Localized skin inflammation during cutaneous leishmaniasis drives a chronic, systemic IFN-γ signature

Camila Farias Amorim ID1, Fernanda O. Novais ID1, Ba T. Nguyen ID1, Mauricio T. Nascimento ID2,3, Jamile Lago ID2,3, Alessandro S. Lago ID2,3, Lucas P. Carvalho ID2,3, Daniel P. Beiting ID1*, Phillip Scott ID1*

1 Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, United States of America, 2 Serviço de Imunologia, Complexo Hospitalar Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Brazil, 3 Laboratório de Pesquisas Clínicas do Instituto de Pesquisas Gonçalo Moniz–Fiocruz, Salvador, Brazil

¤ Current address: Department of Microbial Infection and Immunity, College of Medicine, The Ohio State University, Columbus, Ohio, United States of America

* beiting@upenn.edu (DPB); pscott@upenn.edu (PS)

Abstract

Cutaneous leishmaniasis is a localized infection controlled by CD4+ T cells that produce IFN-γ within lesions. Phagocytic cells recruited to lesions, such as monocytes, are then exposed to IFN-γ which triggers their ability to kill the intracellular parasites. Consistent with this, transcriptional analysis of patient lesions identified an interferon stimulated gene (ISG) signature. To determine whether localized L. braziliensis infection triggers a systemic immune response that may influence the disease, we performed RNA sequencing (RNA-seq) on the blood of L. braziliensis-infected patients and healthy controls. Functional enrichment analysis identified an ISG signature as the dominant transcriptional response in the blood of patients. This ISG signature was associated with an increase in monocyte- and macrophage-specific marker genes in the blood and elevated serum levels IFN-γ. A cytotoxicity signature, which is a dominant feature in the lesions, was also observed in the blood and correlated with an increased abundance of cytolytic cells. Thus, two transcriptional signatures present in lesions were found systemically, although with a substantially reduced number of differentially expressed genes (DEGs). Finally, we found that the number of DEGs and ISGs in leishmaniasis was similar to tuberculosis—another localized infection—but significantly less than observed in malaria. In contrast, the cytolytic signature and increased cytolytic cell abundance was not found in tuberculosis or malaria. Our results indicate that systemic signatures can reflect what is occurring in leishmanial lesions. Furthermore, the presence of an ISG signature in blood monocytes and macrophages suggests a mechanism to limit systemic spread of the parasite, as well as enhance parasite control by pre-activating cells prior to lesion entry.
Author summary

Cutaneous leishmaniasis caused by the protozoan *Leishmania braziliensis* exhibits two dominant inflammatory responses in cutaneous lesions: Interferon-γ (IFN-γ)-mediated signaling, which promotes parasite control, and cytolysis mediated by cytotoxic CD8+ T and NK cells, which promotes increased pathology. To determine if these responses were limited to cutaneous lesions, we performed RNA-seq on the blood of cutaneous leishmaniasis (CL) patients, and detected both transcriptional signatures in the peripheral blood. The presence of interferon stimulated genes, as well as circulating IFN-γ, suggests that protective immune responses are not limited to the lesion site, but are occurring systemically. This may be one mechanism to ensure optimal control of the parasites, both by limiting their systemic spread and by pre-activating cells to kill the parasites prior to entry into the lesions. The cytolytic transcriptional signature was uniquely detectable in the blood of *L. braziliensis* patients when compared to the blood of patients with tuberculosis (TB) or malaria, further emphasizing the importance of this pathway in cutaneous leishmaniasis. Taken together, these data suggest that this localized infection has a systemic component that may have an impact the development of the disease.

Introduction

Cutaneous leishmaniasis is a localized skin infection caused by the protozoan parasite *Leishmania* spp. and transmitted to the host through the bite of infected sand flies. There are no vaccines for cutaneous leishmaniasis and the standard treatment with pentavalent antimony has toxic side effects and is associated with high rate of failure in endemic areas [1–5]. While the clinical forms of the disease are quite diverse, control of the parasite largely depends upon the production of IFN-γ by CD4+ Th1 cells [6,7]. T cells within lymph nodes that drain the site of infection proliferate and differentiate into Th1 cells due to the presence of IL-12. These T cells then transit to the local lesion site, where they produce IFN-γ, leading to macrophage activation and parasite control. During the initial stages of infection recruited monocytes provide a safe haven for parasites [8], but as the infection progresses and immunity develops, infiltrating monocytes are activated by IFN-γ produced by both effector T cells and skin resident memory T cells and contribute to protection [9,10].

Untargeted transcriptomic approaches have expanded our understanding of many infectious diseases. For example, a transcriptional signature in the peripheral blood distinguishes patients undergoing a reversal reaction in leprosy [11], and genes associated with disease progression in tuberculosis (TB) have been identified in a series of studies [12–16]. Several studies also identified the transcriptional response associated with pathological cerebral malaria in humans and experimental mouse models [17]. Recently, peripheral blood gene signature was found to be predictive for treatment outcome in Ethiopian patients with visceral leishmaniasis and HIV [18]. Transcriptional analysis of lesions from patients infected with *L. braziliensis* found a strong ISG signature, as well as a cytotoxic signature [19–21]. These studies in patients, and parallel studies in murine models, defined an immunopathological pathway initiated by cytolysis, leading to NLRP3 inflammasome activation and release of pro-inflammatory IL-1β [22–24]. Importantly, blockade of either NLRP3 or IL-1β ameliorated pathology in murine models suggesting that host-directed therapies may be useful in lessening disease in patients. The significance of this pathway in patients was confirmed by correlating cytolytic gene expression with treatment outcome [19,20,22,24]. An analysis of transcriptional responses in the blood of patients with visceral leishmaniasis identified expression of both an ISG and

Funding: PS is funded by the National Institutes of Health (NIH) R01-AI-149456 (https://grants.nih.gov/grants/funding/r01.htm). LPC is funded by the National Institute of Allergy and Infectious Diseases AI 136032 (https://www.niaid.nih.gov). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.
cytotoxic signature [25], but no studies have fully examined the transcriptional profile in the blood of patients with cutaneous leishmaniasis (CL) [26], where disease is highly localized to the skin lesion.

To investigate the systemic transcriptional signatures in cutaneous leishmaniasis and understand what insights these signatures can provide in understanding the immunological responses developed in this disease, we performed RNA sequencing (RNA-seq) in the peripheral blood of L. braziliensis-infected patients. We observed a consistent and significant monocyte and macrophage-associated Interferon-stimulated gene (ISG) signature in the blood of CL patients. Additionally, a cytotoxic signature including the expression of GZMB, PRF1, and GNLY was also evident. To understand these responses relative to other infections we compared our results with published data from human tuberculosis and malaria. Although a ISG response was common to all three infections, the cytolytic signature was unique to leishmania. Thus, our results indicate that although cutaneous leishmaniasis is a local skin infection, a systemic transcriptional profile is present in patients and mirrors what is seen in the lesion. Furthermore, our studies suggest that a systemic immune response can activate cells prior to entry into leishmanial lesions, thus potentially enhancing parasite control.

Methods

Ethics statement

This study was conducted according to the principles specified in the Declaration of Helsinki and under local ethical guidelines (Ethical Committee of the Medical School, UFBA, Salvador, Bahia, Brazil, and the University of Pennsylvania Institutional Review Board). Informed consent for the collection of samples and subsequent analysis was obtained.

