Molecular Immunology 106 (2019) 36–45

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Molecular variants of *Leishmania (Viannia) braziliensis* trigger distinct patterns of cytokines and chemokines expression in golden hamster

Felipe Dutra Rêgo[a,*], Jordana Rodrigues Barbosa Fradico[b], Andréa Teixeira-Carvalho[b,1], Célia Maria Ferreira Gontijo[a,1]

[a] Grupo de Estudos em Leishmanioses, Instituto René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), Av. Augusto de Lima, 1715 Barro Preto, CEP 30190–002, Belo Horizonte, Minas Gerais, Brazil

[b] Grupo Integrado de Pesquisas em Biomarcadores, Instituto René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), Av. Augusto de Lima, 1715 Barro Preto, CEP 30190–002, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
Cutaneous leishmaniasis
Cytokines
Chemokines
Genetic-variant strains
L. braziliensis

ABSTRACT

Cutaneous leishmaniasis (CL) mainly caused by *Leishmania braziliensis* is a chronic inflammatory disease widely spread in Brazil. Genetic variant strains of this parasite have been associated with atypical clinical manifestations of CL in an endemic area in Brazil. Furthermore, these strains have presented distinct biological behaviors in golden hamster, suggesting differential activation of the immune response. In the present study we proposed to evaluate the localized immune response in golden hamsters infected with known molecular variant strains of *L. braziliensis*, in distinct time points post-infection (PI). Detailed analyses of the mRNA expression of cytokines and chemokines in hamster-skin lesions were performed. Heat map matrix and hierarchical cluster analysis were carried out to segregate the strains due to mRNA expression. Distinct patterns of immune response were found in both time points, more evident in the recent-phase disease (30 days-PI). At this time point, the genetic variant strains expressed high levels of *tnfα*, *il12* and *tgfβ* whilst the non-variant strain expressed *ifnγ*, *il6*, *il4*, *il10, il13* and *ccl17*. The hierarchical clustering highlights this distinct pattern in which all genetic variant strain was grouped in the cluster I and the non-variant strain grouped into the cluster II. At late-phase disease (60 days-PI) all isolates expressed high levels of *il4* and *il10*. The non-variant strain shown a significant reduced expression of *ifnγ*, *il6*, *ccl17*, and *ccl22* whilst distinct patterns were observed for the genetic variant strains. For the first time, a large panel of cytokines and chemokines mRNA-expression was analyzed in experimental trials using golden hamsters as animal model and genetic variant strains of *L. braziliensis*. Our findings suggest that genetic variant strains of *L. braziliensis* are able to trigger differential gene expression of cytokines and chemokines in the skin lesion from infected hamsters. The parasite intrinsic ability to activate distinct pathways in the host-parasite interaction may be associated to the large spectrum of clinical manifestation observed in CL-patients.

1. Introduction

Cutaneous leishmaniasis (CL) caused by *Leishmania* parasites is considered one of the major public health problem in many regions of the world. These protozoan parasites infect human macrophages and cause a wide spectrum of clinical manifestations, dependent upon both the species of the parasite and the immune response to infection by the host (PAHO/WHO, 2017). CL could range from localized cutaneous and mucocutaneous leishmaniasis to diffuse form, characterized by widespread non-ulcerating nodules (Teixeira et al., 2006).

For the last decade, studies have been performed in an endemic focus of CL in the Xakriabá Indigenous Reserve (XIR), state of Minas Gerais, southeast of Brazil. A wide range of vertebrate hosts as well as potential vectors of *Leishmania* parasites have been reported (Quaresma et al., 2011; Rêgo et al., 2014, 2015). Distinct forms of CL can be found in this endemic focus such as localized cutaneous, mucosal, and disseminated leishmaniasis. However, the most striking find is the presence of atypical clinical manifestations that have been associated with genetic variants of *Leishmania braziliensis* (Quaresma et al., 2018). These atypical lesions have been characterized by the presence of unusual lesions such as vegetative, plaque, papule, verrucous, crusted, keloids and lupoid lesions that do not fit the classical well-defined ulcer. Recent CL-lesions have shown an intense inflammatory reaction, characterized by the presence of both mononuclear and

* Corresponding author.
E-mail address: felipedutra04@hotmail.com (F.D. Rêgo).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.molimm.2018.12.013
Received 12 June 2018; Received in revised form 7 November 2018; Accepted 9 December 2018
Available online 18 December 2018

Table 1
Characteristics of the *L. braziliensis* strains selected for this study.

Sample	Patient ID	Age (y)	Gender	Clinical form	Lesion type	# of lesions	*hsp70* PCR-RFLP profile	DNA sequencing
RR410	316	20	M	LCL	Ulcer and papule	2	≠Lb	=Lb
RR412	330	45	F	DL	Ulcer and papule	3	≠Lb	=Lb
RR415	340	13	F	LCL	Plaque	1	≠Lb	=Lb
RR418	346	30	M	LCL	Ulcer	1	≠Lb	=Lb
RR444	426	18	M	LCL	Ulcer	1	=Lb	=Lb

M, male; **F**, female; **LCL**, localized cutaneous leishmaniasis; **DL**, disseminated leishmaniasis; **(+)**, positive test; **≠Lb**, RFLP-profile distinct to *L. braziliensis*; **=Lb**, RFLP-profile similar to *L. braziliensis*. The characteristics of the isolates were extracted from Quaresma et al., (2018).

polymorphonuclear cells, whereas late CL lesions have exhibited a predominance of mononuclear leukocytes (Costa-Silva et al., 2014). Moreover, differential gene expression of cytokines and chemokines has been reported in the skin lesions from CL-patients associated with the time evolution of lesions. Important biomarkers have been associated with recent lesions, such as modulatory cytokines (*tgfβ1*), anti-inflammatory cytokine (*il10* and *il4*), and pro-inflammatory cytokine and chemokines (*tnfα, ifnγ, il12, ccl2, ccl3, ccl5* and *cxcl10*) (Costa-Silva et al., 2014).

This wealth of leishmaniasis outcomes in this endemic area led us to suspect that the intraspecific genetic variation among the *L. braziliensis* isolates could reflect in other aspects in the disease outcome. Previous study reinforces this hypothesis, showing that the genetic variant strains present distinct patterns of biological behavior in golden hamsters (Rêgo et al., 2018). Statistical differences in the parasite burden as well as in the lesion size, onset time of visible lesion and distinct histopathological changes have been reported suggesting that intrinsic aspects of each parasite strain could modulate the host immune response mainly in the early phase of infection (Rêgo et al., 2018). Studies performed with *L. braziliensis* strains from other endemic regions in Brazil support this idea. In an experimental trail using BALB/c mice, a comparative analysis of genetic variant strains of *L. braziliensis* have been performed in which infected popliteal lymph nodes cells have produced high levels of IL-12 and IL-10 for the isolate from the state of Bahia whilst IFN-γ production and *ccl2* expression were higher in cells infected with the isolate from the state of Ceará, leading to diverse cell recruitment and differential inflammatory responses (Indiani De Oliveira et al., 2004; Teixeira et al., 2005). However, in these studies a range of cytokines and chemokines have not been evaluated, which could help to better understand the mechanisms involved in the host-immune response. Furthermore, the golden hamster, the animal model for experimental trails with *L. braziliensis* has not been used (Gomes-Silva et al., 2013), remaining until then, little evidences about the relationship between variant strains and their ability to activate distinct pathways in animal model.

The golden hamster reproduces many of the clinical and histopathological features observed in the human disease (Gomes-Silva et al., 2013; Kahl et al., 1991; Osorio et al., 2003). Furthermore, its outbred genetic background highlights intrinsic characteristics, that could reproduce the heterogeneity of clinical outcomes observed in human CL (Ribeiro-Romão et al., 2014). Recently, Ribeiro-Romão et al., (2016) have been standardized a molecular assay to quantify the gene expression of immunological markers in hamsters infected by *L. braziliensis*. This study provides an important tool to better understand the host-parasite relationship, in an attempt to explain the phenomena that occur in human disease, and to establish parameters to evaluate vaccine and new drug candidates.

In this study, we explore the relationship between variant genetic strains of *L. braziliensis* and immune response in golden hamsters. We evaluated the localized immune response in hamsters infected with genetic variant isolates of *L. braziliensis* from the XIR through the analysis of cytokines and chemokines expression in distinct time points post-infection (PI). The results indicate that mainly in the early-phase disease, the variant strains presented different patterns of mRNA expression and did not clustered with the non-variant strain, as previously observed in the study of biological behavior (Rêgo et al., 2018). Although in the late-phase disease differences in mRNA expression remained evident, the variant and non-variant strains grouped in the same cluster. Our results indicate that genetic variant strains of *L. braziliensis* are able to unleash differential expression of cytokines and chemokines and these distinct patterns might contribute to the wide spectrum of CL presentations as well as in the disease outcome.

