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From the parasite to the host pathogenesis – the historical and biological aspects beyond the *Trypanosoma cruzi* infection

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Revisão sistemática sobre a interação parasito-hospedeiro com enfoque na cepa Y do *Trypanosoma cruzi*

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"From the parasite to the host pathogenesis – the historical and biological aspects beyond the Trypanosoma cruzi infection"

Aos 12 dias do mês de julho de 2019, as 8:30 horas, no sala multimidia do ICEB reuniu-se a Comissão examinadora da Monografia do aluno Breno Luiz Pimenta dos Santos. A defesa pública de monografia iniciou-se pela apresentação oral feita pelo candidato e, em seguida, argüição pelos membros da banca. Ao final, os membros da banca examinadora reuniram-se e declararam a candidata APROVADO, com a nota 10.

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RESUMO

O protozoário hemoflagelado *Trypanosoma cruzi*, agente etiológico da doença de Chagas, apresenta grande variabilidade genética com taxas de alterações em seu DNA de até 48% após seu isolamento e interação com diferentes modelos experimentais. Estas novas populações do parasito, denominadas cepas, são hoje classificadas em 6 Unidades Discretas de Tipagem (DTU), caracterizando o parasito tanto pela sua genética quanto pelas suas características biológicas. A cepa Y do *T. cruzi, classificada como DTU TcII,* é muito utilizada em experimentos laboratoriais pela sua fácil manutenção *in vitro* e *in vivo* e por sua alta virulência. O objetivo desta revisão foi analisar os diferentes estudos publicados na literatura científica, realizados em camundongos de diferentes linhagens, abordando as particularidades biológicas e imunopatológicas inerentes desta relação específica "parasito-hospedeiro", além de situar o leitor no contexto histórico e evolutivo do *T. cruzi*. A pesquisa bibliográfica utilizada para este estudo foi obtida nas fontes ScieLo (*Scientific Electronic Library Online*), PUBMED and *Medline* com o cruzamento dos termos "*Trypanosoma cruzi*", "Y strain" "host-parasite interaction" "inflammation", não se restringindo à temporalidade dos trabalhos nem no impacto das revistas científicas. Este estudo reforçou a variabilidade imunopatológica existe para a cepa Y quando avaliada em modelos animais distintos, fato que reforça a necessidade de cautela nas interpretações das publicações científicas e suas comparações entre si. Da mesma forma que já é preconizado para as populações genéticas do *T. cruzi*, o hospedeiro mamífero também exerce controle na resposta imunopatológica e conduz o curso clínico associado à infecção pela cepa Y do *T. cruzi*.

Palavras-chaves: *Trypanosoma cruzi*, cepa Y, interação parasito-hospedeiro, inflamação.

Sumário

1. Introdução

1.1. História

Em 1909 o médico e pesquisador Carlos Ribeiro Justiniano das Chagas identificou o *Trypanosoma cruzi* e caracterizou a enfermidade causada por este parasito: a Tripanossomíase Americana ou doença de Chagas (Chagas, 1909). Em 1921, Chagas e sua equipe já tinham identificado também o vetor, os reservatórios naturais e os aspectos morfológicos e clínicos da infecção aguda e crônica desta nova doença (Chagas, 1913; Coura, José Rodrigues & Viñas, 2010). Considerada uma doença autóctone favorecida pela miséria e pelo subdesenvolvimento, a doença de Chagas foi negligenciada até os anos 40, quando a zoonose começou a ser reconhecida e controlada por iniciativas políticas no Brasil (J C P Dias, Silveira, & Schofield, 2002). Porém, a história natural entre o ser humano e o *T. cruzi* transcorre os séculos, sendo hoje estudada pelos paleoparasitologistas para o entendimento do ciclo e do vínculo do parasito aos humanos.

Há 6000 anos, a população Andina começava a deixar seus hábitos nômades, se tornando sedentária, mas mantendo os velhos hábitos de caça a mamíferos – fato este que reforçava o contato direto desta população com o sangue fresco dos animais durante o abate. Além disso, novos hábitos como a domesticação de animais e a estocagem de comida favoreciam a presença de animais silvestres, em particular roedores, no peridomicílio (Montoya, Carlos, Dias, & Coura, 2003). Outra espécie atraída por estes novos hábitos nômades e pela permanência do homem próximo à estrutura silvestre foram os triatomíneos hematófagos, conhecidos hoje como os vetores invertebrados do *T. cruzi*. Estes vetores coabitavam cavernas e encostas em busca de refúgio e alimento nesse período da história primitiva. Todas estas evidências/hipóteses corroboram para o possível contato primitivo entre o ser humano e o *T. cruzi* anterior à chegada do "homem branco" às Américas. Além disso há estudos que demonstraram a presença do parasito em tecidos mumificados de populações extintas há 9000 anos e em ossos datados de mais de 12000 anos na região costeira do Chile (Araújo, Castagno, Gallina, Aires, & Elisa, 2009; Aufderheide et al., 2004; Guhl, Jaramillo, & Yockteng, 1997; Guidon, 1991; Machado et al., 2013).

A doença de Chagas ainda é considerada pela Organização Mundial de Saúde (WHO) uma enfermidade negligenciada do ponto de vista farmacológico, mesmo com

a existência de ações para seu controle em alguns países da América Latina (WHO, 2012). Na América do Norte, na Europa e em outras partes do mundo também há um crescente número de indivíduos soropositivos para o *T. cruzi* devido às migrações nas últimas décadas (J. R. Coura & Carlos Pinto Dias, 2009). Estima-se que hoje, cerca de 8 milhões de pessoas encontram-se infectadas pelo parasito em todo o mundo, sendo que sua maioria localiza-se na América Latina (WHO, 2015). A Tabela 1 mostra, epidemiologicamente, como a doença de Chagas se apresenta na América Latina.

Tabela 1 - Mudança na mortalidade, prevalência e incidência da doença de Chagas, através da transmissão vetorial nos países da América Latina dos anos de 1990, 2000, 2006 e 2010.

Parâmetros/estimativa	1990	2000	2006	2010
Número de mortes/ano	>45.000	21.000	12.500	12.000
Número de pessoas infectadas	30.000.000	18.000.000	15.000.000	5.742.167
Casos novos/ano	700.000	200.000	41.200	29.925
População total sob risco	1000.000.000	40.000.000	28.000.000	70.199.360

Fonte: Tabela retirada do II Consenso brasileiro em doença de Chagas, 2015.

Dessa forma, mesmo diante de todos os avanços no combate ao vetor e ao *T. cruzi*, permanece evidente a necessidade de novas medidas de controle para a doença de Chagas na América Latina, de conhecimento sobre seu agente etiológico e de novas propostas de manejo clínico para os indivíduos chagásico. No entanto, os grandes determinantes da transmissão da enfermidade ao homem ainda estão atrelados à questão socioeconômica da sociedade, às mudanças climáticas e ambientais, à manipulação do alimento (açaí artesal) e à grande concentração de pessoas em áreas urbanas (Prata, 2001; WHO, 2012; João Carlos Pinto Dias & Matos, 2013).

1.2. Morfologia e o ciclo evolutivo do *Trypanosoma cruzi*

O *T. cruzi* é um flagelado da família *Trypanosomatidae* que apresenta três estágios evolutivos em seu ciclo biológico: tripomastigota, amastigota e epimastigota. As formas amastigotas são encontrados nos tecidos do hospedeiro vertebrado infectado e apresentam-se como estruturas arredondadas e sem flagelos exteriorizados e desenvolvidos (Alvarenga, 1997). As formas epimastigotas são flageladas e alongadas

e são encontradas no sistema digestório do vetor invertebrado - triatomíneo (De Souza, Gryrtberg, & Nery-Guimarães, 1975; Pérez- Molina & Molina, 2018). Finalmente, as formas tripomastigotas são as formas encontradas na circulação sanguínea (tripomastigota sanguíneo) e na ampola retal do triatomíneo (tripomastigota metacíclico), sendo a forma infectante para os vertebrados mamíferos (Tyler, Olson, & Engman, 2002). O clico evolutivo do *T. cruzi* é complexo (**Figura 1**), envolvendo diferentes espécies de vetores (*Triatoma infestans*, *Triatoma sórdida*, *Triatoma rubrovaria*, *Triatoma pseudomaculata*, *Triatoma brasiliensis*, *Panstrongylus lutzi*, *Panstrongylus megistus*, dentre outros) e de hospedeiros vertebrados (homem, tatu, macacos, cães, gatos, gambás etc) (Rassi Jr, 2010).

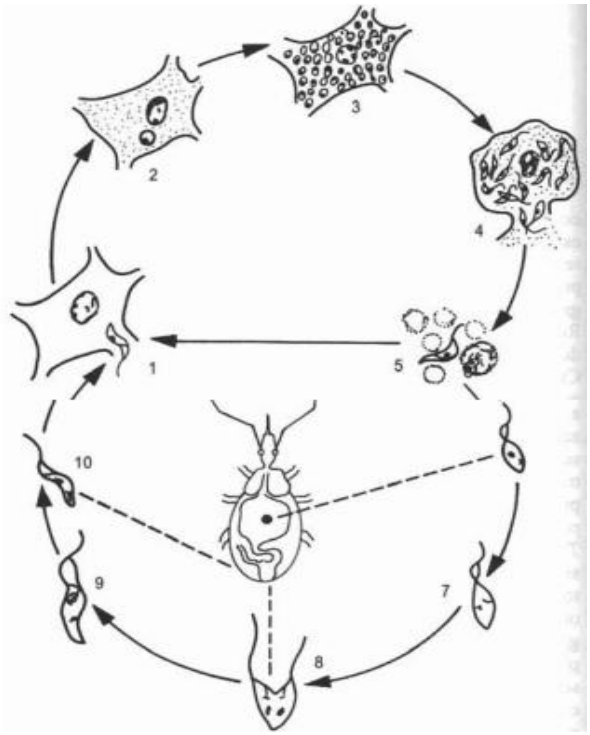

Figura 1- **O ciclo evolutivo do *Trypanosoma cruzi*.** (1) a forma tripomastigota metacíclica infecta a célula vertebrado. (2) mudança para o estágio de amastigota. (3) multiplicação do parasito dentro da célula. (4) lise celular e liberação de diversos tripomastigotas sanguíneos na corrente sanguínea. (5) a forma tripomastigota sanguínea pode infectar outras células (1) ou passar ao corpo do vetor invertebrado pelo aparelho picador-sugador do mesmo. (6) forma tripomastigota dentro do invertebrado. (7) no intestino posterior do invertebrado ocorre a mudança para o estágio epimastigota. (8) multiplicação das formas de epimastigota. (9) no reto do invertebrado ocorre a mudança para o estágio tripomastigota metacíclico (10) nas fezes do triatomíneo, as formas tripomastigotas metacíclicas penetram ativamente a pele do hospedeiro

vertebrado através da lesão causada pela picada do inseto. (Ciclo retirado do livro Parasitologia Básica de Pereira Neves, 2005)