Study design

Fifty patients with cutaneous leishmaniasis (CL patients), with a characteristic skin lesion and positive PCR for L. braziliensis participated in this study. Exclusion criteria included previous anti-leishmanial treatment, individuals under 18 years old, pregnancy, or the presence of other comorbidities. Before treatment, 3 ml of peripheral blood was collected from CL patients and 14 endemic non-L. braziliensis infected controls (healthy subjects, HS) in Corte de Pedra, Bahia, Brazil, and were stored in Tempus Tubes (Applied Biosystems) at -20°C. Eighteen patients from the 50 patients who had peripheral blood drawn also had a 4mm biopsy collected from the border of their cutaneous lesions. 7 biopsy samples were also collected from the skin non-infected individuals to serve as controls. Patients were given standard of care treatment (daily intravenous injections of pentavalent antimony; 20mg/kg/day for 20 days). At day 90, after the start of treatment, patients were evaluated for lesion resolution. Cure was defined as re-epithelialization of lesions in the absence of raised borders. Patients with active lesions at 90 days were defined as failing treatment and were given an additional round of chemotherapy.

Measurement of IFN-γ in the serum of CL patients

Serum from 17 randomly selected CL patients and 10 HS was obtained from peripheral blood by centrifugation and stored at -80°C. Concentration of IFN-γ was determined by Enzyme-linked immunosorbent assay (ELISA—BD biosciences), according to the manufacturer’s instructions. The results are expressed in pg/ml.
Processing peripheral blood of CL patients for RNA-seq

Blood samples were collected and stored in Tempus tubes at -80°C and shipped to the University of Pennsylvania, where RNA extraction, cDNA library preparation, and RNA sequencing were performed. RNA was extracted using the Tempus Spin RNA Isolation Reagent Kit (Applied Biosystems), and RNA quality and quantification were assessed using a Tapestation 4200 (Agilent) and Qubit 3 (Invitrogen), respectively. Whole-transcriptome sequencing libraries were prepared with the TruSeq Stranded Total RNA with Ribo-Zero Globin (Illumina) and sequenced on an Illumina NextSeq 500 to produce 75 bp paired-end reads with a mean sequencing depth of 32 million reads per sample. Raw reads were mapped to the human cDNA reference transcriptome (Ensembl, GRCh38 release 97) using Kallisto, version 0.46.0 [27].

RNA-seq data analysis and visualization

All analyses and visualizations were carried out using the statistical computing environment R version 3, RStudio version 1.2.5042, and Bioconductor version 3.11 [28]. Transcript-level counts were summarized to genes using the TxImport package [29] and human gene annotations from the biomaRt package [30]. Data from CL patients was generated as described above. Publicly-available RNA-seq data from tuberculosis (21 patients with active diseases and 12 healthy subjects from the London cohort) and malaria (65 adult patients with uncomplicated malaria, and 16 healthy subjects) were downloaded from the Sequence Read Archive (SRA). Data were normalized using the Trimmed Mean of M-values (TMM) method in EdgeR package [31]. Genes with $< 1$ count per million (CPM) in $n$ of the samples, where $n$ is the size of the smallest group of replicates, were filtered out. Normalized, filtered data were variance-stabilized using the VOOM function in Limma [32], and batch effect correction was carried out using the Combat function from the sva package [33] or intercepting for the batch factor in the model matrix for differential gene expression (DGE) [32]. DGE testing with Benjamini-Hochberg correction for multiple testing was carried out using Limma. [32]. Permutational multivariate analysis of variance (PERMANOVA) was calculated using the vegan package [34]. GO analysis was carried out using the gprofiler2 package [35]. Gene Set Enrichment Analysis (GSEA) was carried out using GSEA software (Broad Institute, version 4.0.2) and Reactome, KEGG and Biocarta pathways databases [36]. Single sample GSEA (ssGSEA) was carried out using the GSVA package [37] and the 51-gene peripheral blood leishmanial signature. The immuneDEconv package [38] was used to estimate cell abundances from RNAseq data using the MCP-counter method [39]. Type I- or II IFN-stimulated genes were identified using InterferomeDB v2.0 [40]. Spearman’s rho ($\rho$) correlations were performed in the R statistical environment using the ggpubr and Hmisc package. Wilcoxon rank-sum test was performed using the ggpubr and stats package. Coefficient of variation (cV) was calculated as described previously [19].

Results

Transcriptional profiling identifies an Interferon-stimulated gene (ISG) signature in the peripheral blood of patients infected with *Leishmania braziliensis*

To investigate the systemic transcriptional signatures associated with CL, we performed RNA-seq on the peripheral blood collected from 50 CL patients and 14 healthy subjects (HS). Peripheral blood gene expression distinguished CL patients from HS by principal component analysis (PCA; PERMANOVA, F statistic = 0.006) (Fig 1A). GSEA using the Reactome
Pathway Database revealed the Interferon-gamma signaling within the top 10 pathways enriched in CL patients (Fig 1B). Other pathways significantly enriched in CL relative to HS were also associated with Interferon signaling, although annotated as the intracellular antigen processing: ubiquitination and proteasome degradation; antigen presentation: folding, assembly and peptide loading of class I MHC; C-type lectin receptors (clec7a, dectin 1) signaling; activation of NF-kB in B cells, cellular responses to external stimuli (hypoxia) and the metabolism of proteins (UCH proteinases), FDR ≤ 0.01 (Fig 1B). No signatures were significantly enriched in the blood of HS relative to CL, FDR ≤ 0.01.

To further investigate specific-gene signatures present in the peripheral blood of CL patients, we carried out a differential gene expression (DGE) analysis between CL relative to HS. We observed that 60 genes were differentially expressed between the two groups. Included in the 51 genes upregulated in CL compared to HS (FC ≥ 2, and FDR ≤ 0.01) (Fig 1C), were interferon-responsive genes, such as guanylate-binding proteins (GBP1-GBP6 and GBP1P1), STAT1, SOCS1, WARS, Fc gamma receptor genes (FCGR1A, FCGR1B, FCGR1C), the transcription factor ATF3, and GZMB that encodes the cytolytic granule granzyme B. Gene Ontology (GO) enrichment analysis of these 51 genes confirmed a significant enrichment for cellular responses to type II interferon (FDR ≤ 0.0001.)
An Interferon-stimulated type II gene (ISG) systemic signature mirrors the local immune response in the CL lesion

Given the fact that type I interferon (IFN-a/b) and type II interferon (IFN-γ) signal through similar downstream transcriptional responses, and given multiple reports of both interferon responses occurring during protozoan parasite infections [42,43], we next used the Interferome database [40] to more precisely annotate these 51 genes with respect to interferon signaling. 42 out of the 51 genes (82%) were confirmed as Interferon-stimulated genes (ISGs) in Interferome. From this set of 42 ISGs, 5 were annotated as exclusively type II-ISGs (C1QB, PKB, DHRS9, GADD45G, and CADM1), none were exclusively type I-ISGs, and the remaining 37 respond to both type I and type II interferons (Fig 2A and 2B). These data suggest that the ISG signature in the blood of CL patients may be driven by IFN-γ.