2. Material and methods

2.1. Animals and ethics statements

Seventy-two male 4-6-week-old golden hamsters (*Mesocricetus auratus*) weighing 80–100 grams were obtained from Instituto René Rachou/FIOCRUZ Animal Facility, where they were maintained under controlled environmental conditions. All procedures involving experimental animals were approved by the Ethics Committee on Animal Use (CEUA/Fundação Oswaldo Cruz; License number LW 02/16) and were conducted following the guidelines of the Brazilian College for Experiments with Animals (COBEA - Law 11.794/2008). Ethical statements involving the collection of biological samples in humans have been obtained in a previous study (Quaresma et al., 2018). Here, all patient data was anonymized.

2.2. Parasite culture

The *L. braziliensis* strains (Table 1) have been isolated from skin lesions of ACL-patients from XIR after brief passages in culture medium. All isolates have been identified as *L. braziliensis* by Multilocus Enzyme Electrophoresis technique, the *hsp70* PCR-RFLP and genetic sequencing of the same amplicon (Quaresma et al., 2018). In addition to these samples, *L. braziliensis* MHOM/BR/75/M2903 were also used as reference strain in experimental infections. Promastigotes cultures were grown in NNN/LIT medium (Quaresma et al., 2018).

2.3. Inoculation of parasites into golden hamsters

Due the fact that the isolates have been maintained in cryobank since they were characterized as *L. braziliensis*, we perform previous inocula in golden hamster to recover the infectivity of each isolate. Promastigote forms from stationary-phase were inoculated intradermally into the right hind paw of the hamsters at a dose of 1×10^6 parasites in 200 µL of phosphate saline buffer (PBS). After about 40 days, the animals were euthanized and the skin lesions were harvested, macerated in PBS and maintained in NNN/LIT medium at 25 °C ± 1 °C.

In this study, parasites of the third passage *in vitro* after the previous passage in hamster were used with the same conditions described above for the inocula. The infectivity of each parasite strain has been evaluated following distinct parameters (Rêgo et al., 2018). The sample growth curve of each strain was used to define the stationary phase of growth *in vitro* and was performed daily until the twentieth in flow cytometry using size and granularity parameters (Rêgo et al., 2018). To better characterize the dynamic of the immune response after infection

of animals with distinct strains, we have selected two distinct time points to evaluate the both immune profile relate to the early (30 days PI; six animals per strain) as well as late (60 days PI; six animals per strain) immune events triggered after hamster´s infection."

2.4. RNA extraction and reverse transcription

Hamsters-skin lesion fragments (20 to 30 mg per sample) from both PI-periods were collected, immediately mounted in Tissue-Tek® OCT compound (Miles Scientific, IL, USA) and cryopreserved. For the RNA extraction step, the RNeasy® mini kit (Qiagen, Austin, Texas, USA) was used following the manufactory instructions. After the extraction step the RNA was stored at −80 °C until use. The RNA was quantitated using Qubit® RNA HS Assay Kit (Life Technologies, Eugene, Oregon, USA) and then was reverse transcribed (final concentration = 100 ng) using the ImProm-II™ Reverse Transcription System kit (Promega, Maddison, USA). The cDNA concentration was also quantitated using Qubit® ssDNA Assay Kit and adjusted to a final concentration of 100 ng/μL.

2.5. Gene expression analysis by reverse transcription-quantitative real-time PCR (RT-qPCR)

RT-qPCR was performed for relative quantitative assessment based on sequences of primers previously described (Table 2). In the real-time PCR assays, 2 μl of cDNA (concentration = 10 ng/μL) were used in a final reaction volume of 10 μL, with 5.0 μL of Power SYBR Green PCR Master Mix 2X (Life Technologies,CA, USA). RTqPCR was performed with an activation step at 95 °C for 10 min, followed by 40 cycles of denaturation and annealing/extension (95 °C for 15 s and 60 °C for 1 min). Reactions without the reverse transcriptase enzyme (No-RT reactions) were performed to control DNA contamination. A melt curve stage was performed for each specific amplification analysis. All reactions were performed in duplicate in a Step One Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Gene expression was calculated by relative quantitation by comparative Ct method ($^{\Delta\Delta}$Ct), using the housekeeping *gapdh* and γ*actin* as reference genes and uninfected-skin samples of each animal as group control (Livak and Schmittgen, 2001; Ribeiro-Romão et al., 2016).

2.6. Statistical analysis

Statistical analyses were initially performed using GraphPad Prism (GraphPad Software 6.0, San Diego, CA, USA). To check Gaussian distribution, D'Agostino & Pearson omnibus normality test were performed. The comparative analysis of gene expression levels among the strains and between time points PI, was performed by Kruskal-Wallis followed by Dunn's post-test. Pearson's correlation test was performed to analyze positive and negative correlations between variables. For all tests, significant differences were considered at two-tailed $p < 0.05$. The biomarker cluster patterns were defined by heat maps assembled using GraphPad Prism, using the values of relative quantification of cytokines and chemokines normalized by Z-Score. Hierarchical clustering was carried out using the IBM SPSS Statistics software (Chicago, IL, USA) by squared-Euclidean distance to segregate the strains.

3. Results

3.1. Gene expression profile of cytokines and chemokines at 30 days-PI

Thirty days post-infection, cells from RR444-infected hamster expressed the highest levels of both *ifn*-γ and *il-6* ($p < 0.05$). Cells from the infected hamsters with the strains RR410, RR412, RR415 and RR418 showed a significant increase in *tnf-α* and *il-12* expression in contrast to *ifn*γ and *il6* (Fig. 1). Cells from RR444-infected hamster also expressed higher amounts of the regulatory cytokines *il4*, *il10* and *il13* than the other strains, showing a significant upregulation at this time point. The expression of *tgfβ* was higher in animals infected with RR415 and RR418-strains when compared with the other strains ($p < 0.001$), while *il17* gene was markedly expressed in cells from RR410-infected animals ($p < 0.001$). The development of Th2-mediated immune responses by the expression of *il21* was upregulated in cells from RR412 and RR415-infected hamsters, and downregulated in cells from RR418

Table 2
Primers sequences and standard curve parameters used for gene expression analysis by RT-qPCR in golden hamsters infected by *L. braziliensis*-strains.

Gene	Primer sequences (5' - 3')	Primer concentration (μM)	Product length (bp)	Melting temperature	Reaction efficiency (%)	R^2	Reference
gapdh	Fw: GGTTGCCAAACCTTATCAGAAATG Rv: TTCACCTGTTCCACAGCCTTG	10	194	60	100.2	0.98	Ribeiro-Romão et al., 2014
γ*actin*	Fw: ACAGAGAGAAGATGACGCAGATAATG Rv: GCCTGAATGGCCACGTACA	40	70	60	101.7	0.99	Espitia et al., 2010
il4	Fw: ACAGAAAAAGGGACACCATGCA Rv: GAAGCCCTGCAGATGAGGTCT	120	95	60	98.8	0.97	Espitia et al., 2010.
il6	Fw: GGACAATGACTATGTGTTGTTAGAA Rv: AGGCAAATTTCCCAATTGTATCCAG	40	99	60	98.2	0.99	Ribeiro-Romão et al., 2016
il10	Fw: GAGCATTTGAACCTAG Rv: CTTGAAGACGCCTTTCTC	2.5	194	60	97.5	0.98	Moreira, 2012
il12p40	Fw: GACCAGTCCACCTCTACAAC Rv: GCAGCCAAGCAAGATGTG	2.5	88	60	102.9	0.96	Moreira, 2012
il13	Fw: AAATGGCGGGTTCTGTGC Rv: AATATCCTCTGGGTCTTGTAGATGG	40	81	60	96.5	0.98	Espitia et al., 2010.
il17	Fw: AAGGCAGCAGCGATCATCC Rv: GGAACGGTTGAGGTAGTCTGAG	40	92	60	98.3	0.97	Ugur et al., 2014
il21	Fw: GGACAGTGGCCCATAAAACAA G Rv: TTCAACACTGTCTATAAGATGACGAAGTC	40	80	60	97.1	0.99	Espitia et al., 2010.
tnfα	Fw: TGAGCCATCGTGCCAATG Rv: AGCCCGTCTGCTGGTATCAC	40	79	60	99.3	0.99	Espitia et al., 2010.
tgfβ	Fw: GGCTACCACGCCAACTTCTG Rv: GAGGGCAAGGACCTTACTGTACTG	40	81	60	99.5	0.98	Espitia et al., 2010.
*ifn*γ	Fw: TGTTGCTCTGCCTCACTCAGG Rv: AAGACGAGGTCCCCTCCATTC	40	130	60	100.8	0.99	Espitia et al., 2010.
ccl17	Fw: GTGCTGCCTGGAGATCTTCA Rv: TGGCATCCCTGGGACACT	40	89	60	99.5	0.99	Espitia et al., 2010.
ccl22	Fw: TGGTGCCAACGTGGAAGAC Rv: GAAGAACTCCTTCACTACGCGC	40	82	60	98.1	0.99	Espitia et al., 2010.