O *T. cruzi* alterna as diferentes formas morfológicas e funcionais durante o seu ciclo de vida, além de envolver centenas de mamíferos e invertebrados como possíveis reservatórios silvestres (Briones, Souto, & Stolf, 1999). Sua plasticidade biológica permite a sua transmissão tanto para os humanos quanto para outros mamíferos susceptíveis, sendo a infecção por este parasito transmitida, principalmente, pelas fezes contaminas do seu vetor triatomíneo e pela via oral. Também existem "rotas secundárias" para a infecção como as vias congênitas, transfusional, durante transplantes de órgãos e até por acidentes laboratoriais com formas tripomastigotas do parasito (Afonso, Ebell, & Tarleton, 2012; Hidron et al., 2010; Hovsepian, Penas, Mirkin, & Goren, 2012). Como apresentado na Figura 1, inicialmente, os triatomíneos se infectam durante sua alimentação (insetos hematófagos), ingerindo sangue de mamíferos contaminado com formas tripomastigotas (tripomastigotas sanguíneos). Os tripomastigotas, uma vez no estômago do inseto vetor, convertem esta forma à forma epimastigota e, por fissão binária, se multiplicam e aderem as membranas das células intestinais, principalmente com a ajuda de seu flagelo (Tyler et al., 2002). No intestino posterior do triatomíneo, os epimastigotas convertem sua forma em tripomastigota metacíclica, aderindo a região cuticular da ampola retal do inseto. Essas formas evolutivas são exteriorizadas com suas fezes enquanto o invertebrado se alimenta do sangue de mamíferos (inclusive, acidentalmente, o homem). O curso natural da transmissão segue quando o mamífero se infecta com as formas tripomastigotas, encontradas nas fezes do triatomíneo, em contato com o tecido mucoso ou fissuras na pele (até mesmo causada pela própria picada do inseto vetor). Os tripomastigotas metacíclicos invadem células próximas ao local de infecção induzindo um influxo de cálcio com um desarranjo temporário do citoesqueleto celular que permite a migração e fusão dos lisossomos, formando o vacúolo parasitóforo (Caler, Morty, Burleigh, & Andrews, 2000). Inicialmente, as formas tripomastigotas evadem do vacúolo e iniciam nova transformação em formas amastigotas, que são as formas intracelulares replicativas do ciclo de vida do parasito (Dvorak & Howe, 1976; Harth, Andrews, Mills, & Engel, 1993). Livres, dentro do citoplasma, as formas amastigotas iniciam uma intensa replicação, consumindo os nutrientes celulares, até sua nova modificação morfológica para a forma tripomastigota sanguínea. O constante movimento dos novos

parasitos culmina na lise da célula hospedeira e extravasamento de centenas de parasitos para a corrente sanguínea. Estes, por sua vez, infectam outras células dando continuidade ao ciclo biológico do protozoário intracelular (Ley, Andrews, Robbins, & Nussenzweig, 1988; Tyler et al., 2002). O ciclo de transmissão final se conclui quando estes tripomastigotas sanguíneos são levados ao trato digestório de outros triatomíneos durante novo repasto sanguíneo. Células cardíacas, musculares, endoteliais, vasculares, nervosas e todos os tipos de células nucleadas dos mamíferos podem ser parasitadas (Combs et al., 2005; Teixeira, Dutra, & Ota, 2005; Machado et al., 2013).

1.3. Transmissão

A transmissão do *T. cruzi* para o homem pode ser dividida em dois cenários: (i) transmissão primária: sendo o principal modo de transmissão, compreendendo a transmissão vetorial, oral, congênita, transfusional e (ii) transmissão secundária, que compreende acidentes laboratoriais com sangue para testes e análises ou diretamente por acidentes com animais infectados (J. R. Coura, 2015). O boletim epidemiológico feito pelo Ministério da Saúde em 2015 mostrou que no período entre 2000 e 2013 a transmissão oral acidental (alimentos contendo macerado do triatomíneo) representava maior porcentagem nos indivíduos infectados superando a transmissão silvestre típica e prevalente no passado

A mudança nos números e porcentagens dos tipos de transmissão do agente etiológico da doença se dá devido às ações contra o vetor no passado, às mudanças ambientais, da condição socioeconômica da população e da sua instalação urbana em ambientes previamente rurais (Prata, 2001; WHO, 2012; Dias & Matos, 2013)

1.3.1. Transmissão vetorial

Mesmo diante do avanço do controle na transmissão deste parasito no Brasil, a transmissão vetorial ainda se mantém prevalente em alguns países da América Latina. Essa transmissão é dependente do vetor invertebrado hematófago: o triatomíneo, ou popularmente conhecido “barbeiro” ou “bicudo” (Rassi Jr, 2010). Esse inseto é proveniente de zonas tropicais, antes acostumado apenas às áreas florestais e, mais recentemente, adaptado a ambientes domésticos (J. Coura & Borges-pereira, 2012). A condição doméstica é de extrema importância para a transmissão da doença por triatomíneos, já que casas construídas de pau-a-pique e outras estruturas semelhantes possuem abrigos ideias para este vetor (Massad, 2008). Durante o dia, o inseto se

encontra escondido em buracos na estrutura destas residências ou no peridomicílio e, a noite, adentra os recintos em busca de alimento nos residentes (Massad, 2008). Os triatomíneos contaminados picam e sugam o sangue de sua presa, e então, defecam ao redor da picada. Em suas fezes, encontram-se tripomastigotas metacíclicos que penetram ativamente pela porta de entrada criada pelo inseto ou por escoriações feitas pelo próprio hospedeiro infectando-o (Massad, 2008).

No Brasil o controle do vetor invertebrado da doença de Chagas se iniciou com as primeiras campanhas de controle ao triatomíneo no início da década de 50 (Brener, 1979). Desde então a transmissão vetorial do parasito teve brusca queda no cenário mundial, principalmente após 1975, ano em que ações químicas contra o vetor foram instauradas em ambientes domiciliares. Devido a todos os esforços desde a década de 70 no controle ao principal vetor da doença de Chagas, o *Triatoma infestans*, em 2006 o Brasil recebeu a certificação internacional pela interrupção da transmissão de doença de Chagas pela referida espécie do vetor (J. R. Coura & Dias, 2009). Atualmente, o principal desafio do Ministério da Saúde em relação a novos casos de transmissão vetorial do *T. cruzi* está relacionado a novas espécies vetoriais autóctones e microepidemias de certas espécies em ambiente urbano, transmissões endêmicas na região amazônica (Silveira, 2011; J. R. Coura, 2015; II Consenso de Doença de Chagas, 2015).

1.3.2. Transmissão transfusional sanguínea

Outra forma bastante comum para a transmissão em áreas não endêmicas do parasito é por meio da transfusão de sangue. Em 1945, esse meio de transmissão do *T. cruzi* foi sugerida por Dias et al. Posteriormente, vários casos foram reportados e confirmou-se que a transfusão de sangue é um importante meio para a transmissão do *T. cruzi*. Apesar de ser um meio de transmissão, essa via possui uma infectividade menor pela necessidade de um número elevado de parasitos no sangue do doador, quadro encontrado durante a fase aguda da doença (Coura, 2009; Molina, 2018). Importante fator para esta forma de transmissão se é a maioria dos pacientes chagásicos apresentar-se assintomática, mesmo durante a fase aguda, dificultando o diagnóstico e aumentando a transmissão da doença causados por transmissão por transfusão de sangue infectado (J. R. Coura, 2015). Hoje, empregando-se os testes sorológicos anti-

T. cruzi, necessários para a doação de sangue, houve redução na transmissão por transfusões sanguíneas no Brasil (J. R. Coura, 2015).

1.3.3. Transmissão oral

A transmissão oral é muito comum no ciclo silvestre, já que muitos animais possuem os triatomíneos contaminados como fonte diária de sua alimentação no ambiente natural (J. R. Coura, 2015). A infecção oral humana é associada ao consumo de carne de animais infectadas, consumo de frutas e legumes e, principalmente, o creme de açaí. O primeiro caso de infecção oral no Brasil foi reportado em 1965 no Rio Grande do Sul e, desde então, em vários estados brasileiros casos de infecção oral por *T. cruzi* tem sido confirmados (Brener, 1979).

A infecção oral é bem mais agressiva e tem uma mortalidade maior que a infecção vetorial (Silva-dos-Santos et al., 2017). Ambas as formas tripomastigotas são associadas a transmissão oral da doença (Barreto-de-Albuquerque et al., 2015). O *T. cruzi*, ao infectar uma célula gástrica, estabelece uma interação com a mucosa do estomago. Um grupo de glicoproteína gp82, presentes na membrana do parasito, parece promover essa reação, resultando na infecção da célula gástrica através da mobilização de cálcio (Covarrubias, Cortez, Ferreira, & Yoshida, 2007)

1.3.4. Transmissão congênita

Segundo o livro do médico e parasitologista brasileiro, Prof. Dr. Zigman Brener, intitulaldo "*Trypanosoma cruzi* e a doença de Chagas" (1979), devemos não só a descoberta da enfermidade à Carlos Chagas, mas também, a comprovação da transmissão congênita. Essa transmissão, deve-se principalmente ao nível placentário, precisando de alguma alteração morfológica ou funcional, facilitando a entrada do parasito. Outro fator que aumenta a porcentagem de chance da infecção atravessar a barreira placentária é o nível de parasitemia materno, ou seja, mães chagásicas em fase aguda têm mais chances de transmitir o parasito ao feto, devido a sua alta carga parasitária em seu sangue circulante (Brener, 1979).

Os fetos que adquirem o parasito antes do 5º mês de gravidez geralmente são abortados, devido à alta carga parasitaria no indivíduo que ainda está se formando. O coração, esôfago, intestinos, cérebro, pele e a musculatura esquelética são as áreas mais afetadas no feto. Após o parto, os principais sinais clínicos do recém-nascido são a

hepatoesplenomegalia e miocardite aguda, semelhantes aos sintomas dos pacientes com a doença de Chagas adquirida, outros sinais como alterações neurológicas, edemas e outros problemas cutâneos também são observados (Messenger, Miles, & Bern, 2015).

A doença congênita não possui prevenção, porém, com um diagnóstico rápido do recém-nascido, tratamentos podem ser iniciados, alcançando uma taxa de cura próxima a 100%, principalmente com o benznidazol (5 a 10 mg/kg por 30-60 dias) ou com o nifurtimox (10-15 mg/kg por 60 dias) (Massad, 2008). Ambos os medicamentos são nitrocompostos utilizados no tratamento principalmente da fase aguda da doença de Chagas, não sendo muito eficazes quando a doença se cronifica (Mazzeti et al., 2018).

1.4. Quadro clínico

A doença de Chagas é dividida em duas fases: a fase aguda e a fase crônica, sendo que a primeira é caracterizada pela alta presença de parasitos na circulação sanguínea (Chagas, 1909). A infecção humana vem acompanhada de uma febre intensa, edemas, linfonodos hipertrofiados, hepatomegalia, esplenomegalia e, em poucos casos, a insuficiência cardíaca. Por serem manifestações clínicas comuns e semelhantes às manifestações de outras doenças, muitas vezes o diagnóstico é negligenciado (Pinto, Valente, & Valente, 2008; Pérez-molina & Molina, 2018).

Existem também os sinais de porta de entrada da infecção, tais como: sinal de Romaña e o chagoma de inoculação (Rassi Jr, 2010). O primeiro consiste basicamente em um edema bipalpebral e foi considerado uma descoberta muito valiosa para a doença que não tinha um quadro clínico consistente. Depois da descoberta do sinal de Romãna, o diagnóstico da doença de Chagas ficou mais preciso e, consequentemente, os casos começaram a aumentar em diversos estados do Brasil (Prata, 1999). O segundo sinal se trata de uma formação cutânea semelhante a um furúnculo localizado em qualquer parte do corpo e a lesão gera uma úlcera na pele (Rassi Jr, Rassi, & Little, 2000).