Additionally, to understand the degree to which gene expression changes in the blood of CL patients reflect what is happening at the site of infection, in the leishmanial skin lesion, RNA-seq was performed on lesion biopsies obtained from a subset of the same patients analyzed above. As expected, the number of differentially expressed genes in lesions was much higher than in the blood (4269 vs. 60 total DEG, respectively; FDR \( \leq 0.01 \), and logFC \( \geq 1 \)) (Fig 2A). Similarly, 1774 and 42 ISGs were upregulated in lesion and blood, respectively (Fig 2A and 2B). Arbitrary thresholds imposed during DEG analysis can result in poor performance when comparisons are made across tissues where the magnitude of the response differs. In contrast, the threshold-free approach employed by GSEA showed a similar enrichment for IFN signatures in both blood and lesions from CL patients (S1 Fig). Since the number of exclusive type-II ISGs was greater than the number of exclusive type-I ISGs in the blood, and considering that there were no exclusive type-I ISGs upregulated in the blood, we hypothesize that transcriptional changes associated with IFN-γ predominant in CL. To determine if exposure to IFN-γ might be occurring in the blood, we measured IFN-γ protein in CL patients and found elevated levels in the serum (Fig 2C). We also observed a significant correlation between serum levels of IFN-γ protein and the peripheral blood ISG signature in CL (\( \rho = .79 \) and \( P = .0001 \)) (Fig 2D). Taken together, these results show a systemic Th1 response in CL that mirrors the ISG signature observed at the site of the infection in the skin.

A cytotoxicity transcriptional signature is observed in the peripheral blood of patients infected with L. braziliensis

While the gene expression program observed in the peripheral blood of CL patients was enriched for a type II ISG signature, we noticed that GZMB and GZMH were also significantly upregulated in our DEG analysis (FDR \( \leq 0.01 \) and FC \( \geq 2 \)). These genes encode proteins associated with cytolysis by CD8+ cytotoxic T cells (CTL) and NK cells. Cytolytic responses are associated with increased pathology in CL lesions and predict treatment failure [19,22,24]. Therefore, we explored this signature in the peripheral blood of CL patients. Taking a candidate gene approach, we observed that GZMA, PRF1, and GNLY were also upregulated in the blood of CL patients compared to HS (\( P < .05 \)) (Fig 3A). Additionally, three cytotoxicity-related signatures from the Biocarta and KEGG Pathway Databases (NK cells pathway, NK cell-mediated cytotoxicity pathway and CTL pathway) were enriched in the peripheral blood of CL patients relative to HS (FDR = 0.01, 0.03 and 0.06, respectively) (Fig 3B). Leading edge analysis of the peripheral blood DEGs from CL patients showed that Killer cell lectin-like receptor genes from the CD94/NKG2 family, including KLRD1-3, and KLRD1, and components of the cytolytic and antigen presentation and costimulation machinery (GZMB, PRF1, and HLA-A) were enriched and shared between at least two of the signatures (Fig 3B). These
results indicate that, in addition to the strong IFN-γ and ISGs, a cytotoxicity signature is detected in the blood of CL patients. The expression of cytolytic granule genes in CL lesions is dramatically upregulated compared to healthy skin, and the CD8+ T cell-mediated cytotoxicity has a critical pathogenic role in lesion development [22]. We asked whether the magnitude of cytolytic granule gene expression in the lesion was correlated to the magnitude in the blood in the same individual by analyzing 18 samples from CL patients who had paired lesion and blood RNA-seq data; however, we did not observe a significant correlation (P > 0.05) (S2 Fig).

The peripheral blood ISG signature in CL patients is correlated with monocytes

To investigate whether specific cell subtypes in the blood were associated with the ISGs and cytotoxic signature observed systemically in CL patients, we used the MCP-counter method to estimate the abundance of different cell types in our blood RNA dataset [39]. Increased abundance of NK cells, CTLs, monocytes and monocytes/macrophages-like cells were observed in
the blood samples from CL patients compared to HS (P<0.05) (Figs 4A and S3). The majority of the ISGs were positively correlated with MCP-counter cell type abundance scores for monocytes and macrophase/monocyte-like cells (Fig 4B), suggesting that these cells are the potentially the ones that are mainly being activated by the circulating IFN-γ. Interestingly, we also observed a significant positive correlation between GZMB and GZMH expression, and the abundance of NK cells and CTLs (P<0.05) (Fig 4B). Additionally, there was also a significant positive correlation between GZMA, PRF1, GNLY and the abundance of NK cells and CTLs (P<0.05) (S4 Fig), suggesting that the systemic cytolytic signature observed in CL patients is associated with these cytotoxic cells. We previously reported that the expression of cytolytic genes predicted treatment failure in CL patients receiving standard-of-care therapy with pentavalent antimony [19]. Unfortunately, neither systemic expression of GZMB, GNLY, and PRF1 expression nor increased abundance of CTLs or NK cells in the blood (S5B and S5C Fig) were predictive of treatment outcome. In addition, PCA analysis of our blood data and enrichment score for our 51-gene peripheral blood signature failed to distinguish patients that cured versus failed antimony treatment (P>0.05) (S5D and S5E Fig). All of these patients were
treated with antimony after sample collection, and therefore we cannot rule out that differential gene expression in the blood would have influenced disease progression or severity in the absence of treatment.

**Tissue localization may impact the quality and quantity of the peripheral blood response during infection**

To understand our results in the context of other infectious diseases, we compared our findings with data of studies of two other intracellular pathogens. Two publicly available RNA-seq datasets of the whole blood from adult patients with active tuberculosis (TB, n = 21) [41] and uncomplicated malaria (n = 65) [14] were analyzed similarly to the CL blood dataset to investigate the transcriptional signatures when compared to controls (n = 12 for TB, and n = 16 for malaria). This also allowed us to compare the blood signature in a disease with a relatively localized infection (TB) and one where the infection is systemic (malaria). Principal component analysis of the TB and malaria datasets showed that infection contributed to the largest source of variation in each dataset (Fig 5A). Previous analyses of these datasets found an upregulation of ISGs [12–14], and here we sought to compare the relative magnitude of the type I and II ISG transcriptional changes between the CL, TB and malaria dataset by performing DGE analysis. While the 42 ISGs were upregulated in the blood of CL patients (Fig 2A and 2B), 133 ISGs were upregulated in the blood of patients with active TB, in which 85% is composed by type I and type II ISG (Fig 5B). These results are consistent with the previous analysis of this dataset [13,14]. In contrast, malaria was marked by a much larger number of DEGs that included 1,288 ISGs, as well as many genes not annotated as part of the Interferome (Fig 5B) (FDR < 0.01 and FC > 2). In addition, ISGs observed in TB and CL were more strongly induced in the blood of malaria patients. These results indicate that *L. braziliensis* and TB, perhaps due to the tissue-specific nature of these infections, elicit quantitively and qualitatively similar
systemic ISG, although there were more ISGs induced in TB. In contrast, in malaria there was a 10-fold increase in ISGs compared to TB and CL. Single sample GSEA (ssGSEA) allowed us to test each TB and malaria RNA-seq sample for enrichment of the 51-gene peripheral blood leishmanial signature. This analysis showed that the ISGs observed in the peripheral blood of CL patients were also induced in the majority of patients with active TB and malaria relative to their own study controls (P\textless{}0.001) (Fig 5C), indicating that this ISG signatures is likely conserved amongst Th1 inducing pathogens.