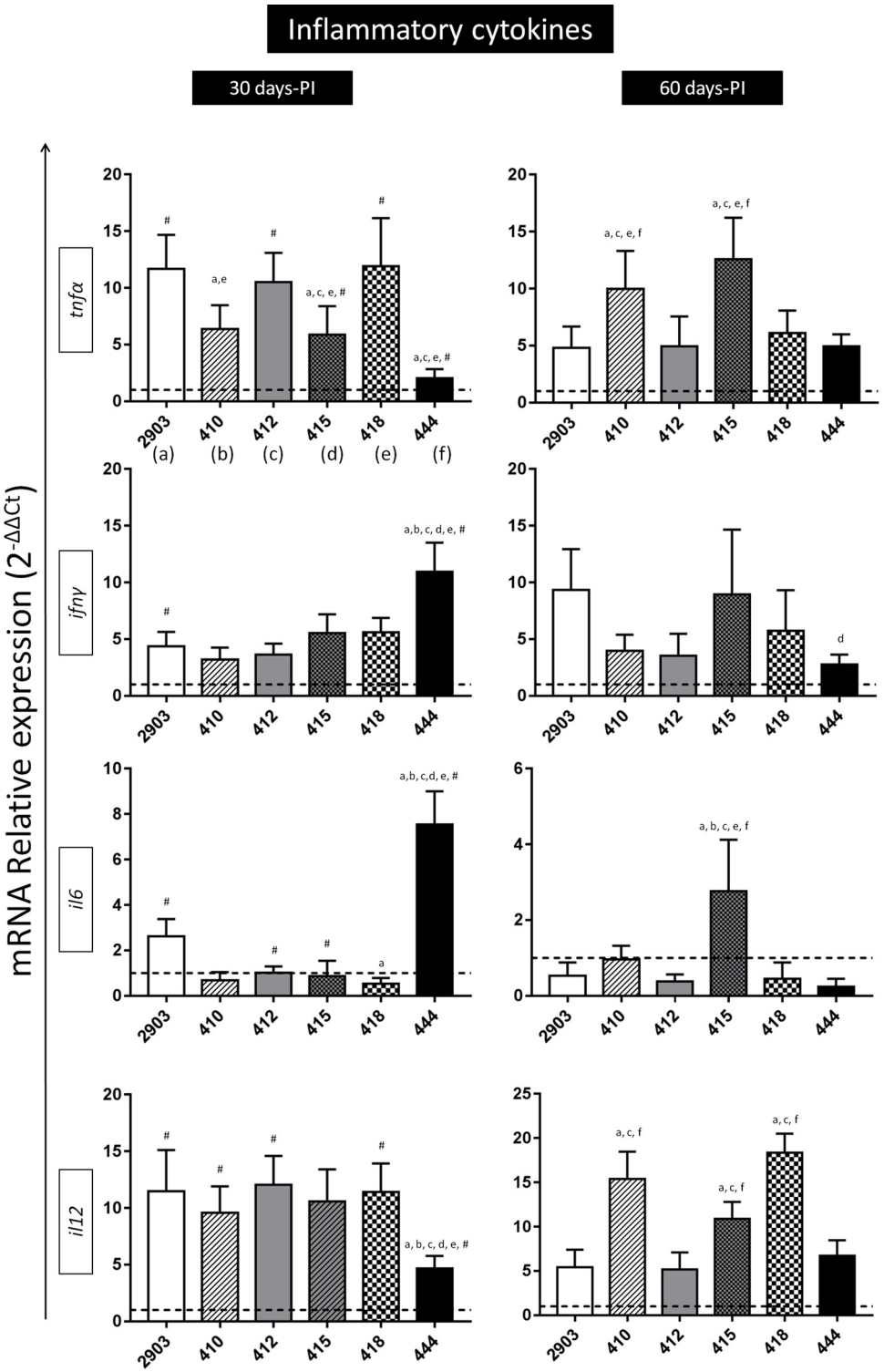


Fig. 1. Gene expression profile of inflammatory cytokines in skin lesions of infected golden hamsters at 30 and 60 days post-infection (30 days-PI and 60 days-PI, respectively). The results are presented in a column chart format and the relative quantification was performed by the comparative Ct method ($^{\Delta\Delta}$Ct), using skin of uninfected hamsters as group control (Fold change = 1), as indicated by the dotted line. Horizontal bars represent median ± standard deviation of $^{\Delta\Delta}$Ct for each strain. Statistical differences between groups were considered significant when $p < 0.05$. In each graph, significant differences among the strains were represented as a specific letters (M2903 = a; RR410 = b; RR412 = c; RR415 = d; RR418 = e; RR444 = f) and differences of gene expression between time point post-infections were represented as "#" symbol.

and RR444 (Fig. 2).

Cells of animals infected with the strain RR412 expressed higher levels of *ccl2* in comparison to the others strains evaluated ($p < 0.05$), and the increase in *ccl5* expression was not different among the strains. *ccl17* was upregulated in cells from RR412 and RR444-infected hamsters ($p < 0.05$) and the expression of high amounts of *ccl22* was observed for RR412, RR415, RR 418 and RR444, with significant difference to RR410 (Fig. 3).

3.2. Gene expression profile of cytokines and chemokines at 60 days-PI

Sixty days post-infection, animals infected with RR410, RR415 and RR418 expressed a markedly Th1 response as shown by the significant expression of *tnfα* and *il12*. Cells from RR415-infcted hamsters expressed high levels of *il6* when compared to the other strains. The expression of *ifn*γ was lower in animals infected with RR444 than from RR415 infected cells (Fig. 1). The regulatory cytokines was markedly upregulated as shown by the expression of *il10* and *il17* for all strains as well as *il13* for RR444. *il4* expression was significant higher in RR410

