Genomic Profiling of Human *Leishmania braziliensis* Lesions Identifies Transcriptional Modules Associated with Cutaneous Immunopathology

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The host immune response has a critical role not only in protection from human leishmaniasis but also in promoting disease severity. Although candidate gene approaches in mouse models of leishmaniasis have been extremely informative, a global understanding of the immune pathways active in lesions from human patients is lacking. To address this issue, genome-wide transcriptional profiling of *Leishmania braziliensis*-infected cutaneous lesions and normal skin controls was carried out. A signature of the *L. braziliensis* skin lesion was defined, which includes over 2,000 differentially regulated genes. Pathway-level analysis of this transcriptional response revealed key biological pathways present in cutaneous lesions, generating a testable 'metapathway' model of immunopathology and providing new insights for treatment of human leishmaniasis.

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INTRODUCTION

Leishmania braziliensis has a spectrum of clinical manifestations, all of which are associated with immunopathology (de liveira and Brodskyn, 2012). Patients develop small nodules at the site of infection that progress to chronic ulcerated lesions. We hypothesize that, although parasite infection acts as an initial trigger for lesion development, it is the immunopathologic response that determines disease severity. Thus, defining the host inflammatory pathways within leishmania lesions is crucial for the development of new treatment modalities.

Many studies have examined the systemic immune response in *L. braziliensis*-infected patients, and show that cells from patients release pro-inflammatory molecules in response to leishmania antigen (Bottrel *et al.*, 2001; Follador *et al.*, 2002; Vargas-Inchaustegui *et al.*, 2010). These responses likely contribute to both the control of the parasites and the pathologic inflammatory response in the lesions (Bosque *et al.*, 1998; Bacellar *et al.*, 2002; de Oliveira and Brodskyn, 2012; Giudice *et al.*, 2012). Although important, these systemic responses may not reflect what is occurring at the site of infection. Indeed, recent studies of lesion biopsies from *L. braziliensis* patients have revealed an unexpected pathologic role for CD8 T cells during disease, which would not have been obvious from studies on systemic responses (Novais *et al.*, 2013; Santos Cda *et al.*, 2013).

Transcriptome analysis has helped elucidate critical genes expressed during interactions between leishmania parasites and human macrophages (Ramirez *et al.*, 2012). In addition, a genomic profiling has been reported for leishmania lesions from patients, in which the authors compared cutaneous leishmaniasis (CL) and mucosal leishmaniasis (Maretti-Mira *et al.*, 2012). To our knowledge, however, ours is the first report to dissect the changes that occur in the skin after infection with leishmania when compared with normal skin. Using a genome-wide transcriptional analysis, we report on the pathways present in *L. braziliensis* lesions and propose a hypothetical 'metapathway' of immunopathology that drives disease.

RESULTS

Comparative transcriptomics of *L. braziliensis* lesions and normal skin

We performed genome-wide transcriptional profiling on 25 biopsies from *L. braziliensis* patients (Supplementary Table S1 online) and 10 normal skin biopsies obtained from non-endemic controls. Principal component analysis (PCA) of the entire data set showed that principal component 1 (PC1) accounted for 54.3% of the variation in the data and resolved samples into two main groups, normal and lesion skin. PC2 accounted for a smaller amount of variation (12.4%) occurring

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Abbreviations: CL, cutaneous leishmaniasis; FC, fold change; GO, Gene Ontology; GSEA, gene set enrichment analysis; HC, hierarchical clustering; MSigDB, Molecular Signatures Database; PCA, principal component analysis

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within both these groups (Figure 1a). The separation of lesion and control samples along a single principal component indicated that differentially expressed genes could be identified with high statistical confidence.

Analysis of *L. braziliensis* lesions compared with normal skin identified 2,028 differentially expressed genes (⩾2-fold, false discovery rate ⩽1%) (Figure 1b). Hierarchical clustering (HC) based on Pearson's correlation delineated two major clusters. Cluster 1 comprises 947 genes whose abundance is decreased in lesions, relative to normal skin. The 10 most ''repressed'' genes from this cluster include genes associated with maintenance of skin barrier function, such as keratin-27 (*KRT27*), filaggrin-2 (*FLG2*), and dermcidin (*DCD*) (Figure 1c). Cluster 2 comprises 1,081 transcripts that were more abundant in lesions compared with normal skin. The most strongly 'induced' members from this cluster included genes associated with inflammatory cell recruitment (*CXCL9, CXCL10,* and *CCL8*) and cytotoxicity (*GZMA, GZMB,* and *GLYN*) (Figure 1d).

