



2ND GLOBAL VIRTUAL
SYMPOSIUM ON
KINETOPLASTID DISEASES

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Developmental and reproductive strategies of *Leishmania*

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Abstract:

Sandfly vectors transmit *Leishmania* through egestion of parasites into the host skin. The transmissible dose is shaped by *Leishmania* development in the sandfly gut, described as a sequential differentiation of promastigote morphotypes and culminating with the transmission of non-dividing metacyclic promastigotes that are thought to be uniquely infective to the vertebrate host. Little is known about the transcriptional programs and molecular markers for the stages colonizing the anterior midgut in mature infections and that may be co-transmitted by the sandfly bite. We generated a single-cell atlas of *Leishmania* major colonizing its natural vector *Phlebotomus duboscqi* to elucidate the transcriptomic complexity of *Leishmania* major, providing markers for three transmissible stages. Contrary to prevailing models, our analyses indicate a non-linear developmental progression, with bifurcation into either replicating and non-replicating metacyclics or attached and detached haptomonads. We demonstrate that haptomonads constitute a key component of the transmitted inoculum, and along with non-dividing metacyclics, are infectious to mice. *Leishmania* development also includes a cryptic sexual cycle. Genetic exchange has been formally demonstrated via the generation of hybrids in the laboratory. Experimentally, genetic exchange in *Leishmania* is nonobligatory, relatively rare, and naturally confined to life cycle stages present in the sandfly midgut. Per whole genome sequencing, allele inheritance is Mendelian and involves meiosis-like recombination of the nuclear genome. Deletion of meiosis- and plasmogamy-related genes reveals their requirement for successful hybridization. Mitochondrial DNA inheritance appears uniparental for maxicircle kinetoplast DNA (kDNA) but biparental for minicircle kDNA. Future studies will need to confirm the existence of haploid gametes, explore the conditions promoting their development, and exploit the generation of sexual recombinants to map genes controlling important traits.

Notes:

Understanding Chagas disease pathogenesis and prospect for immunotherapeutic vaccine development

Eric Dumonteil

Department of Tropical medicine and Infectious Disease, Celia Scott Weatherhead School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA, USA

Abstract:

Chronic infections with *Trypanosoma cruzi* can lead to Chagas disease, which clinical manifestations include cardiac and/or digestive disease associated with severe morbidity and mortality. However, only 20–40% of infected patients will develop clinical disease but we are still unable to predict which ones, which makes patient care challenging. Many years of efforts have evidenced the great biological variability among *T. cruzi* strains, classified into 7 discrete typing units (DTUs), but failed to provide clear associations between their virulence/pathogenicity and their genetic characteristics. However, recent studies with improved genotyping approaches suggest that many natural infections involve multiple parasite strains/DTUs and parasite composition is emerging as a key determinant of the host response to infection and may be associated with disease progression. On the other hand, immunotherapeutic approaches, aimed at increasing the efficacy of the immune response against the parasite and prevent disease progression, have shown promise in pre-clinical models. In particular, a vaccine candidate based on parasite antigens Tc24 and TSA1 formulated with a TLR4 agonist is in advanced stages of development. Tc24 is a flagellar calcium binding protein and TSA1 belongs to the trans-sialidase multigene family of the parasite. Extensive evaluation of therapeutic vaccines in dogs and non-human primates is now underway, and this vaccine may provide new alternatives for disease control.

Notes:

Development of genetically modified *Leishmania* vaccine: update on immunogenicity correlates of protection and clinical studies

Hira Nakhasi

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Abstract:

Leishmaniasis is a neglected tropical disease caused by infection with the *Leishmania* parasite following transmission by an infected sand fly. It results in different clinical presentations ranging from cutaneous leishmaniasis (CL), that can lead to physical disfigurement, to visceral leishmaniasis (VL; Kala-azar) which is fatal if not treated. Millions of people worldwide are at risk of developing leishmaniasis, with an estimated incidence of over 200,000 cases of CL, and over 50,000 cases of VL each year. Although progress has been made in reducing VL burden in Southeast Asia through the elimination program, there are numerous recent outbreaks reported from other countries in Africa and South America and in non-endemic areas. To achieve global elimination of leishmaniasis, new tools are required, including better surveillance and a safe and effective vaccine. Asymptomatic infection or treatment of VL patients confers life-long immunity in most cases. Moreover, the stability of the *Leishmania* parasite genome indicates that the development of an effective vaccine against leishmaniasis might be possible using a live attenuated vaccine strategy. Our laboratory has developed a genetically modified live attenuated dermatotropic *Leishmania* vaccine (major Centrin gene deleted parasite, LmCen-/-) which is safe and effective against both cutaneous and visceral forms of leishmaniasis in preclinical studies. I will discuss the advancement of the LmCen-/- live attenuated vaccine toward human trials, to identify biomarkers of immunity, and use of leishmanin skin test (LST) as a surrogate biomarker of protective cellular immunity.