### A peripheral blood signature of cytotoxicity distinguishes CL from malaria and TB patients

Our observation that the peripheral blood ISG response observed in CL patients was also induced to similar or higher levels in TB and malaria, raised the question of whether there...
were any aspects of the peripheral blood program that were unique to CL. To address this question, we carried out GSEA on the TB and malaria datasets and tested for enrichment of our CL signature. Although most of the CL genes fell within the leading edge for both the TB and malaria datasets, indicating that they are strongly induced during all three infections, several were either not part of the leading edge or were actually downregulated during either TB or malaria. Amongst these were two cytolytic genes (GZMH and GZMB) (S6 Fig). To investigate this further, we expanded our analysis to include other components of the cytolytic machinery and found that GZMA, GNLY, and PRF1 were not upregulated in TB compared to HS (P > 0.05) (Fig 6A). Similarly, in malaria these genes were either only modestly upregulated (GZMA and GZMB) or were downregulated (GZMH, GNLY and PRF1), compared to HS (P < 0.05) (Fig 6A). These data suggest expression of cytolytic components may be a unique aspect of the peripheral blood signature of CL, compared to TB and malaria. To explore whether this difference could be explained by cell composition in the peripheral blood mononuclear cell compartment, we used MCP-counter to estimate cell proportions in all three infections. This analysis revealed that the blood of patients with active TB did not show an increased abundance of CTLs and NK cells relative to HS (P > 0.05), while samples from malaria patients showed significant lower abundances of CTL and NK cells when compared to HS (P < 0.0001) (Fig 6B). These data suggest that the cytolytic profile we observed is driven by an increased relative abundance of cytotoxic cells in the blood of CL patients, rather than simply increased per-cell expression of these genes.

Discussion
Given its relative ease of accessibility, peripheral blood offers an appealing sample type for transcriptional profiling to delineate mechanisms of disease. However, the extent to which blood reflects what is happening at a local site is unclear since there have been few direct comparisons of gene expression in tissues and blood collected from the same individuals. Cutaneous leishmaniasis is primarily a localized infection, with little evidence of disease beyond the skin. Nevertheless, we identified two transcriptional signatures in the blood, interferon and cytotoxicity, that were also present in lesions from L. braziliensis patients. Both of these
responses are central to the outcome of infection. IFN-\(\gamma\) activates phagocytic cells, such as macrophages and monocytes, to limit parasite replication, while cytotoxicity promotes increased inflammation and thus can exacerbate the disease [6,22,23]. The presence of these signatures in the blood indicates that even in a relatively localized infection systemic changes in gene expression are evident.

A large number of genes are stimulated following exposure to IFNs [44,45]. Precisely which ISGs are expressed is determined by several factors, including the type of IFN, the magnitude of the stimulation, and the cell type responding— all of which are influenced by pathogen species and strain [42,46]. Studies of patients with symptomatic visceral leishmaniasis infected \(L.\) \textit{donovani} or \(L.\) \textit{infantum} identified IFNG as upregulated in the peripheral blood when compared with healthy controls [25,47,48]. Blood transcriptional ISG signatures have also been described in tuberculosis, leprosy, influenza, Respiratory syncytial virus and malaria [11,14,17,49,50]. In a recent study, the transcriptional profiles from the blood and lungs of C57BL/6 mice were compared after infection with a variety of pathogens known to induce a full spectrum of T cell responses [42], and found that the magnitude of IFN-related signatures was particularly high in the blood of mice infected with \textit{Toxoplasma gondii} when compared to infection by other microorganisms, and this IFN signature was predominately type II in the lungs [42].

In \(L.\) \textit{braziliensis} patients the ISG response was biased towards type II ISGs, consistent with the critical role IFN-\(\gamma\) plays in leishmaniasis. Furthermore, we showed that serum levels of IFN-\(\gamma\) correlated with peripheral the peripheral ISG signature. The increased expression of ISGs was driven by an increase in monocytes and macrophages in the blood, suggesting that these cells may already be primed to control the infection prior to lesion entry.

In support of the idea that monocytes entering lesions have been shaped by factors in the blood, CL patients from Corte de Pedra, Brazil show increased frequencies of intermediate and non-classical monocytes in the peripheral blood, combined with increased expression of MHC class II, TNF, and matrix metalloproteinase 9 (MMP-9) [51,52]. Furthermore, cells from patients were better able to kill parasites when compared with cells from healthy subjects [53]. The notion of pre-activation of cells before entry into infected tissues extends to other diseases. For example, a similar response has been described in toxoplasma, where IFN-\(\gamma\) from NK cells in the bone marrow primed monocytes even before entering the blood circulation [54]. A common feature of \(L.\) \textit{braziliensis} infections is lymphadenopathy in the lymph nodes draining the site of infection [55]. Parasites and activated T cells are present within these organs, in which the latter are possible sources of the circulating IFN-\(\gamma\) seen in patients [56].

We predict that as a leishmanial infection progresses, this systemic priming of cells will lead to better control of the parasites, promoting more rapid lesion resolution. Given that once activated, macrophages are non-specific, it is possible that other infections dependent on IFN-\(\gamma\) might also be better controlled in these patients. On the other hand, the presence of low levels of IFN-\(\gamma\) leading to a systemic proinflammatory environment could have deleterious effects on patient well-being, a potential negative influence that has yet to be evaluated [57,58]. In contrast to cutaneous leishmaniasis, in visceral leishmaniasis where there is often uncontrolled parasite replication, peripheral blood monocytes were found to have an anti-inflammatory response [59].

Genes specifically induced by type I IFNs were not expressed at higher levels in the blood of patients, suggesting that IFN\(\alpha/b\) may not play a systemic role in \(L.\) \textit{braziliensis} infections. In lesion biopsies, some type 1-exclusive ISGs were upregulated, although their role in the disease is unclear. Some studies indicate IFN-\(\alpha/\beta\) can play a protective role during the first few days of infection [60], while other studies suggest that type I IFNs suppress the immune responses in both cutaneous and visceral leishmaniasis [61–65]. In active TB, type I IFN signaling has an
important role in driving local susceptibility to the \textit{M. tuberculosis} \cite{66}. Moreover, the blood of active TB patients showed an enrichment for type I-ISG-like signatures, but a negative enrichment for a T cell \textit{IFNG-TBX21} signature \cite{42}. These results together indicate that the class of the infectious pathogen is the main feature that determines the type of systemic IFN-related inflammation \cite{42}.