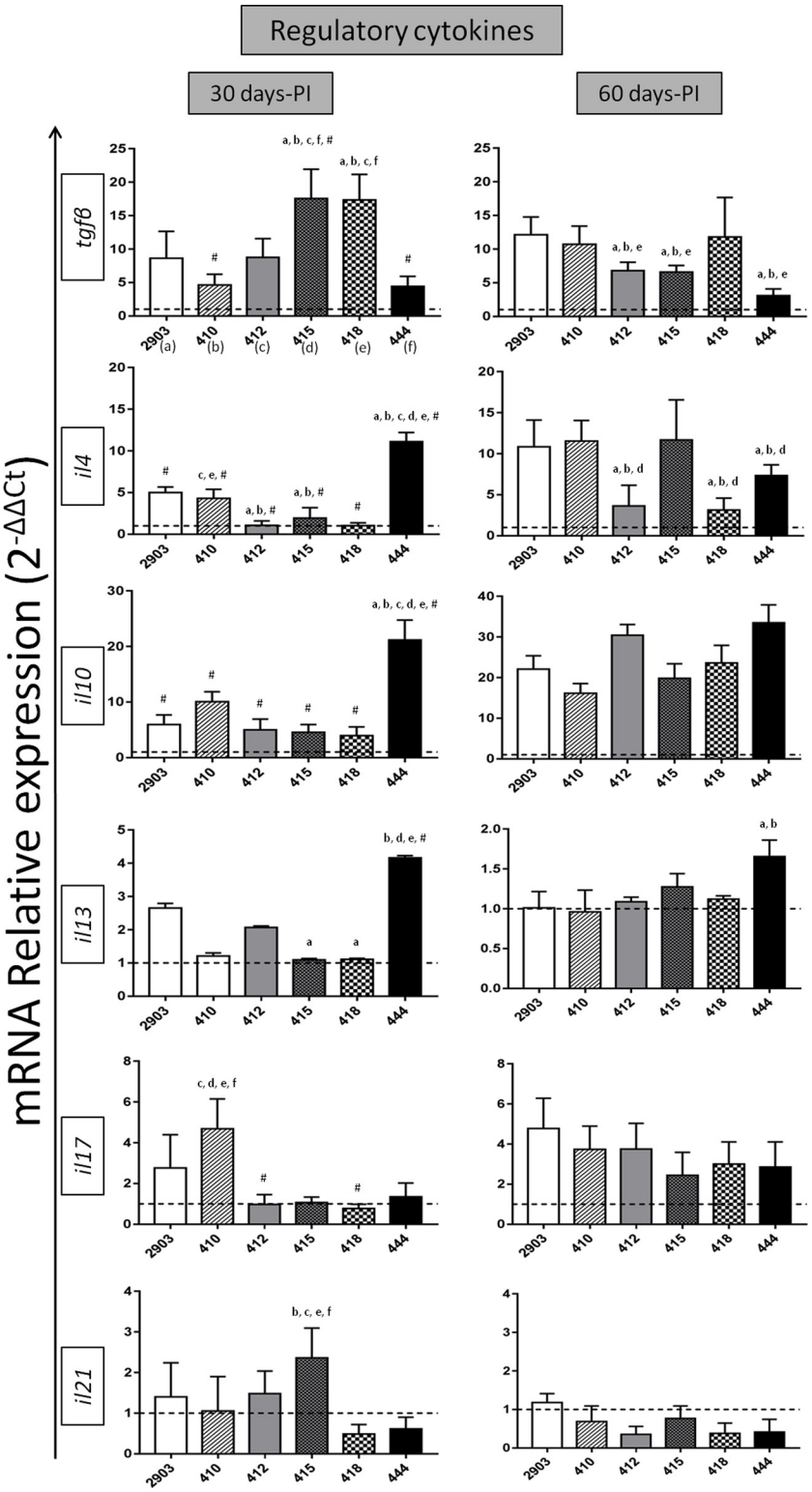


Fig. 2. Gene expression profile of regulatory cytokines in skin lesions of infected golden hamsters at 30 and 60 days post-infection (30 days-PI and 60 days-PI, respectively). The results are presented in a column chart format and the relative quantification was performed by the comparative Ct method ($^{\Delta\Delta}$Ct), using skin of uninfected hamsters as calibrator (Fold change = 1), as indicated by the dotted line. Horizontal bars represent median ± standard deviation of $^{\Delta\Delta}$Ct for each strain. Statistical differences between groups were considered significant when $p < 0.05$. In each graph, significant differences among the strains were represented as a specific letters (M2903 = a; RR410 = b; RR412 = c; RR415 = d; RR418 = e; RR444 = f) and differences of gene expression between time points post-infections were represented as "#" symbol.

and RR415-infected animals ($p < 0.01$). No difference was observed between strains in the *il17* expression and *il21* had its expression downregulated in cells from infected hamster with all strains (Fig. 2).

There was no significant difference between strains in the upregulation of *ccl2* and *ccl5* at this time point, while *ccl17* was not markedly expressed only for RR412 and *ccl22* kept highly expressed in cells from RR415-infected hamsters ($p < 0.001$) (Fig. 3).

3.3. Heat map matrix and hierarchical clustering shown distinct patterns of immune response

A heat map matrix was constructed to evaluate whether distinct genic cytokine and chemokine patterns were associated to *L. braziliensis* strains at distinct time points in experimental trials (Fig. 4). Using this approach, it was possible to segregate distinct immunological profiles between the strains mainly in the early phase of the infection (30 days-PI). At this time point, the genetic variant strains upregulated *tnfα*, *il12*

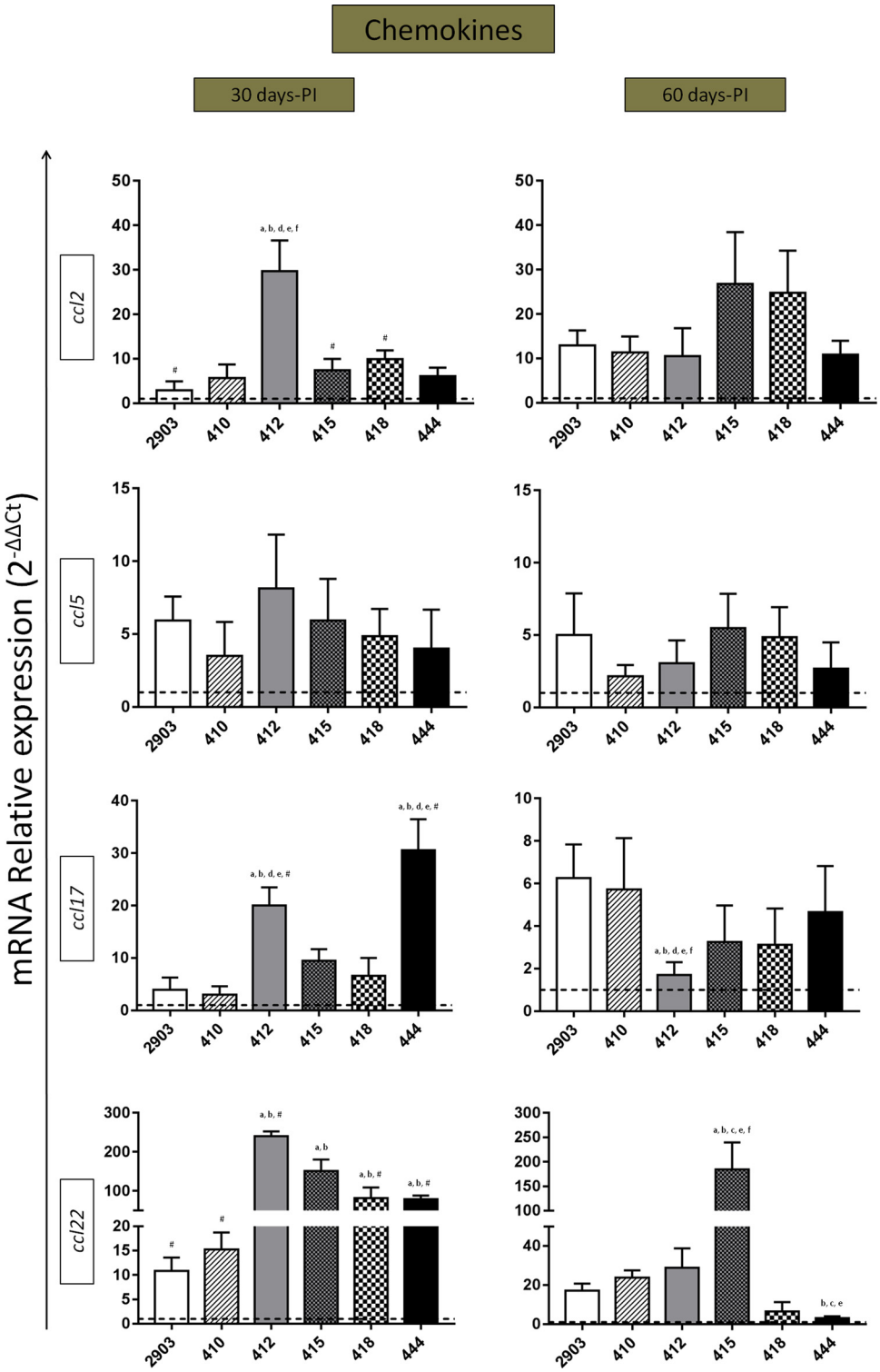
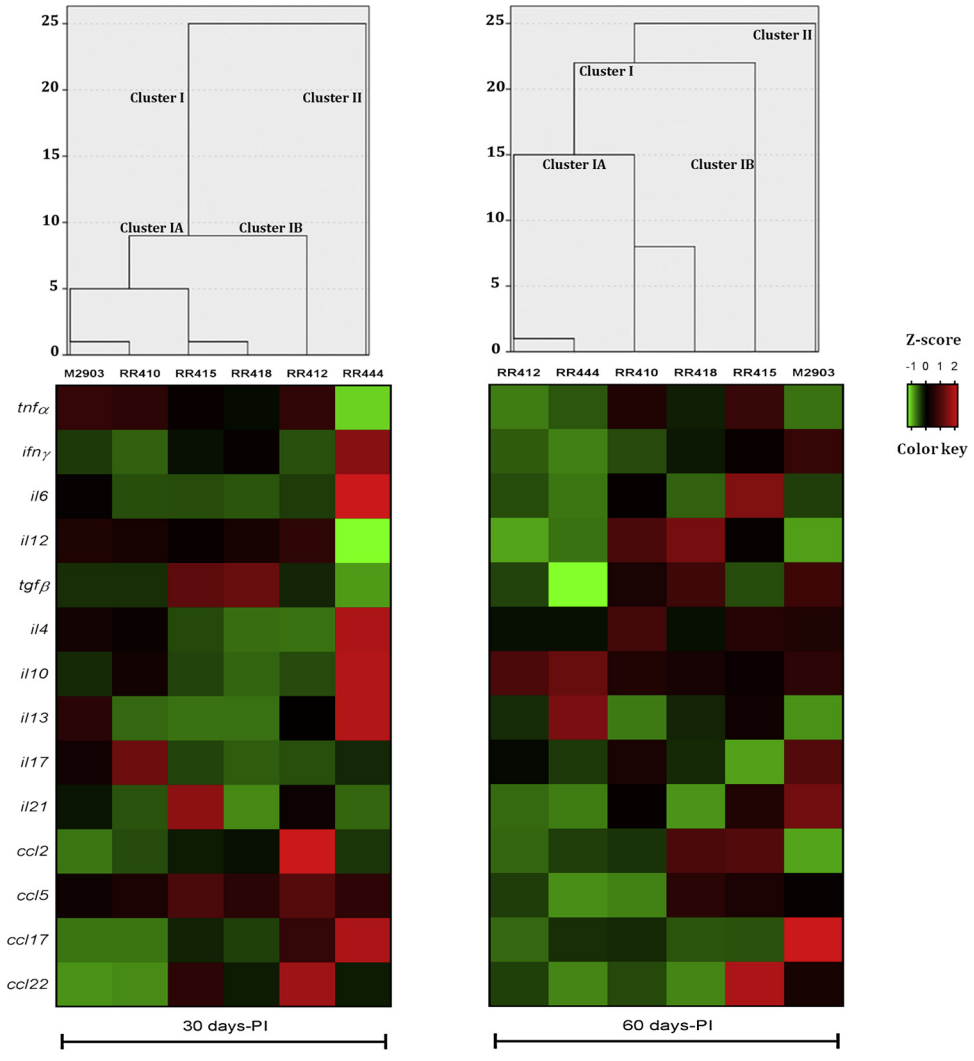