Notes:

From prototype to patient access: Advancing *T. cruzi* LAMP for early diagnosis of congenital chagas disease

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Abstract:

Background: Congenital Chagas disease (cCD) remains a major public health challenge in Latin America. Current diagnostic algorithms rely on microscopy during the first months of life and delayed serology, resulting in limited sensitivity and substantial loss to follow-up. Molecular methods such as real-time PCR (rtPCR) improve early detection but are difficult to implement in resource-limited settings. A previous proof-of-concept study conducted in two rural maternity hospitals in Bolivia showed that *T. cruzi* loop-mediated isothermal amplification (*T. cruzi*-LAMP) achieved diagnostic sensitivity and specificity comparable to rtPCR, supporting its evaluation in a larger multicentre setting.

Methods: A prospective multicentre study was conducted in nine maternity hospitals in endemic areas of Argentina, Bolivia, and Paraguay. Pregnant women were screened for *T. cruzi* infection, and newborns of infected mothers were evaluated by microscopy-based micromethod (MM), *T. cruzi*-LAMP, and rtPCR at birth and at 2 months of age, followed by serology at 9 months. LAMP was performed using rapid PURE DNA extraction and the Eiken LF-160 platform. A substudy in 222 infants evaluated dried blood samples collected on FTA filter paper. Parasite genotyping was performed in rtPCR-positive infants. Implementation was accompanied by on-site personnel training, transfer of the methodology to participating centres, and external quality assessment using panels of artificially prepared samples.

Results: Between December 2021 and December 2022, 7,482 pregnant women were screened, of whom 722 were seropositive for *T. cruzi* (9.7%; 95% CI 9.0–10.3). Overall, 544 infants completed follow-up. Twenty-four congenital infections were confirmed, corresponding to a vertical transmission rate of 4.4% (24/544; 95% CI 2.7–6.1). MM detected 16 cases. All microscopy-positive

confirmed by serology. In the FTA substudy, LAMP results obtained from dried blood showed 100% concordance with those obtained from fluid blood. Among successfully genotyped cases, TcV predominated, with only two mixed TcV/TcVI infections.

Conclusions: *T. cruzi*-LAMP showed greater diagnostic sensitivity than microscopy and clinical performance comparable to rtPCR for the early detection of congenital infection. Its ability to identify infections missed by microscopy, together with full concordance between fluid-blood and FTA-based testing, supports the feasibility of decentralised diagnosis closer to the point of care. Importantly, successful implementation across multiple endemic sites was achieved through local staff training, technology transfer, and external quality assessment. Together, these findings indicate that *T. cruzi*-LAMP could substantially shorten the time to diagnosis, reduce loss to follow-up, and enable earlier curative treatment of congenitally infected infants in settings where conventional molecular diagnostics remain difficult to implement.

Notes:

Gerald F Spath

Institut Pasteur, Paris, France

Abstract:

The emergence of drug-resistant *Leishmania* threatens the efficacy of current antileishmanial chemotherapy and underscores the need for new therapeutic concepts. *Leishmania* displays remarkable genome plasticity, which is characterized by frequent gene and chromosome amplifications, enabling rapid adaptation to environmental pressures, including drug exposure. Consequently, strategies that directly target parasite viability are inherently vulnerable to resistance arising through target modification, altered drug uptake, or amplification of efflux systems. My presentation will be centered on a complementary approach: targeting the host-parasite interaction rather than the parasite alone. *Leishmania* manipulates macrophage signaling, transcriptional regulation, epigenetic state, and metabolism to establish intracellular conditions that favor parasite survival and chronic infection. Based on our previously published results, we propose that host histone-modifying enzymes, which are altered during infection to establish permissive macrophage transcriptional programs, represent promising targets for host-directed, antileishmanial intervention.

