACTTCGGGGT Reverse - GTATGAGATAGCAAATCGGCTGACGG	184	[21]
IL-6	Forward - ATGAACTCCTTCTCCACAAGCGC Reverse - GAAGAGCCCTCAGGCTGGACTG	628	[21]
GAPDH	Forward - GGTGAAGGTCGGAGTCAACGG Reverse - GGTCATGAGTCCTTCCACGAT	520	[21]
CCL5	Forward - CGGGATCCATGAAGGTCTCCGCGGCA Reverse - CGGAATTCCTAGCTCATCTCCAAAGA	297	[21]
CXCL8	Forward - GTGTGAAGGTGCAGTTTTGC Reverse - GCAGTGTGGTCCACTCTCAA	126	[22]
BIRC-3	Forward - GGCAGCAGGTTTACAAAGGAG Reverse - ACCCATGCACAAAACTACCTC	143	[23]
SOD2	Forward - CACTGCAAGGAACAACAGGC Reverse - GGGATCATTAGGGTATGATCAGCA	201	[23]
NFKB1A	Forward - TGCACTTGGCCATCATCCAT Reverse - TCTCGGAGCTCAGGATCACA	190	[23]
ICAM-1	Forward - TATGGCAACGACTCCTTCT Reverse - CATTCAGCGTCACCTTGG	238	[21]
VCAM-1	Forward - ATGACATGCTTGAGCCAGG Reverse - GTGTCTCCTTCTTTGACACT	260	[21]