Functional enrichment and pathway analysis of the *L. braziliensis* lesion

We next carried out a functional enrichment analysis using Gene Ontology (GO) terms (Ashburner *et al.*, 2000). Genes upregulated in lesions were enriched in GO terms related to inflammation, host defense, and chemotaxis (Figure 2a). In contrast, genes downregulated in lesions were associated primarily with fatty acid metabolism and epidermal development (Figure 2a). This enrichment analysis suggests that lesion development is associated with a remodeling of the local skin environment, marked by induction of a potent pro-inflammatory signature and a concomitant loss of epidermal and fatty acid metabolic signatures. Although useful for identifying general functional categories, GO enrichment analysis is biased in that it requires a relatively arbitrary selection of differentially expressed genes as input. Therefore, using gene set enrichment analysis (GSEA) analysis we leveraged manually curated pathway databases, including Reactome, Kyoto Encyclopedia of Genes and Genomes, Biocarta, and the Pathway Interaction Database (Nishimura, 2001; Vastrik *et al.*, 2007; Schaefer *et al.*, 2009; Kanehisa *et al.*, 2014), to identify the key pathways enriched in lesions. Despite our finding that over 2,000 genes were differentially regulated in the *L. braziliensis* lesion, pathway analysis showed that much of this transcriptional response could be explained by a small number of pathways (Figure 2b). GSEA results confirmed a potent repression of fatty acid metabolism in the *L. braziliensis* lesion. To further investigate this altered metabolic profile, we

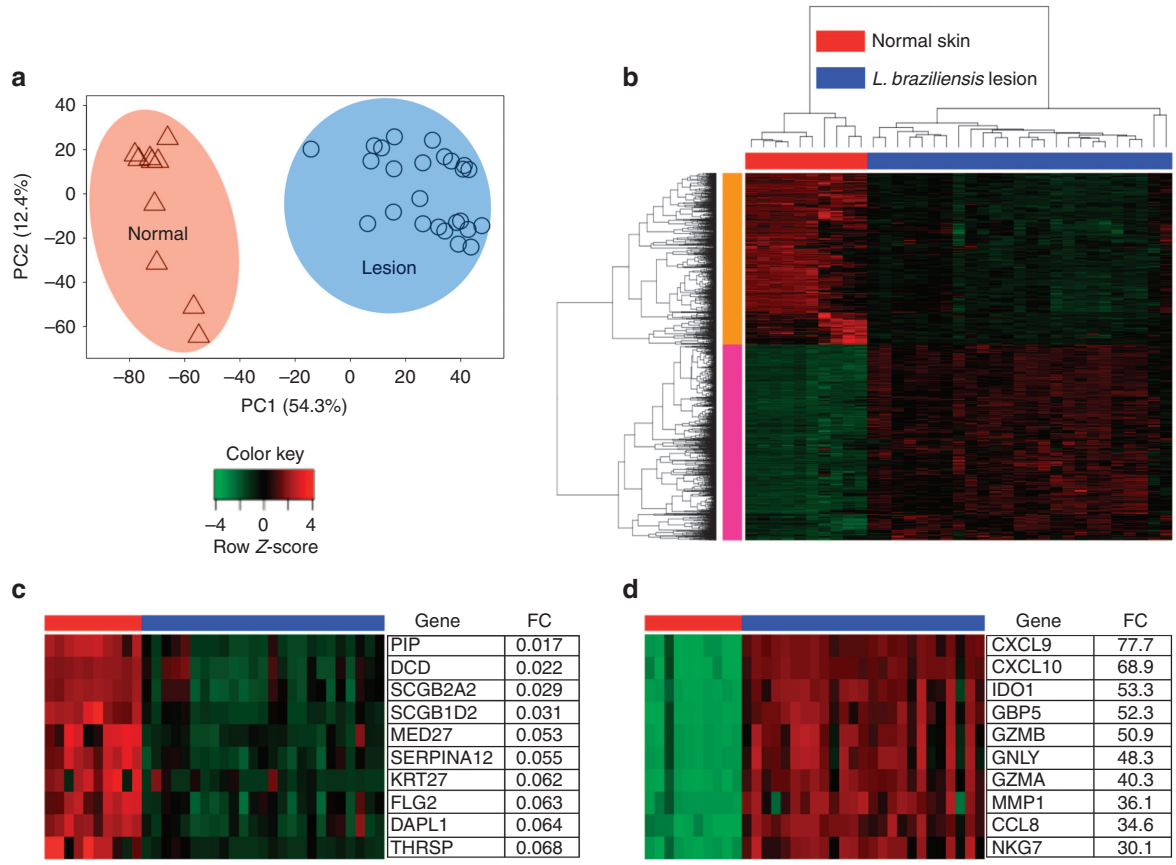

Figure 1. Defining the transcriptome of the human *L. braziliensis* skin lesion. (**a**) Principal component analysis of the entire normalized data set showing PC1 (*x* axis) and PC2 (*y* axis). (**b**) HC of all the differentially expressed genes between normal skin and *L. braziliensis* lesion (FC ⩾2 and FDR ⩽1%). (**c**, **d**) Top 10 'induced' (**c**) and 'repressed' (**d**) genes. (**b**–**d**) Columns represent individual patients and rows represent individual genes, colored to indicate expression levels based on a *Z*-score. FC, fold change; FDR, false discovery rate; HC, hierarchical clustering; PC, principal component.