Our recent single-cell RNA-seq analyses revealed that *Leishmania amazonensis* induces a unique, complex macrophage phenotype combining pro- and anti-inflammatory features. Profound immuno-metabolic remodeling in infected macrophages establishes a cancer-like Warburg effect (aerobic glycolysis) as the primary source of ATP production, with lactate secretion conferring remarkable resistance to multiple forms of regulated cell death. We show that this progressive shift toward glycolytic metabolism is independent of the canonical metabolic master regulator HIF1 α but is regulated at epitranscriptomic levels by the m⁶A reader protein IGF2BP2, similar to certain epithelial cancers. This cancer cell-like phenotype is associated with dampened NF- κ B and NLRP3 inflammasome responses, histone H3K9/14 hypoacetylation, and H3K4 hypotrimethylation at promoters of inflammatory genes. Phenotypic screening of an epigenetic inhibitor library originally developed for cancer therapy identified multiple compounds with host-directed, anti-leishmanial activity that specifically target the macrophage “eraser” enzyme LSD1 (lysine-specific demethylase 1, also known as KDM1A). Reduction of intracellular parasite burden upon LSD1 inhibitor (LSD1-i) treatment correlated with (i) altered LSD1 complex composition, (ii) redistribution of LSD1 promoter occupancy, and (iii) partial reversion of the infection-perturbed macrophage expression profile, notably, rescue of antimicrobial responses, which likely compromises intracellular parasite survival.

These data establish a first proof of principle for host-directed antileishmanial therapy and reveal LSD1 and IGF2BP2 as compelling host therapeutic targets that may be relevant to other intracellular pathogens known to manipulate macrophage functions. Repurposing inhibitors of epitranscriptomic and epigenetic enzymes, several of which already have established clinical use in cancer or autoimmune disease, provides an attractive route toward accelerating antileishmanial drug discovery.

Notes:

Beyond genomics: m6A-mediated epitranscriptomic reprogramming as an early-response mechanism to antimonial resistance in *Leishmania infantum*

Nilmar Moretti

Federal University of Sao Paulo, Brazil

Abstract:

Leishmania parasites lack canonical transcriptional control and depend on post-transcriptional mechanisms to regulate gene expression. However, whether chemical RNA modifications contribute to antimony drug resistance is unknown. Here, we map the N6-methyladenosine (m6A) epitranscriptome across antimony-sensitive (SbS), stably resistant (SbR), and drug-resensitized (P18) *Leishmania infantum* parasites. Global m6A abundance declined dose-dependently upon antimony exposure, recovered fully in P18, and fell again upon drug re-exposure, revealing a dynamic "epitranscriptomic memory." Nanopore Direct RNA sequencing identified 10,012, 8,776, and 10,036 m6A sites across 4,765, 4,593, and 4,921 transcripts in SbS, SbR, and P18, respectively. The dominant motif was GGACU (82.7% of sites; 100% DRACH), invariant among strains and resistance states. Among a curated panel of 25 m6A-modified antimony-resistance genes, differential methylation and differential expression were strongly positively correlated, a coupling 11- to 20-fold enriched over the genomic background. The efflux transporter MRPA acquired a resistance-specific CDS m6A site (position 544) coinciding with transcriptional upregulation, while the drug-uptake channel AQP1 was simultaneously hypomethylated and downregulated in SbR. These findings establish m6A as a rapid, reversible post-transcriptional switch that coordinates antimony-resistance gene expression in *Leishmania* and identify the epitranscriptomic machinery as a potential therapeutic target.

Notes:

Exploiting parasite's metabolism; The good, the bad and the ugly

Terry K. Smith

University of St Andrews, Scotland, UK

Abstract:

My talk will cover three different projects involving three different kinetoplastids.

“UGLY”: Leishmaniasis is an awful protozoan disease caused by Leishmania parasites transmitted by sand fly bites, that occurs throughout the tropics and subtropics worldwide. Here we present a meaningful treatment therapy for Cutaneous Leishmaniasis, utilising photodynamic therapy (PDT).