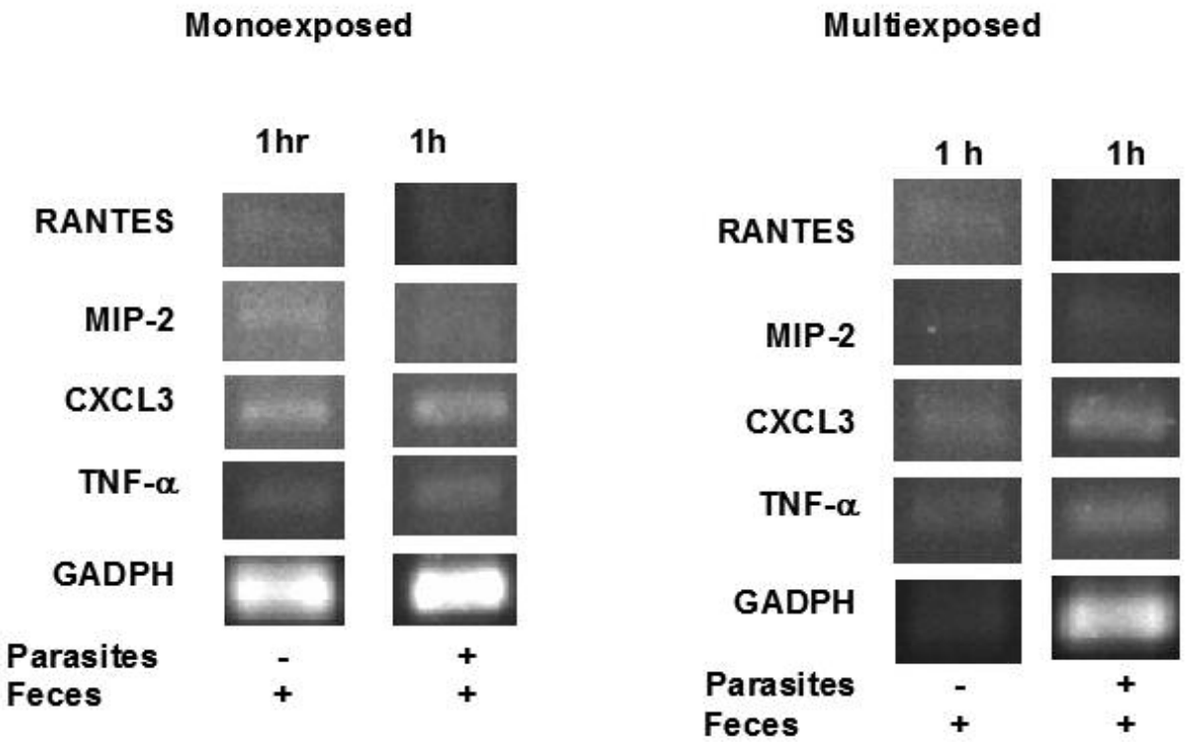

Figure 1. Representative images of RT-PCR products at the site of mono-exposure and multi-exposure to excretions of *T. dimidiata* contaminated or not with *Trypanosoma cruzi* in Balb/c mice. The hindpad of inoculated mice with 5 µl of faeces contaminated or not with 1000 metacyciclic trypomastigotes were processed for RT-PCR for CXCL2, CXCL3, CCL3, TNF-α, IL-β, IL-14, IL-10, IL-17, INF-γ, TGF-β and iNOS. Only those positive are represented.

AG-3' and 5'-GTG CAT TCC GCT TAG CTT TC-3'), CCL5 (5'-TCT TCT CTG GGT TGG CAC ACA C-3' and 5'-CCT CAC CAT CAT CCT CAC TGC A-3'), and GAPDH (5'-GAG GGG CCA TCC ACA GTC TTC-3' and 5'-CAT CAC CCA TCT TTC CAG GAG CG-3'). The cycle reactions were as follows: an initial denaturation step of 95°C for 5 min., followed by 25 cycles of 95°C for 45 s, 55°C for 1 min., 72°C for 1 min., and a final elongation step of 72°C for 7 min. The GAPDH gene was used as an internal control to evaluate the relative expression of the aforementioned cytokine and chemokine genes. The relative expression of genes against GAPDH was determined by densitometry analysis with the IMage Lab 4.1 software (Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, MD, USA).

b) PCR for HeLa cells. The set of primers used for the amplification of genes from HeLa cells are shown in Table 1. PCR conditions were as follows: initial DNA denaturation at 95°C for 5 min. and 35 rounds of denaturation (95°C for 1min.), annealing (54°C for CXCL8, 55°C for IL-1β, IL-6, TNF-α, CCL5, VCAM-1, ICAM-1, and GAPDH, and 58°C for BIRC-3, NFKB1A, SOD2, 1 min. in each case) and extension (72°C for 1 min.) and a final elongation step of 72°C for 5 min. PCR products were separated on 1.8% agarose gels containing 0.5 µg/ml ethidium bromide and photographed under ultraviolet light. Densitometry analysis was done by using the Image J software (Version 1.50i).

Statistical analysis. All data, are representative of at least two independent experiments in the case of animal model and three independent experiment in the case of *in vitro* assay. Data are expressed as the means plus standard deviations. Significant differences between treatments were analysed by one-way analysis of variance (ANOVA) followed by a Dunnett multiple comparison tests, and a Kruskal-Wallis test at $p<0.05$, by using Prism 5.0a (GraphPad, San Diego, CA) software.

Results

Expression of cytokine and chemokine genes at the site of inoculation

Inoculation of *T. dimidiata* faeces in Balb/c mice induces the expression of four mediators out of

Table 2. Cytokine expression profile at the site of mono-exposure to excretions of *T. dimidiata* contaminated or not with *Trypanosoma cruzi* in Balb/c mice

Time after inoculation	CXCL2 MIP-2 (SD)	CCL5 RANTES (SD)	CXCL3 (SD)	TNF-α (SD)
1hr	*0.22 ± 0.05	0.04 ± 0.006	0.4 ± 0.08	*0.19 ± 0.06
24hr	Neg	0.02 ± 0.004	Neg	0.13 ± 0.01
1hr^{p}	*0.08 ± 0.04	0.02 ± 0.005	0.6 ± 0.07	*0.37 ± 0.07
24hr^{p}	Neg	0.02 ± 0.002	*0.08 ± 0.009	0.30 ± 0.009

h = hours; Neg = negative; ^{p} = faeces + metacyclic trypomastigotes; the following cytokines not included in the table were negative: IL-β, IL-14, IL-10, IL-17, INF-γ, TGF-β and iNOS; SD (standard deviation); * = $p<0.05$ (means ± SD) of three experiments.

eleven tested. No expression was detected for IL-β, IL-14, IL-10, IL-17, INF-γ, TGF-β.