The ability of CTLs and NK cells to kill infected cells is essential for the control of several infectious diseases, although in some cases cytotoxicity is not protective and provokes a destructive inflammatory response \cite{67,68}. This is the case in cutaneous leishmaniasis, where excessive cytolysis leads to inflammasome activation and subsequent production of the proinflammatory cytokine IL-1\textbeta \cite{22}. The consequence of this cytolytic response has been shown both in experimental murine models \cite{22,24}, as well as in patients where high levels of \textit{PRF1}, \textit{GZMB} and \textit{GNLY} predict treatment failure \cite{19}. Here, we show that elevated expression of these cytolytic genes in patients was not limited to the lesions, since granzymes (\textit{GZMA}, \textit{GZMB}, and \textit{GZMH}), perforin (\textit{PRF1}) and granulysin (\textit{GNLY}) were expressed at higher levels in the peripheral blood of CL patients compared with healthy subjects. Interestingly, VL patients also exhibit higher expression of \textit{GZMA}, \textit{GZMB}, and \textit{PRF1} in the blood compared to healthy subjects, indicating a potential common cytolytic response in leishmaniasis \cite{25}. Correlating with the presence of these cytolytic genes, our analysis of cell abundance indicated that CTLs and NK cells were present at a higher level in the blood of patients compared with healthy subjects. Consistent with these findings, we previously reported that the percentage of NK cells is elevated in patients, with high levels of perforin and granzyme B \cite{69}.

The cytotoxicity signature seen in CL was not observed in the blood of either the TB or malaria patients. The cytotoxic function of CD8+ T cells and NK cells has been associated resistance in active TB \cite{70,71}. These cells are found in increased frequencies in the lungs, where they control the proliferation of the bacteria efficiently through direct lyse of infected macrophages upon cytotoxic degranulation \cite{72}. It is possible that the lack of the cytotoxic CTL/NK cell signature in the blood of TB patients is due to the migration of these cells to the lungs. In regards to the malaria infection, as in CL, CTLs play a pathogenic role in the attempt to control the protozoan spread especially in severe cases of the disease such as cases of child infection or cerebral malaria \cite{17,67,68}. Since we only included in this study patients with uncomplicated malaria, this could be the explanation for the lack of a remarkable cytotoxic signature.

In the Northeast of Brazil, the failure rate for the first line drug, pentavalent antimony, can be as high as 50\% \cite{73,74}, and patients are only given alternative drugs following one or more full treatment courses. Since we found that high levels of cytolytic gene expression in lesions predicts treatment failure \cite{19}, one of our goals in this study was to determine if analysis of gene expression in the blood might also be used as a predictor of treatment failure. The elevated levels of cytolytic genes in the blood of patients compared with healthy subjects was evidence of the importance of this pathway in cutaneous leishmaniasis. However, the large variation in expression levels that we observed in lesions from patients was not observed in the blood, which may account for our inability to use cytolytic gene expression in the blood as biomarkers to predict treatment failure.

In the present study, we profiled the transcriptional signatures in the peripheral blood of CL patients and observed an enrichment of an ISG signature associated with monocytes as well as a cytotoxic signature associated with CTLs/NK cells. The immune mechanisms associated with these signatures have a crucial impact on the outcome of infection with \textit{Leishmania}, one leading to better protection while the other promoting increased disease. The consequences for the patients exhibiting these changes in gene expression in the blood has yet to be determined. However, it is reasonable to propose that such responses could influence both
protective and pathologic responses to cutaneous leishmaniasis. Furthermore, these results raised the question of how these systemic responses might influence the response to other infections, as well as the overall health of these individuals.

**Supporting information**

**S1 Fig.** The interferon signaling is enriched in both lesion and blood from CL patients. GSEA enrichment plots showing three Interferon-related pathways from the Reactome Pathway Database enriched in the CL peripheral blood (left) and lesion biopsy (right) RNA-seq datasets (IFN signaling, systematic name (sn): M983; IFN gamma signaling, sn: M965; IFN alpha and beta signaling, sn: M973). NES, normalized enrichment score; FDR, false discovery rate. (TIFF)

**S2 Fig.** The expression of cytolytic granule genes in the peripheral blood of CL patients is not correlated with the expression of these genes in the lesions in the same patient. Correlation between the expression of GZMB, GNLY, and PRF1 in peripheral blood and lesions of the same patient. ρ, Spearman’s rho correlation coefficient; ns, non-significant P > .05. CPM, counts per million. (TIFF)

**S3 Fig.** MCP-counter estimates cell type abundance in the CL blood bulk RNAseq dataset. Median of MCP-counter scores for 11 cell types between CL patients (yellow) and HS (blue). Wilcoxon rank-sum test was used for statistical analysis, and P values are represented in the plots. (TIFF)

**S4 Fig.** The expression of genes associated with cytolytic granules correlates with the abundance of CTL and NK cells. Correlation matrix shows individual correlations between GZMB, GZMA, GZMH, GNLY, and PRF1 genes and the abundance of CTL and NK cells obtained with MCP-counter. The Spearman correlation coefficient ρ is for each correlation is represented in a color scale between -1 and 1 (yellow and red). All correlations included in this matrix were statistically significant, P < .001. (TIFF)

**S5 Fig.** The transcriptional cytotoxicity signature is not predictive of clinical outcome in the peripheral blood of CL patients. (A) Coefficient of variation (cV) for GZMB, GNLY, and PRF1 expression amongst CL patients in the peripheral blood and lesion biopsy datasets (blue and yellow, respectively). (B) Expression of cytolytic genes (GZMB, GNLY, and PRF1) in the peripheral blood of patients who Cured and Failed the first round of treatment. CPM, counts per million log2 scale. (C) Median of MCP-counter abundance scores of T cells, T CD8+ cells, NK cells, and CTL between CL patients who failed the first round of treatment with pentavalent antimony (n = 16, yellow) and CL patients who cured (n = 31, blue). Wilcoxon rank-sum test was used for statistical analysis, and P values are represented in the plots. (D) Principal component analysis showing principal component 1 (PC1) and PC2 for RNA-seq data from the peripheral blood of CL patients who failed or cured the lesion after the first round of treatment with pentavalent antimony. (B) Enrichment score (ES) of the CL peripheral blood signature (51 genes) by ssGSEA in the blood of CL patient who Failed or Cured. ns, non-significant by Wilcoxon rank-sum test, ns, non-significant, P > .05. (TIFF)
S6 Fig. GZMB and GZMH from the CL peripheral blood signature are not enriched in the blood of TB and malaria patients. A running enrichment score (ES) plot from a GSEA using the 51 genes in the CL peripheral blood signature as a signature in the TB (left) and uncomplicated malaria (right) datasets. The y-axis shows the running ES from each gene from the peripheral blood leishmanial signature, and the x-axis ranks all the genes in the dataset based on their overrepresentation in the different phenotypes of subjects. The total number of genes in each dataset is indicated in parenthesis. Leading-edge genes included in the left side of the blue vertical line (rank at maximum) are overrepresented in the peripheral blood of patients with active TB and malaria. GZMB and GZMH were highlighted in red the enrichment plots. (TIFF)

Acknowledgments

We thank Ednaldo Lago, Dr. Luiz Guimarães, and Dr. Edgar M. Carvalho’s clinical team in Corte de Pedra, Bahia, Brazil.

Author Contributions

Conceptualization: Camila Farias Amorim, Fernanda O. Novais, Daniel P. Beiting, Phillip Scott.

Data curation: Camila Farias Amorim, Ba T. Nguyen, Mauricio T. Nascimento, Jamile Lago, Alexsandro S. Lago.

Formal analysis: Camila Farias Amorim, Daniel P. Beiting.

Funding acquisition: Lucas P. Carvalho, Phillip Scott.

Investigation: Camila Farias Amorim, Fernanda O. Novais, Lucas P. Carvalho, Daniel P. Beiting, Phillip Scott.