Na maioria das vezes, o desenvolvimento da doença pode seguir um caminho benigno entre 30 e 90 dias após a infecção, porém, principalmente em crianças com menos de três anos e em indivíduos imunossuprimidos, há casos fatais (J. R. Coura, Junqueira, & Ferreira, 2018). A presença do parasito no sangue do paciente desencadeia uma resposta inflamatória em pouco tempo, reduzindo a parasitemia em poucos dias, devido a formação de anticorpos específicos ao *T. cruzi* (Sosa-estani & Leonor, 2006). O diagnóstico da doença de Chagas pela análise do sangue deve ser feito em poucas

semanas, já que com o passar dos dias, a tendência é de que não consiga-se mais detectar parasitos circulantes com técnicas convencionais, tornando o diagnóstico direto inviável (Hernández et al., 2016)

Um dos sintomas que mais levam aos quadros de óbito da doença de Chagas é a miocardite (J. R. Coura & Carlos Pinto Dias, 2009). Na fase aguda, os medicamentos, principalmente os nitrocompostos, são eficazes ao conter a evolução da miocardite, mas falham quando a mesma se cronifica (Cunha-Neto & Chevillard, 2014). Como o diagnóstico da doença é bastante difícil em sua fase aguda, a maioria dos indivíduos infectados a descobrem na fase crônica, tornando difícil seu tratamento. (Ministério da Saúde, 2015).

Após a fase aguda, a doença entra em sua forma crônica, podendo variar em indeterminada, cardíaca ou digestória. Na forma crônica indeterminada o paciente não apresenta sintomatologia clínica aparente aos exames mais sensíveis, por anos ou décadas. Geralmente ela perdura para o resto da vida. Seu diagnóstico se torna mais difícil, visto que existem pouquíssimos parasitos em sangue circulante. A fase indeterminada pode evoluir com o tempo para a chamada forma crônica sintomática, podendo apresentar sintomatologia cardíaca (forma cardíaca), digestiva (forma digestiva) ou ambas (forma cardiodigestiva ou mista) (Andrade, Machado, Chiari, Pena, & Macedo, 1999; Higuchi, Benvenuti, Reis, & Metzger, 2003; Marin-neto & Cunha-neto, 2007).

A contínua infeção pelo protozoário leva a ativação do sistema imune nos pacientes chagásicos (Alvarez et al., 2019). Na cardiopatia chagásica o principal fato clínico é a insuficiência cardíaca congestiva (ICC), isso se deve substituição de área muscular por áreas de fibrose, interrompendo fibras e fascículas, diminuindo assim, a massa muscular cardíaca (Pereira Neves, 2005). Em sua forma digestiva, os principais sintomas são: disfagia, odinofagia, dor retroesternal, regurgitação, pirose, entre outros. O principal fator clínico envolvido com essa fase são representados pelo megaesôfago e megacólon (Pereira Neves, 2005). O tratamento da doença de chagas crônica pelos fármacos Nifurtimox e Benznidazol (ambos utilizados e com alta taxa de sucesso na fase aguda) não têm grande eficácia, além de possuírem efeitos colaterais como estresse gastrointestinal, hipersensibilidade cutânea e sintomas neurológicos (Barry, Versteeg, Wang, Id, & Zhan, 2019).

1.5. As cepas do *Trypanosoma cruzi*

Uma das características mais importantes do *T. cruzi* é a sua grande variabilidade genética, contendo diferenças em seu DNA entre algumas cepas de até 48% (Lewis et al., 2010). Atualmente as cepas do parasito são divididas em seis Unidades Discretas de Tipagem (DTU, sigla em inglês) nomeadas de TcI a TcVI (*T.* cruzi I a VI) e outra DTU chamada TcBat (Brenière, Waleckx, & Barnabé, 2016). Essas DTU's são utilizadas para separar cepas com características diferentes, com diferenças genéticas, parasitológicas, epidemiológicas, entre outras (Brenière et al., 2016). Sabe-se também que cepas de DTU's diferentes podem habitar o mesmo vetor ou até o mesmo hospedeiro (Breniere et al., 1998).

Resumidamente, cada DTU possui características diferentes, por exemplo, TcI tem uma distribuição mais ampla na América, abrangendo do sul dos Estados Unidos até a Argentina, estão mais relacionados a ciclos silvestres, apesar de serem evidentes também no ciclo doméstco. TcII, TcV e Tc VII estão relacionados a ciclos domésticos, principalmente nos países do Cone Sul e Bolívia (Zingales et al., 2012; Barnabe et al., 2013; Perez et al., 2013). TcIII e TcIV possuem maior ciclo silvestre e são encontradas em hospedeiros de florestas tropicais. A última, TcBat, foi descoberta em morcegos, mas já foram encontradas cepas, recentemente, dessa DTU em humanos (Marcili et al., 2009). Essa substancial diferença genética entre as DTU's causa grande impacto nas características epidemiológicas, biológicas e nos fármacos utilizados para o combate de cada cepa (Tibayrenc, 2010) Muitos estudos publicados sugerem que essa grande variabilidade genética de protozoários de cepas diferentes (até em uma mesma DTU), tanto em termos de patofisiologia, virulência, tropismos e respostas imunológicas, geram uma grande dificuldade na produção de vacinas e novas drogas contra a doença (Callejas-Hernández, Rastrojo, Poveda, Gironès, & Fresno, 2018).

Tabela 2 – Listagem de algumas cepas das seis DTU's conhecidas.

Cepa	DTU	Origem	Hospedeiro
X10cl1	TcI	Pará, Brasil	*Homo sapiens*
Cutia c1	TcI	Espirito Santo, Brasil	*Dasyprocta aguti*
Y	TcII	São Paulo, Brasil	*Homo sapiens*
Mas cl1	TcII	Distrito Federal, Brasil	*Homo sapiens*
M6241 cl6	TcIII	Pará, Brasil	*Homo sapiens*
X109/2	TcIII	Makthlawaiya, Paraguai	*Canis familiaris*
CanIII cl1	TcIV	Pará, Brasil	*Homo sapiens*
Dog Theis	TcIV	EUA	*Canis familiaris*
Bug 2148 cl1	TcV	Rio Grande do Sul, Brasil	*Triatoma infestans*
SO3 cl5	TcV	Potosí, Bolívia	*Triatoma infestans*
CL Brener	TcVI	Rio Grande do Sul, Brasil	*Triatoma infestans*
Tula cl2	TcVI	Talahuen, Chile	*Homo sapiens*

Fonte: (Jose et al., 2014)

Pesquisadores têm investigado as diferentes cepas em modelos animais e suas escolhas refletem o modelo que se propõem a estudar. Destaca-se, na Tabela 2, a cepa Y, amplamente descrita na literatura em estudos para triagem de compostos anti-*T. cruzi*. Caracteriza-se como parcialmente susceptível ao Bz, desenvolve uma intensa resposta inflamatória e quadro imunopatologico em modelos experimentais.

1.6. A cepa Y

A cepa Y do *T. cruzi* (pertencende a *DTU Tc II*) foi descoberta e isolada em 1953 pelo médico e infectologista Vicente Amato Neto. Neste ano, residente na Divisão de Moléstias Infecciosas e Parasitárias no Hospital das Clínicas em São Paulo, Vicente Amato recebia uma mulher e sua filha provenientes da cidade de Marília. Ambas mostravam-se estar com febre e logo, o diagnóstico para a doença de Chagas foi realizado. As duas estavam na fase aguda da doença. O médico notou então que os *Trypanosomas cruzi* isolados das pacientes mostravam certa peculiaridade: alta mortalidade e grande virulência em experimentos feitos em modelos animais. Por esses motivos, principalmente, esse protozoário foi alvo de uma caracterização mais ascídua e então, foi dado um nome para a cepa: Y, devido a primeira letra do nome da primeira paciente registrada com esse protozoário (Neto, 2010).

Hoje, a cepa Y do *T. cruzi (DTU Tc II)* é muito utilizada em estudos nas universidades brasileiras em roedores devido a sua virulência (Neto, 2010) e ao mesmo tempo por apresentar parcial susceptibilidade ao benznidazol. Camundongos utilizados em experimentos laboratoriais, geralmente, morrem na ausência de tratamento etiológico em duas semanas, sendo o pico de parasitemia observado para este modelo de estudo, próximo ao 8º dia de infecção (Gatto et al., 2017). Luiz *et al* realizaram um estudo em 1999 mostrando o ciclo de vida da cepa Y em camundongos. A infectividade e histologia, assim como a parasitemia foram medidos dia a dia no estudo de Luiz e colaboradores: ao segundo dia, ainda não foi observada parasitemia, mas já foram encontradas lesões no cérebro, fígado e nos rins; no quinto dia após inoculação já foi avaliado algumas formas do *T. cruzi* no sangue dos animais, principalmente no sangue retirado das cavidades cardíacas, as lesões nos órgãos citados aumentaram, podendo já ser vistos formas amastigotas na histologia. No sétimo e último dia de análise observava-se o pico da parasitemia e ninhos de amastigotas foram observados em capilares próximos ao fígado e ao baço. Esse padrão da infeção por *T. cruzi* da cepa Y é observado até hoje em experimentos feitos com roedores.

Os medicamentos mais utilizados para tratar a doença de Chagas em sua fase aguda são os nitrocompostos benznidazol (Bz) e o nifurtimox (NFX) (Mazzeti et al., 2018). Apesar da relativa eficácia no tratamento da fase aguda da infeção pela cepa Y do *T. cruzi*, os dois fármacos mostram-se pouco eficazes na fase crônica da doença. A diferença na eficácia antiparasitária dos compostos nitro-heterocíclicos nas diferentes fases da doença, talvez possa estar relacionada às propriedades farmacocinéticas desfavoráveis destes compostos, como a meia-vida relativamente curta e a baixa penetração tecidual (Workman, White, & Walton, 1984; Urbina & Docampo, 2003). Bz (100 mg/Kg), já se mostrou eficaz na cura parasitológica da doença de Chagas aguda causada pela cepa Y em quase 100% dos casos (Lourenço, Faccini, Costa, Mendes, & Filho, 2018). O outro composto, o NFX, apesar de ter uma taxa de efetividade menor que o Bz, também se torna um importante medicamento para sua cura parasitológica. Os estudos de Mazzeti et al. (2018) mostraram que o NFX (50mg/Kg por 40 dias) curou cerca de 43% do grupo infectado com a cepa Y do *T. cruzi*.

Entretanto, a diversidade genética tem se caracterizado como um entrave na descoberta de novos compostos eficientes e eficazes contra o *T.cruzi* (Santana et al., 2019). Além disso, a presença do parasito no hospedeiro desencadeia uma resposta

imune que envolve a liberação de mediadores inflamatórios que, em desequilíbrio, pode definir se o indivíduo permanecerá apenas infectado ou desenvolverá a doença.