In the mono-exposure group (mice exposed once to faeces), the TNF-a gene expression was more intense with faeces contaminated with metacyclic trypomastigotes for both experimental times (1h and 24h) than with faeces free of metacyclic trypomastigotes. However, in mice multi-exposed to faeces a significantly reduce expression of TNF-α gene was observed if metacyclic trypomastigotes are present (Fig. 1, Tables 2 and 3).

In the case of macrophage inflammatory protein (MIP)-2 or CXCL2 its expression in mono-exposed group is down-regulated if metacyclic trypomastigotes are present. Whereas in multi-exposed animals there were no differences (Fig. 1).

The expression of CXCL3 and CCL5 was similar between both groups, however expression of CCXL3 was down-regulated in the presence of metacyclic trypomastigotes in multi-exposed mice (Fig. 1).

***T. dimidiata* faeces induce the production of pro-inflammatory molecules in HeLa cells**

The results showed that faeces induce secretion of IL-6, CXCL8 and CCL5 either contaminated or not with *T. cruzi*; however, the Hela cells did not respond to faeces producing IL-1β and TNF-α. Notably, the secretion of CXCL8 was down-regulated if parasites are present (Fig. 2).

The expression of genes of early response to stress such as NFKBIA, mitochondrial superoxide dismutase 2 (SOD2) and BIRC-3 were higher in the presence of faeces either contaminated or not with *T. cruzi* (lanes 4 and 5 and columns 4 and 5) at 6 hours compared with controls and triatoma urine (Fig. 3, lanes 2 and 3).

However, if we compare only faeces at 18 hrs, the expression of BIRC-3 and SOD2 was positively influence in the presence of metacyclic trypomastigotes, but down-regulated for NFKBIA gene expression. Finally, we did not observe changes in VCAM-1 and ICAM-1 expression.

Table 3. Cytokine expression profile at the site of multiexposure to excretions of *T. dimidiata* contaminated or not with *Trypanosoma cruzi* in Balb/c mice

Time after inoculation	CXCL2 (MIP-2)	CCL5 (RANTES)	CXCL3	TNF-α
1hr	0.110 ± 0.05	0.03 ± 0.001	1.2 ± 0.06	*1.13 ± 0.08
24hr	Neg	0.02 ± 0.003	0.19 ± 0.008	0.30 ± 0.04
1hr^{p}	0.14 ± 0.06	0.02 ± 0.003	*0.56 ± 0.01	*0.36 ± 0.01
24hr^{p}	Neg	Neg	Neg	0.31 ± 0.07

h = hours; Neg = negative; ^{p} = faeces + metacyclic trypomastigotes; the following cytokines not included in the table were negative: IL-β, IL-14, IL-10, IL-17, INF-γ, TGF-β and iNOS; SD = standard deviation; * = $p<0.05$ (means ± SD) of three experiments.