Methodology: Camila Farias Amorim, Mauricio T. Nascimento, Daniel P. Beiting.

Project administration: Phillip Scott.

Resources: Phillip Scott.

Software: Camila Farias Amorim.

Supervision: Fernanda O. Novais, Daniel P. Beiting, Phillip Scott.

Validation: Camila Farias Amorim.

Visualization: Camila Farias Amorim, Daniel P. Beiting, Phillip Scott.

Writing – original draft: Camila Farias Amorim.

Writing – review & editing: Camila Farias Amorim, Fernanda O. Novais, Lucas P. Carvalho, Daniel P. Beiting, Phillip Scott.

References

1. Ponte-Sucré A, Gamarro F, Dujardin J-C, Barrett MP, López-Vélez R, et al. (2017) Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. PLoS Negl Trop Dis 11: e0006052. https://doi.org/10.1371/journal.pntd.0006052 PMID: 29240765

2. Costa RS, Carvalho LP, Campos TM, Magalhães AS, Passos ST, et al. (2018) Early Cutaneous Leishmaniasis Patients Infected With Leishmania braziliensis Express Increased Inflammatory Responses After Antimony Therapy. J Infect Dis 217: 840–850. https://doi.org/10.1093/infdis/jix627 PMID: 29216363
3. Lago AS do, Nascimento M, Carvalho AM, Lago N, Silva J, et al. (2018) The elderly respond to anti-
mony therapy for cutaneous leishmaniasis similarly to young patients but have severe adverse reac-
tions. Am J Trop Med Hyg 98: 1317–1324. https://doi.org/10.4269/ajtmh.17-0736 PMID: 29582733

4. Arevalo J, Ramirez L, Adua V, Zimic M, Tuliano G, et al. (2007) Influence of Leishmania (Viannia) spe-
cies on the response to antimonial treatment in patients with American tegumentary leishmaniasis. J
Infect Dis 195: 1846–1851. https://doi.org/10.1086/518041 PMID: 17492601

5. Unger A, O’Neal S, Machado PRL, Guimarães LH, Morgan DJ, et al. (2009) Association of treatment
of American cutaneous leishmaniasis prior to ulcer development with high rate of failure in northeastern
Brazil. Am J Trop Med Hyg 80: 574–579. https://doi.org/10.4269/ajtmh.2009.80.574 PMID: 19346378

6. Scott P, Novais FO (2016) Cutaneous leishmaniasis: immune responses in protection and pathogene-
sis. Nat Rev Immunol 16: 581–592. https://doi.org/10.1038/nri.2016.72 PMID: 27424773

7. Scott P, Arts D, Uzonna J, Zaph C (2004) The development of effector and memory T cells in cutane-
ous leishmaniasis: the implications for vaccine development. Immunol Rev 201: 318–338. https://doi.
org/10.1111/j.0105-2896.2004.00198.x PMID: 15361250

8. Peters N, Sacks D (2006) Immune privilege in sites of chronic infection: Leishmania and regulatory T
cells. Immunol Rev 213: 159–179. https://doi.org/10.1111/j.1600-065X.2006.00432.x PMID: 16972903

9. Glennie ND, Volk SW, Scott P (2017) Skin-resident CD4+ T cells protect against Leishmania major by
recruiting and activating inflammatory monocytes. PLoS Pathog 13: e1006349. https://doi.org/10.1371/
journal.ppat.1006349 PMID: 28419151

10. Hohman LS, Peters NC (2019) CD4+ T Cell-Mediated Immunity against the Phagosomal Pathogen
Leishmania: Implications for Vaccination. Trends Parasitol 35: 423–435. https://doi.org/10.1016/j.pt.
2019.04.002 PMID: 31080088

11. Teles RMB, Lu J, Tió-Coma M, Goulart IMB, Banu S, et al. (2019) Identification of a systemic interfer-
yon inducible antimicrobial gene signature in leprosy patients undergoing reversal reaction. PLoS Negl
Trop Dis 13: e0007764. https://doi.org/10.1371/journal.pntd.0007764 PMID: 31600201

12. Bloom CI, Graham CM, Berry MPR, Rozakeas F, Redford PS, et al. (2013) Transcriptional blood signa-
tures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. PLoS
One 8: e70630. https://doi.org/10.1371/journal.pone.0070630 PMID: 23940611

13. Berry MPR, Graham CM, McNab FW, Xu Z, Bloch SAA, et al. (2010) An interferon- inducible neutrophi-
lic blood transcriptional signature in human tuberculosis. Nature 466: 973–977. https://doi.org/10.1038/natu-
re09247 PMID: 20725040

14. Singhania A, Verma R, Graham CM, Lee J, Tran T, et al. (2018) A modular transcriptional signature
identifies phenotypic heterogeneity of human tuberculosis infection. Nat Commun 9: 2308. https://doi.
org/10.1038/s41467-018-04579-w PMID: 29921861

15. Thompson EG, Du Y, Malherbe ST, Shankar S, Braun J, et al. (2017) Host blood RNA signatures pre-
dict the outcome of tuberculosis treatment. Tuberculosis (Edinb) 107: 48–58. https://doi.org/10.1016/
j.2017.08.004 PMID: 29050771

16. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, et al. (2016) A blood RNA signature for
tuberculosis disease risk: a prospective cohort study. Lancet 387: 2312–2322. https://doi.org/10.1016/
S0140-6736(15)01316-1 PMID: 27017310

17. Lee HJ, Georgiadiou A, Otto TD, Levin M, Coin LJ, et al. (2018) Transcriptomic Studies of Malaria: a
Paradigm for Investigation of Systemic Host-Pathogen Interactions. Microbiol Mol Biol Rev 82. https://
doi.org/10.1128/MMBR.00071-17 PMID: 29695497

18. Adriaensen W, Cuypers B, Cordero CF, Mengasha B, Blesson S, et al. (2020) Host transcriptomic sig-
nature as alternative test-of-cure in visceral leishmaniasis patients co-infected with HIV. EBioMedicine
55: 102748. https://doi.org/10.1016/j.ebiom.2020.102748 PMID: 32361248

19. Amorim CF, Novais FO, Nguyen BT, Msic AM, Carvalho LP, et al. (2019) Variable gene expression and
parasite load predict treatment outcome in cutaneous leishmaniasis. Sci Transl Med 11. https://
doi.org/10.1126/scitranslmed.aax4204 PMID: 31748229

20. Novais FO, Carvalho LP, Passos S, Roos DS, Carvalho EM, et al. (2015) Genomic profiling of human
Leishmania braziliensis lesions identifies transcriptional modules associated with cutaneous immuno-
pathology. J Invest Dermatol 135: 94–101. https://doi.org/10.1038/jid.2014.305 PMID: 25036052

21. Christensen SM, Dillon LAL, Carvalho LP, Passos S, Novais FO, et al. (2016) Meta-transcriptome Pro-
filing of the Human-Leishmania braziliensis Cutaneous Lesion. PLoS Negl Trop Dis 10: e0004992.
https://doi.org/10.1371/journal.pntd.0004992 PMID: 27631090