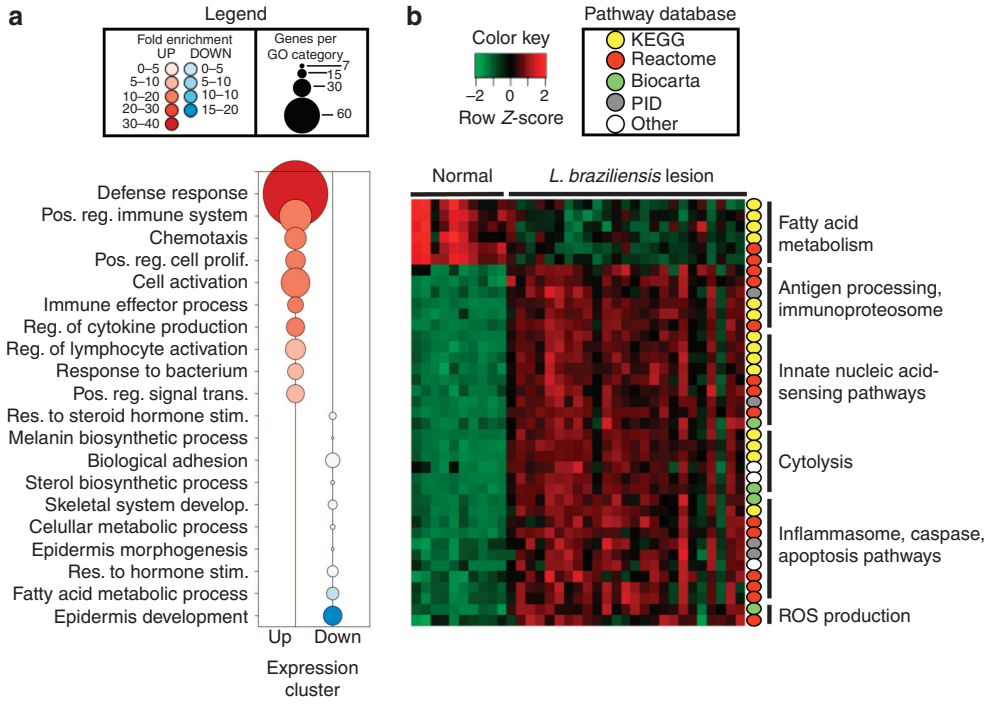

Figure 2. Functional enrichment analysis of *L. braziliensis*-infected skin. (**a**) GO enrichment analysis showing Biological Process terms enriched in induced genes from Figure 1b (red) or repressed genes (blue) in lesions relative to normal skin. (**b**) GSEA showing enriched pathways from the MsigDB C2 canonical pathway collection. Color-coded circles to the right indicate pathway database provenance. Columns represent samples and rows represent individual pathways, colored to indicate expression levels based on a *Z*-score. GO, Gene Ontology; GSEA, gene set enrichment analysis; KEGG, Kyoto Encyclopedia of Genes and Genome; MSigDB, Molecular Signatures Database; PID, Pathway Interaction Database.

examined all genes known to be involved in either cholesterol or triglyceride and free fatty acid metabolism (Supplementary Figure S1 online). Interestingly, we identified a global repression of both cholesterol and free fatty acid biosynthesis (Supplementary Figure S1c—d online), and a significant increase in expression of lipid exporters (Supplementary Figure S1e–f online), suggesting that *L. braziliensis* lesions are characterized by dysregulated lipid biosynthesis. In contrast, lesions showed marked induction of at least five key pathways. As expected, cytotoxicity and pathways involved in the generation of reactive oxygen species were strongly induced in the *L. braziliensis* lesion (Novais *et al.*, 2013, 2014). In addition, this analysis identified at least three other pathways associated with the lesion transcriptome: (1) antigen processing and immunoproteasome activation; (2) nucleic acid sensing; and (3) inflammasome activation and apoptosis.

Identification of unique and conserved pathways associated with skin lesion disease