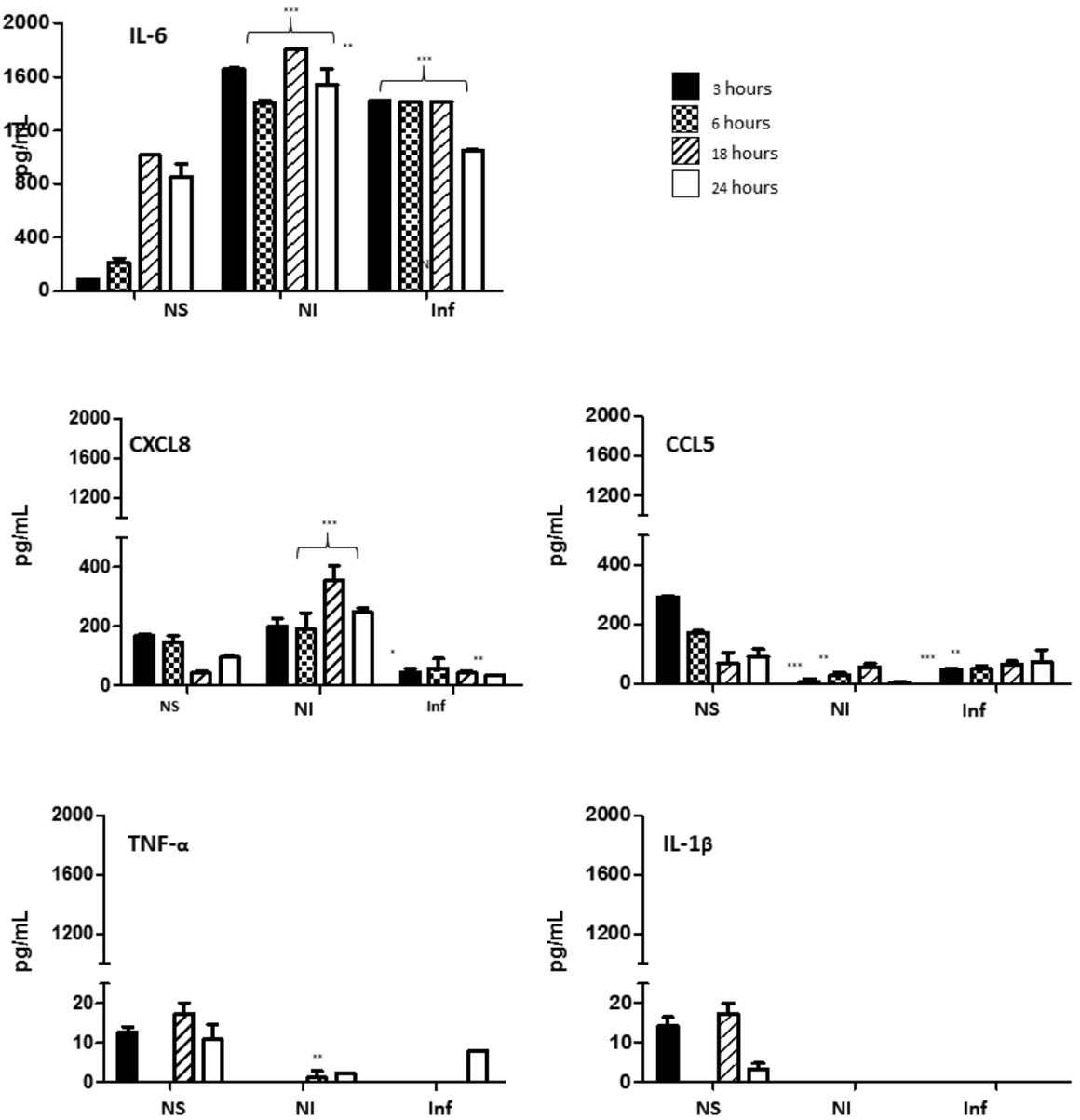

Figure 2. Profile of cytokines and chemokines induced by *T. dimidiata* faeces contaminated or not with *Trypanosoma cruzi* from HeLa cells

HeLa cells were stimulated with faeces from *T. dimidiata* NI (Uninfected), Inf (Infected) and NS (Non-stimulated), for 3, 6, 18 and 24h. Cytokines (IL-1β, IL-6 and TNF-α) and chemokines (CXCL8 and CCL5) were measured in cells culture supernatants by ELISA. Graphs show values in pg/ml (means ± SD) of three experiments run in duplicate.

Discussion

During the transmission of *T. cruzi* parasite as well as in the vector feeding process, the skin plays an important role as a physical barrier and in the inflammation reaction; the chagoma is a local inflammatory response to triatoma's fluids. The skin cells trigger a response that is decisive for the establishment of the infection [7,8].