22. Novais FO, Carvalho AM, Clark ML, Carvalho LP, Beiting DP, et al. (2017) CD8+ T cell cytotoxicity
mediates pathology in the skin by inflammasome activation and IL-1β production. PLoS Pathog 13:
e1006196. https://doi.org/10.1371/journal.ppat.1006196 PMID: 28192528
23. Santos C da S, Boaventura V, Ribeiro Cardoso C, Tavares N, Lordelo MJ, et al. (2013) CD8(+) granzyme B(+) mediated tissue injury vs. CD4(+) IFN-γ mediated parasite killing in human cutaneous leishmaniasis. J Invest Dermatol 133: 1533–1540. https://doi.org/10.1038/jid.2013.4 PMID: 23321919

24. Novais FO, Carvalho LP, Graff JW, Beiting DP, Ruthel G, et al. (2013) Cytotoxic T cells mediate pathology and metastasis in cutaneous leishmaniasis. PLoS Pathog 9: e1003504. https://doi.org/10.1371/journal.ppat.1003504 PMID: 23874205

25. Gardinassi LG, Garcia GR, Costa CHN, Costa Silva V, de Miranda Santos IKF (2016) Blood Transcriptional Profiling Reveals Immunological Signatures of Distinct States of Infection of Humans with Leishmania infantum. PLoS Negl Trop Dis 10: e0005123. https://doi.org/10.1371/journal.pntd.0005123 PMID: 28789896

26. Vivarini A de C, Calegari-Silva TC, Saliba AM, Boaventura VS, França-Costa J, et al. (2017) Systems Approach Reveals Nuclear Factor Erythroid 2-Related Factor 2/Protein Kinase R Crosstalk in Human Cutaneous Leishmaniasis. Front Immunol 8: 1127. https://doi.org/10.3389/fimmu.2017.01127 PMID: 28959260

27. Bray NL, Pimentel H, Melsted P, Pachter L (2016) Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol 34: 525–527. https://doi.org/10.1038/nbt.3519 PMID: 27043002

28. Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, et al. (2015) Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods 12: 115–121. https://doi.org/10.1038/nmeth.3252 PMID: 25635003

29. Soneson C, Love MI, Robinson MD (2015) Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. [version 2; peer review: 2 approved]. F1000Res 4: 1521. https://doi.org/10.12688/f1000research.7563.2 PMID: 26925227

30. Durinck S, Spellman PT, Birney E, Huber W (2009) Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. Nat Protoc 4: 1184–1191. https://doi.org/10.1038/nprot.2009.97 PMID: 19617889

31. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139–140. https://doi.org/10.1093/bioinformatics/btp616 PMID: 19910308

32. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, et al. (2015) limma powers differential expression analysis of digital gene expression data. Bioinformatics 28: 882–883. https://doi.org/10.1093/bioinformatics/bts205 PMID: 22257669

33. Dixon P (2003) VEGAN, a package of R functions for community ecology. Journal of Vegetation Science 14: 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

34. Kolberg L, Raudvere U, Kuzmin I, Vilo J, Peterson H (2020) gprofiler2—an R package for gene list functional enrichment analysis and namespace conversion toolset g:Profiler. F1000Res 9: 709. https://doi.org/10.12688/f1000research.24956.2 PMID: 33564394

35. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 102: 15545–15550. https://doi.org/10.1073/pnas.0506580102 PMID: 16199517

36. Hänzelmann S, Castelo R, Guinney J (2013) GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 14: 7. https://doi.org/10.1186/1471-2105-14-7 PMID: 23323831

37. Sturm G, Finotello F, Petitprez F, Zhang JD, Baumbach J, et al. (2019) Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-ontology. Bioinformatics 35: i436–i445. https://doi.org/10.1093/bioinformatics/btz2363 PMID: 31506600

38. Bacht E, Giraldo NA, Lacroix L, Buttard B, Elarouchi N, et al. (2016) Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol 17: 218. https://doi.org/10.1186/s13059-016-1070-5 PMID: 27765066

39. Rusinova I, Forster S, Yu S, Kannan A, Masse M, et al. (2013) Interferome v2.0: an updated database of annotated interferon-regulated genes. Nucleic Acids Res 41: D1040–D1046. https://doi.org/10.1093/nar/gks1215 PMID: 22890015

40. Yamagishi J, Natori A, Tolba MEM, Mongan AE, Sugimoto C, et al. (2014) Interactive transcriptome analysis of malaria patients and infecting Plasmodium falciparum. Genome Res 24: 1433–1444. https://doi.org/10.1101/gr.158980.113 PMID: 25091627

41. Singhania A, Graham CM, Gabryšlová L, Moreira-Teixeira L, Stavropoulos E, et al. (2019) Transcriptional profiling unveiling type I and II interferon networks in blood and tissues across diseases. Nat Commun 10: 2887. https://doi.org/10.1038/s41467-019-10601-6 PMID: 31253760
43. Gun SY, Claser C, Tan KSW, Rênia L (2014) Interferons and interferon regulatory factors in malaria. Mediators Inflamm 2014: 243713. https://doi.org/10.1155/2014/243713 PMID: 25157202

44. Schoggins JW (2019) Interferon-Stimulated Genes: What Do They All Do? Annu Rev Virol 6: 567–584. https://doi.org/10.1146/annurev-virology-092818-015756 PMID: 31283436

45. Schneider WM, Chevillotte MD, Rice CM (2014) Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol 32: 513–545. https://doi.org/10.1146/annurev-immunol-032713-120231 PMID: 24554742

46. Schoggins JW (2014) Interferon-stimulated genes: roles in viral pathogenesis. Curr Opin Virol 6: 40–46. https://doi.org/10.1016/j.coovi.2014.03.006 PMID: 24713352

47. Fakiola M, Singh OP, Syn G, Singh T, Singh B, et al. (2019) Transcriptional blood signatures for active and amphotericin B treated visceral leishmaniasis in India. PLoS Negl Trop Dis 13: e0007673. https://doi.org/10.1371/journal.pntd.0007673 PMID: 31419223

48. Blackwell JM, Fakiola M, Singh OP (2020) Genetics, Transcriptomics and Meta-Taxonomics in Visceral Leishmaniasis. Front Cell Infect Microbiol 10: 590888. https://doi.org/10.3389/fcimb.2020.590888 PMID: 33324576

49. Parnell GP, McLean AS, Booth DR, Armstrong NJ, Nalos M, et al. (2012) A distinct influenza infection signature in the blood transcriptome of patients with severe community-acquired pneumonia. Crit Care 16: R157. https://doi.org/10.1186/cc11477 PMID: 22894041

50. Herberg JA, Kaforou M, Gormley S, Sumner ER, Patel S, et al. (2013) Transcriptomic profiling in childhood H1N1/09 influenza reveals reduced expression of protein synthesis genes. J Infect Dis 208: 1644–1668. https://doi.org/10.1093/infdis/jit348 PMID: 23901082

51. Campos TM, Passos ST, Novais FO, Beiting DP, Costa RS, et al. (2014) Matrix metalloproteinase 9 production by monocytes is enhanced by TNF and participates in the pathology of human cutaneous Leishmaniasis. PLoS Negl Trop Dis 8: e3282. https://doi.org/10.1371/journal.pntd.0003282 PMID: 25393535

52. Passos S, Carvalho LP, Costa RS, Campos TM, Novais FO, et al. (2015) Intermediate monocytes contribute to pathologic immune response in Leishmania braziliensis infection. J Infect Dis 211: 274–282. https://doi.org/10.1093/infdis/jiu349 PMID: 25139016