2. Justificativa

A diversidade genética, inerente às populações do *T. cruzi*, reflete em seus distintos padrões biológicos e na resistência deste parasito às tentativas quimioterápicas para eliminá-lo. Não suficiente, a diversidade genética também atua, diretamente, na relação imunopatológica/clínica com os distintos hospedeiros mamíferos vinculados ao *T. cruzi*. No entanto, mesmo ciente destas questões, tem-se buscado justificar dados obtidos em laboratório (modelo experimental, principalmente) com padrões genéticos de parasito e modelos de hospedeiros distintos, o que em teoria, mereceria uma reflexão mais apurada. Neste sentido, a presente revisão avaliou os padrões imunopatológicos da cepa Y do *T. cruzi*, frente a diferentes linhagens de camundongos (ou seja, nem haverá alteração de espécie do hospedeiro, mas apenas linhagem), reforçando sobre a importância da comunidade científica discutir resultados sempre "associados ao mesmo padrão genético do parasito" e "ao respectivo hospedeiro". Se assim não o for, incorre-se em possível falha de análise e interpretação dos dados.

3. Objetivos

3.1. Objetivo geral

- Apresentar um panorama histórico/científico relativo à interação parasito-hospedeiro do *Trypanosoma cruzi* caracterizando, em particular, a cepa Y deste parasito no contexto imunopatológico em modelo experimental.

3.2. Objetivos Específicos

- Avaliar a característica do inóculo no comportamento imunopatológico e parasitológico causados pela cepa Y do *T. cruzi* em camundongos.
- Avaliar a questão temporal da infecção nestes animais (variação do dia de infecção até a morte/eutanásia), frente ao comportamento imunopatológico e parasitológico.
- Avaliar se há alteração na expressão e produção dos mediadores inflamatórios em diferentes estudos publicados sobre a cepa Y refletindo no perfil inflamatório no tecido cardíaco destes animais.

4. Medotologia

O proposto projeto trata-se de um estudo de natureza bibliográfica e documental. Os artigos foram selecionados na base de dados de dois sites: o PubMed e o Web of Science, utilizando como palavras-chave os termos: "*Trypanosoma cruzi*", "Y strain", "Chagas disease", "immunopathology", "mouse", e "inflammatory mediators". A partir do cruzamento desses descritores, os títulos e "abstracts" dos artigos foram analisados, e aqueles que contiveram informações relevantes sobre o tema do trabalho foram levados em conta. A análise dos artigos foi focada na fase aguda da doença de Chagas, mas algumas informações sobre a fase crônica da enfermidade também foram analisadas, principalmente a forma indeterminada. A metodologia dos artigos selecionados não fora aprofundada, visando o foco do projeto nos resultados encontrados na literatura sobre o comportamento imunopatológico e parasitológico da cepa Y. Os artigos analisados foram apenas os que contiverem estudos em camundongos (independente da linhagem) como modelo animal, não se restringindo à temporalidade dos trabalhos nem no impacto das revistas científicas.

4.1. A revisão

A revisão da doença de Chagas será realizada através da busca de artigos científicos com a metodologia descrita acima. Na revisão, a cepa Y do *T. cruzi* também será abordada, mas como um exemplo da diversidade genética do parasito. Serão realizadas tabelas e uma linha do tempo para descrever e demonstrar a história natural do protozoário e sua diversidade.

5. Resultados

Short title: ***T. cruzi* and the mammalian host interaction**

From the parasite to the host pathogenesis – the historical and biological aspects beyond the *Trypanosoma cruzi infection*

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Abstract

American trypanosomiasis or Chagas disease is a lifelong and persistent infection caused by the protozoan *Trypanosoma cruzi* and remains the most significant cause of morbidity and mortality in South and Central America. Owing to immigration and to the additional risks of blood transfusion and organ transplantation, the number of reported cases of Chagas disease is recently increasing in Europe and United States of America. The disease is caused by a moderate to intense and lasting inflammatory response by triggering local expression of inflammatory mediators (upregulation of cytokines, chemokines, lipid mediators and others) and favoring activation and recruitment of distinct leukocytes into various tissues aiming parasites elimination. This long-term inflammatory process triggers biochemical, physiological and morphological alterations and clinical disturbances in the digestive (eg. megaesophagus, megacolon), central nervous system (eg. meningoencephalitis, cerebellar syndromes) and cardiac (eg. myocarditis, arrhythmias, congestive heart failure, autonomic derangements and microcirculatory disturbances) systems. Indeed, the pathogenesis of Chagas disease is intricate and multifactorial and the role of parasite and immune response for its starting and maintenance is still unsettled and controversial. In this review, we offer a historical view concerning the *T. cruzi* infection and an update on the current knowledge about "strategies" employed by the parasite to survive into the mammalian host. In this way, parasites from the Y strain genetic population of this parasite was highlighted by their characteristics and their particularities during the experimental infection.

Keywords: *Trypanosoma cruzi*; Y strain; pathogenesis; inflammation; experimental infection; cytokines.

1. *Trypanosoma cruzi* discovery: past and present views

Introduced to the scientific community in 1909, by the Brazilian medical doctor Carlos Ribeiro Justiniano das Chagas, the American Trypanosomiasis or Chagas disease (Chagas, 1909) is still an important zoonotic parasitic disease in American continent and a public health problem around the world (Calvet *et al.*, 2012; Hovsepian *et al.*, 2012; Callerjas-Hernández *et al.*, 2018). By 1921, Chagas and his team of clinicians and researchers have described the etiology, the vector, natural reservoirs and morphological and clinical aspects of the acute and chronic new illness, including raising possible autoimmunity theories about this new entity (Coura, 2010; Cardillo *et al.*, 2015) The new discovery of Carlos Chagas was associated with poor living conditions, particularly dwelling poverty and, regarded by the World Health Organization (WHO) capable to lead to social shame, disability and mortality (Dias, 2013; Prata, 2001; Pérez-Molina & Molina, 2018). In particular, this dwelling poverty walks together with the history of humanity and the close interaction established with wild or domestic mammalians from the past to the present. This interaction has contributed to the attraction of hematophagous triatomines and their maintenance constantly around the human habitation. Flashes of this vector-reservoir-human proximity can be evidenced in different periods in the timeline of human Chagas disease in Americans (Figure 1). Around 6-8 thousands of years ago, Andean population become sedentary maintained the old habits of hunt large and small mammals, which favored close contact with fresh blood, and adopted new ones as domestication of animals and storage of food, particularly grains, which attracted wild small rodents to the human peridomicilium (Dias, 2002). Besides, hematophagous triatomines survived in slopes and caves and, in that period of history, these insect vectors and human cohabited the same rock-shelters, as evidenced by the primitive art in archeaological sites in all Latin American. All together, these evidences can reinforce the primitive contacts between human and *T. cruzi* and in the last decades, paleoparasitological studies employing molecular tools comes to suggest that human infection by *T. cruzi* may exist previously the presence of the humans in the Americans since some studies demonstrated the presence of the parasite in 9,000-year-old mummies tissues from coastal regions of Chile and in 12,000 years-old bones (Araújo *et al.*, 2009; Aufderheide *et al.*, 2004; Guhl *et al*, 1997; Guidon, 1991; Machado *et al.*, 2013; Steberding, 2014).

In our days, it is predictable that Chagas disease affects about 7 million people in South America (Alvarez *et al.*, 2019) and that around 80 million people are exposed to

the possibility of contracting the illness (Coura, 2009; Dias, 2007; Hidron *et al.*, 2010; Malafaia, Guilhem, Talvani, 2013). The vector-borne transmission of *T. cruzi* commonly occurs in individuals that live in poor-quality rural areas when the insect vectors invade the archaic houses and feed on people when they are sleeping (Hidron *et al.*, 2010; Machado *et al.*, 2013). Domestic and wild animals can be infected and operate as reservoirs for the parasite and, in the peridomicilium, these animals also attract hematophagous insects close to the human habitation. After its discovery, Chagas disease remained restricted to the Americas for few decades, but as a recently consequence of the emergent migration movements, a rising number of "imported Chagas disease" have been detected in non-endemic areas such as EUA, Canada, Europe, Australia and Japan (Quijano-Hernandez, 2011; Afonso *et al.*, 2012; J.R. Coura, Carlos & Dias, 2014). The reduction in the frequency of Chagas disease observed in some countries of Latin America was a reflection of a long-term governmental program to control insect vectors and also due the more stricted blood-bank screening (J.R. Coura, 2015). However, even today, the major worry concerning endemic countries still remains to identify screening blood donors and exert adequate clinical management to those chronic patients (Moncayo, 2003; Rassi JR, 2010) and, at least, persist in a discovery of a new less toxic and efficacy therapy anti-*T.cruzi* capable to eliminate the circulating (trypomastigotes) and the tissue (amastigotes) evolutive forms of these parasites (Urbina, 2010; Soeiro & Castro, 2011).

In our current days, there is a characteristic of *T. cruzi* that is always one step ahead all imunoparasitologists: the genetic diversity. Some strains of the parasite have 48% of differences in its DNA (Lewis *et al.*, 2010). The *T. cruzi*'s strains are divided into six discrete typing units (DTU). They are: *T. cruzi* I *to T. cruzi* VI (TcI to TcVI) (Brenière, Waleckx & Barnabé, 2016). The scientific community use this to divide the strains into groups with biology, genetics, epidemiological and parasitological characteristics (Brenière *et al*., 2016). One of the strain most used in studies is the Y strain. Described in the literature in studies with medical drugs against the *T. cruzi*, this strain is considered to be partially susceptible to Benznidazol (Bz) and to evolve an intense inflammatory response (Neto, 2010). The Y strain of the *T. cruzi* will be the target of this research.

Belonging to the DTU TcII, the Y strain was discovered and isolated in 1953 by the physicist and infectologist Dr. Vicente Amato Neto. In this year, resident in the Division of Infectious and Parasitic Diseases (Divisão de Moléstias Infecciosas e

Parasitárias) of the Clinics Hospital of São Paulo, he attended a woman with her daughter complaining about fever and headaches. Later then, the diagnosis of Chagas disease was given for both: they were in acute phase of the pathology. Dr. Amato noticed that the isolated *T. cruzi* of both had some peculiar characteristics such as high mortality and virulence in experiments in animal models. Then, this protozoon was target for a more intense approach and characterization. It was given the name of Y strain, by the initial of the name of the patient zero of this specific protozoon (Neto, 2010).

Then, the natural history of the *T. cruzi* and its strains became an important aspect for studies involving this parasite. The demonstration of a timeline of the history of the parasite and its pathogenesis is proposed to describe the history beyond this protozoan. It starts millions of years ago while South America and Africa was separated in the Middle Crataceous (Haag *et al.,* 1998) and reached human contact around 8,000 years ago with the Andean population (**Figure 1**)

Already knowing the huge genetic diversity in different strains of the *T. cruzi*, another point of these topic must be debated: Is this genetic diversity also related into a single strain? The answer is yes, and the Y strain could be the key to this questioning. This strain has two metacyclic forms (both identified as Y strain, TcII) differ in expression of surface molecules and the infectability of mice by the oral route (Cortez *et al.*, 2012). One of these isolates expresses gp82 on its surface, so, it has been given its name Y82. The other one, expresses only gp30, being named Y30. Cortez and contributors' research found out that both strains have similar mechanisms to enter host cells, but they have different capacities to bind to gastric mucin.

The information presented in **Table 1** reflects about the genetic diversity into the Y strain of the *T. cruzi*. Time of infection, parasite load, animal model and infective route changes the biology of the experiment, also changing many characteristics of the infection by the Y strain of the *T. cruzi*.

Trypanosomes from the Salivary group (*Trypasonoma brucei*) diverged in the Middle Cretaceous when South America separated from Africa (Haag et al. 1998) and family Didelphidae began the dispersion of *Trypanosoma* ancestors through their anal gland secretions and/or urine.