“BAD”: Trypanosma cruzi is the causative agent of Chagas disease, that affects numerous countries in the Americas and beyond. Lipid metabolism within these parasites is vital for their survival, here we are able to elucidate and validate (genetically and chemically) parasite specific drug targets.

“GOOD”: Crithidia is a monoaxenic kinetoplastid cousin to Leishmania, that is noninfectious to vertebrates. We are exploiting this highly malleable metabolic cellular platform to make high value omega-3 and -6 fatty acids and so much more.

Notes:

Twist and shout: Topoisomerase II inhibitors for rapid cure of Trypanosome infections

Manuel Saldivia

Novartis Global Health, Emeryville, CA, USA

Abstract:

Chagas disease affects millions of people, primarily in Latin America, and current therapies are limited by long treatment duration and safety-related discontinuations. A safe, short-course therapy would substantially benefit patients.

This talk will follow the cyanotriazole series from phenotypic hit to optimized candidate. Screening against *Trypanosoma brucei* revealed cyanotriazoles as rapidly acting compounds with sterilizing activity across kinetoplastids, and target ID studies established that they kill parasites by selectively poisoning trypanosomal topoisomerase II. First-generation compounds validated the mechanism in vivo but carried safety liabilities, including lymphopenia and neutrophilia arising from sphingolipid pathway modulation.

Optimization was therefore directed at preserving the class-defining pharmacology while removing these off-target effects, delivering IID432, through a tailored aminopyrrolidine core. IID432 stabilizes the TcTopoII–DNA cleavage complex by covalently engaging a parasite-specific cysteine (Cys477), conferring selectivity over human TOP2A with no off-target activity on human topoisomerases. Brief exposure rapidly induces parasite-specific DNA damage and produces sterilizing activity in vitro and in vivo without recrudescence, and a single oral dose achieves sterile cure in a murine model of chronic *T. cruzi* infection.

The cyanotriazole series illustrates how a phenotypic hit can be resolved to a parasite-specific molecular vulnerability and optimized into a first-in-class candidate with potential to significantly shorten Chagas treatment.

Notes:

Navigating chemical space to develop cruzain inhibitors as potential candidates to treat Chagas disease

Rafaela Salgado Ferreira

Universidade Federal de Minas Gerais, Brazil

Abstract:

Proteases are widely explored drug discovery targets. In our group, we have been combining experimental and structure-based computational approaches to discover and optimize protease inhibitors as therapeutics for neglected and emerging diseases. I will discuss examples of various screening strategies adopted by our group, focusing on the protease cruzain, a target for Chagas disease. Throughout the past decade, we have conducted experimental screening of privileged compound sets, such as the Malaria box and Pathogen box, and virtual screening of commercial and in-house libraries. In ongoing projects, we are combining molecular docking and machine learning to screen ultralarge commercially available virtual libraries against cruzain. During my talk, I will present the different strategies explored by our group, together with the challenges involved and the experimental results we obtained, including the discovery and characterization of potent protease inhibitors with trypanocidal activity.

Notes:

Parasitological cure and impact of benznidazole treatment on the evolution of chronic Chagas disease in cohorts of patients from the Jequitinhonha Valley, Minas Gerais, Brazil

Marta De Lana

Universidade Federal de Ouro Preto, Brazil

Abstract:

Introduction: Chagas disease (CD) is a tropical infection prevalent in rural areas of Latin America. Etiological treatment with benznidazole (BZ) is indicated for patients with CD in the acute and early chronic phases. However, there is still disagreement regarding the criteria for cure and whether the etiological treatment yields real clinical benefits for the patient. Objectives: The objectives were to evaluate the effect of BZ treatment on the results of laboratory tests used to monitor cure, as well as its impact on the clinical progression of the disease, across three cohorts from the Jequitinhonha Valley, Minas Gerais (MG), an important endemic area of this disease in Brazil, in long-term follow-up. Materials and

Methods: A prospective study involving 174 patients with chronic CD, subdivided into three cohorts categorized as treated (BZ) and untreated (NT). These cohorts consisted of groups GR1 (BZ: 21; NT: 21), GR2 (BZ: 39; NT: 36), and GR3 (BZ: 13; NT: 44). Patients were evaluated at two time points (T1 and T2) to assess parasitological cure using hemoculture, PCR, conventional serology (CS)-ELISA, non-conventional serology (NCS) Chagas-Flow ATE, and qPCR (at T2). Results were interpreted according to three cure criteria: one classic (negative Hc, PCR, and ELISA) and two alternative criterias (negative Hc, PCR, ELISA, and NCS; and negative qPCR). Patients also underwent clinical assessment, ECG, echocardiography (ECHO), and chest X-ray.

