Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

The criminal association of *Leishmania* parasites and viruses

Matteo Rossi and Nicolas Fasel

In nature, humans infected with protozoan parasites can encounter viruses, which could alter their host immune response. The impact of viruses on human parasitic diseases remains largely unexplored due to the highly sterilized environment in experimental studies and the difficulty to draw a correlation between co-infection and pathology. Recent studies show that viral infections exacerbate pathology and promote dissemination of some *Leishmania* infections, based on a hyper-inflammatory reaction driven by type I interferons. Thus, not only the infecting parasite species, but also bystander viral infections could be a major determinant of the outcome of *Leishmania* infection. In this review, we focus on the contribution of viral co-infection to the exacerbation of leishmaniasis's pathology and its possible impact on treatment and vaccination strategies.

Address
Department of Biochemistry, University of Lausanne, Epalinges, Switzerland

Corresponding author: Fasel, Nicolas (nicolas.fasel@unil.ch)

Current Opinion in Microbiology 2018, **46**:65–72

This review comes from a themed issue on **Host microbe interactions: parasitology**

Edited by **Pascal Mäser**

https://doi.org/10.1016/j.mib.2018.07.005

Introduction

Leishmaniasis is a disease caused by infection with protozoan parasites of the genus *Leishmania*, transmitted to the human by the bite of an infected sand fly. Affecting over 12 million people worldwide, the outcome of leishmaniasis can vary depending on several factors, ranging from the self-healing cutaneous form (CL) to the more severe and chronic, disseminated or mucocutaneous forms (DCL and MCL, respectively) in 5–10% patients, or to possibly lethal visceral leishmaniasis (VL). In addition, human patients can also develop symptomatic relapses, appearing weeks to years after the healing of the primary infection and often causing more severe symptoms. Despite the major determinant of the disease outcome being the species of the infecting parasite, no conclusive *Leishmania* species-specific virulence factors have been identified. Additionally, no human genetic polymorphisms involving key immune molecules [1] could fully explain the development of more severe forms of leishmaniasis, drug resistance and disease relapses after treatment. This lack of species-specific virulence factors implies the involvement of parasite extrinsic mediators in the determination of the infection outcome.

Viruses not only infect humans but are also found associated to unicellular eukaryotes such as protozoan parasites. This provides evidence of their prevalence throughout evolution and possibly of their importance in human infections. Recent reports on the impact of a viral endosymbiont present in some human protozoan parasites species open up a new field of research focusing on co-infecting viruses. This research aims to explain some of the aggravated symptoms observed in humans infected with *Leishmania* protozoan parasites and possibly in other parasite infections.

Leishmania parasites have developed an arsenal of tools to escape the immune system [2] and limit inflammation [3,4], making it difficult for the host to mount a correct protective immune response. In addition, the concomitant or subsequent infection with a different pathogen could redirect the immune response, thus affecting the course of leishmaniasis. The pathological impact of natural co-infecting pathogens has been widely overlooked because studies are conducted mainly in highly sterilized environments and performed on highly controlled pathogen-free animals harboring defined microbiome and virome. However, in its natural environment, the human body is exposed to bacteria, viruses and parasites, questioning the relevance of studies conducted in sterile environments and limiting the translation of experimental models to humans.

In this review, we focused on the current knowledge of *Leishmania* and viral co-infections focusing on the host immune response and the implication for therapy and vaccination. We provocatively propose that the exposure to viruses and the type I interferon (IFN) response plays an essential role in the development of symptoms observed in endemic regions that could not be explained by differences in the parasites' genome or by genetic polymorphisms of the host. In *Leishmania* infection, pathogens are often transmitted together such as in the salivary reflux of the sand fly, where *Leishmania* parasites are found alongside bacteria and arboviruses [5], although the concomitant infection by alternatively transmitted

pathogens cannot be excluded. To date only few studies report viral co-infection with *Leishmania*, most of which focus on the intracellular *Leishmania* RNA virus (LRV) (the only intrinsic aggravating factor identified thus far) and on HIV. More recently, experimental models using lymphocytic choriomeningitis virus (LCMV) or phleboviruses harbored by the sand fly vector transmitting *Leishmania*, give additional support to the concept that there is a worsening of the symptomatic outcomes in humans caused by exogenous viruses.