53. Carneiro PP, Conceição J, Macedo M, Magalhães V, Carvalho EM, et al. (2016) The Role of Nitric Oxide and Reactive Oxygen Species in the Killing of Leishmania braziliensis by Monocytes from Patients with Cutaneous Leishmaniasis. PLoS One 11: e0148084. https://doi.org/10.1371/journal.pone.0148084 PMID: 26840253

54. Askenase MH, Han S-J, Byrd AL, Morais da Fonseca D, Bouladoux N, et al. (2015) Bone-Marrow-Resident NK Cells Prime Monocytes for Regulatory Function during Infection. Immunity 42: 1130–1142. https://doi.org/10.1016/j.immuni.2015.05.011 PMID: 26070484

55. Barral A, Barral-Netto M, Almeida R, de Jesus AR, Grimaldi Júnior G, et al. (1992) Lymphadenopathy associated with Leishmania braziliensis cutaneous infection. Am J Trop Med Hyg 47: 587–592. https://doi.org/10.4269/ajtmh.1992.47.587 PMID: 1449199

56. Barral A, Guerreiro J, Bomfim G, Correia D, Barral-Netto M, et al. (1995) Lymphadenopathy as the first sign of human cutaneous infection by Leishmania braziliensis. Am J Trop Med Hyg 53: 256–259. https://doi.org/10.4269/ajtmh.1995.53.256 PMID: 7573708

57. Moulton VR, Suarez-Fueyo A, Meidan E, Li H, Mizui M, et al. (2017) Pathogenesis of human systemic lupus erythematosus: A cellular perspective. Trends Mol Med 23: 615–635. https://doi.org/10.1016/j.trends.2017.05.006 PMID: 28623084

58. Boehncke W-H (2018) Systemic inflammation and cardiovascular comorbidity in psoriatic patients: causes and consequences. Front Immunol 9: 579. https://doi.org/10.3389/fimmu.2018.00579 PMID: 29675020

59. Singh N, Kumar R, Chauhan SB, Engwerda C, Sundar S (2018) Peripheral blood monocytes with an antiinflammatory phenotype display limited phagocytosis and oxidative burst in patients with visceral leishmaniasis. J Infect Dis 218: 1130–1141. https://doi.org/10.1093/infdis/jiy168 PMID: 30053070

60. Diefenbach A, Schindler H, Donhauser N, Lorenz E, Laskay T, et al. (1998) Type 1 interferon (IFN-alpha/beta) and type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. Immunity 8: 77–87. https://doi.org/10.1016/s1074-7613(00)80460-4 PMID: 9462513

61. Kumar R, Bunn PT, Singh SS, Ng SS, Montes de Oca M, et al. (2020) Type I Interferons Suppress Anti-parasitic Immunity and Can Be Targeted to Improve Treatment of Visceral Leishmaniasis. Cell Rep 30: 2512–2525.e9. https://doi.org/10.1016/j.celrep.2020.01.099 PMID: 32101732

62. Xin L, Vargas-Inchaustegui DA, RAINER SS, Kelly BC, Hu J, et al. (2010) Type I IFN receptor regulates neutrophil functions and innate immunity to Leishmania parasites. J Immunol 184: 7047–7056. https://doi.org/10.4049/jimmunol.0903273 PMID: 20483775
63. Rossi M, Castiglioni P, Hartley M-A, Eren RO, Prével F, et al. (2017) Type I interferons induced by endogenous or exogenous viral infections promote metastasis and relapse of leishmaniasis. Proc Natl Acad Sci USA 114: 4987–4992. https://doi.org/10.1073/pnas.1621447114 PMID: 28439019

64. Sacramento LA, Benevides L, Maruyama SR, Tavares L, Fukutani KF, et al. (2020) TLR4 abrogates the Th1 immune response through IRF1 and IFN-β to prevent immunopathology during L. infantum infection. PLoS Pathog 16: e1008435. https://doi.org/10.1371/journal.ppat.1008435 PMID: 32210480

65. Khouri R, Bafica A, Silva M da PP, Noronha A, Kolb J-P, et al. (2009) IFN-beta impairs superoxide-dependent parasite killing in human macrophages: evidence for a deleterious role of SOD1 in cutaneous leishmaniasis. J Immunol 182: 2525–2531. https://doi.org/10.4049/jimmunol.0802860 PMID: 19201909

66. Ji DX, Yamashiro LH, Chen KJ, Mukaida N, Kramnik I, et al. (2019) Type I interferon-driven susceptibility to Mycobacterium tuberculosis is mediated by IL-1Ra. Nat Microbiol 4: 2128–2135. https://doi.org/10.1038/s41564-019-0578-3 PMID: 31611644

67. Huggins MA, Johnson HL, Jin F, N Songo A, Hanson LM, et al. (2017) Perforin Expression by CD8 T Cells Is Sufficient To Cause Fatal Brain Edema during Experimental Cerebral Malaria. Infect Immun 85. https://doi.org/10.1128/IAI.00985-16 PMID: 28264905

68. Kaminski L-C, Riehn M, Abel A, Steeg C, Yar DD, et al. (2019) Cytotoxic T Cell-Derived Granzyme B Is Increased in Severe Plasmodium Falciparum Malaria. Front Immunol 10: 2917. https://doi.org/10.3389/fimmu.2019.02917 PMID: 31921176

69. Campos TM, Novais FO, Saldanha M, Costa R, Lordelo M, et al. (2020) Granzyme B produced by natural killer cells enhances inflammatory response and contributes to the immunopathology of cutaneous leishmaniasis. J Infect Dis 221: 973–982. https://doi.org/10.1093/infdis/jiz538 PMID: 31748808

70. Allen M, Bailey C, Cahatol I, Dodge L, Yim J, et al. (2015) Mechanisms of Control of Mycobacterium tuberculosis by NK Cells: Role of Glutathione. Front Immunol 6: 508. https://doi.org/10.3389/fimmu.2015.00508 PMID: 26500648

71. Mazzaccaro RJ, Stenger S, Rock KL, Porcelli SA, Brenner MB, et al. (1998) Cytotoxic T lymphocytes in resistance to tuberculosis. Adv Exp Med Biol 452: 85–101. https://doi.org/10.1007/978-1-4615-5355-7_11 PMID: 9889963

72. Serbina N, Liu C-C, Scanga C, Flynn J (n.d.) 1 CTL from Lungs of Mycobacterium tuberculosis-Infected Mice Express Perforin In Vivo and Lyse Infected Macrophages1.

73. Machado PR, Ampuero J, Guimarães LH, Villasboas L, Rocha AT, et al. (2010) Miltefosine in the treatment of cutaneous leishmaniasis caused by Leishmania braziliensis in Brazil: a randomized and controlled trial. PLoS Negl Trop Dis 4: e912. https://doi.org/10.1371/journal.pntd.0000912 PMID: 21200420

74. Prates FV de O, Dourado MEF, Silva SC, Schriefer A, Guimarães LH, et al. (2017) Fluconazole in the Treatment of Cutaneous Leishmaniasis Caused by Leishmania braziliensis: A Randomized Controlled Trial. Clin Infect Dis 64: 67–71. https://doi.org/10.1093/cid/ciw662 PMID: 27803094