Fig. 3. Gene expression profile of chemokines in skin lesions of infected golden hamsters at 30 and 60 days post-infection (30 days-PI and 60 days-PI, respectively). The results are presented in a column chart format and the relative quantification was performed by the comparative Ct method ($^{\Delta\Delta}$Ct), using skin of uninfected hamsters as calibrator (Fold change = 1), as indicated by the dotted line. Horizontal bars represent median ± standard deviation of $^{\Delta\Delta}$Ct for each strain. Statistical differences between groups were considered significant when $p < 0.05$. In each graph, significant differences among the strains were represented as a specific letters (M2903 = a; RR410 = b; RR412 = c; RR415 = d; RR418 = e; RR444 = f) and differences of gene expression between time points post-infections were represented as "#" symbol.

whilst the non-variant strain (RR444) upregulated *ifnγ*, *il6*, *il4*, *il10* and *il13*. *ccl2* gene was high expressed in cells of animals infected with RR412 and *ccl17* was highly expressed in animals infected with RR412 and RR444. Comparing the mRNA expression among the time points, in general, at 60 days-PI all strain expressed higher levels of *il4* and *il10*. The non-variant strain (RR444) shown a significant reduction in the expression of *ifnγ*, *il6*, *ccl17*, and *ccl22* whist distinct patterns were observed for the genetic variant strains. Some patterns was substantially different such as the expression of *tnfα* for RR412, RR415 and RR418; *tgfβ* for RR410 and RR415; *ccl17* for all genetic variant strain and *ccl22* for RR410, RR412 and RR418 (Fig. 4).

The results of the hierarchical cluster analysis (HCA) are shown in the dendogram (Fig. 4). The HCA could readily identified distinct patterns in the gene expression, highlighting the differences observed in the heat map matrix, in each time points, mainly in the early-phase disease. At 30 days-PI, all genetic variant strain grouped in the Cluster I whilst the non-variant (RR444) grouped in the Cluster II. The expressions of *tnfα* and *il12* were significantly decisive to segregate these clusters (Fig. 4, inserted table A). Still regarding the Cluster I, all genetic variant strain isolated from CL-patients grouped in Cluster IA and



A

Strains	Components
ALL*	*ccl5*
M2903, RR410, RR412, RR415, RR418	*tnfα, il12*
M2903, RR412, RR415, RR418	*tgfβ*
RR412, RR415, RR418, RR444	*ccl22*
M2903, RR412, RR444	*il13*
M2903, RR410	*il17*
RR412, RR444	*ccl17*
RR412	*ccl2*
RR415	*il21*
RR444	*ifnγ, il6, il4, il10*

*Universal

B

Strains	Components
ALL*	*ifnγ, il10, il13, il17, ccl2, ccl5*
M2903, RR410, RR415, RR418, RR444	*ccl17*
M2903, RR410, RR415	*il4*
M2903, RR410, RR418	*tgfβ*
RR410, RR415, RR418	*il12*
RR410, RR415	*tnfα*
RR415	*il6, ccl22*

*Universal

Fig. 4. Patterns of cytokines and chemokines at 30 and 60 days post-infections (30 days-PI and 60 days-PI, respectively) from gold hamsters within strains of L. *braziliensis*. Heat map analysis and Venn diagrams revealed distinct patterns of gene expression in skin lesions. For heat map, values of gene expression of each infected-animal were used. In Venn diagrams, the criterion to discriminate the components was the presence of significant difference in the expression among the strains. Numbers inside the diagrams represent the total of components (cytokines and/or chemokines). Inserted Table A: Stratified analysis of similar and specific components expressed by L. *braziliensis* strains at 30 days-PI. Inserted Table B: Stratified analysis of similar and specific components expressed by L. *braziliensis* strains at 60 days-PI.

the single isolate from DL-patient (RR412) clustered in 1B, in which the expression of *ccl2* was determinant for the formation of these subgroups.

At 60 days-PI, all strains provided from the XIR (Table 1) grouped in the cluster I without distinction of their genetic background, and the reference strain (M2903) used as positive control of experimental trails grouped in the cluster II. There was no clear which component was determinant for this segregation, but the differentiated expression of *ccl17* in animals infected with the reference strain may have had a crucial role. Still about the cluster I, distinct patterns were observed in the expression of cytokines and chemokines in the samples from the XIR, highlighting the expression of *il6* and *cll22* genes in animals infected with RR415 and *il13*gene in animals infected with non-variant strain (RR444).

4. Discussion

L. braziliensis parasite populations are extremely diverse in Brazil with known intraspecific genetic variability (Kuhls et al., 2013; Nolder et al., 2007; Oddone et al., 2009; Rougeron et al., 1990). Some studies have been shown that the wide spectrum of clinical manifestations caused by this parasite may be triggered by a complex interplay between genetically factors of parasite as well as host traits (Campanelli et al., 2010; Indiani De Oliveira et al., 2004; Saravia et al., 1990; Schriefer et al., 2004; Teixeira et al., 2005). Here, we explore the immune features of golden hamster infected with strains of *L. braziliensis* from a well-defined study area where ACL is endemic (Xakriabá Indigenous Reserve, state of Minas Gerais, Brazil) to better understand the ability of genetic variant strains to trigger distinct pathways in the host-immune response.

In the early phase of infection (30 days-PI), the presence of a mixed Th1-Th2 profile was observed in animals infected with the non-variant

strain due to the upregularion of *ifnγ*, *il4* and *il10*, whilst the genetic-variant strains induced variable expressions of *tnfα*. In general, *il10* gene expression was significantly higher in the late-phase disease for all strains, suggesting an important role for this cytokine in limiting tissue damage. These results are consistent with those described by some authors for human disease and in experimental infections with *L.* (*Viannia*) parasites (Belkaid et al., 2002, 2001; Bomfim et al., 2007; de Moura et al., 2005).