Results: Mean ages were: GR1 (BZ: 29.6; NT: 32.6), GR2 (BZ: 35.4; NT: 37.4), and GR3 (BZ: 24.6; NT: 46.6). Females predominated in GR1 and GR2, while males predominated in GR3. The majority of patients in GR1 and GR2 were from rural areas, whereas the majority in GR3 were from urban areas; most patients across all three groups had only primary education. Cure rates based on the classic criterion were lower than those obtained using the two alternative criteria (GR1-BZ: 75.0% vs. 90.0%; GR2-BZ: 63.4% vs. 70.0%; and GR3-BZ: 36.4% vs. 45.5%). The three treated groups showed a lower prevalence of ECG abnormalities suggestive of chronic Chagasic cardiomyopathy (T1 and T2), fewer echocardiographic abnormalities (T2), a lower prevalence of severe cardiomegaly (grades I and II) at T1 and T2, less cardiac involvement (T2), and a higher prevalence of the indeterminate clinical form (T1 and T2). Conclusions: The results revealed high rates of parasitological cure—particularly when considering the alternative criteria—and beneficial effects of BZ treatment regarding the development of Chagasic cardiomyopathy, progression of the clinical forms, disease staging, and mortality.

Notes:

Carlos Robello

Institut Pasteur de Montevideo, Uruguay

Abstract:

Trypanosoma cruzi displays a highly unusual genome organization that reflects its remarkable biological and adaptive versatility. Its nuclear genome is characterized by extensive repetitive sequences, multigene families, and a predominantly polycistronic transcriptional organization, in which genes are arranged in directional clusters separated by strand-switch regions and processed through trans-splicing and polyadenylation. Recent improvements in genome assembly have revealed a 32-chromosome karyotype and provided an increasingly complete view of its complex genomic architecture.

The *T. cruzi* genome is strongly compartmentalized into a core compartment, enriched in conserved and hypothetical genes, and a disruptive compartment, enriched in the highly variable multigene families trans-sialidases, mucins, and MASPs. These compartments differ in nucleotide composition, gene content, synteny, and transcriptional activity. Notably, genes located in the disruptive compartment show lower average expression throughout the parasite life cycle. Nuclear RNA-seq data further indicate that this difference cannot be explained solely by post-transcriptional regulation, pointing to a contribution of nuclear mechanisms in controlling compartment-specific gene expression.

To investigate whether chromatin organization contributes to this regulation, chromosome conformation capture and Hi-C data were analyzed at different genomic scales. Both genomic compartments display distinct chromatin folding domains (CFDs), corresponding to different three-dimensional chromatin environments. The disruptive compartment exhibits increased intra-chromosomal contacts and smaller folding domains than the core compartment. Moreover, CFD boundaries correlate with regions associated with the initiation and termination of polycistronic transcriptional units.

Together, these observations support a model in which the unusual one-dimensional organization of the *T. cruzi* genome is coupled to its three-dimensional chromatin architecture, and in which local chromatin folding contributes to transcription regulation across distinct genomic compartments.

Notes:

Mining the *Trypanosoma cruzi* kinome: Identification of unique protein kinases essential for differentiation

Ana Paula C A Lima

Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro – UFRJ, Rio de Janeiro, Brazil

Abstract:

Protein kinases (PKs) are central regulators of cell physiology, coordinating signaling processes for a wide range of cellular functions. In unicellular pathogens, PKs play critical roles in environmental sensing and in coordinating proliferation, differentiation, and interactions with the host. In *Trypanosoma brucei* bloodstream forms, 42 PKs were validated as essential. Similarly, in *Leishmania mexicana*, there are 44 essential PKs in promastigotes, in addition to several others required for differentiation and mammalian infection. In *Trypanosoma cruzi*, however, the kinome remains largely unexplored. We analyzed the available genomes of *T. cruzi* Dm28 and identified 190 PK genes. Of these, 122 are shared among the three tri-tryps, while *T. cruzi* and *T. brucei* share an additional 35 PK genes, and *T. cruzi* and *Leishmania* share a further 13. In addition, 19 *T. cruzi* PK genes lack orthologues in either *T. brucei* or *Leishmania*. The optimization of CRISPR/Cas9-based approaches for *T. cruzi* has allowed us to use a *T. cruzi* Dm28 line constitutively expressing T7 RNA polymerase and SpCas9, to attempt to generate null mutants for these 19 *T. cruzi*-specific PK genes. Null mutants were successfully obtained for 14 genes. A preliminary phenotypic screen revealed that five of these null mutant lines were unable to complete the *T. cruzi* life cycle in vitro. Three mutants were unable to differentiate from epimastigotes into metacyclic trypomastigotes during metacyclogenesis and were therefore designated TcMKs (*Trypanosoma cruzi* Metacyclogenesis Kinases). The remaining two mutants were unable to differentiate from amastigotes into trypomastigotes in mammalian cells, and designated TcTKs (*Trypanosoma cruzi* Trypomastigogenesis Kinases). Following differentiation with TAU3AAG, parental-line epimastigotes generated approximately 45% metacyclic trypomastigotes, 50% intermediate forms, and 5% remained as epimastigotes. No trypomastigotes were observed for the three TcMK null mutants, paralleled with higher proportion of intermediate forms. Intermediate forms were found at 70% for TcMK1 null mutants, 60% for TcMK2 and 65% for TcMK3 null mutants. These results indicate a strong impairment in metacyclogenesis in all three TcMK null mutants. TcMK1, TcMK2, and TcMK3 null mutants were fully susceptible to lysis by human serum and were unable to establish infection in mammalian cells. Consistent with their impaired differentiation, expression of the metacyclic stage-specific surface proteins gp82 and gp90 was virtually undetectable. These phenotypes were fully rescued in the respective addback lines. Furthermore, a kinase dead TcMK2 addback carrying an Asp266Ala substitution failed to complement the differentiation phenotype, indicating that TcMK2 kinase activity is required for metacyclogenesis. Consistent with this defect, the mutant parasites were also unable to infect mammalian cells, supporting an essential role for functional TcMK1-3 in the transition to the infective metacyclic stage. Finally, metacyclic trypomastigotes lacking TcTK1 or TcTK2 were capable of invading LLCMK2 cells; however, infection remained restricted to a low number of intracellular amastigotes after six days, and no trypomastigotes were observed even after two weeks. Immunofluorescence analysis revealed that, for TcTK2 null mutants, intracellular amastigotes co-localized with LAMP-1, suggesting that the parasites failed to escape the parasitophorous vacuole. Taken together, the results identify five PKs that play essential roles at distinct and critical checkpoints during the *T. cruzi* developmental cycle.

Notes:

Mayra Fernanda Ricci

Federal University of Minas Gerais, Brazil

Abstract:

Chagas disease (CD) remains a serious public health problem, with high morbidity rates and global economic impact. Although effective during the acute phase, Benznidazole (BZ) treatment during the chronic phase has low success rates and causes severe adverse effects. Different studies towards vaccine development have used Trans-sialidase (TS), a key *Trypanosoma cruzi* surface protein involved in immune evasion and parasite survival, as a target antigen. Our group showed that mice immunization with mRNA of TS induces a protective Th1 response that is efficient in a prophylactic immunization protocol. After developing a chronic model of infection in BALB/c mice infected with a Y strain expressing luciferase, we tested a therapeutic protocol consisting of TS mRNA encapsulated into lipid nanoparticle (LNP). Efficacy was assessed through evaluation of parasitemia, survival rates, bioluminescence, cardiac/gastrointestinal histology and immunological analyses. The chronic murine model of infection mimics the cardiac/intestinal alterations of CD. Infected mice were treated with TS mRNA/LNP using a four-dose protocol with or without association with a suboptimal dose of BZ. Both therapeutic protocols induced specific humoral responses anti-TS and increased production of IFN- γ , IL-10, and IL-4. Assessment of cardiac function by electrocardiogram (ECG) recordings showed that treatment with TS mRNA/LNP improved the ECG score compared with the untreated group. Finally, histopathological analyses of the heart and colon of mice vaccinated with TS mRNA/LNP showed a significant reduction of inflammatory infiltrate and tissue and neuronal protection when compared to infected mice. Collectively, these findings showed that TS mRNA vaccination is a promising therapeutic strategy against CD, that induces a balanced immune response, with reduced tissue inflammation as well as cardiac and intestinal dysfunctions that are associated with chronic infection.