Insect´s bites can cause an immediate skin reaction [9], most hematophagous insects secrete a saliva rich in proteins, which has different substances with anticoagulant properties, antihistamines, vasodilators and antiplatelet agents that facilitate their vertebrate host feeding process, and at the same time may favour the transmission of parasites [3]. In another recent paper, it was demonstrated that faeces inoculated in the skin

induces a strong inflammatory reaction characterized by the presence of neutrophils and macrophages at early times after inoculation. This inflammatory reaction seems to play a role diminishing the infection when mice are challenged with metacyclic trypomastigotes [7].

In our work, we found that cells at the inoculation site are able to respond to faeces inducing the expression of cytokines such as TNF-α and chemokines such as CCL5, CXCL2 and CXCL3 that are associated with the recruitment of different immune cells. It is known that chemokine that belongs to the GRO subfamily of CXC chemokines such as GROβ (CXCL2), and GROγ (CXCL3) which is produced by macrophages, monocytes, epithelial cells and assist in the recruitment of polymorphonuclear neutrophils (PMNs) to sites of injury or infection. In addition above-mentioned chemokines the persistence expression of TNF-α at the inoculation site increases the vascular permeability facilitating the recruitment of cells such as neutrophils and monocytes that are predominant in the infiltration foci as previously reported [7].

On the other hand, the CCL5 expression ensures the recruitment of immune cells to the site of exposure such as T lymphocytes, eosinophils and basophils [10]. In our work, we observed a light expression of this chemokine that correlated with the infiltrating cell type observed in previous work [7].

An interesting observation was that the presence of metacyclic trypomastigotes in faeces reduces the expression of CXCL2 and CXCL3 at the inoculation site (Table 2 and 3). However, the expression of TNF-α increased in the presence of the parasite in the mono-exposed group at 1 h post inoculation, but decreased in the multi-exposed group. A possible explanation for this finding may be that in multi-exposed group, the memory immune response is already mounted and the memory effector cells infiltrated at the inoculation site can downregulate the TNF-α production, but this hypothesis deserves proof.

On the other hand, epithelial cells are actively contributing to the immune system secreting several molecules related to the immune response, HeLa cells have been used as a model for studies of interaction with *T. cruzi* in short times [11].

In this work, we used the HeLa cell model to investigate the physical interactions between the epithelial cells and the faeces of *T. dimidiata* and their effect on the immune response. The results