The role of *il6* in CL has been analyzed in distinct scenarios (Moskowitz et al., 1997; Titus et al., 2001) but their role in *L. braziliensis* infections remains unclear. In our study, the non-variant strain RR444, expressed high levels of *il6* at 30 days-PI, suggesting a strong interplay with macrophages in an attempt to parasite elimination (Hatzigeorgiou et al., 1993; Moskowitz et al., 1997). On the other hand, cells infected with the genetic variant strains expressed similar levels comparing to control group (non-infected skin) or even downregulated this cytokine.

tgfβ is able to regulate the inflammatory immune response by suppressing the differentiation of CD4+ effector cells, inhibiting the effect of *ifnγ* suppressing the activity of macrophages, dendritic cells and natural killer (NK) cells (Ding et al., 1990; Tsunawaki et al., 1988; Yoshimura et al., 2010). The upregularion of *tgfβ* observed in animals infected with the genetic variant strains RR415 and RR418 might be blockade the activity of *ifnγ* in parasite elimination at 30 days-PI. It is important to note that the gene expression of this cytokine appeared higher but its real effect to control the parasite population may be blockade (Barral et al., 1993; Silva et al., 1991). The *ifnγ*-blockade effect may also be occurred due to the upregulation of *il4* in animals infected with the non-variant strain RR444 (Lehn et al., 1989; Liew et al., 1989; Liew and O'Donnell, 1993). Still about *tgfβ* we did not observe a clear relationship in its expression and *il10* upregulation as described by Barral et al., (1993), as well as downregulation of *tnfα* (Oliveira et al., 2014).

Experimental studies have shown that more pathogenic strains of *L. braziliensis* expressed high levels of *ccl2* (Teixeira et al., 2005). As we observed, this chemokine was highly expressed in animals infected with RR412 strain at 30 days-PI (Fig. 4). Of note, this strain has been isolated from a patient with disseminated cutaneous leishmaniasis (DL), which importance is widely known due to the severity of the disease as well as the therapeutic challenge and an increasing prevalence (Carvalho et al., 1994; Turetz et al., 2002). Our finding, however, was contrary to described by Machado et al., (2011), in which similar patterns in the immune response was observed in CL and DL-patients. Some authors have been reported that whilst CL-patients present an exacerbated type-1 immune response, an absence of cell-mediated immune response allows parasite multiplication and dissemination have been observed to DL-patients (Turetz et al., 2002). In our study, cells from RR412-infected hamsters did not expressed significant lower levels of *tnfα* and the pattern of *ifnγ* transcription was similar to the other variant strains, differing only from non-variant isolate (RR444; $p < 0.001$).

Cytokines exert an indirect effect on leukocyte recruitment by inducing the expression of several chemokine genes (Ohmori et al., 1993). TNFα and IL1β released from activated neutrophils (polymorphonuclear cells) and macrophages have been implicated in chemokine synthesis in several cell types (Moser et al., 2004). In cutaneous leishmaniasis, IL12 is required for the induction of Th1-related chemokines such as CCL2, which reaches a production peak in the early-phase disease and is not TNF-mediated but depends on the virulence of the *Leishmania* strain. Thus, it appears that *in vivo* the level of *Leishmania*-mediated release of CCL2 could directly, without the involvement of pro-inflammatory TNF, either favor the development of a 'self-healing' course of the disease or result in a 'chronic' form of leishmaniasis.

A correlation between resistance and upregulation of *ccl5* in experimental infections with *L. major* infection has been described (Santiago et al., 2004), although *ccl5*-deficient mice do not show increased susceptibility (Rodriguez-Sosa et al., 2003). Faced with this divergence, here, all samples upregulated *ccl5* at 30 and 60 days PI without statistical significance among them. Mainly at 30 days-PI, this chemokine was the unique common biomarker for all strains, indicating a possible role to the infection control in the early-phase disease (Fig. 4, inserted table A), even with high parasite burden at this time point for all genetic variant strains (Rêgo et al., 2018)

According Nickel et al., (1999) the upregulation of *ccl17* at 30 days-PI in cells from RR444 -infected hamsters could be associated with a polarized Th2-response. We observed a positive correlation between the *ccl17* gene expression and *il4* and *il10* (both $p < 0.001$). Although animals infected with RR412strain also expressed high levels of *ccl17* at this same time point, a type-2 response was not clear, in which the negative correlation between *ccl17* gene expression and *il10* ($p = 0.0418$) and *il4* ($p = 0.0378$) was observed. In chronic phase-disease the expression of *ccl17* in patients with cutaneous leishmaniasis result in an attraction of $CCR3^+$ and $CCR4^+$ cells due to the expression of *il10* and *tgfβ*, controlling the inflammatory reaction (Campanelli et al., 2010). We did not observe a significant increase in the mRNA expression of *ccl17* comparing 30 days to 60 days-PI, although high transcription of *il10* was observed for all strains and a an increase level of *tgfβ* was observed for RR410-infected hamsters.

In ACL, the expression of *ccl22* chemokine has been associated with the upregulation of both *il4* and *il13* in activated macrophages and, on the other hand, the downregulation of *ccl22* is associated to *ifnγ* activity (Bonecchi et al., 1998; Orlofsky et al., 2000). At 30 days-PI, cells from RR444-infected hamsters presented high expressions of *il4*, *il13* and *ccl22* with a positive correlation among them (*ccl22* and *il4* – $p < 0.001$; *ccl22* and *il13* – $p < 0.012$), reinforcing this host-immune network. As mentioned before, although *ifnγ* was high expressed in cells infected with this strain, the effectiveness of the type-1 response may be reduced due *il4* upregulation since we observed a positive correlation among them ($p < 0.0001$). The highest value of ccl22 gene expression at this time point was observed in animals infected with RR412 strain. In this specific case a positive correlation was observed between this chemokine and *ifnγ* ($p < 0.002$), on the other hand, a discreet upregulation of type-2 response was observed for *il4* and *il13*. In an overview, Teixeira et al., (2005) reported that *ccl22* and *ccl5* expression did not show significant modulation in BALB/c mice infected with *L. braziliensis*, in contrast that we observed here.

The heat map matrix revealed distinct patterns of cytokine and chemokine expressions among the strains during the experimental trial that have been highlighted by the HCA (Fig. 4). According to these approaches the evaluated strains of *L. braziliensis* were segregated due to the upregulation of cytokines and chemokines. At 30 days-PI the non-variant strain did not clustered with all genetic variant strains. This pattern of clustering has been also observed for the biological behavior evaluated following several parameters as onset time of visible lesion, lesion size, histopathological changes and parasite burden (Rêgo et al., 2018).

For the first time, a large panel of cytokine and chemokine mRNA-expression was analyzed in experimental trials using golden hamsters as animal model and genetic variant strains of *L. braziliensis*. Our data contribute to better understand immunobiological factors that are involved during the disease progression. In summary, the finding presented here indicate that genetic variant strains of *L. braziliensis* albeit from the same endemic area, induced cytokine and chemokine expression patterns at different paces or intensities, leading us to suggest that distinct and diverse cell recruitment and differential immune response occurred mainly in the early-phase disease. These distinct host-immune features are interconnected with the biological behavior previously observed in golden hamsters (Rêgo et al., 2018) reinforcing that intrinsical genetic characteristics of the parasites are able to trigger distinct pathways in the host-parasite interaction and consequently, implicate in disease progression.

Acknowledgements

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - grant number 479408/2010-6), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG - grant number APQ-00289-10) and European Community LeishEpiNetSA (grant number 015407). C.M.F. Gontijo and A. Teixeira-Carvalho are researcher fellows from CNPq. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. We thank the Programa de Pós Graduação em Ciências da Saúde of the Instituto René Rachou and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/CAPES for the awarded grant.

References

Barral, A., Barral-Netto, M., Yong, E.C., Brownell, C.E., Twardzik, D.R., Reed, S.G., 1993. Transforming growth factor beta as a virulence mechanism for Leishmania braziliensis. Proc. Natl. Acad. Sci. U. S. A. 90, 3442–3446. https://doi.org/10.1073/PNAS.90.8.3442.

Belkaid, Y., Hoffmann, K.F., Mendez, S., Kamhawi, S., Udey, M.C., Wynn, T.A., Sacks, D.L., 2001. The role of interleukin (IL)-10 in the persistence of Leishmania major in the skin after healing and the therapeutic potential of anti-IL-10 receptor antibody for sterile cure. J. Exp. Med. 194, 1497–1506.

Belkaid, Y., Piccirillo, C.A., Mendez, S., Shevach, E.M., Sacks, D.L., 2002. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 420, 502–507. https://doi.org/10.1038/nature01152.