Leishmania and endogenous viruses

Leishmania RNA virus

LRVs are members of the *Totiviridae*, a family of dsRNA viruses infecting a wide range of organisms from yeast to salmon. In *Leishmania* parasites, virus-like particles have been reported first in *L. hertigi* by electron microscopy (EM) and subsequently some have been further molecularly characterized [6]. As a member of the *Totiviridae* family, LRV harbors a 5.3 kb dsRNA genome encoding an RNA-dependent RNA polymerase (RDRP) and a capsid. Thus far, no evidence of extracellular viral particles has been reported and LRV is considered as a viral endosymbiont, uniquely localized in the cytoplasm, not shed nor released. The characterization of LRVs in different *Leishmania* species led to a subdivision in two subgenera. The LRV1 subgenus is found in the New World, where it has been described to infect *L. guyanensis* and *L. braziliensis* parasites [7], while the Old World LRV2 is found in *L. major*, *L. aethiopica* [8] and possibly in *L. infantum* [9]. LRV1 and LRV2 genomes slightly differ whereby the expression of the RNA dependent RNA polymerase (RDRP) is possibly obtained by different frame-shifting mechanisms.

Seemingly, LRV does not affect parasite survival *in vitro*, as there is no sign of retarded growth or virus-induced mortality, therefore suggesting a commensal relationship between the two pathogens. However, LRVs are almost exclusively found in *L. Viannia* species, which contrarily to *L. Leishmania* species possess an RNAi machinery allowing the parasite to control the replication rate of LRV, suggesting a potential parasitotoxic effect in other species [10]. To date, there is no information on the relevance or impact of LRV on the parasite when in the sand fly, to determine whether LRV would provide any survival advantage to parasites. Differently, in the mammalian host, LRV1 presence within *L. guyanensis* parasites was associated with the development of exacerbated lesions and metastasis in defined animal models [11•,12••]. The mechanisms of LRV exacerbation of *Leishmania* infection are related to the modulation of the immune response, rather than to viral-intrinsic properties (see Figure 1). In fact, as reported in the murine model of *L. guyanensis* infection, the recognition of LRV1 dsRNA by Toll-like receptor 3 (TLR3) induces a hyperinflammatory response, which not only enhances tissue destruction, but also increases the survival of infected macrophages through an Akt-dependent pathway, thus favoring the persistence of the parasite [13]. Moreover, the LRV1-dependent production of IL-17 promotes parasites dissemination and the consequent formation of infectious metastasis [12••]. Interestingly, even though the disease-exacerbatory role was evident in wild type animals, the pro-metastatic effect of LRV1 and IL-17 was observed only in absence of IFN-γ, a condition similar to the low IFN-γ production observed in human DCL patients, highlighting the concurrence of multiple factors in the promotion of parasite dissemination. Further, the disease-aggravating role of LRV1 was recently shown to completely depend on early type I IFN signaling, suggesting that other viral co-infections could have a similar impact on leishmaniasis [14••], as detailed in the section below. Thus, the parasite benefits from increased persistence and dissemination, which favors transmission. The viral endosymbiont benefits from the parasite cellular machinery for replication and is protected from the antiviral type I IFN response of the macrophage. This in turn leads to mutual symbiosis with LRV that acts as an additional help for the parasite to modulate the innate immune response of the host to its advantage.

In humans, LRV1 has been associated with an increased risk of developing MCL following infection with different *Leishmania* (*Viannia*) species, as shown in a Brazilian study where over 70% of MCL patients were infected with LRV1 positive parasites [15]. In other studies, the presence of LRV1 was associated with increased treatment failure and disease relapse rates in *L. guyanensis* and *L. braziliensis* infected patients [16,17], strengthening the link between LRV and cutaneous leishmaniasis's severity. The pathology-increasing role of LRV1 could also possibly augment parasite fitness, as suggested by an epidemiological study in French Guyana, where approximately 75% of *L. guyanensis* isolates were infected by LRV1 [18]. At the present time, there is no direct evidence of an implication of LRV2 in the determination of leishmaniasis outcomes. However, LRV2 has been isolated from an Iranian *L. infantum* infected patient who was unresponsive to Glucantime® treatment [9], providing circumstantial evidence of a possible role of LRV2 in drug resistance. However, the appearance of MCL or DCL is not exclusively associated with the presence of LRV as in two other Brazilian studies where the parasites isolated from the majority of MCL patient were LRV negative [17,19]. This suggests the involvement of other factors, such as the exposure of the host's immune system to exogenous co-infecting viral pathogens in the determination of the outcome of the leishmaniasis.

Endogenous viruses in other human protozoan parasites

From the early 1970s, viral endosymbionts have been observed in several other human protozoan parasites, while more recently new viruses have been observed