Our data identified core pathways associated with the *L. braziliensis* lesion; however, it remained an open question as to whether they were a common feature of skin inflammation. To address this question, we compared our data with similar transcriptomic data generated from human psoriasis lesions (Figure 3). With the use of data obtained from 334 paired microarrays from lesion and non-lesion sites in 167 patients (Tian *et al.*, 2012), we quantitatively compared the enrichment of pathways in leishmania lesions with psoriasis (Figure 3). As expected, only the *L. braziliensis* lesion was enriched for ''JAK/signal transducer and activator of transcription signaling'', the ''IFN-γ pathway'', and the ''Leishmaniasis'' Kyoto Encyclopedia of Genes and Genomes pathway, all of which include genes well known to be critical mediators of protection from this parasite. In addition, *L. braziliensis* lesions were uniquely enriched for ''NK-mediated cytotoxicity'' and ''allograft rejection'', whereas our analysis showed that the ''graft versus host disease'' is enriched in both diseases. However, we found it to be much more strongly enriched in *L. braziliensis* lesions, suggesting that this pathologic response is a dominant feature of CL. Similarly, this analysis also identified inflammasome activation as a major pathway activated in *L. braziliensis* lesions but not in psoriasis. Several pathways were preferentially enriched in psoriasis and primarily included cell proliferation and nucleotide metabolism. Finally, several pathways were enriched in both diseases, including IFN-α/β signaling, nucleotide-binding and oligomerization domain-like receptor signaling, cytosolic DNA sensing, defensins, and regulation of apoptosis. Taken together, this comparison indicates that *L. braziliensis* induces a molecular signature of disease distinct from psoriasis.

Early- and late-stage lesions are transcriptionally indistinguishable

Lesions from *L. braziliensis* patients could be classified into two categories based on the clinical stage of the disease, termed early and late (Figure 4 and Supplementary Table S1

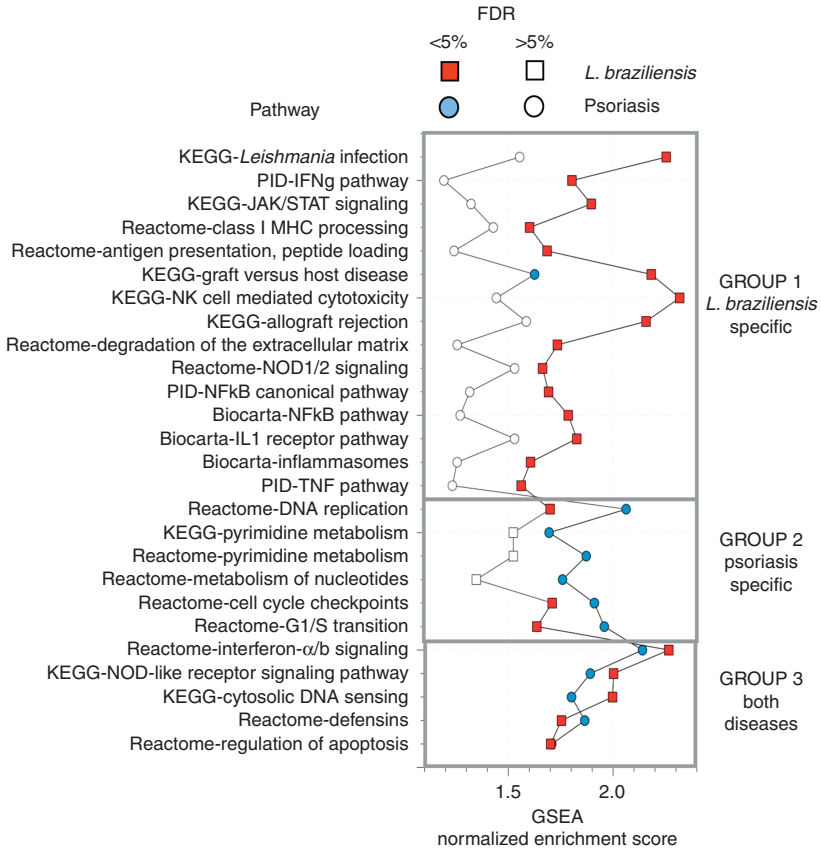

Figure 3. *L. braziliensis* and psoriasis skin lesions are associated with activation of distinct transcriptional modules. Normalized enrichment scores (*x* axis) for select pathways (*y* axis) identified as being significantly enriched in psoriasis (blue squares) and/or *L. braziliensis* (red circles) skin lesions by gene set enrichment analysis. Open symbols indicate nonsignificant enrichment. FDR, false discovery rate; GSEA, gene set enrichment analysis; KEGG, Kyoto Encyclopedia of Genes and Genome; PID, Pathway Interaction Database.

online). Patients with early lesions had a small papule with no evident ulceration, a median lesion size of 38 mm^2 (Figure 4a), and an illness duration of $\leqslant$30 days (Figure 4b). In contrast, patients with late lesions had an illness duration of $\geqslant$30 days, with ulcerated lesions with a median size of 250 mm^2. Despite these marked differences, PCA of the entire transcriptome (data not shown) and an HC of the differentially expressed genes failed to resolve early- and late-stage lesions as transcriptionally distinct disease states (Figure 4c). In addition, our analysis failed to find any significant differentially expressed genes between the two lesion stages, even when less stringent cutoffs were used (fold change = 1.5 and $P \leqslant 0.05$) (data not shown). The observation that *L. braziliensis* lesions at different clinical stages are indistinguishable by gene expression and pathway analysis (data not shown) reveals that the key pathways associated with *L. braziliensis* lesions are evident well before the development of ulcerated skin lesions, and therefore may be promoting cutaneous pathology, rather than simply arising as a consequence of disease.

Identification of genes associated with a molecular signature of skin pathology

We next sought to identify gene signatures that contributed to patient-to-patient variability in the lesion transcriptome. A PCA was carried out using only the 2,028 differentially expressed genes from lesion samples. PC1 accounted for 35.2% of the variability within the group of lesion samples, followed by 11.1% in PC2 (Figure 5a), and PC1 and PC2 together explained almost half of the variation between patients' samples. This analysis showed that patients varied in their induction of this transcriptional program, but this variation was independent of age, sex, drug sensitivity (data not shown), and lesion stage (Figure 5a). To determine which genes had the strongest influence on these two principal components, and therefore contributed the most to variability in the lesion transcriptome between patients, we plotted the PCA 'scores' from all differentially expressed genes for PC1 and PC2 (Supplementary Figure S2 online). This analysis identified a subset of immune and skin barrier function genes, whose expression is variable across the patients. A subset of immune genes and skin barrier genes (Supplementary Figure S2 online) from our PCA score plot was selected for correlation analysis (Figure 5b). As expected, there was a strong positive correlation between functionally related genes (Figure 5b), such as components of the cytolytic granule (*GZMB, GNLY,* and *PRF1*) (Figure 5b), meaning that patients with high levels of granzyme B transcript in the lesion often had high levels of granulysin and perforin. In contrast, the

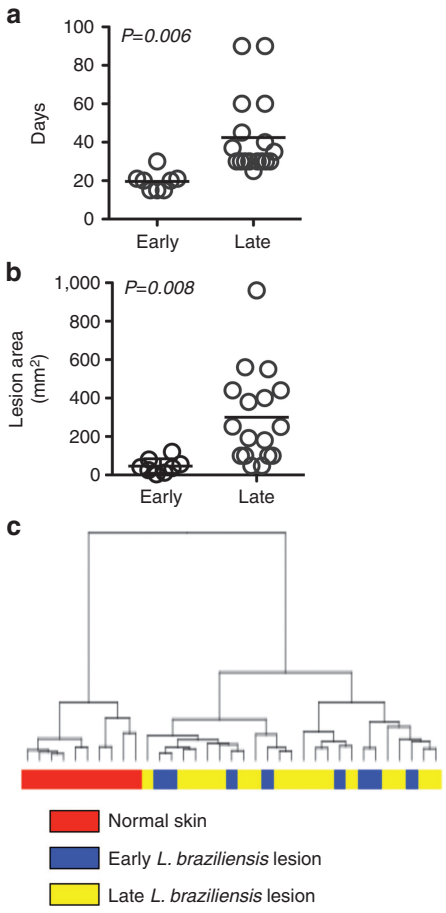

Figure 4. Early and late lesions from *L. braziliensis*-infected skin are transcriptionally indistinguishable. (**a**) Illness duration (days) and (**b**) lesion size from early- and late-stage *L. braziliensis*-infected patients. (**c**) Hierarchical clustering of normal skin and early- and late-stage *L. braziliensis*-infected patients based on differentially expressed genes.

cytolytic genes and components of the inflammasome, as well as *IL8* and *LAG3*, showed a strong negative correlation with skin barrier genes such as filaggrin-1 and -2 and loricrin (*FLG*, *FLG2,* and *LOR*) (Figure 5b). Indeed, the more *GLNY* (Figure 5c) or *IL1B* (Figure 5d) expressed in a lesion, the less *FLG* or *LOR* expressed. We also observed a negative correlation between the expressions of *AIM2* and *LOR* (data not shown). This inverse correlation between immune genes and skin barrier genes was not a general relationship seen with just any strongly induced immune effector gene, as there was no significant correlation between the expression of *NCF1* and skin barrier genes (Figure 5e). Taken together, these results show that patients exhibit variability in induction of specific genes and that a subset of these genes is associated with a more severe molecular signature of skin pathology.

DISCUSSION

Here, we identified key immunological pathways induced following infection and developed a putative model explaining immunopathology in *L. braziliensis* lesions (Figure 6). Activation of CD8 T cells requires recognition of antigens presented via major histocompatibility complex class I, and the immunoproteasome has an important role in this process (Groettrup *et al.*, 2010). We hypothesize that immunoproteasome activation drives cytolytic CD8 T cells in the skin. As cell death leads to the release of danger-associated molecular patterns (DAMPs), we propose that DAMPs act as a positive feedback that potentiates CD8 T–cell (Bonilla *et al.*, 2012; Kim *et al.*, 2014) and inflammasome (Latz *et al.*, 2013) activation. The inflammasome has been implicated in detrimental responses to several inflammatory diseases (Davis *et al.*, 2011), and its definitive role in human leishmaniasis is still unclear. Finally, the most highly induced genes, *CXCL10* and *CXCL9,* are chemokines that recruit T cells, and we propose that excessive expression of these chemokines brings more CD8 T cells to the skin, thereby exacerbating immunopathology.

Genes associated with the development and function of T helper type 1 (Th1) responses were highly expressed in *L. braziliensis* lesions, whereas genes associated with Th2 (Novais *et al.*, 2014) or Th17 responses (data not shown) were not induced. This contrasts with the observations that Th2 and Th17 responses are induced in mucosal disease (Boaventura *et al.*, 2010; Maretti-Mira *et al.*, 2012). Our results are consistent with the strong Th1 response observed systemically in CL patients (Carvalho *et al.*, 2012). Several genes downstream of IFN-γ were upregulated and may contribute to pathology. These data show an increased expression of immunoproteasome genes in CL, which helps in generating major histocompatibility complex class I epitopes from the parasite and ultimately increase CD8 T–cell activation. Also, studies indicate that the immunoproteasome contributes to inflammation (Muchamuel *et al.*, 2009) and CD8 T–cell survival (Moebius *et al.*, 2010). In addition to immunoproteasome-related genes, IFN-γ also induces expression of CXCL10 and CXCL9, both of which recruit activated T cells and NK cells (Dufour *et al.*, 2002). Therefore, we propose that, in addition to its well-known function in parasite control (Kaye and Scott, 2011), IFN-γ participates indirectly in immunopathological responses in *L. braziliensis* infection by inducing the recruitment of CD8 T cells and NK cells to the skin and triggering cytotoxicity by stimulating the immunoproteasome activation and antigen presentation to CD8 T cells.

We found that cytotoxicity is one of the main signatures of disease induced by *L. braziliensis,* a finding consistent with a previous study performed with a smaller number of samples (Novais *et al.*, 2013). Although we find Th1 responses induced in lesions, the dominance of the cytolytic pathway is evident when one compares the fold change in *IFNG* and *GZMB* expression between normal skin and leishmanial lesions. As expected, *IFNG* is increased in expression (8.8 fold change) (Novais *et al.*, 2014), but *GZMB* has a significantly higher fold change (50.9) (Novais *et al.*, 2013). In *L. braziliensis* patients' lesions, CD4 but not CD8 T cells produce IFN-γ, and thus the main function of CD8 T cells in the lesions of patients appears to be cytotoxicity (Santos Cda *et al.*, 2013). We found that cytotoxic CD8 T cells mediated immunopathology in mice, but the mechanism by which cytotoxicity enhanced disease was unclear (Novais *et al.*, 2013). In light of our transcriptome

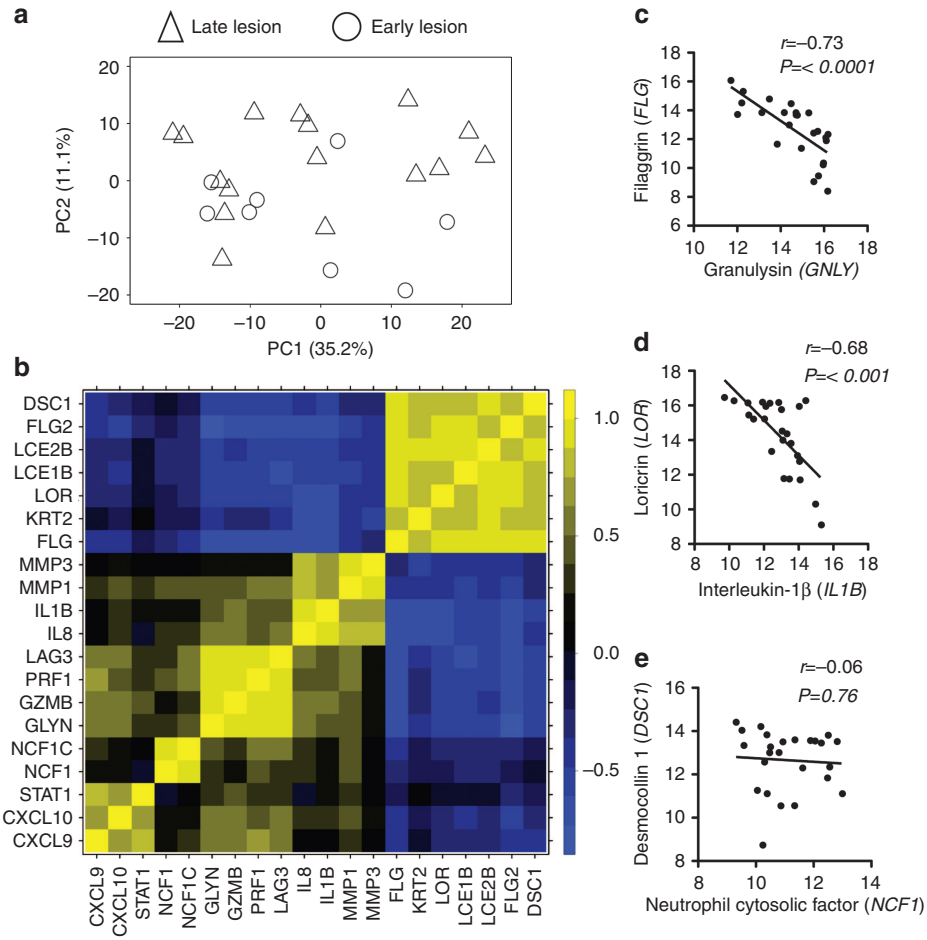

Figure 5. Cytotoxicity and inflammasome-related genes inversely correlate with expression of skin barrier function genes. (**a**) Principal component analysis of differentially expressed genes from *L. braziliensis*-infected patients. (**b**) Correlation heatmap showing selected modules of genes. Columns and rows represent individual genes, colored to indicate the correlation coefficient (*r*). (**c**–**e**) Log2 expression of (**c**) filaggrin and granulysin, (**d**) loricrin and interleukin-1β, and (**e**) desmocolin-1 and neutrophil cytosolic factor-1 in *L. braziliensis*-infected patients.

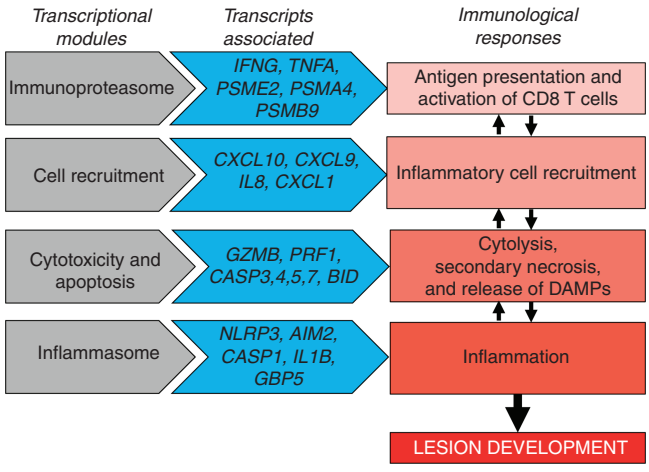

Figure 6. A putative "metapathway" driving immunopathology in cutaneous leishmaniasis. Transcriptional modules (pathways) induced during leishmaniasis (shown on the left), with examples of genes that are differentially expressed in lesions (shown in the center), suggest a testable model of immune responses (shown on the right) that lead to immunopathology within leishmanial lesions, in which cytotoxicity has a central role.

analysis, we now hypothesize that the increased pathology mediated by CD8 T cells is due to activation of the inflammasome by release of DAMPs.

Activation of the inflammasome generates mature IL-1β, which promotes increased inflammation by stimulating the production of chemokines, such as IL-8, and also matrix metalloproteinases, which degrade the extracellular matrix leading to more damage to the skin. Our study indicates that genes associated with the inflammasome pathway (such as *IL1B*, *AIM2*, *NLRP3*, *CASP1*, and *CASP5*) are highly expressed in *L. braziliensis* lesions, suggesting that there is inflammasome activation and secretion of IL-1β during disease. In fact, *ex vivo*-cultured human *L. braziliensis* lesions release IL-1β protein into culture supernatants (Carvalho *et al.*, unpublished data). However, the role that the inflammasome and subsequent IL-1β have in human disease is still unclear. IL-1β mRNA was previously found in lesions from *L. braziliensis* patients (Pirmez *et al.*, 1993), and in individuals infected with *L. mexicana* IL-1β production has been linked to disease severity (Fernandez-Figueroa *et al.*, 2012). Here, we expand those results by demonstrating that genes associated with two

inflammasome pathways, *AIM2* and *NLRP3*, are upregulated in lesions and thus may have a previously unappreciated role in *L. braziliensis* human disease.

Skin diseases can share some characteristics. For example, dysbiosis of the skin has recently been considered a distinctive feature of both CL and psoriasis (Cho and Blaser, 2012; Naik *et al.*, 2012). In addition, IFN-γ has been associated with immunopathology in both diseases, although by different mechanisms. In *L. braziliensis* infection, IFN-γ is thought to induce immunopathology by activating innate cells. In psoriasis, IFN-γ synergizes with other pro-inflammatory cytokines, notably IL-17, and induces activation of keratinocytes. Although Th17 responses have been implicated in *L. braziliensis* infection in mucosal leishmaniasis (Boaventura *et al.*, 2010; Maretti-Mira *et al.*, 2012), we could not detect differences in IL-17 transcripts in *L. braziliensis* patients, suggesting that, unlike psoriasis, *L. braziliensis* CL is not associated with a Th17 response. Our comparison of pathways enriched in these two diseases revealed additional differences. For example, although cytotoxicity has been implicated in both leishmaniasis (Novais *et al.*, 2013) and psoriasis (Yawalkar *et al.*, 2001; Prpic Massari *et al.*, 2007), our data show that cytotoxicity is a more pronounced signature in *L. braziliensis* infection.