Bomfim, G., Andrade, B.B., Santos, S., Clarêncio, J., Barral-Netto, M., Barral, A., 2007. Cellular analysis of cutaneous leishmaniasis lymphadenopathy: insights into the early phases of human disease. Am. J. Trop. Med. Hyg. 77, 854–859. https://doi.org/10.4269/ajtmh.2007.77.854.

Bonecchi, R., Bianchi, G., Bordignon, P.P., D'Ambrosio, D., Lang, R., Borsatti, A., Sozzani, S., Allavena, P., Gray, P.A., Mantovani, A., Sinigaglia, F., 1998. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J. Exp. Med. 187, 129–134.

Campanelli, A.P., Brodskyn, C.I., Boaventura, V., Silva, C., Roselino, A.M., Costa, J., Saldanha, A.C., Rodrigues de Freitas, L.A., de Oliveira, C.I., Barral-Netto, M., Silva, J.S., Barral, A., 2010. Chemokines and chemokine receptors coordinate the inflammatory immune response in human cutaneous leishmaniasis. Hum. Immunol. 71, 1220–1227. https://doi.org/10.1016/J.HUMIMM.2010.09.002.

Carvalho, E.M., Barral, A., Costa, J.M.L., Bittencourt, A., Marsden, P., 1994. Clinical and immunopathological aspects of disseminated cutaneous leishmaniasis. Acta Trop. 56, 315–325. https://doi.org/10.1016/0001-706X(94)90103-1.

Costa-Silva, M.F., Gomes, L.I., Martins-Filho, O.A., Rodrigues-Silva, R., Freire, J., de, M., Quaresma, P.F., Pascoal-Xavier, M.A., Mendes, T.A., de, O., Serakides, R., Zauli, D.A.G., Campi-Azevedo, A.C., Melo, M.N., Gontijo, C.M.F., Peruhype-Magalhães, V., Teixeira-Carvalho, A., 2014. Gene expression profile of cytokines and chemokines in skin lesions from Brazilian Indians with localized cutaneous leishmaniasis. Mol. Immunol. 57, 74–85. https://doi.org/10.1016/J.MOLIMM.2013.08.008.

de Moura, T.R., Novais, F.O., Oliveira, F., Clarencio, J., Noronha, A., Barral, A., Brodskyn, C., de Oliveira, C.I., 2005. Toward a novel experimental model of infection to study American cutaneous leishmaniasis caused by leishmania braziliensis. Infect. Immun. 73, 5827–5834. https://doi.org/10.1128/IAI.73.9.5827-5834.2005.

Ding, A., Nathan, C.F., Graycar, J., Derynck, R., Stuehr, D.J., Srimal, S., 1990. Macrophage deactivating factor and transforming growth factors-beta 1 -beta 2 and -beta 3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-gamma. J. Immunol. 145, 940–944.

Espitia, C.M., Zhao, W., Saldarriaga, O., Osorio, Y., Harrison, L.M., Cappello, M., Travi, B.L., Melby, P.C., 2010. Duplex real-time reverse transcriptase PCR to determine cytokine mRNA expression in a hamster model of New world cutaneous leishmaniasis. BMC Immunol. 11, 31. https://doi.org/10.1186/1471-2172-11-31.

Gomes-Silva, A., Valverde, J.G., Peralva, R., Plácido-pereira, R.M., Da-cruz, A.M., 2013. Golden hamster (Mesocricetus auratus) as an experimental model for Leishmania (Viannia) braziliensis infection. Parasitology 140, 771–779. https://doi.org/10.1017/S0031182012002156.

Hatzigeorgiou, D.E., He, S., Sobel, J., Grabstein, K.H., Hafner, A., Ho, J.L., 1993. IL-6 Down-modulates the cytokine-enhanced antileishmanial activity in human macrophages. J. Immunol. 151, 3682–3692.

Indiani De Oliveira, C., Teixeira, M.J., Teixeira, C.R., Ramos De Jesus, J., Rosato, Bomura, A, Silva, Da, J.S., Brodskyn, C., Barral-Netto, M., Barral, A., 2004. Leishmania braziliensis isolates differing at the genome level display distinctive features in BALB/c mice. Microbes Infect. 6, 977–984. https://doi.org/10.1016/j.micinf.2004.05.009.

Kahl, L.P., Byram, J.E., David, J.R., Comerford, S.A., Lichtenberg, Fvon, 1991. Leishmania (Viannia) braziliensis comparative pathology of golden hamsters infected with isolates from cutaneous and mucosal lesions of pacientes residing in Tres Bracos, Bahia, Brazil. Am. J. Trop. Med. Hygyene 44, 218–232.

Kuhls, K., Cupolillo, E., Silva, S.O., Schweynoch, C., Côrtes Boité, M., Mello, M.N., Mauricio, I., Miles, M., Wirth, T., Schönian, G., 2013. Population structure and evidence for both clonality and recombination among Brazilian strains of the subgenus Leishmania (Viannia). PLoS Negl. Trop. Dis. 7, e2490. https://doi.org/10.1371/journal.pntd.0002490.

Lehn, M., Weiser, W.Y., Engelhorn, S., Gillis, S., Remold, H.G., 1989. IL-4 inhibits H2O2 production and antileishmanial capacity of human cultured monocytes mediated by IFN-gamma. J. Immunol. 143, 3020–3024.

Liew, F.Y., O'Donnell, C.A., 1993. Immunology of Leishmaniasis. Adv. Parasitol. 32, 161–259. https://doi.org/10.1016/S0065-308X(08)60208-0.

Liew, F.Y., Millott, S., Li, Y., Lelchuk, R., Chan, W.L., Ziltener, H., 1989. Macrophage activation by interferon-γ from host-protective T cells is inhibited by interleukin (IL) 3 and IL 4 produced by disease-promoting T cells in leishmaniasis. Eur. J. Immunol. 19, 1227–1232. https://doi.org/10.1002/eji.1830190712.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25, 402–408. https://doi.org/10.1006/METH.2001.1262.

Machado, P.R., Rosa, M.E.A., Costa, D., Mignac, M., Silva, J.S., Schriefer, A., Teixeira, M.M., Bacellar, O., Carvalho, E.M., 2011. Reappraisal of the immunopathogenesis of disseminated leishmaniasis: in situ and systemic immune response. Trans. R. Soc. Trop. Med. Hyg. 105, 438–444. https://doi.org/10.1016/j.trstmh.2011.05.002.

Moreira, Ndas D., 2012. História Natural da leishmaniose visceral em hamster Mesocricetus auratus experimentalmente infectados por duas cepas de Leishmania infantum com perfis distintos de virulência e patogenicidade. Universidade Federal de Ouro Preto.

Moser, B., Wolf, M., Walz, A., Loetscher, P., 2004. Chemokines: multiple levels of leukocyte migration control. Trends Immunol. 25, 75–84. https://doi.org/10.1016/j.it.2003.12.005.

Moskowitz, N.H., Brown, D.R., Reiner, S.L., 1997. Efficient immunity against Leishmania major in the absence of interleukin-6. Infect. Immun. 65, 2448–2450.

Nickel, R., Beck, L.A., Stellato, C., Schleimer, R.P., 1999. Chemokines and allergic disease. J. Allergy Clin. Immunol. 104, 723–742.

Nolder, D., Roncal, N., Davies, C.R., Llanos-Cuentas, A., Miles, M.A., 2007. Multiple hybrid genetypes of Leishmania (Viannia) in a focus of mucocutaneous Leishmaniasis. Am. J. Trop. Med. Hyg. 76, 573–578. https://doi.org/10.4269/ajtmh.2007.76.573.

Oddone, R., Schweynoch, C., Schönian, G., de Sousa, C., dos, S., Cupolillo, E., Espinosa, D., Arevalo, J., Noyes, H., Mauricio, I., Kuhls, K., 2009. Development of a multilocus microsatellite typing approach for discriminating strains of Leishmania (Viannia) species. J. Clin. Microbiol. 47, 2818–2825. https://doi.org/10.1128/JCM.00645-09.

Ohmori, Y., Wyner, L., Narumi, S., Armstrong, D., Stoler, M., Hamilton, T.A., 1993. Tumor necrosis factor-alpha induces cell type and tissue-specific expression of chemoattractant cytokines in vivo. Am. J. Pathol. 142, 861–870.

Oliveira, W.N., Ribeiro, L.E., Schrieffer, A., Machado, P., Carvalho, E.M., Bacellar, O., 2014. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of human tegumentary leishmaniasis. Cytokine 66, 127–132. https://doi.org/10.1016/J.CYTO.2013.12.016.