100 million years

1,8 million years

In Cenozic Era (quaternary period) there was dispersion of *Homo sapiens* and predominance of small mammalians responsible to mantain natural cycle of T*rypanosoma* by possible oral and vector transmission.

8,000 years

Primitive Andean population adopted sedentary habits with grain storage which causes proximity of rodents and hematophagous triatomines. There was starting domesticalization of small rodents for comsumption and for rituals. Possible "origen of Chagas disease in humans".

1492

Christopher Columbus and his fleet reach the New World "American Continent" on October, 12.

1500

Brazil was officially disvoovered by the Portuguese navigator and explorer Pedro Alvares Cabral and by his squadron sighted Mount Pascoal, called "discovery coast" (Bahia), on April 22 .

1879

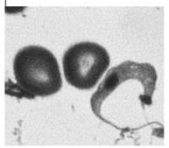
Carlos Justiniano Ribeiro das Chagas was born on July 9th in a coffe farm in Oliveira, MG, Brazil.

1908

After his initial efforts to the control of Malaria and yellow Fever, Carlos Chagas described a new form of *Trypanosoma* in Triatomines and *Callithrix penicilata* (Lassance, MG, Brazil)

1909

Carlos Chagas published, In the 1st volume of "Memórias do Inst. Oswaldo Cruz" his classical article about the 'new human Trypanosomiasis'



1911

Carlos Chagas presented at The National Academy of Medicine (RJ, Brazil) the first description of a congenital transmission of the new disease.

1912

Carlos Chagas announced At the National Academy Of Medicine (RJ, Brazil) The possibly sylvatic cycle In armadillos. In this year, an international jury awarded him the Shaudinn Prize, given to the best study on protozoology and microbiology.

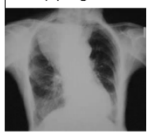
Carlos Chagas was nominated for the Nobel Prize in Medicine.

1913

& Carlos Chagas was, oncemore , nominated for the Nobel Prize in Medicine * and, in this year, received the title "*Honoris causa*" from Harvard University, EUA.

1916

Carlos Chagas firstly raised the involvement of the digestive system related to megasophagus and dysphagia



1921

1923

Villela recognize d and reinforcesed the concecption of the "indeterminate form" already put foward by Chagas (1916) . This concept will be validadted at the Applied Research Meeting on Chagas disease held in Araxá, MG,Brazil (Anonymus 1985).

1934

Carlos Chagas (55) died in Rio de Janeiro (Brazil), from an acute heart attack. In this year, Emmanuel Dias reinforced the main features of *T.cruzi* life cycle in the invertebrate hosts.

1935

Emmanuel Dias and Evandro Chagas described the oftalmo glanglionar complex named "Romanã Signal", caused by local hypersensibility reaction after *T.cruzi* infection

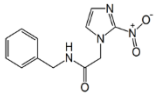
1936

The possibilityof Chagas disease be transmitted by transfusions was first raised (Mazza et al. 1936)

1940

In Bambuí, MG state, there was discovered important foci of Chagas disease, by Almícar Vianna Martins. For decades, Bambui become a source of Chagas clinical research with Emmanuel Dias and colegues.

1943



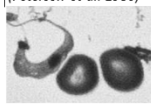
Specific chemotherapy for Chagas disease was empiracally introduced for clinical use (i) Nifurtimox and (ii) Benznidazole (in detail)

1953

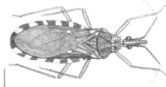
Dr Amato Neto discovered and isolated the Y strain of the *Trypanosoma cruzi in Marília, São Paulo*

1986

Cloning *Trypanosoma cruzi (Peterson et al. 1986)*



2006



International Committee of the OPA said Brazil free transmission of Chagas disease by *Triatoma infectans*

& Since 1920, Dr. Júlio Afrânio Peixoto and other members of the "National Academy of Medicine" have started an untimely polemic about the true relation between "*T.cruzi* and Chagas disease" (Coutinho et al. 1999) and due this , nobody was granted the with Nobel Prize in medicine in 1921 since Chagas was the only real candidate for this prize.

Figure 1 – Natural history's timeline of *Trypanosoma cruzi*. A timeline showing the possible diversification of *T. cruzi* specie to the Brazil's certificate of free transmission of Chagas disease by *Triatoma infestans,* going through the disease discovery, discovery of its chemotherapy agents and the Y strain discovery.

Table 1 – References of studies with the Y strain of the *T. cruzi.* Several characteristics of some Y strain studies was analyzed such as: (i) parasite load at inoculation; (ii) period of infection; (iii) animal model (mice lineage); (iv) inflammatory mediators' analysis; (v) histopathology; (vi) route of infection; (vii) if any drugs were used; (viii) percentage of parasitemia level of the animals; (ix) negative results in PCR (Caldas, 2012).

References	Parasitary load (trypomastigote forms)	Time of infection	Animal model	Inflammatory mediators	Histopathology	Infective route	Drug	Parasitemia clearance	Negative results in PCR
(Mazzeti *et al*., 2018)	5.0 x 10^3 bloodstream	40 days	Swiss	None taken.	None taken.	i.p.	Bz	100%	100%
(Gatto *et al*., 2017)	1.0 x 10^4 bloodstream	11 days	Balb/c	↑ IFN- γ , IL-17, IL-10 and TGF-β.	None taken.	i.p.	Bz	100%	0%
(Mateus *et al*., 2019)	1.0 x 10^5	30 days	Balb/c	↑ Ag-specific responses of TCD4+ and TCD8+ ↓ IFN-γ, TNF-α and IL-2.	Parasite detected in colon, heart, liver, skeletal muscle and blood. Average inflammatory infiltrate was higher in colon and liver. Necrosis was observed in the heart and skeletal muscle.	i.p.	None	100%	None taken
(Luiz *et al*., 1999)	1.0 x 10^5	7 days	Balb/c	None taken.	Amastigotes nests appearing in capillaries and sinusoids of the liver and spleen. Parasites found on the sternal bone marrow in young blood cells.	i.p.	None	0%	None taken
(Diniz *et al*., 2018)	5.0 x 10^3	20 days	Swiss	None taken	None taken	i.p.	E1224 -Bz	100%	83%

(Oliveira *et al.*, 2012)	1.0 x 10^5	20 days	Balb/c	None taken	The parasite tropism led them to infect the kidneys and liver, mainly. Heart and lung were also infected.	i.p.	None	0%	None taken
(Shrestha *et al.*, 2017)	1.0 x 10^2 bloodstream	12 days	C57BL/6	↑ CCL2 and CCL5 normal ratio TNF, IL-17 and IL-10.	Normal heart/body weight ratio.	i.p.	None	0%	None taken
(Novaes *et al.*, 2015)	5.0 x 10^3	16 days	C57BL/6	↓ TNF-α and IFN- γ by Bz treatment. ↑ ALT and AST.	Liver microscopic structure modification.	i.p.	Bz	100%	None taken

i.p.: intraperitoneal; Bz: benznidazole;

2. Natural history and life cycle of *Trypanosoma cruzi*

Paraphrasing the popular question "Which came first: the chicken or the egg?" we are faced with another an evolutionary question whether the ancestral host of Trypanosomatidae was invertebrate or vertebrate. The define conclusion has been still unclear for the scientific community and tending one way or the other depending on the evolutionary evidence provided by the new applied technology tolls, but there is no doubt that *Trypanosomatidae* protozoans isolation or spread around Americans was limited by the distribution of their mammalian hosts and vectors. In the particular case of *T. cruzi*, the natural history conducts our thoughts to a small number of old mammalians species (prior to human contacts) that probably conducted the first chapters of this history, such as marsupials, rodents, bats, primates and hematophagus insects (Pérez-Molina & Molina, 2018). In this way and returning to the question about "the chicken or the egg", particular attention should be given to the family *Didelphidae* where some representatives (opossum *Didelphis marsupialis*) are able to keep the two proliferative cycles of the *T. cruzi*, including epimastigote forms in the light of their scent glands as well as intracellular amastigotes in different tissues. It means that opossum may serve, in concomitance, as a reservoir and as a vector to *T. cruzi* in the natural environmental (Deane, 1984; Carreira *et al.*, 2001). It is also proposed that during the Cenozoic period the ancestors of the family *Didelphidae* began their dispersion with the intra-species transmission of the ancestors of *T. cruzi* through the anal gland secretions and/or urine. Based on this hypothesis emerges a primitive association between *Trypanosoma* and marsupials of the *Didelphis* genus and, the possible wellspring of Chagas disease in the America (Stevens, 2001). Besides, some other reports reinforce the old interaction of human and marsupials which possible contributed to the introducing of the Chagas disease to the *Homo sapiens*: (i) marsupials had widely distribution in the Americas, (ii) these animals were well adapted to the linings of the houses, hollow of trees and other shelters close to the human housing, (iii) marsupials have survived front of the human predatory hunting for food and, (iv) they become well adapted to human actions in their natural environment

In the present time, the natural history of Chagas disease still persists as an enzootic infection of wild animals in endemic areas (Rassi Jr, 2010). Although paleoparasitological studies have pointed the presence of *T. cruzi* in the prehistoric mummies or bones (J.R. Coura & Dias, 2009; Rassi Jr, 2010), as discussed before, endemic Chagas disease started through the deforestation caused by human measures over the last 2-3 centuries (Coura, 2007; Steverding, 2014). As a consequence of

woodland clearance for agriculture and livestock rearing in Latin America the hematophagous triatomines carrying parasites, which were left without their natural reservoirs, started to colonize areas around human domicile. They personalized to this new place, including feeding on the blood of domestic animals as well as humans (Pérez-Molina & Molina, 2018)

T. cruzi alternates between different morphological and functional forms during its life cycle and involves more than hundred species of mammalian vertebrates as well as invertebrate hosts (Rassi Jr, 2010). Its biological plasticity allows its transmission to humans and other susceptible hosts, mainly through the feces of infected hematophagous *triatominae* ("kissing bugs") or by secondary routes such as oral transmission, blood transfusion, from mother to fetus (congenital infection), tissue transplantation or by accidents with people who work with the live parasites in laboratory (Hidron *et al.*, 2010; Afonso *et al.*, 2012; Hovsepian *et al.*, 2012). Initially, the kissing bugs become infected when they take a meal from infected mammalian host with trypomastigotes forms circulating in the blood.

The trypomastigotes once in the stomach of the insect vector, convert into epimastigotes and spheromastigotes and, by binary fission, epimastigotes multiply and attach to the perimicrovillar membranes of the intestinal cells predominantly through their flagellum (Tyler *et al.*, 2002). In the next, at the insect posterior digestive region, part of the epimastigotes convert again into metacyclic trypomastigotes adhering to the cuticle lining the epithelium of the rectum and rectal sac. These forms are next leaved with the vector urine or feces during blood meals. After that, the course of a natural transmission to a new mammalian host appears when the parasite-laden feces contaminate nasal or oral mucous membranes, the conjunctivae or injured skin, as well as local vector bites. The metacyclic trypomastigotes invade local host cells attaching to the macrophage surface predominantly or cells-like, inducing an intracellular influx of calcium with temporary disarrangement of the cytoskeleton which allows the migration and fusion of lysosomes to form the parasitophorous vacuole (Caler *et al.*, 2000). Inside the vacuole, while membrane of parasitophorus vacuole suffers digestion, trypomastigote form starts an internalization process transforming into the amastigote, the intracellular replicative form of parasite (Dvorak & Howe, 1976; Fernandes & Andrews, 2013). Free inside the cytoplasm, amastigote forms replicate several times consuming cellular nutrients and

changing environment until such time that start a new morphological change into trypomastigote form. The constant kinetic movement of hundred new parasites into a weakened host cell culminates in its disruption and scape of infective forms of *T. cruzi* to the extracellular environment and bloodstream. Each new parasite can march into the other healthy cells (Ley *et al.*, 1988; Tyler *et al.*, 2002). The cycle transmission is concluded when circulating trypomastigotes are taken up in blood meals by reduviid bugs. Cardiac myocytes, peripheral muscle cells, endothelial and vascular smooth muscle cells, cells of the central and peripheral nervous systems, cells of the reticuloendothelial system, adipocytes and all types of nucleated mammalian cells can be parasitized (Combs *et al.*, 2005; Machado *et al.*, 2013).

