Dynamics of *Trypanosoma cruzi* kDNA integration into host genome

Aline Moraes[a], Nadjar Nitz[a], Luísa Rodrigues[a], Luciana Hägstrom[a], Mariana Hecht[a]

[a] Interdisciplinary Laboratory of Biosciences, [b] Laboratory of Animal Welfare, School of Medicine, University of Brasilia, Campus Universitário Darcy Ribeiro, Brasília, Federal District, Brazil. Zip Code: 70.910-900.

Abstract

Chagas disease is endemic throughout Latin America. Its etiological agent, *Trypanosoma cruzi*, infects about eight million people worldwide. Lateral transfer of *T. cruzi* kinetoplastid minicircle DNA (kDNA) to the vertebrate host genome has been linked to the pathogenesis of autoimmunity-mediated, chronic Chagas disease. However, the machinery involved in kDNA integration and the mechanisms underlying autoimmunity have yet to be elucidated. We developed a quantitative PCR (qPCR) protocol to quantify *T. cruzi* kDNA minicircle integration into the mammalian host genome. A steadily increase of integrated kDNA minicircles was detected during the course of infection. Variation in melting curve patterns as kDNA insertions accrued allowed us to measure changes in the composition or size of integrated fragments. Amplicon sequencing revealed nucleotide loss in integrated kDNA minicircle variable regions. Treatment with benznidazole eliminated infection but did not prevent integration and accumulation of parasite kDNA. In contrast, a retrotransposition inhibitor (zidovudine) effectively controlled integration, and deacetylation inhibitors (sodium butyrate and trichostatin A) prevented kDNA nucleotide excision. Suppressors of DNA repair pathways did not affect kDNA integration. *In vivo* experiments confirmed *in vitro* findings, showing kDNA integration in mice with chronic, but not acute, CD. In conclusion, this report demonstrates that *T. cruzi* kDNA minicircles are transferred to the host genome, where they integrate and accumulate over time. Integrated kDNA sequences undergo topological rearrangement as the infection becomes chronic. *T. cruzi* kDNA integration seems to depend mainly on retrotransposition machinery. Our results suggest possible markers of chronic Chagas disease prognosis and potential new targets for treatment. More generally, our data illustrate a striking instance of horizontal gene transfer between a protozoan parasite and its vertebrate host; the evolutionary significance and consequences of this phenomenon remain to be fully elucidated.