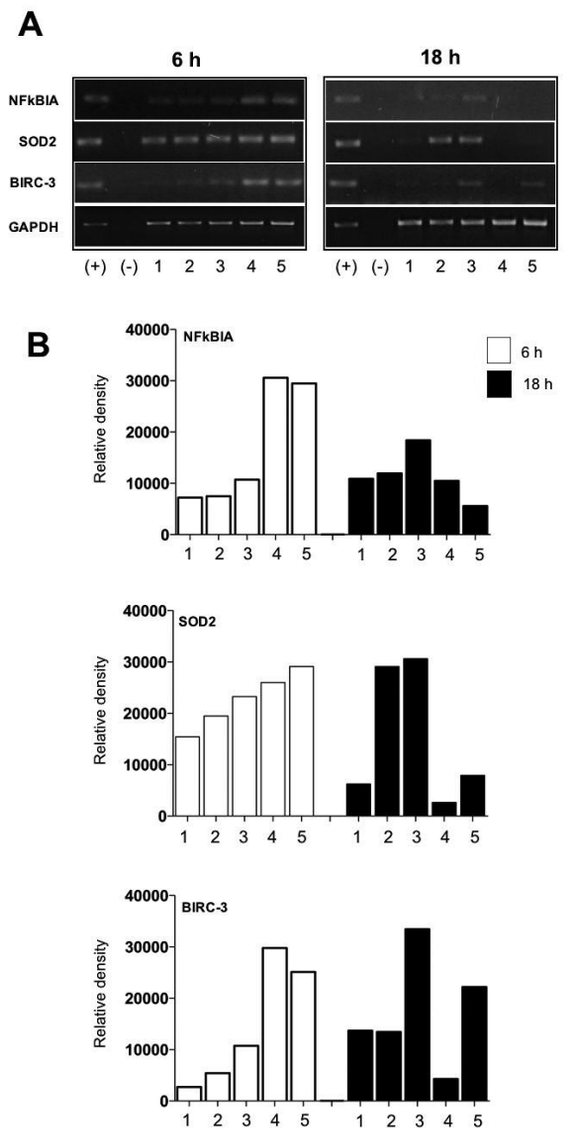

Figure 3. Effect of faeces from *T. dimidiata* on the expression of cellular stress genes
A. RT-PCR analysis of NFkBIA, SOD2, and BIRC-3 mRNAs in HeLa cells was performed as described. HeLa cells were stimulated with faeces for 6 and 18h; (+) positive control, (-) negative control (non-DNA); 1) Non-stimulated; 2) Uncontaminated bug urine; 3) Bug urine contaminated with *T. cruzi*; 4) Uncontaminated bug faeces; 5) Bug faeces contaminated with *T. cruzi*.
B. Densitometric analysis. The intensities of each band were quantified and plotted from the gels shown in panel A corresponding to the expression of genes, the expression was expressed as relative density. GAPDH was used as control housekeeping gene.

showed that the interaction of the fluids with the cells did not induce the production of IL-1β and TNF-α. *Triatoma* faeces contain many bacterial, LPS can initiate acute inflammatory responses that are

typical of the host reaction to tissue injury or infection [12], so it is expected that the production of these cytokines and chemokines may be induced by the presence of the bacteria or their components in the insect fluids. The production of TNF-α is significantly correlated with signalling through TLR4/MD2. However, HeLa cells express TLR4 but not TLR2 or MD2, which is necessary for the response by TLR4 [13]. The above information may explain our findings in HeLa cells.

On the other hand, CXCL8 chemokine was stronger stimulated either in the presence or not of *T. cruzi*, it is known that CXCL8 is a potent stimulator of angiogenesis and capable of directing the migration of neutrophils and can be active for a long period of time [14].

Furthermore, from the above the role of epithelial cells as sentinels and triggering of the inflammatory process is observed [7].

The CCL5 plays an active role in recruiting a variety of leukocytes into inflammatory sites including T cells, macrophages, eosinophils and basophils. [15], and is a major component in chronic inflammatory processes [16]. In our work, we found in *in vitro* assay of Hela cells, that CCL5 secretion was not stimulated with faeces free of *T. cruzi* at early times, but the presence of *T. cruzi* induces its expression, probably *T. cruzi*-derived molecules such as trypomastigote-derived glycosylphosphatidylinositol (tGPI) anchors of mucin-like glycoproteins can play a role as reported [17]. This finding suggests that epithelial cells can respond to *T. cruzi* antigens as seen in our *in vivo* model.

We observed expression of genes of early response to stress such as NFKBIA at short times (6h), this upregulation in the expression of this gene could be associated with a defence mechanism regulating the inflammatory response in the cell due to an excessive stimulus provided by the content of the faeces. The expression of the SOD2 gene may be involved in protection against cell death [18]. As a result, this protein plays an antiapoptotic role against oxidative stress, ionizing radiation, and inflammatory cytokines [19]. On the other hand, the BIRC3 gene codes for the apoptosis-2 inhibitory protein (cIAP-2) which together with cIAP-1 are influential cell survival proteins. The expression of these genes in our model suggests a balance between activation of defence mechanism by the generation of oxygen reactive compounds and the cell survival. IAPs not only regulate caspases and apoptosis but also modulate inflammatory signalling and immunity [20], but gene expression of early response to stress markedly declined at 18h and solely buffered by the presence of parasites.