3. Molecular mechanism of *T. cruzi* invasion

The major function of innate immunity is the early elimination of invasive microorganisms. *T. cruzi* has developed multifaceted and redundant mechanisms to compose successful cell invasion. Some particular aspects of cell invasion differ across cell types, including surface-surface interactions, enzymatic events, trafficking of donor membranes, trafficking of host membranes, calcium-mediated signaling, cytoskeletal assistance to parasite uptake and cytoplasmic access via escape from the parasitophorous vacuole (Tardieux *et al.*, 1992; Rodriguez *et al.*, 1995; Burleigh, 1998; Yoshida, 2006; Calvet *et al.*, 2012; Barrias *et al.*, 2013) Inoculated infective metacyclic trypomastigotes usually infect local macrophages, fibroblasts and other mesenchymal tissues at the site of primary infection (Epting, Coates & Engman, 2010). It is well established that the parasite has intrinsic tissue tropism, first described by Melo and Brener and supported by results of experimental infection using two isolates strain of the parasite, in which one was found localized in the heart and the other one to the gastrointestinal tract (Epting, Coates & Engman, 2010; Barrias *et al.*, 2013; Borges *et al.*, 2016). The molecular or immunologic elucidation for apparent tissue tropism is not complete and the characteristics of clinical disease emerge from results of a complex interaction among parasite and host genetic variation, immunity and inflammation.

There are many cells with important roles in innate immunity such as dendritic cells, macrophages and natural killer (NK) cells and are important elements in the initial control of *T. cruzi* replication. Resident tissue macrophages are supposed to play a significant role *in vivo* as one of the first host cells to be invaded by *T. cruzi*. In the beginning,

trypomastigote and epimastigote forms were competently internalized by macrophages and later experiments discovered their presence inside phagolysosomes (Tecia & Carvalho, 1989; Nagajyothi *et al.*, 2013) but only the trypomastigotes could escape from the phagolysosome and multiply in the cytosol (Nogueira & Cohn, 1976; Barrias *et al.*, 2013). Also, *T. cruzi* trypomastigotes are capable of directly invading professional phagocytes and nonphagocytic cells. Surrounded by professional phagocytes, tissue resident macrophages are essential targets for initial infection, where they start a robust innate immunity and the systemic anti-parasite inflammatory response. Also, the professional phagocytes have been recognized both as crucial cellular targets and as a defense instrument for the host (Nagajyothi *et al.*, 2013). For the cellular procedure of phagocytosis, reviews are suggested (Mauel, 1982; Thorne, 1983).

Two major pathways have been characterized to expose the infection of non-phagocytic cells. The first one depends on a calcium-mediated signaling at the surface for lysosomal trafficking to offer donor membranes for the vacuole in a dependent manner on actin polymerization and microtubules (Schenkman *et al.*, 1991; Tardieux *et al.*, 1992; Tardieux *et al.*, 1994; Tyler *et al.*, 2002; Epting, Coates & Engman, 2010; Fernandes & Andrews, 2013). The second pathway is characterized by a plasma membrane-mediated invagination involving PI_3 Kinase signaling and independent of actin polymerization (Souza *et al.*, 2002; Andrade *et al.*, 2005; Burleigh, 2005; Epting, Coates & Engman, 2010). It is important to notice that the capacity for cell invasion is not limited to metacyclic or cell-derived trypomastigotes. The dividing amastigotes (Mortara *et al.*, 1999) and insect stage epimastigotes (Florencio-Martínez *et al.*, 2010) are adapted to determine infections. The amastigote forms are progressively more recognized to share similar infectivity to trypomastigotes.

The mechanism used by the parasite to egress from the bloodstream into the tissues needs to be well known. The abundant number of surface proteases proposes that enzymatic digestion between the endothelial cell and into the original connective tissues is a direct process driven by the parasite. Cruzipain is one of these essential protease used during cellular invasion (McKerrow *et al.*, 1993; Mcgrath *et al.*, 1995; Stoka *et al.*, 1995) Uehara *et al.*, 2012) and is fundamental to allow passage through the unbroken endothelium as well the extracellular matrix. Also, the modifications in the surface residue through trans-sialidase contribute to endothelial cell interactions (Dias W, 2008)

but more studies are required to address this elementary step in parasite propagation through escape from the vascular compartment. A considerable and assorted group of surface glycoproteins and proteases can interact with host cells and extracellular matrix. Many of the glycoproteins share the GPI (glycosylphosphatidylinositol) moiety and the GPI-anchored proteins are first synthesized in the ER, resulting in extracellular membrane-associated proteins (Cardoso *et al.*, 2013). The structures and functions of these proteins are different such as adhesion, paracrine signaling, surface enzymes and cell differentiation (DosReis *et al.*, 2002; Fujita & Jugami, 2008). Many GPI-anchored proteins of *T. cruzi* are connected in the host response and macrophage infection (Ropert *et al.*, 2002; DosReis, 2011).

Analysis of GPI anchors isolated from trypomastigote-derived mucin-like glycoproteins (GPI-mucins) repeal their capacity to activate macrophages and elicit the production of proinflammatory cytokines (Campos *et al.*, 2001; DosReis, 2011). Also, genomic DNA from *T. cruzi* can stimulate macrophages and dendritic cells. The protozoan genomic DNA has enough levels of CpG motifs to cause moderate activation of host cells and their treatment with methylase or DNAse obliterates the DNA pro-inflammatory activity on dendritic cells and macrophage (Shoda *et al.*, 2001).

For many years, the molecular mechanism of invasion by *T. cruzi* associated with regulatory pathways has received attention. Numerous mammalian host cell receptors such as toll-like receptors (TLRs), kinins, receptor tyrosine kinases, TGF and EGF receptors, interacts with *T. cruzi* and the activity of these receptors is necessary for optimal parasite binding and/or invasion (Caradonna, 2011). TLRs are primary means by which the innate immune system recognizes and respond against microorganism. However, an excessive activation of these primitive receptors can induce an uncontrolled inflammatory process as observed in septic shock induced by the pyrogenic lipopolysaccharide (LPS) from Gram-negative bacteria infection.

T. cruzi-derived components are recognized by TLR2 (GPI-anchor and Tc52), TLR4, and TLR9 (genomic DNA). The TLR-mediated MyD88 signaling pathway induces pro-inflammatory cytokines such as IL-12p40 in phagocyte cells orchestrating an inflammatory response mediated by inflammatory mediators (IFN- γ, TNF-α, chemokines and others) in the mammalian hosts (Bafica *et al.*, 2019).

TLR9 has been show to mediate the proinflammatory activity of *T. cruzi* DNA and infection with *T. cruzi* trigger high levels of nuclear factor kB (NF-kB) via TLR9 while TLR2 has participation in the cardiomyocyte hypertrophy (Petersen *et al.*, 2005; Junqueira *et al.*, 2010). Importantly, *T. cruzi* can also activate innate immune responses independently of TLRs (Chessler *et al.*, 2009; Kayama *et al.*, 2009). Host cell receptor LDLr (Low Density Lipoprotein receptor) has been show to be used by *T. cruzi* for their internalization and fusion (Nagajyotchi *et al.*, 2011). The mechanisms involved in LDLr endocytosis look similar to that used by the protozoan *T.cruzi* during its internalization and involves calcium mobilization, acid environment and fusion with endosomes/lysosomes. Based on this, it was proposed that parasite might binds to mammalian cell membrane receptors and activates a cascade of proteins that are also described as positive regulators of LDLr transcription, such as transcription factors, PI3Kinase, TLRs and TGF-β (Nicholson; Hajjar, 1992). Besides, the parasite *T. cruzi* has expanded the mechanisms of escaping the immune response and suppressing host apoptosis by modulating the expression of host cell receptors, signaling molecules and secreted factors.

4. Genetic characteristics of the Y strain

One of the most challenging characteristics of the *T. cruzi* is the high number of different proteins and its functions. Furthermore, the parasite still has a large percentage of hypothetical proteins (proteins that its function is not well known), in other words, there is more proteins that we do not know its functionality than the ones we know (**Table 2**).

Table 2 – Percentage of hypothetical protein content across *T. cruzi* annoted strains by Cellejas-Hernandéz *et al.*, 2018.

Strain	**HP(%)**
Dm28c	64.26
SylvioX10	49.50
Y	**56.94**
Bug2148	53.25
BEL	51.53
BNEL	51.55
B7	50.60

Hp: hypothetical protein

The information on the table 2 show us that the Y strain of the *T. cruzi* has 56.94 of proteins with unknown function. Callejas-Hernandéz (2018) also analyzed trans-sialidase (TS) activity. Those proteins are located on the membrane of metacyclic and bloodstream trypomastigote, besides intraccelular amastigote. They are the principal protein's family involved with host parasite interaction processes (Freitas *et al.*, 2011). The mainly process of these proteins are the catalysis of the transference of sialic acid molecules from host glycol-conjugates to parasite surface's acceptor molecules (Cellejas-Hernandéz et al., 2018). According to Cellejas-Hernandéz group, the Y strain's most TS proteins belongs to a subfamily of proteins that is associated to antigenic variation, allowing the parasite to adapt to the host environment.

5. Clinical manifestations of Chagas' disease

Chagas' disease is clinically divided in the acute and chronic phases. Symptoms range from mild to severe, although many people do not experience symptoms even in the acute then in the chronic stage (Rassi Jr, 2010; Hovsepian *et al.*, 2012) The acute phase remains 4-8 weeks and is associated with unspecific symptoms or clinical signals such as prolonged fever, headache, anorexia, vomiting, drowsiness, malaise, hyperemia and edema at the portal of entry (Romaña sign or inoculation chagoma) splenomegaly and enlarged lymph nodes (Teixeira *et al.*, 2011; Hovsepian *et al.*, 2012; Pérez-Molina, 2012;). During the acute phase, the most severe manifestations are myocarditis accompanied by arrhythmias, congestive heart failure and, more rarely, meningoencephalitis (Rassi Jr, 2010). Echocardiogram (ECG) abnormalities include sinusal tachycardia, first-degree atrioventricular block, sinus tachycardia, QRS low voltage, and primary alterations of the T-wave (Rassi Jr, 2010; Teixeira, 2011). Chest X rays may show an enlargement of the cardiac shape (Teixeira, 2011). The signs and symptoms of acute Chagas disease resolve spontaneously in one or two months in about 90% of infected individuals even if untreated. However, in children above three years old or immunosuppressed individuals there are cases of death (Coura, Junqueira & Ferreira, 2018) Most of the individuals that survive in the acute phase (60-70%) advance to a chronic stage of the disease and, 60-70% of them stay in an asymptomatic clinical form (named indeterminate form) that is characterized by a positive serological test with a specific IgG antibody, absence of clinical manifestations (signs and symptoms), absence

of electrocardiographic abnormalities and normal-sized heart, esophagus and colon without alterations by X-ray inspection (Andrade *et al.*, 1999; Rassi JR *et al.*, 2001; Rassi JR, 2010; Teixeira *et al.*, 2011). This indeterminate form may last months to a full lifetime. The remaining 30-40% of infected individuals will expand the chronic phase of the infection characterized by cardiac and/or digestive form months to decades after the initial infection, usually 10 to 30 years (Nagajyotchi *et al.*, 2013). If cardiac form of Chagas disease is responsible for laboral incapacity, low quality of life and death among chronic infected individuals, cardiac clinical evaluation requires care and knowledge on the part of the clinician. Even today, the auscultation, the electrocardiography (ECG) and the thoracic X-ray are useful and essential in endemic areas or in hospitals to diagnosis mild or severe cardiac disturbances. It is well recognized the importance of the anticipated diagnosis when Chagas heart is installed due the necessity to start specific pharmacotherapy or impose immediately changes in the routine of chagasic individuals (eg. suspension of laboral activities) aiming the survival of those individuals. In the last decades, new approaches have emerged to improve the quality of the diagnosis of Chagas heart disease such as imaging of the heart employing echocardiography, microPET and cardiac magnetic resonance imaging (Palomino *et al.*, 2000; Machado *et al.*, 2013).

The cardiac form is therefore the most serious and common manifestation of chronic Chagas disease. Generally, intitulated "Chagas heart disease", it is associated with abnormalities of the conduction system, arrhythmias, apical aneurysms, thromboembolism, congestive heart failure, autonomic derangements and others (Acquatella, 2007; Hidron *et al.*, 2010; Barry *et al.*, 2019). Many of these events are common even in younger individuals such as sudden death, heart failure and thrombo-embolic disorders (Coura, Junqueira & Ferreira, 2018). There are several ECG abnormalities such as right bundle branch block left anterior fascicular block, ST-T changes, ventricular premature beats, abnormal Q waves and low voltage of QRS. Another hallmark of the disease is the sustained ventricular tachycardia. The heart failure is the latest manifestation of the Chagas heart disease and is associated with higher mortality (Marín-Neto *et al.*, 1999; Rassi Jr, 2010). The annual mortality of Chronic Chagas' cardiomyopathy (CCC) is 4% (Barry *et al.*, 2019). Sudden death can also occur abruptly and unexpected in patients who were earlier asymptomatic, and it is the most important cause of death in patients with Chagas heart disease. Usually is associated with

ventricular tachycardia and fibrillation or, more not often, with complete atrioventricular block or sinus node dysfunction (Rassi Jr, 2010). Chagasic-cardiomyopathy-associated with heart failure can occur in a period of 7 months to 2 years. It is important to notice that the major microscopic finding in the heart of a chagasic patient who succumbs to Chagas' disease is an inflammatory infiltrate (lymphocytic) that destroy parasite-free neurons and cardiac fibers (Teixeira *et al.*, 2011).

Another important clinical form of chronic Chagas disease involves the digestive system and it is characterized by alterations in the secretory, motor and absorptive functions of the esophagus and gastrointestinal tracts. This form is rare in northern South America, Central America and Mexico, but is almost exclusively in south of the Amazon basin especially in Brazil, Argentina, Chile and Bolovia (Miles *et al.*, 2003; Rassi Jr 2010). In chronically infected individuals, the gastrointestinal dysfunction develops in 10-15% and involves dysphasia with odynophagia, combined with epigastric pain, regurgitation, ptyalism and malnutrition in the megaoesophagus case (Rassi Jr 2010). Megacolon involves the sigmoid segment, rectum or descending colon and produces extended obstipation, abdominal distention and large bowel obstruction. The longtime of solid feces retention incites the dilatation of the colon causing discomfort and pain (Kannen *et al.*, 2018). Based on X-ray findings, megacolon is classified in stages: stage I, natural elimination of fecal matter; stage II, without natural elimination of fecal matter and stage III, with completely obstruction and impossibility of elimination after pharmacological motivation. Individuals with megacolon present long-term waves and hypercontraction of muscle fibers and sigmoid colons show low mobility with higher wave frequency rates when compared with healthy individuals (Rassi Jr, 2010; Teixeira, 2011). Megasyndromes are not commonly observed in kids and surgery is indicated in advanced cases.

The cardiodigestive form is a junction of heart disease with megacolon, megaesophagus or both. In most cases, the development of megaesophagus precedes heart and colon disease, but the prevalence of the cardiodigestive form is unknown.

Congenital *T. cruzi* infection is an acute infection that affects newborns and is diffused by vertical transmission from an infected mother to infant. Most of them are asymptomatic or have mild symptoms, however, if left untreated, may lead to chronic disease later in life (Carlier *et al.*, 2011; Pérez-Molina 2012). Studies from

epidemiological data in Latin America indicates that the number of congenital cases of *T. cruzi* infection is superior than 15,000 per year (Carlier, 2011; Sánchez & Ramírez, 2013). The transmission has been described in endemic and non-endemic areas and the demonstration of symptomatic congenital Chagas disease consist of low birth weight, fever, hypotonicity, prematurity, hepatosplenomegaly, low Apgar scores, meningoencephalitis, respiratory insufficiency, anemia and thrombocytopenia (Carlier, 2011; Perez-Molina, 2012; Sánchez & Ramírez, 2013; Messenger, Miles & Bern, 2015). Abortion and placentitis are related with infection in the uterus. Treatment with anti-parasitic drugs is not recommended, but there are specific measures to be taken to prevent congenital infection for infected pregnant women (Carlier, 2011). Techniques in cord blood or placental tissues such as PCR, show amplified sensitivity over traditional parasitological methods for diagnosis of congenital infection (Mejia *et al.*, 2011).

Finally, after the 80's decade and the spread of HIV infection around the world, and important issue concerning human Chagas disease was came up: if immune system controls parasite blood replication and drive them to tissues and organs, how would be the clinical behavior of a HIV- immunosuppressed individual during a chronic phase of Chagas disease? It was already known that patients with chronic Chagas disease who became immunosuppressed for any reason (medication, transplantation, etc) have a reactivation of the infection. Patients with Chagas disease/ HIV co-infection may present unusual clinical manifestations such as involvement of central nervous system and/or serious cardiac lesions and cutaneous lesions related to the reactivation of the infection (Vaidian *et al.*, 2004). In those individuals are described fever, headaches, focal neurological deficits and vomiting, all classical clinical signals of acute meningoencephalitis. Besides, there is identification of trypomastigote forms of *T. cruzi* in cerebrospinal fluid reinforcing the conception of the neuronal commitment in the presence of both infection agents.

6. Pathology and pathogenesis

During the *T. cruzi* infection, its control depends on innate and acquired immune responses triggered during early infection and both are decisive for host survival. During the acute phase of the infection, the load of the parasites will dictate the magnificence of the inflammatory response and the damage caused by the parasite itself and by the host's

immune response to different tissues and organs (Andrade, 1999; Hidron, 2010; Rassi Jr, 2010).

Several studies from experimental models of *T. cruzi* infection recommended that a Th1- immune response profile mediated by $CD4^+$ and $CD8^+$ T cells is important to control the parasitism by the production of cytokines such as interferon-gama (INF- γ), tumor necrosis factor (TNF-α), interleukin (IL)-12 and others (Silva *et al.*, 1998 Mateus *et al.*, 2019). Numerous *T. cruzi*-derived molecules including GPI mucins from trypomastigotes and DNA stimulate the synthesis of inflammatory cytokines by macrophages and phagocytic cells (Camargo, 1997; Almeida 2001; Shoda *et al.*, 2001 Machado, Tyler, Brandt, 2013). This inflammatory profile has a protective role mostly through the synthesis of nitric oxide, which has a strong trypanocidal action (Cardoni *et al.*, 1990; Ribeiro, 1993; Chandra *et al.*, 2002; Gutierrez *et al.*, 2009) Importantly, during *T. cruzi* infection, IFN- γ also support migration of T cells and establishment of myocarditis by inducing expression of chemokines such as CCL5 (RANTES), CCL2 (MCP-1), CXCL10 (chemokine C-X-C motif ligand 10) and CXCL9 (MIG), and adhesion molecules such as ICAM (intercellular adhesion molecule and VCAM (vascular cell adhesion molecule) (Talvani, 2000). A second signal is provided by TNF-α stimulating NO production and anti-*T.cruzi* activity in IFN-γ-activated macrophages.

Production of interleukin 10 and transforming growth factor γ negatively regulate NO production (Gutierrez, 2009; Machado, 2012) and these down-regulatory cytokines (TGF-α and IL-10) appear to be related to parasite replication by inhibition of macrophage trypanocidal activity (Reed *et al.*, 1994; Silva *et al.*, 1991 Rassi Jr, 2010; Esper *et al*, 2015). Neutralization of endogenous IL-10 conducts to an increased *T. cruzi*-induced IFN-γ production and parasite killing (Reed, 1994; Cardillo *et al.*, 1996). Together, these results suggest that during infection in mice, IL-10 may act as a potent inhibitor of IFN-γ production and the initial resistance to infection is a result of the balance between IL-10 and IFN-γ (Cardillo, 1996; Chevillard *et al.*, 2018). During the acute phase of the infection when cellular and possibly humoral immune response eventually control, but fail to totally eliminate the parasite, a variable long asymptomatic phase then occurs and factors such as the parasite strain, the quality of immune response, the parasite load and the presence or absence of reinfection all might influence the course of chronic disease (Lima *et al.*, 1999; Hidron, 2010; Chevillard *et al.*, 2018).

The role of *T. cruzi* in the pathology of acute phase of Chagas disease and the importance of etiological treatment in that stage is extensively accepted (Brener, 1997) but the participation of the parasite in the pathogenesis of chronic Chagas disease has received controversies during decades (Cunha-Neto *et al.*, 1995; Tarleton, 2001). There are numerous theories to explain the etiology of chronic cardiac lesions that is related with the parasite persistence, the immune reaction to the parasite and autoimmunity elicited directly and indirectly (Engman & Leon, 2002; Hidron *et al.*, 2010; Tarleton, 2003; Teixeira *et al.*, 2011). The product of chronic cardiac lesions is neuronal damage, various degrees of necrosis, microvascular damage and fibrosis (Cunha-Neto & Chevillard, 2014; MARIN-NETO *et al.*, 2007). There are several independent studies showing that the functional and anatomical parasympathetic divisions are involved in neuronal damage in Chagasic patients (Amorim *et al.*, 1995); Lewis *et al.*, 2017). Other issue collectively with neuronal damage is that microcirculatory changes leading to ischemia have been implicated in the pathogenesis of chronic cardiomyopathy (Marin-Neto, 2007). Mediators that promote vasospasm and platelet aggregation, occlusive platelet thrombi in intramural coronary arteries and increased production of cytokines have been previously established in experimental models of Chagas disease (Morris *et al.*, 1992; Rossi *et al.*, 2010).

