AN INSIGHT INTO THE LEISHMANIA RNA VIRUS

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Abstract

Leishmania RNA virus is an ancient virus that has coevolved with its protozoan host. The purpose of this article is to convey current understanding of Leishmania RNA virus as it has emerged over the past decade. The potential of the virus to play a role in modulating parasite virulence is also discussed.

Key words: Leishmania RNA virus, totivirus, dsRNA

After the discovery in 1960 of virus like particles (VLPs) in the parasitic protozoan Entamoeba histolytica, researchers began to report similar structures in an ever-expanding list of unicellular eukaryotes. The wide distribution of VLPs in lower eukaryotes such as Leishmania spp, Trypanosoma spp, Trichomonas vaginalis, Giardia lamblia should not come as a surprise because the vast majority, if not all, of other living systems have proved susceptible to viral infection. The International committee on taxonomy of viruses currently recognizes six families of ds RNA viruses, five of which infect eukaryotes and one (Cystoviridae) that infects bacteria. The largest known family of eukaryotic ds RNA viruses is Reoviridae whose members infect a wide spectrum of plant and animal species. In contrast, members of Partitiviridae and Birnaviridae exhibit a bisegmented genome and infect fungi as well as various plant and animal species. Members of the Hypoviridae and Totiviridae, on the other hand, exhibit an unsegmented genome and are known to infect only unicellular eukaryotes, including both protozoa and fungi.

The family Totiviridae currently encompasses three genera of the ds RNA viruses: Totivirus, Giardiaivirus and Leishmaniaivirus. The classified Totiviruses [Sc VL-A, Helminthosporium victORIAE, 190 S virus (Hv 190 SV) and UmV] all infect fungi while Giardiaivirus, T. vaginalis viruses (GLV and TVV) and Leishmaniaivirus (types LRV 1 and LRV 2) infect only protozoa. Viruses with non-segmented ds RNA genomes of approximately 5200 bp in length have been identified in over 13 strains of the new world parasitic protozoa, Leishmania braziliensis and in one strain of the old world parasite, L. major. These viruses have recently been placed in the genus Leismania virus, in the family Totiviridae.

Origin of Leishmania RNA Virus (LRV)

Two surveys of new world parasites identified LRV only in those strains that originated from the Amazon River basin of South America. The apparent narrow geographical distribution and the strong nucleotide identity (>90%) observed between two independent LRV isolates might reflect a recent origin of these viruses. However, the more recent discovery of a similar virus in the old world parasite L. major, in combination with the lack of an infectious phase for these viruses suggests that LRV arose prior to the divergence of old and new world parasites. Genetic recombination is considered unlikely because reproduction in Leishmania spp. is predominantly asexual. Comparative analyses of restriction fragment length polymorphisms (RFLPs) also support a long history of co-evolution between individual LRV isolates and their respective parasite host strains. Together the findings support a current view that LRV are ancient viruses.

The observation that virus infected parasites grow more readily in culture than do their uninfected cohorts raised concern that LRV may actually arise during laboratory manipulation due to a positive effect of virus-infection on parasitic growth in vitro. Conflicting reports on the infection status of at least one parasite strain when grown in different laboratories were consistent with the hypothesis. However, the laboratory of Dr. Patterson recently identified LRV1 plus-strand RNA in tissue biopsy material from human patients with cutaneous leishmaniasis. The result formally proves that LRV are not an artifact of laboratory culture but rather occur naturally in the field.

Genome Structure and Organization

Stuart and coworkers were the first to report a complete cDNA sequence for the ds RNA genome of an LRV isolate. The prototype virus, termed LRV1-1, was obtained from a laboratory clone (1A) derived from the New World parasite L. guyanensis. Complete cDNA sequences are now available for a second new world virus isolate (LRV1-4) and a diverged virus (LRV2-1) isolated from L. major. Presently over 15 different LRVs have been reported in various new world parasite species, corresponding to an estimated infection rate of about 20% among the strains tested. Several of the isolates appeared to derive from parasitic infections that occurred...
outside the Amazon basin. No systematic survey has yet been attempted in Old World parasites.1