The results obtained in this work show that immediately after the contact of faeces of *T. dimidiata* and the skin cells. They induce the expression of early response genes involved in cell defence such as TNF-α, CXCL2, CXCL3 and CXCL8.

Activation of transcription factors that are able to regulate activities such as inflammation, immune response and apoptosis, but on the other side, the presence of metacyclic trypomastigotes can interfere with this inflammatory response.

In conclusion, the triatomine' fluids participate in the development of early immune response of the host that combined with the presence of the parasite, the outcome of infection can be modified.

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References

[1] Guhl F., Pinto N., Aguilera G. 2009. Sylvatic triatominae: a new challenge in vector control transmission. *Memórias do Instituto Oswaldo Cruz* 104 (Suppl. 1): 71-75. doi:10.1590/S0074-02762009000900012

[2] Farfán-García A.E., Angulo-Silva V.M. 2011. Conducta alimentaria de poblaciones de *Triatoma dimidiata* (Hemiptera: Reduviidae: Triatominae) en una zona endémica y sus implicaciones epidemiológicas [*Triatoma dimidiata* populations' (Hemiptera: Reduviidae: Triatominae) feeding behaviour in an endemic zone and related epidemiological implications]. *Revista de Salud Pública* 13: 163-172 (in Spanish with summary in English). doi:10.1590/S0124-00642011000100014

[3] Mesquita R.D., Carneiro A.B., Bafica A., Gazos-Lopes F., Takiya C.M., Souto-Padron T., Vieira D.P., Ferreira-Pereira A., Almeida I.C., Figueiredo R.T., Porto B.N., Bozza M.T., Graça-Souza A.V., Lopes A.H.C.S., Atella G.C., Silva-Neto M.A.C. 2008. *Trypanosoma cruzi* infection is enhanced by vector saliva through immunosuppressant mechanisms mediated by lysophosphatidylcholine. *Infection and Immunity* 76: 5543-5552. doi:10.1128/IAI.00683-08

[4] Lima M.S., Carneiro A.B., Souto-Padron T., Jurbergd J., Silva-Neto M.A.C., Atella G.C. 2018. *Triatoma infestans* relies on salivary lysophosphatidylcholine to enhance *Trypanosoma cruzi* transmission. *Acta Tropica* 178: 68-72. doi:10.1016/j.actatropica.2017.10.022

[5] Monteón V.M., Furuzawa-Carballeda J., Alejandre-Aguilar R., Aranda-Fraustro A., Rosales-Encina J.L., Reyes P.A. 1996. American trypanosomiasis: *in situ* and generalized features of parasitism and inflammation kinetics in a murine model. *Experimental Parasitology* 83: 267-274. doi:10.1006/expr.1996.0074

[6] Monteon V., Hernández O., López R., Reyes P.A. 2009. Cytokine expression at the inoculation site and nearby tissues in an animal model infected with metacyclic trypomastigotes of *Trypanosoma cruzi*. *Tropical Medicine and Health* 37: 141-147. doi:10.2149/tmh.2009-03

[7] Monteon V., Quen-Rámirez E., Macedo-Reyes V., Lopez R., Acosta-Viana K., Pennigton P., Ramos-Ligonio A. 2016. 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. *Annals of Parasitology* 62: 209-219. doi:10.17420/ap6203.54

[8] Mendes M.T., Carvalho-Costa T.M., da Silva M.V., Anhç A.C.B.M., Guimarães R.M., da Costa T.A., Ramirez L.E., Rodrigues V., Oliveira C.J.F. 2016. Effect of the saliva from different triatomine species on the biology and immunity of TLR-4 ligand and *Trypanosoma cruzi*-stimulated dendritic cells. *Parasites and Vectors* 9: 634. doi:10.1186/s13071-016-1890-x

[9] Fradin M.S., Carroll S.P. 2012. Chapter 47: Protection from blood-feeding arthropods. In: *Wilderness Medicine*. (Ed. P.S Auerbach). 6th ed. Elsevier Saunders, Philadelphia, PA, USA: 872-882.