After *T. cruzi* infection, autoimmunity is another topic that has been suggested to be a reasonable etiology for the chronic myocarditis in infected patients (Engman, 2002; Cunha-Neto, 2006; Marin-Neto, 2007; Lewis *et al.*, 2017). In this view, antibody-dependent cytotoxicity and/or the direct activation of autoreactive T cells is an aspirant instrument for the triggering of autoimmunity (Leon & Engman, 2001; Engaman & Leon, 2002; Bonney *et al.*, 2011). There are many antigens from *T. cruzi* that cross-react with cardiac and noncardiac host components such as serum from chronic chagasic patients that contain cross-reactive antibodies between human and *T. cruzi* proteins (Cunha-Neto *et al.*, 1995; Bonney & Engman, 2015) and with more attention to antibodies that cross-react with cardiac myosin heavy chain and the *T. cruzi* antigen B13, because they were identified in most sera from patients with chronic Chagas' cardiomyopathy than in asymptomatic infected individuals (Cunha-Neto, 1995; Marin-Neto, 2007; Bonney & Engman, 2015); in addition, $CD4^+$ T cells clones derived from biopsy of patients with Chagas' cardiomyopathy were found to be reactive with both cardiac myosin heavy chain

and the B13 *T. cruzi* protein (Cunha-Neto *et al*, 1996; Bonney & Engman, 2015). The importance of inducible T regulatory (iTreg) and Th17 cells has received attention in the development and progression of inflammatory autoimune disease (Weaver, 2007; Ma & Zhou 2009). Treatment of naive peripheral CD4 T cells with TGF-α plus IL-2 and a TCR stimulant enhance Treg in the thymus; iTreg are involved in immune modulation and deceleration of autoimmunity by recomposing self-tolerance (Afzali *et al.*, 2007; Zhu *et al.*, 2012). Autoimmune reactivity is essential in the description of an autoimmune disease and it is detected in otherwise healthy individuals. There are some questions that remain to be explained such as whether the autoantibody and autoreactive T-cell responses are pathogenic and whether any pathogenic responses can be maintained in the absence of infection. It is not clear why the presence of mononuclear cells in the heart causes damage and their connection with release of auto-antigens and production of autoantibodies as well what describe them to the heart and whether they can be preserved in the nonattendance of infection (Tarleton, 2007; Monteiro, 2007; Machado, 2012). The autoimmune theory of Chagas' disease continues to be confronted in different ways because the anti-self-direct mechanism triggering the inflammatory effectors lymphocyte is not known (Kierszenbaum, 2005) and the autoimmune humoral factors might be sources of heart disease (de Leon, 2003; Cihakova, 2008; Teixeira, 2011).

The Y strain of the *T. cruzi* has high virulence, as previously described and it is partially susceptible to the benznidazol (Bz) therapy (Neto, 2010). In animal model its pathogenicity is already described by different groups (**Table 1**), which is usually followed by an intense inflammatory infiltration in the committed organs (eg. skeletal and cardiac muscles). The parasitemia peak for the Y strain occurs around the 7^{th} and 8^{th} day in murine model of infection, which is followed by a high number of blood parasites in a dependency on the load of the parasites during the inoculum (Melo & Brener, 1978; Luiz *et al.*, 1999). The Y strain is characterized by a rapid multiplication, a high parasitemia during the initial stage of infection and a high mortality on days 15-20 post-infection (Oliveira *et al.*, 2012).

7. Diagnosis and treatment

The diagnosis during the acute Chagas disease can be performed by observation of the trypomastigote forms in a fresh blood smear by microscopic examination. A thick

and thin blood smear stained allows parasite visualization by Giemsa-stained (Gomes *et al.*, 2009; Kirchhoff, 2011)

During the chronic phase, because low and intermittent parasitemia, the presence of IgG antibodies against *T. cruzi* antigens needs to be detected in more than one method. Ezyme-linked immunosorbent assay (ELISA), indirect immonofluorescence, or indirect haemagglutination (IHA) are most normally employed (Gomes, 2009; Mucci *et al.*, 2017). For a final diagnosis, two positive tests are suggested. PCR is not recommended in routine diagnosis, however, because of its heightened sensitivity compared with other parasitological methods, could be useful to confirm diagnosis in cases of inconclusive serology and as an auxiliary method to monitor treatment (Britto, 2009; Rassi Jr, 2010). To assess newly diagnosed patients with chronic *T. cruzi* infection is necessary steps that include complete medical history, physical examination and a resting 12-lead electrocardiogram. Asymptomatic patients with a normal ECG and no gastrointestinal tract or cardiovascular symptoms have a positive prognosis (Ianni *et al.*, 2001; Rosa *et al.*, 2018) and should be followed up every 12-24 months. Patients with ECG changes consistent with CCC should undergo a routine cardiac measurement to establish the stage of the disease such as 24-h Holter monitoring (detect arrhythmias), combined chest radiography and 2D (two-dimensional) echocardiography. In this view, clinicians can stratify patients by risk and implement appropriate treatment.

The better method of choice to identify congenital infection is microhaematocrit examination because it is very sensitive and a small amount of blood needed (Freilij 1995). The infant should be tested for anti-*T. cruzi* IgG antibodies at 6-9 months of age if the premature results are continually negative or if the test is not done early in life (Gomes, 2009; Raimundo, Massad & Yang, 2010).

The goal of treatment is to eliminate the parasite and target the signs and symptoms of the disease, however, is not adequate and involves parasite-specific therapy and adjunctive therapy for the management of the clinical manifestations (Machado & Dutra 2012). There are only two drugs, benznidazole (Bz) (Lafepe, Brazil) and nifurtimox (Lampit, Bayer 2502) that are recommended for Chagas' disease treatment (Mazzeti *et al.*, 2018). Both drugs are United States Food and Drug Administration approved, but are available under investigational use protocols. Anti-trypanosomal treatment is strongly recommended for all cases of acute, congenital and reactivated infection (Meymandi *et*

al., 2018), for children with infection and for patients up to 18 years of age with chronic disease. In other hand, antitrypanosomal treatment is not recommended during pregnancy, in patients with intense renal or hepatic insufficiency and to patients with advanced Chagas heart disease or megaesophagus (Rassi Jr, 2010; Meymandi *et al.*, 2018). The Bz has been extensively investigated because it has the best and safety efficacy and it is used for first-line treatment and is better tolerated overall (Viotti *et al.*, 2009 Ademar *et al.*, 2017). For adults, the doses are 5 to 7 mg/kg (Bz) per day for 60 days, or 8 to 10 mg/kg (nifurtimox) per day for 60 to 90 days. Children should be given 5 to 10 mg/kg (Bz) in 2 or 3 divided doses per day for 60 days, or 15 mg/kg (nifurtimox) in 3 divided doses per day for 60 to 90 days and both drugs should be given after meals. The drugs have variable efficacy, must be taken for extended periods, and patients may experience severe side effects such as vomiting, nausea and anorexia (Chatelain, 2015). These drugs are most effective for treatment of acute and congenital infection and the parasitological cure is believed to occur in 60-85% of persons with acute infection who complete a full course of either drug. The management of patients in the indeterminate stage to prevent transition to the chronic phase and whether they should receive these drugs is the focus on ongoing studies and currently there is no standard for the management of these indeterminate cases. It is important to know that both drugs, Bz and nifurtimox, are mutagenic (Gorla *et al.*, 1989; Diniz *et al.*, 2012). The intricate natural history of the *T. cruzi* infection and scarce tools to assess cure have made it not easy to define appropriate intervals and end points to be followed. In the acute phase, in the early congenital or reactivated *T. cruzi* infection, hemoculture and direct examination of blood or the buffy coat have been suggested for monitoring response to treatment. In the chronic phase of the infection, there is no assessing of confirmed value for documentation of responses. Because of recently blood bank screening, amplified community understanding and demographic alterations, clinicians are able to encounter further patients with Chagas diseases in the near future. It is important to notice that the treatment based on the nitroaromatic compounds (nifurtimox and Bz) offer unsatisfactory results and considerable effects. The development of new drugs to treat this neglected tropical disease is an urgent need (Chatelain, 2015). In the past few years the progresses and understanding in the biology and biochemistry of *T. cruzi* have permitted the identification of multiple new targets for Chagas disease chemotherapy. Among these

new targets for antiparasitic drugs are: cruzipain, trypanothione synthesis, ergosterol biosynthesis inhibitors and thiol dependent redox metabolism (Duschak, 2011).

In the last two decades' studies it has been demonstrated that *T. cruzi* needs specific sterols for cell viability and proliferation in the entire stages of its life cycle and the ergosterol biosynthesis pathway has been chemically confirmed in different steps *in vitro* (Urbina, 2002; Urbina *et al.,* 2003; Vannier-Santos *et al.*, 2019). Many studies have shown that the commercially available ergosterol biosynthesis inhibitors (EBI) have suppressive but not curative activity against *T. cruzi* infections in experimental animals or in humans and they can fail to stop the progression of the disease (Urbina, 2002; Urbina, 2003; Vannier-Santos *et al.*, 2019). Recent studies with posaconazol (POS) have shown that this compound can eliminate intracellular amastigote forms of *T. cruzi* from cultured cardiomyocytes (Silva *et al.*, 2006); Maclean *et al.*, 2018). Other studies have established that the anti-*T. cruzi* activity of POS in a murine model of acute Chagas disease is less dependent on IFN- γ than Bz (Ferraz *et al.*, 2007). POS was registered in 2005 in the European Union and Australia for treatment of invasive fungal infections, in 2006 in the USA for treatment azole-resistant candidiasis the prophylaxis of invasive fungal infections and under clinical trials for the specific treatment of chronic Chagas disease in the beginning of 2010 (Urbina, 2010). Additional triazoles (TAK-187, UR-9825 and ravuconazole) have been shown trypanocidal activity *in vivo* and *in vitro* (Ademar *et al.*, 2017).

As discussed before, *T. cruzi* contains cruzipain (a cathepsin L-like cysteine protease) also named gp51/57 and cruzain (recombinant enzyme) that is in charge for the main proteolytic activity of all stages of the parasite life cycle (Cazzulo, 2002; Urbina, 2003; Uehara *et al.*, 2012). From these results, cruzipain is an important and confirmed target for anti-*T. cruzi* chemotherapy. In the other hand, the potential of peptide-like inhibitors as anti-*T. cruzi* drugs remains to be investigated. The Y strain of the *T. cruzi* is partially susceptible to actual drugs used to combat the disease such as Bz and nifurtimox (Ademar *et al.*, 2017). In animal model, the success rate of those drugs on acute phase against the Y strain is high (Mazzeti *et al.,* 2018). These characteristics of this strains reinforce its importance in chemotherapy studies

8. Conclusions

Despite current advances, many points remain regarding the innate and acquired immunological mechanism associated with the resistance and the pathogenesis of Chagas disease. In the past few years, the relevance in Neglected Tropical Diseases has amplified as a result of numerous developments including new approaches to the control or abolition of these diseases. There is no vaccine or drug for prophylaxis for American trypanosomiasis. Preventive measures are targeted at minimizing contact with vectors. However, is necessary to understand the immune mechanisms involved in the control of these infection and thus new candidate therapeutic or prophylactic targets that are effective and substantially free of side effects. In fact, there are a lot of unanswered points requiring scientific examination. Biomarkers for both diagnosing infection and assessing parasitological cure are examples that lead from the acute to chronic disease, determining whether anti-parasitic therapy reduces the possibility of developing chronic disease.

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