Notes:

The potential impact of co-circulation and co-infection of *Leishmania* and Phlebovirus in sandfly vectors and reservoir hosts on the transmission and development of leishmaniasis

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3 Vaccine Control Unit, National Drug Control Laboratory, Tunis, Tunisia

4 Laboratory of Transmission, Control, and Immunobiology of Infections. Institute Pasteur de Tunis. Tunisia

5Department of Basic Sciences, Higher Institute of Biotechnology of Sidi Thabet, University of Manouba, Manouba, Tunisia

Abstract:

Phlebotomus perniciosus is the main sandfly vector of *Leishmania infantum*, etiological agent of zoonotic visceral leishmaniasis (ZVL) affecting humans and dogs in the Western Mediterranean basin. *Phlebotomus perniciosus* is also the main vector of Toscana virus (TOSV), a sandfly borne phlebovirus that exhibits neurotropism and may lead to aseptic meningitis and meningo-encephalitis in the Mediterranean basin. TOSV is responsible for one third of summer meningitis in the Western Mediterranean basin. Since both pathogens are transmitted by the same sandfly vectors, human and dog populations living in Western Mediterranean countries are at high risk for *L. infantum* and TOSV infections. While dogs are the main reservoir hosts for *L. infantum*, their role as reservoir hosts for TOSV remains undetermined. This study investigated TOSV and *L. infantum* infections in one healthy and three infected dogs with ZVL (A, B, C) following natural exposition to sandfly bites in a focus of ZVL located in Northern Tunisia from June to October 2020. At the end of the exposition period, infected and healthy dogs were returned to the dog kennel. They were examined for TOSV and *L. infantum* infections by xenodiagnosis using a laboratory-colonized *P. perniciosus*. Pools of freshly engorged *P. perniciosus* at days 0 and those at days 7 post-feeding were screened for TOSV and *L. infantum* by nested PCR in the polymerase gene and kinetoplast minicircle DNA, respectively. In the exposure site, *P. perniciosus* is the most abundant sandfly species. The minimum infection rates of sandflies with TOSV and *L. infantum* were 0.10 and 0.05%, respectively. *Leishmania infantum* DNA and TOSV RNA were detected in *P. perniciosus* females fed on dog B and C, respectively. The isolation of TOSV in Vero cells was achieved from two pools containing *P. perniciosus* fed on dog C. No pathogens were detected in *P. perniciosus* females fed on dog A and on the healthy dog. We report for the first time the reservoir competence of dog with ZVL in the transmission of TOSV to sandfly vectors in natural settings, in addition to its role as a main reservoir host of *L. infantum*.

In 2021, we evaluated the impact of the co-circulation of *L. infantum* and TOSV in sandfly vector on the co-infection of dogs with these pathogens on the development of ZVL under natural settings. Fifty two dogs were exposed in the same study site from August to October 2021. The minimum infection rates of sandflies with TOSV and *L. infantum* were similar to those reported in 2021. At the end of the exposure period, dogs were examined for *L. infantum* and TOSV infections and were monitored for clinical symptoms until 2023. The prevalence of infected dogs with only *L. infantum* was 3.8% (2/52). The prevalence of infected dogs with only TOSV was 28.8% (14/52). The majority of exposed dogs (67.3%) were not infected neither by *L. infantum* nor by TOSV. Only one dog was co-infected with TOSV and *L. infantum*, which have developed clinical symptoms related to ZVL two years later. The remaining of dogs did not show any symptoms related to ZVL. Our results strongly suggest that TOSV is a co-factor in the development of ZVL in canine. Further investigations are needed to study the impact of co-infection of humans with TOSV and *L. infantum* in the development of ZVL.

Notes:

Elimination of Human African Trypanosomiasis in Guinea: Many reasons to be optimistic some reasons to stay awake

Jean-Mathieu Bart

Institut de Recherche pour le Developpement, France

Abstract:

TO BE UPDATED

Notes: