Leishmania RNA virus as a possible determinant of pathogenicity in American Tegumentary Leishmaniasis: Detection in clinic samples

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Background

Tegumentary Leishmaniasis (TL) is a neglected disease affecting rural and low-income populations in whom mucosal involvement (ML) may develop, leading to morbidity even after treatment. ML has been associated with infection by members of Viannia complex, most commonly L. (V) braziliensis, but there is no clear explanation why only 5–10% of individuals with cutaneous leishmaniasis will develop ML. Individuals with ML have high levels of pro-inflammatory cytokines and elevated cytotoxic T-cell activity; however, this phenotype is just a consequence more than the cause of the metastatic process. The Leishmania RNA virus (LRV) has been recently implicated as a possible modulator agent in the pathogenesis of leishmaniasis since its presence seems to link the primary infection with the subsequent development of metastatic disease. The present study aims to identify LRV in clinical samples from patients with cutaneous, disseminated, and mucosal leishmaniasis to test the hypothesis that LRV is more frequently identified in individuals with metastatic disease.

Patients and Methods

Fifty-six patients with a confirmed diagnosis of TL were enrolled at the Leishmania Clinic, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru, after IRB approval from UPCH. A biopsy was obtained for strain identification and measurement of parasite load and identification of LRV dsRNA. Strain identification was performed as described by Veland et al. Clinical specimens for LRV detection were minced with a sterile scalpel, and then homogenized with 0.75 mL of Trizol Ls in a Tenbroeck Homogenizer (Bellco). RNA was isolated according to the manufacturer’s instructions. Then, 0.2–1.0 µg of RNA treated with the RQ1 RNase-Free DNase (Promega) was used for cDNA synthesis with the Transcriptor First Strand cDNA Synthesis Kit (Roche) according to the manufacturer’s instructions. 5 µL of cDNA (1/10) was added to 25 µl quantitative real-time PCR (qPCR) reactions, which contained 1X iQ SYBR Green Supermix (Bio-Rad) and 300 nM of both primers, namely LRV F 5-GAG TGG GAG TCC CCC ACA T-3, and LRV R 5-TGG ATA CAA CCA GAC GAT TGC T-3. Each run included a positive control sample (cDNA from promastigotes of the strain MHOM/BR/75/M4147), a negative control (cDNA from peripheral blood mononuclear cells), a blank (no-template control) and a standard curve. The standard curve was prepared in the range of 1 × 10⁶ to 1 × 10¹ copies/reaction with serial dilutions of a linearized plasmid (containing a cloned segment of the 5’ UTR of the LRV capsid). The ‘Abs Quant/Fit Points’ mode of the LightCycler 480 software v1.5.0 was used to calculate the crossing point cycle (CP). The ‘melting curve genotyping’ mode of the LightCycler software was used to generate the melting curves. A clinical sample was considered positive for LRV if one or more reaction replicates had a CP value and only if their melting curves had the same profile as those of the standards included in the same experiment. A sample was quantified when it had a CP value falling within the range of the standard curve. The highest dilution of template of the standard curve was defined as the limit of quantification (LOQ).

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Results and Discussion

Individuals were predominantly males (85.7%) and median age was 39 years. Disease was acquired in jungle regions (91%) mainly during agricultural activities (62.3%). Enrolled individuals were clinically classified as follows: a) Localized cutaneous Leishmaniasis (LCL, 42.9%); b) Metastatic mucosal disease with inactive cutaneous scar (CML, 37.5%); c) Mucosal disease without clinical or epidemiological evidence of prior cutaneous disease (PML, 5.4%); d) Metastatic mucosal disease with active cutaneous lesion (MCL, 7.1%) and e) Disseminated disease (DisL, 7.1%). LRV was detected in 6 individuals: two patients with CML (10% of CML cases), two with DisL (50% of DisL cases) and two with LCL (9% of LCL cases). HIV infection was identified in one patient with DisL (LRV negative) whereas subclinical TB infection in two patients with DisL, one of them with detectable LRV.

Detection of LRV in clinical samples can be particularly challenging although qRT-PCR has the advantage of being highly sensitive but requires the identification of highly conserved frames to identify genetic polymorphisms. Our results suggest that LRV may be associated with acute parasitic dissemination described by Ives et al in mouse models: detection of LRV was infrequent and similar in CML and LCL but more frequent in DisL. Future studies are necessary to support our findings and identification of frequent immune-modulator conditions like subclinical TB infection need to be adequately addressed. Our findings in human leishmaniasis suggest that LRV may generate DisL but not necessary CML in humans. Current results in LRV however, cannot be extrapolate from animal models to human disease until more robust research will be performed although LRV-1 may be a conditional (but not a necessary) contributor for spread of parasitic infection.

References