The LRV genome typically encodes two large open reading frames (ORFs) on one strand while the strand of opposite polarity lacks any recognizable coding potential. The two large ORFs are preceded by a 5' untranslated region (UTR) whose length varies from 340 nt in LRV2-1 to 449 nt in LRV1-4. The 5' UTR encodes the most strongly conserved nucleotide sequence in the viral genome and contains one or more short ORFs. There are no reports that these small ORFs encode gene products.1

Parasite Phenotype Modulation

Members of the Totiviridae family have been shown to alter phenotypic expression of its host. L-A virus of the yeast S. cerevisiae has been linked to a killer toxin, encoded by a satellite ds RNA M, which is lethal to strains not infected with the virus. Unlike the helper Totiviruses associated with the yeast and killer systems, the member viruses in the Totiviridae that infect filamentous fungi and parasitic protozoa are not known to be associated with killer phenotypes. In protozoan virus systems where isogenic strains are available, the results suggest that virus infection can affect parasite phenotype, at least in vitro. Loss of the virus equates to a loss of phenotypic variation. Occurrence of TVV has been shown to correlate with ability of T. vaginalis to undergo phenotypic variation through up-regulated surface expression of a prominent cellular immunogen P.27 Virus infected strains exhibit qualitative and quantitative changes in the expression of other, mostly unidentified, cellular proteins and also show altered growth kinetics in vitro. GLV infection, when established at sufficiently high levels, can reduce parasite attachment to artificial surfaces and induce cessation of division in cultured cells. The importance of these phenotypic changes to infection and disease remains to be described in vivo.1 The precedence of Totiviruses altering host phenotype in combination with the enigmatic pathophysiology of cutaneous leishmaniasis raises an intriguing possibility that LRV may confer a state of hypovirulence or hypervirulence on the host parasite.8

One possibility is that the observed variability in diseased pathology reflects at least in part, some inherent differences in parasite virulence. The presence of LRV could alter parasite phenotype in ways that affect virulence and ultimately disease pathogenesis, as has been reported for other ds RNA viruses of simple eukaryotes. A strategy to determine if there is a correlation between the presence of LRV and phenotypic variation of the host would be to assay biopsy samples for the presence of the virus and determine if the virus is linked to either the mucocutaneous or cutaneous form of the disease. Early attempts to establish an association between virus infection and altered virulence provided inconclusive results.8

Another approach to understanding virus-parasite relationship would be to use an infected and uninfected isogenic parasite in macrophage infectivity studies to determine if the virus plays a role in parasite entry. Unfortunately, LRV particles are unable to produce an infection in uninfected parasites. Ro et al have generated a virus cured Leishmania strain that could be used to circumvent this issue.9 It remains possible that the virus-infected parasites may not demonstrate an altered ability to enter macrophages, in which case, cytokine profiles of infected bone marrow derived macrophages could be analyzed. For example, suppression of cytokines that promote a Th1 response may lead to a more severe clinical outcome. Conversely, enhanced secretion of Th1 promoting cytokines may facilitate clearance of the parasite and a mild leishmaniasis.8

A lack of isogenic parasite strains has hindered a direct examination of the relationship between the LRV and virulence in Leishmania spp. While early studies have demonstrated the ability to transfer infection with whole virus particles, those infections were transient and the virus was quickly lost after a short time in culture. Instability of a complete virus genomic cDNA sequence in bacteria is a problem faced by many attempting to generate infectious clones of RNA viruses. Recently, however LRV was successfully eliminated from a previously infected strain by growing the parasite in culture medium containing the translation inhibitor, hygromycin B.9 The paired strains offer a possibility to test for a role of virus infection in parasite phenotype and disease pathogenesis in an animal model.

Recently, a Taqman detection assay has been developed by Carrion, O’Halleron and Patterson (unpublished data). The preliminary results of a study using this tool to detect virus in swabs from leishmaniasis patients in Brazil is revealing a greater than 80% viral infection rate. Upon completion of this study, it should be possible to delineate the correlation between LRV infection and virulence modulation.8 The existence of a correlation between virus infection and disease pathogenesis would provide a valuable prognostic indicator in the clinical setting and may suggest novel avenues for treatment of human leishmaniasis.

References

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January-March 2007

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Source of Support: Nil. Conflict of Interest: None declared.

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