[10] Krensky A.M., Ahn Y.T. 2007. Mechanisms of disease: regulation of RANTES (CCL5) in renal disease. *Nature Clinical Practice Nephrology* 3: 164-170. doi:10.1038/ncpneph0418

[11] Caradonna K.L., Burleigh B.A. 2011. Chapter 2: Mechanisms of host cell invasion by *Trypanosoma cruzi*. *Advances in Parasitology* 76: 33-61. doi:10.1016/B978-0-12-385895-5.00002-5

[12] Martich G.D., Boujoukos A.J., Suffredini, A.F. 1993. Response of man to endotoxin. *Immunobiology* 187: 403-416. doi:10.1016/S0171-2985(11)80353-0

[13] Wyllie D.H., Kiss-Toth E., Visintin A., Smith S.C., Boussouf S., Segal D.M., Duff G.W., Dower S.K. 2000. Evidence for an accessory protein function for Toll-like receptor 1 in anti-bacterial responses. *Journal of Immunology* 165: 7125-7132. doi:10.4049/jimmunol.165.12.7125

[14] Russo R.C., Garcia C.C., Teixeira M.M., Amaral F.A. 2014. The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. *Expert Review of Clinical Immunology* 10: 593-619. doi:10.1586/1744666X.2014.894886

[15] Lv D., Zhang Y., Kim H.-J., Zhang L., Ma X. 2013. CCL5 as a potential immunotherapeutic target in triple-negative breast cancer. *Cellular and Molecular Immunology* 10: 303-310. doi:10.1038/cmi.2012.69

[16] Marques R.E., Guabiraba R., Russo R.C., Teixeira M.M. 2013. Targeting CCL5 in inflammation. *Expert Opinion on Therapeutic Targets* 17: 1439-1460. doi:10.1517/14728222.2013.837886

[17] Campos M.A.S., Almeida I.C., Takeuchi O., Akira S, Valente E.P., Procópio D.O., Travassos L.R., Smith J.A., Golenbock D.T., Gazzinelli R.T. 2001. Activation of toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. *Journal of Immunology* 167: 416-423. doi:10.4049/jimmunol.167.1.416

[18] Pias E.K., Ekshyyan O.Y., Rhoads C.A., Fuseler J., Harrison L., Aw T.Y. 2003. Differential effects of superoxide dismutase isoform expression on hydroperoxide-induced apoptosis in PC-12 cells. *Journal of Biological Chemistry* 278: 13294-13301. doi:10.1074/jbc.M208670200

[19] Becuwe P., Ennen M., Klotz R., Barbieux C., Grandemange S. 2014. Manganese superoxide dismutase in breast cancer: from molecular mechanisms of gene regulation to biological and clinical significance. *Free Radical Biology and Medicine* 77: 139-151. doi:10.1016/j.freeradbiomed.2014.08.026

[20] Gyrd-Hansen M., Meier P. 2010. IAPs: from caspase inhibitors to modulators of NF-κB, inflammation and cancer. *Nature Reviews Cancer* 10: 561-574. doi:10.1038/nrc2889

[21] Morán-Utrera Y., López-Monteon A., Rosales-Encina J.L., Méndez-Bolaina E., Ramos-Ligonio A. 2012. *Trypanosoma cruzi* SSP4 amastigote protein induces expression of immunoregulatory and immunosuppressive molecules in peripheral blood mononuclear cells. *Journal of Tropical Medicine* 2012: 829139. doi:10.1155/2012/829139

[22] Moazzezy N., Oloomi M., Bouzari S. 2014. Effect of shiga toxin and its subunits on cytokine induction in different cell lines. *International Journal of Molecular and Cellular Medicine* 3: 108-117. http://ijmcmed.org/article-1-148-en.html

[23] Chiribao M.L., Libisch G., Parodi-Talice A., Robello C. 2014. Early *Trypanosoma cruzi* infection reprograms human epithelial cells. *Biomed Research International* 2014: 439501. doi:10.1155/2014/439501

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