Orlofsky, A., Wu, Y., Prystowsky, M.B., 2000. Divergent regulation of the murine CC chemokine C10 by Th1 and Th2 cytokines. Cytokine 12, 220–228. https://doi.org/10.1006/CYTO.1999.0535.

Osorio, Y., Melby, P.C., Pirmez, C., Chandrasekar, B., Guarín, N., Travi, B.L., 2003. The Site of Cutaneous Infection Influences the Immunological Response and Clinical Outcome of Hamsters Infected With Leishmania panamensis. pp. 139–148.

PAHO/WHO, 2017. Leishmaniasis. Epidemiological Report of the Americas. http://iris.paho.org/xmlui/handle/123456789/34112. http://iris.paho.org/xmlui/handle/123456789/34856.

Quaresma, P.F., Rêgo, F.D., Botelho, H.A., da Silva, S.R., Moura, A.J., Neto, R.G.T., Madeira, F.M., Carvalho, M.B., Paglia, A.P., Melo, M.N., Gontijo, C.M.F.F., 2011. Wild, synanthropic and domestic hosts of Leishmania in an endemic area of cutaneous leishmaniasis in Minas Gerais State, Brazil. Trans. R. Soc. Trop. Med. Hyg. 105, 579–585. https://doi.org/10.1016/j.trstmh.2011.07.005.

Quaresma, P.F., de Brito, C.F.A., Rugani, J.M.N., Freire, J., de, M., Baptista, R., de, P., Moreno, E.C., Gontijo, R.C., Rêgo, F.D., Diniz, J.E., Melo, M.N., Gontijo, C.M.F., 2018. Distinct genetic profiles of Leishmania (Viannia) braziliensis associate with clinical variations in cutaneous-leishmaniasis patients from an endemic area in Brazil. Parasitology 1–9. https://doi.org/10.1017/S0031182018000276.

Rêgo, F., Shimabukuro, P.H., Quaresma, P., Coelho, I., Tonelli, G., Silva, K.M., Barata, R., Dias, E., Gontijo, C.M., 2014. Ecological aspects of the phlebotominae fauna (diptera: psychodidae) in the Xakriabá indigenous reserve, Brazil. Parasit. Vectors 7, 220. https://doi.org/10.1186/1756-3305-7-220.

Rêgo, F.D., Rugani, J.M.N., Shimabukuro, P.H.F., Tonelli, G.B., Quaresma, P.F., Gontijo, C.M.F., 2015. Molecular detection of leishmania in phlebotomine Sand flies (diptera: psychodidae) from a cutaneous Leishmaniasis focus at xakriabá indigenous reserve, Brazil. PLoS One 10, e0122038. https://doi.org/10.1371/journal.pone.0122038.

Rêgo, F.D., da Rocha Lima, A.C.V.M., Pereira, A.A.S., Quaresma, P.F., Pascoal-Xavier, M.A., Shaw, J.J., Gontijo, C.M.F., 2018. Genetic variant strains of Leishmania (Viannia) braziliensis exhibit distinct biological behaviors. Parasitol. Res. 1–12. https://doi.org/10.1007/s00436-018-6014-4.

Ribeiro-Romão, R.P., Moreira, O.C., Osorio, E.Y., Cysne-Finkelstein, L., Gomes-Silva, A., Valverde, J.G., Pirmez, C., Da-Cruz, A.M., Pinto, E.F., 2014. Comparative evaluation of lesion development, tissue damage, and cytokine expression in golden hamsters (Mesocricetus auratus) infected by inocula with different Leishmania (Viannia) braziliensis concentrations. Infect. Immun. 82, 5203–5213. https://doi.org/10.1128/IAI.02083-14.

Ribeiro-Romão, R.P., Saavedra, A.F., Da-Cruz, A.M., Pinto, E.F., Moreira, O.C., 2016. Development of real-time PCR assays for evaluation of immune response and parasite load in golden hamster (Mesocricetus auratus) infected by Leishmania (Viannia) braziliensis. Parasit. Vectors 9, 361. https://doi.org/10.1186/s13071-016-1647-6.

Rodriguez-Sosa, M., Rosas, L.E., Terrazas, L.I., Lu, B., Gerard, C., Satoskar, A.R., 2003. CC chemokine receptor 1 enhances susceptibility to Leishmania major during early phase of infection. Immunol. Cell Biol. 81, 114–120. https://doi.org/10.1046/j.0818-9641.2002.01132.x.

Rougeron, V., Meeûs, T., Hide, M., Waleckx, E., Bermudez, H., Arevalo, J., Llanos-Cuentas, A., Dujardin, J.-C., Doncker, S.De, Ray, D.Le, Ayala, F.J., Bañuls, A.-L., 1990. Extreme inbreeding in Leishmania braziliensis. Proc. Natl. Acad. Sci. U. S. A. 87, 2414–2418. https://doi.org/10.1073/pnas.87.7.2414.

Santiago, Hda C., Oliveira, C.F., Santiago, L., Ferraz, F.O., de Souza, Dda G., de-Freitas, L.A.R., Afonso, L.C.C., Teixeira, M.M., Gazzinelli, R.T., Vieira, L.Q., 2004. Involvement of the chemokine RANTES (CCL5) in resistance to experimental infection with Leishmania major. Infect. Immun. 72, 4918–4923. https://doi.org/10.1128/IAI.72.8.4918-4923.2004.

Saravia, N.G., Segura, I., Labrada, L.A., Weigle, K., Giannini, S.H., Pacheco, R., Goncalves, A., 1990. Recurrent lesions in human Leishmania braziliensis infection-reactivation or reinfection? Lancet 336, 398–402. https://doi.org/10.1016/0140-6736(90)91945-7.

Schriefer, A., Schriefer, A.L.F., Góes-Neto, A., Guimarães, L.H., Carvalho, L.P., Almeida, R.P., Machado, P.R., Lessa, H.A., de Jesus, A.R., Riley, L.W., Carvalho, E.M., 2004. Multiclonal Leishmania braziliensis population structure and its clinical implication in a region of endemicity for American tegumentary Leishmaniasis. Infect. Immun. 72, 508–514.

Silva, J.S., Twardzik, D.R., Reed, S.G., 1991. Regulation of Trypanosoma cruzi infections in vitro and in vivo by transforming growth factor beta (TGF-beta). J. Exp. Med. 174, 539–545. https://doi.org/10.1084/JEM.174.3.539.

Teixeira, M.J., Fernandes, J.D., Teixeira, C.R., Andrade, B.B., Pompeu, M.L., Barral-netto, M., Barral, A., 2005. Distinct Leishmania braziliensis isolates induce different paces of chemokine expression patterns. Infect. Immun. 73, 1191–1195. https://doi.org/10.1128/IAI.73.2.1191.

Teixeira, M.J., Teixeira, C.R., Andrade, B.B., Barral-Netto, M., Barral, A., 2006. Chemokines in host–parasiteinteractions in leishmaniasis. Trends Parasitol. 22, 32–40. https://doi.org/10.1016/J.PT.2005.11.010.

Titus, R.G., DeKrey, G.K., Morris, R.V., Soares, M.B., 2001. Interleukin-6 deficiency influences cytokine expression in susceptible BALB mice infected with Leishmania major but does not alter the outcome of disease. Infect. Immun. 69, 5189–5192. https://doi.org/10.1128/IAI.69.8.5189-5192.2001.

Tsunawaki, S., Sporn, M., Ding, A., Nathan, C., 1988. Deactivation of macrophages by transforming growth factor-β. Nature 334, 260–262. https://doi.org/10.1038/334260a0.

Turetz, M.L., Machado, P.R., Ko, A.I., Alves, F., Bittencourt, A., Almeida, R.P., Mobashery, N., Johnson Jr, W.D., Carvalho, E.M., 2002. Disseminated leishmaniasis: a new and emerging form of Leishmaniasis observed in northeastern Brazil. J. Infect. Dis. 186, 1829–1834. https://doi.org/10.1086/345772.

Ugur, M., Schulz, O., Menon, M.B., Krueger, A., Pabst, O., 2014. Resident CD4+ T cells accumulate in lymphoid organs after prolonged antigen exposure. Nat. Commun. 5, 4821. https://doi.org/10.1038/ncomms5821.

Yoshimura, A., Wakabayashi, Y., Mori, T., 2010. Cellular and molecular basis for the regulation of inflammation by TGF-. J. Biochem. 147, 781–792. https://doi.org/10.1093/jb/mvq043.