A surprising finding of our study was that the transcriptional profile of non-ulcerated lesions was similar to those of patients with ulcerated lesion. This result suggests that, early after infection, inflammatory pathways are activated in the skin, which may explain why lesions often develop despite early detection and treatment (Machado *et al.*, 2002). Although our data are based on a fraction of the total lesion, as biopsies were collected from the border of the ulcer, we believe the results appropriately reflect the ongoing immune response as the ulcer is mainly composed of dead cells. As disease signatures are present before the ulcer develops, our data position cytotoxicity, immunoproteasome, and inflammasome as potential causes of lesion development, rather than as simply arising as consequence of disease.

Therapies that target the inflammatory response, without affecting mechanisms that kill the parasites, would be an ideal adjunct to drug treatment in leishmaniasis. Here, we have identified a hypothetical metapathway that leads from CD8 T–cell activation and cytolysis to IL-1β production. As cytotoxicity does not control *L. braziliensis* parasites (Novais *et al.*, 2013; Santos Cda *et al.*, 2013), nor does IL-1β appear to be protective in humans (Fernandez-Figueroa *et al.*, 2012), blocking the major components of this metapathway should limit pathology without affecting parasite control.

MATERIALS AND METHODS

Ethics statement

This study was conducted according to the principles specified in the Declaration of Helsinki and under local ethical guidelines, and this study was approved by the Ethical Committee of the Federal University of Bahia (Salvador, Bahia, Brazil)(010/10) and the University of Pennsylvania IRB (Philadelphia, PA) (813390). All patients provided written informed consent for the collection of samples and subsequent analysis.

Patients and biopsies

All CL patients were seen at the health post in Corte de Pedra, Bahia, Brazil, which is a well-known area of *L. braziliensis* transmission. The criteria for diagnosis were a clinical picture characteristic of CL in conjunction with parasite detection or a positive delayed-type hypersensitivity response to leishmania antigen. Prior to therapy, biopsies were collected at the border of the lesions using a 4-mm punch before therapy. Normal skin samples were taken from volunteers who were living in a non-endemic area without a history of leishmaniasis.

Transcriptional profiling and functional enrichment analysis

Microarrays and data analyses were carried out as previously described (Beiting *et al.*, 2014). Briefly, Illumina HumanHT-12 version-4 beadchips (Illumina, San Diego, CA) were hybridized with biotin-labeled cRNA generated from 10 normal skin, 8 early, and 17 late lesion samples. Data analyses were carried out using the statistical computing environment, R (v3.0.2), the Bioconductor suite of packages for R, and RStudio (v0.97; Boston, MA). Probesets that were differentially regulated $\geqslant$2-fold (false discovery rate $\leqslant$1%), after controlling for multiple testing using the Bonferroni–Hochberg method (Reiner *et al.*, 2003), were used for HC and heatmap generation. Data have been deposited on the GEO database for public access (GSE# GSE55664). GSEA (Mootha *et al.*, 2003; Subramanian *et al.*, 2005) was carried out using the Broad Institute's MSigDB (v4.0) and either the GSVA bioconductor package (Figure 2) (Hanzelmann *et al.*, 2013) or the GSEA preranked tool (Figure 3) to query the ''C2: Canonical Pathways'' collection in the MSigDB, which consists of 1,310 gene sets, or ''signatures'', representing annotated pathways.

Comparison of *L. braziliensis* and psoriasis lesion transcriptomes

L. braziliensis data were compared with the MAD-3 human psoriasis data set (Tian *et al.*, 2012), a meta-analysis of three independent psoriasis gene expression studies including 334 paired samples (lesion and non-lesion biopsies) from 167 patients (Yao *et al.*, 2008; Gudjonsson *et al.*, 2009; Suarez-Farinas *et al.*, 2012). The MAD-3 data set was first filtered to remove nonspecific Affymetrix probesets (probeset identifiers ending in ''_x_at''). Genes with multiple probesets were used to calculate a mean fold change relative to non-lesion controls. A total of 17,061 genes in common between the psoriasis MAD-3 data set and our *L. braziliensis* lesion data were used for carrying out a competitive GSEA analysis. Both data sets were rank ordered by Log2 fold change in expression between lesion and control and used as input for the GSEA preranked algorithm (Mootha *et al.*, 2003; Subramanian *et al.*, 2005). GSEA results were explored using the network analysis software Cytoscape (Cline *et al.*, 2007; Smoot *et al.*, 2011) and the Enrichment Map plugin (Merico *et al.*, 2010), in order to identify common and unique pathways.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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